Trophic relationships of three deep-sea crustacean species (*Aristeus varidens, Plesionika martia and Funchalia woodwardi*) off Namibia

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Abstract

Trophic ecology of deep-sea crustaceans in the marine waters off Namibia is not well understood, although they form part of diets of important commercial species off Namibia. Study of their trophic relationships enhances our understating of the functioning of marine food webs and avail information needed for consideration of trophic interaction in fisheries management decisions. This study aimed at investigating the trophic relationships of striped red shrimp (*Aristeus varidens*), golden shrimp (*Plesionika martia*) and Woodward's pelagic shrimp (*Funchalia woodwardi*) using stable isotope (nitrogen $\delta^{15}N$) measurements of their muscle tissues. Samples were collected during the 2010 annual hake biomass survey off Namibia. Significant differences were observed in the mean trophic levels and nitrogen (δ^{15}) measurements of the three species. *Funchalia woodwardi* showed a negative linear relationship between size and δ^{15} and between depth and δ^{15}. *Aristeus varidens* and *P. martia* showed no significant linear relationship between δ^{15} and depth or size. This work is the first to examine trophic relationships of the three deep sea crustaceans in the Northern Benguela current ecosystem using stable isotope analysis.

Keywords: Trophic level, stable isotope, *Funchalia woodwardi*, *Aristeus varidens*, *Plesionika martia*, Namibia, Benguela current.

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1 Introduction

The Namibian marine ecosystem is characterized by the Benguela current upwelling ecosystem (BCUE) that is rich in both primary and secondary production. The high productivity in the BCUE supports abundant pelagic and demersal species (Iitembu et al. 2012). Crustaceans are key components of the Benguela current upwelling ecosystem. They have been found in the diet of commercial demersal fish, squids, birds and whales (Roel and Macpherson, 1988; Pillar and Wilkinson 1995; Farina et al. 2008; Hünerlage et al. 2011). A change in the biomass of crustaceans have the potential of modifying the energy transferred between lower and upper trophic levels and may have effects on commercial fish stock (Werner et al.2011). *Funchalia woodwardi* occurs at depths between 50 and 3000m and can grow up to a length of 16cm while *Aristeus varidens* occurs at depths between 400-824m and can grow up to 20 cm in length whereas *Plesionika martia* occurs at depths between 300 and 700m (Bianchi et al. 1999). Both *Aristeus varidens* and *Plesionika martia* are commonly found in the Northern part of Namibia down to 24°S, and *Funchalia woodwardi* is distributed southward from the central part. All three species are in the crustacean order Decapoda (Levinton, 2001) characterized by 5 pairs of pereiopods.

In a marine ecosystem, species and populations interact according to the trophic levels they occupy in a food web (Vrede 1998). A trophic level of a particular species represents its energetic interactions or the role it plays within a food web and it is dependent on the variation in the body size of the organism (Carscallen et al. 2012). Stable isotope analysis (SIA) is widely used in ecology to improve our understanding of ecological systems (Haines 1976; West et al. 2006). The SIA is based on the understanding that there is an isotope enrichment that occurs at a predictable enrichment factor per trophic level (Minagawa and Wada 1984; Peterson and Fry 1987). Due to metabolic processes in an organism, heavier isotopes such as ^{15}N and ^{13}C are retained within the animal tissue (Boyle et al. 2012). As a result there is a constant increase in the isotopic value as trophic level increases. Each trophic level has a specific nitrogen isotope signature based on nitrogen isotope value in an identified organism's tissue (Minagawa and Wada 1984). Analysis of stable isotopes from an animal's tissue can therefore determine the isotopic value of that animal and it can be compared to other parts of the trophic systems to determine its trophic level (Boyle et al. 2012).

