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Abstract

The link between trophic ecology and metal accumulation in marine fish species was investigated through a multi-tracers approach combining fatty acid (FA) and stable isotope (SI) analyses on fish from two contrasted sites on the coast of Senegal, one subjected to anthropogenic metal effluents and another one less impacted. The concentrations of thirteen trace metal elements (As, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sn, U, and Zn) were measured in fish liver. Individuals from each site were classified into three distinct groups according to their liver FA and muscle SI compositions. Trace element concentrations were tested between groups revealing that bioaccumulation of several metals was clearly dependent on the trophic guild of fish. Furthermore, correlations between individual trophic markers and trace metals gave new insights into the determination of their origin. Fatty acids revealed relationships between the dietary regimes and metal contamination that were not detected with stable isotopes. In the region exposed to metallic inputs, the consumption of benthic preys was the main pathway for metal transfer to the fish community while in the unaffected one, pelagic preys represented the main source of contamination. Within pelagic sources, metallic transfer to fish depended on the phytoplankton phylum on which the food web was based, suggesting that microphytoplankton (*i.e.*, diatoms and dinoflagellates) were a more important source of contamination than nano- and picoplankton. This study confirmed the influence of diet in the metal accumulation of marine fish communities, and proved that FAs are very useful and complementary tools to SIs to link metal contamination in fish with their trophic ecology.

Keywords: Trace elements, biochemical markers, diet, contamination, Senegal, tropical fish.

Abbreviations:

CCLME: Canary Current Large Marine Ecosystem

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

FA(s): fatty acid(s)

FAME: fatty acid methyl esters

KW: Kruskal-Wallis

MUFA(s): mono-unsaturated fatty acid(s)

PCA: principal component analysis

PUFA(s): poly-unsaturated fatty acid(s)

SI(s): stable isotope(s)

SIMPER: similarity of percentages analyses

SFA(s): saturated fatty acid(s)

1. Introduction

Increasing concerns regarding metal contamination in marine ecosystems, from both natural and anthropogenic sources, require a better comprehension of the mechanisms that drive their accumulation in organisms. Marine fish are exposed to metals via two major pathways, and even if they can assimilate dissolved metals through their gills (Jeffree et al. 2006), the main pathway is thought to be through feeding (Mathews and Fisher 2009). Because marine organisms display a wide range of contamination patterns, trophic metal inputs therefore depend on the type of prey consumed by marine fish. Furthermore, it has been reported that fish sharing the same habitat do not necessarily share the same levels of metal contamination (Barhoumi et al., 2009; Siscar et al., 2013), which suggests that considering the feeding habitat (e.g., benthic *vs.* pelagic and coastal *vs.* oceanic) of fish is not a sufficient approach to understand how metals are introduced to fish communities. More accurate methods aiming to characterise the trophic ecology of fish communities are therefore necessary to understand the factors affecting metal contamination. Studies investigating the link between trophic ecology and metal contamination report that an organism's metal content was not only dependant on trophic groups, but this relationship was variable according to the metal considered (Metian et al., 2013). Moreover, some species can present intraspecific differences in diet, which are reflected in metal accumulation (Das et al., 2000). This highlights the need to apply trophic studies at the individual level to better understand the contamination drivers.

Over recent years, stable isotope analysis has become a very popular approach to investigate the structure of marine food webs (Valiela, 2015). Among the different strengths of this method for fish communities is the possibility to characterise trophic levels using nitrogen isotopes ($\delta^{15}N$) or to discriminate benthic *vs.* pelagic or continental *vs.* oceanic inputs to the food webs using carbon isotopes ($\delta^{13}C$).

Because stable isotope analysis only provides a two-dimensional discrimination and sometimes fails to discriminate among isotopically similar sources, coupling this approach with fatty acid (hereafter FA) composition analysis has recently been suggested as a solution for a thorough understanding of marine fish trophic ecology (Couturier et al., 2013; Farias et al., 2014; Stowasser et al., 2009). Because different primary producers synthesise different fatty acids and consumers cannot efficiently synthesise them, the composition of FAs reflects the basis of food webs. FA composition analysis has therefore allowed identification of the respective roles of diatoms, dinoflagellates, bacteria or plant detritus in marine food webs (Dalsgaard et al., 2003; Iverson, 2009; Kelly and Scheibling, 2012). Although a number of studies linking stable isotope composition with metal-contaminated species have been published (Chouvelon et al., 2012; Das et al., 2000; Domi et al., 2005; Pethybridge et al., 2012), to our knowledge, no study has ever tried to link the metal content in fish tissues with FA trophic markers. Furthermore, in contrast to stomach content analysis, these tracers can provide time-integrated information on the dietary habits of the fish for the last few months (Beckmann et al., 2014; Buchheister and Latour, 2010). This time scale is thus more relevant to study the chronic trophic metal contamination because trace elements can take several weeks to accumulate (Berntssen et al., 2001; Kim et al., 2006).

To investigate the link between trophic ecology and metal accumulation, a case study of the Canary Current Large Marine Ecosystem (CCLME) in Western Africa was chosen. This ecosystem is one of the world's major cold-water upwelling currents and includes several countries from Morocco to Guinea including Senegal. It ranks third in the world in terms of primary productivity (Chavez and Messié, 2009) and supports one of the largest fisheries among African large marine ecosystems. These fisheries provide food to local populations but also to foreign countries through the attribution of fishing licences and exportation. This

marine ecosystem is prone to metal contamination due to urban effluents and industrial activities (Auger et al., 2015; Diop et al., 2015), including phosphate extraction, which is of special importance for this region (Jasinski, 2015).

Although metals of anthropogenic origin are known to accumulate in marine sediments, they can become available to marine organisms through resuspension processes, in particular due to upwelling activity. Several studies have reported the presence of metals, such as cadmium, in invertebrates from Morocco (Banaoui et al., 2004), Mauritania (Everaarts et al., 1993; Sidoumou et al., 1999) and Senegal (Bodin et al., 2013). Concerning fish communities, although some data are available for the northern part of the CCLME (Chahid et al., 2014; Roméo et al., 1999; Sidoumou et al., 2005), only recent studies have investigated the metal content in fish from the coast of Senegal (Diop et al., 2016a, 2016b). Improved knowledge in this area is of special importance because coastal sediments and waters from this area are known to be impacted by toxic metals such as Cd, Cr, Ni and Pb (Bodin et al., 2013; Diop et al., 2015; Diop et al., 2014, 2012).

In the present study, a multi-tracers approach combining fatty acid and stable isotope analyses was used to investigate the trophic ecology of different fish species from the coast of Senegal. In addition, a metal content analysis was performed on the liver, which is known to be an organ highly involved in metal bioaccumulation by marine fish (Berntssen et al., 2001; Kim et al., 2006; Siscar et al., 2014).

The main objective of this work was to study the repartition of metals between different fish groups characterised by different trophic marker compositions. In addition, correlations between these tracers and trace metal elements were investigated to better understand the pathways leading to the contamination of fish communities.

2. Material and methods

2.1. Study area and sampling

Two sites that were presumed to be impacted differently by metallic contamination were selected (Fig. 1).

The first one was located in the offshore area of Dakar Bay, where urban and industrial wastewaters are directly discharged into the bay (Diop et al., 2015; Diop et al., 2014, 2012).

The second one was located off the Casamance River Estuary, at the extreme southern area of Senegal. Although there are no existing data on metal contamination in marine organisms for this region, this place was considered to be less impacted because of the absence of large cities and/or significant industrial activity.

The samples were collected during the AWA project (Ecosystem Approach to the management of fisheries and the marine environment in West African waters) scientific cruise in March 2014 aboard the RV Thalassa. The fish were caught with a bottom trawl net, packed in plastic bags and frozen on board at -20 °C. Once at the laboratory, the fish were weighed (wet weight) and measured (total length). They were then dissected with ceramic tools to avoid metal contamination, and the liver and a piece of dorsal muscle (a standardised cut on the dorsal muscle just behind the head) were collected. The liver was split into two samples, one for trace metal analysis and one for the fatty acid composition analysis. Stable isotope analyses were conducted on dorsal muscle samples only. Five replicates were analysed for each species and for each type of analysis.

2.2. Trace metal analysis

Liver samples for trace metal analysis were freeze-dried, ground, and stored in individual plastic vials. Approximately 80 mg of the dried samples were digested in a mixture of 7 ml of 70% HNO_3 and 1 ml of 30% H_2O_2 (both of ultrapure quality) in Teflon vessels.

Mineralization was performed in a microwave oven (Ethos One, Milestone) during 15 min with increasing temperature until 200 °C, followed by 15 min at 200 °C (1500 W) and 90 min of cooling. The samples were then evaporated on a hotplate at 100 °C, resolubilised with 2 ml of HNO_3, and diluted to 50 ml with Milli-Q quality water (Merck Millipore). A total of 13 elements (As, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sn, U, and Zn) were analysed using an ICP Q-MS (X Series 2, Thermo Scientific) at the Pôle de Spectrométrie Océan (PSO, Plouzané, France) with an internal solution of Rhodium.

Reference materials (fish protein DORM-4 and lobster hepato-pancreas TORT-2, NRCC) were treated and analysed in the same way as the samples. The results for reference materials displayed mean element recoveries ranging from 84% to 132% for DORM-4 and 89% to 131% for TORT-2. Blanks were included in each analytical batch. The detection limits ($\mu g \cdot g^{-1}$ dry wt) were 0.001 (Ni), 0.003 (Co), 0.016 (As, Pb), 0.017 (U), 0.018 (Cr), 0.054 (Li), 0.133 (Cu), 0.15 (Cd), 0.19 (Mn), 0.278 (Zn), 1.61 (Sn) and 17.83 (Fe). All of the element concentrations (ppm) are provided as a dry weight basis ($\mu g \cdot g^{-1}$ dry wt).

2.3. Stable isotopes

The muscle samples for the analysis of stable isotopes were freeze-dried and ground into a fine and homogeneous powder. Approximately 350 µg of powder was then weighed in tin capsules for isotopic analysis. Because only pure muscle tissues were analysed, no lipid extraction was performed, but all of the C/N ratios of the samples were measured and none exceeded 5.

