



# Seasonal variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fish and squid in the Cabo Frio upwelling system of the southwestern Atlantic

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**ABSTRACT:** To determine whether shifts occurred in the food web associated with upwelling at the Brazilian western boundary South Atlantic Central Water (SACW), we measured isotopic composition ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of 38 fish and 3 squid species from the Cabo Frio food web. Sampling was performed during the austral summer upwelling period (February 2002) and the austral winter non-upwelling period (July 2001). Mean lipid-corrected  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{cor}}$ ) values ranged from  $-20.17$  to  $-15.02\%$ , and mean  $\delta^{15}\text{N}$  ranged from 10.28 to 16.08‰. An analysis of covariance performed using length as a covariate provided evidence of seasonal effects on the mean fish  $\delta^{13}\text{C}_{\text{cor}}$  and  $\delta^{15}\text{N}$ , with higher values during the non-upwelling period than during the upwelling period. These effects are most likely related to biophysical processes that occurred 1 to 6 mo earlier. The data furnish circumstantial evidence of a link between the dynamics of the SACW and the trophic structure of Cabo Frio. The trophic level of the species of fish and squids ranged between the theoretical trophic levels of 3.6 and 5.1, but there were no discrete steps, rather a continuous gradient in isotopic values. This suggests that these food webs are unstructured and the component species have mixed diets.

**KEY WORDS:** Food web · Stable isotope · Upwelling system · South-eastern Brazil

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## INTRODUCTION

The shelf system off the coast of Cabo Frio ( $23^{\circ}\text{S}$ ,  $43^{\circ}\text{W}$ ) is the main Brazilian western boundary coastal upwelling of the South Atlantic Central Water (SACW). Although the SACW contribution occurs intermittently over the shelf and throughout the year, inputs of relatively deep cold water are more frequent during the warm seasons (spring and summer). The upwelling of nutrient-rich waters to the euphotic zone is therefore more common during the warmer months (Castro & Miranda 1998, Silveira et al. 2000, Mahiques et al. 2005). During the upwelling season, nitrate concentrations can range from 2.8 to 7.7  $\mu\text{M}$ , and the chlorophyll *a* (chl *a*) level reaches up to 25.5  $\text{mg m}^{-3}$  (Moser & Giancesella-Galvão 1997).

During the colder months (autumn and winter), when upwelling seldom occurs, the temperature profiles are vertically homogeneous and the surface waters are typically oligotrophic (Valentin et al. 1987, Gonzalez-Rodriguez et al. 1992, Castro & Miranda 1998, Pereira et al. 2009). The concentrations of dissolved inorganic nitrogen during the winter were half those measured during the summer, and the concentrations of chl *a* varied from 5 to 12  $\text{mg m}^{-3}$  (Gonzalez-Rodriguez et al. 1992). Seasonal variation in upwelling thus leads to marked seasonal shifts in primary production, as the nutrient-rich water of the SACW upwelling favours higher phytoplankton biomass and productivity during the warmer months than the colder months.

Seasonal changes in the composition of fish (Fagundes-Netto & Gaelzer 1991), cephalopods (Costa & Fernandes 1993), benthic microbial communities (Sumida et al. 2005) and benthic megafauna (De Léo & Pires-Vanin 2006) have been associated with the seasonality of the upwelling. These variations may be associated with trophic changes, and may affect population dynamics.

Previous analysis of gut contents of some fish species from the study area revealed that seasonal changes in diet composition were related to either prey availability or predator size (Vera & Soares 2008, Muto & Soares 2011). However, gut content analysis only reveals recent meals, and stable isotope signatures may furnish a more reliable record of a time-integrated measure of the diet based on the body tissues (Hobson & Welch 1992).

The temporal discontinuity of primary biological production may be transferred throughout the food web, causing changes in the components of the community and a shift in trophodynamics. Defining such changes may be best performed by the application of stable isotope measures (Hadwen & Arthington 2007, Bowen & Valiela 2008, McKinney et al. 2010, Layman et al. 2012). The  $\delta^{13}\text{C}$  values of the consumers within a food web provide an indication of the carbon sources that support the food web; the  $\delta^{15}\text{N}$  values provide an indication of the trophic level of a species (Minagawa & Wada 1984, Peterson & Fry 1987, McCutchan et al. 2003, Guelinckx et al. 2007).

Biogeochemical processes and the food web in marine upwelling ecosystems have been studied using stable C and N isotopes (Wu et al. 1999, Holmes et al. 2002, Loick et al. 2007, Madigan et al. 2012). Wu et al. (1999) found significant upwelling-linked shifts in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in sinking particulate matter off Vancouver Island, Canada, with these shifts most likely being governed by changes in abundance of nanophytoplankton and diatoms in surface water. Holmes et al. (2002) found a close positive correlation between  $\delta^{15}\text{N}$  values of sinking particulate matter and sea surface temperature in the Benguela offshore region upwelling, where a lower value of  $\delta^{15}\text{N}$  of particulate nitrogen was found in nutrient-rich, cold surface water, and elevated productivity. Loick et al. (2007) found that in the Vietnam upwelling, nitrogen-fixation by phytoplankton provided up to 13% of the nitrogen reaching the higher trophic levels and the remainder was furnished by the upwelling. We expected that upwelling-linked seasonal shifts would impact the Cabo Frio food web because there was evidence that seasonal changes in isotopic values do occur in certain fish (Muto & Soares 2011).

In this paper, we report a first application of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  methods to delineate trophic interactions of 38 fish and 3 squid species representative of the food web of the Cabo Frio upwelling off the coast of Brazil. We used these isotopic data to define the sources of organic matter in the food web, the relative trophic position of the fishes and squids, and the linkage of the food web to the seasonal occurrence of upwelling. Our results may be useful for understanding the food web structure and energy flow for future trophic modelling studies, as well as for forecasting the potential impacts of natural and human-induced declines in prey or predator populations (Madigan et al. 2012).