Although deep-sea crustaceans are important in food webs of the marine waters off Namibia, there is little understating of their trophic relationships. In this study we used nitrogen isotopic values ($\delta^{15}N$ (‰) to determine the trophic level of the deep sea crustaceans (*Aristeus varidens, Plesionika martia* and *Funchalia woodwardi*). The relationships between depth, size (length), trophic levels and nitrogen isotopic values ($\delta^{15}N$ (‰) were also assessed. Our study contributes towards efforts to generate scientific knowledge that can aid in integration of ecosystem considerations into the management of these fishery resources off

Namibia.

2 Materials and methods

2.1 Study Area

The study was carried out within the boundary of the Namibian Exclusive Economic Zone (EEZ) stretching from southern border with South Africa (29°12'S) to the northern part of Namibian waters (19°12'S)(Fig 1). Samples from *Aristeus varidens, Funchalia woodwardi* and *Plesionika martia* were collected over a depth range from 90 to 600 m.

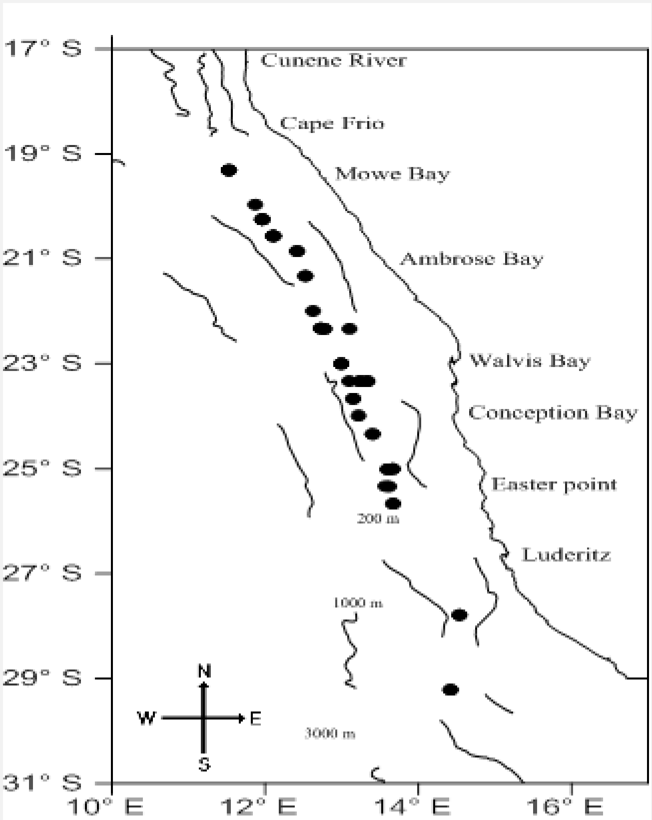

Figure 1: The study area showing sampling stations for the three crustacean species off Namibian coast.

2.2 Sample collection

Samples of crustacean species were collected from the annual hake biomass survey conducted by the National Marine Information and Resource Centre (NatMIRC) using the *MFV Blue Sea 1* research vessel from the 12th January to the 21st February, 2010. The survey follows predetermined sampling sites using a *Gisund Super two-panel* bottom trawl (head length 31m, footrope 47m and the vertical net opening 4.5 to 5.5m) used to catch hake at a speed of about three knots. Sampling was opportunistic with a general of goal of getting a wider size selection of each species and collecting sample at the same trawls station. At each station where sampling was done, individual's crustacean collected where identified and measured (Total length). Samples were rinsed with distilled water, put in plastics, frozen at -20°C on board the vessel and taken to the laboratory at NatMIRC for preparation of stable isotope analysis. The preparation of samples for isotopic analysis was done following a method used by Jardine et al. (2003).

