Adenosine Triphosphate (ATP) and Protein Data in Some Species of Deep-Sea Corals in Newfoundland and Labrador Region (Northwest Atlantic Ocean)

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ADENOSINE TRIPHOSPHATE (ATP) AND PROTEIN DATA IN SOME SPECIES OF DEEP-SEA CORALS IN NEWFOUNDLAND AND LABRADOR REGION (NORTHWEST ATLANTIC OCEAN)

by

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ABSTRACT

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Despite the fact that deep sea corals are recognized as a crucial part of the deep marine ecosystem, little information is available on the general physiology and biochemistry of these species. In this study we measured ATP and protein amounts in deep-sea corals sampled around the coast of Newfoundland. Information found in literature, as well as results obtained in this study highlight the need for a standardization of methods used for protein extraction and overall sample preparation prior to ATP measurement to allow comparisons between deep and shallow water corals. The overall results obtained after decision tree analysis of ATP and protein data show strong differences between species with low seasonality in the data (despite some non-significant variations). This suggests a weak influence of reproductive cycles on general metabolism. Nonetheless, the absence of trend with depth and season in protein data remains surprising considering its link with food availability and some of the observations obtained after previous work on lipids in deep-sea corals.

RÉSUMÉ

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Malgré le fait que les coraux profonds soient reconnus aujourd'hui pour leur importance écologique, il existe peu de données sur leur physiologie et biochimie. Nous avons mesuré, lors de cette étude, les quantités d'ATP et de protéines contenues dans les tissus de coraux prélevés le long de la côte Terre-neuvienne. Nos résultats ainsi que ceux décrits dans la bibliographie concernant les coraux peu profonds, montrent la nécessité d'une standardisation des méthodes d'extraction des protéines ainsi que la préparation de tissus pour les mesures d'ATP. L'application d'arbres décisionnels statistiques aux valeurs obtenues montre d'importantes différences entre espèces avec peu d'effets de la saison de prélèvement. Ceci suggère une faible influence des cycles de reproduction sur les valeurs d'ATP et les quantités de protéines tissulaires. Néanmoins, cette absence d'effet de la saison et de la profondeur sur nos données reste surprenante étant donné l'association entre les mesures effectuées et la présence ou absence potentielle de nutriments, de plus ceci est en contradiction avec des résultats obtenus précédemment sur les lipides des coraux profonds.

INTRODUCTION

Deep sea corals are recognized as a crucial part of the deep marine ecosystem (e.g. Mortensen et al. 1995). Deep-sea corals provide important habitat for a variety of fish species, including some commercial fish species (Jensen and Frederiksen 1992; Buhl-Mortensen and Mortensen 2004). Little information is available on the general physiology and biochemistry of deep-sea corals, and numerous questions remain unanswered.

ATP is the most basic and universal energy-supplying molecule. ATP exists only in living cells. Its ratio to ADP and AMP, the energy charge index, generally reflects the vitality of the cell. Because 99.99% of a coral sample is mineral material, ATP content can be used to estimate living biomass as a parameter to indicate the physiological health of a coral colony (Fang et al. 1989). Effect of tidal flows (Fang et al. 1987), as well as light (Al-Horani et al. 2003), have impacted ATP levels in shallow water corals. Al-Horani et al. (2003) highlighted the link between ATP levels and calcification processes thus proving that ATP can bring direct information on coral vitality. Increased sedimentation due to trawling can impact this vitality. Corals can remove sediment from their surface in a variety of ways, including using tentacles and cilia to move debris, and trapping particles in mucus, which is subsequently sloughed off (Hubbard and Pocock 1972). These cleansing processes are not without energetic cost (ATP levels), and therefore contribute to coral stress (Bak 1978).

To our knowledge, no work has been conducted on ATP levels in deep water corals. ATP can bring information on energy budgets of coral and allow us to conduct interspecies comparisons as well as investigate potential impacts of fishing activities (and pollution) on species sampled in areas under different fishing pressures. Tissue protein amounts were also evaluated in this study and seasonal, depth and/or effect of sampling site were explored, as well as any meaningful species-related trend in protein data distribution. Total protein amounts are another conventional coral biomass parameter (in addition to tissue weight) (Fang et al. 1987), providing therefore information on living biomass in coral samples. Moreover, Grover et al. (2002) showed that well fed coral nubbins had higher protein amounts, thus reflecting vitality of the colony.

MATERIALS AND METHODS

SAMPLE COLLECTION

Coral samples were gathered opportunistically from three sources; the Fisheries and Oceans Canada (DFO) multispecies stock assessment survey, the northern shrimp stock assessment survey (DFO and Northern Shrimp Research Foundation), and the Fisheries Observer Program (DFO). All specimens were assigned a species code,

bagged with locator number and frozen. Samples in this study were collected in the fall, summer and spring of 2005 and the fall of 2007 (Fig. 1). Samples collected in 2005 were frozen at -20°C directly while those sampled in 2007 were first frozen in liquid nitrogen then transferred at -20°C. Sampling depths varied between 50-1500 m, but not all species were represented at all depths. Sampling sites comprised Cape Chidley (61°N, 63°W), Southwest Grand Banks (43°N, 53°W), Southeast Grand Banks (45°N, 49°W) and the Flemish Cap (47°N, 47°W).