The samples were analysed by continuous flow on a Thermo Scientific Flash EA 2000 elemental analyser coupled to a Delta V Plus mass spectrometer at the Pôle de Spectrométrie Océan (PSO, Plouzané, France). The results are expressed in standard δ notation based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for

204 $\delta^{15}N$) following the equation $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 10^3$ (in ‰), where R is
205 $^{13}C/^{12}C$ or $^{15}N/^{14}N$.
206 International isotopic standards of known $\delta^{15}N$ and $\delta^{13}C$ were used: IAEA-600 Caffeine,
207 IAEA-CH-6 Sucrose, IAEA-N-1 and IAEA-N-2 Ammonium Sulphate. A home standard
208 (Thermo Acetanilide) was used for experimental precision (based on the standard deviation of
209 the replicates of the internal standard) and indicated an analytical precision of ± 0.11‰ for
210 $\delta^{13}C$ and ± 0.07‰ for $\delta^{15}N$.
211
212 **2.4. Fatty acids**
213 2.4.1. Lipid extraction
214 After dissection, the liver samples (approximately 300 mg each) were immediately put in
215 glass tubes previously heated for 6 h at 450 °C and containing 6 mL of a chloroform/methanol
216 mixture (2/1, v/v), flushed with nitrogen and stored at -20 °C before analysis. The samples
217 were then manually ground in the mixture with a Dounce homogeniser and vortexed.
218 2.4.2. Separation
219 The neutral lipids were isolated following the method used by Le Grand et al. (2014). An
220 aliquot of total lipid extract (1 mL) was evaporated to dryness under nitrogen, recovered with
221 three 0.5-mL washings of chloroform/methanol (98/2, v/v) and deposited at the top of a silica
222 gel micro-column (Pasteur pipette of 40 mm × 5 mm i.d., plugged with glass wool and filled
223 with silica gel 60, which were both previously heated for 6 h at 450 °C and deactivated with
224 6% water by weight). Only the neutral lipids (NL), including triglycerides, free fatty acids and
225 sterols, were eluted with 10 mL of chloroform/methanol (98/2, v/v) and collected in 20-mL
226 glass vials. After evaporation to a dryness under nitrogen, the NL fraction was recovered and
227 transferred to 7-mL vials with three 1-mL washings of chloroform/methanol (98/2, v/v).
228 2.4.3. Transesterification

After the addition of tricosanoic acid as an internal standard and evaporation to dryness under nitrogen, the fatty acid methyl esters (FAME) were obtained using a method modified from Le Grand et al. (2014). A total of 0.8 mL of a sulphuric acid solution (3.8% in methanol) was added, vortexed and heated for 10 min at 100 °C. Before GC analysis, 0.8 mL of hexane was added and the organic phase containing FAME was washed three times with 1.5 mL of hexane-saturated distilled water. The organic phase was finally transferred to tapering vials and stored at -20 °C.

2.4.4. Gas chromatography analysis

FAMEs were analysed in a Varian CP 8400 gas chromatograph (GC) equipped with a split/splitless injector and a flame-ionization detector. FAMEs were identified using two different capillary columns (ZBWAX - 30 m × 0.25 mm i.d., 0.25-µm thickness, Phenomenex; and ZB-5HT - 30 m × 0.25 mm i.d., 0.25-µm thickness, Phenomenex) by means of a standard 37-component FAME mix (Sigma) and other known standard mixtures. The FAs were expressed as the molar percentage of the total FA content.

2.5. Data analyses

Only FAs accounting for ≥0.5% of total FA in at least one fish sample were included in the data analyses. The groups inferred by stable isotope and fatty acid analyses were derived from the result of a hierarchical cluster analysis (Ward's clustering method). Similarity of percentages analyses (SIMPER) were used to identify the fatty acids that were the most discriminant between the groups. Principal component analyses (PCA) were performed to investigate the variation in fatty acids profiles between individual fish from the same community. The groups derived from the result of the clustering and FAs accounted for more than 75% of the dissimilarity contribution between the groups in the SIMPER routine and were shown in the PCA.

All of the data submitted to the statistical tests were first checked for normality (Shapiro-Wilks test) and for homogeneity of variances (Bartlett test). When these conditions were satisfied, parametric tests were used in the subsequent analysis; otherwise, non-parametric analogues were used. Pearson and Spearman correlation coefficient tests were used to investigate the correlation between the variables (stable isotopes, fatty acids and metal concentrations). A Kruskal-Wallis (KW) test followed by a Conover-Iman multiple comparison test with Bonferroni's adjustment method was performed to test differences between groups in muscle $\delta^{13}C$ and $\delta^{15}N$ values. One-way ANOVAs followed by Tukey's HSD tests or KW tests followed by multiple comparison tests were performed to test differences between groups in the metal concentration for each element. All of the statistical analyses were performed using the free software R (R Development Core Team 2010).

3. Results

3.1. Stable isotopes

The stable isotope ($\delta^{13}C$ and $\delta^{15}N$) composition of fish from the region of Dakar highlighted three main groups that significantly differed in their stable isotope composition (KW tests, $p<0.001$; Fig. 2). The first one contained only individuals of the chub mackerel *Scomber japonicus*. This group displayed lower $\delta^{15}N$ (from 7.8 to 8.5‰) than the other groups and lower $\delta^{13}C$ (-17.3 to -16.2‰) than group 3. The second group was characterised by higher $\delta^{15}N$ (9.9 to 11.7‰) than group 1 but lower $\delta^{13}C$ (-17.0 to -14.9‰) than group 3. This second group included mostly individuals of the species *Boops boops* and *Trachurus trecae* and only one individual of *Caranx rhonchus* and *Pseudupeneus prayensis*. The third group showed higher $\delta^{15}N$ (10.5 to 12.9‰) than group 1 and higher $\delta^{13}C$ (-16.5 to -14.4‰) compared to groups 1 and 2. This third group included all *Diplodus bellottii* individuals, most *P. prayensis* and *C. rhonchus* individuals, and 1 individual of *B. boops*.

Regarding the intra-specific variability: 3 species contained individuals sharing the same group (*S. japonicus, T. trecae,* and *D. bellottii),* whereas 3 others had 1 individual belonging to a different trophic group than its conspecifics (*P. prayensis, C. rhonchus,* and *B. boops).*

Samples from Casamance were also classified into three groups (KW tests, $p<0.001$; Fig. 3). The first one had lower $\delta^{13}C$ (-18.6 to -16.4‰) than other groups and lower $\delta^{15}N$ (12.3 to 13.7‰) than group 2. This last group was characterised by higher $\delta^{13}C$ (-16.2 to -14.7‰) than group 1 and higher $\delta^{15}N$ (13.7 to 14.7‰) than the other groups. Group 3 had similar $\delta^{15}N$ (12.4 to 13.7‰) but higher $\delta^{13}C$ (-15.8 to -15.1‰) than group 1 and similar $\delta^{13}C$ but lower $\delta^{15}N$ than group 2.

The intra-specific variability was high within this fish community in terms of the isotopic signature because individuals from all species were split into at least two groups.

3.2. Fatty acid analysis

For the fish community of both Dakar and Casamance, a total of 34 fatty acids represented more than 0.5% of the total FA in at least one sample and were therefore considered for further analysis.

A hierarchical cluster analysis (Ward's clustering method) allowed the clear separation of three groups in Dakar based on their global FA composition. Group 1 was characterised by a lower proportion of saturated fatty acids (SFAs) and a higher proportion of poly-unsaturated fatty acids (PUFAs), which accounted for half of the total fatty acids, than the other groups (Table 2). Group 2 presented an intermediate amount of SFAs but the highest content of mono-unsaturated fatty acids (MUFAs) and the lowest of PUFAs. Group 3 showed the highest content of SFAs and intermediate levels of MUFAs. The FA distribution within SFAs and PUFAs did not differ strongly between the different groups. The most important

individual FAs were 16:0, 18:0, and 14:0 for SFAs and 20:5n-3, 22:6n-3, and 22:5n-3 for PUFAs. However, MUFA partitioning was rather different with 16:1n-7, 18:1n-9, and 22:1n-11 dominating group 1, whereas 18:1n-9, 16:1n-7, and 18:1n-7 were the most abundant in groups 2 and 3.

The principal component analysis (Fig 4) shows the separation between the three groups in Dakar and the fatty acids responsible for the inter-group differences. Group 1 contained all *S. japonicus* and was characterised by an abundance of 22:1n-11 and n-3 PUFAs such as 20:5n-3, 22:5n-3 and 22:6n-3. The second group was mostly characterised by its high amount of 16:0, 18:1n-9 and to a lesser extent 22:1n-11 and 16:1n-7. It included all of the *T. trecae* and *C. rhonchus* and 3 individuals from *B. boops.* The third group showed a dominance of various fatty acids such as 16:0, 18:0, 18:1n-7 and 20:4n-6. All of the *D. bellottii* and *P. prayensis* individuals were in this group, along with the two individuals of *B. boops*. All of the individuals of the same species were classified in one group, except *B. boops,* which was split between two groups.

Fish from Casamance were also clearly separated into three groups by the hierarchical cluster analysis. The repartition of fatty acids between the different classes was less variable from one group to another in this region. Indeed, similar contents of SFAs were found between the groups with group 1 only differing from the others by a lower amount of MUFAs and higher amount of PUFAs (Table 3). The dominant FAs within the SFA class were 16:0, 18:0 and 14:0, and within the MUFAs, they were 18:1n-9, 16:1n-7 and 18:1n-7. The PUFAs were mostly composed of 22:6n-3, 20:5n-3 and 22:5n-3 in groups 1 and 2 and of 20:5n-3, 22:6n-3 and 20:4n-6 in group 3.

The principal component analysis (Fig. 5) highlighted that the first group was discriminated by the n-3 PUFAs 22:6n-3, 20:5n-3 and 22:5n-3 and to a lesser extent by 18:0. Group 2 was

mostly characterised by 18:1n-9. Group 3 was discriminated from the other groups by its contents of 16:1n-7, 18:1n-7 and 16:0. Only two species, *C. chrysurus* in group 1 and *G. decadactylus* in group 3, were classified in only one group. This demonstrates the high intra-specific variability in terms of trophic markers existing within the species sampled at this location.

3.3. Trace metal analysis

For some of the trace elements analysed, the concentrations in the liver of the fish sampled in Dakar varied significantly between the groups identified from the stable isotope (SI groups) and the fatty acid (FA groups) analyses (Fig. 6A). In the SI groups, a higher level of Pb and Zn was found in group 3 than in group 2. Fe was more abundant in group 3 than in group 1. These differences were also found between the FA groups, which also showed many other differences in the concentrations of trace elements. A higher amount of As, Cd, Co, Fe, Ni, and Sn was found in group 3 than in group 2. Likewise, group 2 was characterised by the highest amounts of Mn, Pb, and Zn and group 3 was characterised by more As, Fe, and Pb than group 1.