To achieve our goal, our analysis needed to consider the isotope time turnover of the food web components. The isotope time turnover data available for plankton range within a period of days (Montoya et al. 1991), and for fish muscle tissue, within a period of months (Lorrain et al. 2002, Herzka 2005, Guelinckx et al. 2007, Buchheister & Latour 2010, Fanelli & Cartes 2010).

Our study was part of a broad, multidisciplinary effort (SW Atlantic Continental Shelf Ecosystem Dynamics Project) aimed at clarifying the impact of the cold SACW on the marine ecosystem. The main purposes of this work were (1) to analyse the temporal changes of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and (2) to estimate the trophic levels of representative fish and squid species.

## MATERIALS AND METHODS

### Study area

The study site ( $22^{\circ}57.91' - 23^{\circ}06.4'S$ ,  $41^{\circ}59.0' - 42^{\circ}07.9'W$ ; Fig. 1) on the continental shelf off the coast of Cabo Frio is located in central-southern Brazil. Cabo Frio has a dry climate (mean annual rainfall = 823 mm), with a higher rainfall rate during the austral summer (December, the wettest month, has a rainfall rate of <114 mm) and low rainfall rate during winter (August, the driest month, has a rainfall rate of <42 mm) (Barbieri & Coe Neto 1999). There may be some anthropogenic influence on the area owing to wastewater effluent discharge from the coastal residential area (Sella et al. 2006).

The shelf is relatively narrow (~50 km wide), with a few rivers contributing terrigenous materials (Ekau & Knoppers 2003). This system is subjected to the intermittent N-NE-wind-driven western boundary coastal upwelling of the SACW (temperature = 14–15°C; salinity = 35.6–35.8), which is stronger in austral

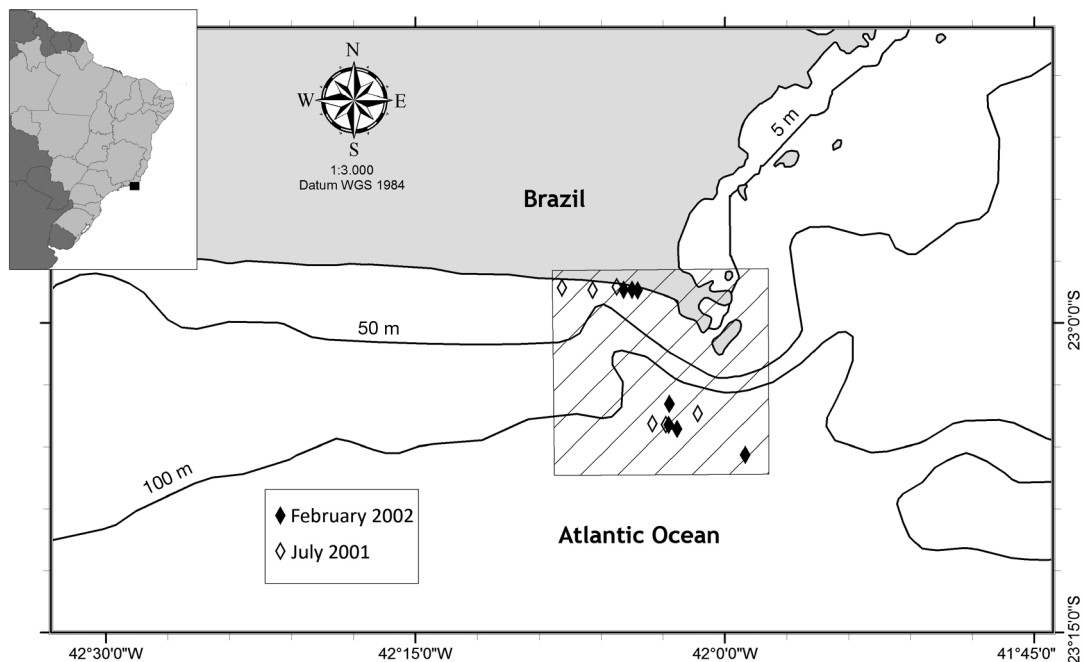


Fig. 1. Southeastern coast of Brazil, showing the sampling sites on the continental shelf off the coast of Cabo Frio during the non-upwelling (July 2001; empty symbols) and the upwelling season (February 2002; filled symbols)

spring and summer seasons and brings colder, nutrient-rich waters to the surface (Castro & Miranda 1998). The 50–100 m isobaths are less than 5 km from the shore, favouring the upwelling of the deep water up to 20 km from the coast (Valentin et al. 1987).

According to Gonzalez-Rodriguez et al. (1992), there are 3 phases during the upwelling between September and April (austral spring to autumn). First, the upwelling of cold, nutrient-rich water occurs. Second, increases in the surface water temperature, phytoplankton biomass and productivity occur, accompanied by a decrease in the nutrient concentrations. Third, a subsidence phase occurs, characterized by a return to oligotrophic conditions. During the S-SW winds (June to August; austral winter) and prevailing southern cold fronts, upwelling is inhibited and the oligotrophic condition persists. During the non-upwelling season, nutrient sources essential to the food web depend on regeneration from the detritus.

During our sampling period, the usual upwelling dynamics occurred. Kampel (2003) found no coastal upwelling during July 2001, a week before our sampling, but reported SACW at depths of 50 to 100 m on the continental shelf. During February 2002, also a week before our sampling, SACW upwelled to the 25 m isobath.

Large surface chlorophyll values were registered during 2001 and 2002, with higher peaks in austral

spring 2001 and summer 2002, which is the time of the usual upwelling of SACW in the study area. This enhanced primary productivity is reflected in the sediments by microbial biomass and labile organic matter content, which are available as a food source for the benthic fauna (Sumida et al. 2005).

### Field and laboratory work

Sampling was performed during 2 cruises on board the RV 'Prof. W. Besnard' during July 2001 (austral winter; non-upwelling season) and February 2002 (austral summer; upwelling season) at sites in the shallow area and the deeper area (Fig. 1). Fish and squid were collected using an otter trawl, samples of water for collecting suspended particulate matter (on the surface, in the thermocline range and at the bottom, below the thermocline), were obtained using a Niskin bottle, and samples for surface sediment organic matter (SOM) analysis were collected with a box-corer. Decayed leaves of grass plants and macroalgae (red algae, *Ulva* sp. and *Sargassum* sp.) were found in the otter trawl samples.