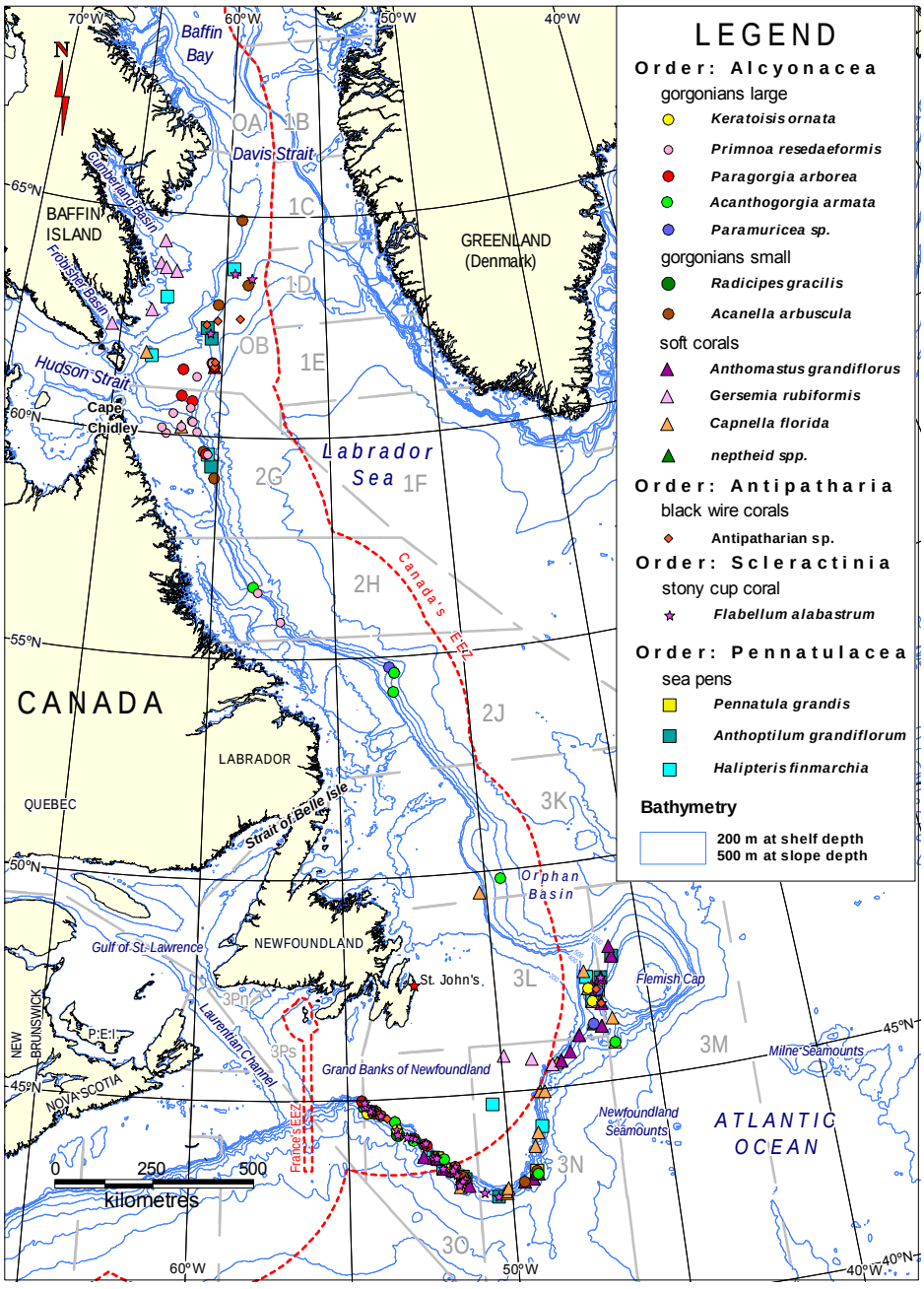

Figure 1. Map of sample sites and species collected.

A total of 18 species were sampled for analyses (not all species were sampled both years). The coral samples acquired belonged to the order Alcyonacea which is subdivided into three informal groups, including soft corals with polyps contained in massive bodies, gorgonians with a consolidated axis, and gorgonians without a consolidated axis (Bayer 1981). Soft corals consisted of one alcyoniid (*Anthomastus grandiflorus* (Ag), Verrill 1878) and two nephtheids (*Gersemia rubiformis* (Gr), Ehrenberg 1834 and *Capnella florida* (Cf), Verrill 1869). When nephtheids were difficult to identify, they were defined by the general term of nephtheids (nepht). Six species of gorgonians with a consolidated axis were also considered for this study: *Acanella arbuscula* (Aa) (Johnson 1862), *Paramuricea* spp. (Ps) (*P. grandis*, Verrill 1883, *P. placomus*, L.), *Primnoa resedaeformis* (Pr) (Gunnerus 1763), *Keratoisis ornata* (Ko) (Verrill 1878), *Acanthogorgia armata* (Aar) (Verrill 1878), and *Radicipes gracilis* (Rg) (Verrill 1884). One species of gorgonian without axis was analyzed: *Paragorgia arborea* (Pa) (L.).

The order Antipatharia was represented by (*Bathypathes* spp. (Bs) (Brooke 1889). In one instance, one sample was defined as Anti due to identification difficulties. The order Pennatulacea was represented by three species *Anthoptilum grandiflorum* (Agr) (Verrill 1879), *Halipteris finmarchia* (Hf) (Sars, 1851), and *Pennatula grandis* (Pg) (Ehrenberg 1834). Similarly than nephtheids, some samples were designed by the common name: sea pen (Ss) because of some identification difficulties. Finally, the order Scleratinia was represented by one species *Flabellum alabastrum* (Fa) (Moseley 1876).

ATP ASSAY

Polyps from the coral samples were removed, minced, weighed and put in an extraction vessel with sulfuric acid on ice. A volume of 0.6N H_2SO_4 acid, usually 5ml, was added to the sample and this mixture and was left on ice (Fang et al. 1987). The volume of 0.6N H_2SO_4 used was determined according to the amount of coral tissue available for extraction, 5 l of H_2SO_4 was used for 5 g of tissue or less, a volume of 10ml H_2SO_4 for more than 5 g tissue. The actual amount of tissue and volume of acid used were recorded for each coral. The correlation between amounts of tissue (g) and ATP amounts was explored using *Capnella florida* samples only as not enough tissue was available for other species. An aliquot, either 0.7 ml or 1.0 ml, of the extraction mix was removed from the extraction vessel and transferred to a small disposable culture tube and the pH was adjusted to approximately pH 7 with the addition of 1-2 drops of 30% NaOH. This mixture was also kept on ice prior to being assayed. The aliquot was removed at 10, 15, and 20 minutes after the start of the extraction. Initially an aliquot was also removed at 5 minutes after the start of the extraction but the presence of ATP was not detected at this time interval in any of the samples assayed. The pH adjustment was necessary to prevent the acid from inhibiting the ATP bioluminescence (SIGMA 2004). The assay was prepared by adding to the wells of the reaction plate, 100 µl of ATP assay mix (full strength), followed by either 100 µl of extraction mixture or 100 µl of water or of ATP standard + water to equal 100 µl (Yang et al. 2004). Readings were

taken at 10, 15, and 20 minutes; the time that showed the greatest amount of ATP had that assay repeated twice. ATP was expressed as 10^{-13} moles/g of tissue (wet weight).

