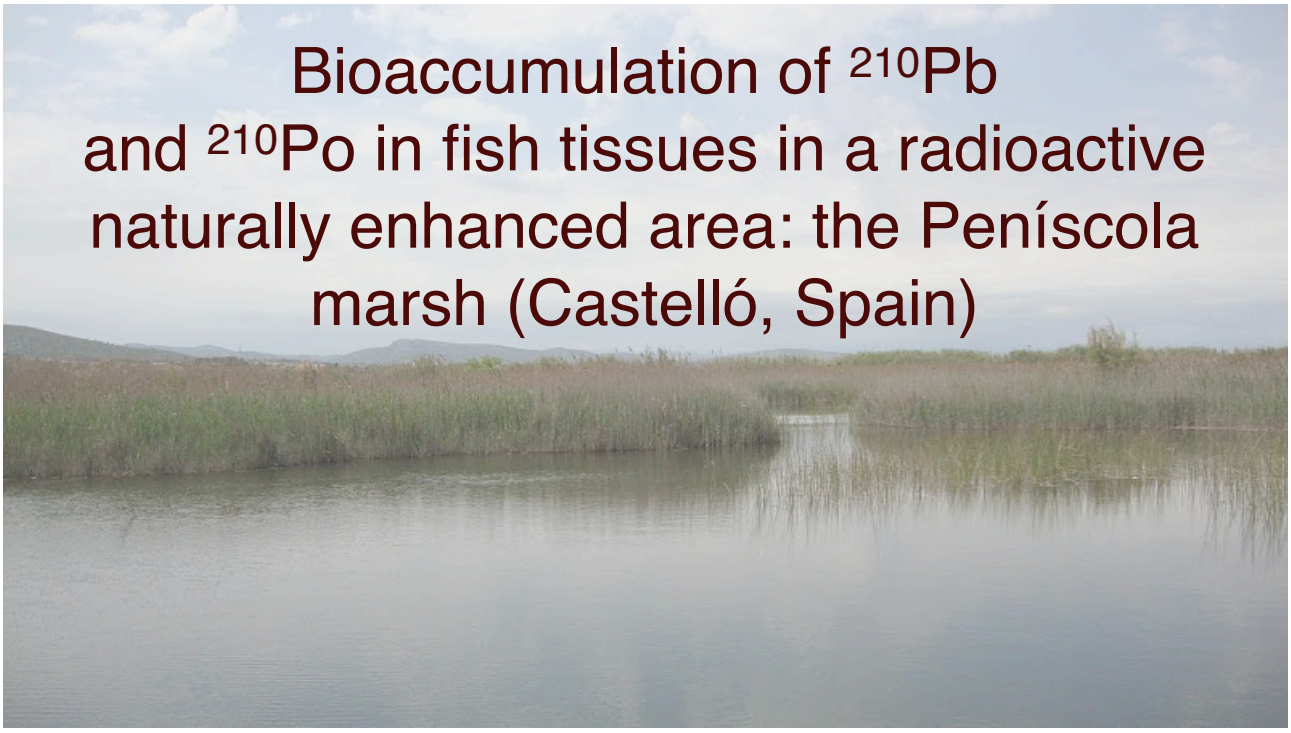
Universitat Autònoma de Barcelona - Facultat de Ciències

Llicenciatura de Ciències Ambientals

PROJECTE DE FINAL DE CARRERA

Curs 2010-2011

Bioaccumulation of ^{210}Pb and ^{210}Po in fish tissues in a radioactive naturally enhanced area: the Peníscola marsh (Castelló, Spain)



Ester López Castillo

Directors: Dr. Jordi Garcia-Orellana
Dra. Núria Casacuberta Arola

La imatge de la portada correspon a un ullal de la marjal de Penyíscola. La fotografia va ser realitzada per *Karina Kammer Attisano*.

Aquest projecte ha estat imprès en paper lliure de clor i utilitzant una font que permet un estalvi de tinta aproximat del 25% respecte les fonts tradicionalment usades.

I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale.

Maria Skłodowska-Curie (1867-1934)

Acknowledgments

En primer lloc voldria agrair als directors del meu projecte, en Jordi Garcia-Orellana i la Núria Casacuberta Arola, per haver-me donat la oportunitat de realitzar aquest projecte i haver-me proposat realitzar-ho en anglès. Ha sigut tot un repte de superació personal!

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Vull agrair al Consejo de Seguridad Nuclear per haver financiat aquest projecte, en el marc del sub-projecte "Medidas de vigilancia radiológica ambiental en la marjal de Peñíscola" del projecte principal "Estudio de la instrumentación de vigilancia radiológica ambiental y de medida de radón en condiciones ambientales extremas" fundat pel CSN al 2009. Agrair també al projecte "Evaluación de la descarga de agua subterránea al mar desde el acuífero regional Jurásico de la Unidad Hidrogeológica de El Maestrazgo (Castellón) mediante isótopos de Ra" (EDASMAR) (Codi: CGL2006-09274/HID)

També agrair a en Gerard Carmona i en Lluís Benejam per haver-nos acompanyat de campanya i haver fet la feina bruta de pescar els peixets, i per ensenyar-nos que a Penyíscola no només s'hi pot anar de campanya, sinó també a gaudir de l'oci local! A en Gerard, altra vegada, i a tota la colla de l'Emili García-Berthou també els he d'agrair que m'acollissin al seu laboratori, m'ensenyessin l'art de la dissecció (sobretot amb les gambúsies, allò sí que és un art!) i no es queixessin gaire quan els ocupava les poiates o les estufes!

D'una manera molt especial, agrair a tots els companys del Laboratori de Radioactivitat Ambiental, tant els que hi són com els que han marxat, per acollir-me al grup de manera tan càlida, pels dinars on hem rigut tant, per tot el que he après de vosaltres al laboratori , despatx i a les campanyes... i sobretot, per tot el que he crescut com a persona amb vosaltres.

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Finalmente, agradecer a mi familia el apoyo incondicional que me brindan en todo momento, sea cual sea mi decisión.

Contents

1. Preface

Until recently, human health was the major focus of radiation protection practices, and it was understood that, if standards were set to protect human health, no other species would be threatened as a population even if individuals of the species were harmed. However, awareness about the vulnerability of the marine and terrestrial environment has grown, also the need of protecting the environment against anthropogenic pollutants including radionuclides. Consequently, radiation protection philosophy has begun to evolve, increasing the emphasis on protecting biotic populations other than man from the potential effects of radiation (Pentreath, 1999).

Although the major contribution to the radiation exposure received by all types organisms comes from natural sources, the effect of radionuclides in terrestrial and marine organisms has been studied from artificial radioactive sources such as ^{137}Cs or ^{90}Sr (i. e. Bukovac *et al.*, 1965, Krouglov *et al.*, 1997). Natural radioactivity refers to those radioactive sources that have not been originated due to human activities (Ortega and Jorba, 1994). Hence, natural radioactivity includes radiation from primordial radionuclides and their decay products, cosmic rays and the products of their interaction with stable nuclides in the atmosphere - neutron activation and spallation products (Eisenbud and Gesell, 1997; Shaheed *et al.*, 1997).

Primordial radionuclides have half-lives similar to Earth's age, so they exist since the formation of the planet. Nevertheless, those with relatively short half-lives ($T_{1/2}<10^8$ y) in comparison with Earth's age ($4.5\cdot10^9$ y) have disappeared from Earth (Eisenbud and Gesell, 1997). One example of that case is the ^{237}Np decay chain: with a half-life of $2\cdot10^6$ y, which has already disappeared from Earth's crust (Ivanovich, 1992b).

Natural radionuclides can be classified into two groups, those that are primordial radionuclides (^{238}U, ^{232}Th and ^{235}U) and those that decay into stable elements, not forming radioactive decay chain products (i.e. ^{40}K). However, most terrestrial radionuclides belong to decay chains.

Some of the natural radionuclides of the ^{238}U series (^{226}Ra, ^{210}Pb and ^{210}Po) and others of the ^{232}Th series (^{228}Ra) are considered crucial either for their toxicological significance or for their special accumulation behaviour in the environment (Shaheed *et al.*, 1997). The existing database regarding interactions of biota with naturally occurring radionuclides is slightly limited and considers a few isotopes whose half-lives and chemical characteristics make them interesting and suitable for different uses, such as tracers of productivity and carbon flux in the ocean (i.e. Murray *et al.*, 2005), which are scientifically used as chronometers of biogenic processes (i.e. Turekian *et al.*, 1979) or act as sources of ionising radiation for marine organisms (Cherry and Heyraud, 1982). For this latest reason, it is also important to have information about the levels of these radionuclides, as they contribute a substantial fraction of the radiation dose to natural ecosystems (Holtzman, 1966).

There is far more information about the concentrations of ^{210}Po and ^{210}Pb in marine organisms than all other natural isotopes. First studies started in the 1960s and 1970s, establishing that ^{210}Po was the source of most of the α radiation detected in plankton (Cherry, 1964; Shannon and Cherry, 1967; Shannon *et al.*, 1970; Heyraud *et al.*, 1976; Heyraud and Cherry, 1979). Oceanographers started considering the use of ^{210}Po and its relationship to its grandparent ^{210}Pb as tracers of particle flux in the ocean (i.e.

Radakovitch *et al.*, 1999). More recently, investigations studied the behaviour of ^{210}Po in marine organisms and food chains, focusing on invertebrates such as mussels, squids and shrimps (i.e. Skwarzek and Falkowski, 1988; Heyraud *et al.,* 1994). Lately, they investigated the accumulation of ^{210}Po in higher levels of the food chains - fishes and large predators (i.e. Carvalho, 2011). Durand *et al.* (1999) analysed ^{210}Po in fish livers and found that polonium associates with metallothioneins[1] and ferritin[2] but is not accompanied by a similar strong binding of ^{210}Pb, which explains the generally very high ^{210}Po/^{210}Pb ratio observed in fish tissues and, in particular, fish liver. Recently, the behaviour of ^{210}Po in the upper levels of food chains has become clearer, but not totally understood. It is relatively well studied the preferential assimilation of ^{210}Po over ^{210}Pb in the soft tissues of marine organisms despite the higher concentration of ^{210}Pb in hard parts like bone and shell (i.e. Carvalho and Fowler, 1993), and also the assimilation and possible biomagnification of ^{210}Po but not ^{210}Pb as the nuclides pass through higher trophic levels (Shaheed *et al.*, 1997; Stewart *et al.*, 2005).

There is very few data regarding accumulation of ^{210}Pb and ^{210}Po in terrestrial ecosystems (i.e. Brown *et al.*, 2010) and there is even less data about interactions in freshwater or brackish environments (i.e. Clulow *et al.*, 1998; NKS, 2009).

This work is carried out in the frame of the sub-project “Medidas de vigilancia radiológica ambiental en la marjal de Peñíscola” of a main project “Estudio de la instrumentación de vigilancia radiológica ambiental y de medida de radón en condiciones ambientales extremas” funded by the Consejo de Seguridad Nuclear in 2009. This research is complemented with another independent study about the interactions between ^{210}Pb and ^{210}Po with the flora in the same study area (Vilarrasa Nogué, 2011: Distribució i Transferència de ^{210}Pb i ^{210}Po en plantes: La Marjal de Peníscola). With these two studies, the purpose is to reach a deeper level in the knowledge regarding the interaction and pathways of these two radionuclides to the biota in brackish water environments.

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[1] Metallothioneins (MT) are a family of cysteine-rich, low molecular weight proteins, localized to the membrane of the Golgi apparatus. MT have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals. MTs function is not clear, but experimental data suggest MTs may provide protection against metal toxicity, be involved in regulation of physiological metals (Zn and Cu) and provide protection against oxidative stress (Sigel *et al.*, 2009).

[2] Ferritin is an ubiquitous intracellular protein that stores iron and releases it in a controled fahsion. (www.chemistry.wustl.edu)

2. Objectives

The research carried out in this project has been focused on the study of polonium (^{210}Po) and radioactive lead (^{210}Pb) accumulation in aquatic organisms and the transfer to fish tissues for understanding isotope dynamics in the aquatic environment.

Most of the research done about the interactions of ^{210}Pb and ^{210}Po with biota has been performed exclusively in marine environments. This pair of radionuclides has attracted the attention of scientists because of their relatively high concentrations in marine organisms in comparison with those in terrestrial organisms (Carvalho, 2011). Furthermore, it is of special relevance the greater accumulation of ^{210}Po in marine biota compared to its grandparent ^{210}Pb, as the former one might lead to greater human doses in case of ingestion of the organisms which accumulated it (Cherry and Shannon, 1974; Parfenov, 1974).

The main objective of this project was to determine the bioaccumulation in ^{210}Pb and ^{210}Po, in both, different fish species and fish tissues from samples collected in a Mediterranean coastal wetland (the Peníscola wetland, Castelló, Spain) characterised by having high levels of ^{226}Ra ($T_{1/2}$= 1600 y) and ^{222}Rn ($T_{1/2}$= 3,8 d) due to high values of radium in sediments and water (^{226}Ra=2 - 3·10^{3} Bq·m^{-3}, ^{222}Rn =6.7·10^{2} - 6.2·10^{5} Bq·m^{-3} in water and ^{226}Ra=2.2·10^{2} - 7.8·10^{2} Bq·kg^{-1} in sediments linked to the groundwater discharge from the Maestrat aquifer (Rodellas-Vila, 2009). Due to these high concentrations of natural radionuclides and because both ^{226}Ra and ^{222}Rn belong to the ^{238}U decay chain - so they are grandfathers of ^{210}Pb and ^{210}Po (the radionuclides which this research focus on) this area represents an ideal location for the study of the interaction between ^{210}Po and ^{210}Pb and the fishes of this brackish environment.

This main objective was divided into several specific objectives:

- Analyse the different accumulation of ^{210}Pb and ^{210}Po in tissues (i.e. kidney, muscle, etc.).
- Identify the major route of entry of ^{210}Pb and ^{210}Po into the fish body.
- Study the dependence of ^{210}Pb and ^{210}Po accumulation with size and species.
- Analyse bioaccumulation factors (BAF), in order to determine the correlation between concentration of both radionuclides in tissues and its concentration in its food as well as through direct content in water.

3. ^{210}Pb and ^{210}Po

3.1. Lead (Pb)

Lead (Pb) has 38 known isotopes, of which four are stable isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb). ^{204}Pb is entirely a primordial radionuclide and is not a radiogenic nuclide. The other three are the ending products of the U and Th decay chains (^{238}U, ^{235}U and ^{232}Th respectively). However, they also exist as primordial isotopes.

From the radioactive isotopes of lead, the longest-lived radioisotopes are ^{205}Pb, with a half-life of $15.3 \cdot 10^6$ years and ^{202}Pb, with a half-life of $53 \cdot 10^3$ years. Of naturally-occurring radioisotopes, the longest is ^{210}Pb, with a half-life of 22.3 years. Half-life of the other radioisotopes is shorter than a day.

^{210}Pb is a naturally occurring radionuclide of the uranium series (Ivanovich, 1992b). It is a daughter product in the ^{238}U decay chain. The environmental ^{210}Pb arises mainly due to the decay of ^{222}Rn gas emanating from the earth's soil into the atmosphere. ^{222}Rn gas decays to ^{210}Pb via short-lived particulate nuclides (^{218},^{214}Po, ^{214}Pb, ^{214}Bi) (Eisenbud and Gesell, 1997).

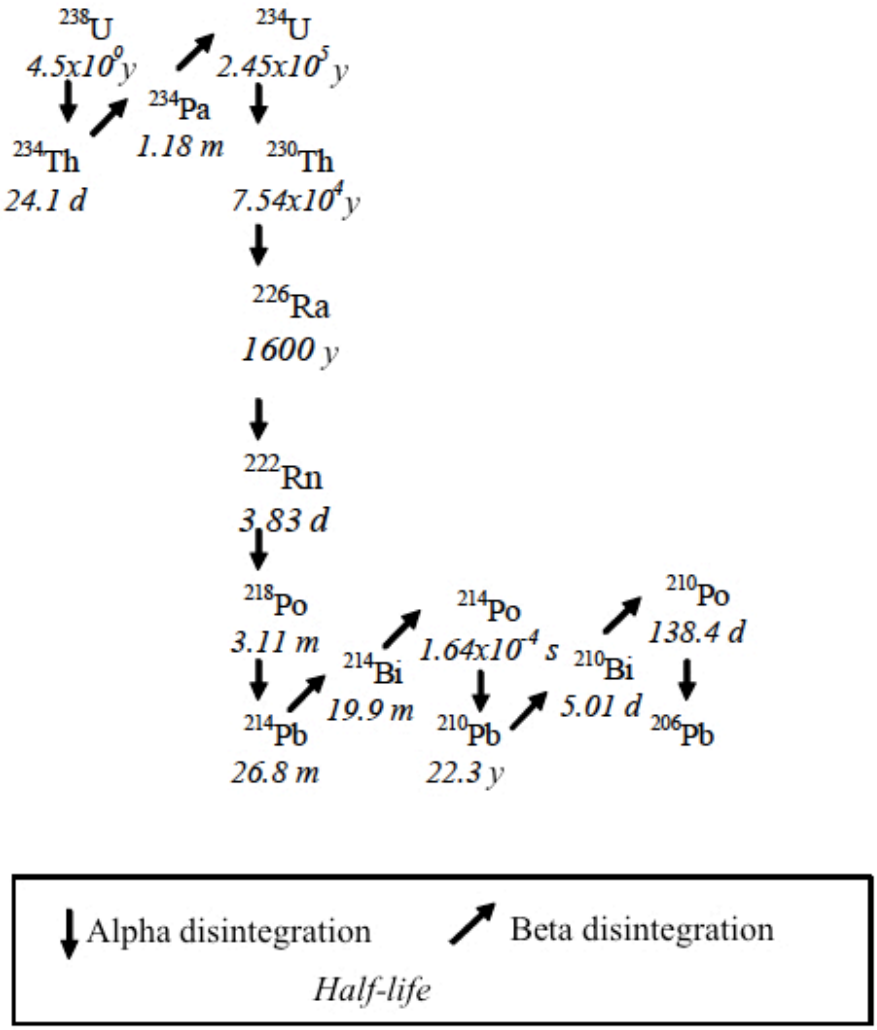

Figure 1. The ^{238}U decay chain. Source: Adapted from Rodellas-Vila, 2008.

^{210}Pb is a β emitter and decays into ^{210}Bi with emission energies of 16.93 and 63.5 keV (Smith *et al.*, 2008). It is also a gamma emitter with emission energy of 46.5 keV. It has a specific activity of 325.6 GBq g^{-1}.

