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Comparative bioaccumulation of trace elements between *Nautilus pompilius* and *Nautilus macromphalus* (Cephalopoda: Nautiloidea) from Vanuatu and New Caledonia

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ABSTRACT

The concentrations of 16 trace elements were investigated and compared for the first time in the digestive and excreting tissues of two *Nautilus* species (Cephalopoda: Nautiloidea) from two geologically contrasted areas: (1) *N. macromphalus* from New Caledonia, a region characterized by its richness in nickel ores and its lack of tectonic activities and (2) *N. pompilius* from the Vanuatu archipelago showing high volcanic and tectonic activities. In both *Nautilus* species, results clearly highlighted that the digestive gland played a key role in the bioaccumulation and storage of Ag, Cd, Ce, Co, Cu, Fe, La, Nd, V, and Zn whereas As, Cr, Mn, Ni, Pb, and Se were accumulated in a greater extent in the excreting tissues (i.e. pericardial and renal appendages). Despite contrasting environments, no significant difference ($p<0.05$) was found between the two *Nautilus* species in the concentrations of most of the essential and non-essential elements, including Ni and associated metals in Ni ores (i.e. Co and Mn). As nautilus lives on the outer shelf of barrier reefs, these results strongly support the hypothesis that the New Caledonian lagoon traps the major amount of the trace elements derived from natural erosion and the intense mining activities conducted on land. In contrast, the concentrations of the rare earth elements (Ce, La, and Nd) were significantly higher in *N. pompilius* than in *N. macromphalus*, probably as a result of the local enrichment of Vanuatu waters by specific environmental processes, such as volcanism or upwelling.

1. Introduction

Marine organisms are exposed to trace elements that are present in their diet and dissolved in seawater. This double exposure results in element accumulation in their different tissues whether or not these elements are essential to the metabolism (Rainbow, 2002). This process, widely described as bioaccumulation can vary greatly according to the specificities of the organisms such as age/size, sex, and lifestyle, and according to the relative bioavailability of the metal in diet and seawater (Rainbow and Wang, 2001). Comparative analysis of trace element concentrations between closely related species living in different geographical areas can therefore allow at assessing the influence of the environment on their bioaccumulation.

Among the marine species, cephalopods belong to a molluscan group comprising ca. 700 species (Boyle and Rodhouse, 2005), in which high concentrations of trace elements such as Ag, Cd, Cu or Zn have generally been recorded (Martin and Flegal, 1975; Finger and Smith, 1987; Miramand and Bentley, 1992; Yamada et al., 1997; Bustamante et al., 1998; Dorneles et al., 2007). Indeed, several studies have demonstrated that the digestive and the excretory organs of cephalopods play a major role in the bioaccumulation of trace elements, as these organs are deeply involved in the assimilation processes, detoxification, and storage of both essential and non-essential metals (Miramand and Guary, 1980; Miramand and Bentley, 1992; Bustamante et al., 2000, 2002a, b, 2004).

In the present work, we focused on the bioaccumulation of trace elements in the *Nautilus* genus. Nautiluses are scavenger cephalopods, inhabiting the outer shelf of barrier reefs in the Indo–Pacific area, generally at a depth of 300–500 m (O'Dor et al., 1993; Norman, 2000). In spite of its evolutionary interest (Nautilus is the last representative of ectocochleate cephalopods, i.e. considered as a "living fossil"; Boyle and Rodhouse, 2005) and its physiological specificities (slow digestion and most of the excretory processes taking place in the pericardial appendages;

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Schipp et al., 1985; Westermann et al., 2002), only very few studies deal with the bioaccumulation of trace elements in this ancestral genus. High concentrations of several metals and metalloids were reported in the digestive and excretory tissues of *Nautilus macromphalus* (Bustamante et al., 2000), a species endemic to the New Caledonian waters. These authors suggested that the high metal concentrations may be due to a high exposure in the metal-rich conditions in the surrounding New Caledonian waters. Indeed, the largest resources of Ni as laterites in the world are present in New Caledonia where open-cast mining is conducted since the 20th century, leading to a dramatic increase of metal concentrations in coastal waters and, subsequently, the metal burdens in marine biota (Labrosse et al., 2000; Metian et al., 2008). However, it is not clear whether metal accumulation observed in *N. macromphalus* reflected the impact of metal contamination through human activities on the outer shelf waters of New Caledonia or either if it is more due to a natural progressive bioaccumulation during nautilus long life span (*viz*. 10–15 yr).