2.3 Laboratory Analyses

The stable isotope analysis (SIA) was done at the ISO-Environmental CC Laboratory in Rhodes University (Grahamstown, South Africa). The analyses were done using a Europa Scientific Elemental Analyzer coupled to a 20-20 Isotope Ratio Mass Spectrometer (IRMS). Beet sugar, ammonium sulfate and casein were used as in-house standards calibrated against International Atomic Energy Agency (IAEA) standards CH-6 and N-1. The $^{12}C/^{13}C$ and $^{14}N/^{15}N$ isotope measurements were expressed in the delta notation relative to the levels of ^{13}C in Pee Dee Belemnite and ^{15}N in atmospheric nitrogen (N2), according to the following equation: $\delta X = \{(R_{sample}/R_{standard}) - 1\} \times 1000$, where X is ^{13}C or ^{15}N and R is the ratio of the heavy to light isotope for the sample (R_{sample}) and standard ($R_{standard}$) in units of parts per thousand (‰).

2.4 Trophic level calculations

Trophic levels (TL) of crustaceans were calculated following Post (2002): $[(\delta^{15}N_{consumer}$-$\delta^{15}N_{base})/\Delta\delta^{15}N] + 2.0$, where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ value of the consumer, $\delta^{15}N_{base}$ is the baseline $\delta^{15}N$ value of the food web, $\Delta\delta^{15}N$ is the trophic enrichment factor and 2.0 is the trophic level of the baseline organism used to establish the $\delta^{15}N_{base}$. The trophic enrichment factor set at 3.4 ‰was used (following Iitembu et al. 2012; Boyle et al. 2012). For the baseline $\delta^{15}N_{base}$ value we used 9.2‰established by Kohler et al. (2011) from the mean of two mytilid bivalve species (*Choromytilus meridionalis* and *Mytilus galloprovincialis*) studied in 2009 off Luderitz, Namibia. The use of bivalves to establish the $\delta^{15}N_{base}$ is based on their feeding technique (filter feeders) and their isotopic turnover rates that can adapt to seasonal variability (Lorrain et al. 2002; Iitembu et al. 2012).

2.5 Statistical analyses

Single factor analysis of variance (ANOVA) was used to determine if there are significant differences in the trophic levels and nitrogen values of the deep sea crustacean's species (*Aristeus varidens, Plesionika martia* and *Funchalia woodwardi*). For each species, simple linear regression analysis was used to determine the relationship between the sizes (length) and trophic levels. Simple linear regression analysis was further used to determine the relationship between water depths at which the crustacean species were found and their trophic levels. A correlation analysis was run to test the strength of the relationships. The ANOVA and regression analyses were carried out at $\alpha = 0.05$. We adjusted the significance level for post hoc test (Tukey) to $\alpha = 0.0167(0.5/3)$ to control the type I error.

3 Results

3.1 Trophic levels and nitrogen isotopes

Aristeus varidens had the highest mean *TL* and δ^{15}N (mean length = 6.8) while *P. martia* had the lowest mean *TL* and δ^{15}N (mean length = 8.5) (Table 1). The results from comparison of mean trophic levels and mean nitrogen values of the three species (*A. varidens, P. martia* and *F. woodwardi*) from one way analysis of variance (ANOVA) showed that there was a significant difference among the mean *TL* ($p < 0.05$), and mean δ^{15}N ($p = 0.012$). The post-hoc comparisons of mean trophic levels using Tukey HSD test indicated that *P. martia* was significantly different from *A. varidens* ($p < 0.0167$) but not *F. woodwardi* ($p > 0.0167$). No significant differences were found between *A. varidens* and *F. woodwardi* ($p > 0.0167$). In terms of nitrogen values (post-hoc Tukey, HSD test), *P. martia* and *A. varidens* were significantly different from each other ($p = 0.014$) while *A. varidens* and *F. woodwardi* were not significantly different ($p = 0.852$). *F. woodwardi* was also not different from *P. martia* ($p = 0.06$).

Table 1: Species ($n =$ sample size) mean trophic levels ($\pm$ s.e.), mean^{15}N value ($\pm$ s.d.) and mean length (cm).