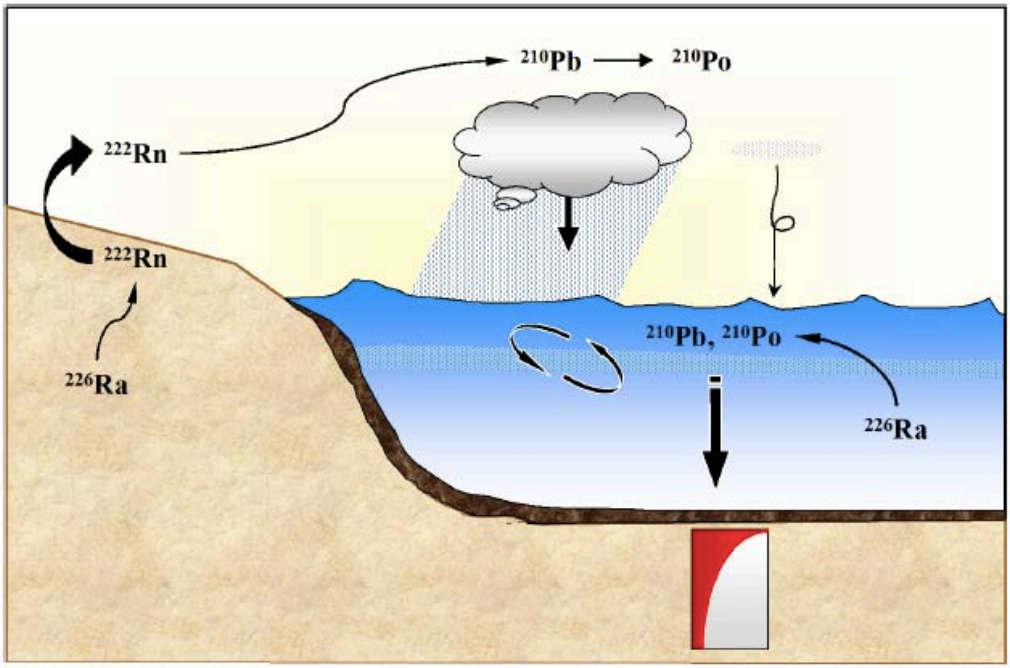

Figure 2. The ^{210}Pb cycle. Source: Laboratori de Radioactivitat Ambiental, UAB

The average activity concentration of ^{210}Pb in the atmosphere of the northern hemisphere, reported by the United Nations was 0.5 mBq·m^{-3} (UNSCEAR, 2000). As only a little fraction of ^{222}Rn can escape from earth's soil to the atmosphere before it decays, a fraction of ^{210}Pb is formed *in situ* as a product of this disintegration - the supported ^{210}Pb. In secular equilibrium, activity of supported ^{210}Pb is the same as ^{226}Ra activity. The fraction of ^{210}Pb that comes from direct atmospheric deposition is unsupported ^{210}Pb (excess). ^{210}Pb originated by ^{222}Rn decay is highly reactive, binding rapidly to aerosols, being transported and eliminated from the atmosphere by wet and dry deposition. Its removal time in the atmosphere ranges from 1 to 10 days (Turekian *et al.,* 1977). Once unsupported ^{210}Pb is deposited, its concentration decreases with time due to radioactive disintegration. It is possible to establish a relation between initial deposited concentration and concentration displayed in each soil layer. In geochronology, this characteristic is used in order to date events that have happened in the last 100-150 years.

^{210}Pb activity concentrations in filtered seawater and suspended particulate matter in the North-East Atlantic Ocean ranged from 0.85 to 2.27 mBq L^{-1}, and particulate ^{210}Pb from 0.09 to 1.00 mBq L^{-1} (Carvalho, 2011)

3.2. Polonium (Po)

Polonium was discovered by Pierre and Marie Curie in 1898 in the course of research of the radioactivity of uranium and thorium mineral (Figgins, 1961). Polonium has 25 known radioactive isotopes with mass numbers of 192-218, of which only the 208, 209 and 210

isotopes have half lives longer than 1 day (Connan *et al.*, 2007). Among these three isotopes, ^{210}Po is of the most interest from a marine environmental impact viewpoint (Alam and Mohamed, 2011). This is because it is considered an important source of internal radiation dose to marine organisms (Cherry and Shannon, 1974; Cherry and Heyraud, 1982).

^{210}Po is a naturally occurring radionuclide of the uranium series (Ivanovich *et al.*, 1992). It is a daughter product in the ^{238}U decay chain. Alpha-emitting ^{210}Po with a half-life of 138 days is a daughter of ^{210}Bi and a granddaughter of ^{210}Pb (Eisenbud and Gesell, 1997). ^{210}Po can also be produced by neutron activation of ^{209}Bi (NKS, 2009).

^{210}Po is a high alpha particle emitter (Cherry and Shannon, 1974) and among natural radionuclides occurring in the ocean, alpha emitters are considered to be the most important (Hernandez *et al.*, 2002) because of their high mass and charge, are more damaging and so are accorded a "radiation weighting factor" of 20 (ICRP, 1990). ^{210}Po has a specific activity of 166 TBq $\cdot g^{-1}$ (NKS, 2009). Hence, ^{210}Po can have a toxic effect even in small concentrations due to its high-energy alpha radiation (Moroz and Parfenov, 1972).

Polonium is known to be sometimes a volatile element and has also been shown to become volatile in both fresh and marine waters by the action of microorganisms (Momoshima *et al.*, 2001, Momoshima *et al.*, 2002). Both, ^{210}Pb and ^{210}Po are found at elevated levels in the sea-surface microlayer (Bacon *et al.*, 1980).

The average activity concentration of ^{210}Po in the atmosphere of the northern hemisphere, reported by the United Nations was 0.05 mBq$\cdot m^{-3}$ (UNSCEAR, 2000). These ^{210}Po nuclides are deposited on terrestrial and marine surfaces with aerosol particles. (Lee *et al.*, 2009).

^{210}Po activity concentrations in filtered seawater and suspended particulate matter in the North-East Atlantic Ocean ranged from 0.35 to 1.70 mBq L^{-1} and particulate ^{210}Po from 0.24 to 1.12 mBq L^{-1} (Carvalho, 2011).

Owing to its high radiotoxicity, ^{210}Po has been of great concern from the viewpoint of a radiation protection to the human body. ^{210}Po ingested with foodstuffs is considered to be among the largest contributors to the internal radiation dose to man (Lee *et al.*, 2009). More than half of the internal radiation dose that man receives originates from this natural radionuclide as a result of seafood consumption (Aarkog *et al.*, 1997). Through the ingestion pathway, ^{210}Pb and ^{210}Po deliver about 83% of the annual effective dose to humans (UNSCEAR, 2000)

3.3. ^{210}Pb and ^{210}Po bioaccumulation

The bioaccumulation of ^{210}Pb or ^{210}Po refers to a process by which these radionuclides are accumulated in various tissues of a living organism. The level to which a radionuclide is accumulated in an organism depends on its chemical characteristics and speciation in water or sediment, as well as biological processes, including rates of uptake from water or diet, excretion, and metabolic transformation. These in turn, may be influenced directly by the physiology of the organism which is, of course, affected by diverse biological, physical and chemical factors, such as habitat, feeding behaviour and species (Stewart *et al.*, 2008).

First of all, it is important to note that neither lead nor polonium have any known biological function; hence, organisms would not actively be 'seeking' to incorporate them through enzymatic action or through specific membrane channels or other transport mechanisms, as happens with essential metals (Williams, 1981; Simkiss and Taylor, 1995). Besides, the concentrations of these radionuclides are generally so low that organisms would have to expend impractical amounts of energy to concentrate them from the surrounding water (Stewart *et al.*, 2008).

Lead and polonium, which speciate as cations in seawater, display very strong binding to particle surfaces, including organisms (Stewart *et al.*, 2008). Pb associates largely with dissolved carbonates (Bruland, 1983). In consequence, these metals become enriched in single-celled organisms (i.e., phytoplankton) to which they largely adsorb and the concentration factors that represent the degree of enrichment relative to ambient seawater are commonly 10^4-10^6 (Fisher, 1986; Stewart and Fisher, 2003a).

Lead is an oxygen-seeking metal that frequently associates with mineral fractions of organisms (i.e. bone, shell and structure) (Nieboer and Richardson, 1980). In single-celled organisms, which provide the largest surface areas for these metals to bind to, lead associates with cell walls. Smaller cells with higher surface-to-volume ratios tend to show highest concentration factors (Fisher and Reinfelder, 1995).

Despite the numerous studies on ^{210}Po, the specific mechanism of uptake remains unclear. Because the uptake is unaffected by light or temperature, and there is no biological requirement for this element, it appears that it is taken up inadvertently as an analogue of some needed element (Stewart and Fisher, 2003a).

However, ingestion is the main route of entry of this radionuclide (Carvalho and Fowler, 1994). ^{210}Po is readily assimilated by marine primary producers (Fisher *et al.*, 1983) and further concentrated along the food chain, a behaviour that has been linked to sulphur uptake (Cherry and Shannon, 1974).

Due to its position in group VI of the periodic table and its known association with protein, it has been suggested that Po acts as a sulphur-analogue like Se or binding to sulphur ligands (Schwarz, 1976; Cherrier *et al.*, 1995; Church and Sarin, 2008). so in living organisms, polonium associates with proteins (Cherry and Heyraud, 1981, Fisher *et al.*, 1983; Stewart and Fisher, 2003).

However, when Cherrier *et al.* (1995) followed the uptake and partitioning of Po and radioactive ^{35}S in bacterial cells, they found that the kinetics of uptake were different between the two elements, despite their very similar eventual localization within the cells. Many studies have found high concentrations of Po associated with metallothionein and cysteine in invertebrate and vertebrate livers (Durand *et al.*, 1999) and a link between Po and S-containing amino acids in sinking organic matter (Stewart *et al.*, 2007). Potentially, polonium can replace selenium in selenocysteine, a protein which is present in several enzymes. Also selenium and polonium have similar distribution pattern depending on the internal organs of marine vertebrates and fish (Heyraud and Cherry, 1979). Unlike Pb, Po can penetrate into the cytoplasm of cells (Fisher *et al.*, 1983; Stewart and Fisher, 2003).

Both radionuclides are particle reactive elements and therefore, once associated with single cells, there is the possibility that they can be assimilated into the tissues of animals that ingest those phytoplankton cells (i.e. molluscs, zooplankton). However, the efficiency with which ingested elements are assimilated in herbivores appears to be directly related

to the extent to which they can penetrate into the cytoplasm of phytoplankton cells (Reinfelder and Fisher, 1991; Stewart and Fisher, 2003b). As Po is the only radionuclide of these pair that does penetrate into the cytoplasm of cells, it shows appreciable assimilation in zooplankton, with efficiencies of approximately 40% (Stewart and Fisher, 2003b). Assimilated Po pass through trophic chain, from herbivores to carnivores that consume them. Then, Po is bioconcentrated in the tissues of diverse marine animals at higher trophic levels.

First studies of ^{210}Po accumulation in marine fishes (Heyraud and Cherry, 1979) deduced that the ingestion of food should play a major role in the accumulation of ^{210}Po. Shannon (1973) reported that pelagic fish, (i.e. mackerel, *Scomber scombrus*), contained five times more ^{210}Po than demersal species such as plaice (*Pleuronectes platessa*). Then, the potential use of this radionuclide as a natural tracer of the diet of marine organisms was suggested (Carvalho, 1988; Heyraud *et al.*, 1988; Cherry *et al.*, 1989).

Durand *et al.*, (1999), studying the accumulation of ^{210}Po in liver of teleost marine fishes, found that polonium binds to metallothioneins and ferritin. Among the subcellular fractions from liver of mackerel (*Scomber scombrus*), 80% of the ^{210}Po was accumulated in the cytosolic fraction, 11.7% in light lysomal and mitochondrial fraction and the rest in the other fractions.

The unassimilated Po is egested by the zooplankton in fecal pellets and by the fishes and other marine animals in feces, where nearly all of the ingested Pb becomes packaged. Pb and Po can remain bound to fecal pellets or feces for long enough periods to allow the sinking fecal matter to transport these elements to deep waters (Stewart *et al.*, 2005).

With regards to other species apart from fishes, Skwarzec and Fabisiak (2007) studied the accumulation of ^{210}Po in marine birds, finding that polonium is non-uniformly distributed in the marine birds. The results showed that the polonium is non-uniformly distributed in this birds. The highest activities of ^{210}Po were observed in feathers, muscles and liver and the lowest in skin and skeleton. Furthermore, species of birds that eat crustaceans, mollusks, fish and plants accumulated more polonium than species that eat mainly fish or plants. The high accumulation on feathers suggested an external source of polonium such as the air, meaning that the adsorption of ^{210}Po on the feather surface may be an important transfer from air to water.

Regarding to terrestrial environments, Brown *et al.* (2010), studied activity concentrations of ^{210}Po in fauna (invertebrates, mammals and birds). They found that concentrations ranged between 2 and 123 Bq kg^{-1} dry weight and in plants and lichens between 20 and 138 Bq kg^{-1} dry weight. They also found that humus is an important reservoir for ^{210}Po and that fauna, in close contact with this media, may also exhibit elevated levels of ^{210}Po. Focusing on small mammals, activity concentrations fell within a range from 23 to 85 Bq kg^{-1} dry weight. ^{210}Po/^{210}Pb ratios were higher than ratios from other organisms studied, appearing to be indicative of a preferential uptake or prolonged retention of ^{210}Po relative to ^{210}Pb for this group of mammals in this particular environment.

NKS (2009) analyzed ^{210}Po and other radionuclides in a terrestrial freshwater environment. Average concentrations of ^{210}Po in lake waters was 1.9 mBq kg^{-1}. Regarding to fishes, values of ^{210}Po concentration in whole fish ranged from 1.0 to 6.5 Bq kg^{-1} fresh weight. They analyzed edible parts and other parts separately, finding that in edible parts, concentration was one order of magnitude lower.

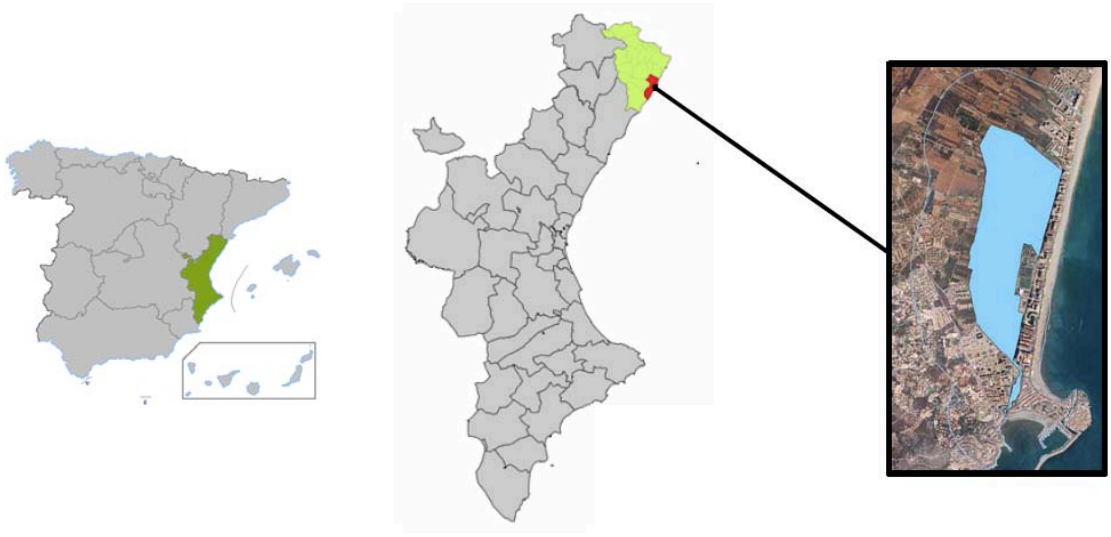

Figure 3. Location of Peníscola (in red) in the region of Baix Maestrat (light green). Valencian Community is shown in green in the map of Spain. On the right, the area occupied by the Peníscola marsh according to the list of wetlands of the Valencian Community. Source: Rodellas-Vila, 2008.

The Peníscola marsh is an important wetland in the municipality of Peníscola, in Baix Maestrat (Valencian Community, Spain). It is considered as a Special Protection Area (SPA) for the European Union legislation (or 'Lugar de Importancia Comunitaria (LIC)' as an equivalent in Spanish legislation), 'Zona Húmeda' from the 'Catálogo Valenciano de Zonas Húmedas' (approved by Decision of the Valencian Government on september 2002, developing the provisions of the Ley 11/1994, de Espacios Naturales Protegidos de la Comunidad Valenciana) and as a flora micro-reserve (www.cma.gva.es, www.marm.es).

With an extension of 105.49 ha, it is placed in the southern edge of the Vinaròs-Peníscola coastal flat, above the Vinaròs-Peníscola hydrogeological system. It also limits with the Maestrat hydrogeological system. These two aquifers cause the groundwater discharge in the marsh (Mejias *et al.*, 2006).

The main land uses in Peníscola marsh are agricultural (20%) and natural marsh (80%), surrounded by residential uses. Despite the urban development pressure caused by the municipality of Peníscola, the marsh preserves in great measure its natural characteristics. Water uptake and seawater intrusion in the marsh influence areas have caused aquifer and marsh salinization (Cherta Val, 2008, Rodellas-Vila *et al.,* 2009).

Peníscola marsh has a great importance in terms of biodiversity because of the habitats that can be found there. The habitats presenti in the LIC are shown in Table 1.

Table 1. Habitats present in the LIC of Peníscola marsh.

Habitats	Vegetation	Relative area occupied (%)
Lagoons		30%
Juncetalia maritimi	Mediterranean saline scrubs with tall grass and reeds	20%
Arthrocnemetalia fruticosae	Mediterranean and thermo-Athlantic halophilic scrubs	20%
Calcareous oligo-mesotrophic waters	Bentic vegetation and formations of charophyte algae	20%
Molinion-Holoschoenion	Mediterranean grasslands with tall grass and reeds	10%

Source: www.cma.gva.es

The Peníscola marsh has great populations of Valencia toothcarp - samaruc (*Valencia hispanica*, considered as one of the most critically endangered species in the world) and Spanish toothcarp - fartet (*Aphanius iberus*, endangered) as endemic populations (www.iucnredlist.org). Since 2004, Peníscola marsh is included into 'Recovery and Retrieval Plans' of these two species, in order to avoid their extinction. Nevertheless, in the last ten years, *A. iberus* and *V. hispanica* have been threatened by the massive presence of the mosquitofish (*Gambusia holbrooki*) and the degradation of their habitat by the presence of the Red-eared slider (*Trachemys scripta elegans*), the Red-rimmed melania - also called Malaysian trumpet snail (*Melanoides tuberculata*), the Red-swamp crawfish (*Procambarus clarkii*), and the carp (*Cyprinus carpio*) (Crivelli, 2006). Small populations of European pond turtle (*Emys orbicularis*) and Spanish pond turtle (*Mauremys leprosa*) can also be found there (www.cma.gva.es). Different species of birds nest in the marsh, like the Little grebe (*Tachybaptus ruficollis*), Mallard (*Anas platyrhynchos*) and the Common moorhen (*Gallinula chloropus*) (Orta *et al.*, 1992).

In the Peníscola marsh, three main channels can be observed: Sèquia Templera, Sèquia del Rei and Sèquia de la Sangonera. These three channels confluence before flowing into the Mediterranean Sea and their flow depend on groundwater discharge, carried out from the Vinarós-Peníscola aquifer and from the Maestrat aquifer in a diffuse way through sinkholes. On wet months of the year, a sheet of water covers a great part of the marsh, facilitating the development of the aquatic and swamp vegetation that dominates this ecosystem (Rodellas-Vila, 2008).