In the fish community from Casamance, significant differences also existed between the different groups in terms of the metal concentration in the liver (Fig. 6B). According to the SI groups, only one element varied depending on the trophic preferences. Li was indeed more abundant in group 1 than in group 3. More differences were found in the metal contents between the FA groups. Group 1 displayed higher levels of Co, Cu, Fe, Li, Sn and Zn than group 3. Group 1 also contained more Li than group 2. Finally, group 2 showed more Fe and Zn than group 3.

3.4. Correlations between metals and trophic markers

Considering only the relationships that indicated high correlations (arbitrarily determined for correlation coefficients > 0.5), very few metals were correlated with stable isotope ratios. Fe was positively correlated with $\delta^{15}N$ for fish from Dakar (Table 3), whereas Li was negatively correlated with $\delta^{15}N$ in Dakar and with $\delta^{13}C$ in Casamance (Table 4). Pb was positively correlated with $\delta^{13}C$ at both stations, and this same relationship existed for Sn in Dakar.

However, all of the trace metal elements were positively correlated with at least one fatty acid, except Cr in Dakar (Table 3) and As, Cr and Sn in Casamance (Table 4). Zn, Mn and Cu were found to be negatively correlated with 18:1n-9 in Dakar, whereas Fe and Zn were negatively correlated with 16:1n-7 in Casamance.

4. Discussion

4.1. Stable isotope analysis

In marine ecosystems, stable isotopes are commonly used to infer trophic levels through $\delta^{15}N$ values and offshore *versus* inshore, or pelagic *versus* benthic inputs through $\delta^{13}C$ values. Moreover, the position of individuals in bivariate isotope space (the isotopic niche) is considered a convenient proxy for the trophic niche (Layman et al., 2012).

Individual fish from Dakar occupied three distinct trophic niches. The first one (group 1) was characterised by low $\delta^{15}N$, which reflected a low trophic position within the fish community and a low $\delta^{13}C$ that indicated pelagic/offshore dietary habits. The second group, which showed similar $\delta^{13}C$ but higher $\delta^{15}N$, may also feed on pelagic prey but at a higher trophic level. Finally, the third group occupied a niche characterised by similar $\delta^{15}N$ to group 2 but lower $\delta^{13}C$. The fish in this group may feed on more benthic/coastal prey than other groups.

In the fish community of Casamance, three main isotopic niches were also observed. Group 1 contained fish with both low $\delta^{13}C$ and $\delta^{15}N$, which suggested that the fish were feeding on

pelagic prey of lower trophic levels. In contrast, other groups displayed higher $\delta^{13}C$, which showed a more benthic/coastal origin of the prey. Among them, the $\delta^{15}N$ signature was higher in group 2, which suggested a higher trophic level than the fish from group 3.

4.2. Fatty acid analysis

Fish sampled offshore of Dakar were structured in three major groups based on their FA composition. The first group only included the chub mackerel *S. japonicus*, which also differed from the other species based on its composition of stable isotopes. This species was characterised by its high amount of n-3 PUFAs (∑ n-3, Table 2). Because 20:5n-3 (eicosapentaenoic acid , EPA) is commonly considered a marker of diatoms (e.g., Alfaro et al., 2006; Kharlamenko et al., 2001; Meziane and Tsuchiya, 2000) and 22:6n-3 (docosahexaenoic acid, DHA) is a marker of dinoflagellates, the ratio of these two FAs has been used to determine the predominant taxa in phytoplankton (Parrish et al., 2000). The EPA/DHA ratio in this first group was higher than 1, which suggests that *S. japonicus* relies on higher proportions of a diatoms-based food web (Alfaro et al., 2006). The first group was also characterised by a higher content of 22:1n-11 than group 3. This FA is known to be synthesised in high proportions by copepods and has been commonly used to evaluate the reliance of fish species on copepod-based pelagic food webs (Stowasser et al., 2012, 2009). Therefore, the FA composition of this group fits with the previously described diet of immature chub mackerels, which have been reported to rely on copepods and euphausiids in the North-West African shelf (Castro, 1993). Considering these observations, *S. japonicus* from the region of Dakar may therefore rely on herbivorous zooplankton feeding on diatoms.

The second group was characterised by its high content of 16:0, which is a ubiquitous FA that most organisms are able to synthesise *de novo*. Even if its abundance varies among taxa, it is

405 difficult to attribute its presence to a specific type of prey. This group also showed high
406 amounts of 18:1n-9, a major FA in most marine animals, which are able to biosynthesise it *de*
407 *novo* by chain elongation from 18:0 (Kelly and Scheibling 2012). In benthic environments,
408 this FA has been identified in various dietary sources, such as brown algae, mangrove detritus
409 or animal material (Alfaro et al., 2006; Bachok et al., 2003; Jaschinski et al., 2011). In pelagic
410 ecosystems, it has been used as a marker of carnivory (Pethybridge et al., 2014). However,
411 this FA can also be abundant in phytoplankton species (Dalsgaard et al., 2003; Escribano and
412 Pérez, 2010), and its presence in higher trophic levels may depend more on its abundance on
413 basic components of the food web than on the trophic level of the consumer. At high and
414 temperate latitudes, the seasonal food supply induces the storage of lipid reserves by
415 zooplankton (Kattner and Hagen, 2009). Zooplankton from these regions are known to
416 display high proportions of 18:1n-9 in these reserves (Lee et al., 2006). In tropical and
417 subtropical waters, copepods from the oligotrophic epipelagic environment are characterised
418 by continuous feeding and generally do not accumulate lipids in significant amounts in the
419 upper 250 m of the water. In contrast, in deeper environments, copepods are exposed to lower
420 prey densities and feed mainly through episodic sedimentation events, which results in higher
421 energy storage than for epipelagic copepods including higher 18:1n-9 levels (Teuber et al.,
422 2014). Upwelling regions are characterised by periodic high productivity events, which are
423 comparable to the seasonal food input at temperate and high latitudes and may thus result in
424 similar lipid storage strategies. It has been reported that copepods feeding on pico- and
425 nanoplankton also displayed higher 18:1n-9 levels than those feeding on microplankton,
426 which can be free from this FA (Escribano and Pérez, 2010). Here, 18:1n-9 was strongly
427 negatively correlated with EPA (diatoms marker) and DHA (dinoflagellates marker)
428 (Supplementary material, Table 1), which where the least abundant in group 2. As diatoms
429 and dinoflagellates are the major components of microplankton (Anabalón et al. 2014), this

suggests that this group relies on small-sized fractions of phytoplankton. Moreover, this group was also characterised by a high content of 16:1n-7, which is generally considered a diatoms marker (e.g., Richoux and Froneman 2008) but has also been reported as abundant in bacteria (Kharlamenko et al. 2001) and is abundant in marine sediments (Perry et al. 1979). Here, the fact that 16:1n-7 is negatively correlated with the dinoflagellates marker 22:6n-3 suggests a benthic origin.

Thus, the FA compositions for these individuals were in accordance with the reported diets of the species included in this group. Indeed, for the class size sampled here (see Table 1), *T. trecae* is known to feed on planktonic crustaceans, and *C. rhonchus* presents a diet composed of small planktivorous fish and planktonic and benthic crustaceans (Boëly et al., 1973; Sley et al., 2008), whereas *B. boops* shows an omnivorous diet composed of fish, benthic crustaceans and zooplanktonic prey (Derbal and Hichem Kara, 2008). In group 2, the abundance of 18:1n-9 may reflect the consumption of pelagic prey, zooplankton or small fish, whereas the 16:1n-7 signature may result from the ingestion of benthic crustaceans.

The third group is discriminated by 18:0, also a ubiquitous FA synthesised through elongation of 16:0. This group was also characterised by 18:1n-7, which is commonly used to assess the bacterial contribution in the marine food web (Alfaro et al., 2006; Kharlamenko et al., 2001; Meziane and Tsuchiya, 2000), and 20:4n-6. The latter has been reported to be abundant in brown and red algae (Kelly and Scheibling, 2012) but can be found in high proportions in marine invertebrates from sites devoid of macroalgae, which is the case for most of the Senegal coastline. Therefore, the authors hypothesised that this FA may results from feeding upon drift algae, phytodetritus or microbial mats (Cook et al., 2000). Many other primary producers are also able to synthesise this FA, such as diatoms (Dunstan, et al. 1993), fungi and protozoa (Kharlamenko et al., 2001; Kim et al., 1998). It has also been shown in

controlled feeding and *in situ* studies that this FA can be selectively retained or biosynthesised *de novo* by bivalves and crustaceans (Budge et al., 2001; Kelly et al., 2009; Soudant et al., 1996). Finally, it has been used to identify the consumption of benthic prey in marine fish (Stowasser et al., 2009). In the present study, it was positively correlated with 22:4n-6 alongside the 22:5n-6 (Supplementary material, Table 1), which can all be synthesised from 20:4n-6. Because this group is also characterised by a high bacterial marker content, such as 15:0, it is likely that high amounts of (n-6) PUFAs in this group come from the assimilation of phytodetritus and the microphytobenthos. Again, this is in accordance with the described ecology of the species included in this group, which is dominated by two benthic fish. *D. bellottii* shows an omnivorous diet composed of algae, bivalves and crustaceans (Horta et al., 2004), whereas *P. prayensis* feeds mainly on benthic invertebrates (Caverivière, 1993).

In the fish sampled in Casamance, the first group was mainly discriminated from others by its high content of n-3 PUFAs (Fig. 5). As observed in group 1 from Dakar, this group may also rely on a pelagic food web. However, an EPA/DHA ratio lower than 1 indicates that dinoflagellates were more important than for fish from Dakar. This group is dominated by *C. chrysurus*, which is known to be a pelagic particulate feeder (Faye et al., 2011), and *C. rhonchus.* The latter is an opportunistic piscivorous species for the size class sampled in Casamance (see Table 1) and feeds at almost 70% on planktivorous fish, mainly on anchovies *Engraulis encrasicolus* and to a lesser extent on clupeids such as *Sardinella aurita* (Boëly et al., 1973; Sley et al., 2008). Anchovies are known to contain high levels of DHA (Pethybridge et al., 2014), which seems to confirm this species as the dominant prey for *C. rhonchus.*
Two individuals of the barracuda *S. guachancho* were also found in this group. This piscivorous species feeds mainly on small pelagic fish such as *Sardinella spp., T. trecae* and *C. chrysurus* for the size class of individuals sampled (see Table 1; Akadje et al., 2013).

