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Trimethylamine oxide accumulation as a function of depth in Hawaiian mid-water fishes

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ABSTRACT

Trimethylamine oxide (TMAO) is a common osmolyte and counteracting solute. It is believed to combat the denaturation induced by hydrostatic pressure as some deep-sea animals contain higher TMAO levels than their shallow water counterparts. It has also been proposed that TMAO may accumulate passively during lipid storage resulting in a correlation between lipid content and TMAO levels in some groups. Previous research showed that lipid content decreased with depth in species of Hawaiian fishes presenting a novel test of these competing hypotheses. TMAO ranged from 20.4 to 92.8 mmol/kg. Lipid content ranged from 0.50 to 4.7% WW. After completing a comprehensive search for depths available in the literature, provided here, we analyzed TMAO and lipid as a function of average, minimum and maximum depth of occurrence for 27 species of fishes from nine orders. We found that TMAO is positively correlated with all measures of habitat depth (hydrostatic pressure) but the relationship is strongest with average depth. We further showed using phylogenetic independent contrasts that this relationship was not influenced by the evolutionary relatedness of these species. Interestingly, we found that lipid content increased with depth, in direct contrast to previous studies. TMAO is thus also positively correlated with lipid content. While we are unable to distinguish between these hypotheses, we show that TMAO is strongly correlated with depth in mid-water fishes.

1. Introduction

Trimethylamine oxide (TMAO) is an important cellular component in a wide range of taxa, from bacteria to humans (Chen et al., 2011; Treacy et al., 1995). It was first described in marine organisms (Bickel, 1969 in ref. Suwa, 1909; Norris and Benoit, 1945) as a prominent osmolyte (Cholette and Gagnon, 1973; Forster and Goldstein, 1976). Later, it was shown to be a strong counteracting solute, (Yancey and Somero, 1979) protecting protein structure (Yancey and Siebenaller, 1999; Qu and Bolen, 2003) and function (Baskakov et al., 1998) from various environmental perturbants including, hydrostatic pressure (Gillett et al., 1997), urea and ammonia toxicity (Yancey and Somero, 1980; Miñana et al., 1996), and temperature stress (Treberg et al., 2005; Villalobos and Renfro, 2007).

TMAO increases with habitat depth inter- and intraspecifically in benthic fishes and skates as well as some invertebrate groups (Kelly and Yancey, 1999; Yancey et al., 2001; Yancey et al., 2002; Laxson et al., 2011; Samerotte et al., 2007), suggesting that this molecule is used to combat the increasing stress of hydrostatic pressure. Most recently, Yancey et al. (2014) showed a hadal snailfish at 7000 m with a TMAO content of 386 mmol/kg, almost eight times higher than the average fish in the euphotic zone. These observed correlations with depth have been further supported by evidence that TMAO prevented hydrostatic pressure denaturation in vitro (Yancey and Siebenaller, 1999).

However, not all taxa show an increase in TMAO with depth (Seibel and Walsh, 2002). Some shallow-living squids have TMAO levels that approach that reported for the hadal snailfish. These authors suggest a novel mechanism of TMAO synthesis leading to accumulation as a byproduct of lipid metabolism and storage and that TMAO is not necessarily retained as a specific adaptation to high hydrostatic pressure. This hypothesis was supported by a strong correlation between total lipid content and TMAO in cephalopods as well as anecdotal evidence in a variety of other groups. For example, lipid content is often higher in deep-living and polar species, which may explain the tendency of species in those habitats to accumulate large quantities of TMAO. However, a subsequent study did not find a relationship between mean TMAO and triacylglycerol content in fishes (Samerotte et al., 2007), perhaps due to the differing time courses of accumulation and retention that resulted in differing size-scaling relationships of these two compounds.

Furthermore, an evolutionary relationship has been suggested for TMAO synthetic capacity between elasmobranchs and

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chimaeras (Treberg et al., 2006), which may impose inherited limitations on accumulation potential. If phylogeny also plays a role in TMAO accumulation in teleosts, it is possible that depth-related differences are driven by evolutionary history rather than environmental selection or substrate availability. Alternatively, a relationship to phylogeny may coexist and mask environmental trends making analyses between distantly related taxa difficult.

In (1990), Childress et al. examined a population of Hawaiian mid-water fishes that exhibited decreasing lipid content with increasing habitat depth (and hydrostatic pressure). Here, we examine TMAO and lipid content in 27 species of Hawaiian fishes from the same region studied in Childress et al. (1990) to test the competing hypotheses of hydrostatic pressure and lipid content on TMAO accumulation. A stronger relationship to hydrostatic pressure should elicit an increase in TMAO with habitat depth while a decrease with depth may be seen if TMAO is primarily accumulated as a by-product of lipid metabolism. Alternatively, an increase in lipid and TMAO with depth could represent a situation in which fishes accumulate TMAO passively during lipid storage with deeper fishes retaining the molecule for further pressure counteraction.

2. Materials and methods

2.1. Collection and sampling

Fishes were collected aboard the *R/V Kilo Moana* (University of Hawaii) in June 2012 off the west coast of Oahu in the Hawaiian Islands. Specimens were captured using a modified opening-closing Mother Tucker trawl with 3 m^2 mouth (Childress et al., 1978) between depths of 50–2000 m. Animals were recovered in a 30-l thermally insulated cod end and immediately processed for later analysis. Individuals from 17 different species were gently blotted dry then flash frozen whole for determination of total lipid content. Additionally, muscle tissue was excised from similar specimens of the same and additional species, for a total of 27 species, and flash frozen for subsequent analysis of TMAO. All samples were collected in accordance with IACUC #AN12-07-026 and stored at −80 °C until experimentation was conducted. Representatives of each species were preserved in 5% formalin or photographed for later identification using taxonomic and identification references available in the literature.