	A. varidens $n = 17$	*F. woodwardi* $n = 15$	*P. martia* $n = 17$
Mean trophic level	2.8$\pm$ 0.07	2.7 $\pm$0.13	2.4 $\pm$ 0.08
Mean δ^{15}N(‰)	11.9$\pm$0.32	11.6$\pm$0.51	10.6$\pm$ 0.31
Mean total length (cm)	6.8	9.3	8.5

3.2 Depth and nitrogen isotope

Plesionika martia and *A. varidens* did not show any significant relationships between depth and δ^{15}N ($p = 0.140; p = 0.718$). However, *F. woodwardi* displayed a significant negative linear relationship ($p = 0.003$) between depth and δ^{15}N (Fig.2). *F. woodwardi* also had the strongest correlation between δ^{15}N and depth with $r = -0.715$ as compared to *P. martia* ($r = 0.373$) and *A. varidens* ($r = -0.095$).

3.3 Size and nitrogen isotope

Aristeus varidens and *P. martia* did not show significant linear relationships ($p = 0.902; p = 0.344$) between size and nitrogen measurements. A significant negative linear relationship was displayed ($p = 0.011$) between nitrogen and size for *F. woodwardi* (Fig.3). *Aristeus varidens* displayed a weak correlation between size and δ^{15}N ($r = 0.032$) (Fig. 3). *Plesionika martia* and *F. woodwardi* correlation coefficients between size and δ^{15}N were: $r = 0.244$ and $r = -0.636$, respectively.

4 Discussion

This study was aimed at investigating the trophic relationships in relation of the three deep sea crustaceans: *Aristeus varidens*, *Plesionika martia* and *Funchalia woodwardi*, off the coast of Namibia. We observed significant differences between the mean trophic levels and nitrogen values of the three crustaceans. A significant relationship between size and δ^{15}N and between depth and δ^{15}N were observed in *F. woodwardi* but not in *P. martia* and *A. varidens*.

Aristeus varidens had the highest δ^{15}N (11.9) of all the crustacean species in this study. This can be an indication that it feeds at a higher trophic level than *Plesionika martia* which