Hence, it is not surprising to find this species alongside *C. chrysurus*, which confirms that the FA composition of a predator reflects that of its prey. Therefore, group 1 mostly contains phytoplankton-feeding species and their direct predators, which share a similar FA composition.

The second group in Casamance was discriminated from others by 18:1n-9 and from group 1 by 16:1n-7, which is similar to what was observed for fish from Dakar. This group was dominated by *Brachydeuterus auritus*, which is known to feed on a variety of sources, including copepods and *Selene dorsalis*, which feeds on pelagic fish and crustaceans (Caverivière, 1993; Diouf, 1996). It also included three barracudas that may feed on different species of forage fish than their conspecifics belonging to group 1. Because *C. chrysurus* and *Sardinella* species are characterised by high levels of n-3 PUFAs (Njinkoué et al., 2002), it can be hypothesised that barracudas from group 2 fed on *T. trecae* instead, which showed a high level of 18:1n-9 in Dakar.

In summary, group 2 gathers secondary and tertiary consumers and belongs to a pelagic food web based on phytoplankton phyla other than diatoms and dinoflagellates.

G. decadactylus was the only species classified in the third group for fish sampled in Casamance. This species was characterised by its content of the benthic marker 16:1n-7 and 18:1n-7. The latter has been linked to diatoms (Dalsgaard et al., 2003) and was thus used as an herbivory marker for copepods in the Benguela upwelling system (Schukat et al., 2014). It has also been found alongside 16:1n-7 in high amounts in the surface sediments of the Humbolt upwelling ecosystem (Gutiérrez et al., 2012) and is commonly used to assess the bacterial contribution in marine food webs (Alfaro et al., 2006; Kharlamenko et al., 2001; Meziane and Tsuchiya, 2000). Moreover, in this study, this FA was positively correlated to

505 iso17:0 (Supplementary material, Table 2), which is known to be predominantly synthesised
506 by bacteria (Volkman et al., 1980) and used as a bacterial marker in sediments (Rajendran et
507 al., 1993, 1992). This suggests that here, 18:1n-7 must be more associated with the benthic
508 ecosystem than with the pelagic one. This is in accordance with the described diet of *G.*
509 *decadactylus,* which feeds mainly on benthic crustaceans (Caverivière, 1993).
510
511 In this fish community, only two species were classified exclusively in only one group. This
512 demonstrates the high intra-specific variability in terms of dietary habits and highlights the
513 need to consider them at the individual level rather than at the specific one.
514
515 **4.3. Metal concentration differences between trophic groups**
516 Some studies have successfully associated the metal contents with the isotopic niche of
517 marine fish species (Das et al., 2000; Domi et al., 2005). In the present study, the trophic
518 groups inferred by the analysis of dual stable isotopes poorly explained the variability in
519 terms of contamination. In Dakar, the association of the coastal/benthic food intake with a
520 higher trophic level led to the largest Fe, Pb and Zn contamination, whereas in Casamance,
521 only Li was dominant in fish showing offshore/pelagic habits combined with low trophic
522 levels.
523
524 To our knowledge, there is no published study using fatty acids to infer the dietary origin of
525 metal contamination in a marine fish community. In this study, we attempted to demonstrate
526 how bioaccumulation of several trace metal elements relates to the trophic preferences of the
527 fish using the FA composition.
528 In Dakar, the consumption of benthic prey led to a higher contamination of Cd, Ni, Pb, Fe,
529 Co, Zn, As, and Sn (Fig. 6A). This was highlighted by the positive correlation found between

these elements and at least one FA from a benthic origin (Table 3). These FAs included bacterial markers, such as iso17:0, ant17:0 and 15:0 (Volkman et al., 1980), which are characteristic of sediment communities (Rajendran et al., 1993, 1992) and were more abundant in group 3 (∑ BAFA). This benthic FA pool also contained n-6 PUFAs from phytodetritus or the microphytobenthos, which were, like the BAFAs, more abundant in group 3 (∑ n-6). Within the pelagic ecosystem, the microphytoplankton-based food web seemed to be a major pathway for the transfer of Mn, Pb and Zn to fish as revealed by the negative correlation between Mn and Zn and the nano- and picoplankton marker 18:1n-9 (Table 3), which may be less impacted by these elements. Indeed, taxonomic differences in the plankton metal concentration can exist as shown between diatoms, flagellates and picophytoplankton (Twining et al., 2015; Twining and Baines, 2013). These differences can also vary between ocean basins and the elements considered.

Conversely, in the community from Casamance, feeding on pelagic prey seemed to induce a higher accumulation of Fe and Zn (Fig. 6B) as indicated by the negative correlation between these elements and the benthic marker 16:1n-7 characterising group 3 and the positive correlation between Zn and DHA, a dinoflagellates marker that was found in higher proportions in groups 1 and 2. Furthermore, fish belonging to the microphytoplankton-based food web were more exposed to Co, Cu and Sn than the benthic feeders. This is suggested by the correlation found between Co and Cu and n-3 PUFAs, which were dominant in group 1. Indeed, Cu was positively correlated with DHA and Co with 22:5n-3, which is formed through a 2-carbon chain elongation of EPA. Co was also correlated with 20:4n-3 FA, which can be used to characterise either fungi, protozoa or algae (Kharlamenko et al., 2001). In this case, it was present alongside the dinoflagellates marker DHA, suggesting a pelagic origin. Finally, the diatom-feeding fish or their predators from group 1 accumulated more Li than other species. Contamination of this group by Li can also be linked to the planktonic n-3

555 PUFAs (20:4n-3 and 22:5n-3) and to 18:3n-3. The production of 18:3n-3 from 18:1n-9 is only
556 realised by primary producers and requires delta 12 and delta 15 desaturase enzymes,
557 respectively (Dalsgaard et al., 2003). 18:3n-3 is commonly used to characterise sea grass,
558 green macroalgae or vascular plants (Alfaro et al., 2006; Kharlamenko et al., 2001; Meziane
559 and Tsuchiya, 2000; Richoux and Froneman, 2008). Thus, primary producers seem to be
560 particularly involved in Li transfer to the fish community.
561
562 Although not responsible for the differences among groups, some correlations between
563 individual trophic markers and trace metals can give new insights into the determination of
564 their origin near the coastline of Senegal (Tables 4 and 5).
565 In the Dakar fish community, Sn was positively correlated with $\delta^{13}C$, which suggests an
566 increase with coastal/benthic habits and is in accordance with the predominance of this
567 element in the benthic predators group inferred by FAs. U was likewise correlated with 14:0,
568 which is the dominant FA in marine cyanobacteria (Carpenter et al., 1997; Merritt et al.,
569 1991) and suggests the benthic origin of this metal. In contrast, Cu and Li seemed to result
570 from the consumption of pelagic organisms. Indeed, Cu was positively correlated with the
571 diatoms marker EPA, and negatively with the nano- and picoplankton marker 18:1n-9,
572 revealing dominance in the microphytoplankton. Moreover, Li was positively correlated with
573 the planktonic 20:4n-3 and negatively with $\delta^{15}N$, which reveals a stronger presence in the low
574 trophic level species that feed on planktonic prey. It was also negatively correlated with
575 17:1n-8 and 18:2n-4, two FAs synthesised in large amounts by bacteria (Dalsgaard et al.,
576 2003; Pond et al., 1997). Along with the results of the Li distribution in Casamance, it can be
577 concluded that this element may be predominant in the pelagic environment.
578 In Casamance, Pb was associated with the coastal/benthic isotope signature inferred by $\delta^{13}C$
579 and 20:4n-6, which was likely the result of phytodetritus consumption. This corroborates

observations in Dakar, which indicate that Pb may be derived from coastal anthropogenic sources (Diop et al., 2015). Cd and U were found to be associated with both the planktonic FA 22:5n-3 and a bottom linked FA: the bacterial FA 17:0 for Cd and the phytodetritus marker 22:4n-6 for U. Conversely, for observations in Dakar where these two elements had only a benthic origin, they seemed to result from a larger variety of sources in this case. Finally, Mn and Ni were only linked to n-3 PUFAs, which suggests a pelagic origin.

4.4. Differences in the contamination patterns between communities

Physiological characteristics, such as subcellular differences in handling metals (Eyckmans et al., 2012), can lead to differences in metal accumulation between species. However, even if only one species, *C. rhonchus*, was present in both of the stations sampled, general patterns can be drawn from the two fish communities.

In Dakar, among the 13 elements analysed, only two metals (Cu and Li) were only associated with planktonic markers, whereas eight of them (As, Cd, Co, Fe, Ni, Pb, Sn and Zn) were exclusively related to bacterial or phytodetritus/microphytobenthos markers. Concerning Cd, Ni and Pb, these results are in accordance with previous studies regarding the metallic contamination of sediments from the Dakar area and where high levels of these metals have been reported (Diagne et al., 2013; Diop et al., 2015; Diop et al., 2012). Furthermore, it is in accordance with the theory concerning the distribution of metals between the particulate and dissolved fraction. Indeed, Co and Pb are scavenged in greater amounts by the solid phase in the water column from the Dakar coast, which explains the fact that these elements sink to the sediments (Diop et al., 2014). Surprisingly, these authors also found high amounts of Cr, whereas in this study, no correlation was established between this element and any marker. Even if extremely high concentrations have been reported in mussels from the harbour of Dakar (up to 298 ppm dry weight, Diagne et al., 2013), Cr showed the lowest affinity to the

bioavailable fraction in sediments from this area among the other metals (Diop et al., 2015). The authors concluded that Cr cannot be remobilised into the aquatic environment under normal biogeochemical conditions, which could explain the low Cr levels observed in fish. Likewise, the sediments presented an elevated contamination of Cu, whereas in the present study, it was associated with the pelagic environment. Finally, only Mn was found to correlate with the markers characteristic of both the pelagic and the benthic environment.

Because the Dakar area is exposed to urban and industrial rejects (Diop et al., 2015; Diop et al., 2014, 2012), the contamination may occur near the coast as shown with the stable isotope analysis (Fig 6). Thus, the trace metal elements may be rapidly trapped in the sediments, leading to a high contamination of benthic organisms in this region. For example, the green algae *Ulva lactuca* presented higher levels of Pb than other potential prey, and the Cd amount in this species was higher in Dakar than at other locations (M. Diop et al., 2015). Moreover, the mussel, *Mytilus galloprovincialis,* from Dakar is known to be highly contaminated with Cd and Pb (Diagne et al., 2013), and the other mussel species, *Perna perna,* also showed a greater amount of Cd than in Morocco and Mauritania (M. Diop et al., 2015; Sidoumou et al., 2006). Finally, the two sand-living species, *Cardita ajar* and *Dosinia isocardia,* also presented very high levels of Cd (Sidoumou et al., 2006). Here, the sediment compartment seemed to be the major source for the transfer of metals up to the fish community.

