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^{210}Po and ^{210}Pb trophic transfer within the phytoplankton–zooplankton–anchovy/sardine food web: a case study from the Gulf of Lion (NW Mediterranean Sea)

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Abstract :

The transfer of ^{210}Po and ^{210}Pb in the food web of small pelagic fishes (from phytoplankton and zooplankton to anchovy Engraulis encrasicolus and sardine Sardina pilchardus) is investigated in the Gulf of Lion (GoL). We present original data of ^{210}Po and ^{210}Pb activity concentrations, C and N stable isotope ratios, measured (i) from different size classes of phytoplankton and zooplankton during spring and winter in different environments of the GoL, and (ii) in two fish species. Significant spatial patterns based on ^{210}Po, ^{210}Pb activity concentrations and ^{210}Po/^{210}Pb ratios in the different plankton size classes are evidenced by hierarchical clustering, both in spring and winter. This variability, also observed for C and N stable isotopes ratios, is connected to local specific pelagic habitats and hydrodynamics. The sampling strategy suggests that ^{210}Po bioaccumulation in the GoL remains at a constant level from the first (dominated by phytoplankton) to the second trophic level (zooplankton), while ^{210}Pb bioaccumulation shows an increase in winter. Based on stable N isotope ratios and ^{210}Po activity concentrations measured in anchovies and sardines, we evidence ^{210}Po bio-magnification along the trophic food web of these two planktivorous pelagic fishes.

Highlights

► ^{210}Po and ^{210}Pb activity concentrations in plankton vary up to a factor of two in the Gulf of Lion (East vs West). ► ^{210}Po and ^{210}Pb variability is connected to local specific pelagic habitats. ► Bio-magnification of ^{210}Po is evidenced in anchovy/sardine foodwebs.

Keywords : ^{210}Po, ^{210}Pb, Phytoplankton, Zooplankton, Pelagic fishes, Mediterranean Sea

1. Introduction

Lead-210 (^{210}Pb, half-life: 22.4 yrs) and Polonium-210 (^{210}Po, half-life 138 days) are non-conservative natural radionuclides produced from the ^{238}U decay chain whose distribution and behavior in the ocean have been closely investigated to explore (i) marine particle fluxes/exchanges that are essential for contaminant transport and carbon cycling (Bacon et al., 1976, 1985; Moore and Dymond, 1988; Cochran et al., 1990; Radakovitch et al., 1999; Masqué et al., 2002) and (ii) for toxic reasons owing to ^{210}Po accumulation in marine organisms (e.g. Cherry and Shannon, 1974; Heyraud and Cherry, 1979) and health concerns via the radiation dose received by humans through fish and seafood ingestion (e.g. Carvalho, 1995; Al Masri et al., 2000). Many inquiries focus on ^{210}Po and ^{210}Pb accumulation in planktonic species at the bottom of the marine food chain that are essential for trapping and removing nutrients and abiogenic particles in surface waters (e.g. review of Fowler, 2011) and in fishes (e.g. review of Carvalho, 2011; Stewart et al., 2008). Investigations on bioaccumulation across the food web mostly rely on laboratory experiments (Carvalho and Fowler, 1993, 1994; Stewart et al., 2005; Stewart and Fisher, 2003; Stewart et al., 2008), in particular to discriminate uptake from food ingestion (trophic transfer) and/or water. Zooplankton organisms play a major role in these pelagic food webs by transferring phytoplankton productivity to upper consumer levels like fishes (Saiz et al., 2007; Bănaru et al, 2013a) and allowing contaminants to accumulate from planktonic low-level organisms up to high-level predators in the pelagic food webs (Cossa et al., 2012). Carvalho (2011) notes that both ^{210}Po activity concentrations and ^{210}Po/^{210}Pb ratio may depend upon the number of trophic levels in the food chain, and suggested that enhancement of ^{210}Po activity concentrations is very pronounced in small zooplankton feeding upon bacteria and phytoplankton. However, the amplitude of this enrichment varies widely because of its reliance on numerous parameters such as food type, taxonomic composition, assimilation efficiency or trophic conditions (Fowler, 2011, Jeffree & Szymczak, 2000). Due to such variations, Fowler (2011) concludes in a review that *"additional effort is needed to expand the database on ^{210}Po levels in different phytoplankton groups with the corresponding second trophic level zooplankton samples to which they are being compared*". He also underlines that *"more detailed ^{210}Po analyses of pelagic organisms within well-defined food chains*

sampled from the same water mass will help clarify some of the trophic level differences in ^{210}Po noted in previous studies".

Here we examine the transfer of ^{210}Po and ^{210}Pb in the food webs of small pelagic fishes in the Gulf of Lion (hereafter GoL) as part of the COSTAS program (Contaminants in the trophic system: phytoplankton, zooplankton, anchovy, sardine). This multidisciplinary program aims at studying organic (PCB and PBDE) and inorganic (trace metals, ^{210}Po and ^{210}Pb) contaminant transfer within the food webs of two dominant planktivorous teleosts, the sardine, *Sardina pilchardus*, and the European anchovy, *Engraulis encrasicolus*, two species that rely entirely on planktonic food resources (Costalago et al., 2012). Stable isotope ratios of carbon and nitrogen have been widely used to analyze marine food webs and determine the trophic level of organisms (Fry and Sherr, 1984; Costalago et al., 2012). They represent also a precious tool to investigate contaminant biomagnification along food webs (Cossa et al., 2012; Harmelin-Vivien et al., 2012). We present ^{210}Po and ^{210}Pb activities as well as C and N stable isotope ratios measured in the GoL from (i) various size classes of phytoplankton and zooplankton in spring and winter and (ii) sardines and anchovies fished during spring.

Our purpose is to (i) investigate seasonal and spatial variability of ^{210}Po and ^{210}Pb activities in plankton in the GoL, (ii) determine ^{210}Po and ^{210}Pb bio-accumulation from phytoplankton to zooplankton trophic levels and (iii) define ^{210}Po bio-magnification from plankton to fishes thus providing a rare opportunity to compare in situ measurements to experimental data.

2. Material and methods

2.1. Study area

The Gulf of Lion (GoL), in the northwestern Mediterranean Sea, presents complex hydrological dynamic patterns defined by : (i) the cyclonic Northern Current that flows along the continental slope, (ii) a combination of wind-driven processes such as coastal upwelling and dense shelf water formation and (iii) freshwater dynamics associated with the large Rhone River discharge (Millot, 1999). The Rhone River has the largest mean annual discharge (1700 $m^3.s^{-1}$) in the Western Mediterranean basin. It is responsible for 83% of the suspended particulate matter flux to the French Mediterranean coast

(Gairoard et al., 2012) and 50% of the primary production of the GoL (Lochet and Leveau, 1990). The very low tidal range in the Mediterranean allows the Rhone riverine plume to expand westward into the GoL. This plume is particularly evidenced in the first two meters of the water column (Lorthiois et al., 2012). The influence of suspended particulate matter (SPM) from the Rhone River on both surface water and sediment is observed westward all over the gulf area (Durrieu de Madron et al., 2000; Espinasse et al., 2014a).

In the GoL, the diversity of zooplankton communities is generally lower in coastal waters than offshore while its biomass is higher near the coast due to riverine inputs of nutrients (Champalbert, 1996; Gaudy et al., 2003). Different zooplankton habitats are distinguished based on both biological (species composition, size structure) and physical (depth, salinity, wind, currents) variables (Espinasse et al., 2014a). Phytoplankton and zooplankton communities display conspicuous seasonal variations of composition and structure that are reflected in their related isotopic signatures (Harmelin-Vivien et al., 2008; Bănaru et al., 2013a, Espinasse et al., 2014a, 2014b). Phytoplankton spring bloom generally occurs from March to June in the GoL (Alekseenko et al., 2014).

2.2. Sampling and handling

2.2.1. Seawater, SPM and plankton

Seawater, SPM and plankton were sampled in May 2010 and February 2011 onboard RV "L'Europe" in the GoL, at seven sites along an east-west transect (Fig. 1). A chlorophyll-*a* (Chl-*a*) concentration profile was obtained at each sampling site using a CTD probe fitted to a fluorimeter to determine the maximum Chl-*a* concentration depth at which further sampling was performed. Seawater was pumped at Chl-*a* maximum using a Teflon coated pump system and was directly filtered through a 0.45µm pre-weighed cellulose acetate filter in a trace metal dedicated laboratory under laminar flow clean hoods. The Chl-*a* maximum depth was always observed below the river SPM plume near the Rhône River (ST2), suggesting low particle content for samples collected at this location and depth. Filtered seawater was acidified with HCl at pH 2 and kept at 4°C in the dark in acid-cleaned LDPE 1.5 L bottles. Filters were also kept at 4°C for SPM analysis. To obtain a sufficient amount of SPM, 10 to 15 L of seawater were filtered. Plankton was collected by two means according to particle size: pumping

or trawling. Small plankton organisms were sampled by pumping seawater *in-situ* at the Chl-*a* maximum depth with a 8 cm diameter tubing and filtered onboard through 200 µm, 60 µm and 6 µm mesh size plankton nets (see Harmelin-Vivien et al., 2008 for more details). Two small plankton size fractions were retained (i.e. [6-60 µm] and [60-200 µm]). The trawling system used to get larger plankton organisms (200 µm mesh) was towed at 2-3 knots during 30 min near the Chl-*a* maximum depth. The samples were immediately sieved onboard in our trace metal laboratory through four different filter meshes: 2000 µm, 1000 µm, 500 µm and 200 µm. We obtained four large plankton fractions: [200-500 µm], [500-1000 µm], [1000-2000 µm] and [> 2000 µm] that were kept in acid pre-cleaned PE tubes and frozen at -18° C onboard the ship. These fractions were then freeze-dried and kept in the dark at room temperature in the laboratory. An aliquot of each plankton fraction was kept apart for stable isotope analysis.

2.2.2. Anchovies and sardines

Anchovies (*Engraulis encrasicolus)* and sardines (*Sardina pilchardus*) were collected in March 2011 by professional fishermen in two areas of the GoL corresponding to eastern and western zooplankton stations (Fig. 1). Fishes (anchovies n=26 and sardines n=8) were dissected to extract muscles, liver, gonads and the body remains (i.e. skin, head and skeleton). Fishe's samples were pooled by area according to species and organs, in order to gather enough material for analyses. All dissected fishes were 10 to 13 cm long in total length (TL). The time lag of one month between plankton and fish sampling in 2011 (due to the availability of the research vessel) does not modify data interpretation for stable isotope signatures, since the time of integration of prey into fish muscles ranges from 1 to 3 months (Maruyama et al., 2001; Guelinkx et al., 2007). It also do not modify data interpretation for 210Po as its biological half life varied from 40 days in living organisms (ICRP 1968) to 35 days in phytoplankton (Stewart and Fisher).