Fish and squid samples were frozen at  $-20^{\circ}\text{C}$  and were later thawed for sorting, identified to species and measured to determine their total length and mantle length (nearest mm), respectively. Samples of muscle tissue were excised from individual fish and

squid, and these samples were pooled to create representative composite samples, aiming for a minimum of 5 individuals per pool.

Suspended particulate matter (SPM), consisting of phytoplankton and other particulates, was collected by gently filtering seawater—first, using a 125 µm mesh net, then onto pre-combusted (400°C for 1 h), 47 mm, GF/F glass fibre filters (0.7 µm nominal pore size), until the flow of water decreased to a slow drip. The filters were stored frozen at –20°C. The leaves of the plants and macroalgae were gently washed with distilled water and stored frozen. Samples of sediment were kept frozen at –20°C.

All samples were freeze-dried (–60°C; 48 h), ground to a homogenous powder with a mortar and pestle and stored in sealed vials. Subsamples of approximately 2 mg were packaged into 5 mm × 8 mm tin capsules and stored over desiccant until the analysis could be performed ashore. The samples were analysed at the University of California, Davis Stable Isotope Facility. Isotope ratios were measured in a continuous flow isotope ratio mass spectrometer (IRMS) used for high precision analysis of combusted solid samples at natural abundance (Europa Scientific Hydra 20/20 IRMS; Europa Scientific). The standard reference materials were Vienna PeeDee Belemnite for δ<sup>13</sup>C and atmospheric N<sub>2</sub> for δ<sup>15</sup>N. Inorganic carbon was removed by HCl fumigation before analysis of δ<sup>13</sup>C. Total organic carbon and total nitrogen elemental composition were measured with the IRMS, using size standards with our samples.

Stable isotope ratios are expressed using the delta (δ) notation, which is defined in units of per mil (‰), according to the following relationship:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 \quad (1)$$

where  $X = {}^{13}\text{C}$  or  ${}^{15}\text{N}$  and  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$  (Peterson & Fry 1987).

### Data analysis

The bulk δ<sup>13</sup>C data of fish and squid species were lipid-normalized (δ<sup>13</sup>C<sub>cor</sub>) according to Kiljunen et al. (2006), to avoid the confounding effect of lipid storage on the interpretation of the results.

To verify the effect of season on the mean δ<sup>13</sup>C<sub>cor</sub> and δ<sup>15</sup>N isotopes of the pooled fish and squid species, we used a Minitab® 16.1.0 routine to perform *t*-tests and general linear model (GLM) analyses of covariance (ANCOVA), using specimen length as the covariate. This technique attempts to make allowances between groups to adjust length imbal-

ances among samples, to verify the effect of season independent of any length differences between seasons that may exist. We also performed *t*-tests and GLM ANCOVA for the 7 species with adequate sample sizes (≥3) in the 2 periods. All data were normal (non-significant, Kolmogorov-Smirnov test), or according to Minitab® assistant, normality was not an issue because sample sizes were at least 15. Only the GLM ANCOVA results are presented because both tests showed the same results. To verify the effect of season on SPM and SOM, we used a *t*-test after verifying the homogeneity of variances.

The trophic level (TL) of each species was calculated according to the equation of Jennings et al. (2002) (TL = [δ<sup>15</sup>N species – δ<sup>15</sup>N bivalve]/3.4) + 2.5), considering the δ<sup>15</sup>N (July 2001 = 8.18‰; February 2002 = 6.69‰) of the local suspensivore bivalves (T.N. Corbisier pers. comm.), and a trophic enrichment factor (TEF) of 3.4‰ for the δ<sup>15</sup>N (Minagawa & Wada 1984, Søreide et al. 2006).

For the interpretation of the results, we also considered a TEF of 1‰ for δ<sup>13</sup>C (Vander Zanden & Rasmussen 2001). Additionally for consumers, we considered a lag time of 1 to 6 mo before sampling, based on the biological turnover data available for fish muscle tissue (Lorrain et al. 2002, Herzka 2005, Guelinckx et al. 2007, Buchheister & Latour 2010, Fanelli & Cartes 2010). Feeding habits of the studied species are summarized in the Supplement at [www.int-res.com/articles/suppl/m512p009\\_supp.pdf](http://www.int-res.com/articles/suppl/m512p009_supp.pdf).

## RESULTS

### Seasonal variability

The mean values of δ<sup>13</sup>C, δ<sup>13</sup>C<sub>cor</sub>, δ<sup>15</sup>N and bulk C:N ratios for fish and squids in July 2001 (non-upwelling season) and February 2002 (upwelling season) are described in Table 1 (feeding habits of the species are available in the Supplement). Stable isotope analyses were performed on 38 fish species and 3 squid species that are common representatives of the food web composition within the Cabo Frio upwelling system. Twenty-two fish species were found in both seasons.

Mean δ<sup>13</sup>C<sub>cor</sub> values of fish ranged from –17.92 to –15.02‰ in the non-upwelling season and from –20.17 to –16.77‰ in the upwelling season. Mean δ<sup>15</sup>N values of fish ranged from 12.13 to 16.08‰ in the non-upwelling season and from 10.28 to 15.51‰ in the upwelling season. The squid values ranged from –18.20 to –17.23‰ and from 10.97 to 12.83‰, for δ<sup>13</sup>C<sub>cor</sub> and δ<sup>15</sup>N, respectively (Table 1).



The values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for SPM, SOM, macroalgae and grass leaves are also shown in Table 1. SPM  $\delta^{15}\text{N}$  values did not vary between seasons ( $t = 0.27$ ,  $p = 0.793$ ,  $df = 11$ ), and SPM  $\delta^{13}\text{C}$  values were lower in July (non-upwelling season) than in February (upwelling season;  $t = -5.10$ ,  $p = 0.000$ ,  $df = 11$ ). SOM  $\delta^{15}\text{N}$  values did not vary between seasons ( $t = 0.51$ ,  $p = 0.623$ ,  $df = 10$ ).