Interestingly, in the Casamance region, metal contamination seems to be less dictated by the fish relationship with the sediments. Indeed, only Pb was exclusively related to a microphytobenthos, whereas the other elements (Co, Li, Mn and Ni) were only associated with planktonic markers or both benthic and pelagic markers (Cd, Cu, Fe, U, and Zn). The contamination is not likely to be the result of local anthropogenic rejects because this region is not subjected to industrial activities or heavy urban development. However, it may occur through fish migrations from another contaminated areas or the transport of elements by the

hydrodynamic process. The Senegalese coast is subjected to wind-driven upwelling, which occurs mainly in winter and is stronger from February to April. It induces a coastal jet arising from the geostrophic adjustment of the surface density gradient between the cold upwelled coastal waters and the warmer open ocean waters (Allen, 1973). This upwelling jet is confined nearshore and flows southward alongside the coast of Senegal (Auger et al., 2015). Furthermore, recent studies have shown that the coastal waters of southern Senegal come from the upwelling area of Dakar Bay through advection processes (Ndoye, 2016). Because the fish were collected during the active upwelling season when the coastal current is the strongest, it strengthens the hypothesis of a transport of the metals from the Dakar area subjected to industrial effluents of this region.

Finally, this study suggests that microphytoplankton (*i.e.*, diatoms and dinoflagellates) were a more important source of contamination than the smaller size fraction species. Whereas nanoplankton are dominant in the phytoplankton community of the Dakar area, microplankton are the almost exclusive component in Casamance (Donval *personal communication*). As physical and biological factors seem to converge to induce a strong contamination of the marine pelagic environment in Casamance, further investigations are required to evaluate the impact of northern anthropogenic rejects in this region.

This case study of the coastal environment from Senegal showed that anthropogenic activities seemed to drive the input pathways of trace metal elements in the marine food webs. It also highlights the need to consider the trophic relationships to understand the contamination patterns of the marine fish communities.

5. Conclusion

This study demonstrated the implication of trophic ecology in the accumulation of several trace metal elements in fish communities. The individual approach revealed high intraspecific

variability in dietary preferences and metal contamination for some species. Stable isotopes (SIs) and fatty acids (FAs) were both relevant tools to discriminate trophic groups within the fish community, but FAs proved to be more discriminant to link metal contamination in fish with their trophic ecology. Because most previous studies trying to investigate metallic contamination in the marine biota in relation to their trophic ecology used stable isotope analysis, we suggest that extending fatty acid analysis to heavy metal contamination studies would provide new perspectives.

In the region subjected to high anthropogenic metallic inputs, local rejects led to a higher contamination of the benthic environment. In contrast, in the unaffected region, contamination was more associated with the pelagic ecosystem, where the metallic transfer to fish depended on the phytoplankton phylum on which the food web was based. This study suggests that microphytoplankton (*i.e.*, diatoms and dinoflagellates) were a more important source of contamination than nano- and picoplankton species.

In the present study, bioaccumulation was clearly dependent on the trophic preferences of fish, as inferred through SI signature and FA composition, for several trace metal elements. Moreover, fatty acids revealed many relationships between the dietary regimes and metal contamination that were not visible with stable isotopes.

Acknowledgements: The authors thank greatly Sébastien Hervé for the design of the Fig. 1 and the graphical abstract, and Jean Raffray for the identification and the dissection of the fish. Samples used in this study were collected during a scientific cruise of the AWA project in West Africa. This article benefited from the comments of two anonymous reviewers. This work was financially supported by the French National Research Agency project ANR-11-CEPL-0005 EPURE.

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Figure captions

Figure 1 A map showing the two sample stations along the coast of Senegal.

Figure 2 Muscle $\delta^{13}C$ and $\delta^{15}N$ values (‰) of individuals from six species from Dakar. The groups (circled) were derived from the result of a hierarchical cluster analysis (Ward's method).

Figure 3 Muscle $\delta^{13}C$ and $\delta^{15}N$ values (‰) of individuals from six species from Casamance. The trophic groups (circled) were derived from the result of a hierarchical cluster analysis (Ward's clustering method).

Figure 4 Principal component analysis (PCA) of the fatty acid composition of the liver of six fish species from Dakar. The trophic groups (circled) were derived from the result of a hierarchical cluster analysis (Ward's clustering method). Fatty acids that account for more than 75% of the contribution of dissimilarity between the groups in the similarity of percentages analysis (SIMPER) are shown.

Figure 5 Principal component analysis (PCA) of the fatty acid composition of the liver of six fish species from Casamance. The trophic groups (circled) were derived from the result of a hierarchical cluster analysis (Ward's clustering method). Fatty acids that account for more than 75% of the contribution of dissimilarity between the groups in the similarity of percentages analysis (SIMPER) are shown.

Figure 6 Comparison of the trace element concentrations ($\mu g \cdot g^{-1}$ dry wt) in the liver of fish from Dakar (A) and from Casamance (B) according to their trophic group. SI group: trophic groups inferred by stable isotopes; FA group: trophic groups inferred by fatty acids. Different letters indicate significant differences between groups (ANOVAs followed by Tukey's HSD tests or KW tests followed by multiple comparison tests).

Supplementary material captions

Table 1 Correlations (561) between fatty acids in the liver of fish from Dakar.

Table 2 Correlations (561) between fatty acids in the liver of fish from Casamance.

Table 1 Summary (mean ± standard deviation) of the biological parameters of fish from Dakar and Casamance. n: number of individuals; Total length in cm; Wet weight in g.

Station	Species	n	Total length (cm)	Wet weight
Dakar	*Boops boops*	5	18.7 ± 1.6	63.7 ± 2.2
	Caranx rhonchus	4	19.9 ± 1.4	91.3 ± 13.1
	Diplodus bellottii	5	21.1 ± 1.8	77.9 ± 8.7
	Pseudupeneus prayensis	5	21.4 ± 1.1	120.6 ± 15.0
	Scomber japonicus	5	16.2 ± 0.6	32.9 ± 5.2
	Trachurus trecae	5	21.8 ± 0.4	100.0 ± 4.8
Casamance	*Brachydeuterus auritus*	5	20.8 ± 0.4	127.0 ± 5.0
	Chloroscombrus chrysurus	5	20.3 ± 1.2	72.2 ± 10.6
	Caranx rhonchus	5	32.9 ± 3.0	334.4 ± 68.8
	Galeoides decadactylus	5	17.6 ± 1.3	63.0 ± 16.1
	Selene dorsalis	5	24.4 ± 1.3	170.0 ± 22.7
	Sphyraena guachancho	5	34.4 ± 3.3	197.0 ± 53.6

Table 2 Fatty acid composition (mean ± SD, %) of liver tissue of the three groups derived from the result of a hierarchical cluster analysis (Ward's method) from Dakar. Only FAs accounting for ≥0.5% of total FA in at least one fish sample are shown. Different letters indicate significant differences between the groups (ANOVAs followed by Tukey's HSD tests or KW tests followed by multiple comparison tests).

	Group		
FA	**1**	**2**	**3**
TMTD	0.5 ± 0.2^{b}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}
iso17:0	0.5 ± 0.2^{a}	0.4 ± 0.1^{b}	1.1 ± 0.5^{a}
ant17:0	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.3 ± 0.2^{b}
14:0	2.8 ± 0.6	2.8 ± 0.7	2.7 ± 1.8
15:0	0.3 ± 0.1^{a}	0.2 ± 0.1^{b}	0.7 ± 0.2^{c}
16:0	14.5 ± 1.5^{b}	20.5 ± 2.3^{a}	22.7 ± 1.4^{a}
17:0	0.9 ± 0.0^{a}	0.4 ± 0.1^{b}	1.3 ± 0.5^{a}
18:0	4.3 ± 1.0^{a}	6.0 ± 0.9^{a}	7.9 ± 1.7^{b}
∑ **SFA**	**23.9** ± 1.6^{a}	**30** ± 2.5^{b}	**36.8** ± 4.0^{c}
16:1n-7	5.1 ± 1.2^{a}	8.6 ± 3.0^{b}	5.9 ± 2.4^{a}
17:1n-8	0.3 ± 0.3^{a}	0.3 ± 0.1^{ab}	0.5 ± 0.2^{b}
18:1n-11	0.9 ± 0.6^{a}	0.0 ± 0.0^{b}	0.2 ± 0.1^{a}
18:1n-9	3.8 ± 1.2^{a}	24.9 ± 3.1^{b}	8.5 ± 3.3^{c}
18:1n-7	3.0 ± 0.5^{a}	4.0 ± 1.0^{ab}	4.6 ± 1.0^{b}
20:1n-9	1.8 ± 0.6^{a}	1.4 ± 0.9^{ab}	0.6 ± 0.5^{b}
22:1n-11	3.4 ± 1.6^{a}	1.6 ± 1.7^{a}	0.3 ± 0.6^{b}
22:1n-9	0.5 ± 0.2^{b}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}
∑ **MUFA**	**18.7** ± 3.6^{a}	**41.1** ± 3.9^{b}	**20.8** ± 5.4^{a}
16:2n-4	0.5 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
16:3n-4	0.4 ± 0.3	0.4 ± 0.2	0.2 ± 0.1
16:3n-6	0.2 ± 0.0	0.1 ± 0.1	0.4 ± 0.5