2.3. Laboratory analyses

2.3.1. ^{210}Po and ^{210}Pb

2.3.1.1. SPM, plankton and fishes

^{210}Po and ^{210}Pb analyses were performed on 60 mg aliquots of dried and homogenized plankton and fish's organs, as well as SPM's filters. First, ^{209}Po tracer was added to each sample to determine recovery efficiency. After equilibrium (12 hours), a sequential dissolution/evaporation with continuous stirring steps was applied using HNO_3 (60° C, 30 min), $HNO_3/HClO_4$ (60° C, 30 min), HF 7% (60° C) and HCl (60° C, 30 min), but only HNO_3 and HCl acid steps were used to oxidize fish samples. The last evaporation step was stopped when few milliliters of solutions remained. They were completed with 250 ml of 0.4 N HCl, and heated up to 90°C with addition of ascorbic acid (10 mg). Then, a pre-cleaned silver disc was placed into the beaker for the spontaneous deposition of ^{209}Po and ^{210}Po (60° C during at least 4 hours). After plating, potential ^{209}Po and ^{210}Po traces were removed from solution onto an Ag foil scrap with continuous stirring overnight. To determine ^{210}Pb from ^{210}Po ingrowth, sample solutions were stored at least for 6 months and a second Po deposition with a new addition of ^{209}Po tracer and a new measurement of polonium isotope α emission was performed. The ^{210}Po activity concentration was measured with ORTEC α spectrometers equipped with PIPS detectors (surface active area 450 mm^2). The mean counting time was 140 hours, and the background activity of detector (3 to 5 counts per day) was subtracted from the total count of the ^{210}Po region of interest. The minimum detectable activity concentration was ~ 4 Bq kg^{-1}.

The equations used for the calculation of activities included chemistry efficiency, associated uncertainties and propagation errors related to radionuclide ingrowth, decay and recovery. The accuracy of analyses on particulate samples was confirmed by our result in a recent international calibration (Church et al., 2012).

2.3.1.2. Seawater

Seawater was collected using 10 L Niskin PVC bottles, filtered through 0.45 µm PTFE filter, and stored in a 1.5 L HDPE plastic bottle at 4° C after acidification to pH 2. Dissolved ^{210}Po and ^{210}Pb were analyzed according to the method described in Radakovitch et al. (1998). At first, ^{209}Po and stable Pb were added to the samples to determine recovery efficiency. After equilibrium (12 hours), 1.5 L of water were mixed with APDC (AmmoniumPyrrolidinoDithioCarbonate, 13 mL, 4 %) and MIBK (Methyl-IsoButylKetone, 75 mL) in a separating funnel. After 30 minutes, the solvent was

recovered in a beaker and evaporated on a hot plate, at 110° C. Then, sequential dissolution/evaporation with continuous stirring steps was performed on the samples using HNO_3 (60° C, 30 min), $HNO_3/HClO_4$ (60° C, 30 min) and HCl (60° C, 30 min). The same steps as described previously for SPM, plankton and fishes were followed to determine radionuclide activities. Then, few mL of the 0.4 N HCl solution were collected before Po deposition for total Pb concentration analyses (ICP-MS), in order to estimate Pb recovery (97 ± 7%).

2.3.2. Total Pb concentrations

Total Pb was analyzed on SPM, phytoplankton and zooplankton fractions at the IFREMER-Nantes laboratory. This laboratory is in charge of the analyses for environmental quality of marine environment at the national level and regularly participates to intercalibration studies (www.quasimeme.org). Samples were totally digested within hermetically sealed Teflon bombs in a mixture of HNO_3 and HCl for phytoplankton and zooplankton and a mixture of HCl, HNO_3, and HF for SPM. All acids were SupraPur® from Merck (protocol described by Chiffoleau et al., 2004). Concentrations were determined using an ICP-MS (Thermo Electron Corporation, Element X Series®). Accuracy was determined using Certified Reference Materials (CRM) MESS-3 and BCSS-1 from the NRCC (Ottawa) for SPM and CRM 278 from the BCR (Brussels) for biota. A blank sample and CRM were included within each batch of 15 samples for the total digestion procedure and the analyses. Blank values were always below the detection limits and the CRM within the certified concentrations.

2.3.3. Stable isotope analysis

As plankton fractions may contain carbonates, acidification of the samples was necessary to remove ^{13}C-enriched carbonates (De Niro and Epstein, 1978; Sarakinos et al., 2002). Subsamples were acid-dripped with 1% HCl, rinsed and dried, to determine $\delta^{13}C$ (Bănaru et al., 2013b; Espinasse et al., 2014b). The remaining untreated sample was used for $\delta^{15}N$ analysis. Three replicates were performed on each plankton fraction for each site and season for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope measurements were carried out with a continuous-flow isotope-ratio mass spectrometer (Delta V

Advantage, Thermo Scientific, Bremmen, Germany) coupled to an elemental analyzer (Flash EA1112 Thermo Scientific, Milan, Italy). Results are expressed in δ notation (per mil ‰) relative to international standards, Vienna PeeDee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$, according to the equation δX (‰) = $[(R_{sample} / R_{standard}) -1] \times 10^3$, where X is ^{13}C or ^{15}N and R is the isotope ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. For both $\delta^{13}C$ and $\delta^{15}N$, a precision < 0.1‰ was achieved with replicate measurements of internal laboratory standards of acetanilide. Percentage of organic C and organic N were obtained with the elemental analyzer and were used to calculate the sample's C/N ratios.

2.3.4. Plankton composition

Prior to stable isotope analyses, the taxonomic composition of plankton fractions was estimated visually under a binocular microscope. The dominant groups of organisms were determined in each size fraction and the results were compared to those provided by more specific studies performed in the GoL on phytoplankton (Harmelin-Vivien et al., 2008) and zooplankton size-classes (Bănaru et al., 2013b; Espinasse et al., 2014b).

2.4. Data analysis

A hierarchical clustering was implemented to average ^{210}Po and ^{210}Pb mean activity concentrations in plankton fractions using complete linkage and Euclidean distances to investigate radionuclide variations between sites, and potential spatial structuring in zones in the GoL with season. Differences in mean activity concentrations of ^{210}Po, ^{210}Pb and $^{210}Po/^{210}Pb$ ratios with size per season were tested by a non-parametric Kuskal-Wallis procedure (for non-normalized data). Two-way crossed ANOVAs were run on ^{210}Po and ^{210}Pb activity concentrations, and on $\delta^{13}C$ and $\delta^{15}N$ values, to assess the effects of the zone and size parameters for plankton fractions > 60 µm for each sampling season. When differences were significant, post-hoc comparisons of means were performed with Tukey tests. To test for bioaccumulation processes across plankton fractions and within the two analyzed pelagic fish species, linear regressions were run between the Log_{10} of ^{210}Po activity concentrations and $\delta^{15}N$ values of plankton fractions and fishes in the western part of the GoL in winter. Because stable isotope ratios

of fishes were not performed, we used the mean $\delta^{15}N$ values of sardines (8.69 ± 0.41 ‰) and anchovies (8.07 ± 0.33 ‰) given by Costalago et al. (2012) for similar size classes of these two fish species collected in winter in the GoL. Statistical analyses were done with Statististica 9.1 software.

3. Results

3.1. Taxonomic composition of plankton size-classes

Whatever the season, the analysis of the size-fractionated plankton samples indicates that the smaller-size class [6-60 µm] is a mixture of detritus and phytoplankton, while the [60-200 µm] size class is dominated by phytoplankton (mainly diatoms and dinobionts), but also contains a few heterotrophic microzooplankton (copepod eggs, nauplii and copepodits) (Table 1). The [200-500 µm], [500-1000 µm] and [1000-2000 µm] size-fractions are composed of zooplankton and dominated by copepods (*Clauso/Paracalanus* spp., *Calanus* spp., *Oithona* spp., *Acartia* spp.; Espinasse et al., 2014b), while the largest fraction [>2000 µm] mainly consists of gelatinous organisms (salps, siphonophores and chaetognaths).

3.2. ^{210}Po and ^{210} Pb in seawater, SPM and plankton

^{210}Po, ^{210}Pb activity concentrations and ^{210}Po/^{210}Pb ratios in seawater, SPM and in the different plankton size classes at each site and season are shown in Table 2. Significant spatial patterns are evidenced by hierarchical clustering of mean ^{210}Po and ^{210}Pb activity concentrations in plankton, both in spring (May 2010) and winter (February 2011; Fig. 2). At each season, except for ^{210}Pb in winter, ^{210}Po and ^{210}Pb activity concentrations are significantly different ($P < 0.01$) in the eastern (ST1 and ST10, ST2, ST3) and the western (ST4, ST5, ST6, ST7) GoL.

3.2.1. Spring

In May 2010, the highest mean activity concentrations tend to be in SPM and the [6-60µm] fraction for both ^{210}Po and ^{210}Pb, while the lowest values are measured in the dissolved phase (Table 3). However, these results should be taken with caution as these small-sized fractions were not sampled at all stations. Mean ^{210}Po activity concentrations do not significantly differ among plankton fractions from [60-200 µm] to [1000-2000 µm], while lower ^{210}Po values are measured in the largest size

fraction [> 2000 µm] (Table 3). Mean ^{210}Pb activity concentrations tend to be higher in the [6-60 µm] and [60-200 µm] plankton fractions than in the other fractions. ^{210}Po/^{210}Pb ratios increase from the dissolved fraction to phytoplankton and zooplankton, with a maximum ratio found in the [200-500 µm] size-fraction (Table 3). As SPM and [6-60 µm] plankton fractions are dominated by detritus and were not sampled at all stations, they were not considered for further analyses and discussion. A two-way ANOVA indicates that, in May 2010, ^{210}Po activity concentrations in plankton significantly vary with both zone and size-fraction (Table 4). ^{210}Po activity concentrations are higher in the eastern zone than in the western zone of the GoL (Fig. 3a). This geographic pattern is consistent among all fractions, as indicated by the absence of interaction between zone and size. In contrast, no differences in ^{210}Pb activity concentrations are observed between the eastern and western zones (Table 4, Fig. 3b), leading to higher ^{210}Po/^{210}Pb ratios in the eastern region of the GoL (Fig. 3c).

3.2.2. Winter

In February 2011, mean ^{210}Po activity concentrations do not differ in SPM and among plankton fractions when data are available, while ^{210}Pb activity concentrations tend to be higher in SPM (Table 3). Like in spring, these results should be taken with caution as these small-sized fractions were not sampled at all stations. ^{210}Po/^{210}Pb ratios increase with fraction size, with a maximum in the [200-500 µm] fraction, and a slight drop in the largest size-fraction [> 2000 µm]. Like in spring, ^{210}Po activity concentrations are higher in the eastern zone than in the western zone of the GoL for all plankton fractions (Table 4 and Fig. 4a). The same spatial distribution is observed for ^{210}Pb activity concentrations (Table 4, Fig. 4b). However, in contrast to spring, ^{210}Po/^{210}Pb ratios are significantly higher in the western zone of the GoL (Fig. 4c).