PROTEIN DETERMINATION

Prior to protein extraction, decalcification was carried out according to Yamashiro et al. (1999) without use of formaldehyde. Approximately 1 g of coral tissue was added to a labeled glass tube containing 10 ml of 10% acetic acid and left at room temperature for 48 hours. After 48 hours, proteins were extracted according to Yamashiro et al. (2005) with some modifications. The mixture of coral and acetic acid was homogenized for 2-5 minutes with a polytron at a speed of ~10000 rpm. The polytron probe was cleaned after each coral sample with acetic acid. 10 ml of boiling 0.5 M NaOH was added to the digested and homogenized sample and was homogenized again for 2-5 minutes with the polytron at 10000 rpm. The liquid phase was decanted into clean, labeled centrifuge tubes. The solid phase had another 10 ml of 0.5 M NaOH (boiling) added to it and the homogenization was repeated. The liquid phase was decanted and combined with the previous liquid in the centrifuge tubes. The solid phase had another 10 ml of 0.5 M NaOH (boiling) added to it and the homogenization was repeated. The liquid phases were combined for each sample and centrifuged for 10 minutes at 2000g, 4^{o}C. The supernatant from the extraction was decanted into a tube and held for protein determination according to Lowry et al. (1951). Similarly to ATP amounts, protein data was expressed in mg per g of wet weight. The correlation between wet weight and protein amounts was assessed using *Capnella florida* samples.

STATISTICAL ANALYSIS

Decision tree analysis (DTA) was carried out to explore trends and differences in ATP and protein data attributable to variations in water depth, sampling site location and species. DTA allows the formulation of relationships between one response (i.e., dependent) variable and several predictor (i.e. independent) variables by dividing a data set recursively into smaller, increasingly homogeneous portions. The final result constitutes a division of the original data set into mutually exclusive and exhaustive sub-sets (Morgan and Sonquist 1963; Kass 1980; Hawkins and Kass 1982; Breiman et al. 1984; Quinlan et al. 1987; Biggs et al. 1991; Safavian and Langrebe 1991). DTA was carried out using the procedure described by Breiman et al. (1984). At every level of the tree, stepwise splitting is performed by examining each of the predictor variables in turn and selecting the predictor resulting in the smallest within-group sum-of-squares for a binary split. The splitting criterion is expressed as proportional reduction in error (PRE), with a minimum PRE of 0.05 required for a split to result for any given predictor variable. The procedure supports both continuous and categorical variables. Categorical predictor variables used in the analysis include sampling site location (“Location”), coral species (“Species”), season of sampling (“Season”), as well as sampling year (“Year”). Water depth measured in meters (“Depth”) was included as continuous predictor variable. The risk of overfitting was avoided by specifying a

minimum number of cases, or stop size, for the creation of new nodes (Puestow et al. 2001). That is, if a given node contained fewer observations than the specified stop size it was not further partitioned. A stop size of 5 was selected for all tree models. DTA was applied to the entire dataset to examine the relationship between the predictor variables and ATP ("ATP"), as well as between variables and protein amounts ("PROT").

RESULTS

ATP

The ATP extraction procedure in acid revealed variations in extraction times (10, 15 or 20 minutes) resulting in the maximum ATP value. ATP values are expressed per g of wet weight of tissue. The correlation between weight and moles of ATP (done only for one species, all other samples did not have enough material) shows linearity starting after 0.5 g of tissue (Fig. 2).

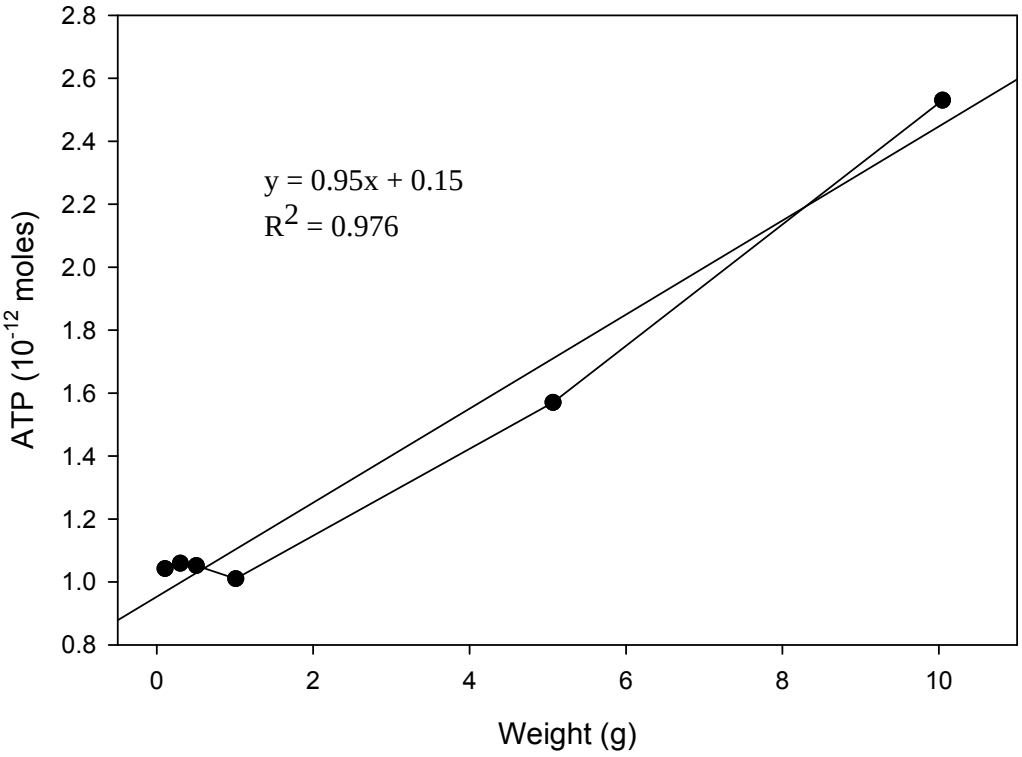

Figure 2. Correlation between ATP (10^{-12} moles) and crude weight (g) of *Capnella florida*.