The aim of the present study was therefore to determine the bioaccumulation of a large range of elements in the genus *Nautilus* comparing two species from geologically contrasted areas: (1) *N. macromphalus* from New Caledonia which is characterized by its richness in nickel ores and its lack of tectonic activities and (2) *N. pompilius* from the Vanuatu archipelago which shows high volcanic and tectonic activities (Harrison et al., 1996). The analysed elements comprised 11 metals (Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn), two metalloids (As and Se) and three rare earth elements (Ce, La, and Nd). These elements were selected for comparison with other species and other published works concerning Nautilus (e.g. Miramand and Bentley, 1992; Bustamante et al., 2000; Ichihashi et al., 2001). The concentrations and relative contents of these different trace elements were determined in the digestive and the excretory tissues (i.e. pericardial and renal appendages) and compared between both nautilus species. The specificities and the potential influence of the environment on bioaccumulation of these trace elements in the *Nautilus* genus are discussed.

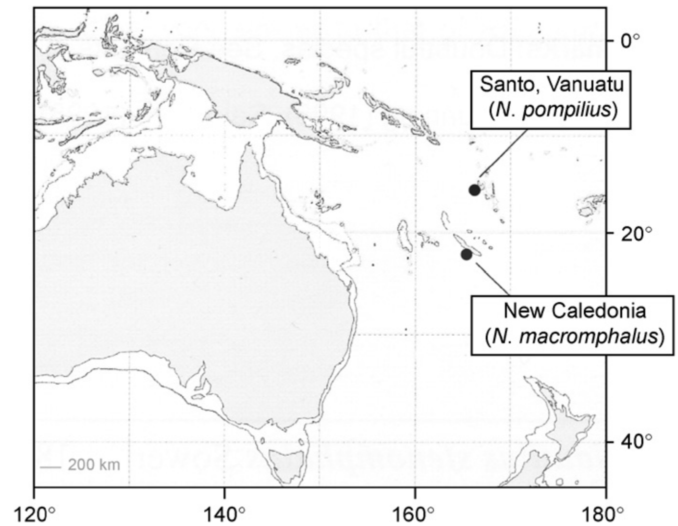

Fig. 1. Location of *Nautilus pompilius* and *N. macromphalus* specimens' collection.

Table 1
Characteristics of the sampled specimens of *Nautilus pompilius* and *N. macromphalus*

Species	Sex	Length (mm)	Weight (g)
N. pompilius			
1	Male	177	825
2	Male	151	660
3	Male	171	796
N. macromphalus			
1	Male	164	766
2	Female	162	676
3	Male	173	870
4	Male	160	758
5	Male	140	480

2. Materials and methods

2.1. Biological material

Three *N. pompilius* and five *N. macromphalus* specimens were collected, respectively, off Santo island coast (Vanuatu, 15°40′522″S, 167°02′838″E, depth: 334 m) and on the outer shelf of New Caledonia (22°20′500″S, 166°13′400″E, depth: 300 m) in May 2006 (Fig. 1). Each individual was measured and weighted prior to dissection. The main features of the eight specimens sampled are reported in Table 1.

All specimens were dissected aseptically and for each specimen one pericardial appendage, one renal appendage and a fraction of the digestive gland were immediately frozen in liquid nitrogen and stored at −80 °C for metal analyses.

2.2. Analytical procedure

Tissue samples were freeze-dried. Aliquots of the samples ranging from 20 to 300 mg were digested using a 3:1 v:v nitric-hydrochloric acid mixture with 65% ultrapure HNO_3 and ultrapure 37% HCl. The acidic digestion was performed overnight under ambient temperature and then heated in a microwave during 30 min, with increasing temperature until 105 °C, and 15 min at 105 °C (1200 W). After the mineralisation process, each sample was diluted to 30 or 50 ml with milli-Q quality water, according to the volume of acid added to the mineralisation (3 and 4.5 ml, respectively).