The  $\delta^{13}\text{C}_{\text{cor}}$  or  $\delta^{15}\text{N}$  values of fish were consistently higher during the non-upwelling season relative to the upwelling season (Fig. 2, Table 2). The squid species sampled in the upwelling season were pooled as *Doryteuthis* spp., for comparison between periods. There was no evidence that  $\delta^{13}\text{C}_{\text{cor}}$  or  $\delta^{15}\text{N}$  means differed with season (Table 2).

ANCOVA results for the 7 fish species with at least 3 measurements are listed in Table 3. We found an

effect of season on  $\delta^{15}\text{N}$  values (higher in the non-upwelling season) for 4 species (*Gymnothorax conpersus*, *Lophius gastrophysus*, *Mullus argentinae*, *Porichthys porosissimis* and *Raneya brasiliensis*), and no effect for *Etropus longimanus* and *Trachurus lathami*. Regarding  $\delta^{13}\text{C}_{\text{cor}}$ , there was an effect of season on 4 species (higher in the non-upwelling season), and no effect was observed for *G. conpersus* and *R. brasiliensis*.

### Sources of organic matter for fish and squid

To identify sources that furnish organic matter to the consumers within the Cabo Frio food web, and to better depict the structure of the food web, we plotted  $\delta^{13}\text{C}_{\text{cor}}$  versus  $\delta^{15}\text{N}$  of the consumers we collected (Fig. 3).

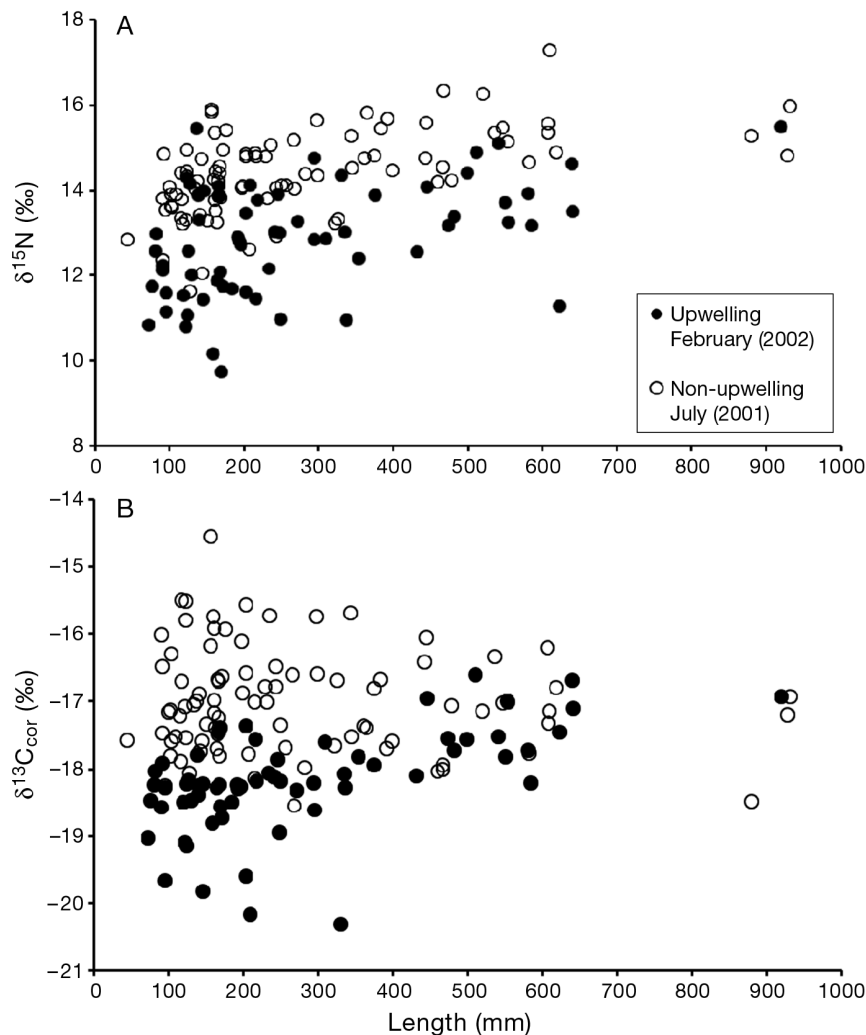


Fig. 2. Means of (A)  $\delta^{15}\text{N}$  and (B) lipid-normalized  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{cor}}$ ) values against the size of fish (all species pooled) from the Cabo Frio upwelling system in the non-upwelling (July 2001; empty symbols) and the upwelling season (February 2002; filled symbols). Statistical results are provided in Table 2. The length-adjusted regression equation for  $\delta^{15}\text{N}$  is  $y = 12.853 + 0.0029x$ ,  $R^2 = 0.47$ , and for  $\delta^{13}\text{C}_{\text{cor}}$   $y = -17.847 + 0.008x$ ,  $R^2 = 0.37$ . Both equations are significant at  $p < 0.001$

Table 2. Results of the general linear model analysis of covariance (factor: period; covariate: length) for stable isotopes of the pooled fish and squid species from the Cabo Frio system in July 2001 (non-upwelling season) and February 2002 (upwelling season). \*p < 0.05, \*\*\*p < 0.001;  $\delta^{13}\text{C}_{\text{cor}}$ : lipid-normalized  $\delta^{13}\text{C}$ ; adj: adjusted; ns: non significant; n: number of samples

	July 2001	February 2002	Length <i>F</i>	Period <i>t</i>
<b>FISH</b>				
All species (df = 153)	n = 88	n = 66		
Mean length = 274.2 mm ( $\pm 188.5$ SD)				
Adj mean $\delta^{15}\text{N}$ (SE)	14.42 (0.11)	12.89 (0.12)	46.45***	-9.43***
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-16.99 (0.09)	-18.27 (0.10)	4.72*	-9.38***
Species in both periods (df = 120)	n = 66	n = 55		
Mean length = 268.2 mm ( $\pm 180.0$ SD)				
Adj mean $\delta^{15}\text{N}$ (SE)	14.35(0.12)	12.99 (0.13)	39.94***	-7.42***
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-17.09 (0.10)	-18.29 (0.11)	14.23***	-8.34***
<b>SQUID (df = 7)</b>				
Mean length = 75.35 mm ( $\pm 30.95$ SD)	n = 4	n = 4		
Adj mean $\delta^{15}\text{N}$ (SE)	12.80 (0.43)	11.74 (0.43)	0.07 ns	-1.66 ns
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-17.25 (0.30)	-17.97 (0.30)	0.04 ns	-1.68 ns

