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# Trophic structures in tropical marine ecosystems: a comparative investigation using three different ecological tracers

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ABSTRACT

We looked at how three ecological tracers may influence the characterization and interpretation of trophic structures in a tropical marine system, with a view to informing tracer(s) selection in future trophic ecology studies. We compared the trophic structures described by stable isotope compositions (carbon and nitrogen), the total mercury concentration (THg) and levels of essential fatty acids (EFA) at both the individual and species level. Analyses were undertaken on muscle tissue samples from fish and crustacean species caught in the waters surrounding the Seychelles. The carbon isotope composition ($\delta^{13}C$) correlated to the proportion of arachidonic acid (ARA), whereas the nitrogen isotope composition ($\delta^{15}N$) correlated to the proportion of docosahexaenoic acid (DHA) and THg. At the individual level, trophic position obtained with these three last tracers are similar. In contrast, the eicosapentaenoic acid (EPA) was not clearly correlated to any of the tracers. At the species level, the use of EFA (ARA and DHA), as compared to stable isotopes, resulted in slight structural modifications, mainly in the middle trophic levels. For example, the EFA overestimated the trophic positions of *Thunnus alalunga* and *Etelis coruscans* but underestimated the trophic positions of other snappers and groupers. While ARA mainly originates from coastal/benthic areas, DHA is conserved throughout the food web and may be used as a proxy indicator of trophic position. However, metabolic disparities can affect ecological tracers and in turn, distort the trophic structures derived from their results. This is especially true for species with close trophic ecologies. Despite these caveats, we think that analysing at the individual level the wealth of ARA, DHA and THg data that has already been obtained through earlier nutrition or food security studies would enhance our understanding of trophic structures.

## 1. Introduction

Understanding the intricate flows of energy and nutrients through food webs is an essential component of both fundamental and applied ecology. In marine systems, comparisons of these flows are made to assess the effects of anthropogenic pressures such as fishing, to trace the transfer of pollutants through food webs and to quantify the impacts of global climate change (e.g., Litzow et al., 2006; Hebert et al., 2008). In tropical marine systems, however, this type of quantitative information is often lacking, despite their sensitivity to climate change and exposure to high levels of fishing pressure and coastal habitat destruction (Munday et al., 2009).

Trophic ecology studies on marine species commonly use intrinsic ecological tracers. These tracers are biogeochemical compounds found within organisms, including stable isotopes, biomagnifying pollutants and fatty acids (Ramos and González-Solís, 2012). Nitrogen and carbon are two elements commonly used as stable isotope tracers. With nitrogen, the $^{15}N/^{14}N$ ratio generally increases in fish species from one trophic level to the next and consequently, it can be used to indicate trophic position (Vander Zanden and Cabana Rasmussen, 1997). With carbon, the $^{13}C/^{12}C$ ratio can be used to determine the sources of primary production in a food web (i.e., benthic *vs.* pelagic inputs) (France, 1995). Examples of biomagnifying pollutants include methyl-mercury (MeHg) and organic polychlorobiphenyls. Chemically stable and persistent, these compounds accumulate in the tissues of marine organisms and their concentrations are regulated by factors such as exposure to chemical compounds, including dietary exposure (Kelly et al., 2007). As these compounds do not tend to degrade, their concentrations generally increase with each trophic level (Lavoie et al., 2013; Chouvelon et al., 2014). Finally, fatty acids are long carbon chains constituting lipids that

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are necessary for a variety of physiological functions. A subset of these, known as 'essential fatty acids' (EFA), govern key functions such as growth, development and immune response (Arts et al., 2001). EFA and their precursors are mainly found in marine plants and algae and cannot be readily synthesized by all consumers. Thus, these EFA are incorporated into consumers in a conservative manner and can therefore be used to track different primary production sources and predators-prey relations throughout food webs (Dalsgaard et al., 2003; Piché et al., 2010). Three EFA are recognized as being essential for consumers: docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (ARA; 20:4n-6) (Arts et al., 2001). In marine ecosystems, these three EFA are commonly used as biomarkers to understand trophic structure and called 'fatty acids trophic markers' (e.g., Müller-Navarra et al., 2000; Budge et al., 2006; Iverson, 2009). For example, DHA increases with the trophic position of Coral Sea-caught albacore tuna *Thunnus alalunga* (Parrish et al., 2015) and with nitrogen stable isotope values across a Mediterranean lagoon food web, from sediment organic matter to fish (Koussoroplis et al., 2011). Significant positive correlations were also highlighted between DHA/EPA ratios, nitrogen stable isotope signatures and degree of carnivory in coastal calanoid copepods (El-Sabaawi et al., 2008).

An advantage of ecological tracers is their ability to reflect the integration of consumers' diets over a relatively long timeframe. However, their main limitation is that they are influenced by both environmental (abiotic) and biological (metabolic) processes (Ramos and González-Solís, 2012). For example, stable isotope compositions are affected by growth rates and baseline changes; local pollution sources and the transfer of contaminants to specific organs (for accumulation or excretion) can affect outputs from pollutant tracers; and the turn-over and modifications that EFA may undergo within consumers remain largely unknown (Iverson, 2009; Ramos and González-Solís, 2012). To overcome these limitations and provide complementary insights, the combined use of different ecological tracers and/or tissues is a growing area of research in the field of trophic ecology. This combined approach allow researchers to better characterize contaminant and nutrient flows within food webs and better detect differences in dietary habits among sympatric species (e.g., Hebert et al., 2009; Le Croizier et al., 2016; Sardenne et al., 2016). However, although informative, this approach multiplies the number of expensive and time-consuming analyses to be undertaken, requires adapted statistical approaches, and can lead to interpretation challenges when each tracer leads to a different conclusion. For example, the trophic level attributed to the outcomes of a stable isotope analysis can be uncorrelated with the mercury concentrations detected (Bond, 2010), as mercury bioaccumulation also depends on dietary exposure and size/age of organisms.