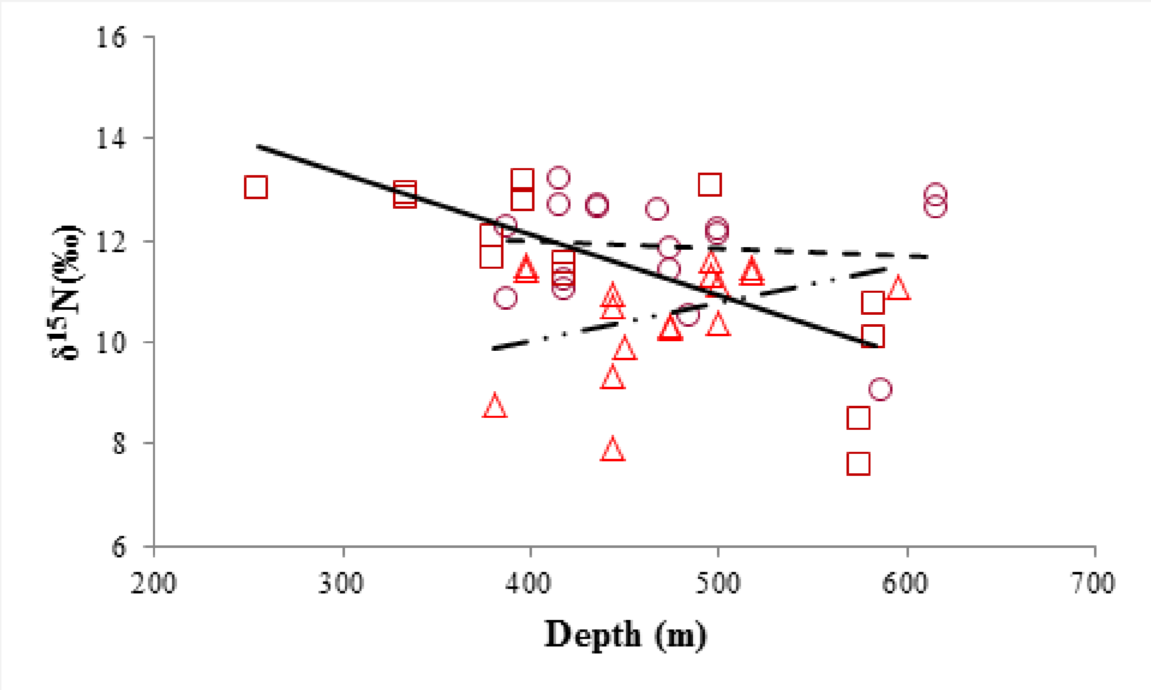

Figure 2: The plot of δ^{15}N with depth (m) for *A. varidens* (◯), *P. martia* (Δ) and *F. woodwardi*(□). The dashed line signifies a linear line ($R^2 = 0.009$) for *A. varidens*. The straight line signifies a linear line ($R^2 = 0.511$) for *F. woodwardi* and the long dash dot dot line signifies the linear line ($R^2 = 0.14$) for *P. martia*.

had the lowest δ^{15}N (10.6). There was, however, no significant differences ($p > 0.0167$) observed in the mean δ^{15}N (‰) of *A. varidens* and *Funchalia woodwardi*, an indication that they feed at the same trophic level. In terms of trophic levels of *A. varidens* and *F. woodwardi*, there were also no significant differences observed, which confirms the similarity in δ^{15}N. The observed similarity in the mean trophic levels of the three crustacean species as compared to δ^{15}N found in their tissues is due to the fact that trophic levels are calculated based on nitrogen isotope measurements (Post 2002). If one assume that *TL* of 3.0 represent complete zooplanktivory as in Iitembu et al. (2012), our results indicate that the three crustaceans (*A. varidens (TL=2.8), P. martia (TL=2.4)* and *F. woodwardi (TL=2.7))* are zooplanktivorous. Trophic levels and δ^{15}N values of *A. varidens*, mean length 6.8cm (2.8; 11.9‰) from our study matches with that of small *Trachurus capensis*, mean length 18.7 cm (2.8; 11.9 ‰) from Iitembu et al. (2012), indicating that the two species feed at the same trophic level. Juveniles of *T. capensis* and *A. varidens* possibly feed on the same zooplanktons given their *TL* of 2.8. Trophic levels and δ^{15}N measures of *F. woodwardi* (2.7; 11.6‰) are closer to that of *Pasiphaeidae* (2.7; 11.5‰). The variations in trophic levels and nitrogen values between the species can also be attributed to various factors such as poor