Table 3. Results of the general linear model analysis of covariance for the isotope signatures of the fish species (factor: period; covariate: length) from the Cabo Frio system in July 2001 (non-upwelling season) and February 2002 (upwelling season). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001; ns: non significant; nd: not done (non-normal data); adj: adjusted; n: number of samples;  $\delta^{13}\text{C}_{\text{cor}}$ : lipid-normalized  $\delta^{13}\text{C}$

Species	July 2001	February 2002	Length <i>F</i>	Period <i>t</i>
<i>Etropus longimanus</i> (df = 6)				
Mean length (CV) = 112.0 mm (17.4)	n = 3	n = 4		
Adj mean $\delta^{15}\text{N}$ (CV)	12.90 (4.3)	12.35 (0.60)	0.81 ns	-0.60 ns
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-16.95 (0.75)	-18.94 (0.65)	nd	nd
<i>Gymnothorax conspersus</i> (df = 6)				
Mean length (CV) = 605.6 mm (26.1)	n = 4	n = 3		
Adj mean $\delta^{15}\text{N}$ (SE)	15.05 (0.09)	13.46 (0.11)	33.11**	-11.24***
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-17.24 (0.21)	-17.14 (0.25)	2.18 ns	0.31 ns
<i>Lophius gastrophisus</i> (df = 10)				
Mean length (CV) = 410.0 mm (40.3)	n = 4	n = 7		
Adj mean $\delta^{15}\text{N}$ (SE)	16.27 (0.30)	13.57 (0.22)	7.46*	-7.24***
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-17.20 (0.23)	-18.12 (0.17)	0.25 ns	-3.20*
<i>Mullus argentinae</i> (df = 7)				
Mean length (CV) = 154.2 mm (22.0)	n = 4	n = 4		
Adj mean $\delta^{15}\text{N}$ (SE)	13.82 (0.25)	12.42 (0.25)	4.57 ns	-3.96*
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-16.73 (0.18)	-18.09 (0.18)	17.64**	-5.33**
<i>Porichthys porosissimus</i> (df = 10)				
Mean length = 199.1 mm (39.9)	n = 6	n = 5		
Adj mean $\delta^{15}\text{N}$ (SE)	13.58 (0.24)	11.56 (0.27)	5.02 ns	-5.53**
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-17.84 (0.27)	-18.85 (0.29)	1.45 ns	-2.54*
<i>Raneya brasiliensis</i> (df = 6)				
Mean length (CV) = 192.6 mm (19.5)	n = 3	n = 4		
Adj mean $\delta^{15}\text{N}$ (SE)	13.68 (0.40)	12.16 (0.35)	1.23 ns	-2.79*
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-16.87 (0.40)	-18.04 (0.35)	0.00 ns	-2.17 ns
<i>Trachurus lathami</i> (df = 6)				
Mean length (CV) = 125.6 mm (26.1)	n = 4	n = 3		
Adj mean $\delta^{15}\text{N}$ (SE)	13.69 (0.28)	12.60 (0.32)	0.05 ns	-2.59 ns
Adj mean $\delta^{13}\text{C}_{\text{cor}}$ (SE)	-17.76 (0.07)	-18.25 (0.08)	0.20 ns	-4.85**

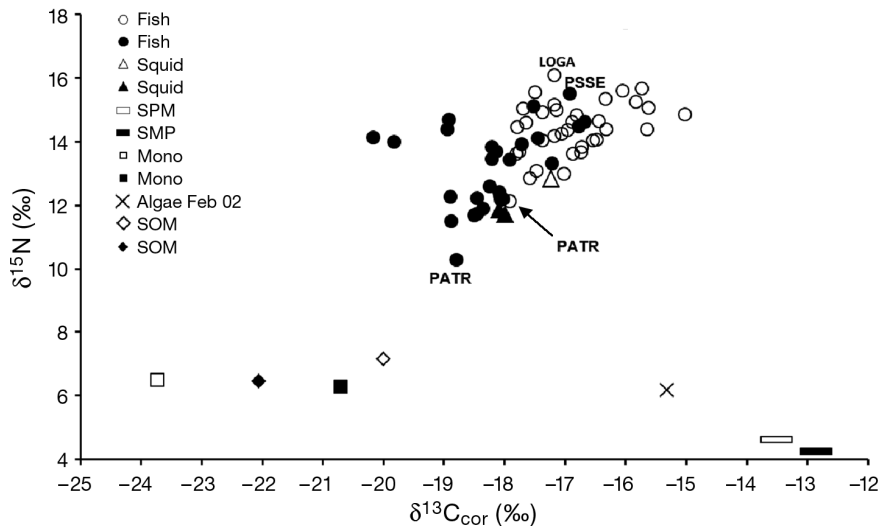


Fig. 3. Biplot of the mean lipid-normalized  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{cor}}$ ) and  $\delta^{15}\text{N}$  values of fish and squid species from the Cabo Frio upwelling system in the non-upwelling (July 2001; open symbols) and the upwelling seasons (February 2002; filled symbols).  $\delta^{13}\text{C}$  values are given for suspended particulate matter (SPM), sediment organic matter (SOM), algae, and monocotyledonous plants (Mono). LOGA: *Lophius gastrophysus*; PATR: *Paralichthys triocellatus*; PSSE: *Pseudoperca semifasciata*

The range of the  $\delta^{13}\text{C}_{\text{cor}}$  values fell within  $-20$  and  $-15$ ‰, a range that spans the gap between the potential sources (SPM, SOM, macroalgae, grass). Benthic and pelagic feeders overlapped in their  $\delta^{13}\text{C}_{\text{cor}}$  range in both seasons (Fig. 3, Table 1; also see the Supplement). Taking into account the  $\delta^{13}\text{C}$  TEF of 1‰ at a trophic step, and the range of  $\delta^{13}\text{C}_{\text{cor}}$ , it appears that SPM and SOM jointly support the consumers.