In this study, we looked at how the choice of ecological tracer can influence the characterization of trophic structure (i.e. on two dimensions) and trophic positions in the food chain (i.e. on one dimension) in a tropical marine ecosystem, with a view to improving tracer(s) selection in future studies. Drawing on the fundamental theories associated with each tracer, as previously outlined, we hypothesized that independent ecological tracers should yield similar results. For example, nitrogen isotope composition, DHA and mercury contents theoretically increase with the trophic position and thus should be correlated and provide the same trophic information. To test this hypothesis, we compared the results of three independent ecological tracers tested on 17 species of market fish and crustaceans collected within the Seychelles' surrounding waters. These species, all of which belonged to trophic level 3 or above, were selected to capture a wide range of phylogenetic differences (i.e., different families), vertical distributions (i.e., pelagic, demersal and benthic) and habitat type (i.e., oceanic and coastal) (Table 1). The three tracers used were the stable isotope compositions of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), total mercury concentrations (THg) and EFA levels (DHA, EPA and ARA). Our investigation focused on identifying the correlations present between the three tracers and comparing the trophic structures (i.e., relative proximities among individuals and species on two dimensions) described by each tracing method. In the absence of information on baselines compositions of the Seychelles' marine ecosystem, the present study focused on the relative trophic position of individuals/species in the food web instead of absolute trophic level.

## 2. Material and methods

### 2.1. Fish and crustacean sampling

A total of 92 individuals (17 fish and crustacean species; Table 1) were collected from the Plateau of Mahé, Seychelles (3.68°S to 6.51°S and 53.93°E to 57.14°E) between April 2014 and February 2015 using either purse-seine, hand-line, long-line, snorkelling, trap or tangle net during scientific surveys of the Seychelles Fishing Authority. To limit the influence of size on the ecological tracers (Dang and Wang, 2012), similarly sized individuals were selected for each species (i.e., intra-species coefficient of variation < 25%). For each individual, fork length (fish) or total length (crustaceans) was recorded using callipers and a sample of white muscle tissue (~20 g) was taken from the front dorsal region and stored at −80 °C shortly after collection. For the stable isotope analysis, a subsample of each muscle sample was freeze dried and ground into a fine homogeneous powder using a Retsch Mixer Mill MM200. Another subsample was keep frozen for the THg and EFA analyses.

### 2.2. Stable isotope analysis

The stable isotope analysis was performed on samples of lipid-free powder (0.4 ± 0.1 mg) that were packed into tin capsules. The lipids were removed, as they are known to influence $\delta^{13}C$ values (Post et al., 2007; Bodin et al., 2009). They were extracted from the powdered samples (approximately 350 ± 100 mg) following the method set out in Bodin et al. (2009) (12 ml of dichloromethane at 100 °C under 1900 psi for 20 min using an Dionex Accelerated Solvent Extractor 200) that allows simultaneous C and N stable isotope analysis without an unwanted effect on $\delta^{15}N$ values. Lipid extracts were evaporated and weighed to the nearest 0.1 mg to determine the total lipid content, expressed as a percentage of the dry weight (dw). The lipid-free powders were analyzed using an Elemental Analyser (Flash EA 1112; Thermo Scientific) coupled to an Isotope Ratio Mass Spectrometer (Delta V Advantage with a Conflo IV interface; Thermo Scientific) at the LIENSs Stable Isotope facility (La Rochelle, France). Results were reported in the δ unit notation and expressed as parts per thousand (‰), relative to international standards (atmospheric $N_2$ for nitrogen and Vienna-Pee Dee Belemnite for carbon). Calibration was completed using reference materials (IAEA-$N_2$, $-NO^-_3$, −600 for nitrogen; USGS-24, IAEA-CHE, −600 for carbon). Analytical precision, based on replicate measurements of acetanilide (Thermo Scientific) was < 0.15‰ for both $\delta^{15}N$ and $\delta^{13}C$. The effectiveness of the chemical extraction was checked by examining the C:N ratio from the percent element weight (C:N < 3.5; Post et al., 2007).

### 2.3. Total mercury concentration

The biomagnifying form of Hg (MeHg) constitutes most of the THg in the upper trophic levels (Cai et al., 2007; McMeans et al., 2015). Thus, for analytical convenience, we only measured THg. The analysis was performed on fresh white muscle samples (10–50 mg) using a Direct Mercury Analyser DMA-80 Dual Cell (Milestone) at the Seychelles Fishing Authority (Victoria, Seychelles). The results were reported in parts per million (ppm), which equates to $\mu g\ g^{-1}$ in wet weight. Calibration blanks were run in between each sample to ensure Hg levels were reset to 0.1 ng. Analytical performance was checked every 15–20 samples against two laboratory control analyses (performed on large