Ag, As, Cd, Ce, Co, Cr, Cu, Fe, La, Mn, Nd, Ni, Pb, Se, V, and Zn were analysed either by ICP-OES (Varian® Vista-Pro) and ICP-MS (Varian® Ultra Mass 700). Reference tissues, dogfish liver DOLT-3 (NRCC), lobster hepatopancreas TORT-2 (NRCC) and bush branches and leaves (GBW 07602) were treated and analysed in the same way as the samples. Results were in good agreement with the certified values, and the standard deviations were low, proving good repeatability of the method. The results for standard reference materials displayed recoveries of the elements ranging from 81% to 119% ($n = 10$). For each set of analyses, blanks were included in each analytical batch. The detection limits ($\mu g\,g^{-1}$ dry wt) for ICP-OES were 8.3 (As, Fe, Zn), 3.3 (Ag, Se), 1.67 (Pb, V), 0.83 (Cd, Co, Cr, Cu, Mn, Ni), and for ICP-MS, they were 0.150 (Ni, V), 0.065 (Cd, Co, Cr, Cu, Mn, Pb), 0.033 (Ag), 0.017 (Nd), 0.008 (Ce, La). Average element concentrations are presented with standard deviations. All trace element concentrations are given on a dry weight basis ($\mu g\,g^{-1}$ dry wt).

2.2.1. Data analyses

Statistical analyses were performed using MINITAB 13.2 Software. Comparison of trace element concentrations in nautilus tissues between sites was tested by 1-way ANOVA. Hypothesis of normal distribution was tested using the Anderson–-Darling test and equality of variance by the Bartlett test. Pearson coefficients were calculated between trace elements in each tissue. The significance level for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1. Trace element concentrations in the tissues

Metal concentrations in the digestive gland, the renal appendages and the pericardial appendages of *N. macromphalus* and *N. pompilius* are reported in Table 2. La, Ce, and Nd concentrations fell below the detection limit in the renal appendages. Among the three tissues analysed, the digestive gland was the major site of accumulation for Ag, Cd, Ce, Co, Cu, Fe, La, Nd, V, and Zn (Table 2). The digestive gland also concentrated As and Ni at concentrations close to the highest ones recorded in the other tissues. As, Cr, Mn,

Table 2
Trace elements concentrations (Mean ± SD; $\mu g\,g^{-1}$ dry wt) in the tissues of Nautiloidae from this study and from the literature