3.3. ^{210}Po and ^{210}Pb in small pelagic fishes

Anchovies were fished from both the eastern and the western zones of the GoL, while sardines were fished only in the western zone. As no spatial difference in mean ^{210}Po and ^{210}Pb activity concentrations in anchovy muscles are observed (t-test, $P > 0.05$), mean values on all individuals are considered for anchovies. Mean lengths (TL) and weights (W) of anchovies (TL: 11.9 ± 0.9 cm; W:

11.0 ± 2.5 g) and sardines (TL: 11.8 ± 0.9 cm; W: 11.0 ± 3.3 g) are not significantly different (t-tests, $P > 0.05$). Sardines and anchovies are 1 or 2 years old (age estimates from otoliths observation, C. Munschy, IFREMER, pers. comm). ^{210}Po activity concentrations in fish tissues follow the order liver~gonads>fish remains>muscles for both species (Table 5). Activity concentrations are significantly different between species, with higher activity concentrations measured in all organs in anchovies (t-tests, $P < 0.01$). ^{210}Pb activity concentrations are also significantly different between species, with higher values measured in anchovies, especially in liver and gonads (t-tests, $P < 0.01$). However, no difference in ^{210}Pb activity concentrations between fish species is recorded in muscles (t-tests, $P > 0.05$). For anchovies, higher ^{210}Pb activity concentrations are measured in liver than in gonads and remains, with very low values in muscles (Table 5). For sardines, ^{210}Pb activity concentrations in liver and gonads are one order of magnitude lower than in anchovies, while the highest activity concentrations are determined in fish remains. Radionuclide activity concentrations in the whole fish are calculated as the sum of the mean ^{210}Po and ^{210}Pb activity concentrations in each tissue, in relation to the weight percentage of this tissue (Table 5). Higher activity concentrations are measured in whole anchovies than in whole sardines, the difference between species being higher for ^{210}Po (x 2.3) than for ^{210}Pb (x 1.4).

3.4. C and N stable isotope ratios

Mean δ^{13}C of plankton, all sizes and stations combined, is significantly lower in spring (-23.18 ± 0.31 ‰) than in winter (-21.92 ± 0.40 ‰) ($P < 0.001$), while mean δ^{15}N does not significantly differ with season (3.56 ± 0.76 ‰ in spring and 2.79 ± 0.95 ‰ in winter, $P > 0.05$). During both the spring and winter cruises, δ^{13}C and δ^{15}N of plankton are more elevated in the eastern GoL (Table 6, Fig. 5). However, divergences in isotopic signatures with size differ with parameters (C *vs* N) and seasons. In spring, δ^{13}C does not vary significantly among plankton size fractions, while δ^{15}N is markedly lower in the largest size class [> 2000 µm] (Table 6). The absence of interactions between geographical zones and fractions for the two parameters indicates that both δ^{13}C and δ^{15}N values are higher in the eastern zone in all plankton fractions. In winter, higher δ^{13}C are observed in the two largest fractions, [1000-2000 µm] and [> 2000 µm], while the highest δ^{15}N are measured in the [1000-2000 µm]

fraction and the lowest in the [60-200 μm] fraction. . In all fractions, $\delta^{13}C$ and $\delta^{15}N$ values are higher in the eastern zone in winter, except for $\delta^{15}N$ of the [> 2000 μm] fraction (Table 6).

3.5. Relationships between radionuclide activities and stable isotope ratios

Significant positive linear curve fits are observed between $Log_{10}{}^{210}Po$ (y) and $Log_{10}\delta^{15}N$ (x) for plankton size fractions in spring ($y = 1.81x + 1.19$, $R^2 = 0.85$, $P < 0.001$) and winter ($y = 0.64x + 2.15$, $R^2 = 0.59$, $P < 0.01$). However, the slope and accuracy of the relationships are higher in spring than in winter. In order to investigate a possible bio-magnification of ^{210}Po in the food webs of sardines and anchovies, activities in whole fishes are used with mean radionuclide activity concentrations in plankton fractions in the western zone of the GoL in winter, where both fish species were sampled. A significant positive linear relationship between $Log_{10}{}^{210}Po$ and $Log_{10}\delta^{15}N$ ($p < 0.05$) is observed from plankton to these two zooplanktivorous fish species (Fig. 6), confirming ^{210}Po bio-magnification along the food webs, in relation to the trophic level of organisms as determined from $\delta^{15}N$.

4. Discussion

4.1. ^{210}Po and ^{210}Pb spatial variability in the Gulf of Lion

The GoL hydrodynamic is characterized by a cyclonic Northern Current that influences the transport of SPM originating from the Rhone River, westward within the Gulf with a highly variable dilution zone (Durrieu de Madron et al., 2000; Estournel et al., 2001). We do not measure any gradient of ^{210}Po and ^{210}Pb activity concentrations in SPM, seawater or plankton westward along the cyclonic Northern Current, but two distinct zones, East versus West, are evidenced by hierarchical clustering based on mean ^{210}Po and ^{210}Pb values in plankton in spring and winter (Fig. 2). This specific spatial difference is also seen from the various planktonic size-fractions in the eastern and western zones of the GoL, during spring and winter (Fig. 3 and 4). Such geographical variations may originate either from different ^{210}Po input in the GoL (difficult to prove owing to the lack of available data from riverine and atmospheric input), or from different planktonic community/ecology, or from specific hydrodynamic features in the GoL. Higher $\delta^{13}C$ and $\delta^{15}N$ are also measured in the eastern zone of the GoL in the different plankton size classes collected during spring and winter (Table 6, Fig. 5), suggesting

different origins for the organic matter assimilated by planktonic communities (Fry and Sherr, 1984) in the eastern and western GoL pelagic food webs. Espinasse et al. (2014b) demonstrate that the history of the water masses, a couple of weeks before sampling, results in two main patterns with different isotopic signatures of zooplankton, persistent in spring and winter. The water masses sampled in our eastern sites (ST1/ST10, ST2 and ST3) are carried onto the shelf from off-shelf region by the Northern Current and are characterized by high $\delta^{13}C$ and $\delta^{15}N$ values. On the contrary, the water masses sampled in the western sites of the present study (ST4, ST5, ST6 and ST7) already resided on the shelf and show lower $\delta^{13}C$ and $\delta^{15}N$ values These results corroborate the finding of Espinasse et al. (2014b) on the role of the origin of water masses on plankton characteristics. In addition, Espinasse et al. (2014a) show significant correlations between hydrophysical and biological features in the GoL during spring 2010 and winter 2011. They demonstrate the presence of different planktonic habitats (a practical concept aiming at representing in a synthetic way the distribution of zooplankton communities as a function of environmental conditions (Zarauz et al., 2007)) in the GoL with similar characteristics thorough all seasons. The characterization of the different habitats by Espinasse et al. (2014a) and the importance of water masses origin (Espinasse et al., 2014b) provide complementary insights on the influence of hydrodynamics on the observed „East versus West" variability of $\delta^{13}C$ and $\delta^{15}N$ values and ^{210}Po and ^{210}Pb bio-accumulated in plankton. In our study, sites ST1/ST10 present contaminant and isotopic characteristics that are closer to those of sites ST2 and ST3 than to the western sites (Fig. 5). These two sites are characterized by specific $\delta^{13}C$ and $\delta^{15}N$ features (higher $\delta^{13}C$ and $\delta^{15}N$, Fig.5 and also Bănaru et al., 2013b), likely due to inputs of organic matter from terrestrial and anthropogenic origins, as shown by hydrodynamic studies performed in the Bay of Marseille by Pradal and Millet (2006). Their characteristics, closer to those observed in front of the Rhone River, can also be related to an occasional intrusion of the Rhone River plume into the Bay of Marseille during our sampling periods (Fraysse et al., 2013).

4.2. ^{210}Po bioaccumulation in plankton

Apart from higher activities in the eastern zone, ^{210}Po activity concentration is rather constant among plankton size-fractions, except during spring for the largest fraction ([> 2000 μm]; Tables 3 and 4).

These results diverge from previous investigations off the Cape of Good Hope (Shannon et al., 1970) and from laboratory experiments (Stewart et al., 2005). Radionuclide activity concentrations in the [60-200 μm] fraction (mainly composed of phytoplankton, Table 1) during the COSTAS project are higher than those measured in phytoplankton from the SE Atlantic Ocean (Shannon and Cherry, 1967; Shanon et al., 1970).

Dissolved ^{210}Po activity concentrations range from 2.5 to 5.2 Bq m^{-3} during the COSTAS project. They are higher than those measured in the NW Mediterranean sea by Heyraud and Cherry (1979) (0.8 to 1.9 Bq m^{-3}) and Bojanovski et al. (1983) (0.7 to 2.7 Bq m^{-3}) but similar to those measured by Tateda et al. (2003) (3.1 to 19.7 Bq m^{-3}, with a geometric mean of 6.1 ± 0.3 Bq m^{-3}) in a coastal site near Monaco. The range of ^{210}Po activity concentrations for surface open and coastal waters reported by Fowler (2011) for all seas is 0.4-3.1 Bq m^{-3}. The activity concentration measured at ST1 is unusually high (15 ± 0.8 Bq m^{-3}) but similar to some values reported by Tateda et al (2003). Seven samples were collected exactly in the same area from March to December 2010 in the framework of a parallel project (POTOMAC) that show elevated ^{210}Po dissolved activity concentrations at the Chl-*a* maximum depth (ranging from 6.8 to 20.6 Bq m^{-3}, with a median near 10 Bq m^{-3}) with no clear explanation.

In zooplankton ([200-500 μm] to [> 2000 μm]), ^{210}Po activity concentrations (27-461 Bq kg^{-1} dry wt) are in the lowest range of reported values for unidentified zooplankton species in different oceans (30-800 Bq kg^{-1} dry wt; review by Fowler, 2011; Stewart et al., 2008) and are generally higher in winter than in spring (Fig. 3 and 4). This disparity in ^{210}Po activity concentrations may be due to changes in zooplankton composition and/or the relative proportion of various species (Table 1) as previously seen for other species in different environments (copepods: 20-3200 Bq kg^{-1} dry wt, and euphausiids: 15-630 Bq kg^{-1} dry wt in Fowler, 2011). In the largest plankton fraction [> 2000 μm], ^{210}Po activity concentrations significantly shift from low to high activity concentrations in spring and winter respectively ($P < 0.001$) (Fig. 3 and 4). The predominance of salps observed during the spring cruise corroborates this observation (Table 1), as salps typically exhibit low ^{210}Po activity concentration (Krishnawami et al., 1985) and low trophic level position (Fig. 5; Rodriguez y Baena et al., 2007). To summarize, the Gulf of Lion is characterized by elevated ^{210}Po bio-accumulation in the first trophic

levels, SPM and phytoplankton (due to both adsorption on surface particles and absorption by living cells), and lower bio-accumulation in higher zooplankton levels.