All extractions conducted with less than 0.5 g were therefore not considered in this data analysis. ATP values (all species considered) varied from 7.93 10^{-15} to 513.00 10^{-13} moles of ATP/g of coral tissue (wet weight) with a mean of 27.00 10^{-13} moles/g. All ATP means are summarized in Table 1. DTA on all values of ATP shows

the influence of the sampling year on ATP (Fig. 3A) with ATP levels being higher the second year of sampling (2007). This result shows the necessity of considering 2 sets of data (one for each year) when researching trends or patterns in ATP data. When considering ATP data for each year of sampling, both DTAs show the importance of the predictor “Species” in determining differences in ATP values (Fig. 3B, 3C). Species with higher (or lower) ATP values do not seem to be consistent in 2005 and 2007 rendering it difficult to conclude on definite trends on ATP differences between species. To try to see if a “Depth”, “Season”, and/or “Location” effect could have been masked by the “Species” effect, DTAs were also determined for species with 10 or more samples (with enough samples for every case: season, location) for every sampling year.

Table 1. ATP and protein data of sampled deep-sea corals (mean ± standard deviation).

Species	ATP (10^{-13} moles/g) Year 2005	ATP (10^{-13} moles/g) Year 2007	Proteins (mg/g) Year 2005	Proteins (mg/g) Year 2007	Depth (m) Min- max
Ag	0.23±0.42 (n=7)	70.44±73.82 (n=18)	288.26±146.64 (n=7)	331.95±471.67 (n=18)	212-1347
Gr	0.28±0.38 (n=10)		405.96±174.79 (n=10)		65-1415
Cf	0.37±0.62 (n=11)	60.23±80.65 (n=18)	662.43±322.43 (n=11)	899.63±341.07 (n=18)	231-1375
Nepht		41.64±29.07 (n=5)		485.86±293.42 (n=5)	852-1233
Aa	0.27±0.42 (n=18)	0.65±0.08 (n=2)	276.21±143.61 (n=18)	229.78±2.48 (n=2)	274-1277
Ps	0.27±0.30 (n=2)	153.66±110.45 (n=2)	184.83±47.47 (n=2)	183.13±91.39 (n=2)	195-852
Pr	0.78±0.83 (n=14)		445.75±306.67 (n=14)		162-1157
Ko		20.56±11.95 (n=3)		125.62±22.32 (n=3)	1183-1233
Aar	2.00±1.73 (n=11)	24.11±14.09 (n=2)	264.99±197.00 (n=11)	138.14±5.02 (n=2)	195-1415
Rg		7.60 (n=1)		291.57 (n=1)	1355
Pa	0.58±0.65 (n=9)		262.27±95.85 (n=9)		370-1277
Bs		9.50 (n=1)		908.41 (n=1)	969
Anti		13.18 (n=1)		545.69 (n=1)	938
Agr	0.07±0.05 (n=4)	124.47±142.88 (n=9)	382.55±427.97 (n=4)	151.00±127.85 (n=9)	578-1422
Hf	1.09±2.14 (n=4)	113.52±180.85 (n=7)	238.08±128.20 (n=4)	704.75±270.34 (n=7)	473-1337
Pg		73.80±77.56 (n=5)		405.53±149.45 (n=5)	823-969
Ss	0.29±0.35 (n=6)		277.00±193.38 (n=6)		193-1223
Fa	0.16±0.25 (n=21)	8.71±11.73 (n=11)	360.08±156.68 (n=21)	395.18±161.34 (n=11)	296-1422

Alcyonacea: *Anthomastus grandiflorus* (Ag), *Gersemia rubiformis* (Gr), *Capnella florida* (Cf), other nephtheids (nepht), *Acanella arbuscula* (Aa), *Paramuricea spp.* (Ps), *Primnoa resedaeformis* (Pr), *Keratoisis ornata* (Ko), *Acanthogorgia armata* (Aar), *Radicipes gracilis* (Rg), *Paragorgia arborea* (Pa). Antipatharia: *Bathypathes spp.* (Bs). Pennatulacea: *Anthoptilum grandiflorum* (Agr), *Halipteris finmarchia* (Hf), *Pennatula grandis* (Pg), other seapens (Ss). Scleratinia: *Flabellum alabastrum* (Fa).