Tissue	Rare earth elements			Non essential metals			Essential metals and metalloids									
	La	Ce	Nd	Ag	Cd	Pb	As	Co	Cr	Cu	Fe	Mn	Ni	Se	V	Zn
N. macromphalus (Bustamante et al., 2000)																
Digestive gland	NA	NA	NA	4.45±2.68	45.1±13.2	0.46±0.17	166±26	7.8±3.6	4.4±1.1	106±46	666±231	10.1±1.7	16.3±7.8	NA	8.8±2.0	672±208
Pericardial appendages	NA	NA	NA	20.3±6.2	2.16±1.63	1.00±0.82	260±111	1.31±0.80	8.25±1.55	27.2±33.4	78±46	25.1±23.0	5.64±3.54	NA	0.18±0.09	22.4±26.6
Renal appendages	NA	NA	NA	1.80±2.22	1.87±1.23	1.06±0.88	112±65	0.43±0.37	2.54±2.88	7.1±10.2	343±160	77.1±54.1	22.7±13.9	NA	1.02±0.72	11.0±16.8
N. macromphalus (present study)																
Digestive gland	0.30±0.07	0.25±0.11	0.19±0.07	9.42±3.91	51.5±3.5	0.98±0.15	257±19	4.69±1.51	2.95±0.35	263±78	353±63	10.9±1.3	14.0±1.04	2.65±0.90	14.8±2.28	388±61
Pericardial appendages	0.11±0.02	0.17±0.05	0.15±0.05	1.82±0.54	1.93±0.76	1.57±0.11	169±19	0.65±0.09	9.59±3.35	73.7±9.9	19±1	12.7±2.9	6.93±1.37	3.72[a]	5.62±2.26	56.5±1.8
Renal appendages	<dl	<dl	<dl	0.44±0.12	3.39±1.16	2.11±0.51	152±17	0.18±0.03	2.57±0.36	27.6±4.2	20±3	68.2±6.2	19.7±2.39	9.84±0.94	1.54±0.13	35.2±2.8
N. pompilius (present study)																
Digestive gland	1.00±0.14	1.58±0.32	0.92±0.10	5.65±1.04	74.3±6.8	0.62±0.07	210±16	3.85±1.04	1.37±0.24	311±61	625±51	15.4±1.7	9.77±0.07	2.35±0.84	8.42±0.57	470±52
Pericardial appendages	0.24±0.03	0.42±0.05	0.31±0.01	1.75±0.07	2.39±0.37	1.24±0.24	323±53	0.79±0.07	9.30±3.87	99.7±2.4	21±3	24.1±4.9	7.02±0.79	2.49[a]	4.94±0.74	58.2±2.7
Renal appendages	<dl	<dl	<dl	0.35±0.03	5.93±0.31	0.99±0.21	292±80	0.18±0.03	1.98±0.08	53.3±4.6	24±3	307±83	19.6±0.97	13.3±6.12	1.82±0.02	42.7±1.5

dl: detection limit; NA: not analysed.
[a] Only one value.

Ni, Pb, and Se concentrations were remarkable in excreting tissues. More specifically, the renal appendages exhibited the highest Mn and Se concentrations, whereas As, Ni, and Pb were concentrated at similar concentrations within both renal and pericardial appendages. Finally, the pericardial appendages were the major site of accumulation for Cr.

3.2. *Comparison between species*

Generally, the pattern of trace element concentrations was the same in both *N. macromphalus* and *N. pompilius* tissues. However, there were few exceptions and more particularly in the digestive gland: firstly, Ce, Fe, La, and Nd concentrations were significantly ($p<0.05$) higher in *N. pompilius;* secondly, Cd and Cr concentrations were significantly ($p<0.05$) higher in *N. macromphalus* (Table 3).

In the case of the pericardial appendages, As, La, and Nd concentrations were significantly higher ($p<0.05$) in *N. pompilius* (Table 3). For the renal appendages, Cu was the only element for which a significant difference was detected, *N. pompilius* having significantly higher concentrations than *N. macromphalus* (Table 3).

3.3. *Relative proportion of trace elements between tissues*

Among the three tissues considered, the digestive gland contained the largest proportions of most of the trace elements with 73–81% of Ag, 90–91% of Cd, 61–79% of Ce, 80–86% of Co, 67–72% of Cu, 90–93% of Fe, 72–81% of La, 55–75% of Nd, 56–67 of V, 81–82% of Zn in both *Nautilus* species (Fig. 2). The only exceptions were As, Cr, Mn, Ni, Se, and Pb which were mainly contained in the excreting tissues, i.e. renal and pericardial appendages (Fig. 2). The renal appendages were the major site of accumulation of Mn, Ni, and Se (i.e. 74–89% for Mn, 49–54% for Ni; 61–73% for Se) while the pericardial appendages accumulated the highest relative proportion of Cr (i.e. 63–74%). Although the concentrations of some of the trace elements were high in the excretory organs, these tissues contained in fact low amounts of metals because of their small masses.

3.4. *Correlations between metals*

Table 4 shows the correlation between trace elements within the three tissues. It is noteworthy that the rare earth elements were correlated between each other within the digestive gland ($r>0.985$ and $p<0.001$ in all cases) and the pericardial appendages ($r>0.922$ and $p<0.003$ in all cases). In the digestive gland, rare earth elements varied positively with Fe and Mn ($r>0.918$ and $r>0.807$ and $p<0.004$ and $p<0.028$, respectively) but negatively with As ($r>-0.726$ and $p=0.047$). Cu correlated positively with Zn ($r=0.834$ and $p=0.020$ in all cases) but negatively with Se in this tissue ($r=-0.809$ and $p=0.028$ in all cases).