16:4n-1	0.8 ± 0.3[a]	0.5 ± 0.3[a]	0.2 ± 0.1[b]
18:2n-6	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.2
18:2n-4	0.5 ± 0.1[a]	0.3 ± 0.1[b]	0.4 ± 0.1[a]
18:3n-4	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
18:3n-3	0.6 ± 0.1[a]	0.4 ± 0.2[b]	0.3 ± 0.1[b]
18:4n-3	1.0 ± 0.2[a]	0.8 ± 0.3[a]	0.5 ± 0.2[b]
18:5n-3	1.3 ± 0.7[b]	0.4 ± 0.3[a]	0.3 ± 0.1[a]
20:4n-6	2.0 ± 1.1[a]	0.8 ± 0.2[b]	2.9 ± 0.8[a]
20:4n-3	1.2 ± 0.2[b]	0.7 ± 0.2[a]	0.7 ± 0.2[a]
20:5n-3	20.0 ± 1.5[a]	9.2 ± 1.4[b]	13.3 ± 2.3[c]
21:5n-3	0.7 ± 0.3	0.5 ± 0.2	0.4 ± 0.1
22:4n-6	0.5 ± 0.3[a]	0.2 ± 0.1[b]	0.7 ± 0.3[a]
22:5n-6	0.3 ± 0.2[a]	0.2 ± 0.1[b]	0.6 ± 0.2[c]
22:5n-3	4.9 ± 1.2[a]	2.3 ± 0.6[b]	3.1 ± 0.6[c]
22:6n-3	14.2 ± 4.2[a]	7.5 ± 1.6[b]	11.1 ± 3.3[a]
∑ PUFA	**50.4** ± 3.0[a]	**25.2** ± 4.0[b]	**36.4** ± 5.4[c]
∑ BAFA	1.8 ± 0.1[a]	1.0 ± 0.2[b]	3.4 ± 1.0[c]
∑ n-3	55.4 ± 4.0[b]	33.22 ± 6.7[a]	37.8 ± 3.9[a]
∑ n-6	3.7 ± 1.7[a]	1.7 ± 0.3[b]	5.3 ± 1.3[a]
20:5n-3/22:6n-3	1.4	1.2	1.2

Table 3 Fatty acid composition (mean ± SD, %) of liver tissue of the three groups derived from the result of a hierarchical cluster analysis (Ward's method) from Casamance. Only FAs accounting for ≥0.5% of total FA in at least one fish sample are shown. Different letters indicate significant differences between groups (ANOVAs followed by Tukey's HSD tests or KW tests followed by multiple comparison tests).

	Group		
FA	**1**	**2**	**3**
iso17:0	0.6 ± 0.2^{a}	0.2 ± 0.2^{b}	0.7 ± 0.2^{a}
14:0	1.9 ± 1.2^{a}	1.7 ± 0.5^{a}	4.0 ± 1.0^{b}
15:0	0.4 ± 0.2^{a}	0.2 ± 0.1^{b}	0.3 ± 0.0^{a}
16:0	19.7 ± 4.2	19.2 ± 4.0	23.8 ± 4.6
17:0	0.7 ± 0.3^{b}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}
18:0	6.4 ± 2.1^{a}	5.8 ± 1.2^{ab}	4.5 ± 0.5^{b}
∑ **SFA**	**29.8** ± 6.8	**27.4** ± 4.7	**33.7** ± 5.5
14:1n-5	0.1 ± 0.1^{a}	0.2 ± 0.3^{ab}	0.5 ± 0.2^{b}
16:1n-13t	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.0
16:1n-9	0.4 ± 0.2	0.5 ± 0.4	0.3 ± 0.1
16:1n-7	6.1 ± 2.6^{a}	10.3 ± 3.7^{b}	16.3 ± 3.5^{c}
16:1n-5	0.2 ± 0.1^{a}	0.1 ± 0.1^{a}	0.7 ± 0.1^{b}
17:1n-8	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1
18:1n-9	13.4 ± 4.1^{a}	28.0 ± 3.4^{b}	11.5 ± 1.6^{a}
18:1n-7	4.2 ± 0.8^{a}	3.8 ± 1.0^{a}	9.9 ± 2.2^{b}
20:1n-9	1.0 ± 0.3^{a}	0.8 ± 0.3^{ab}	0.5 ± 0.1^{b}
20:1n-7	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	0.5 ± 0.1^{b}
22:1n-11	0.5 ± 0.4	0.4 ± 0.4	0.2 ± 0.1
∑ **MUFA**	**26.7** ± 5.5^{a}	**45.0** ± 4.7^{b}	**41** ± 1.6^{b}
16:2n-4	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.0
16:3n-4	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1

16:4n-3	0.2 ± 0.1[a]	0.1 ± 0.0[b]	0.2 ± 0.2[ab]
16:4n-1	0.2 ± 0.1[a]	0.1 ± 0.1[b]	0.1 ± 0.0[ab]
18:2n-9	0.1 ± 0.1[a]	0.6 ± 0.4[b]	0.2 ± 0.1[ab]
18:2n-6	1.3 ± 0.3[b]	0.6 ± 0.3[a]	0.7 ± 0.1[a]
18:3n-4	0.3 ± 0.1[a]	0.2 ± 0.1[b]	0.2 ± 0.1[ab]
18:3n-3	0.7 ± 0.3[b]	0.2 ± 0.1[a]	0.3 ± 0.1[a]
18:4n-3	1.3 ± 1.6[a]	0.4 ± 0.2[b]	0.7 ± 0.2[ab]
18:5n-3	0.2 ± 0.3	0.2 ± 0.1	0.4 ± 0.1
20:4n-6	2.0 ± 0.8[a]	1.5 ± 0.4[ab]	1.1 ± 0.2[b]
20:4n-3	0.9 ± 0.3[b]	0.5 ± 0.2[a]	0.4 ± 0.1[a]
20:5n-3	9.5 ± 1.6[a]	6.8 ± 1.8[b]	9.2 ± 2.4[ab]
21:5n-3	0.5 ± 0.1[a]	0.2 ± 0.1[b]	0.4 ± 0.1[a]
22:4n-6	0.5 ± 0.4[a]	0.5 ± 0.2[a]	0.2 ± 0.1[b]
22:5n-3	3.5 ± 1.5[b]	1.8 ± 0.7[a]	1.0 ± 0.3[a]
22:6n-3	14.2 ± 2.9[a]	9.0 ± 2.7[b]	4.7 ± 1.1[c]
∑ **PUFA**	**35.9** ± 4.1[b]	**23.2** ± 5.3[a]	**20.1** ± 4.7[a]
∑ **BAFA**	1.8 ± 0.7[a]	0.7 ± 0.3[b]	1.4 ± 0.3[a]
∑ **n-3**	30.9 ± 4.3[b]	19.4 ± 4.8[a]	17.3 ± 4.2[a]
∑ **n-6**	3.8 ± 1.0[b]	2.6 ± 0.6[a]	2.0 ± 0.4[a]
20:5n-3/22:6n-3	0.7	0.8	2

Tabl**e 4** Correlations (42) between trace metals and tracers in Dakar. Only correlations with a coefficient >0.5 are shown. ** p<0.01; *** p<0.001.

FA	As	Cd	Co	Cu	Fe	Li	Mn	Ni	Pb	Sn	U	Zn
$\delta^{15}N$					0.552 **	-0.678 ***						
$\delta^{13}C$									0.556 **	0.518 **		
14:0											0.507 **	
15:0	0.520 **	0.544 **			0.663 ***		0.604 ***	0.611 ***	0.684 ***			0.745 ***
ant17:0							0.614 ***		0.669 ***			0.776 ***
iso17:0									0.686 ***			0.633 ***
17:0	0.512 **											
18:0												0.510 **
17:1n-8						-0.522 **						
18 :1n-9				-0.551 **			-0.569 **					-0.594 **
18:1n-11								0.533 **				
16:3n-6									0.567 **			
18:2n-4						-0.578 **			0.524 **	0.518 **		
20:4n-6					0.580 **		0.561 **		0.638 ***			0.656 ***
22:4n-6	0.609 ***								0.559 **			
22:5n-6			0.529 **		0.529 **				0.783 ***			
20:5n-3				0.637 ***								
20:4n-3						0.564 **	0.596 **					

Tabl**e 5** Correlations (28) between trace metals and tracers in Casamance. Only correlations with a coefficient >0.5 are shown. ** p<0.01; *** p<0.001.

FA	Cd	Co	Cu	Fe	Li	Mn	Ni	Pb	U	Zn
$\delta^{13}C$					-0.660 ***			0.510 **		
17:0	0.613 ***									0,531 **
18:0				0,594 **						
16:1n-7				-0,539 **						-0,519 **
16:2n-4					0,584 **					
16:4n-1		0,507 **								
18:3n-3					0,593 **					
20:1n-9		0,554 **								
20:4n-3		0.659 ***			0.725 ***					
20:4n-6			0.693 ***					0,535 **		0,557 **
21:5n-3		0,594 **			0.667 ***					
22:4n-6				0,554 **					0,513 **	
22:5n-3	0.621 ***	0.800 ***			0.640 ***		0,502 **		0,533 **	
22:6n-3			0,503 **			0,507 **				0,598 **

Figure 1

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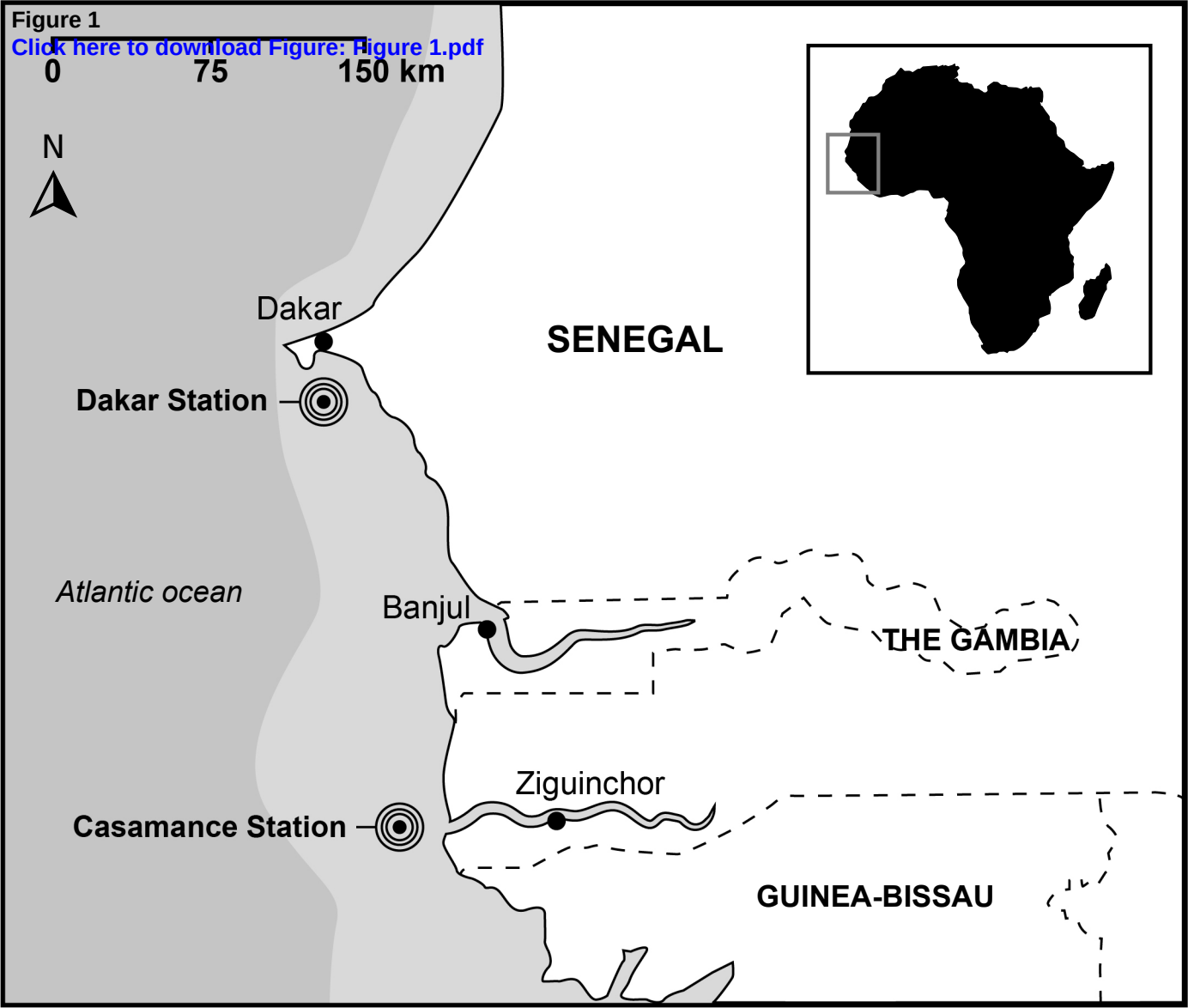