4.3. ^{210}Pb bioaccumulation in plankton

^{210}Pb activity concentrations measured in seawater during the COSTAS project are higher than those previously measured in the Mediterranean Sea, but show similar ^{210}Po/^{210}Pb ratios (i.e. less than 1; Fowler, 2011). ^{210}Pb bio-accumulation in plankton reveals contrasting patterns between spring and winter. During spring, ^{210}Pb exhibits no spatial difference, a contrasted bio-accumulation in the smallest plankton fraction mainly composed of phytoplankton (high) and the larger ones composed of zooplankton (low), and the absence of trophic transfer as demonstrated experimentally by Stewart et al. (2005). In contrast, we observe spatial differences in winter with higher activity concentrations in the eastern GoL and, for the first time, ^{210}Pb activity concentrations that increase with trophic levels (δ^{15}N) in zooplankton (Fig. 4b). Analytical biases cannot account for these observations since (i) samples are analyzed through several mixed series, and (ii) total Pb concentrations show the same patterns with a significant linear correlation between ^{210}Pb and total Pb in plankton (Fig. 7). We also dismiss the presence of fecal pellets, enriched in ^{210}Pb compared to living plankton (Fisher et al., 1983; Stewart et al., 2005; Rodriguez y Baena et al., 2007), in zooplankton size classes, as plankton organisms were carefully sieved and thoroughly rinsed with filtered seawater.

Change in ^{210}Pb activity concentration within plankton size-fractions is likely the cause for the decrease in the ^{210}Po/^{210}Pb ratios during winter (Fig. 4c). This pattern contrasts to that observed across three trophic levels by Stewart et al. (2005) with laboratory experiments where the ^{210}Po/^{210}Pb ratios increase 5- to 12-fold with each trophic level represented by *Artemia sp.* (size: 500-1200 μm) and euphausiids *Meganyctiphanes norvegica* (size: > 2000 μm). These authors suggest that the ^{210}Po/^{210}Pb ratios could be used to tentatively assess the trophic structure of plankton community. When ^{210}Po/^{210}Pb is measured in defined plankton species, experimentally (Stewart et al., 2005) or from sea-sampled organisms (Rodriguez y Baena et al., 2007), the ratio is a good diagnostic tool to evaluate the trophic structure of plankton community. However, in our case, each plankton size class is composed of organisms with different feeding behaviors (herbivores, omnivores and carnivores) and there is no

direct prey-predator relationship between small and large plankton size classes. Since the patterns of variation of $^{210}Po/^{210}Pb$ ratio among size classes is not consistent and differ with season, this ratio cannot be directly used to estimate the trophic structure of the plankton community when based on size class analysis.

4.4. ^{210}Po transfer within the sardine and anchovy food webs

Anchovies and sardines are both planktivorous species (Costalago et al., 2012). They display the typical pattern of ^{210}Po and ^{210}Pb distribution in fishes with higher activity concentrations in all the organs and tissues (especially bone and gut, Carvalho, 2011) with the exception of muscle (Table 5) (Carvalho, 2011; Desideri et al., 2010). Yet, activity concentrations vary between the two species with higher ^{210}Po and ^{210}Pb in anchovies (Table 5). The ^{210}Po concentration ratios (CR; based on fish muscle wet weigh) are 3×10^4 for anchovies and for sardines. The trophic factor from zooplankton to fishes ranges from 0.3 to 0.5 (dry wt, equivalent to 0.08-0.1 wet wt).

For the first time *in-situ*, we evidence ^{210}Po ability to bio-magnify in the food webs of small pelagic fishes (Fig. 6). Accordingly, we assume that the fish diet could explain observed differences between the two species (Table 5), as suggested by Carvalho (2011). In fact, despite being planktivorous, anchovy and sardine diets differ (Costalago et al., 2012) and can be characterized as zooplanktivorous for anchovies (Plounevez and Champalbert, 2000) and zooplanktivorous to phytoplanktivorous for sardines (Bode et al., 2004; Nikolioudakis et al., 2012). Considering almost similar ^{210}Po activity concentrations in phytoplankton and zooplankton, a higher ^{210}Po bioaccumulation in anchovies is not consistent with sardine and anchovy respective diets. Differences in life strategy and lipid content may be considered to explain a higher ^{210}Po bio-accumulation in anchovies relative to sardines. Total lipid contents of muscle and liver are higher (x2.3 and x1.4 respectively) and vary more seasonally in sardine than in anchovy (Pethybridge et al., 2014). Since ^{210}Po seems associated with protein in animal tissues (Carvalho and Fowler, 1994; Durand et al., 1999), then the lower lipid content of anchovy compared to that of sardine may explain higher ^{210}Po concentrations. We suggest that different energetic strategies could influence the bioaccumulation of contaminants in sardine and anchovy and that emphasis should be made in the future on the relationships between energy transfer, lipid reserve,

bio-accumulation of contaminants (Pethybridge et al., 2013, 2014) and protein transfer (Carvalho, 2011).

5. Conclusions

In-situ investigations within the food web of two small pelagic fishes, *Sardina pilchardus* and *Engraulis encrasicolus,* in the Gulf of Lion reveal new insights on ^{210}Po and ^{210}Pb transfer and distribution in the marine ecosystem. We demonstrate that ^{210}Po and ^{210}Pb activity concentrations in plankton can vary spatially at a relative small scale (e.g. Gulf of Lion), and that this variability is connected to local specific pelagic habitats. Furthermore, we show that ^{210}Po bioaccumulation can remain constant from the first (dominated by phytoplankton) to the second (zooplankton) trophic levels, while ^{210}Pb bioaccumulation can increase. Finally, we evidence a ^{210}Po bio-magnification along the trophic food webs of these two planktivorous pelagic fishes.

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7. References

Alekseenko, E., Raybaud, V., Espinasse, B., Carlotti, F., Quéguiner, B., Thouvenin, B., Garreau, P., Baklouti, M., 2014. Seasonal dynamics and stoichiometry of the planktonic community in the NW Mediterranean Sea : a 3D modeling approach. Ocean Dynamics 64, 179-207.

Al-Masri, M.S., Mamish, S., Budeir, Y., Nashwati, A., 2000. ^{210}Po and ^{210}Pb concentrations in fish consumed in Syria. J. Environ. Radioact. 49, 345-352.

Bacon, M.P., Spencer, D.W., Brewer, P.G., 1976. $^{210}Pb/^{226}Ra$ and $^{210}Po/^{210}Pb$ disequilibria in sea water and suspended particulate matter. Earth Planet. Sci. Lett. 32, 277-296.

Bacon, M.P., Huh, C., Fleer, A.P., Deuser, W.G., 1985. Seasonality in the flux of natural radionuclides and plutonium in the deep Sargasso Sea. Deep-Sea Res.II 32, 273-286.

Bănaru, D., Mellon-Duval, C., Roos, D., Bigot, J. L., Souplet, A., Jadaud, A., Beaubrun, P., Fromentin, J. M., 2013a. Trophic structure in the Gulf of Lion marine ecosystem (north-western Mediterranean Sea) and fishing impacts. ICES J. Mar. Sys. 111-112, 45-68.

Bănaru, D., Carlotti, F., Barani, A., Gregori, G., Neffati, N., Harmelin-Vivien, M., 2013b. Seasonal variation of stable isotope ratios of size-fractionated zooplankton in the Bay of Marseille (NW Mediterranean Sea), J. Plankton Res. 36, 145-156.

Bode, A., Alvarez-Ossorio, M.T., Carrera, P., Lorenzo, J., 2004. Reconstruction of trophic pathways between plankton and the North Iberian sardine (*Sardina pilchardus*) using stable isotopes. Sci. Mar. 68, 165-178.

Bojanowski, R., Ballestra, S., Fukai, R., 1983. Natural radionuclides in the north-western Mediterranean Sea. Rapp. Comm. Int. Mer Médit 28, 205-207.

Carvalho, F.P., 1995. 210Po and 210Pb intake by the Portuguese population: the contribution of seafood in the dietary intake of 210Po and 210Pb. Health Physics 69 (4), 469e480.

Carvalho, F.P., 2011. Polonium (210Po) and lead (210Pb) in marine organisms and their transfer in marine food chains. J. Environ. Radioact. 102, 462-472.

Carvalho, F.P., Fowler, S.W., 1993. An experimental study on the bioaccumulation and turnover of polonium-210 and lead-210 in marine shrimp. Mar. Ecol. Prog.Ser. 102, 125-133.

Carvalho, F.P., Fowler, S.W., 1994. A double-tracer technique to determine the relative importance of water and food as sources of polonium-210 in marine prawns and fish. Mar. Ecol. Prog. Ser. 103, 251-264.

Champalbert, G., 1996. Characteristics of zooplankton standings stock and in the western Mediterranean Sea: relations to hydrology. Sci. Mar. 60 (2), 97–113.

Cherry, R.D., Shannon, L.V., 1974. The Alpha radioactivity of marine organisms. Atomic Energy Review 12, 3-45.

Chiffoleau, J.F., Chavaud, L., Amouroux, D., Barats, A., Dufour, A., Pécheyran, C., Roux, N., 2004. Nickel and vanadium contamination of benthic invertebrates following the "Erika" wreck. Aquat. Living Resour., 17, 273–80.

Church, T., Rigaud, S., Baskaran, M., Kumar, A., Friedrich, J., Masque, P., Puigcorbé, V., Kim, G., Radakovitch, O., Hong, G., Choi, H., Stewart, G. 2012. Intercalibration studies of ^{210}Po and ^{210}Pb in dissolved and particulate seawater samples. Limnol & Oceanogr: Methods. 10, 776-789.

Cochran, J.K., Mc Kibbin-Vaughan, T., Dornblaser, M.M., Hirschberg, D., Livingston, H.D., Buesseler, K.O., 1990. ^{210}Pb scavenging in the North Atlantic and North Pacific oceans. Earth Planet. Sci. Lett. 97, 332-352.

Cossa, D., Harmelin-Vivien, M., Mellon-Duval, C., Loizeau, V., Averty, B., Crochet, S., Chou, L., Cadiou, J.F., 2012. Influences of bioavailability, trophic position, and growth on methylmercury in hakes (*Merluccius merluccius*) from northwestern Mediterranean and northeastern Atlantic. Environ. Sci. Technol. 46, 4885-4893.

Costalago, D., Navarro, J., Álvarez-Calleja, I., Palomera, I., 2012. Ontogenetic and seasonal changes in the feeding habits and trophic levels of two small pelagic fish species. Mar. Ecol. Prog. Ser. 460, pp. 169-181.

Desideri, D., Meli, M. A., Roselli, C., 2010. A biomonitoring study: 210Po and heavy metals in marine organisms from the Adriatic Sea (Italy). J Radioanal Nucl Chem. 285, 373-382.

De Niro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta. 42, 495-506.

Durand, J.P., Carvalho, F.P., Goudard, F., Pieri, J., Fowler, S.W., Cotret, O., 1999. ^{210}Po binding to metallotioneins and ferritin in the liver of teleost marine fish. Mar. Ecol. Progr. Ser. 177, 189-196.