The flatfish *Paralichthys triocellatus* exhibited the lowest  $\delta^{15}\text{N}$  value in July 2001 (non-upwelling) and February 2002 (upwelling), 12.13 and 10.28‰, respectively. The highest  $\delta^{15}\text{N}$  values were observed in the predators *Lophius gastrophysus* (16.08‰) in July and *Pseudoperca semifasciata* (15.51‰) in February (Fig. 4, Table 1).

#### Trophic positions of fish and squid

Assuming a  $^{15}\text{N}$ -enrichment factor of 3.4‰ between 2 successive TLs, fish fell into at least 2 TLs, ranging from 3.7 to 4.8 in the non-upwelling season, and from 3.6 to 5.1 in the upwelling season (Table 1). The TLs did not differ between the 2 periods ( $t = -0.73$ ,  $p = 0.470$ ,  $df = 60$ ). The TL for squids ranged from 3.8 to 4.2 in the upwelling season.

The species assemblages were quite similar in the 2 seasons (Fig. 4). Species preying on zooplankton and benthic polychaetes were more numerous at TL4 (tertiary consumers), and those preying on fish were more abundant at TL5 (quaternary consumers). Amongst the 22 fish species occurring in both seasons, there was a shift up and down in the hierarchy, and 8 species changed TL, expressing their changes in mixed diets due to prey availability and population dynamics.

## DISCUSSION

### Seasonal variability

The fish in the Cabo Frio food web showed higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values during the non-upwelling season (July 2001, austral winter) relative to the upwelling season (February 2002, austral summer). According to their feeding habits, the analysed fish were composed of species belonging to both pelagic and benthic food webs. Our isotope results suggest that SPM and SOM, derived from a mix of low- and high- $\delta^{13}\text{C}$  materials, are the primary organic carbon sources supporting fish and squid in the Cabo Frio food web.

We consider our SPM values to be representative of our sampling time, as the isotope time turnover of plankton ranges within a period of days (Montoya et al. 1991). The lower SPM  $\delta^{13}\text{C}$  values during the non-upwelling season are most likely associated with the nanophytoplankton fractionation under oligotrophic conditions (Wu et al. 1999). Although not statistically significant, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of SOM showed the same seasonal pattern as the fish, i.e. higher in the non-upwelling season than in the upwelling season.

Temporal shifts in the isotopic composition at the bottom of the food web will propagate to higher TLs of the ecosystem (Montoya 2007). Nevertheless, the stable isotope ratios of the consumers reflect the assimilated portion of their diet integrated over a period of time preceding the sampling period and do not necessarily reflect the consumers' recent diet. Based on the biological turnover data available for muscle tissue (Lorrain et al. 2002, Herzka 2005, Guelinckx et