Figure 2

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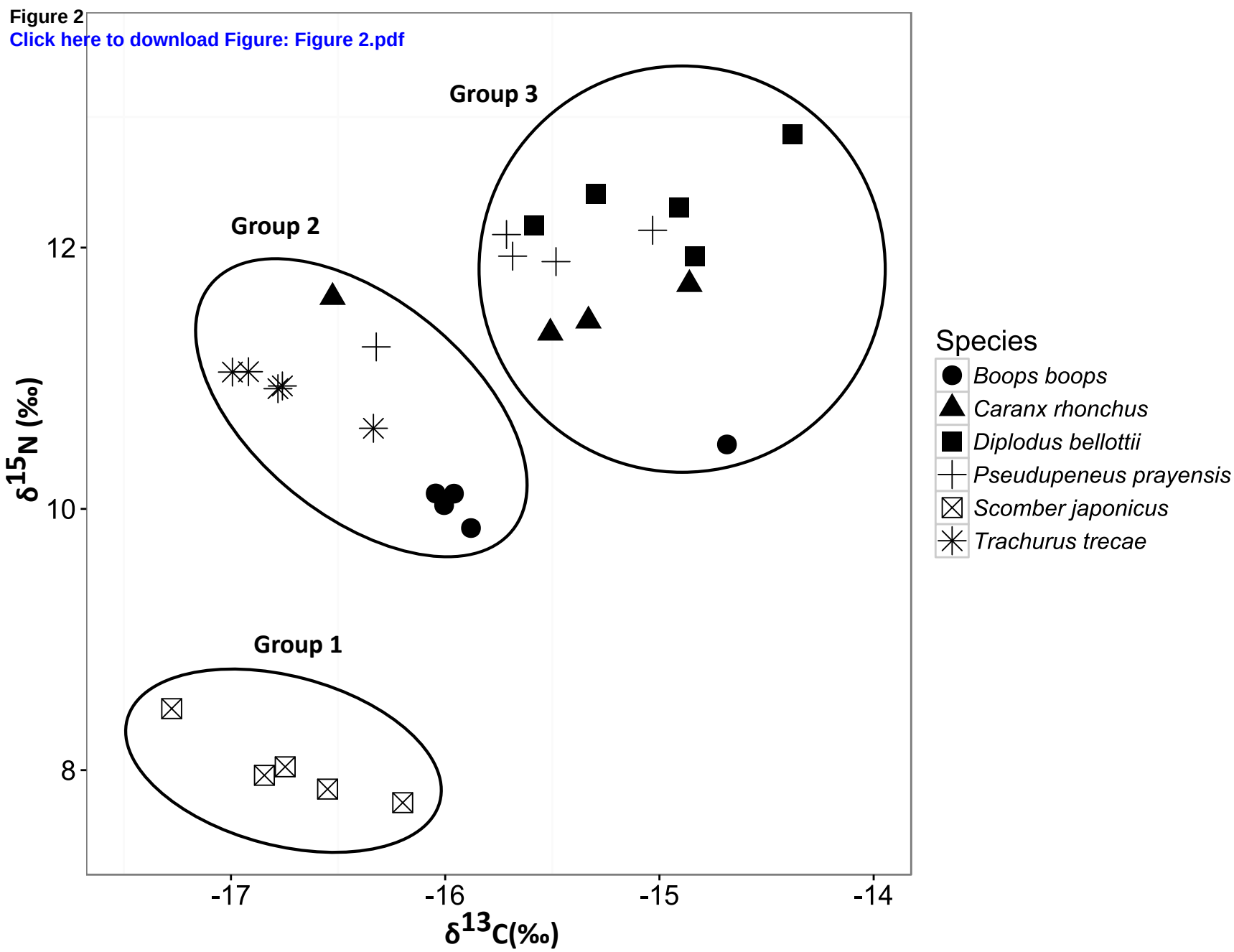

Figure 3

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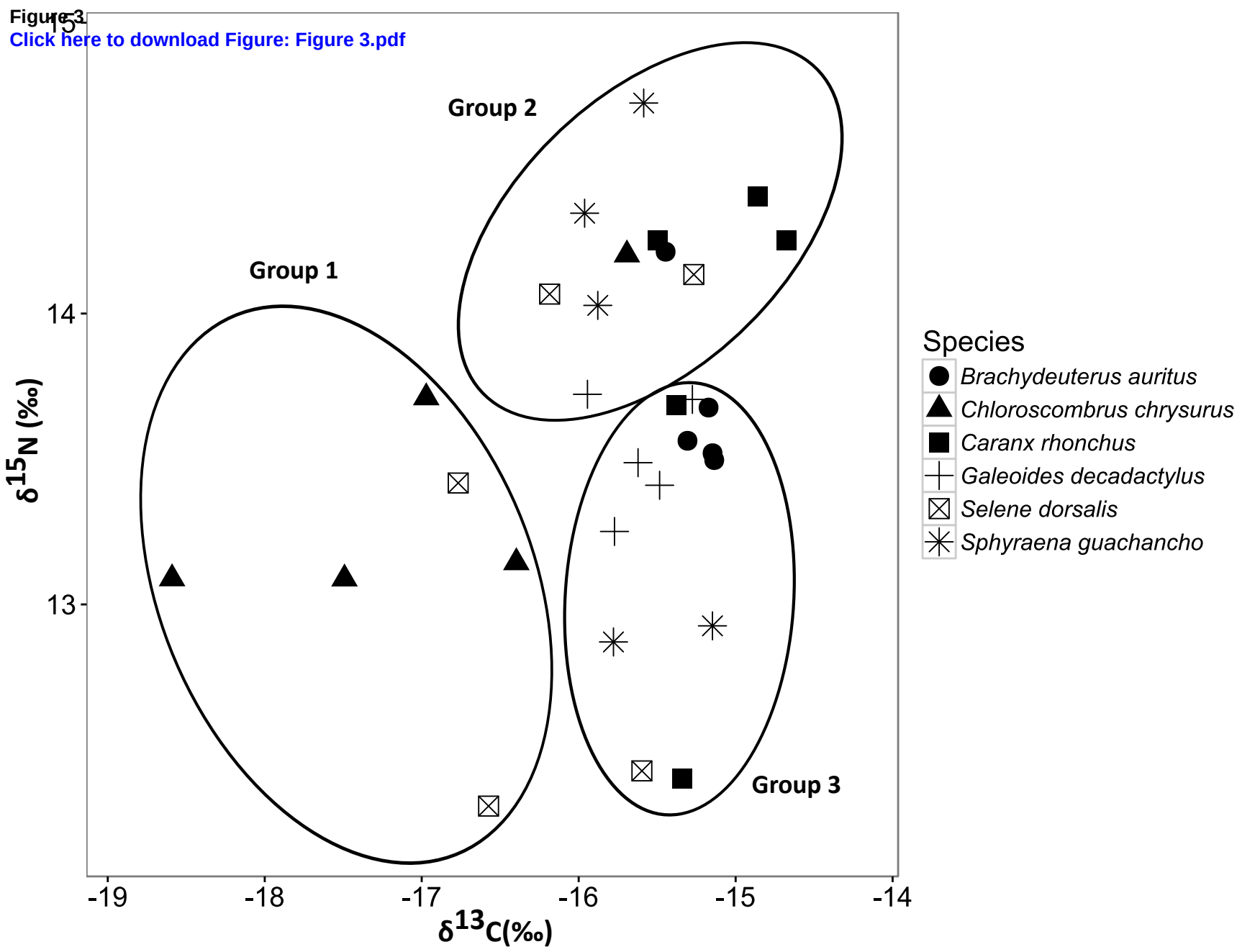