Durrieu de Madron, X., Abassi, A., Heussner, S., Monaco, A., Aloisi, J.C., Radakovitch, O., Giresse, P., Buscail, R., Kerhervé, P., 2000. Particulate matter and organic carbon budgets of the Gulf of Lion (NW Mediterranean). Oceanol. Acta 23, 717-730.

Espinasse, B., Carlotti, F., Zhou, M., Devenon, JL., 2014a. Defining zooplankton habitats in the Gulf of Lion (NW Mediterranean Sea) using size structure and environmental conditions. Mar. Ecol. Progr. Ser. 506, 31-46.

Espinasse, B., Harmelin-Vivien, M., Tiano, M., Guilloux, L., Carlotti, F., 2014b. Patterns of variations in C and N stable isotope ratios in size-fractionated zooplankton in the Gulf of Lion, NW Mediterranean Sea. J. Plankton Res., 36, 1204-1215.

Estournel, C, Broche, P, Marsaleix, P, Devenon, JL, Auclair, F. 2001. The Rhone River plume in unsteady conditions: numerical and experimental results. Est. Coast. Shelf Sci. 53, 25–38.

Fisher, N. S., Burns, K. A., Cherry, R. D., Heyraud, M., 1983. Accumulation and cellular distribution of 241Am, 210Po and 210Pb in two marine algae. Mar. Ecol. Prog. Ser 11, 233-237.

Fowler, S. W., 2011. 210Po in the marine environment with emphasis on its behaviour within the biosphere. J. Environ. Radioact. 102, 448-461.

Fraysse, M., Pinazo, C., Faure, V.M., Fuchs, R., Lazzari, P., Raimbault, P. Pairaud, I., 2013. Development of a 3D coupled physical-biogeochemical model for the Marseille coastal area (NW Mediterranean Sea): What complexity is required in the coastal zone? PloS ONE 8(12): e80012. doi:10.1371/journal.pone.0080012.

Fry, F., Sherr, E., 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contr. Mar. Sci. 27, 13–47.

Gairoard, S., Radakovitch, O., Eyrolle, F., Ludwig, W., Cossa, D. 2012. Flux de matières solides et liquides des bassins versants français à la Méditerranée. Report for Rhone-Mediterranee Corse Water Agency, 88 pp.

Gaudy, R., Youssara, F., Diaz, F., Raimbault, P., 2003. Biomass, metabolism and nutrition of zooplankton in the Gulf of Lion (NW Mediterranean). Oceanol. Acta 26, 357–372.

Guelinkx, J, Maes, J, Van Den Driessche, P, Geysen, B, Dehairs, F, Ollevier, F., 2007. Changes in $d^{13}C$ and $d^{15}N$ in different tissues of juvenile sand goby *Pomatoschistus minutus*: a laboratory diet-switch experiment. Mar. Ecol. Progr. Ser. 341, 205-215.

Harmelin-Vivien, M., Bodiguel, X., Charmasson, S., Loizeau, V., Mellon-Duval, C., Tronczyński, J., Cossa, D,. 2012. Differential biomagnification of PCB, PBDE, Hg and Radiocesium in the food web of the European hake from the NW Mediterranean. Mar. Poll. Bull. 64, 974-983.

Harmelin-Vivien, M., Loizeau, V., Mellon, C., Beker, B., Arlhac, D., Bodiguel, X., Ferraton, F., Hermand, R., Philippon, X., Salen-Picard, C. 2008. Comparison of C and N stable isotope ratios

between surface particulate organic matter and microphytoplankton in the Gulf of Lions (NW Mediterranean). Cont. Shelf Res. 28, 1911-1919.

Heyraud, M., Cherry, R.D., 1979. Polonium-210 and lead-210 in marine food chains. Mar. Biol. 52, 227-236.

ICRP, 1968. Evaluation of Radiation Doses to Body Tissues from Internal Contamination due to Occupational Exposure. Annals of the ICRP/ICRP Publication, 10, 1-94.

Jeffree, R.A., Szymczak, R. 2000. Enhancing effect of marine oligotrophy on environmental concentrations of particle-reactive trace elements. Env. Sci. Technol. 34, 1966-1969.

Krishnaswami, S., Baskaran, M., Fowler, S.W., Heyraud, M., 1985. Comparative role of salps and other zooplankton in the cycling and transport of selected elements and natural radionuclides in Mediterranean waters. Biogeochemistry 1, 353-360.

Lochet, F., Leveau, M., 1990. Transfers between an eutrophic ecosystem, the river rhône, and an oligotrophic ecosystem, the north-western Mediterranean Sea. Fish. Oceanogr. 207, 95-103.

Lorthiois, T., Doxaran, D., Chami, M., 2012. Daily and seasonal dynamics of suspended particles in the Rhone river plume based on remote sensing and field optical measurements. Geo-mar Lett. 32, 89-101.

Maruyama, A., Yamada, Y., Rusuwa, B., Yuma, M., 2001. Change in stable nitrogen isotopes ratio in the muscle tissue of a migratory goby, *Rhinogobius* sp., in a natural setting. Can. J. Fish. Aquat. Sci. 58, 2125-2128.

Masqué, P., Sanchez-Cabeza, J.A., Bruach, J.M., Palacios, E., Canals, M., 2002. Balance and residence times of ^{210}Pb and ^{210}Po in surface waters of the northwestern Mediterranean Sea, Cont. Shelf Res. 22, 2127–2146.

Millot, C., 1999. Circulation in the western Mediterranean Sea. J. Mar. Syst. 20 (1–4), 423–442.

Moore, W.S., Dymond, J., 1988. Correlation of 210Pb removal with organic carbon fuxes in the Pacific Ocean. Nature 331, 339-341.

Nikolioudakis, N., Isari, S., Pitta, P., Somarakis, S., 2012a. Diet of sardine *Sardina pilchardus*: an "end-to-end" field study. Mar. Ecol. Prog. Ser. 453, 173-188.

Pethybridge, H., Roos, D., Loizeau, V., Pecquerie, L., Bacher ,C., 2013. Responses of European anchovy vital rates and population growth to environmental fluctuations: An individual-based modeling approach. Ecol. Model. 250, 370-383.

Pethybridge, H., Bodin, N., Arsenault-Pernet, E.J., Bourdeix, J.H., Brisset, B., Bigot, J.L., Roos, D.,Peter, M., 2014. Temporal and inter-specific variations in forage fish feeding conditions in the NW Mediterranean: lipid content and fatty acid compositional changes. Mar. Ecol. Progr. Ser. 512, 39-54.

Plounevez, S., Champalbert, G., 2000. Diet, feeding behaviour and trophic activity of the anchovy (*Engraulis encrasicolus* L.) in the Gulf of Lions (Mediterranean Sea). Oceanol. Acta, 23(2): 175-192.

Pradal, M. A., Millet, B., 2006. Hétérogénéité spatiale du forçage du vent: fonctionnement des récifs artificiels et circulation des eaux dans la baie sud de Marseille. C. R. Biol. 329, 541–550.

Radakovitch, O., Cherry, R.D, Heyraud, M., Heussner, S., 1998. Unusual 210Po/210Pb ratios in the surface water of the Gulf of Lions. Oceanol. Acta 21, 459-468.

Radakovitch, O., Cherry, R.D, Heussner, S., 1999. ^{210}Pb and ^{210}Po: tracers of particle transfer on the Rhône continental margin (NW Mediterranean). Deep-Sea Res. I 46, 1539-1563.

Rodriguez y Baena, A.M., Fowler, S.W., Miquel, J.C., 2007. Particulate organic carbon: natural radionuclide ratios in zooplankton and their freshly produced fecal pellets from the NW Mediterranean (MedFlux 2005). Limnol Oceanogr. 52, 966-974.

Saiz, E., Calbet, A., Atienza, D., Alcaraz, M., 2007. Feeding and production of zooplankton in the Catalan Sea (NW Mediterranean). Prog. Oceanogr. 74, 313–328.

Sarakinos, H.C., Johnson, M.L., Vander Zanden, M.J., 2002. A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. Can. J. Zool. 80, 381- 387.

Shannon, L.V., Cherry, R.D., 1967. Polonium-210 in marine plankton. Nature 216,352-353.

Shannon, L.V., Cherry, R.D., Orren, M.J., 1970. Polonium-210 and lead-210 in the marine environment. Geochim. Cosmochim. Acta 34, 701-711.

Stewart, G.M., Fisher, N.S., 2003. Experimental studies on the accumulation of polonium-210 by marine phytoplankton. Limnol. Oceanogr 48, 1193-1201.

Stewart, G.M., Fowler, S.W., Teyssie, J.-L., Cotret, O., Cochran, J.K., Fisher, N.S., 2005. Contrasting transfer of polonium-210 and lead-210 across three trophic levels in marine plankton. Mar. Ecol. Prog. Ser. 290, 27-33.

Stewart, G.M., Fowler, S.W, N.S. Fisher, 2008. "The bioaccumulation of U-Th series radionuclides in marine organisms." U-Th series nuclides in aquatic systems 13 (2008): 269-305.

Tateda, Y., Carvalho, F.P., Fowler, S.W., Miquel, J.C., 2003. Fractionation of 210Po and 210Pb in coastal waters of the NW Mediterranean continental margin. Cont. Shelf Res. 23, 295-316.

Zarauz, L, Irigoien, X, Urtizberea, A, Gonzalez, M., 2007. Mapping plankton distribution in the Bay of Biscay during three consecutive spring surveys. Mar. Ecol. Progr. Ser. 345, 27-39.

Figure captions

Fig.1. Location of the sampling sites in the Gulf of Lion, northwestern Mediterranean Sea. Black dots = plankton sampling sites; dotted circles = pelagic fish sampling areas.

Fig. 2. Hierarchical clustering based on mean ^{210}Po and ^{210}Pb activity concentrations in plankton at each GoL site during spring (May) and winter (February) COSTAS expeditions.

Fig. 3. Mean (± SD) activity concentrations of ^{210}Po (a) and ^{210}Pb (b), and ^{210}Po/^{210}Pb ratios (c) in the different size-fractions of plankton in the eastern and western zones of the GoL in May 2010. East-West differences are significant in all fractions for ^{210}Po and ^{210}Po/^{210}Pb ratios.

Fig. 4. Mean (± SD) activity concentrations of ^{210}Po (a) and ^{210}Pb (b), and ^{210}Po/^{210}Pb ratios (c) in the different size-fractions of plankton in the eastern and western zones of the GoL in February 2011. East-West differences are significant in all fractions for ^{210}Po (except for the fraction >2000 μm) and ^{210}Pb.

Fig. 5. Stable isotope ratios of size fractionated plankton in the eastern and western zones of the GoL during spring (May 2010) and winter (February 2011) COSTAS expeditions. Plankton size-fractions 1: [60-200 μm], 2: [200-500 μm], 3: [500-1000 μm], 4: [1000-2000 μm], 5: [>2000 μm]. Standard deviations are not indicated for clarity. Circles were arbitrarily drawn to clearly show spring and winter sampling periods.