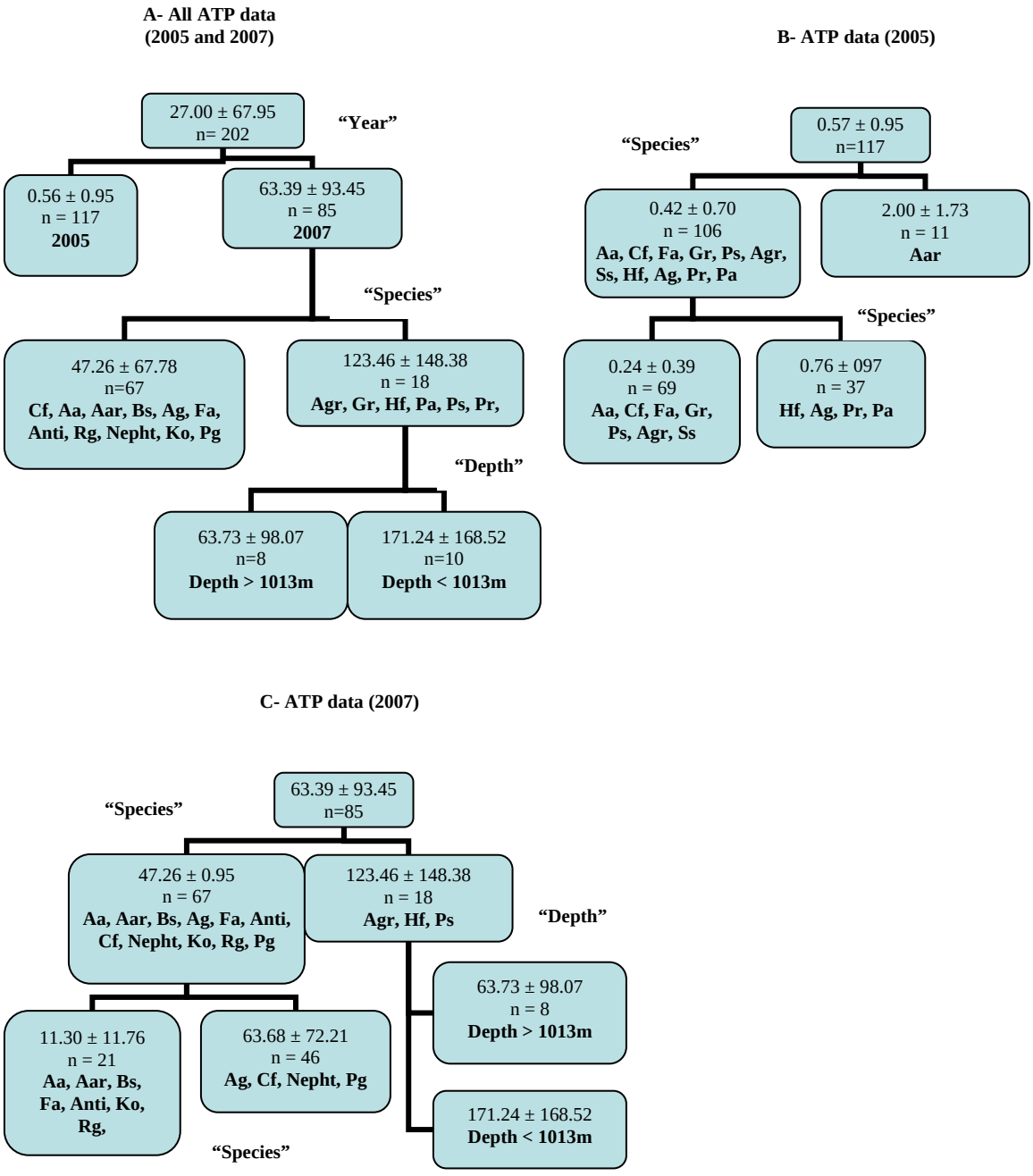

Figure 3. Decision tree analysis for **(A)** ATP (2005+07), **(B)** ATP (2005), **(C)** ATP (2007) with 4 predictors: **Depth, Species, Location, Year**. *Anthomastus grandiflorus* (Ag), *Gersemia rubiformis* (Gr), *Capnella florida* (Cf), other nephtheids (nepht), *Acanella arbuscula* (Aa), *Paramuricea* spp. (Ps), *Primnoa resedaeformis* (Pr), *Keratoisis ornata* (Ko), *Acanthogorgia armata* (Aar), *Radicipes gracilis* (Rg), *Paragorgia arborea* (Pa). Antipatharia: *Bathypathes* spp. (Bs), *Anthoptilum grandiflorum* (Agr), *Halipteris finmarchia* (Hf), *Pennatula grandis* (Pg), other sea pens (Ss). *Flabellum alabastrum* (Fa).

All data is expressed as mean ± standard deviation (10^{-13} moles of ATP/g).

Anthomastus grandiflorus (Ag) DTA for 2005 shows no split in data while a "Depth" effect can be seen in 2007 with an increase in ATP after 938m (Fig. 4A). *Acanella arbuscula* (Aa) samples were >10 only in 2005 showing an effect of sampling site with higher values of ATP in Cape Chidley (Fig. 4B). To the contrary of Ag, DTA (n>10 only in 2007) of *Capnella florida* (Cf) reveals a decrease in ATP after 939m (Fig. 4C). *Flabellum alabastrum* (Fa) sampled in 2005 shows no split in ATP values while in 2007, ATP decreases after 1034m (Fig. 4D). No split in data was seen for *Primnoa resedaeformis* in 2005. All these results show difficulty in establishing any trend with depth and/or location in ATP values.

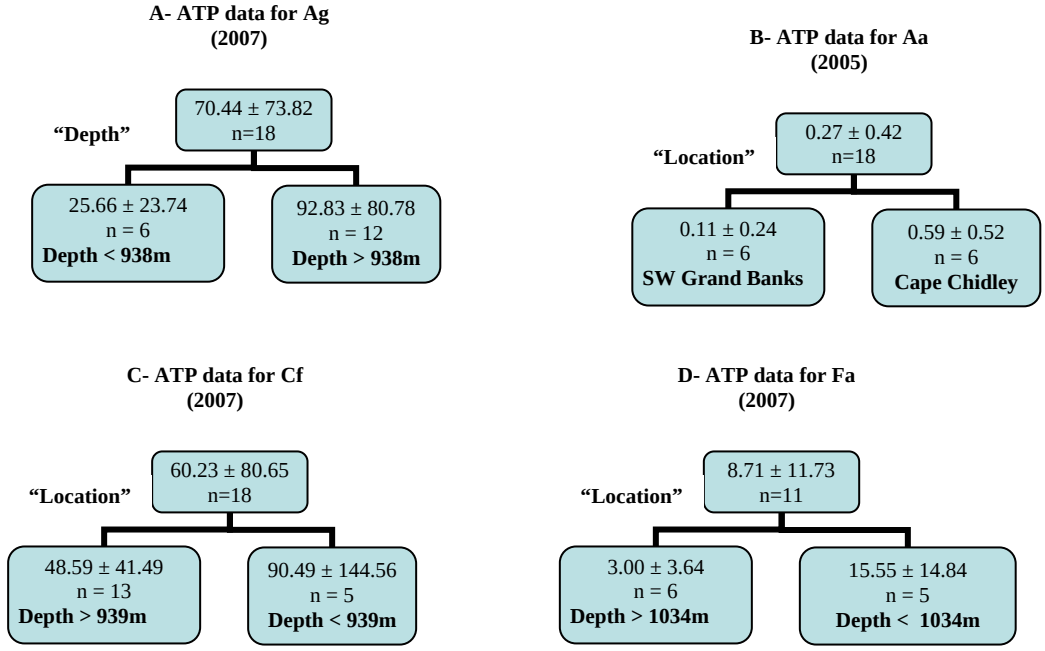

Figure 4. Decision tree analysis for **(A)** ATP in *Anthomastus grandiflorus* (Ag) (2007), **(B)** ATP in *Acanella arbuscula* (Aa) (2005), **(C)** ATP in *Capnella florida* (Cf) (2007), **(D)** ATP in *Flabellum alabastrum* (Fa) (2007) with 3 predictors: **Depth, Location, Season.** All data is expressed as mean ± standard deviation (10^{-13} moles of ATP/g).