5. Species

5.1. *Gambusia holbrooki* (Girard, 1859) - Mosquitofish

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Cyprinodontiformes
Family: Poeciliidae
Genus: *Gambusia*
Species: *G. holbrooki*

Figure 4. Female (top) and male (bottom) *Gambusia holbrooki*.

Description and biology

Highly invasive species, Gambusia holbrooki (mosquitofish) is a small fish characterized by being a generalist predator, its mouth being provided with teeth at both lower and upper position. Mouth shape allows the fish to feed from the top of the water column, where the species that G. holbrooki feed live.

Female are the majority sex, (Pérez-Bote *et al.*, 2005; Moreno-Valcárcel, 2008) reaching 1:5 ratios (Da Franca *et al.*, 1953) in the western coast of the Iberian Peninsula and 1:4 in the east (Vargas *et al.*, 1996). However, in southern populations has been found proportions where males were the majority sex (Fernández-Delgado *et al.*, 1997).

G. holbrooki normally lives 4 years (females) and 3 years (males) (Moreno Valcárcel, 2009) .The maximum age that mosquitofish can reach is 5 years for females (4+)[3] and 4 for males (3+) but those ages have never been found by direct observation of the rings in scales.

The species has a high sexual dimorphism, mainly manifested in the body size and the morphology of the anal fin. Males stop growing when they reach sexual maturity, reaching an average of 30-40 mm (Fernández-Delgado *et al.*, 1997; Vargas *et al.*, 1996; Pérez-Bote *et al.*, 2004). Females continue their growing throughout their lives (Krumholz, 1948) with lengths ranging from 60 to 70 mm (TL)[4], reaching 80 mm in some populations (Vargas *et al.*, 1996). Growth rate is maximum the first months of life, between lately spring and early summer, with the rise of temperatures and food availability (Vargas, 1993; Cabral *et al.*, 1999; Pérez-Bote *et al.*, 2005).

[3] 0+, 1+, 2+, 3+ and 4+ refer to the different cohorts of the species that can be found. 0+ refers to those individuals with ages from 0 to 1 year, 1+ refers to those with ages from 1 to 2 years, etc.

[4] Total length (TL) refers to the length from the tip of the snout to the tip of the longer lobe of the caudal fin, usually measured with the lobes compressed along the midline. It is a straight-line measure, not measured over the curve of the body.

In males, anal fin is modified in order to form a copulatory organ or gonopodium by elongation and transformation of the radii 3 to 5. The end of the gonopodium has hooks for an easy attachment to the female during copulation. During the breeding season, females show a black spot in the ventrolateral area located between the ventral and anal fins (Doadrio, 2002; Pyke, 2005).

G. holbrooki is an ovoviviparous species, with internal fecundation and precocious sexually - they can reach maturity at 6 weeks after being born. Size in maturity depends on the sex and latitude: in males the size range varies between 16.9 and 21.3 mm and in females between 14.1 and 25.1 mm (Moreno Valcárcel *et al.*, 2009). Northern populations reach maturity with smaller sizes (Benejam *et al.*, 2008). G. holbrooki female make several lays throughout a single reproductive period and the gestation period lasts between 21 and 28 days with approximately 50 individuals produced.

The reproductive cycle length is determined by the photoperiod and water temperature. The reproductive period in Iberian Peninsula extends from April-May to September, and coincides with the period of autochthonous cyprinodontids *Aphanius iberus* and *Valencia hispanica*.(Vargas *et al.*, 1996; Fernández-Delgado *et al.*, 1997; Pérez-Bote *et al.*, 2005)

Population dynamics

In the Iberian Peninsula populations of *G. holbrooki* has been found similar age structures: 0+, 1+ and 2+ for females, 0+ and 1+ for males, being 0+ more than the 60% of the population (Fernández-Delgado, 1989; Moreno Valcárcel, 2008). Mosquitofish populations are structured in two main cohorts: 0+ and 1+, with a general replacement in the middle of the reproductive period. At the end of that period, population is composed mainly by the newborn individuals because 1+ cohort dies as a consequence of the reproductive effort. In the case of females, cohort 1+ survives the reproductive period and the winter, dying the following reproductive period (Fernández-Delgado, 1989; Vargas *et al.*, 1996; Fernández-Delgado *et al.*, 1997; Pérez-Bote *et al.*, 2005).

Trophic ecology

G. holbrooki feeding habits have a great plasticity. The basis of mosquitofish diet is zooplankton (cladocerans, ostracods and copepods). They also eat insects, molluscs, worms, plants, algae, rotifers, diatoms, detritus and even smaller fish (Vargas Pera, 1993; Gisbert *et al.*, 1996; Cabral *et al.*, 1998; García-Berthou, 1999; Blanco *et al.*, 2004).

Predators

Little research has been done to determine all of Gambusia holbrooki predators, due to its own predatory nature. In areas where it has been introduced, Gambusia holbrooki has been known to cause top down trophic effects due to the fact that they eat larvae of some top predators such as frogs and other fish (Blanco *et al.*, 2004). Largemouth bass (*Micropterus salmoides*) (Godinho *et al.*, 1997) and otter (*Lutra lutra*) (Adrian y Delibes, 1987) are two of the most important Iberian predators.

Habitat

Mosquitofish is able to colonize very different environments, from the middle and lower areas of the rivers to lakes, reservoirs, artificial ponds and irrigation canals, coastal

marshes and bogs (Meffe *et al.*, 1989). They prefer sections with low stream, shallowness, abundant vegetation and dark substrate (Pyke, 2005). *G. holbrooki* is a benthopelagic species and normally lives in freshwater but there are also populations in brackish environments (Hubbs, 2000; Alcaraz *et al.*, 2007). This species is very tolerant to extreme environmental conditions, living normally in waters between 12-29 ^{o}C, tolerating temperatures until 42 ^{o}C (Al-Johany *et al.*, 1993; Condon *et al.*, 2006).

This species tolerates low dissolved oxygen concentrations (0.28 gL^{-1}) because is able to obtain it from the upper strata of the water column (Pyke, 2005). It also tolerates pH ranging from 6 to 8.8 and high levels of pollution (Brown-Peterson *et al.*, 1990).

In relation to salinity, females reach sexual maturity earlier in saline water than in freshwater, and the reproductive effort is greater (Brown-Peterson *et al.*, 1990).

G. holbrooki is usually the predominant species in the habitats where they live (about the 80% of the total species) with densities ranging from 2 to 10 individuals per square meter and 50-90 individuals per cubic meter (Pyke, 2008). Densities vary throughout the year, reaching the maximum in autumn and the minimum in spring, and also vary with latitude, observing higher abundances in southern populations in the Iberian Peninsula (Benejam *et al.*, 2008).

Geographical distribution

Its natural distribution is the east coast of the United States, but in the first decades of the 20th century the species was introduced on all continents except in Antarctica in order to fight diseases like malaria. In 1921, *G. holbrooki* was introduced in Spain (Lozano Rey, 1935) to eliminate the Anopheles mosquito larvae and nowadays mosquitofish lives in almost all the slow water bodies in the Peninsula. In the Valencian Community mosquito fish can be found in all river basins and most of the marshes, ponds, canals, streams and water springs.

By 2002, the Marsh of Peníscola was the only major water body in the Valencian Community not invaded by mosquitofish, but nowadays the species can be found there too (Jiménez *et al.*, 2002).

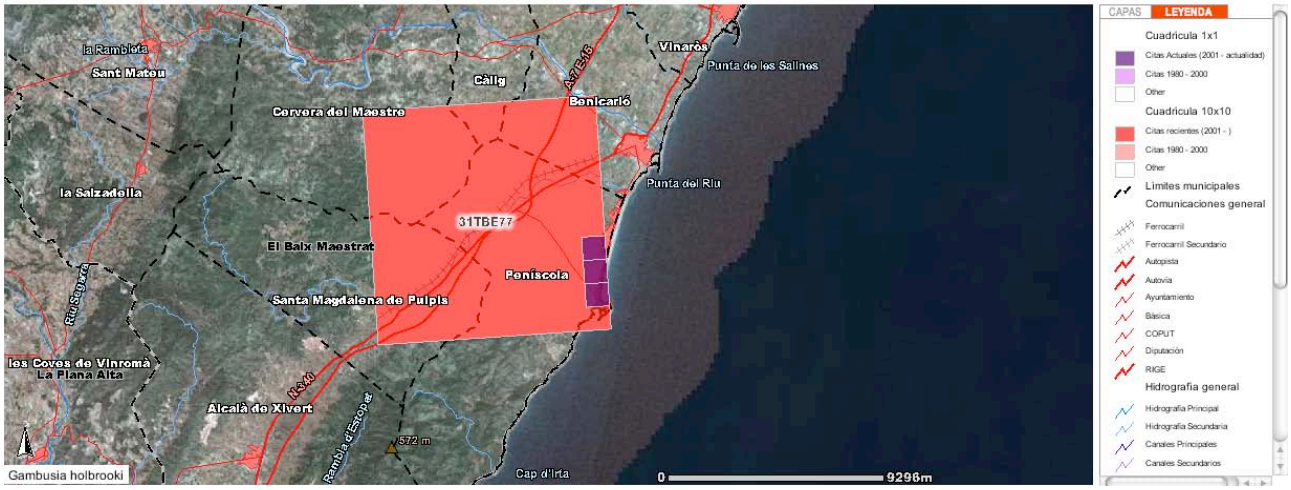

Figure 5. Distribution of *G. holbrooki* in Peníscola marsh. Populations of this species were found on red spots (10 km^2) and more specifically in purple spots (1 km^2) in a biological inventary done by the Database of Biodiversity of the Valencian Community in 2001. Source: database of biodiversity of the Valencian Community (http://bdb.cma.gva.es).

Conservation and legal status

IUCN Global Category: Not cataloged.

Currently mosquitofish is included in the list of the 100 most harmful invasive species of the world, created by the Invasive Species Specialist Group (ISSG) of the IUCN[5]. It has also been included into the list of the 20 exotic species with higher impact in Spain by the Biological Invasions Specialist Group (GEIB, 2006)

G. holbrooki is included in the 'Decreto 213/2009, de 20 de noviembre, del Consell, por el que se aprueban medidas para el control de especies exóticas invasoras en la Comunitat Valenciana - Annex I'.

Its presence is one of the main threatening factors for the last populations of *Valencia hispanica* and *Aphanius iberus* by trophic and space competition, and also depredation of the small fry[6] by the mosquitofish (Rincón *et al.*, 2002; Caiola *et al.*, 2005; Alcaraz *et al.*, 2008).

High densities of G. holbrooki may cause ecosystem damage, altering macroinvertebrate communities, decomposition of phytoplankton, water turbidity and eutrophication.

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[5] IUCN: International Union for Conservation of Nature. It is an international organization dedicated to finding "pragmatic solutions to our most pressing environment and development challenges". It is the world's oldest and largest global environmental network. It supports scientific research, manages field projects all over the world and brings governments, non-government organizations, United Nations agencies, companies and local communities together to develop and implement policy, laws and best practice. (www.iucn.org)

[6] Fry: very small young fish

5.2. *Cyprinus carpio* (Linnaeus, 1758) - Common carp

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Cyprinus*
Species: *C. carpio*

Figure 6. Female *Cyprinus carpio.*

Description and biology

Cyprinus carpio is an introduced species, characterized by two pairs of fleshy sensory barbels project downwards at either side of the mouth. *C. carpio* often grows 30 to 70 cm (TL) (Jiménez *et al.*, 2002) in length and weigh 0.5 to 4 kg (Tomelleri *et al.*, 1990). It is not uncommon for common carp to reach 15 to 20 kg (McCrimmon, 1968).

Males are usually distinguished from females by the larger ventral fin. Carp is characterized by its deep body and serrated dorsal spine (Nelson, 1984). The mouth is terminal[7] on the adult and subterminal[8] on the young (Page *et al.*, 1991). Colour and proportions are extremely variable, but scales are always large and thick.

Males typically become sexually mature at 3 to 5 years and females at 4 to 5 years (Froese *et al.*, 2002; McCrimmon, 1968). Carp lives up to 50 years and usually spawns every year -in May-June at temperatures above 18°C- and age of maturity is related to latitude and altitude. Adults often make considerable spawning migrations to suitable backwaters and flooded meadows. A typical female (about 45 cm) may produce 300,000 eggs, with some estimates as high as one million over the breeding season. Fry average 5 to 5.5 mm in total length. Temperature, stocking density, and availability of food influence individual growth. By the time the fish reach 8 mm the yolk has disappeared and they begin to actively feed. Reproductive success is restricted to years when the water level starts rising in May and when high temperatures and flooding of terrestrial vegetation last for a long period during May and June (Freyhof *et al.,* 2008).

By gulping air at the surface, carp is able to tolerate periods with low oxygen concentrations. In winter, individuals go into deeper waters that tend to be somewhat warmer than shallow water (Kottelat *et al.*, 2007).

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[7] Terminal mouth: Mouth that opens at anterior end of head with upper and lower jaws equal.

[8] Subterminal mouth: Mouth posterior to the tip of the snout. Sometimes also referred to as inferior mouth.

This species is able to hybridize with other species such as goldfish (*Carassius auratus*) (Jiménez *et al.*, 2002).

Trophic ecology

This species is primarily selective benthic omnivorous that specialize on invertebrates that live in the sediments (Lammens *et al.*, 1991). Larvae and juveniles feed mostly on very small zooplankton (rotifers and copepods) and algae (McCrimmon, 1968). Young of year carp feed on a variety of macroinvertebrates including chironomids, caddis flies, mollusks, ostracods, and crustaceans (McCrimmon, 1968). Adult feeds on a wide variety of benthic organisms - aquatic crustaceans, insects, annelids, mollusks, fish eggs, fish remains- and plant material - aquatic plants, algae and seeds (Freyhof *et al.*, 2008, Kottelat *et al.*, 2007, Lammens *et al.*, 1991, McCrimmon, 1968). Its feeding technique, of grubbing around in the sediment and straining food from the mud, has caused problems in areas where the carp has been introduced. As well as uprooting submerged vegetation, it also increases the cloudiness of the water, which can have detrimental effects on native wildlife (Kottelat *et al.*, 2007).

Ecosystem Roles

The method of feeding employed by common carp has important ecological implications. The feeding of carp has been shown to decimate macrophytes and decreases overall water quality (Drenner *et al.*, 1996). Carp tends to reduce macrophyte biomass by bioturbation (Carp often uproot aquatic macrophytes when is feeding). This species also reduces macrophyte biomass by direct consumption (they have been known to feed on tubers and young shoots) and indirectly by increasing turbidity, which reduces the available sunlight (Lougheed *et al.*, 1997, Fletcher *et al.*, 1985). Carp has been shown to decrease water quality by increasing turbidity by re-suspending sediments and increasing the amount of nutrients and thus increasing phytoplankton in the water column (Lamarra, 1975; Brabrand *et al.*, 1990). Carp acts as "nutrient pump" when it consumes the nutrient rich benthic sediments and then excretes those nutrients back into the water column in a form that is available to other organisms (Drenner *et al.*, 1996). This tendency to cause a general decay in water quality and the high fecundity of the carp has caused them to be generally regarded as a nuisance in the environment where they live (McCrimmon, 1968; Page *et al.*, 1991).

Predators

Predators on young carp are large fish species such as northern pike, muskellunge, walleye, and largemouth bass.(Froese *et al.*, 2002; Baldry, 2000). Although birds, such as great blue herons probably also eat them. Adults have no predators other than people (Baldry, 2000; Froese *et al.*, 2002) and the main uses are commercial in fisheries and sport fishing.

Habitat

This species lives in warm, deep, slow-flowing and still freshwaters, such as lowland rivers, swamps, reservoirs and large, well vegetated lakes. They can also live in brackish water as coastal lagoons and marshes. Carp prefer water with muddy bottom because on winter they bury themselves to hibernate. They can tolerate high levels of pollution in water (Jiménez *et al.*, 2002).

It has been introduced in all types of water bodies, reaching high densities that result in massive mortalities in case of contamination or sudden dryings (Jiménez *et al.*, 2002).

Geographical distribution

Common carp is native to the River Danube, in Europe, but has been widely introduced and is now found worldwide except for the poles and northern Asia (Froese *et al.*, 2002; Nelson, 1984). In the Valencian Community, the species has colonized by repopulations almost all reservoirs and rivers. Carp can be found in almost all coastal wetlands and many irrigation ponds.

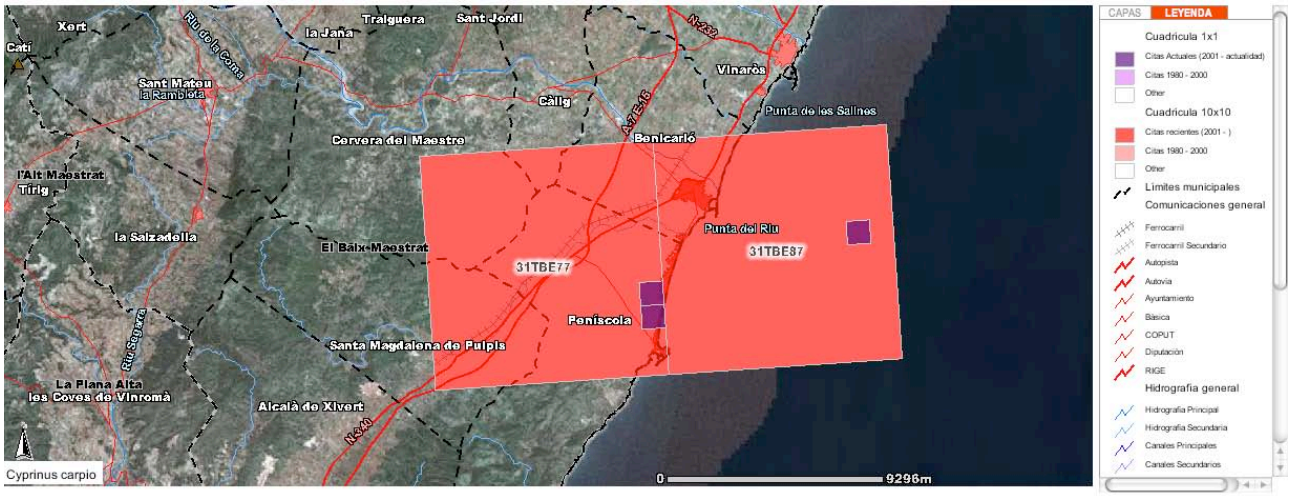

Figure 7. Distribution of *C. carpio* in Peníscola marsh. Populations of this species were found on red spots (10 km^2) and more specifically in purple spots (1 km^2) in a biological inventary done by the Database of Biodiversity of the Valencian Community in 2001. Source: database of biodiversity of the Valencian Community (http://bdb.cma.gva.es).

Conservation and legal status

The original population in River Danube is now under threat and classified as Vulnerable A2ce by the IUCN Red List of threatened species (Freyhoff *et al.*, 2008).

5.2. *Carassius auratus* (Linnaeus, 1758) - Wild goldfish

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Carassius*
Species: *C. auratus*

Figure 8. Two individuals of *Carassius auratus* and three individuals of *G. holbrooki* below a petri dish.