Fig. 6. Bio-magnification of ^{210}Po in the food webs of two small pelagic zooplanktivorous fishes, *Sardina pilchardus* (sardine) and *Engraulis encrasicolus* (anchovy) based on Log_{10} of mean values of δ^{15}N and ^{210}Po activity concentration in the western zone of the GoL in winter.

Fig. 7. Relationship between ^{210}Pb activity concentrations and total Pb concentrations for a) fractions >6 μm and b) fractions >200 μm.

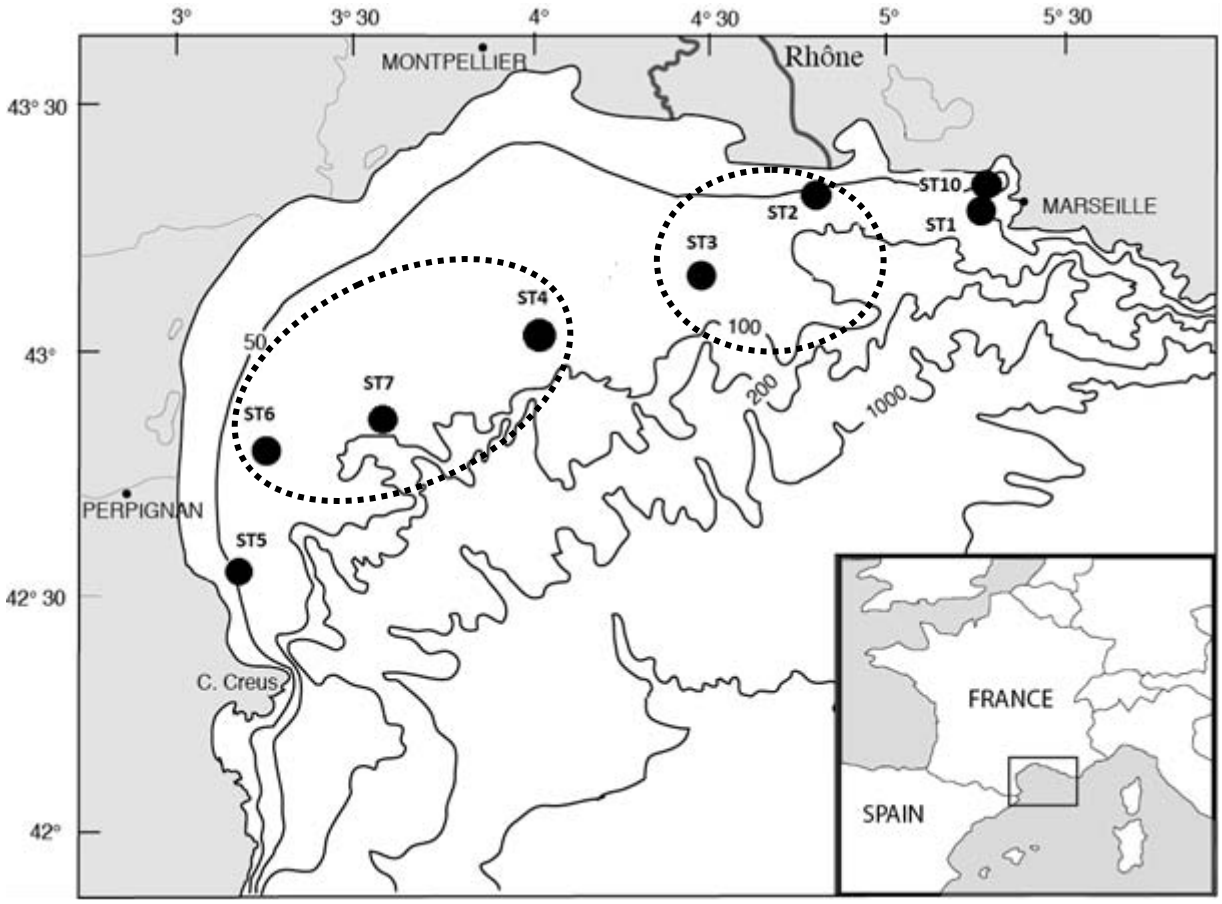
3°
3° 30
4°
4° 30
5°
5° 30
MONTPELLIER
Rhône
43° 30
43°
42° 30
42°
ST10
ST2
ST1
MARSEILLE
ST3
ST4
50
100
200
1000
ST7
ST6
PERPIGNAN
ST5
C. Creus
FRANCE
SPAIN

Fig. 1

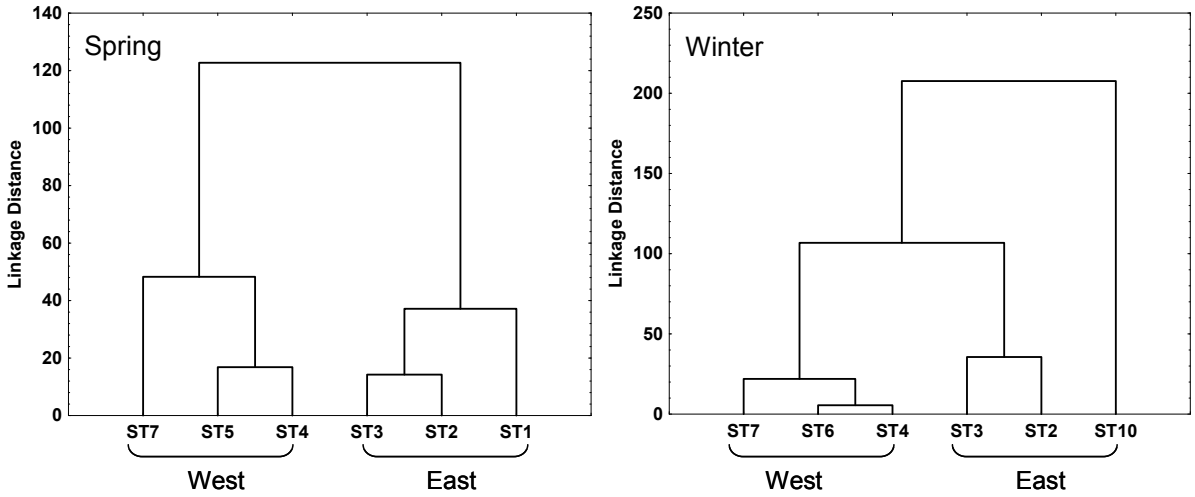
Spring
Linkage Distance
140
120
100
80
60
40
20
0
ST7
ST5
ST4
ST3
ST2
ST1
West
East
Winter
Linkage Distance
250
200
150
100
50
0
ST7
ST6
ST4
ST3
ST2
ST10
West
East

Fig. 2

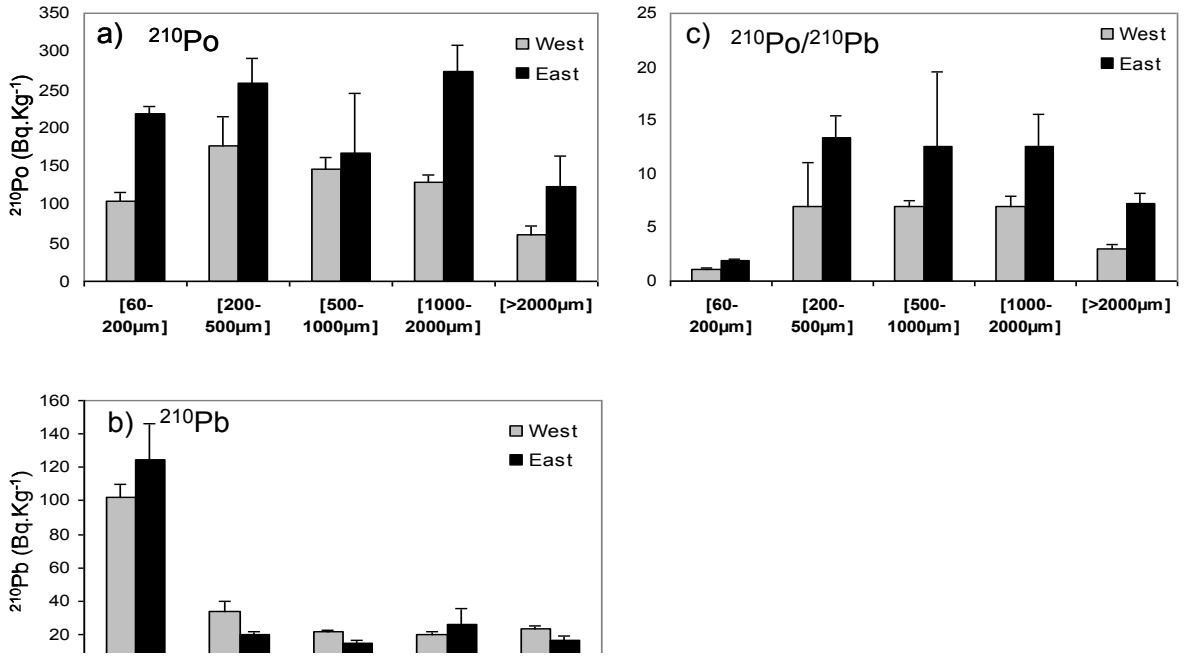
a) 210Po
West
East
210Po (Bq.Kg-1)
350
300
250
200
150
100
50
0
[60-200µm]
[200-500µm]
[500-1000µm]
[1000-2000µm]
[>2000µm]
c) 210Po/210Pb
West
East
25
20
15
10
5
0
[60-200µm]
[200-500µm]
[500-1000µm]
[1000-2000µm]
[>2000µm]
b) 210Pb
West
East
210Pb (Bq.Kg-1)
160
140
120
100
80
60
40
20