In the renal appendages, correlations were weak, Mn varied positively with Cd ($r=0.833$, $p=0.010$) and Zn ($r=0.735$, $p=0.038$), Fe with Zn ($r=0.833$, $p=0.010$) and Pb with Cr ($r=0.936$, $p=0.001$) but negatively with V ($r=-0.753$, $p=0.031$; Table 3).

In the pericardial appendages, Cd concentrations correlated positively with Cr ($r=0.897$, $p=0.006$), Ni ($r=0.965$, $p<0.001$), and *V* ($r=0.931$, $p=0.002$).

Table 3
One-way ANOVA comparison of trace element concentrations in *N. pompilius* (Np) and in *N. macromphalus* (Nm)

Element	Digestive gland	Pericardial appendages	Renal appendages
Rare earth elements			
Ce	Np > Nm ($F = 19.58$; $p = 0.007$)	NS	–
La	Np > Nm ($F = 23.40$; $p = 0.005$)	Np > Nm ($F = 0.609$; $p = 0.025$)	–
Nd	Np > Nm ($F = 25.13$; $p = 0.004$)	Np > Nm ($F = 6.54$; $p = 0.051$)	–
Essential elements			
As	NS	Np > Nm ($F = 9.58$; $p = 0.027$)	NS
Co	NS	NS	NS
Cr	Nm > Np ($F = 11.34$; $p = 0.020$)	NS	NS
Cu	NS	NS	Np > Nm ($F = 15.58$; $p = 0.008$)
Fe	Np > Nm ($F = 10.09$; $p = 0.025$)	NS	NS
Mn	NS	NS	NS
Ni	NS	NS	NS
Se	NS	NS	NS
V	NS	NS	NS
Zn	NS	NS	NS
Non essential elements			
Ag	NS	NS	NS
Cd	Nm > Np ($F = 0.740$; $p = 0.023$)	NS	NS
Pb	NS	NS	NS

NS: not significant.