Description and biology

Exotic freshwater species, the common goldfish has two sets of paired fins - the pectoral fins and pelvic fins, and three single fins - the dorsal, caudal, and anal fin. The dorsal and anal-fin rays are strong and serrated posteriorly. They lack barbels on the upper jaw, and lack scales on the head. They have 27-31 scales along their lateral lines. Goldfish have exceptionally large eyes and acute senses of smell and hearing. They have pharyngeal teeth in their throats that they use to crush food, rather than true teeth (Street, 2002)

Goldfish can grow up to 3 kg and 45 cm long (TL) but are usually much smaller (Street, 2002). The species does not have sexual dimorphism (Street, 2002).

In captivity, lifespan of goldfishes range from 10 years in aquariums and 25 years for goldfishes kept in a pond. In the wild, lifespan is lower than 10 years. The maximum longevity found in wild has been 41 years (Carey *et al.*, 2002).

Population dynamics

Goldfish usually mature in their second year but this varies with diet, water temperature and other environmental influences. In the wild, breeding occurs during the summer. Mature female goldfish will become rounder during breeding, males develop tubercles (small bumps) on their heads, operculi and pectoral fins. Males chase the females for several days before spawning occurs. Females can produce several thousand eggs per spawning period every 8 to 10 days. Eggs are not guarded. Goldfish eggs hatch in about 4-5 days at 18-20 ºC (Street, 2002).

Trophic ecology

In the wild, goldfish are omnivores. They eat plants, insects such as mosquito larvae, small crustaceans, zooplankton, and detritus.

In captivity, goldfish are commonly fed dried flake or pellet food(Street, 2002).

Predators

Carassius auratus is prey of testudines (turtles), *Stizostedion vitreum* (walleye), *Ardea herodias* (the great blue heron), *Butorides virescens* (green heron), *Larus delawarensis* (ring-billed gull) and *Ceryle alcyon* (Belted Kingfisher) (EOL, 2011).

Habitat

In the wild, goldfish can be found in slow-moving, freshwater bodies of water -lakes, ponds, rivers and streams. As with their close relative the carp, they thrive in slightly sludgy water. Goldfish lives in a depth range between 0.1 and 6 metres, and they will survive in water temperatures ranging from freezing to 30 ºC. Goldfish use to live in waters its pH being of 6.5-8.5 (Street, 2002).

Goldfish can hunt in murky environments because they are able to detect far-red and infrared light (Shuker, 2001).

Geographical distribution

Although goldfishes originated in China, they have now spread worldwide in aquariums, ornamental pools, and into the wild (Street, 2002).

Goldfish was been introduced in the Iberian Peninsula in the 18th century. Nowadays the species can be found in all Spanish basins. In the Valencian Community, the species is mentioned in Turia River, Magro River, Algar River and Segura River (Jiménez *et al.*, 2002). The species can also be found in the ditches of the Peníscola Marsh and Jaraco and in some lagoons in Hondo de Elche. The species was mentioned by first time in the Albufera of València, at the end of the 19th century (Jiménez *et al.*, 2002).

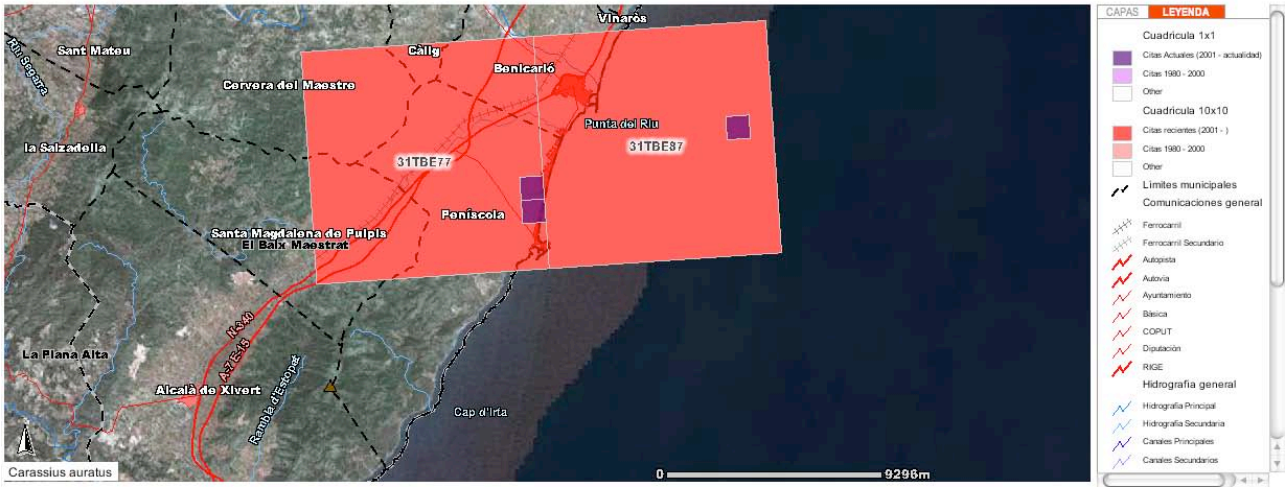

Figure 9. Distribution of *C. auratus* in Peníscola marsh. Populations of this species were found on red spots (10 km^2) and more specifically in purple spots (1 km^2) in a biological inventary done by the Database of Biodiversity of the Valencian Community in 2001. Source: database of biodiversity of the Valencian Community (http://bdb.cma.gva.es).

Conservation and legal status

Goldfish are not endangered.Goldfish should not be released into ponds in the wild because they breed quickly and are capable of crowding out native fish species. They are considered pests in most places where they have been introduced (Street, 2002).

5.4. *Chelon labrosus* (Cuvier, 1829) - Thicklip grey mullet

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Mugilidae
Genus: *Chelon*
Species: *C. labrosus*

Figure 10. *Chelon labrosus.*

Description and biology

Autochthonous Iberian Peninsula species, *Chelon labrosus* has a cylindrical elongate body up to 70 cm in length - maximum length: 75 cm male/unsexed SL[9] (Muus *et al.*,. 1999), common length: 32 cm male/unsexed SL (Thomson, 1990).

The maximum published weight for Chelon labrosus is 4.5 kg and the maximum reported age is around 25 years (Muus *et al.*,.1999).

The species has a broad head that is flattened on top. It has a small upturned mouth and fine eyelids that do not exceed the iris (Jiménez *et al.*, 2002; Morvan Barnes, 2008). The upper lip is very deep, approx. until half the diameter of the eye. It has two well separated dorsal fins, the first one with 4 slender spines and the second one with one spine and 8-9 soft rays. The pectoral fin is quite high on the body. The pelvic fins are situated midway between the pectoral base and the origin of the first dorsal. The tail is large and forked. It is grey-blue above and silvery on the flanks and the belly. Dark longitudinal stripes are present along the scale rows (Morvan Barnes, 2008).

Population dynamics

The length at first maturity (L_m)[10]for Chelon labrosus is 29.5 cm. *C. labrosus* has a medium ability for resilience: a minimum population can double size in a period of 1.4 to 4.4 years.

C. labrosus is an oviparous species. Reproduction occurs in the sea during winter (Billard, 1997). Eggs and larvae are pelagic.

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[9] Standard length (SL) refers to the length of a fish measured from the tip of the snout to the posterior end of the last vertebra or to the posterior end of the midlateral portion of the hypural plate (modified last vertebra, to which caudal fin rays attach). This measurement excludes the length of the caudal fin.

[10] L_m: Length at which 50% of the fish are mature.

Trophic ecology

The species feed mainly on benthic diatoms, epiphytic algae, small invertebrates and detritus (Ben-Tuvia, 1986). *C. labrosus* preys on *Enteromorpha* and *Ulva* detritus.

Predators

Phalacrocorax carbo and *Ardea cinerea* are two species of marine birds that prey on *C. labrosus.*

Habitat

Demersal and catadromus species, *C. labrosus* migrate occasionally from fresh water to the sea to spawn (McDorwall, 1997), and tend to move northward in summer-time as the temperatures rise. Migrations should be cyclical and predictable and cover more than 100 km. (McDorwall, 1997). It is usually found in shallow inshore waters or entering brackish lagoons and freshwater. *C. labrosus* lives in a depth range between 9 and 40 meters.

In Valencian Community, *C. labrosus* usually lives in shallow water, coastal lagoons and areas next to estuaries. They are always in schools between 10 and more than a hundred individuals.

Geographical distribution

Chelon labrosus can be found in Baltic Sea, North Sea, Mediterranean Sea, Black Sea, and in the eastern Atlantic Ocean: from Norway and Iceland to Senegal, including Azores, Madeira, Canary Islands and Cape Verde Islands.

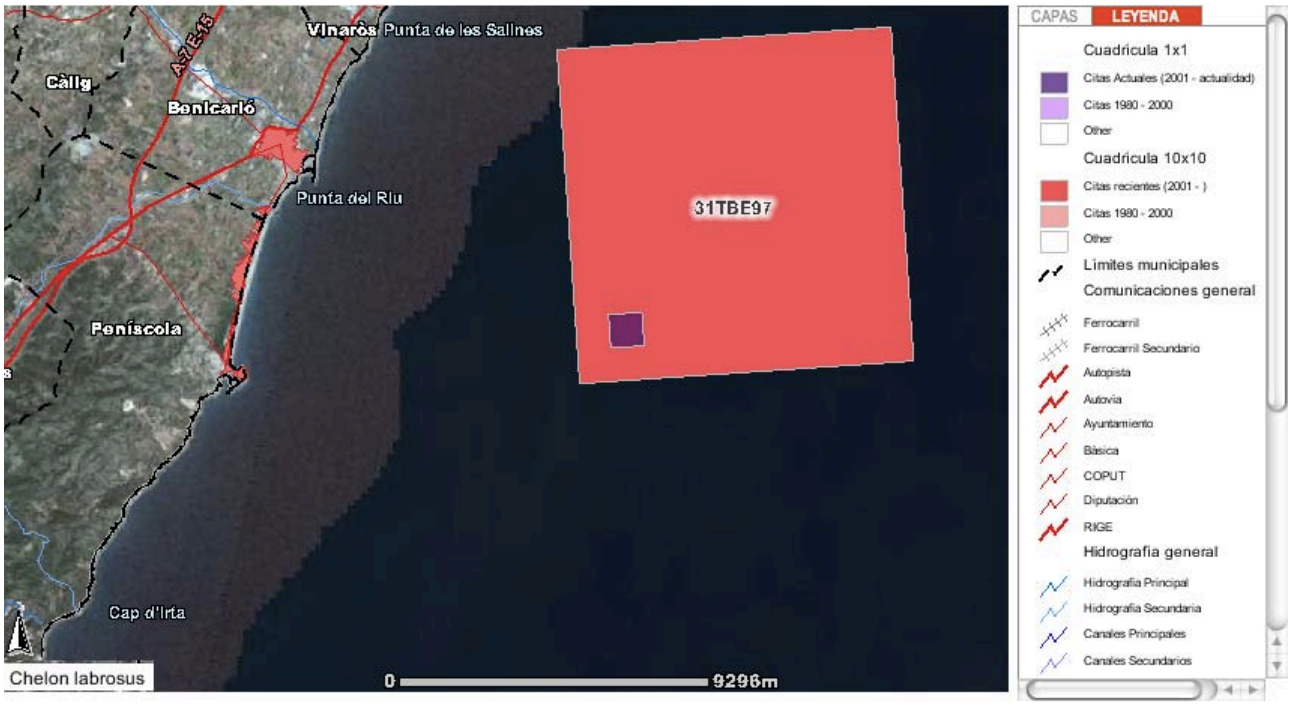

Figure 11. Distribution of *C. labrosus* in Peníscola marsh. Populations of this species were found on red spots (10 km^2) and more specifically in purple spots (1 km^2) in a biological inventary done by the Database of Biodiversity of the Valencian Community in 2001. Source: database of biodiversity of the Valencian Community (http://bdb.cma.gva.es).

Conservation and legal status:

The IUCN status for *C. labrosus* is Least Concern (LC) (IUCN, 2006).

The main threats for C. labrosus are overfishing in lagoons and estuaries and the destruction of those habitats.

The species does not have an assigned minimum catch size. As all the species of Mugilidae, in Spanish Mediterranean Sea the minimum size for fishing is 16 cm.

6. Materials and methods

6.1. Sampling methodology

The main objective of the sampling campaigns was to obtain different species of marsh fishes and have statistically significant values. Samples of *Chelon labrosus*, *Carassius auratus*, *Cyprinus carpio* and *Gambusia holbrooki* were fished between May 27th and 28th 2010.

A second sampling campaign was conducted between November 14th and 17th 2011. This campaign was aimed to catch some fish blank samples as to compare its radioactive concentrations in tissues with those results obtained in Peníscola marsh.

Water samples were collected on 1st December 2010 in order to evaluate bioaccumulation factors (BAF) from water to fish tissues.

Biological samples

Three stations were selected (as shown in Figure 12) under the hypothesis that ^{210}Pb and ^{210}Po accumulation in organisms would be potentially different due to the different ^{210}Pb and ^{210}Po concentrations in water (Rodellas-Vila, 2008). Table 2 shows the coordinates of each fish sampling station.

Some individuals of the species *Chelon labrosus, Cyprinus carpio* and *Carassius auratus* were caught in each station using trammel nets and bread as bait.

Gambusia holbrooki individuals were caught using a net cage, with bread as bait in station 1. For stations 2 and 3, individuals were captured using a small fishing net similar to a butterfly net. The reproductive period of *G. holbrooki* in the Iberian Peninsula extends from April-May to September and they can reach sexual maturity at six weeks after being born (Pérez-Bote and López, 2005). It is supposed that all the individuals caught had already reached maturity.

All the individuals were properly labelled and packaged by species and sampling station. Then they were frozen for storage until further processing of the samples.

Table 2. Coordinates of fish sampling stations in the Peníscola marsh.

Sample code	Coordinates	
St 2	40º 22' 30.90" N	0º 24' 03.28" E
St 3	40º 22' 08.00" N	0º 24' 02.65" E
St 6	40º 22' 33.15" N	0º 24' 00.87" E

Figure 12. Sampling stations for the biological samples. Source: Google Earth.

Water samples

In the case of water samples, five stations of surface water were sampled (Figure 13). Table 3 shoes the coordinates from each sampling station. 3 L of water were collected and each container was properly labeled. Temperature, conductivity and salinity were measured in the field in all samples with a multi-parameter probe YSI 556.

Table 3. Coordinates of water sampling stations in the Peníscola marsh.

Sample code	Coordinates	
Wst 2	40º 22' 30.90" N	0º 24' 03.28" E
Wst 3	40º 22' 23.55" N	0º 24' 08.45" E
Wst 4	40º 22' 16.60" N	0º 24' 14.00" E
Wst 5	40º 21' 50.00" N	0º 23' 56.80" E
Wst 6	40º 22' 33.15" N	0º 24' 00.87" E

Samples were filtered at 1µm pore by using a sandwich filter and a peristaltic pump, in order to separate particulate and dissolved fraction. Subsequently, samples were spiked with ^{209}Po and Pb^{2+} as yield tracers and acidified to stabilize it.

Figure 13. Sampling stations for the water samples. Source: Google Earth.

6.2. Analysis procedure

Biological samples

Sample pre-treatment

Biological samples were stored until they were processed. Five individuals collected at each station were selected when possible. After arriving at the laboratory, the total length (TL), standard length (SL)[11] and body weight of the selected fishes were determined. Some scales between the lateral line and the dorsal spine fin were picked, labelled and stored in the freezer to allow further studies of the individual's ages.

Fishes were subsequently dissected obtaining from each individual gonads, kidney, hepatopancreas and gut. Gut content was removed from the gut by squeezing and was deposited in a Petri dish and weighed. The gut was washed with water in order to remove any remaining content.

Before taking the spine, arch gill and muscle sample, the whole interior of the fish was cleaned of viscera and weighed as the eviscerated weight. The whole two gonads were weighted together as gonadal weight.

After a portion of each tissue was obtained, samples were deposited in a Petri dish and dried at 60 °C for 24h as to obtain fresh weight and dry weight.

^{210}Pb-^{210}Po Radiochemical procedure

^{210}Po and ^{210}Pb were determined by α-spectrometry. For this purpose, 0,250 g. were weighed and transferred into teflon beakers, spiked with (0,703 ± 0,014 Bq mL^{-1}) ^{209}Po as

[11] Standard length (SL) refers to the length of a fish measured from the tip of the snout to the posterior end of the last vertebra or to the posterior end of the midlateral portion of the hypural plate (modified last vertebra, to which caudal fin rays attach). This measurement excludes the length of the caudal fin.

a yield tracer and digested with 40 mL of nitric acid at 75ºC of temperature overnight. The residue was dissolved by adding hydrogen peroxide. After a digestion, the solution was evaporated to dryness to remove the HNO_3 and subsequently converted to a hydrochloric form by adding 2-3 mL of concentrated HCl and evaporated to dryness. This step was repeated three times (García-Orellana, 2004).

The dried sample was dissolved in 80 mL of 1M HCl and placed on a magnetic stirrer with thermostat control at a temperature of 75ºC. With the addition of ascorbic acid to reduce Fe^{3+} to Fe^{2+} (until the solution was colourless), thus eliminating interference in the deposition of polonium.

^{210}Po and ^{209}Po from the solution was spontaneously deposited onto a silver disc (25 mm diameter) suspended in the sample solution by means of a nylon thread taped to the beaker. One face of the disc was lacquered with urethane in order to avoid ^{210}Po isotope to deposit into this face.

The silver disc was kept spinning at that temperature for a period of 6 hours with the aid of the stirrer. At the end of the plating period, the disc was taken out, rinsed with Milli-Q water and dried.

After plating, the solution was stored for 6 months to allow ingrowth of ^{210}Po from ^{210}Pb, and then the ^{210}Po platting step was repeated.

Water samples

Once at the laboratory, 2 mL of Fe^{3+} carrier were added to the filtered and acidified sample. Po isotopes were pre-concentrated with iron hydroxides ($Fe(OH)_3$ precipitation) by slow addition of concentrated ammonium hydroxide with rapid stirring until the pH reached 9. The precipitate was evaporated and deposited following the same procedure as the one described for biological samples (Holm and Fukai, 1977).

Filters were transferred into teflon beakers, spiked with ^{209}Po as a yield tracer and digested with 70 mL of concentrated HNO_3 and 30 mL of HCl. Digestion and deposition was made following the same procedure as biological samples.

6.3. Detection systems

Alpha spectrometry

Po isotopes activities were measured with an alpha-spectrometer equipped with a silicon surface barrier and ion implanted silicon detector (active area: 450 mm^2) (Canberra, Model: Alpha Analyst with Alpha PIPS detectors) and a semiconductor silicon surface barrier detector EG&G ORTEC Mod. 450. The Minimum Detectable Activity (MDA) was in the range of 0.50 - 5.58 mBq for ^{210}Po for a 400,000 seconds counting time.

^{210}Pb was measured through deposition of its grand-daughter ^{210}Po after 6 months ingrowth. Ingrowth and decay corrections were applied to calculate activities of both, ^{210}Pb and ^{210}Po at sampling date.

The quality assurance of radio-analytical measurements was ensured through analysis of certified reference materials.

Pb^{2+} chemical recovery

As Po recovery was performed by calculating the total ^{209}Po obtained compared to the ^{209}Po added, chemical recovery of ^{210}Pb was carried out by the addition of stable Pb (Pb^{2+}). Aliquots of the filtered sample were therefore taken from the platting solution and analyzed by a inductively coupled plasma - optical emission spectrometer (ICP-OES) Perkin-Elmer, mod. Optima 4300DV.