Fig.3

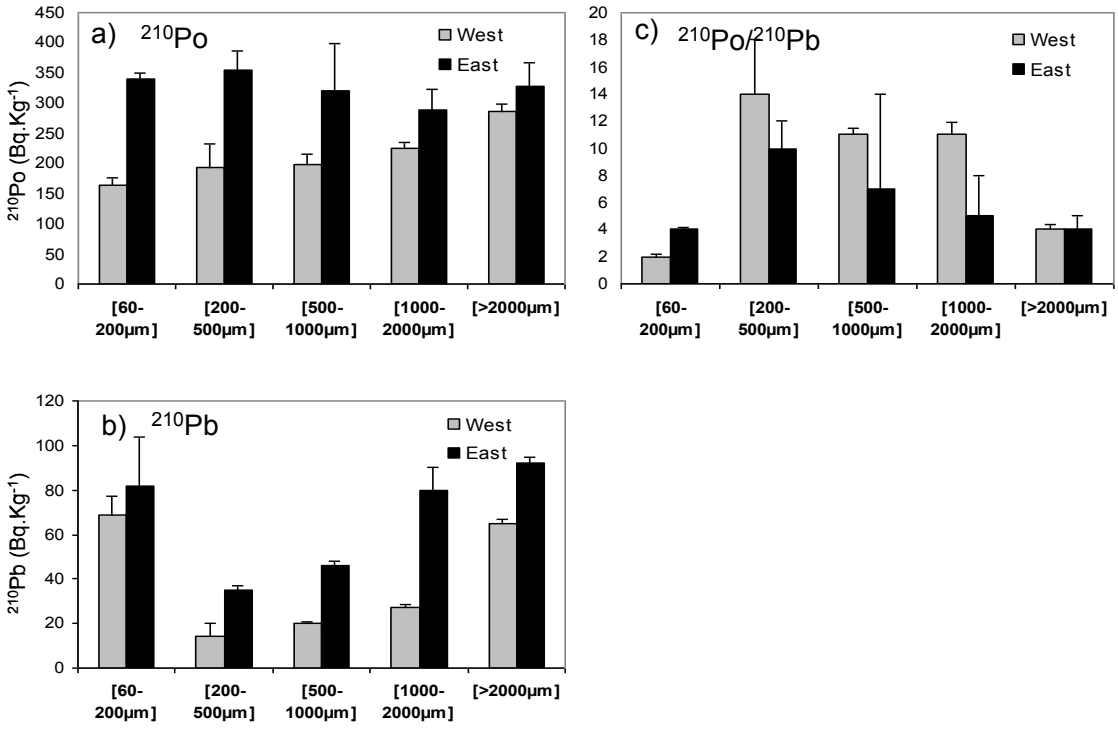
a) 210Po
West
East
210Po (Bq.Kg-1)
450
400
350
300
250
200
150
100
50
0
[60-200µm]
[200-500µm]
[500-1000µm]
[1000-2000µm]
[>2000µm]
c) 210Po/210Pb
West
East
20
18
16
14
12
10
8
6
4
2
0
[60-200µm]
[200-500µm]
[500-1000µm]
[1000-2000µm]
[>2000µm]
b) 210Pb
West
East
210Pb (Bq.Kg-1)
120
100
80
60
40
20
0
[60-200µm]
[200-500µm]
[500-1000µm]
[1000-2000µm]
[>2000µm]

Fig. 4

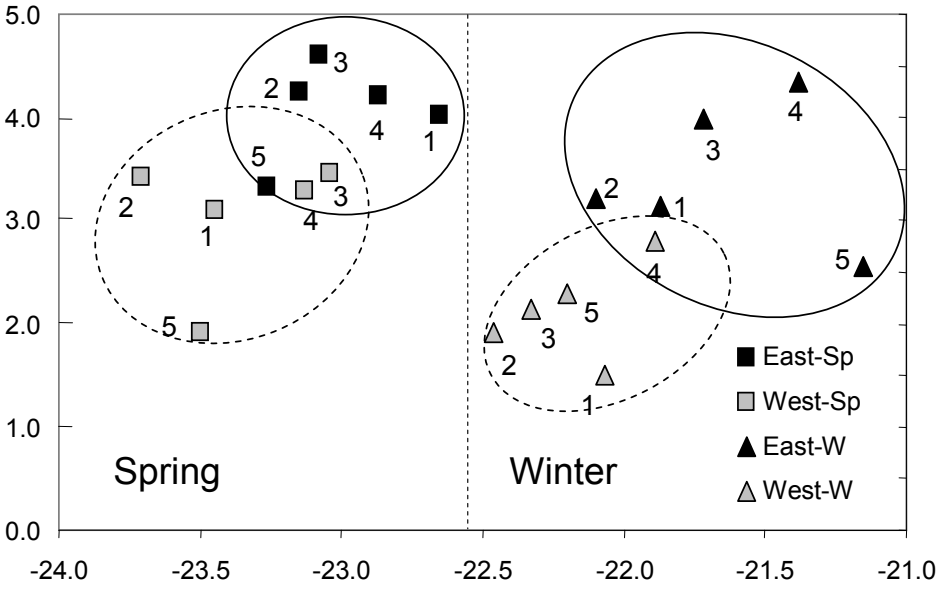
5.0
4.0
3.0
2.0
1.0
0.0
-24.0
-23.5
-23.0
-22.5
-22.0
-21.5
-21.0
Spring
Winter
East-Sp
West-Sp
East-W
West-W

Fig. 5

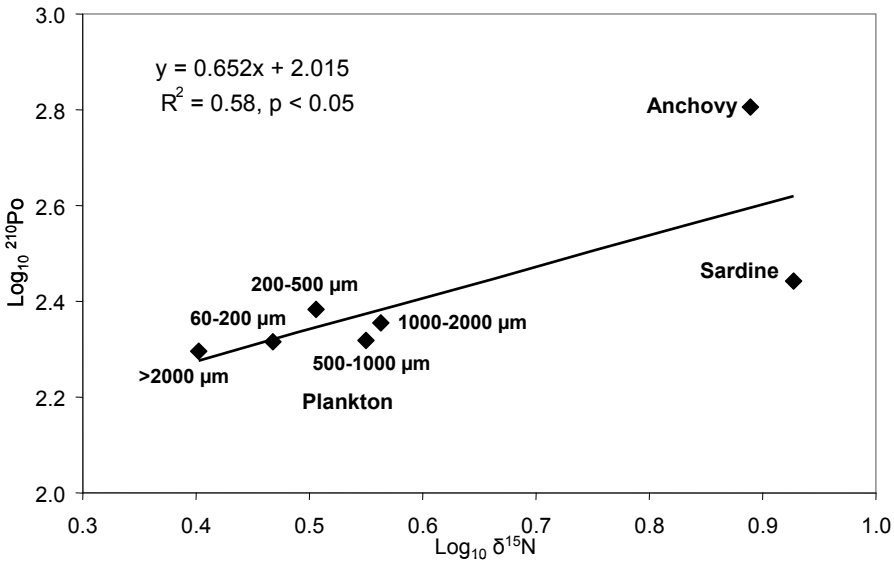
y = 0.652x + 2.015
R2 = 0.58, p < 0.05
Anchovy
Sardine
200-500 µm
60-200 µm
1000-2000 µm
500-1000 µm
>2000 µm
Plankton
Log10 210Po
Log10 δ15N
3.0
2.8
2.6
2.4
2.2
2.0
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Fig. 6

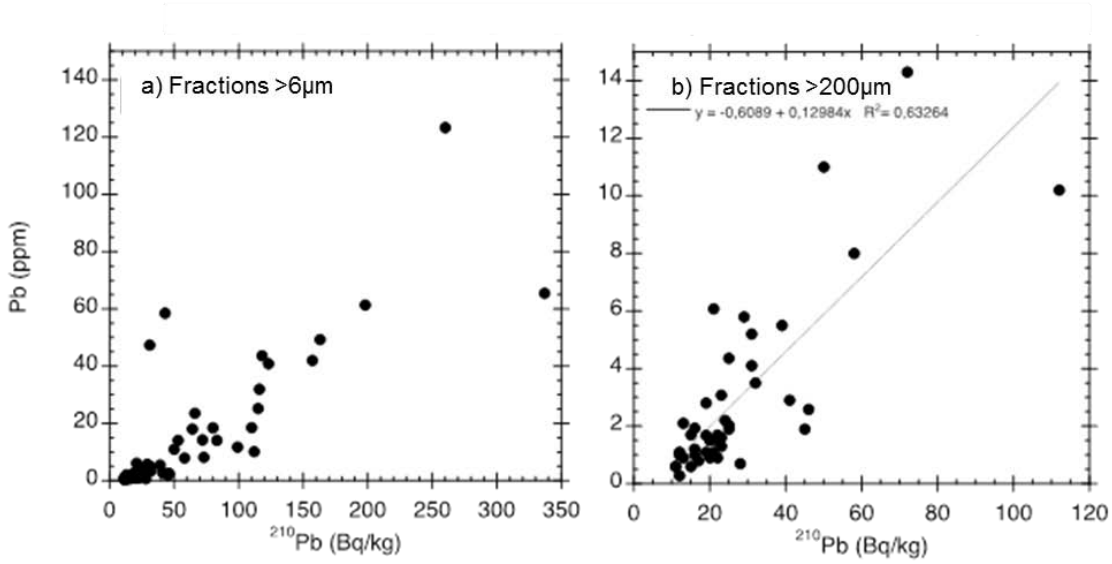
a) Fractions >6µm
Pb (ppm)
^{210}Pb (Bq/kg)
b) Fractions >200µm
y = -0,6089 + 0,12984x R^2= 0,63264
^{210}Pb (Bq/kg)

Fig7

Table 1

Composition of plankton size classes by decreasing order of group importance (dominant groups in bold characters).

Size class (μm)	Main groups
[6-60]	**Detritus, phytoplankton** (diatoms, dinobionts)
[60-200]	**Phytoplankton** (diatoms, dinobionts), copepods (eggs, nauplii and copepodits), detritus
[200-500]	**Copepods (copepodits)**, appendicularians, cladocerans, larvae (mollucs, crustaceans)
[500-1000]	**Copepods,** appendicularians, larvae (mollucs, crustaceans), euphausiids, teleost eggs, chaetognaths, pteropods
[1000-2000]	**Copepods,** pteropods, chaetognaths, appendicularians, larvae (mollucs, crustaceans), euphausiids, teleost eggs
[>2000]	**Salps**, siphonophores, copepods, chaetognaths, pteropods, larvae (crustaceans)

Table 2

^{210}Po and ^{210}Pb activity concentrations in the dissolved (Bq m^{-3}), particulate (Bq kg^{-1}) and plankton (Bq kg^{-1}) fractions and associated ^{210}Po/^{210}Pb ratios, from sampling sites analysed in spring (May 2010) and winter (February 2011) in the Gulf of Lion.