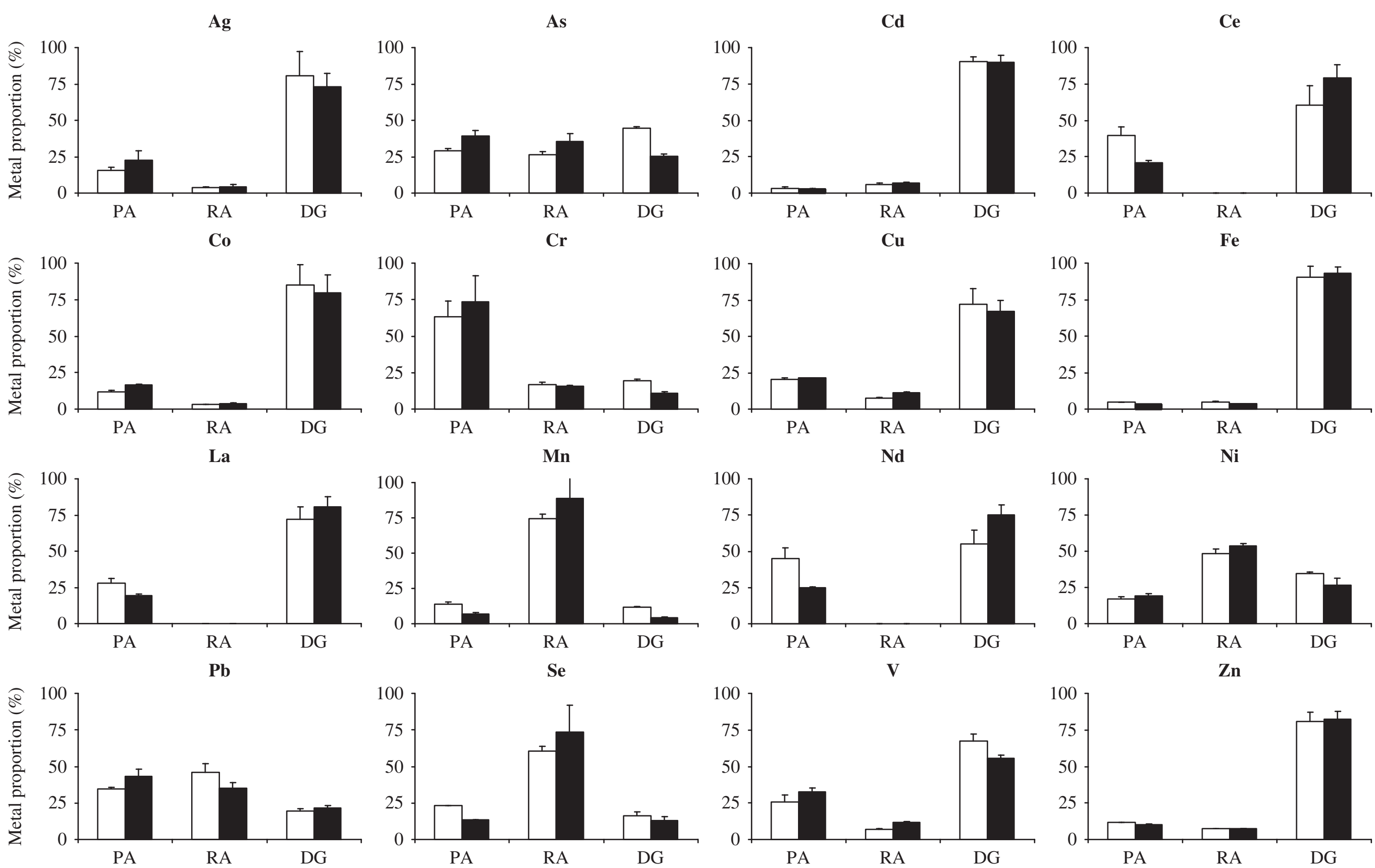

Fig. 2. Relative distribution of the trace elements between the digestive gland (DG), pericardial appendages (PA) and renal appendages (RA) of *Nautilus pompilius* from Vanuatu (black) and *N. macromphalus* from New Caledonia (white). Scale bars represent 1 standard error.

4. Discussion

The present extensive analysis of 16 trace element concentrations in *N. macromphalus* and *N. pompilius* digestive and excreting tissues clearly highlighted that these organs constitute important tissues for the bioaccumulation of rare earth elements, metals, and metalloids. Such results support the hypothesis of efficient regulation processes for non-essential metals in Nautilidae (Bustamante et al., 2000) even if, among the eight studied specimens, the variability of metal concentrations was relatively high (Table 2).

Table 4
Correlations between trace elements within the different tissues of nautilus

Elements	Digestive gland	Renal appendages	Pericardial appendages
La	+Ce, +Nd, −As, +Fe, +Mn	<dl	+Ce, +Nd, +Mn
Ce	+Nd, −As, +Fe, +Mn	<dl	+Nd, +Co, +La
Nd	+Fe, +Mn	<dl	+Ce, +Co, +La
Cu	+Zn, −Se		
Fe	+Mn, −V, +La, +Ce, +Nd	+Zn	+Ag, +Mn, +Cr
Pb	+V	−V, +Cr	
Mn	+La, +Ce, +Nd, +Fe	+Cd, +Zn	+La, +Fe
Cd		+Mn	+Ni, +V, +Cr

Not underlined: $p<0.05$; underlined: $p<0.001$.
<dl: concentrations below the detection limit.