7. Results and discussion

7.1. Concentrations of ^{210}Pb and ^{210}Po in Peníscola marsh water

Radionuclide activity concentrations determined in the water samples are shown in Table 4. Results are shown separately, ^{210}Pb and ^{210}Po in the particulate and dissolved fractions.

Table 4. Concentrations of ^{210}Pb and ^{210}Po (in Bq m^{-3}) in the marsh water.

Water sample	Dissolved fraction		Particulate fraction	
	^{210}Pb	^{210}Po	^{210}Pb	^{210}Po
Wst 2	13.29 ± 0.62	2.46 ± 0.27	4.40 ± 0.28	1.54 ± 0.18
Wst 3	22.47 ± 0.89	4.58 ± 0.5	2.50 ± 0.19	0.83 ± 0.11
Wst 4	13.53 ± 0.64	3.95 ± 0.35	4.93 ± 0.34	1.86 ± 0.21
Wst 5	17.43 ± 0.75	2.94 ± 0.36	5.38 ± 0.32	1.57 ± 0.19
Wst 6	14.03 ± 0.62	3.34 ± 0.33	7.61 ± 0.58	3.77 ± 0.40

Values of ^{210}Pb in dissolved fraction ranged from 13.3±0.6 to 22.5±0.9 Bq m^{-3}, and were higher than the values from ^{210}Po in this fraction, that ranged from 2.5±0.3 to 4.6±0.5 Bq m^{-3}. On the contrary, values of ^{210}Pb in the particulate fraction ranged from 2.5±0.2 to 7.6±0.6 Bq m^{-3} and ^{210}Po ranged from 0.8±0.1 to 3.8±0.4 Bq m^{-3}. In general terms, it is observed that there was more concentration of ^{210}Pb in water rather than ^{210}Po, and higher concentrations of both radionuclides in the dissolved fraction rather than in the particulate fraction, not reaching the secular equilibrium in any case.

As can be observed on Figure 14, there is no gradient from north to the south in the mars waters. Although, the five samples were taken at different points according to the different radioactivity concentration observed in soils in previous studies (Rodellas-Vila, 2008; Rodellas-Vila, 2009). Results showed that there is no significant differences between stations and gradient between Wst 2, located at the northern part of the marsh, was supposed to be the sample with the lowest concentrations, and Wst 6 the one with the highest concentration, due to the levels of ^{226}Ra and ^{222}Rn in sediments and soils.

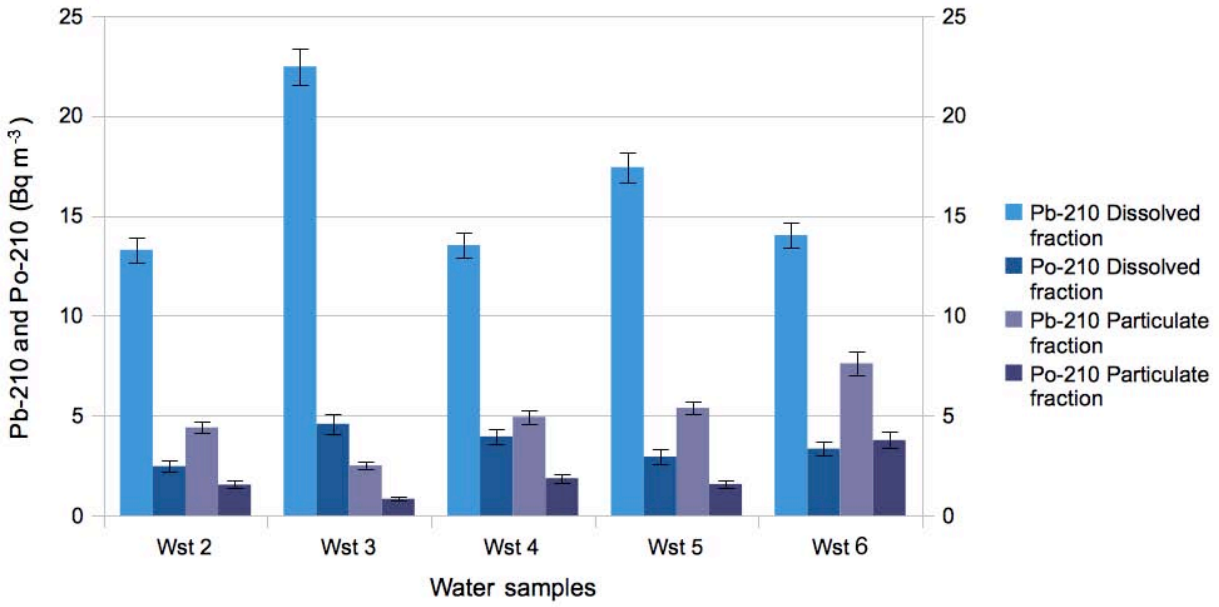

Figure 14. Concentrations of ^{210}Pb and ^{210}Po (in Bq m^{-3}) in Peníscola marsh water samples.

7.2. Concentrations of ^{210}Pb and ^{210}Po in each fish species

7.2.1. *Gambusia holbrooki*

Results of concentration of ^{210}Pb and ^{210}Po (Bq kg^{-1}, dry weight) in whole fish of G. holbrooki are shown in Table 5. Values of ^{210}Pb ranged from 12±1 to 61±3 Bq kg^{-1} and ^{210}Po ranged from 91 to 310 Bq kg^{-1}. As can be observed in Figure 15, values of ^{210}Pb were higher in fishes from the sampling station 3 (with an average value of 35.3±16.4 Bq kg^{-1}) than those from the sampling stations 2 and 6 (with average values of 17.0±6.3 and 19.3±3.6 Bq kg^{-1} respectively). These results agree with the higher concentration of ^{210}Pb found in the water samples 3 and 5, collected in the same channel than the individuals of G. holbrooki from the sampling station 2.

^{210}Pb and ^{210}Po did not show secular equilibrium in *G. holbrooki*, with greater values of ^{210}Po compared with ^{210}Pb concentration. Variability in the concentration of each radionuclide between individuals from the same sampling station was greater in sampling station St 3 and St 6 than in the results from St 2.

Table 5. Biological measures of *G. holbrooki*, concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in whole fish and ^{210}Po/^{210}Pb ratio.

Individuals	Sampling Station	Sex	SL (mm)	Body Weight (g)	Dry:wet weight ratio	^{210}Pb	^{210}Po	^{210}Po/^{210}Pb
1		F	30.53	0.68	0.29	28 ± 2	220 ± 7	7.9±0.1
2		F	28.33	0.51	0.26	12 ± 1	204 ± 8	17.0±0.1
3	2	F	27.30	0.47	0.24	16 ± 2	190 ± 8	11.9±0.1
4		M	33.09	0.74	0.29	12 ± 1	159 ± 7	13.3±0.1
5		M	29.08	0.52	0.29	17 ± 1	236 ± 11	13.9±0.1
6		F	22.13	0.21	0.29	18 ± 2	190 ± 8	10.6±0.1
7		M	16.84	0.09	0.27	30 ± 6	161 ± 11	5.4±0.2
8	3	F	19.03	0.14	0.30	28 ± 3	310 ± 14	11.1±0.1
9		F	29.79	0.76	0.30	61 ± 3	241 ± 14	4.0±0.1
10		F	33.05	1.11	0.25	41 ± 2	188 ± 12	4.6±0.1
11		F	37.12	1.02	0.31	26 ± 1	178 ± 6	6.9±0.1
12		F	29.37	0.67	0.26	17 ± 1	111 ± 5	6.5±0.1
13	6	F	28.82	0.56	0.29	17 ± 2	91 ± 4	5.6±0.1
14		F	29.85	0.76	0.29	18 ± 1	131 ± 7	7.3±0.1
15		F	32.29	0.96	0.27	19 ± 1	206 ± 11	10.8±0.1

The ^{210}Po/^{210}Pb was calculated and results showed that ^{210}Po was accumulated with a range of 5.5 to 16.5 times more than ^{210}Pb. The highest accumulation was found on the individuals from the sampling station 2. Those from St 3 and St 6 showed similar values although rather lower than the ones obtained in St 2.

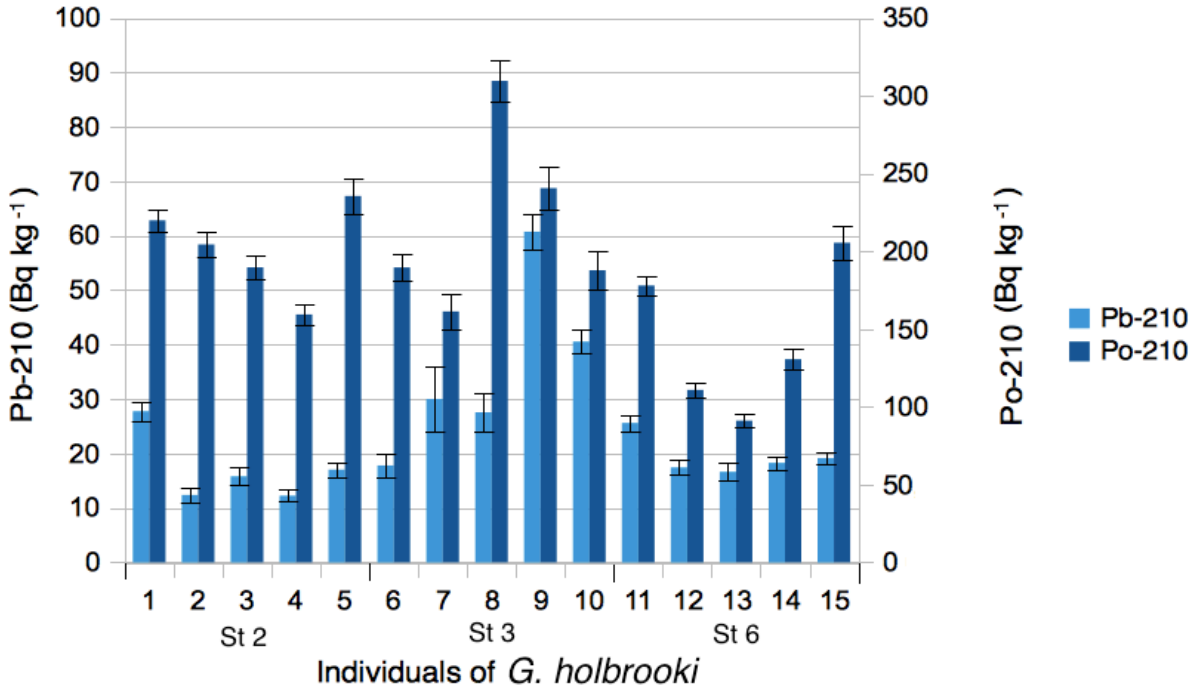

Figure 15. Concentration of ^{210}Pb and ^{210}Po in dry weight in each individual of *G. holbrooki* analyzed. Concentrations are shown in dry weight.

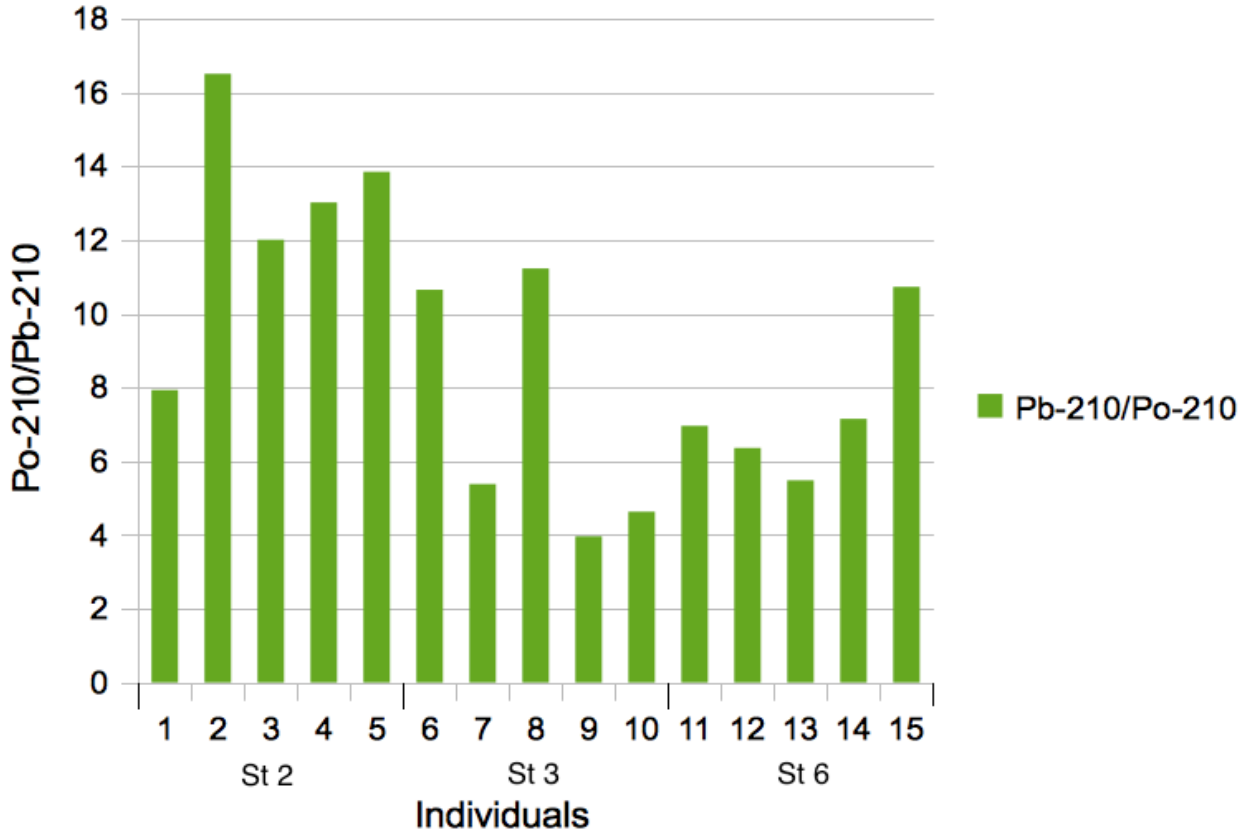

Figure 16. ^{210}Po/^{210}Pb ratio in the tissues of individuals of *G. holbrooki* from the three sampling stations.

In general terms, activity concentrations of ^{210}Pb in samples from station 6 showed lower values than the samples from the other stations, and concentrations of ^{210}Po were similar to those from St 2. This result show that there is no correspondence between ^{210}Po accumulation in fish and ^{210}Po concentration in water, as the water at St 6 had the highest ^{210}Po values of all water samples.

One of the hypothesis was the different accumulation of ^{210}Po depending on the size of weight of the organism. However, R^2 values were calculated and the results obtained showed no relation (i.e. R^2 ranging from 0 to 0,05 for both radionuclides, when considering SL and body weight, respectively).

7.2.2. *Carassius auratus*

Concentrations of ^{210}Po and ^{210}Pb in tissues of *C. auratus* (n=7) are shown in Table 6. Results (see Figure 17) shown that muscle had a concentration of ^{210}Pb that ranged from 6±1 to 21±29 Bq kg^{-1} with an average concentration of 12±8 Bq kg^{-1}. ^{210}Po concentration of muscle ranged from 69±34 to 343±9 Bq kg^{-1} with an average concentration of 164±155 Bq kg^{-1}. Spine showed a concentration of ^{210}Pb that ranged from 194±7 to 351±359 Bq kg^{-1} with an average of 253±85 Bq kg^{-1}. Concentrations of ^{210}Po in spine ranged from 92±9 to 245±228 Bq kg^{-1} and the average of 167±77 Bq kg^{-1}. Concentration of ^{210}Pb in gonads ranged from 6±2 to 22±2 Bq kg^{-1} and the average value was 14±8 Bq kg^{-1}. In comparison, concentration of ^{210}Po was much higher, with a range of 327±227 to 1189±35 Bq kg^{-1} with an average of ^{210}Po concentration of 635±481 Bq kg^{-1}. Concentration of ^{210}Pb in gills ranged from 169±7 to 216±174 Bq kg^{-1} with an average of 194±23 Bq kg^{-1}. Concentration of ^{210}Po was in a range of 517±237 to 844±33 Bq kg^{-1}.

Concentration of ^{210}Pb in hepatopancreas ranged from 25±5 to 40±3 Bq kg^{-1}. On the other hand, concentration of ^{210}Po was in a range of 474±16 to 1966±47 Bq kg^{-1} with an average of 1144±758 Bq kg^{-1}. In kidney, ^{210}Pb concentration ranged from 64±7 to 105±7 Bq kg^{-1} with an average of 88±21 Bq kg^{-1}, and ^{210}Po from 1814±1033 to 3805±137 Bq kg^{-1} with an average of 2488±1141 Bq kg^{-1}. Concentration of ^{210}Pb in gut ranged from 41±9 to 79±5 Bq kg^{-1} with an average of 65±21 Bq kg^{-1}, and ^{210}Po concentration was in the range from 1386±38 to 5621±161 Bq kg^{-1} with an average value of 3261±2158 Bq kg^{-1}. Finally, ^{210}Pb concentration in gut content ranged from 228±10 to 599±20 Bq kg^{-1}, with an average of 384±193 Bq kg^{-1}. ^{210}Po concentration in gut content ranged from 2800±1598 to 5494±140 Bq kg^{-1} with an average of 3833±1452 Bq kg^{-1}. Highest concentration of ^{210}Pb was found in gut content, followed by spine and gills. The lowest ^{210}Pb concentration was found on muscle, gonads and hepatopancreas. On the other hand, the highest ^{210}Po concentration was found on gut content, followed by gut and kidney. The lowest concentrations of ^{210}Po were found on muscle and spine.

When comparing fishes from different sample stations, can be observed that muscle and spines showed higher values of ^{210}Pb concentration in St 2 (21±29 Bq kg^{-1} and 351±359 Bq kg^{-1} for muscle and spine, respectively in St 2, while in St 3 and St 6 were 8±1 and 6±1 Bq kg^{-1} in muscle and 215±8 and 194±7 Bq kg^{-1} in spine, respectively). In muscle, this result indicates that concentration in St 2 is 2 and 2.3 times higher in comparison with St 3 and St 6, respectively. There was little variability on ^{210}Pb concentration in gills, kidney, hepatopancreas and gut between the three sample stations. However, there were significant differences between ^{210}Pb concentrations in gut content among the three stations, reaching in the St 3 (599±20 Bq kg^{-1}) two times the concentration of these tissues in samples collected at St 2 and St 6 (324±300 and 228±10 Bq kg^{-1} respectively).

Concentrations of ^{210}Po in the different tissues of fish samples collected at St 6 were in general higher than those in St 2 or St 3, doubling the concentration of those sites, except in the spine, which was higher in individuals from St 2 (245±228 Bq kg^{-1} in St 2, in comparison with 92±9 and 163±11 Bq kg^{-1} in St 3 and St 6 respectively). Values of ^{210}Po concentration in gonads, gills and kidney were very similar for St 2 and St 3. In the case of gut, there was a high variability between the three stations: Concentration at St 6 (5621±161 Bq kg^{-1}) reached two times the concentration at St 2 (2778±1663 Bq kg^{-1}) and four times the concentration at St 3 (1386±38 Bq kg^{-1}). There was also variability among the concentrations shown in gut content. While values at St 2 and St 3 (2800±1598 and 3206±121 Bq kg^{-1}) were very similar, the concentration at St 6 (5494±140 Bq kg^{-1}) was almost twice the concentration of the other stations.