SPRING		PHASE	^{210}Po ± SD	^{210}Pb ± SD	^{210}Po/^{210}Pb
ST1	DISSOLVED		15 ± 0.8	18 ± 1.0	0.8
	SPM		1969 ± 173	937 ± 155	2.1
	PLANKTON	[6-60µm]	252 ± 51	337 ± 50	0.7
		[60-200µm]	239 ± 17	163 ± 9	1.5
		[200-500µm]	322 ± 12	20 ± 2	16
		[500-1000µm]	*n.d.* *n.d.*	*n.d.* *n.d.*	*n.d.*
		[1000-2000µm]	342 ± 19	45 ± 2	7.6
		[>2000µm]	200 ± 8	23 ± 2	8.8
ST2	DISSOLVED		3.1 ± 0.6	10 ± 0.6	0.3
	SPM		*n.d.* *n.d.*	*n.d.* *n.d.*	*n.d.*
	PLANKTON	[6-60µm]	42 ± 23	116 ± 11	0.4
		[60-200µm]	206 ± 23	123 ± 8	1.7
		[200-500µm]	226 ± 18	22 ± 3	10
		[500-1000µm]	272 ± 19	11 ± 2	24
		[1000-2000µm]	227 ± 17	13 ± 3	17
		[>2000µm]	104 ± 11	15 ± 3	7.0
ST3	DISSOLVED		5.2 ± 0.5	7.7 ± 0.7	0.7
	SPM		179 ± 38	316 ± 43	0.6
	PLANKTON	[6-60µm]	*n.d.* *n.d.*	*n.d.* *n.d.*	*n.d.*
		[60-200µm]	257 ± 20	31 ± 5	8.2
		[200-500µm]	228 ± 15	16 ± 1	14
		[500-1000µm]	216 ± 9	17 ± 1	13
		[1000-2000µm]	252 ± 16	19 ± 2	13
		[>2000µm]	65 ± 6	11 ± 2	5.8
ST4	DISSOLVED		*n.d.* *n.d.*	*n.d.* *n.d.*	*n.d.*
	SPM		*n.d.* *n.d.*	*n.d.* *n.d.*	*n.d.*
	PLANKTON	[6-60µm]	881 ± 88	157 ± 25	5.6
		[60-200µm]	110 ± 15	115 ± 7	1.0
		[200-500µm]	*n.d.* *n.d.*	*n.d.* *n.d.*	*n.d.*
		[500-1000µm]	182 ± 11	24 ± 3	7.7

		[1000-2000µm]	102	±	9	22	±	3	4.7
		[>2000µm]	69	±	7	25	±	3	2.7
ST5	DISSOLVED		5.6	±	0.7	7.2	±	0.5	0.8
	SPM		293	±	336	413	±	118	0.7
	PLANKTON	[6-60µm]	*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
		[60-200µm]	128	±	11	99	±	7	1.3
		[200-500µm]	87	±	8	22	±	3	4.0
		[500-1000µm]	132	±	10	23	±	3	5.7
		[1000-2000µm]	101	±	5	20	±	2	5.0
		[>2000µm]	31	±	3	12	±	2	2.5
ST7	DISSOLVED		*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
	SPM		386	±	45	219	±	65	1.8
	PLANKTON	[6-60µm]	*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
		[60-200µm]	69	±	14	110	±	7	0.6
		[200-500µm]	349	±	22	23	±	5	15
		[500-1000µm]	163	±	9	21	±	3	7.8
		[1000-2000µm]	143	±	9	25	±	3	5.7
		[>2000µm]	27	±	5	21	±	3	1.3

WINTER		**PHASE**	**^{210}Po ± SD**			**^{210}Pb± SD**			**^{210}Po/^{210}Pb**
ST10	DISSOLVED		2.5	±	0.4	14	±	1.1	0.2
	SPM		*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
	PLANKTON	[6-60µm]	527	±	36	198	±	10	2.7
		[60-200µm]	480	±	26	118	±	6	4.1
		[200-500µm]	366	±	18	31	±	2	11
		[500-1000µm]	363	±	16	39	±	2	9.3
		[1000-2000µm]	297	±	16	112	±	4	2.7
		[>2000µm]	461	±	27	120	±	5	3.8
ST2	DISSOLVED		2.2	±	0.4	9.2	±	0.7	0.2
	SPM		233	±	28	216	±	37	1.1
	PLANKTON	[6-60µm]	*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
		[60-200µm]	187	±	14	64	±	3	2.9
		[200-500µm]	378	±	18	43	±	2	8.8
		[500-1000µm]	354	±	19	69	±	3	5.1
		[1000-2000µm]	282	±	25	101	±	7	2.8
		[>2000µm]	244	±	15	106	±	4	2.3
ST3	DISSOLVED		3.5	±	0.3	11.0	±	0.8	0.3
	SPM		239	±	23	226	±	28	1.1
	PLANKTON	[6-60µm]	*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
		[60-200µm]	350	±	26	66	±	5	5.3

		[200-500µm]	316	±	16	31	±	1	10
		[500-1000µm]	244	±	13	32	±	2	7.7
		[1000-2000µm]	286	±	17	29	±	2	9.9
		[>2000µm]	278	±	13	50	±	2	5.5
ST4	DISSOLVED		3.2	±	0.3	12.2	±	0.9	0.3
	SPM		109	±	18	125	±	20	0.9
	PLANKTON	[6-60µm]	210	±	34	260	±	23	0.8
		[60-200µm]	175	±	11	80	±	4	2.2
		[200-500µm]	183	±	12	12	±	1	15
		[500-1000µm]	180	±	9	12	±	1	15
		[1000-2000µm]	212		10	13		1	16
		[>2000µm]	312	±	19	72	±	3	4.4
ST6	DISSOLVED		3.3	±	0.3	12	±	1.1	0.3
	SPM		102	±	19	137	±	27	0.7
	PLANKTON	[6-60µm]	*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
		[60-200µm]	207	±	14	73	±	3	3
		[200-500µm]	169	±	9	15	±	1	113
		[500-1000µm]	183	±	10	28	±	1	7
		[1000-2000µm]	236	±	16	41	±	3	6
		[>2000µm]	257	±	17	58	±	2	4
ST7	DISSOLVED		3.8	±	0.3	13	±	1.1	0.3
	SPM		123	±	25	184	±	35	0.7
	PLANKTON	[6-60µm]	*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
		[60-200µm]	113	±	11	53	±	2	2.1
		[200-500µm]	232	±	13	16	±	1	15
		[500-1000µm]	235	±	14	19	±	1	12
		[1000-2000µm]	*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*
		[>2000µm]	*n.d.*		*n.d.*	*n.d.*		*n.d.*	*n.d.*

Table 3

Mean (± SD) ^{210}Po and ^{210}Pb activity concentrations in the dissolved (Bq m^{-3}), particulate and plankton fraction (Bq kg^{-1}), and ^{210}Po/^{210}Pb ratios in the dissolved, particulate and plankton fractions analysed in spring (May 2010) and winter (February 2011) in the GoL. Letters (a to c) indicate significantly different means as assessed by Kruskal-Wallis post-hoc tests with "a" being the highest mean.

SPRING	^{210}Po ± SD				^{210}Pb ± SD				^{210}Po/^{210}Pb ± SD			
Dissolved	5	±	5	d	11	±	4	c	0.5	±	0.3	b
SPM	707	±	846	a	471	±	320	a	1.3	±	0.8	ab
[6-60 µm]	392	±	436	b	203	±	118	ab	2.0	±	2.4	ab
[60-200 µm]	153	±	64	b	111	±	26	ab	1.4	±	0.6	ab
[200-500 µm]	217	±	111	b	25	±	11	bc	10	±	6.1	a
[500-1000 µm]	156	±	82	b	19	±	4	bc	9.2	±	7.4	a
[1000-2000 µm]	187	±	89	b	23	±	10	bc	8.9	±	4.6	ab
[>2000 µm]	83	±	38	c	18	±	6	bc	4.5	±	2.7	ab

WINTER	^{210}Po ± SD				^{210}Pb ± SD				^{210}Po/^{210}Pb ± SD			
Dissolved	3	±	0.6	b	12	±	1.7	c	0.3	±	0.1	c
SPM	144	±	91	a	167	±	62	a	0.8	±	0.3	b
[6-60 µm]	368	±	224	a	229	±	44	a	1.7	±	1.3	ab
[60-200 µm]	252	±	136	a	76	±	23	b	3.3	±	1.2	ab
[200-500 µm]	274	±	92	a	24	±	12	c	12	±	2.4	a
[500-1000 µm]	260	±	81	a	33	±	20	c	9.4	±	3.8	a
[1000-2000 µm]	263	±	37	a	59	±	45	bc	7.5	±	5.8	ab
[>2000 µm]	311	±	88	a	81	±	31	b	4.1	±	1.2	ab

Table 4
Results of two-way ANOVAs performed on ^{210}Po and ^{210}Pb activity concentrations in the different size fractions (from 60 to >2000µm) of plankton in spring (May 2010) and in winter (February 2011).

F: Statistics; P: probalility; Post-hoc: comparison of means. *: P<0.05; **: P<0.01; ***: P<0.001; ns: not significant.

SPRING Parameter / Factor	F	P	Post-hoc
^{210}Po			
Zone	13.4	0.001 **	East > West
Fraction	3.39	0.025 *	200=1000=60=500>2000
Zone x Fraction	0.64	0.514 ns	
^{210}Pb			
Zone	0.05	0.832 ns	
Fraction	59.4	<0.001 ***	500=2000=1000=200<60
Zone x Fraction	1.53	0.226 ns	
WINTER Parameter / Factor	F	P	Post-hoc
^{210}Po			
Zone	16.8	0.001 **	East > West
Fraction	0.48	0.749 ns	
Zone x Fraction	0.85	0.511 ns	
^{210}Pb			
Zone	9.33	0.007 **	East > West
Fraction	5.69	0.004 **	60=2000>1000=500=200
Zone x Fraction	0.50	0.733 ns	

Table 5

Weight percentage of tissue related to total fish wet weight (%W) and activity concentrations of ^{210}Po and ^{210}Pb (Bq kg^{-1}) in each tissue analysed (^{210}Po-Tissue, ^{210}Pb-Tissue) and in a whole fish according to the relative weight percentage of each tissue (^{210}Po-Fish, ^{210}Pb-Fish).

Anchovy	%W	^{210}Po-Tissue	^{210}Po-Fish	^{210}Pb-Tissue	^{210}Pb-Fish
Muscle	8.8	106	179	15	1
Liver	1.3	2035	29	8	0
Gonad	3.5	2235	4	1	0
Remains	86.4	493	426	8	7
Whole fish	100.0		638		8
Sardine	%W	^{210}Po-Tissue	^{210}Po-Fish	^{210}Pb-Tissue	^{210}Pb-Fish
Muscle	5.4	52	3	51	3
Liver	1	888	9	51	1
Gonad	0.4	535	2	3	0
Remains	93.2	282	263	9	9
Whole fish	100.0		277		12

Table 6

Results of two-way ANOVAs performed on $\delta^{13}C$ and $\delta^{15}N$ in the different size fractions (from 60 to >2000µm) of plankton in spring (May 2010) and in winter (February 2011).

F: Statistics; P: probalility; Post-hoc: comparison of means. *: P<0.05; **: P<0.01; ***: P<0.001; ns: not significant.

SPRING Parameter / Factor	F	P	Post-hoc
$\delta^{13}C$			
Zone	5.53	0.027 *	East > West
Fraction	1.40	0.264 ns	
Zone x Fraction	0.88	0.490 ns	
$\delta^{15}N$			
Zone	43.0	<0.001 ***	East > West
Fraction	9.59	<0.001 ***	500=200=1000=60>2000
Zone x Fraction	0.44	0.778 ns	
WINTER Parameter / Factor	F	P	Post-hoc
$\delta^{13}C$			
Zone	29.9	<0.001 ***	East > West
Fraction	5.90	<0.001 ***	1000=2000>60=500=200
Zone x Fraction	1.90	0.119 ns	
$\delta^{15}N$			
Zone	233	<0.001 ***	East > West
Fraction	29.0	<0.001 ***	1000>500>200=2000=60
Zone x Fraction	9.05	<0.001 ***	2000 East = 2000 West. East > West in all other fractions