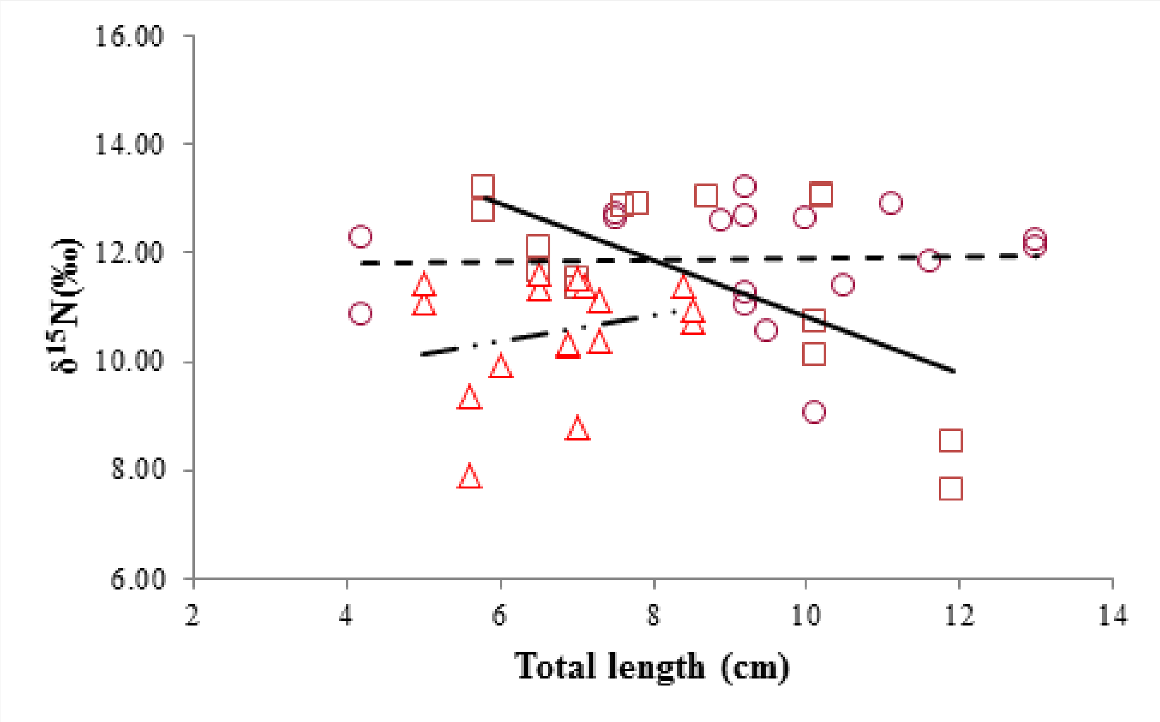

Figure 3: The plot of δ^{15}N with total length for *A. varidens* (◯), *P. martia* (Δ) and *F. woodwardi*(□). The dashed line signifies a linear line ($R^2 = .001$) for *A. varidens*. The straight line signifies a linear line ($R^2 = .0.404$) for *F. woodwardi* and the long dash dot dot line signifies the linear line ($R^2 = .060$) for *P. martia*.

food quality and isotopic routing (Gannes et al. 1997; Peterson 1999).

In term of depth, *Plesionika martia* and *A. varidens* did not show any significant relationships between depth and δ^{15}N ($p = 0.140; p = 0.718$). According to Bianchi et al.(1999), the two species inhabit water depths of between 300m to 800m. The lack of a relationship between depth and δ^{15}N can be an indication that the feeding patterns of these two species does not change with depth. However the observed significant negative linear relationship between size and δ^{15}N ($p = 0.001$) and between depth and δ^{15}N ($p = 0.003$) in *F. woodwardi* (Fig.2), can be an indication that its feeding pattern changes with size (growth) and depth. *Funchalia woodwardi* has been documented to occur at depths between 50 and 3000m (Bianchi et al. 1999), with such a wider depth range, it is possible that its different life stages inhabit different depth ranges. Furthermore, the diets of many species changes throughout their life cycle, as the increasing size improve their ability to feed on larger prey. Different species have been observed to shifts diet as they grow (Mittelbach and Persson, 1998; Iitembu et al. 2012). The wide distribution depth ranges of *F. woodwardi* can also be an indication of the diversity of resources available to this species. Positive correlation between size and depth observed in our study can be indications of ontogenetic trophic shifts in this species. *Aristeus varidens* and *P. martia* showed no significant linear relationships ($p = 0.902; p = 0.344$) between size and nitrogen measurements. The above is an indication that their feeding pattern do not change with growth.

While our study only looked at the trophic relationships between the three crustacean species, the data herein can be used for comparison of these crustacean species with other marine important species available in the literatures. The three species are important prey for many marine organisms including hakes and monkfish, important commercial fish species in Namibia. The isotopic values in this study can also be used as a benchmark for studies of trophic relationships in the Benguela current ecosystem. This study only focused on three crustacean species and data do not include seasonal variations for comparisons. Temporal differences in the trophic relationships of these species are likely to occur, because isotopic signatures of their food sources possibly vary according to season. Future studies need to incorporate other factors such as seasonal variability and other crustaceans species that were not examined in this study. This study has only used 15N isotope measurements, other isotopes like that of carbon, sulfur and oxygen can be used in future studies to improve our isotope-based inferences of the trophic relationships of important crustaceans.

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