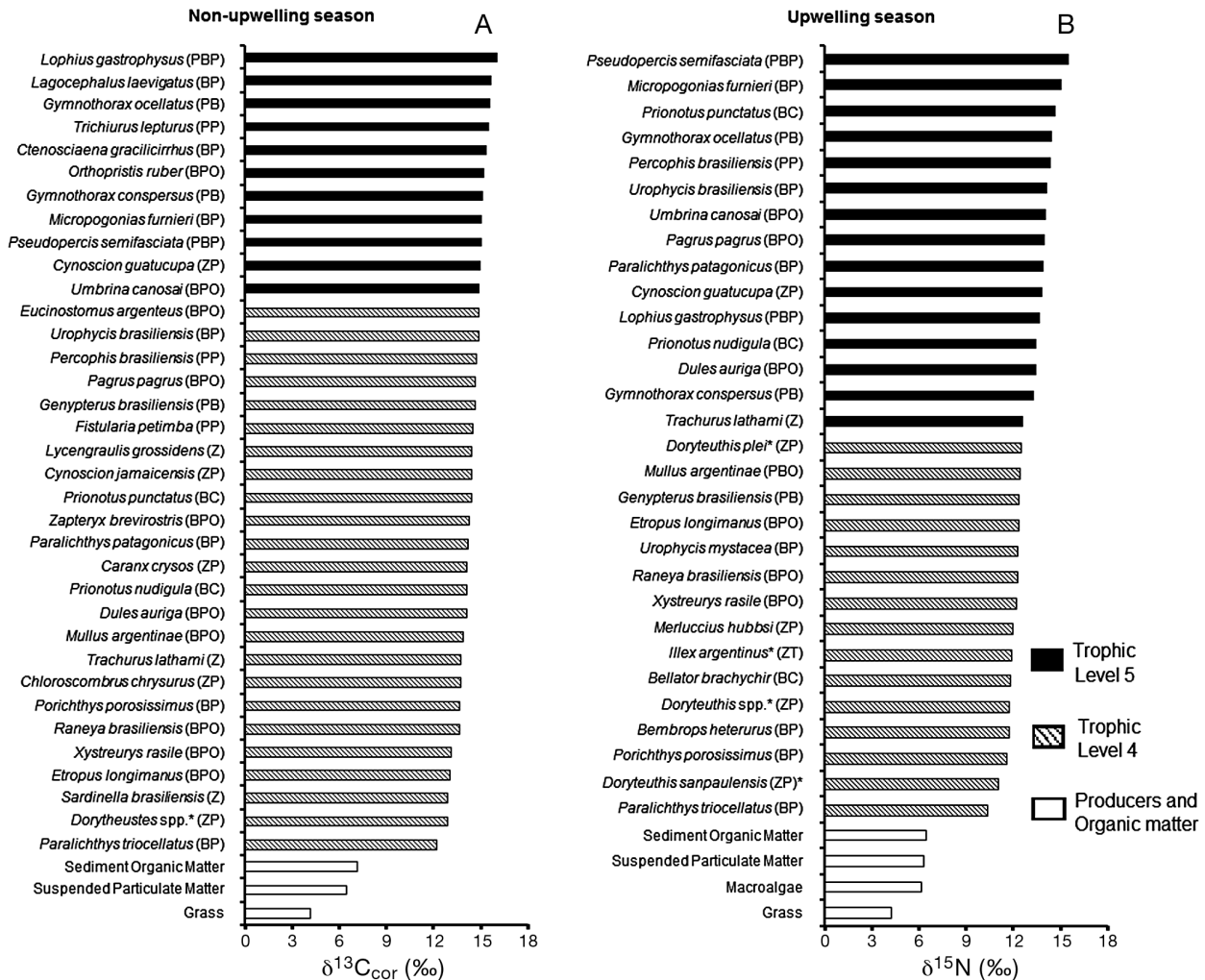


Fig. 4. Mean  $\delta^{15}\text{N}$  values of fish and squid species from the Cabo Frio upwelling system in (A) the non-upwelling (July 2001) season and (B) the upwelling season (February 2002). Feeding-habit codes are given in parentheses. BC: benthic invertebrate feeder (crustacean); BP: benthic invertebrate feeder and piscivore; BPO: benthic invertebrate feeder (polychaetes and ophiuroideans); PB: benthic piscivore; PBP: benthopelagic piscivore and invertebrate feeder; PP: pelagic piscivore; Z: zooplanktivore; ZP: zooplanktivore and piscivore. \*Squid species

al. 2007, Buchheister & Latour 2010), we consider a time lag of 1 to 6 mo preceding the samplings for isotope analyses. Therefore, the consumer isotope signatures recorded during the non-upwelling season (winter 2001) may possibly reflect the time-integrated measure of the assimilated diet in the muscle tissue from the preceding austral autumn 2001, and the consumer isotope signatures recorded during the upwelling season (summer 2002) are representative of the preceding austral spring of 2001.

According to Wu et al. (1997), the  $\delta^{15}\text{N}$  of suspended particulate organic matter (SPOM) depends on the balance between the biological uptake of nitrogen and the physical supply of nutrients; when  $[\text{NO}_3^-]$  is higher than the demand of the phytoplankton, the  $\delta^{15}\text{N}$  of the SPOM is low due to the preferen-

tial uptake of  $^{14}\text{N}$ , thereby lowering the  $\delta^{15}\text{N}$  values of the pelagic food web. As  $[\text{NO}_3^-]$  decreases, the phytoplankton can become more enriched due to the assimilation of the heavier residual nitrate. The further higher value of the deep  $\delta^{15}\text{N}$  nitrate compared to that of the surface biota of the oligotrophic ocean is due to preferential uptake of  $^{14}\text{N}$ , production of  $\text{NH}_4$  with lower  $\delta^{15}\text{N}$  values and  $\text{N}_2$ -fixation, which exports  $^{15}\text{N}$  out of the surface waters (Montoya et al. 2002, Montoya 2007). These findings are in line with our results because during the austral spring and summer seasons in the study area, the reduced forms of nitrogen are the major source of phytoplankton production, with  $\text{NH}_4^+$  contributing, on average, from 56 to 81 % of the total nitrogen utilised (Metzler et al. 1997). The substantial contribution of a  $^{15}\text{N}$  depleted

nitrogen-source may be explained by the dominant  $N_2$ -fixers, such as *Trichodesmium*, in the south-eastern Brazilian oligotrophic waters (Metzler et al. 1997, Carvalho et al. 2008).

Unfortunately, we do not have information on the natural distribution of the stable isotope inputs and the fate of nutrients in the study area. We expected that the higher signatures exhibited by the consumers during the non-upwelling season (July 2001, austral winter) may be partially explained by the fractionation of the higher  $\delta^{15}N$  phytoplankton approximately 1 to 6 mo previously (autumn and early winter seasons), with this signal being passed on to the consumers throughout the trophic web. During this period, the SeaWiFS chlorophyll anomaly exhibited low values of approximately 0 (Sumida et al. 2005). The lower signature values of consumers during the upwelling season (February 2002, austral summer) may also be partially explained by the conditions during the previous spring and early summer 2001, when the SeaWiFS chlorophyll anomaly exhibited peaks at higher values, reflected by the lower  $\delta^{15}N$  values.

In summary, we speculate that the isotope signatures of pelagic and benthic consumers during the non-upwelling season (July 2001) resulted from the lower  $[NO_3^-]$  surface condition (uptake of heavier nitrate by phytoplankton) and  $^{15}N$ -enriched sediment during the previous autumn and early winter 2001, which was reflected throughout the food web. In contrast, the lower signatures in the upwelling season (February 2002) might be the result of higher  $[NO_3^-]$  in the surface waters (preferential uptake of  $^{14}N$  by phytoplankton) and higher levels of  $N_2$  fixation during the previous austral spring and early summer of 2001. The low  $\delta^{15}N$  values ( $-2$  to  $+2\text{‰}$ ) of the SOM across the continental slope off the coast of Marseille, France, were effectively explained by  $N_2$  fixation (Carlier et al. 2007).

We can also consider the input of nitrogen as a result of anthropogenic activity through the land-derived sources of nutrients into coastal areas, carrying nitrogen-enriched wastewater ( $\delta^{15}N = 10\text{--}20\text{‰}$ ), usually in the form of  $NH_4^+$ , as indicated by McClelland et al. (1997), as well as high organic  $NH_4^+$  produced through the microbial loop of the food web (Montoya 2007). Nevertheless, the influence of domestic wastewater in our coastal study area may be low in contrast to the upwelling event, due to its local characteristics described above.

Although we have strong evidence that the  $\delta^{15}N$  signature changes observed throughout the Cabo Frio food web are linked to the upwelling dynamics,

studies on the natural distribution of stable isotopes would be required to trace the inputs and fate of N in order to explain the entire ecosystem process.