The season of sampling (for 2005) did not seem to have a consistent effect on ATP data as ATP values in summer increased for some species and decreased for others (Fig. 5). Statistical differences between seasonal ATP values were found only for *Capnella florida* (Cf).

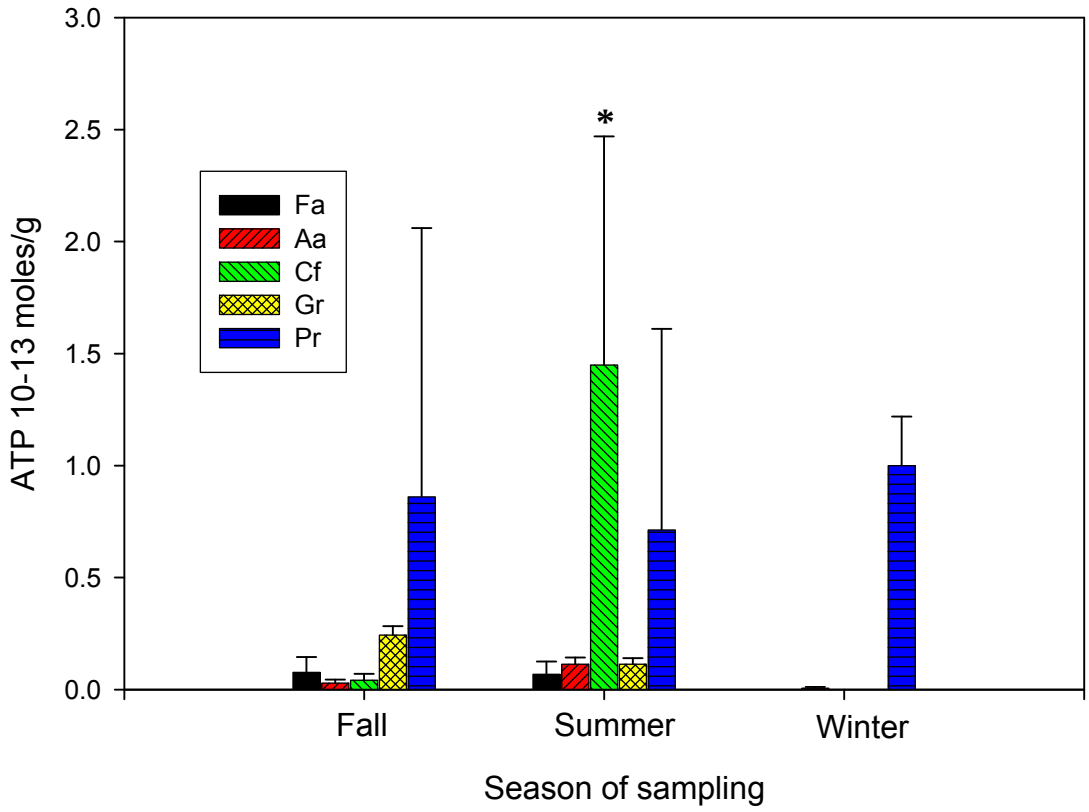

Figure 5. Seasonal ATP values in *Flabellum alabastrum* (Fa), *Acanella arbuscula* (Aa), *Capnella florida* (Cf), *Gersemia rubiformis* (Gr), and *Primnoa resedaeformis* (Pr) for 2005. All data is expressed as mean ± standard deviation (10^{-13} moles of ATP/g), not all species were sampled during the 3 seasons.
* significant difference between fall and summer.

PROTEINS

Correlation between amounts of proteins extracted and tissue weights shows perfect linearity and correlation in weight and mg of proteins (Fig. 6).

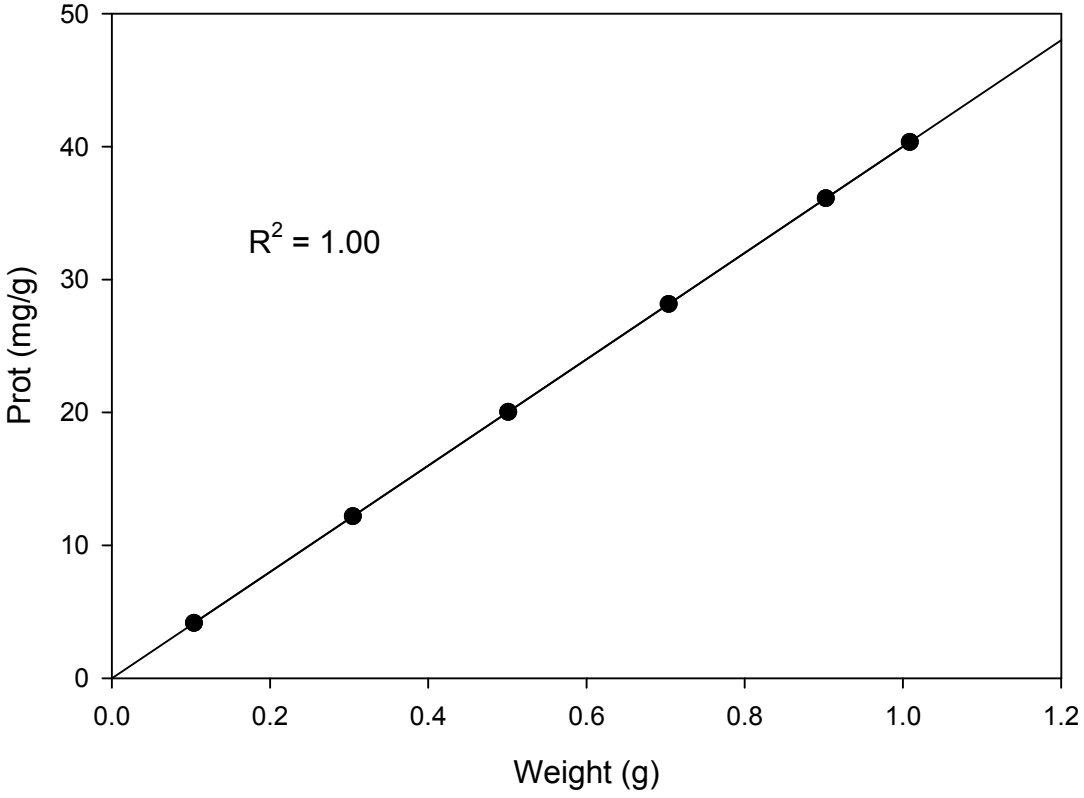

Figure 6. Correlation between protein content (mg) and crude weight (g) of *Capnella florida*.