Figure 4

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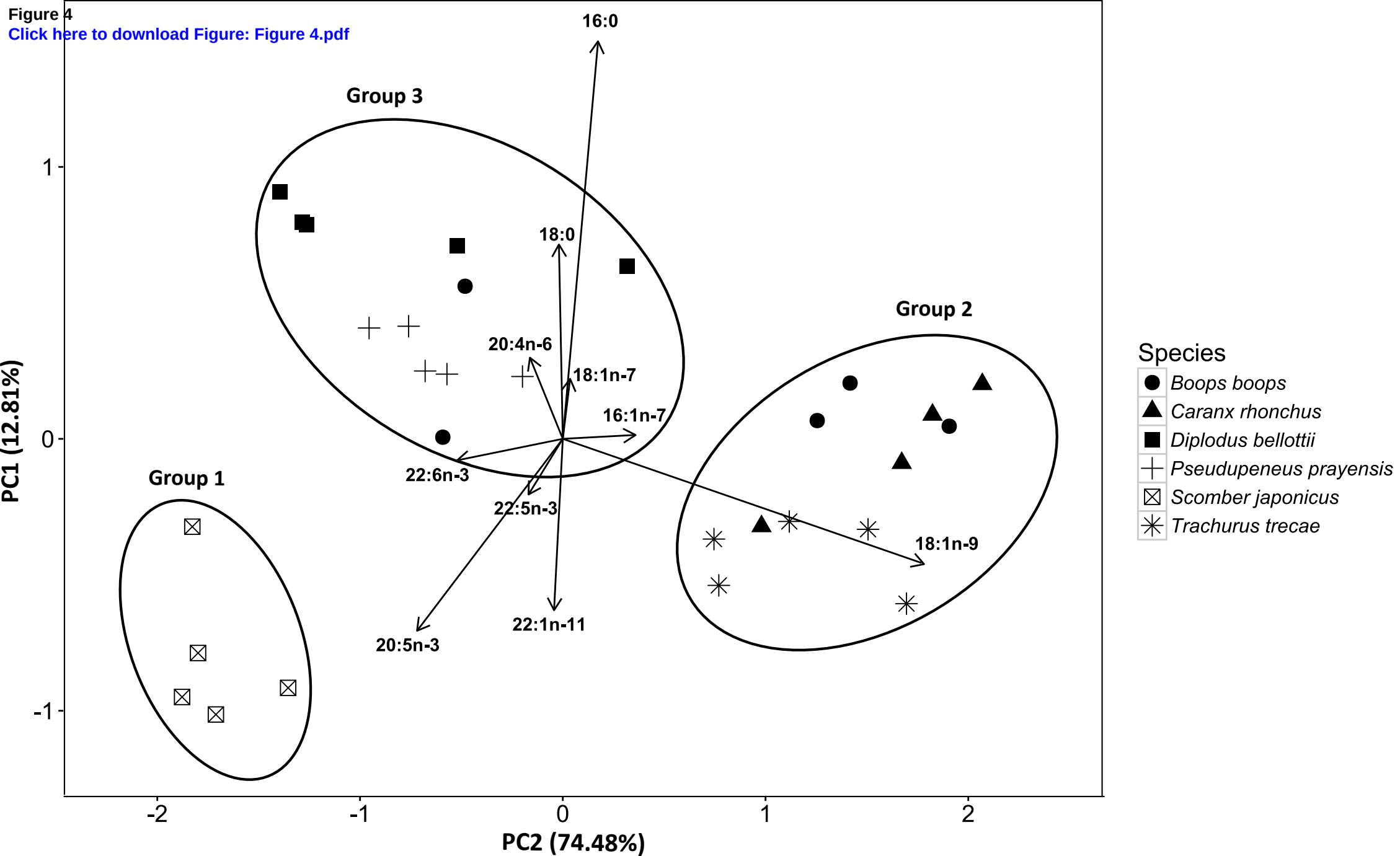

Figure 5

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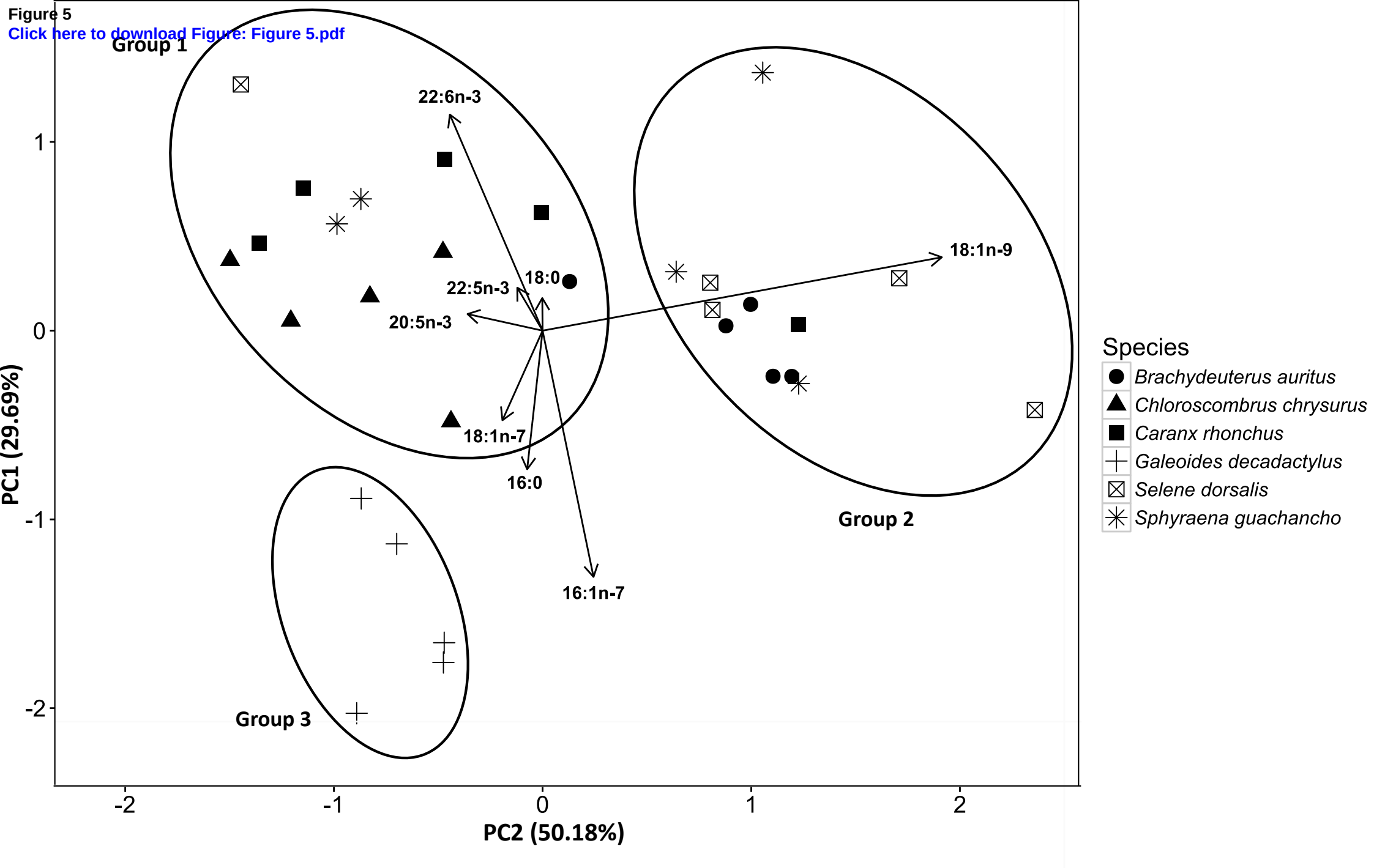

Figure 6

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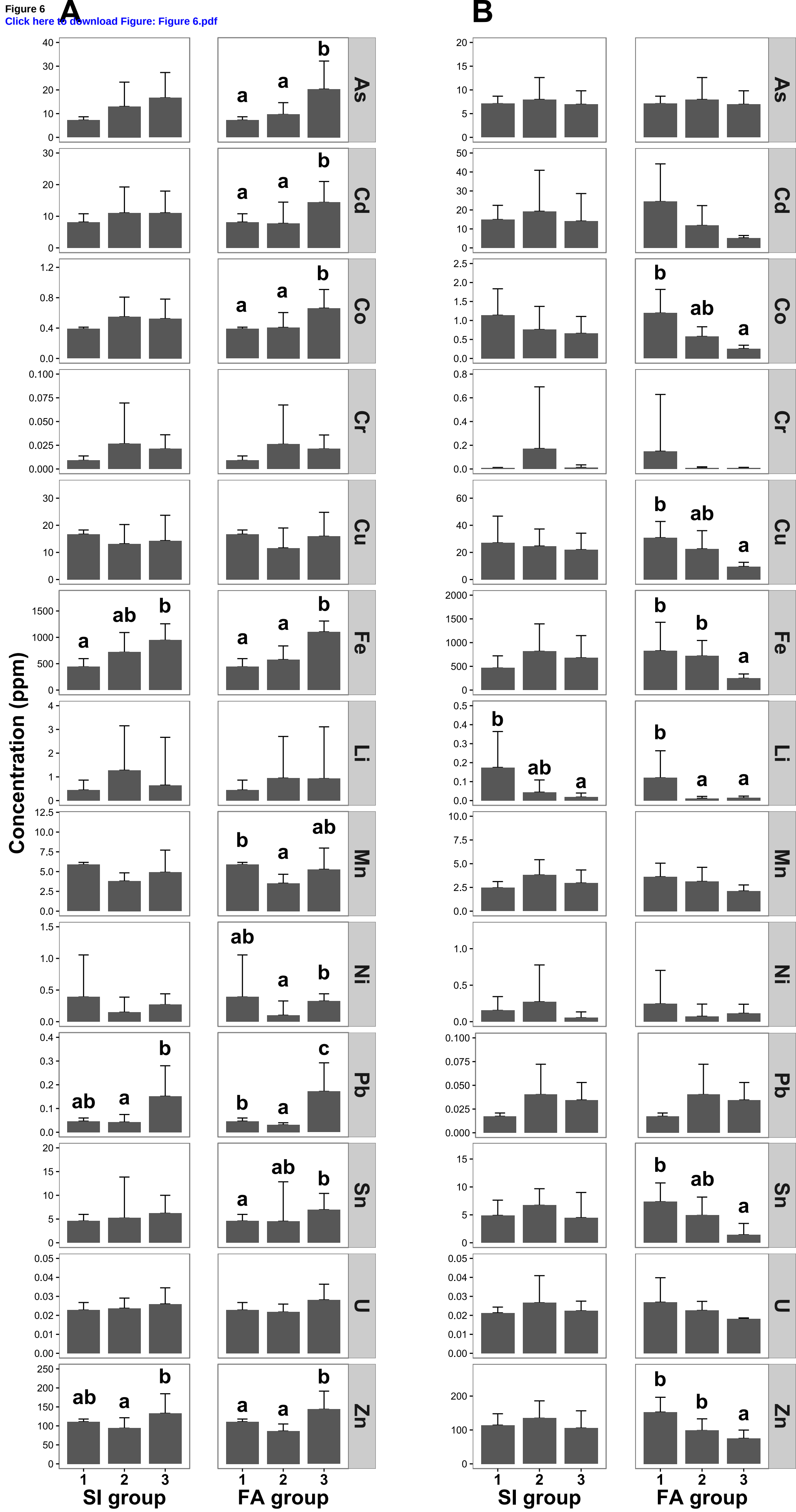

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