In cephalopods, the digestive system plays a major role in both the digestive and the detoxification processes: on the one hand, the digestive gland synthesizes the digestive enzymes and is a place of absorption and assimilation of nutrients (Boucaud-Camou and Boucher-Rodoni, 1983; Boucher-Rodoni and Boucaud-Camou, 1987); on the other hand, this organ is involved in the storage and detoxification of trace elements (Bustamante et al., 2002a, b, 2004). Our investigations on trace element concentrations also confirmed the key role of the digestive gland of both *N. macromphalus* and *N. pompilius* in the metabolism of most of the analysed trace elements (i.e. Ag, Cd, Ce, Co, Cu, Fe, La, Nd, V, and Zn), as it is also the case in more recent cephalopods, i.e. belonging to the Coleoidae (Ghiretti-Magaldi et al., 1958; Martin and Flegal, 1975; Miramand and Guary, 1980; Smith et al., 1984; Finger and Smith, 1987; Miramand and Bentley, 1992; Seixas et al., 2005a; Pierce et al., 2008).

Our results also stressed the potential of excreting tissues (i.e. renal and pericardial appendages) to accumulate several elements such as As, Cr, Mn, Ni, Pb, and Se. In contrast to the Coleoidae, in nautiluses the renal appendages are specialized in the storage of calcium and the pericardial appendages play a major role in the evacuation of the metabolic wastes (Schipp and Martin, 1987). These organs are known to harbour a dual bacterial symbiosis (Pernice et al., 2007) involving spirochaetes and betaproteobacteria. The exact mechanisms by which these bacterial symbionts are involved in nautilus excretion are still enigmatic. Nevertheless, it is widely admitted that micro-organisms can play an important role in the incorporation of trace elements (Worms et al., 2006). Accumulation of Co, Fe, and Ni by betaproteobacterial symbionts of the pericardial appendages of *N. macromphalus* has already been observed by electronic microscopy (Pernice, unpublished data).

However, it remains unclear if this accumulative process is only passive (viz. through adsorption processes) or active (viz. effective absorption of trace elements). Indeed, the betaproteobacterial symbionts present in nautilus tissues could play a role in the detoxification and storage of some metals such as Co, Fe, and Ni (Table 2). Such processes could involve enzymatic reduction and/or complexation with organic compounds (Pernice et al., 2006), leading to the storage of these metals at high concentrations in the excreting tissues, independently of the *Nautilus* species. The role of this bacterial symbiosis on trace element bioaccumulation and detoxification in nautilus excreting tissues clearly deserves further investigations.

Although Nautilidae have been previously reported for their special abilities to accumulate heavy metals at relatively elevated concentrations in their tissues (Bustamante et al., 2000), this study is the first comparative approach aiming to compare trace element concentrations in two *Nautilus* species from two geographically close areas (viz. distant from less than 1000 km). Both species potentially share common trophic features but, at the same time, they live in geologically different environments (richness in nickel ores and lack of tectonic activities in New Caledonia and high volcanic and tectonic activities in Vanuatu), potentially resulting in a different degree of "natural" contamination. New Caledonia is currently the third largest producer of Ni in the world (Dalvi et al., 2004) and mining activities associated to a strong natural erosion have resulted in the enrichment of Ni and associated metals in Ni ores (i.e. Co, Cr, Cu, Fe, and Mn) and some other extracted metals (i.e. Ag and Pb) in the coastal waters and consequently in the lagoon food webs (Monniot et al., 1994; Fernandez et al., 2006; Hédouin et al., 2006; Metian et al., 2008). Conversely, it is likely that the seawater concentrations of most of these metals are very low in Santo islands waters and around Vanuatu in general according to the absence of industrial and mining activities in this area (Harrison et al., 1996). Surprisingly, in the present study, very little difference appeared in the sixteen trace element concentrations between *N. macromphalus* from New Caledonia and *N. pompilius* from Vanuatu. Interestingly, there was no significant difference between both *Nautilus* species for Ag, Co, Mn, Ni, Pb, Se, V, and Zn (Table 3) indicating that the geographical origin does not seem to be a major factor contributing to interspecific variations of these trace element concentrations. As nautilus lives on the outer shelf of barrier reefs and rarely penetrates into lagoons (Saunders and Ward, 1987; Norman, 2000), our results strongly supported the hypothesis that the New Caledonian lagoon traps the major amount of the trace elements derived from the island (Ambatsian et al., 1997). To the best of our knowledge, there are no published data in the open literature concerning trace elements in organisms from the oceanic waters off New Caledonia, except for two marine mammal species (Bustamante et al., 2003). These authors reported very low concentrations of Co, Cr, and Ni in the marine mammal tissues which suggested that mining activity in New Caledonia does not seem to be a significant source of contamination in the oceanic domain. Overall, the global geologic contagion due to the mining activity in New Caledonia seems to be limited to the shoreline waters and to the inner lagoon (Fernandez et al., 2006).