Table 6. Average values of concentration of ^{210}Pb and ^{210}Po (in Bq kg^{-1}dry weight) in tissues and gut content of *C. auratus* (n=7) TL and body weight are average values when more than one individual was collected.

C. auratus		Tissue	Dry:wet weight ratio	^{210}Pb	^{210}Po	^{210}Po/^{210}Pb
Sampling station	2	Muscle	0.32	21 ± 29	69 ± 34	3,3 ± 1,5
		Spine	0.71	351 ± 359	245 ± 228	0,7 ± 1,4
Number of individuals	3	Gonads	0.25	6 ± 2	327 ± 227	57,2 ± 0,8
		Gills	0.20	216 ± 174	517 ± 237	2,4 ± 0,9
TL (cm)	26±9	Kidney	0.21	93 ± 39	1814 ± 1033	19,5 ± 0,7
		Hepatopancreas	0.17	26 ± 4	990 ± 358	38,3 ± 0,4
Body weight (g)	393±377	Gut	0.15	41 ± 9	2778 ± 1663	68,1 ± 0,6
		Gut content	0.18	324 ± 300	2800 ± 1598	8,6 ± 1,1
Sampling station	3	Muscle	0.22	8 ± 1	81 ± 3	9,6 ± 0,1
		Spine	0.67	215 ± 8	92 ± 9	0,4 ± 0,1
Number of individuals	2	Gonads	0.26	22 ± 2	389 ± 14	17,5 ± 0,1
		Gills	0.19	197 ± 8	540 ± 22	2,7 ± 0,1
TL (cm)	22±4	Kidney	0.19	105 ± 7	1844 ± 62	17,5 ± 0,1
		Hepatopancreas	0.18	40 ± 3	474 ± 16	11,8 ± 0,1
Body weight (g)	194±124	Gut	0.14	79 ± 5	1386 ± 38	17,5 ± 0,1
		Gut content	0.19	599 ± 20	3206 ± 121	5,3 ± 0,1
Sampling station	6	Muscle	0.22	6 ± 1	343 ± 9	55,5 ± 0,1
		Spine	0.59	194 ± 7	163 ± 11	0,8 ± 0,1
Number of individuals	2	Gonads	0.24	14 ± 1	1189 ± 35	84,0 ± 0,1
		Gills	0.17	169 ± 7	844 ± 33	5,0 ± 0,1
TL (cm)	19±1	Kidney	0.20	64 ± 7	3805 ± 137	59,0 ± 0,1
		Hepatopancreas	0.24	25 ± 5	1966 ± 47	79,9 ± 0,2
Body weight (g)	128±4	Gut	0.12	74 ± 6	5621 ± 161	75,5 ± 0,1
		Gut content	0.29	228 ± 10	5494 ± 140	24,1 ± 0,1

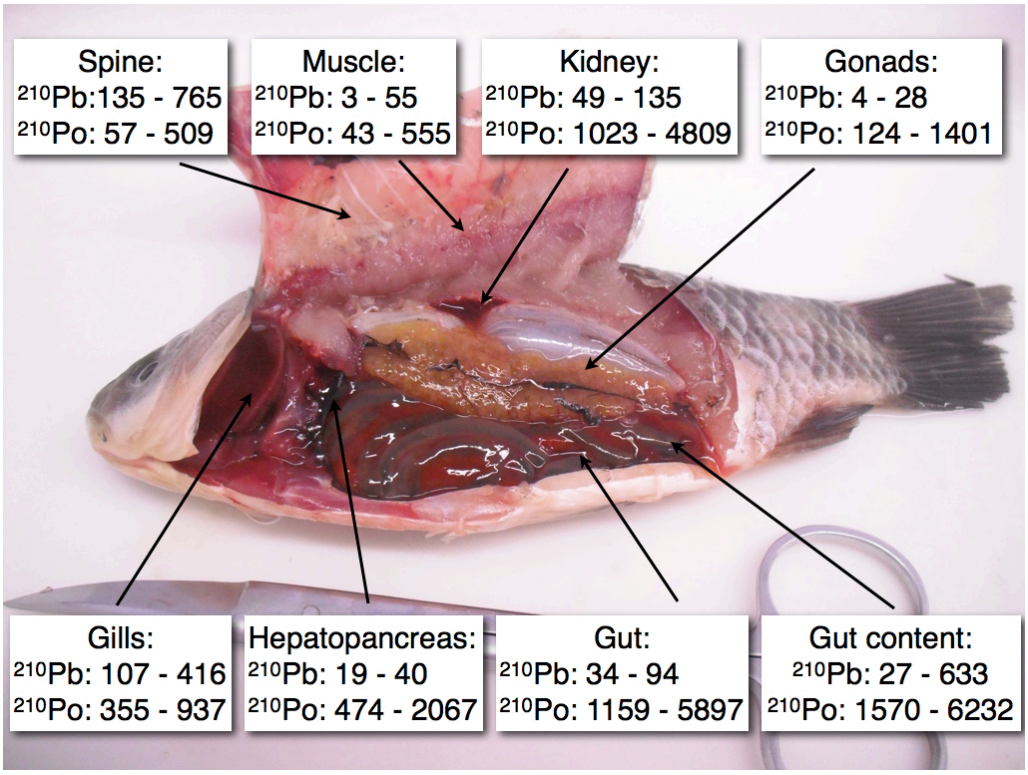

Figure 17. Ranges of values of concentration of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. auratus* (n=7).

Results from Table 6 are shown in three different figures due to the great amount of factors contained in the table. By separating each sample station in one different figure, all the labels can be shown, allowing an easier comprehension of the information.

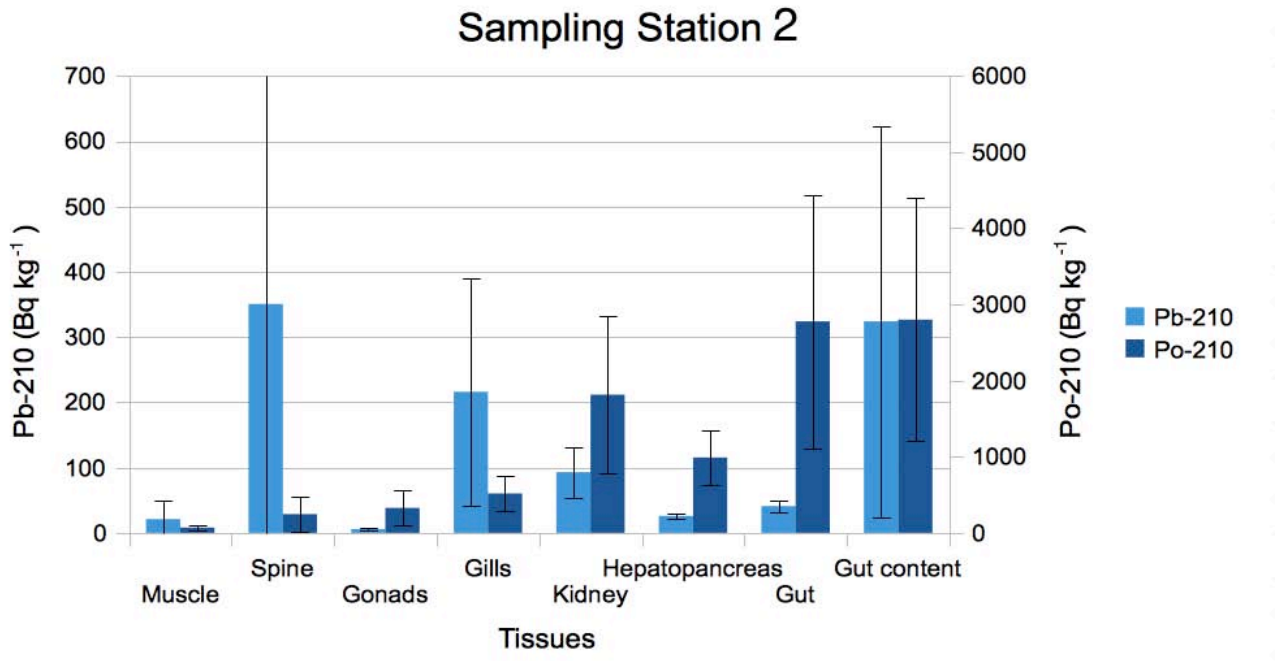

Figure 18. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. auratus* (n=7) from the sampling station 2.

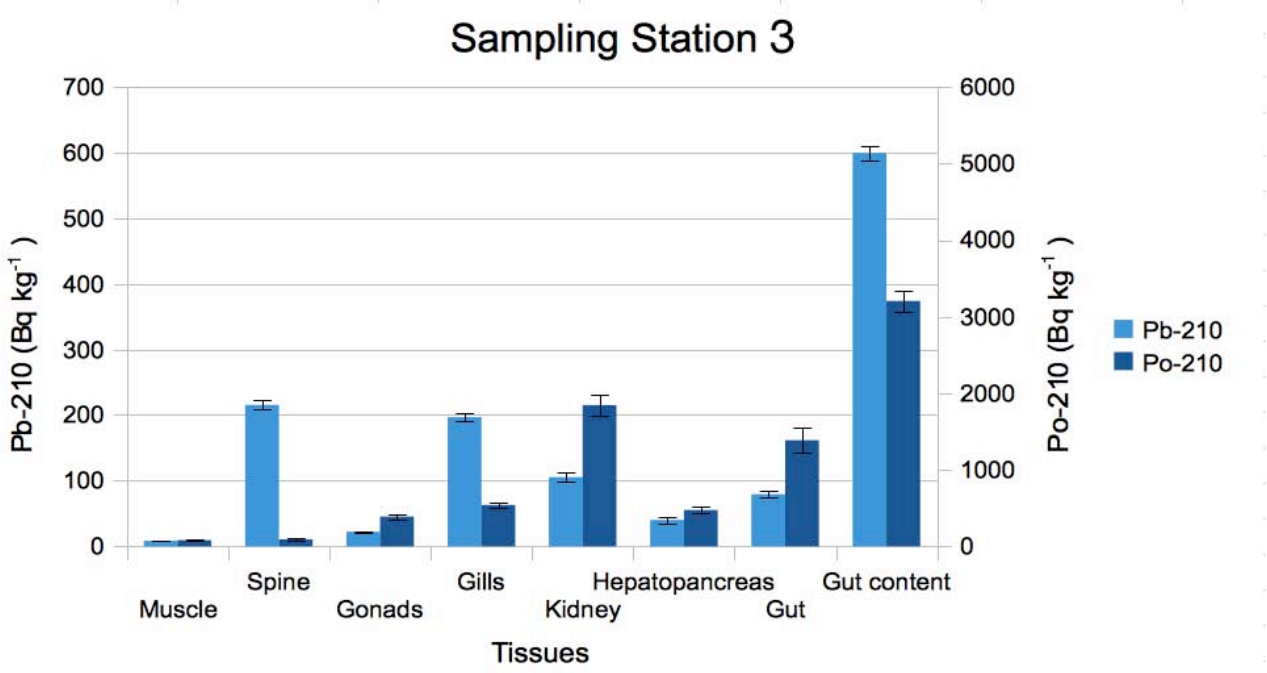

Figure 19. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. auratus* (n=7) from the sampling station 3.

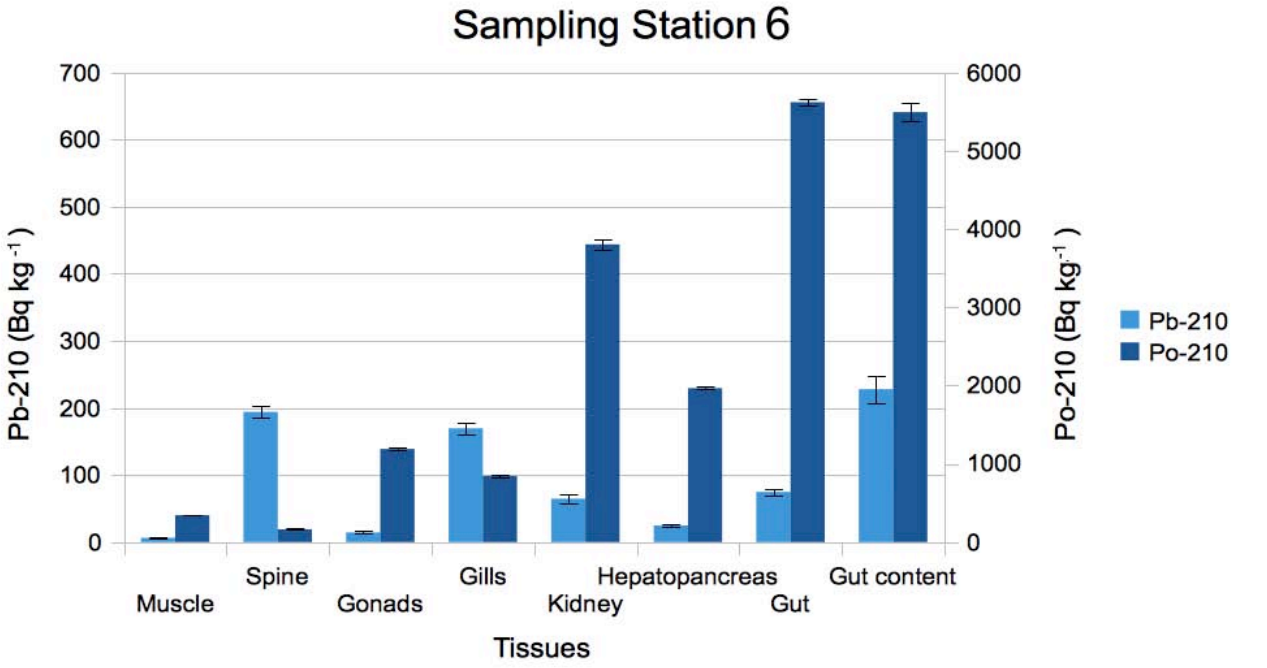

Figure 20. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. auratus* (n=7) from the sampling station 6.

Results from individuals collected at St 2 showed highly inter-individual variations within the same species resulting in a wide range of ^{210}Pb and ^{210}Po concentrations and therefore some mean values with a standard deviation higher than the concentration value itself. These variations seemed to be related to the physiological condition of fish, where length and weight play an important role. Nevertheless, individuals collected at St 3 also showed variability on weight and length between individuals and there was less variability on the values obtained. Hence, not only physiological factors could be conditioning the accumulation of ^{210}Pb and ^{210}Po. These variations could also be caused due to patterns of animal behavior, such as the mobility of individuals from St 2 to the

other areas. Hence, they could be exposed to different concentrations of ^{210}Pb and ^{210}Po in waters.

There was a high variation among the ^{210}Po/^{210}Pb ratio calculated for the tissues analyzed (Figure 21). Results showed a broad range of ^{210}Po accumulation, depending on the tissue analyzed. The lowest values (from 0.4±0.1 to 0.7±1.4)were observed in spine, where ^{210}Pb accumulated in greater proportion than ^{210}Po. Furthermore, ratios on gills, and muscle of individuals in the three sampling stations were also low (from 2.4±0.9 to 9.6±0.1) in stations 3 and 6, where accumulation of ^{210}Po was lower than ten times the concentration of ^{210}Pb, in comparison to the other ratio values. However, ^{210}Po/^{210}Pb ratio for the muscle in St 6 shows a very high value. Highest values corresponded to gonads, hepatopancreas and gut from sites 2 and 6, and also kidney in site 6.

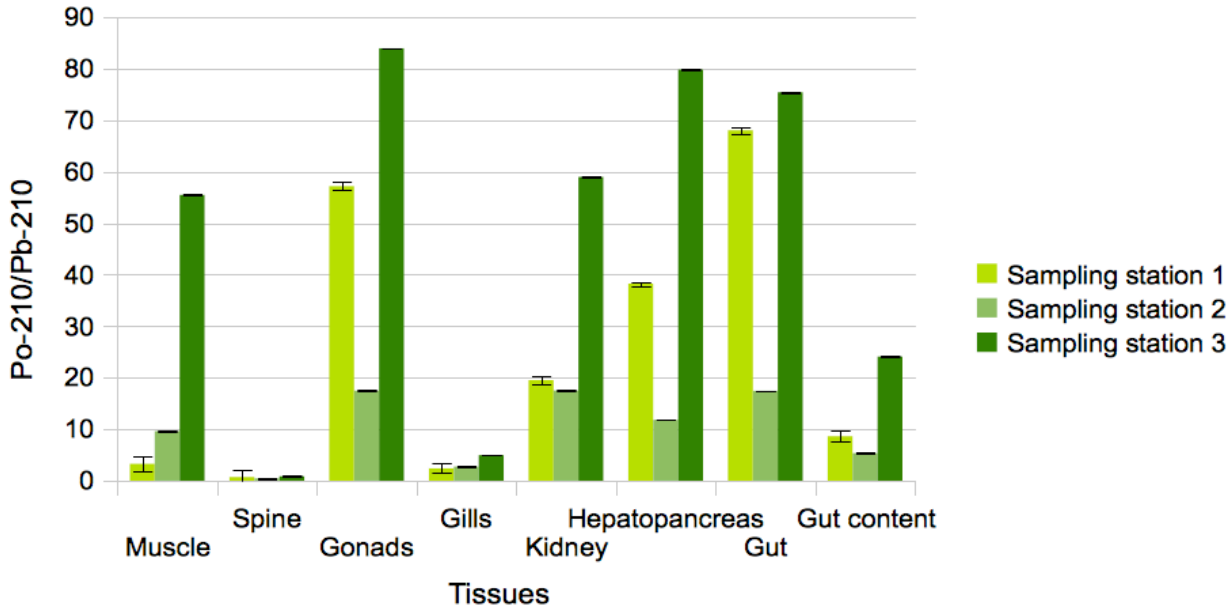

Figure 21. ^{210}Po:^{210}Pb ratio in the tissues of individuals of *C. auratus* from the three sampling stations.

In general terms, concentrations of ^{210}Po in tissues of fishes from the St 6 were higher than those from other sampling stations, doubling the values in most of the tissues This result could be related to ^{210}Po concentration in water from the Wst 6, which had the highest concentrations of ^{210}Po both, particulate and dissolved fraction, between water samples. Concentration of ^{210}Po was 1.3 and 1.8 times higher than concentration at St 2 and St 3 respectively.

Regarding fish size, there is no apparent relation between ^{210}Po concentration and weight or length. Individuals from St 6, the ones with the highest ^{210}Po concentrations in tissues coincide to be, generally, the smallest ones. Despite the high variability shown in TL and weight from individuals of St 2 and St 3, their ^{210}Po concentration in tissues are very similar.

More to this point, there are no significant differences between concentration of ^{210}Pb in tissues among individuals of different size or weight.