2.2. Analytical techniques

Total lipid content for whole body was measured using a similar method to the 2:1 chloroform to methanol extraction described by Bligh and Dyer (1959) paired down for small sample mass (Lee et al. 1996). Muscle tissue samples were deproteinated and homogenized in 5x volume 5% trichloroacetic acid (TCA) followed by spectrophotometric determination of TMAO using the ferrous sulphate-EDTA assay (Wekell and Barnett, 1991). All values represent averages taken from replicate individuals from n=1 to 12.

2.3. Depth analysis

Habitat range was determined according to currently published literature values describing the depth distribution of each species. Average depth is reported as the median of the habitat range, especially in highly migratory species or as average depths specifically reported in the literature. The average depth of a species can be considered as the depth at which the fish spends most of its time (in non-migratory animals) or as a depth that represents the average level of depth stress (e.g. hydrostatic pressure) encountered by the species (migratory species). Minimum depth of occurrence (MDO) is defined as the depth below which 90% of the population of each species can be found (Childress and Nygaard, 1973). Here, MDOs were taken directly from the literature. Where no MDO was available, the shallowest reported depth for the species was used, substituting 10 m for those reported at the surface. TMAO, lipid and size were further analyzed against capture depth with no correlations found (data not shown).

Due to the limited amount of data available for these fishes, references were taken from studies conducted circumglobally (Supplementary Table 1). For some species, reported depths vary widely between publications; in such cases, the depths chosen for use in this study were based on the most recent and regionally specific data available. Occasionally a species vertical distribution changes with size, where smaller fish are frequently found at shallower depths (Collins et al., 2008). In these instances, reported depths are specific to the size of fish analyzed in this study; therefore, authors should be cautious when reporting these listed depths elsewhere.

2.4. Phylogenetic comparison and statistical analysis

TMAO data were subjected to independent contrasts phylogenetic analysis (PIC) to determine if the phenotypic trends seen in this study could be explained by evolutionary relationships among fish species (Felsenstein, 1985; Seibel and Carlini, 2001). The phylogenetic tree used for this analysis was a compilation of trees previously published in the literature (Stiassny et al., 1996; Harold, 1998; Miya and Nishida, 1998; DeVaney, 2008; Davis, 2010; Kenaley, 2010; Betancur-R, 2013; Denton, 2014). The tree was further rooted in the outgroup Chondrichthyes; however, this group is not included in the analysis as elasmobranch values deviate significantly from all teleost values. All data concerning TMAO, lipid, depth of occurrence and weight were further analyzed using regression analysis to assess whether any statistically significant relationships occurred. Statistics and graphs were generated using GraphPad Prism 6.0 and the phylogenetic tree used for PIC was made with statistical package R. Estimated TMAO and depth values were calculated for all ancestral nodes assuming equal branch lengths (punctuated model) and included in Supplementary Fig. 1. Further, contrast values were calculated for each node, which indicate both TMAO and depth after points have been made independent by accounting for any phylogenetic signal.

3. Results

3.1. Fish collection

We collected 27 species of mid-water fishes from 15 trawls ranging in depth from 50 to 2000 m. The species represent 12 families from 9 orders. The habitat depths of each species (average, minimum and maximum) are listed in Table 1.

3.2. TMAO vs. depth

Average TMAO content ranged between 20 and 93 mmol/kg wet mass (Table 1), which is consistent with values reported for fishes elsewhere (Carr et al., 1996). TMAO content increased linearly with all measures of habitat depth. The relationship was strongest with a species' average depth ($r^2=0.5309$, $p<0.0001$; Fig. 1a) but was also significant as a function of MDO ($r^2=0.5074$, $p<0.0001$; Fig. 1b) and maximum depth ($r^2=0.2520$, $p=0.0076$; Fig. 1c). Separating fishes into non-migrating and vertically migrating species did not strengthen the trend with depth and variance between these groups was not significantly different (data not shown). Additionally, a phylogenetically independent analysis of the data (Phylogenetic Independent Contrasts) also resulted in a significant positive relationship between TMAO and habitat depth (($r^2=0.4036$, $p=0.0009$; Fig. 2), which suggests the trend is independent of any phylogenetic relationships across these 27 species.

3.3. Lipid vs. depth and TMAO

Lipid content ranged between 0.5 and 4.7% wet weight in these fishes. Lipid values showed a significant increase with increasing average depth ($r^2=0.2888$, $p=0.0261$) in the 17 species analyzed for lipid in this study. Additional lipid values taken from the literature (n=6) strengthened this relationship ($r^2=0.2496$, $p=0.0152$; Table 1, Fig. 3). Lipid values from the literature were only included for species in this study where lipid was not measured directly. Lipid also significantly increased with MDO but not maximum depth (data not shown). When divided into non-migrating and vertically migrating species, groups did not exhibit significant differences in variance (data not shown). Further, lipid was positively correlated with size in the family Myctophidae ($r^2=0.4145$, $p=0.0009$) and negatively correlated with size in the species, *Sternoptyx diaphana* ($r^2=0.8834$, $p=0.0175$; Fig. 4). However, size was not related to any measure of habitat depth for these species (data not shown). TMAO increased linearly with increasing lipid content ($r^2=0.2744$, $p=0.0309$) across the 17 fish species analyzed. Adding lipid values from the literature (n=6) strengthened this relationship ($r^2=0.4328$, $p=0.0006$; Fig. 5).

4. Discussion

4.1. TMAO vs. depth

TMAO increases with a species' habitat depth in a number of different clades including, anemones (Yancey et al., 2004), crustaceans (Zerbst-Boroffka et al., 2005), Chondrichthyes (Laxson et al., 2011) and teleosts (Kelly and Yancey, 1999; Yancey et al.,