More interestingly, the concentrations of rare earth elements were significantly different between the digestive gland and the pericardial appendages of *N. macromphalus* and *N. pompilius*. F-transition elements of rare earths have similar physicochemical properties, and it is therefore not surprising that they correlate to each other (Table 4). Our results suggested that rare earth elements would reflect the characteristics of the surrounding environment. Very little is known about the concentrations of Ce, La, and Nd in cephalopods, which varied from 0.045 to 1.95 μg g^{-1} d wt in the digestive gland of the squid *Stenoteuthis oualaniensis* (Ichihashi et al., 2001). In fact, rare earth elements have chemistry and sorption/complexation characteristics analogous to transuranic elements such as americium or plutonium (Choppin Torres and Choppin, 1984; Kim et al., 1989; Buckeau et al., 1992).

In Coleoid cephalopods, transuranic elements also accumulate in the digestive and excreting tissues, in particular in the branchial heart and their appendages (Guary et al., 1981; Guary and Fowler, 1982; Bustamante et al., 2006). It is therefore not surprising that they accumulate at relatively high concentrations in nautilus digestive gland and pericardial appendage.

Besides Ce, La, and Nd, the concentrations of As, Cu, and Fe were also higher in *N. pompilius* than in *N. macromphalus*. It is difficult to ascertain the reasons of such a difference but this might also be linked to geological features reflecting a potential enrichment of Vanuatu waters by local environmental processes, such as volcanism or upwelling (Malinovsky and Markevich, 2007). In fact, only two trace elements, Cd and Cr, displayed significantly higher concentrations in *N. macromphalus* than in *N. pompilius* tissues (Table 2). Therefore, the reasons of such interspecific differences in bioaccumulation, i.e. higher concentrations of trace elements in one species, remain unclear whereas an absence of difference could be explained by the similar physiology of these two species (Ward, 1988; Norman, 2000).

As for other marine organisms, in cephalopods, trace element concentrations may vary with biological and environmental factors such as temperature, season, age (size), sex, lifestyle and geographical origin (e.g. Monteiro et al., 1992; Bustamante et al., 1998; Raimundo et al., 2004; Seixas et al., 2005b; Pierce et al., 2008). Further investigations are needed to understand how exactly these factors affect their concentrations in the studied species. However, among these factors, individual size/age is known to be of primary importance in invertebrates (Warnau et al., 1995; Bustamante and Miramand, 2005; Hédouin et al., 2007). In the present study, the size as well as the two others main features measured (sex and weight) were globally homogenous among the eight studied specimens. Because nautiluses are long lived animals, it would be striking to determine the influence of the size/age on trace elements bioaccumulation.

Sources of trace elements to nautilus can be (1) seawater, as it passes through the skin and through the gills during respiration, and (2) food, which is now considered as a major route for metal accumulation in marine animals, including both invertebrates and fish (Koyama et al., 2000; Wang, 2002; Bustamante et al., 2002a, 2004). Knowledge of trace element speciation in the direct environment of nautilus is a key factor to ascertain the relative importance of each uptake pathways. Concerning the first pathway (i.e. seawater), further aquatic toxicity studies are needed to determine the fraction of total metal dissolved in seawater (the 'toxic fraction') that react with, and is transported across biological membranes such as nautilus gills. Concerning the second pathway (i.e. food), our comparative analysis suggested that the metal discharges due to intense mining activities conducted on land in New Caledonia are not easily transferred in the food web of nautilus. Therefore, to assess the impact of mining activities on cephalopods in the region of New Caledonia, further studies should be conducted on more coastal species known to be resident in the impacted areas, such as Sepiidae, Sepiolidae or Octopodidae spending all of their life in the waters of the New Caledonian lagoon.

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