Protein values varied from 22.11 mg to 2179.52 mg/g with a mean value of 410.42 mg/g wet weight. DTA for all protein values shows no effect of sampling year suggesting that data can be combined when looking for trends (Fig. 7A). DTA reveals species differences with *Capnella florida* (Cf), and *Bathypathes* spp. (Bs) (n=1) having the highest values (Fig. 7A). In 2005, only *Capnella florida* (Cf) seems to stand out in terms of its high protein value (Fig. 7C). In 2007, *Capnella florida* (Cf), *Halipteris finmarchia* (Hf), and *Bathypathes* spp. (Bs) (n=1) had the highest protein amounts (Fig. 7B).

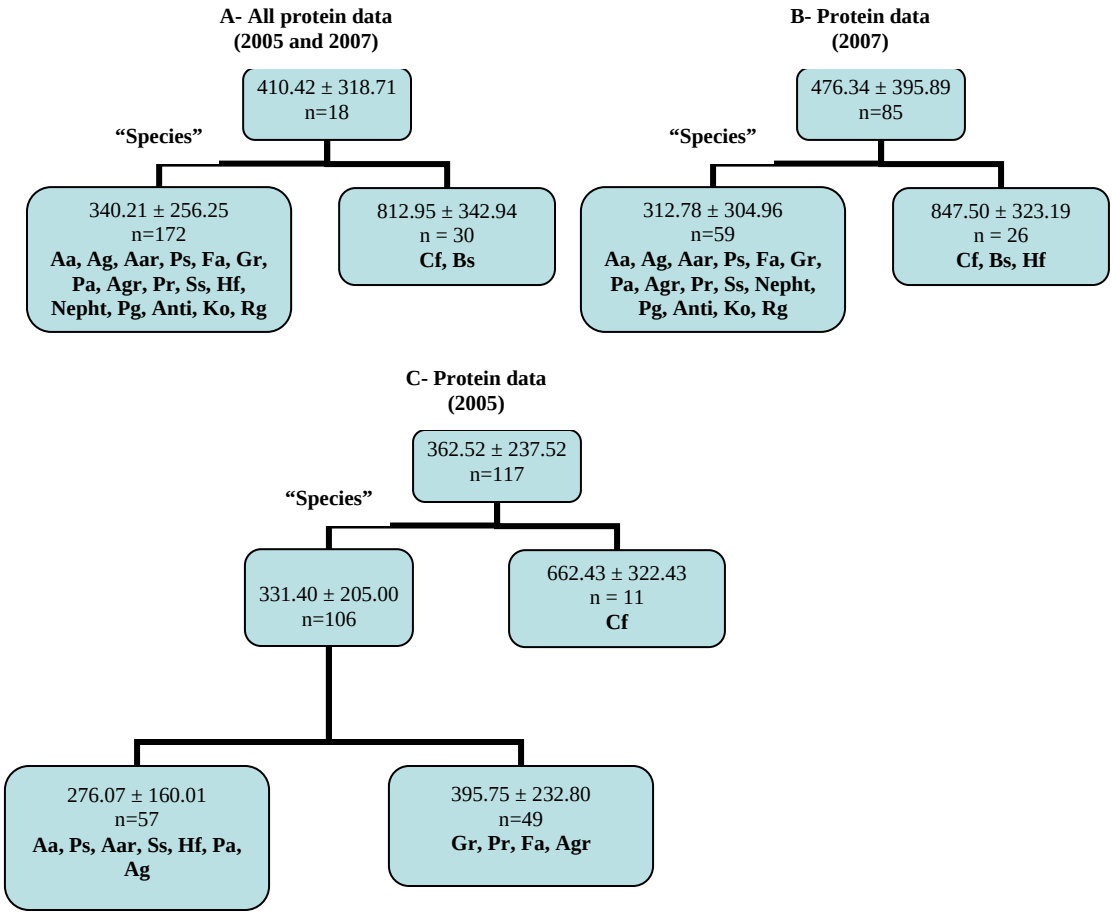

Figure 7**.** Decision tree analysis for **(A)** Proteins (2005+07), **(B)** Proteins (2007), **(C)** Proteins (2005) with 4 predictors: **Depth, Species, Location, Year (only for A)**. *Anthomastus grandiflorus* (Ag), *Gersemia rubiformis* (Gr), *Capnella florida* (Cf), other nephtheids (nepht), *Acanella arbuscula* (Aa), *Paramuricea* spp. (Ps), *Primnoa resedaeformis* (Pr), *Keratoisis ornata* (Ko), *Acanthogorgia armata* (Aar), *Radicipes gracilis* (Rg), *Paragorgia arborea* (Pa). Antipatharia: *Bathypathes* spp. (Bs), *Anthoptilum grandiflorum* (Agr), *Halipteris finmarchia* (Hf), *Pennatula grandis* (Pg), other sea pens (Ss). *Flabellum alabastrum* (Fa). All data is expressed as mean ± standard deviation (mg proteins/g).

When exploring species trends (those with n>10, not presented), DTAs showed an influence of "Depth" with no consistent pattern as some species had protein amounts increasing with "Depth" (Aa in 07, Ag in 07, Fa in 05), while others had decreasing values when "Depth" increased (Aa in 05, Fa in 07). No statistical differences were found between seasonal protein data of Gr, Pr, Aa, Fa, and Cf sampled in 2005 (samples were collected only in the fall for 2007). Similarly to seasonal variations in ATP data, no common pattern was found in these species (those with n>10).