7.2.4. *Cyprinus carpio*

Concentrations of ^{210}Po and ^{210}Pb in tissues of *C. carpio* are shown in Table 7. Results (see Figure 22) shown that muscle had a concentration of ^{210}Pb that ranged from 6±0 to 13±1 Bq kg^{-1} with an average concentration of 8±4 Bq kg^{-1}. ^{210}Po concentration of muscle ranged from 19±3 to 32±2 Bq kg^{-1} with an average concentration of 28±8 Bq kg^{-1}. Spine displayed a concentration of ^{210}Pb that ranged from 122±6 to 221±11 Bq kg^{-1} with an average of 169±50 Bq kg^{-1}. Concentrations of ^{210}Po in spine ranged from 64±7 to 94±23 Bq kg^{-1} and the average of 76±16 Bq kg^{-1}. Concentration of ^{210}Pb in gonads ranged from 14±1 to 18±1 Bq kg^{-1} and the average value was 16±2 Bq kg^{-1}. In comparison, concentration of ^{210}Po was much higher, with a range of 92±7 to 711±26 Bq kg^{-1} with an average of ^{210}Po concentration of 398±310 Bq kg^{-1}. Concentration of ^{210}Pb in gills ranged from 72±3 to 167±10 Bq kg^{-1} with an average of 112±49. Concentration of ^{210}Po was in a range of 201±8 to 343±11 Bq kg^{-1}. Concentration of ^{210}Pb in hepatopancreas ranged from 29±4 to 181±209 Bq kg^{-1}. On the other hand, concentration of ^{210}Po was in a range of 292±14 to 788±28 Bq kg^{-1} with an average of 595±266 Bq kg^{-1}. In kidney, ^{210}Pb concentration ranged from 24±4 to 181±209 Bq kg^{-1} with an average of 90±82 Bq kg^{-1}, and ^{210}Po from 1005±53 to 2102±64 Bq kg^{-1} with an average of 1641±569 Bq kg^{-1}. Concentration of ^{210}Pb in gut ranged from 18±1 to 33±3 Bq kg^{-1} with an average of 28±8 Bq kg^{-1}, and ^{210}Po concentration was in the range from 1525±64 to 2033±65 Bq kg^{-1}. Finally, ^{210}Pb concentration in gut content ranged from 60±4 to 208±11 Bq kg^{-1}, with an average of 155±82 Bq kg^{-1}. ^{210}Po concentration in gut content ranged from 3288±144 to 4691±221 Bq kg^{-1} with an average of 3781±788 Bq kg^{-1}. Highest concentration of ^{210}Pb was found in gut content, followed by gills and spine. The lowest ^{210}Pb concentration was found on muscle and gonads. On the other hand, the highest ^{210}Po concentration was found on gut content, followed by gut and kidney. The lowest concentrations of ^{210}Po were found on muscle and spine.

Among the different sample stations, spine concentrations of ^{210}Pb were similar between stations but slightly higher (140%) at St 2 than at St 3 or St 6. Gills showed the same pattern, with a concentration at St 2 two times higher than St 6, whereas St 3 showed an intermediate value. Kidney showed a great difference between sampling stations, St 2 (181± 209 Bq kg^{-1}), despite its high uncertainty, almost tripled the activity of St 3 (66±4), the same that did St 3 with St 6 concentration (24±2 Bq kg^{-1}). In the case of hepatopancreas, St 2 showed the highest values (129±9 Bq kg^{-1}), 3.6 and 4.5 times higher than concentrations at St 3 and St 6 respectively (36±3 and 29±4 Bq kg^{-1}). For gut content, at St 2 and St 6 high concentrations (208±11 and 197±11 Bq kg^{-1}) could be found, whereas at St 3 concentration was 3.5 times lower (60±4 Bq kg^{-1}). In muscle, concentrations were similar among the three sample stations and the values were low (ranging from 5.7±0.4 to 13±1 Bq kg^{-1}).

Concentrations of ^{210}Po in tissues showed a similar pattern between the three sites. Concentrations in hepatopancreas at St 2 and St 3 were almost 2.5 times higher than concentration at St 6 (706±40 Bq kg^{-1}, 788±28 Bq kg^{-1} and 292±14 Bq kg^{-1} respectively). Concentration in gonads from St 3 was higher than concentration at St 2 or St 6 (711±26 Bq kg^{-1}, 92±7Bq kg^{-1} and 390±21 Bq kg^{-1} at different stations, respectively).

Table 7. Average values of concentration of ^{210}Pb and ^{210}Po (in Bq kg^{-1}dry weight) in tissues and gut content of *C. carpio* (n=4). TL and body weight are average values when more than one individual was collected.

C. carpio		Tissue	Dry:wet weight ratio	^{210}Pb	^{210}Po	^{210}Po/^{210}Pb
Sampling station	2	Muscle	0.23	13 ± 1	19 ± 3	1.5 ± 0,2
		Spine	0.54	221 ± 11	94 ± 23	0.4 ± 0,3
Number of individuals	1	Gonads	0.22	14 ± 1	92 ± 7	6.8 ± 0,1
		Gills	0.19	167 ± 10	240 ± 24	1.4 ± 0,1
TL (cm)	26	Kidney	0.19	181 ± 209	1815 ± 2039	10.0 ± 1,6
		Hepatopancreas	0.18	129 ± 9	706 ± 40	5.5 ± 0,1
Body weight (g)	295	Gut	0.15	33 ± 3	1677 ± 85	51.3 ± 0,1
		Gut content	0.19	208 ± 11	4691 ± 221	22.5 ± 0,1
Sampling station	3	Muscle	0.20	5,7 ± 0,4	32 ± 1	5.7 ± 0,1
		Spine	0.14	163 ± 5	64 ± 7	0.4 ± 0,1
Number of individuals	2	Gonads	0.26	16 ± 2	711 ± 26	45.1 ± 0,1
		Gills	0.17	96 ± 4	343 ± 11	3.6 ± 0,0
TL (cm)	31±10	Kidney	0.19	66 ± 4	2102 ± 64	31.8 ± 0,1
		Hepatopancreas	0.20	36 ± 3	788 ± 28	22.1 ± 0,1
Body weight (g)	516±460	Gut	0.18	32 ± 2	2033 ± 65	63.6 ± 0,1
		Gut content	0.18	60 ± 4	3366 ± 117	56.2 ± 0,1
Sampling station	6	Muscle	0.19	7 ± 0	32 ± 2	4.8 ± 0,1
		Spine	0.50	122 ± 6	70 ± 8	0.6 ± 0,1
Number of individuals	1	Gonads	0.21	18 ± 1	390 ± 21	21.9 ± 0,1
		Gills	0.16	72 ± 3	201 ± 8	2.8 ± 0,1
TL (cm)	21	Kidney	0.18	24 ± 2	1005 ± 53	42.4 ± 0,1
		Hepatopancreas	0.19	29 ± 4	292 ± 14	10.1 ± 0,1
Body weight (g)	156	Gut	0.13	18 ± 1	1525 ± 64	82.5 ± 0,1
		Gut content	0.19	197 ± 11	3288 ± 144	16.7 ± 0,1

The ^{210}Po and ^{210}Pb concentration in kidney from the individual of the St 2 (Figure 23) represents the average from results obtained from 3 replicates of the same sample. Concentrations showed a standard deviation higher than the average value (181±209 Bq kg^{-1} of ^{210}Pb and 1815±2039 Bq kg^{-1} of ^{210}Po). This sample was analyzed three times, because the tissue was big enough to allow three sub-samples from the same sample, by taking every time a piece of the tissue. The high deviation obtained in the results showed the low homogeneity of ^{210}Po and ^{210}Pb concentration in this organ.

In this species there was also a high variation among the ^{210}Po/^{210}Pb ratio calculated in the analyzed tissues (Figure 26). The lowest values were shown in spine (ranging from 0.4±0.1 to 0.6±0.1), where once again, ^{210}Pb accumulated more than ^{210}Po. Furthermore, ratios on gills and muscle of the three sampling stations and hepatopancreas from sites 1 and 3 were also low (ranging from 1.4±0.1 to 5.7±0.1), where accumulation of ^{210}Po was

more than ten times lower than the concentration of ^{210}Pb, in comparison to the other ratio values. Highest values corresponded to gut (from the three sampling stations), gut content, and gonads from St 3 and kidney and gonads from individuals at St 6.

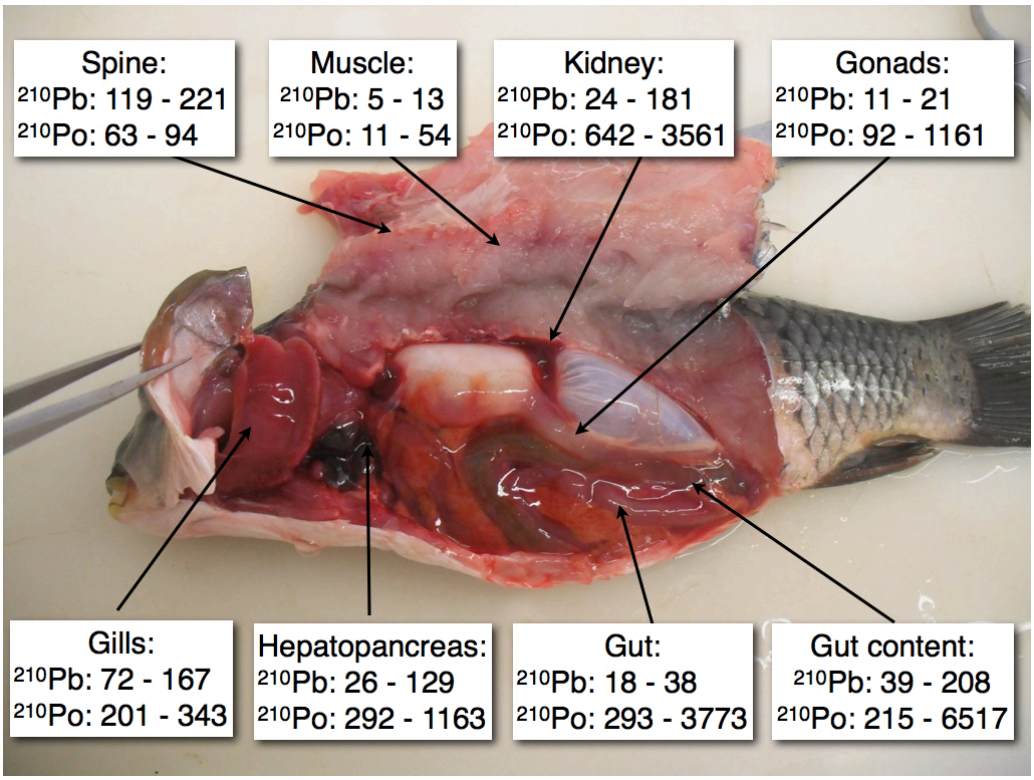

Figure 22. Ranges of values of concentration of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. carpio* (n=4).

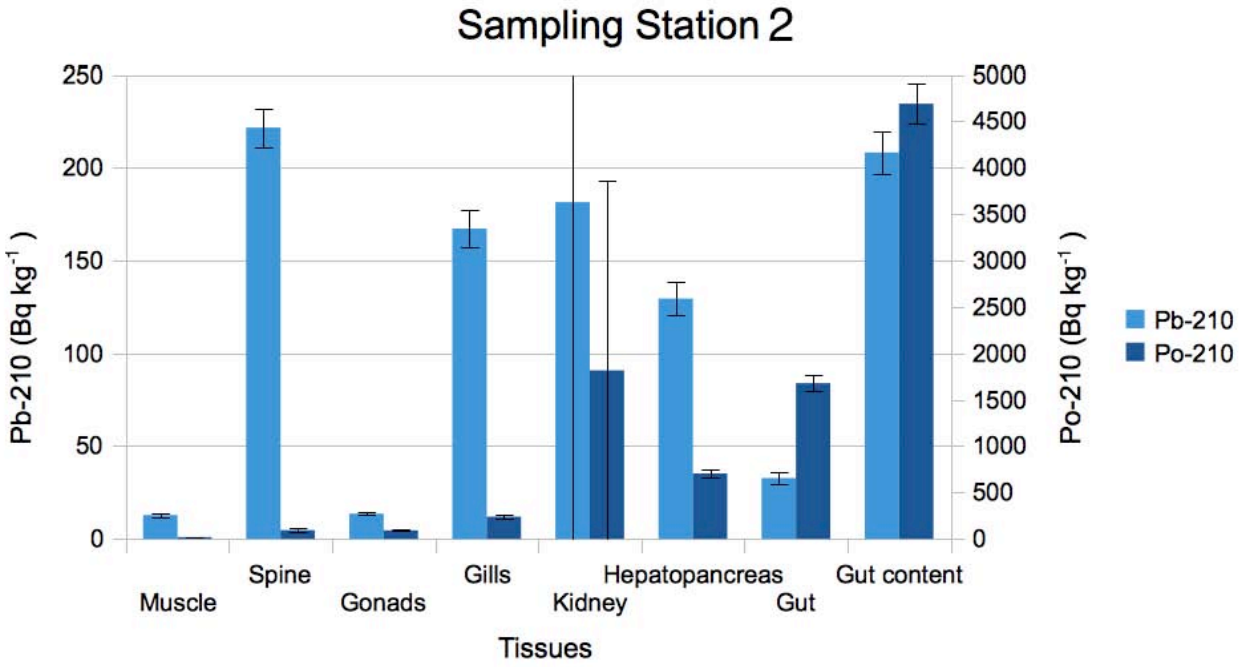

Figure 23. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. carpio* (n=4) from the sampling station 2.

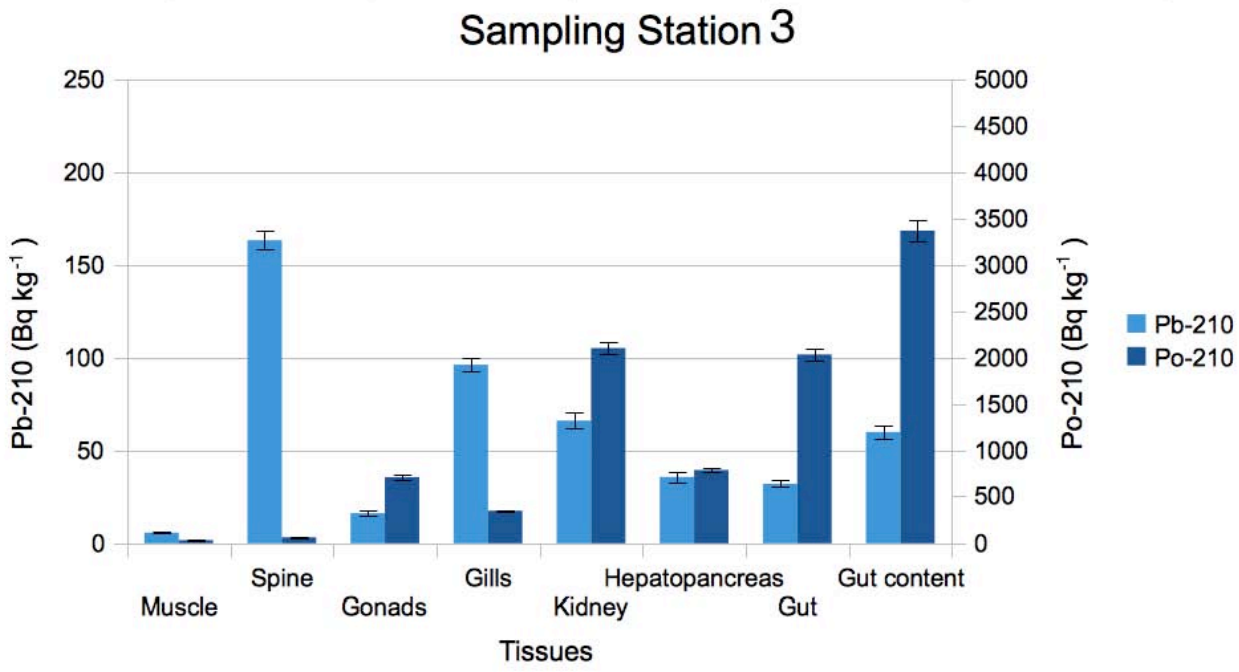

Figure 24. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. carpio* (n=4) from the sampling station 3.

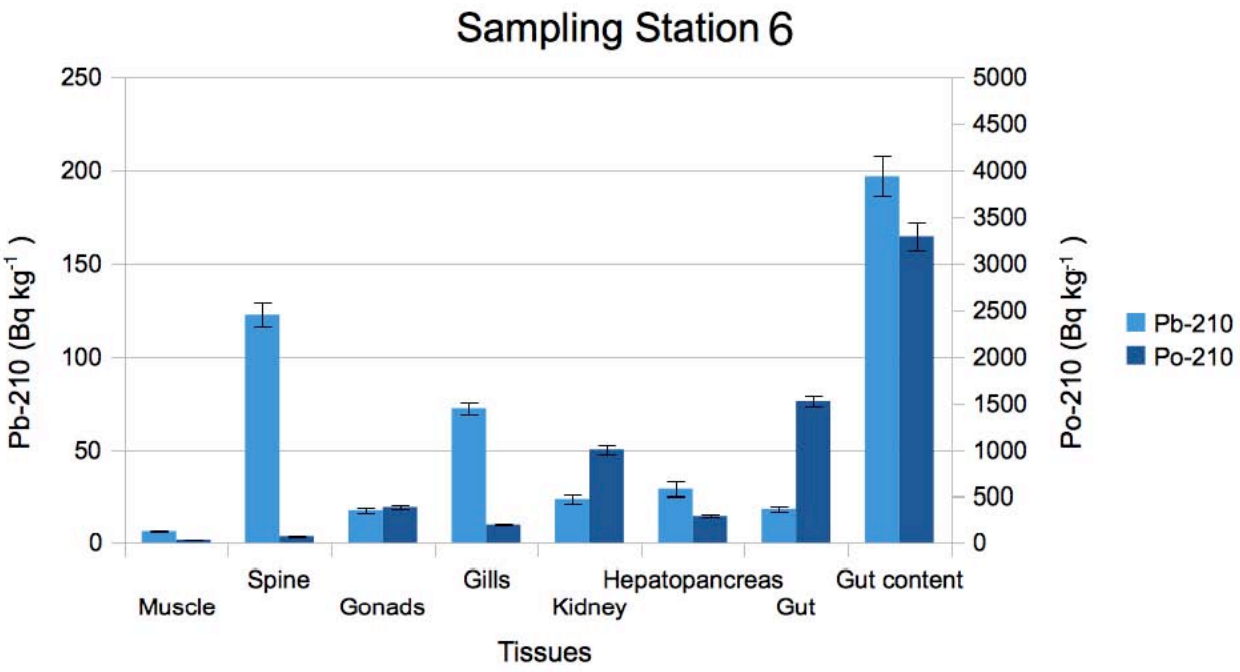

Figure 25. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. carpio* (n=4) from the sampling station 6.