The changes in  $\delta^{15}N$  may also be related to the changes in diet composition due to prey availability (Davenport & Bax 2002). *Porichthys porosissimus*, a benthic invertebrate and fish feeder (Muto 2004), *Lophius gastrophysus*, a benthopelagic piscivore (Pucci 2005), and *Raneya brasiliensis*, a benthic invertebrate feeder (Vera & Soares 2008), undergo dietary seasonal changes in the study area. Nevertheless, these comparisons might not be straightforward, as has been indicated by Herzka (2005), due to the complexity of the metabolism of young and old fish and the different rates of turnover (McCutchan et al. 2003).

The same seasonal pattern found in fish, i.e. higher values in July 2001 (winter) than in February 2002 (summer), was observed for chl *a* in the sediments from the study site (Sumida et al. 2005), and those authors speculated that this trend reflects the sea surface anomalies during winter 2001. The authors indicated a delay of 1 wk between the deposition of the phytodetritus and the microbial activity, and they also observed peaks of microbial communities 4 mo after a major upwelling event, suggesting that the enhanced primary productivity by upwelling is reflected in the sediments and consequently will propagate to the higher trophic levels of the ecosystem.

### Trophic interactions

To understand the trophic positions of organisms in a food web and their variability, information on the tissue turnover rates and the isotope TEF is essential. Isotopic shifts associated with the TL may depend on several factors, such as taxon, the main biochemical form of the nitrogenous waste and the type of diet (Guelinckx et al. 2007). Due to the number of species (41) analysed, we used the accepted TEF ( $\delta^{15}N = 3.4\text{‰}$ ) of Minagawa & Wada (1984), which is considered to be robust and valid (Sørense et al. 2006) for estimating the TL. Additionally, we used this factor to make comparisons with other ecosystems described in published papers (Carlier et al. 2007, Miller et al. 2010, Faye et al. 2011).

$\delta^{13}C$  and  $\delta^{15}N$  values found in Cabo Frio are in the same range as those of the California Current upwelling ecosystem (Miller et al. 2010, Madigan et al. 2012). The range of values is also similar to those of the Newfoundland and Labrador continental shelves (Sherwood & Rose 2005). The similar biophysical

response with the similar hydrodynamic conditions of the systems and the similar nutrient baseline, supported mainly by SPM, most likely explains this finding.

For a coastal area of Ubatuba, south of our study area,  $\delta^{13}\text{C}$  values ( $-17.2$  to  $-12.8\text{‰}$ ) are typical of fauna based on marine phytoplankton (Corbisier et al. 2006).  $\delta^{13}\text{C}$  values in the Bay of Banyuls-sur-Mer, in the Mediterranean Sea, suggest continental inputs to the food chain, and the lower  $\delta^{15}\text{N}$  values of coastal fish ( $6.7$ – $12.6\text{‰}$ ) could reflect the use of SPOM, surface sediment organic matter and microphytobenthos (Carlier et al. 2007). Fish that represented the highest TL in the complex deep-sea food web of Balearic Basin, NW Mediterranean, also showed lower  $\delta^{15}\text{N}$  values ( $6.1$ – $12.63\text{‰}$ ) than our results (Fanelli et al. 2013). According to these authors, the species were mainly linked to vertical inputs of marine snow in the inland slope and less directly to primary production over the mainland slope. This was the same  $\delta^{15}\text{N}$  trend as reported for benthopelagic fish ( $7.27$ – $11.31\text{‰}$ ) from the middle continental slope of the Catalan Sea in the Balearic Basin, associated with  $^{15}\text{N}$ -depleted sediment due to the input of terrigenous matter and matter exported from the shelf (Papiol et al. 2013). The fish data from the Bay of Biscay in the northeast Atlantic showed signals from coastal, oceanic and deep-sea areas, where river discharges appeared to be the primary factor in terms of influencing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of fish (Chouvelon et al. 2012).

Our study indicated that fish and squids function as the tertiary (TL4) and quaternary (TL5) consumers. Miller et al. (2010) found 5 TLs (including sea lion species) in the Northern California Current pelagic food web, where fish had values ranging from 2.7 to 3.8, which were lower than our results. Ranges lower than our observations were also registered in a tropical estuary off the coast of Senegal (2.6–4.7; Faye et al. 2011), and for the Bay of Banyuls-sur-Mer (France), where the benthic food web was composed of 4 TLs, with fish distributed between TL3 and TL4 (Carlier et al. 2007). These authors used the  $\delta^{15}\text{N}$  of pelagic copepods or of average primary consumer values (Post 2002), while we used the  $\delta^{15}\text{N}$  of bivalves for calculating the TL (Jennings et al. 2002).

The heterogeneity of the  $\delta^{15}\text{N}$  TEF values is an issue in food web studies and complicates the assessment of TLs, as has already been argued by several authors (e.g. Jennings et al. 2002, Sherwood & Rose 2005, Carlier et al. 2007, Fanelli & Cartes 2010, Layman et al. 2012). Use of several baselines has been suggested for calculating TLs from  $\delta^{15}\text{N}$  values

(Martínez del Río et al. 2009, Fanelli & Cartes 2010, Chouvelon et al. 2012). Fanelli et al. (2009) also discussed the suitability of TL ( $\delta^{15}\text{N}$  values) indicators of fishing disturbance compared to the diet composition of a benthic fish in the southern Tyrrhenian Sea, western Mediterranean.

Our isotope values did not fall into discrete trophic steps. Rather, we found a nearly continuous gradient of values, akin to the trophic spectrum described by Darnell (1961), which is characteristic of unstructured oceanic food webs (Isaacs 1973). These results suggest mixed diets and TL omnivory. Similar results were reported for pelagic food webs of the California Current upwelling (Miller et al. 2010) and the Southern California Current upwelling (Madigan et al. 2012).

Our results are a first delineation of the upper TLs of the Cabo Frio food web, and suggest that there are seasonal effects associated with the degree of upwelling occurring in the region. The mechanisms involved in the seasonal shifts remain to be elucidated, but their definition would furnish new information regarding the hydrodynamic and biological controls affecting upwelling regions. Detailed measurements of isotopic values of the nutrients, primary producers and prey species, as well as the physical dynamics, would augment the initial data provided here to improve our understanding of the complexities governing upwelling ecosystems.

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