DISCUSSION

Maximum ATP values were recorded at variable times during the extraction procedure. This might have been influenced by differences in size of the coral pieces used for extractions, despite efforts made to standardize mass of chopped pieces to avoid variations in coral surface area/acid contact. This lability of ATP during extraction has been previously observed by Fang et al. (1991). These authors found that an ATP reading would drop to one-tenth of its normal value if a sample was thawed in air and then put into H_2SO_4 for extraction, rather than being thawed directly in H_2SO_4. ATP values reported in this study are relevant as they are representative of the maximum ATP measured for a given tissue despite ATP lability. The high standard deviation observed in this study was also reported by other authors (Fang et al. 1989; 1991) and is also reflective of ATP lability.

The highest value obtained in our study was 513.00 10^{-13} moles of ATP/g; this value is lower than levels obtained by other authors in shallow water corals. Fang et al. (1987) obtained an average of 697.96 10^{-13} moles/g in the species *Acropora gravida*. In Fang et al. (1991), 4 species of *Acropora* as well as *Montipora aequituserculata* and *Porites nigrescens* revealed ATP levels between 14.30 10^{-9} to 91.80 10^{-9} moles/g. Shallow-water corals are essentially photoautrophic with respect to carbon, and have high rates of growth compared to deep-water corals (Huston 1985). Shallow water corals have therefore higher energy requirements than deep-sea species which may be reflected in higher ATP levels. Moreover, conservation of samples could have impacted ATP levels measured in this study. Samples were conserved at -20ºC in 2005 and in liquid nitrogen first than at -20ºC in 2007. In most studies described in the literature, shallow water coral samples used for ATP measurements were fresh or conserved in liquid nitrogen. It is important that sampling should minimize handling stress before freezing in liquid nitrogen (Fang et al. 1991). Corals samples obtained for this study were acquired opportunistically in a manner not always minimizing handling stress of samples. Therefore, any comparisons with ATP values in shallow water corals should be interpreted with caution.

In this investigation, data was combined from different species with different requirements in terms of depth and/or temperature. Decision tree analysis was applied to explore the effect of depth, sampling location and season on ATP and protein data. In the DTA used in this study, splitting is performed by selecting the predictor resulting in the smallest within-group sum-of-squares for a binary split. The objective is to partition data into homogeneous groups, while keeping the tree reasonably small (De'Ath and Fabricius 2000). These groups, even when not significantly different, bring important information as they reveal trends in often complex and unbalanced ecological data. Differences were found between 2005 and 2007 data with higher values the second year of sampling. These differences are probably due to differences in sample conservation. It is well known that ATP quickly disappears following mortality and is decreased during freezing (e.g. Cappeln and Jessen 2001). Initial freezing of coral samples in liquid nitrogen in 2007 could have slowed down ATP degradation leading to highest ATP amounts that year. DTA shows that ATP differences between species are

variable and do not show an association between ATP values and a particular group of corals.

Protein amounts obtained after extraction of deep-sea coral tissues were higher than values found in the literature for shallow water corals. Harithsa et al. (2005) found values between 3 and 8 mg/g tissue in two coral species from the Arabian Sea (*Porites lutea* and *Acropora formosa*). Similarly, Fang et al. (1987) found around 2 mg of protein/g of tissue in another shallow water species *Acropora gravida*. Values found in this study varied between 22.11 mg and 2179.52 mg/g, although, differences in extraction technique could also explain these dissimilarities. In Harithsa et al. (2005), homogenization in a saline solution followed by a centrifugation was used. Fang et al. (1987) used a one step boiling in NaOH to extract protein. In both cases, no decalcification was conducted. In this study, decalcification was performed in 10% acetic acid to ensure access to tissue for protein extraction. The extraction technique performed in this study on deep-sea corals was more "aggressive" (multi-step process), and resulting in more protein being extracted from the tissue. Other authors have used different extraction processes; Shick et al. (2005) measured proteins in methanol extracted colonies solubilized in boiling NaOH, while Grover et al. (2002) similarly to Fang et al. (1987) had tissues directly dissolved in NaOH. Both these authors expressed protein data in mg protein/cm^2.

Proteins amounts were higher in *Capnella florida*, *Keratoisis ornata*, *Radicipes gracilis* (1 specimen only), and *Bathypathes* spp. (n=1) showing no clear association with one group of corals as all these species belong to different orders. An effect of the predictor "Depth" was found on individual species. Nonetheless, this effect was different among species showing the importance of the "Species" predictor in explaining differences in proteins. Lipid analysis performed on these species has revealed a link between coral groups and differences in total lipid percentages with the following sequence: total lipids in gorgonians < soft corals and sea pens < Order Antipatharia (Hamoutene et al. 2008). In this study, antipatharians represented by *Bathypathes* spp. also show high levels of proteins suggesting that this genus could have a high potential for storage. Antipatharians are considered a deep-water species with mean depths usually greater than 1000 m (Kramp 1932; Pax 1932; Gass 2005; Gass and Willison 2005; Wareham and Edinger 2007). Antipatharians could have adapted to deep sea environments where sources of energy are unreliable and unpredictable. Having sufficient protein and lipid storage would enable antipatharian colonies to maintain basic physiological processes during periods of low food availability. When considering only the "Season" effect on the most represented 2005 species no common pattern was found as some species had higher protein amounts in summer versus fall while others showed the opposite trend. Despite the slow growth of deep-sea corals (Watling and Norse 1998) and increased longevity (Sherwood et al. 2005a), it is expected that coral tissue which turns over in something less than 1 year, should represent a unique seasonal signature (Sherwood et al. 2005b). Any decrease in food availability should be reflected in protein data, as starvation in shallow water corals has caused a significant decrease in protein concentration over time (Shick et al. 2005). Despite seeing some

changes in protein amounts with season, no significant differences were found (other than for Cf) and no common pattern was seen in species considered.

The overall results obtained using DTA of ATP and protein data show strong differences between species with low seasonality in the data (despite some non-significant variations). Strong differences were found in reproductive strategies between three deep-water species (*Caryophyllia ambrosia*, *C. cornuformis*, *C. sequenzae*) examined by Rhian et al. (2005) even though they are congeners, showing the importance of the "Species" predictor in explaining differences. Moreover, Rhian et al. (2005) found asynchronous, cyclical hermaphroditism with no evidence of seasonality in these three deep-sea species, suggesting less influence of reproductive cycles on general metabolism. Nonetheless, the absence of trend with depth and season in protein data remains surprising considering its link with food availability and some of the observations found on lipid data in deep-sea corals by Hamoutene et al. (2008).

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