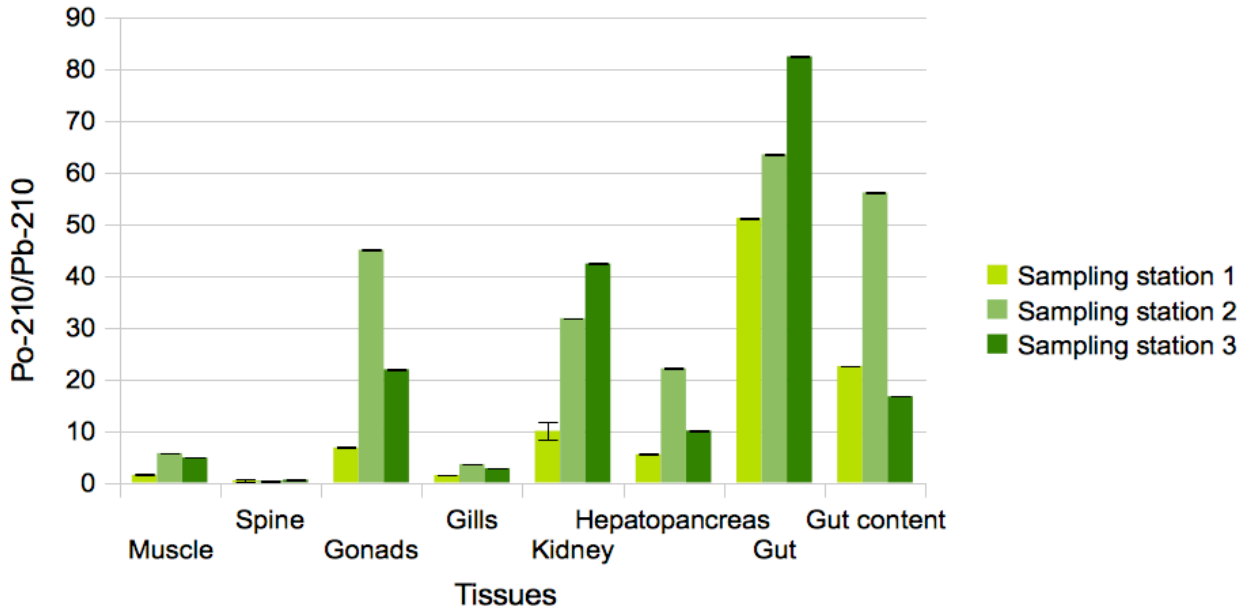

Figure 26. ^{210}Po/^{210}Pb ratio in the tissues of individuals of *C. carpio* (n=4) from the three sampling stations.

In general terms, concentrations of ^{210}Po in tissues of fishes from the St 3 were higher than those from other sampling stations. If accumulation of ^{210}Po had depended of concentrations in water, highest concentrations would have been seen on St 6. The same situation was repeated with ^{210}Pb concentration. Highest values of ^{210}Pb were shown in samples from St 2, where ^{210}Pb concentration in water was the lowest.

Regarding fish size, there is no apparent relation between ^{210}Po concentration and weight or length. This statement is confirmed by the individuals from St 3. Both individuals show high differences in length and weight, as it is shown in the average size and weight: 31±10 cm and 516±460 g. If accumulation of ^{210}Po or ^{210}Pb had depended of size and length, ^{210}Po and ^{210}Pb concentrations would have had high uncertainties in the different tissues.

7.2.4. *Chelon labrosus*

Concentrations of ^{210}Po and ^{210}Pb in tissues of *C. labrosus* are shown in Table 8. As it is shown in Figure 27, in the fish organism, muscle had a concentration of ^{210}Pb that ranged from 5.8±0.3 to 98±3 Bq kg^{-1} d.w. with an average concentration of 38±53 Bq kg^{-1} . ^{210}Po concentration of muscle ranged from 25±2 to 122±4 Bq kg^{-1} with an average concentration of 61±54 Bq kg^{-1}. Spine showed a concentration of ^{210}Pb that ranged from 368±14 to 622±21 Bq kg^{-1} with an average of 461±140 Bq kg^{-1}. Concentrations of ^{210}Po in spine was lower than those of ^{210}Pb, ranging from 258±41 to 366±20 Bq kg^{-1} and the average of 310±55 Bq kg^{-1}. Concentration of ^{210}Pb in gonads ranged from 18±1 to 34±4 Bq kg^{-1} and the average value was 26±8 Bq kg^{-1}. In comparison, concentration of ^{210}Po was higher, with a range of 128±12 to 541±17 Bq kg^{-1}. The average of ^{210}Po concentration was 310±55 Bq kg^{-1}. Concentration of ^{210}Pb in gills was in a range of 347±15 to 907±28 Bq kg^{-1} with an average of 706±311 Bq kg^{-1}. On the other hand, concentration of ^{210}Po was in a range of 376±38 to 982±31 Bq kg^{-1} and an average of 682 ±303 Bq kg^{-1}. Concentration of ^{210}Pb in hepatopancreas ranged from 53±4 to 123±4 Bq kg^{-1} with an average value of 93±36 Bq kg^{-1}. On the other hand, concentration of ^{210}Po

was in a range of 1000±95 to 1186±30 Bq kg^{-1} with an average of 1103±95 Bq kg^{-1}. In kidney, ^{210}Pb concentration ranged from 74±6 to 366±14 Bq kg^{-1} with an average of 248±154, and ^{210}Po from 1195±73 to 2205±99 Bq kg^{-1} with an average of 1544±573 Bq kg^{-1}. Gut concentration of ^{210}Pb ranged from 52±2 to 135±5 Bq kg^{-1} with an average of 63±68 Bq kg^{-1}, and ^{210}Po concentration was in the range from 2542±76 to 3311±141 Bq kg^{-1}. Finally, ^{210}Pb concentration in gut content ranged from 92±6 to 1014±38 Bq kg^{-1}, with an average of 475±481 Bq kg^{-1}. ^{210}Po concentration in gut ranged from 4685±232 to 15920±703 Bq kg^{-1} with an average of 8558±6378 Bq kg^{-1}. Highest concentration of ^{210}Pb was found in spine, followed by gut content and gills. The lowest ^{210}Pb concentration was found on muscle and gonads. On the other hand, the highest ^{210}Po concentration was found on gut content, followed by gut and kidney. The lowest concentrations of ^{210}Po were found on muscle and spine.

When comparing sampling sites, it is observed that gill concentrations of ^{210}Pb were the highest ones, showing similar values to the individuals from St 3 and St 6 although the concentration in St 2 is 2.5 times lower. The highest concentration of ^{210}Pb was found on gut content from the St 3, and showed high differences with those from St 2 and St 6, being 11 and 3.2 times higher, respectively. Spines also showed higher concentrations, ranging from 368 to 622 Bq kg^{-1}. The rest of the tissues showed lower concentrations. Kidney presented similar concentrations in St 3 and St 6 (303±10 Bq kg^{-1} and 366±14 Bq kg^{-1}), being 4 and 5 times higher than concentrations in individuals from St 2. Despite its low value, concentrations of ^{210}Pb in muscle at St 3 were 17 and 11 times higher than those at St 2 and St 6, respectively.

Concentrations of ^{210}Po were generally low, except for gut content, which individuals from St 3 reached 15 920 Bq kg^{-1}. Values of ^{210}Po in gut content in species collected in St 2 and St 6 were also high, but three times lower (4685±232 and 5070±171 Bq kg^{-1} respectively). Values of concentration in gut were also high, showing similar concentrations in individuals from St 3 and St 6 (3311±141 and 2542±76 Bq kg^{-1} respectively). Concentrations of ^{210}Po in hepatopancreas and kidney showed similar results for all the sampling stations, being ^{210}Po concentration in kidney from the St 3 slightly higher. In this species, the general pattern of concentration of ^{210}Po seemed to be: higher concentrations at St 3, followed by St 6 and being St 2 the sample station with the lowest concentration.

Table 8. Average values of concentration of ^{210}Pb and ^{210}Po (in Bq kg^{-1}dry weight) in tissues and gut content of *C. labrosus*. TL and body weight are average values when more than one individual was collected.

C. labrosus		Tissue	Dry:wet weight ratio	^{210}Pb	^{210}Po	^{210}Po/^{210}Pb
Sampling station	1	Muscle	0.22	5,8 ± 0,3	25 ± 2	4.3 ± 0.1
		Spine	0.77	368 ± 14	258 ± 41	0.7 ± 0.2
Number of individuals	1	Gonads	0.68	28 ± 3	128 ± 12	4.6 ± 0.1
		Gills	0.22	347 ± 15	376 ± 38	1.1 ± 0.1
TL (cm)	30	Kidney	0.22	74 ± 6	1195 ± 73	16.1 ± 0.1
		Hepatopancreas	0.23	53 ± 4	1124 ± 65	21.2 ± 0.1
Body weight (g)	244	Gut	0.16	-	-	- -
		Gut content	0.19	92 ± 6	4685 ± 232	51.2 ± 0.1
Sampling station	2	Muscle	0.22	98 ± 3	122 ± 4	1.2 ± 0.05
		Spine	0.58	622 ± 21	366 ± 20	0.6 ± 0.1
Number of individuals	2	Gonads	0.21	34 ± 4	541 ± 17	16.1 ± 0.1
		Gills	0.21	863 ± 23	982 ± 31	1.14 ± 0.04
TL (cm)	32±3	Kidney	0.21	303 ± 10	2205 ± 99	7.3 ± 0.1
		Hepatopancreas	0.18	123 ± 4	1186 ± 30	9.64 ± 0.04
Body weight (g)	314±119	Gut	0.20	52 ± 2	3311 ± 141	63.4 ± 0.1
		Gut content	0.29	1014 ± 38	15920 ± 703	15.7 ± 0.1
Sampling station	3	Muscle	0.28	8,9 ± 0,5	36 ± 1	4.0 ± 0.1
		Spine	0.55	394 ± 13	305 ± 23	0.8 ± 0.1
Number of individuals	2	Gonads	0.21	18 ± 1	327 ± 10	18.5 ± 0.1
		Gills	0.25	907 ± 28	689 ± 30	0.8 ± 0.1
TL (cm)	35±4	Kidney	0.34	366 ± 14	1233 ± 38	3.4 ± 0.05
		Hepatopancreas	0.32	103 ± 3	1000 ± 25	9.7 ± 0.05
Body weight (g)	469±221	Gut	0.20	135 ± 5	2542 ± 76	18.8 ± 0.05
		Gut content	0.32	320 ± 10	5070 ± 171	15.8 ± 0.05

Great variation was found among the ^{210}Po/^{210}Pb ratio calculated for the tissues analyzed (Figure 31). As observed in the other species, spines showed higher accumulations of ^{210}Pb above ^{210}Po. In this species, the ^{210}Po/^{210}Pb ratio was also lower for the gills (with values that range from 0.8±0.1 to 1.1±0.1), where in species from St 6 there was a greater accumulation of ^{210}Pb, instead of ^{210}Po as observed in spines and individuals collected at St 2 and St 3 where there was almost the same accumulation of ^{210}Pb than ^{210}Po. Values for muscle were low in comparison to the ratio of other tissues, concentrating only from 1.25 to 4.30 times more ^{210}Po than ^{210}Pb. Depending on the sampling station, values for gonads, kidney and hepatopancreas were also low. For instance, gonads from individuals in St 2 accumulated 4 times less ^{210}Po than gonads from the other sampling stations. Highest ratios were found on gut and gut contents where gut from individuals from St 3 accumulated 63 times more ^{210}Po than ^{210}Pb, gut contents from St 3 and St 6 accumulated almost 16 times more ^{210}Po and the ratio ^{210}Po/^{210}Pb of the gut content from samples collected at St 2 was 51.

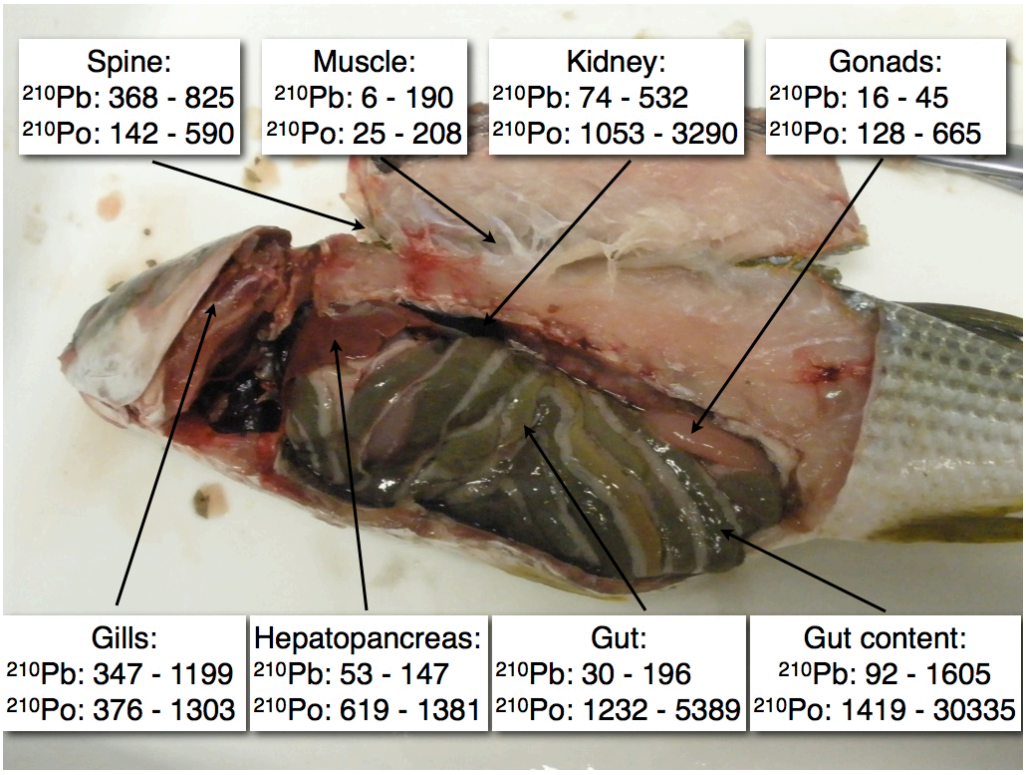

Figure 27. Ranges of values of concentration of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. labrosus* (n=5). Values are shown in dry weight.

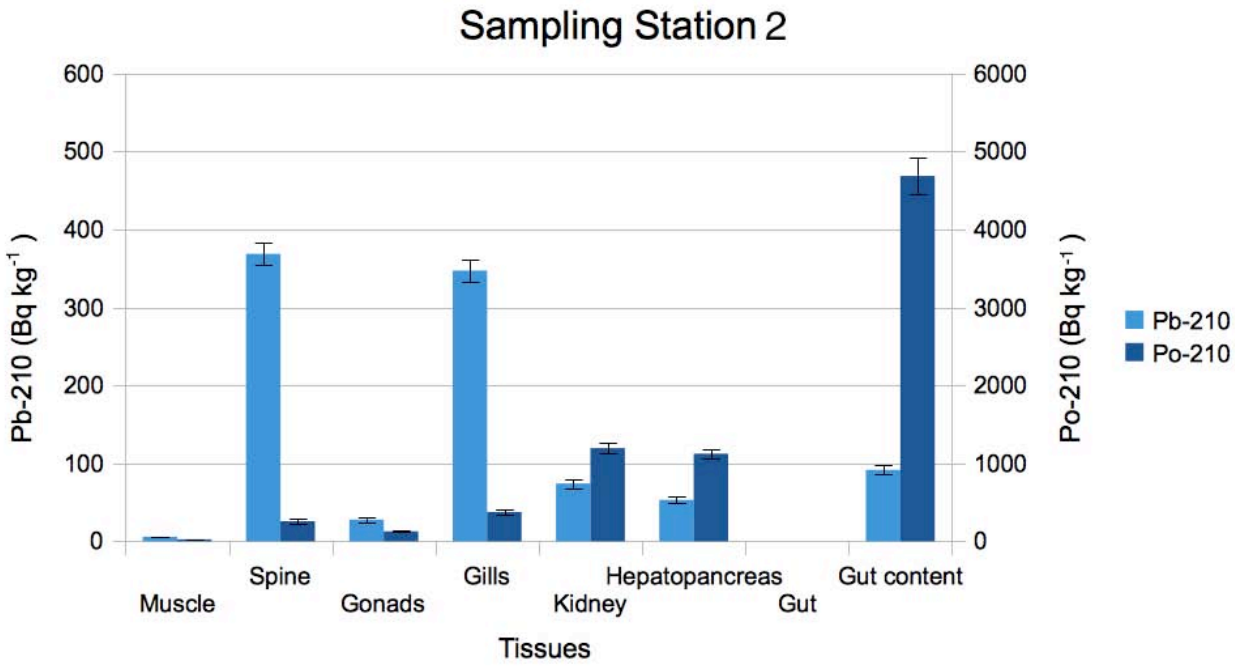

Figure 28. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. labrosus* (n=5) from the sampling station 1. Results are shown in dry weight.

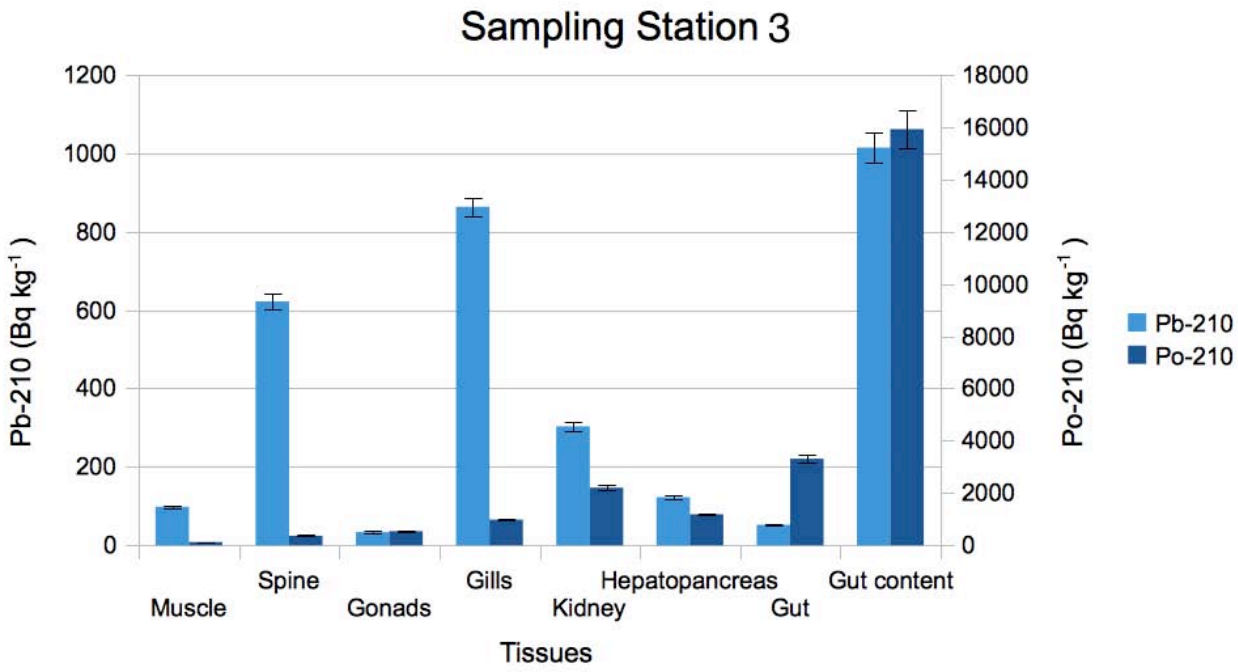

Figure 29. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. labrosus* (n=5) from the sampling station 2. Results are shown in dry weight.

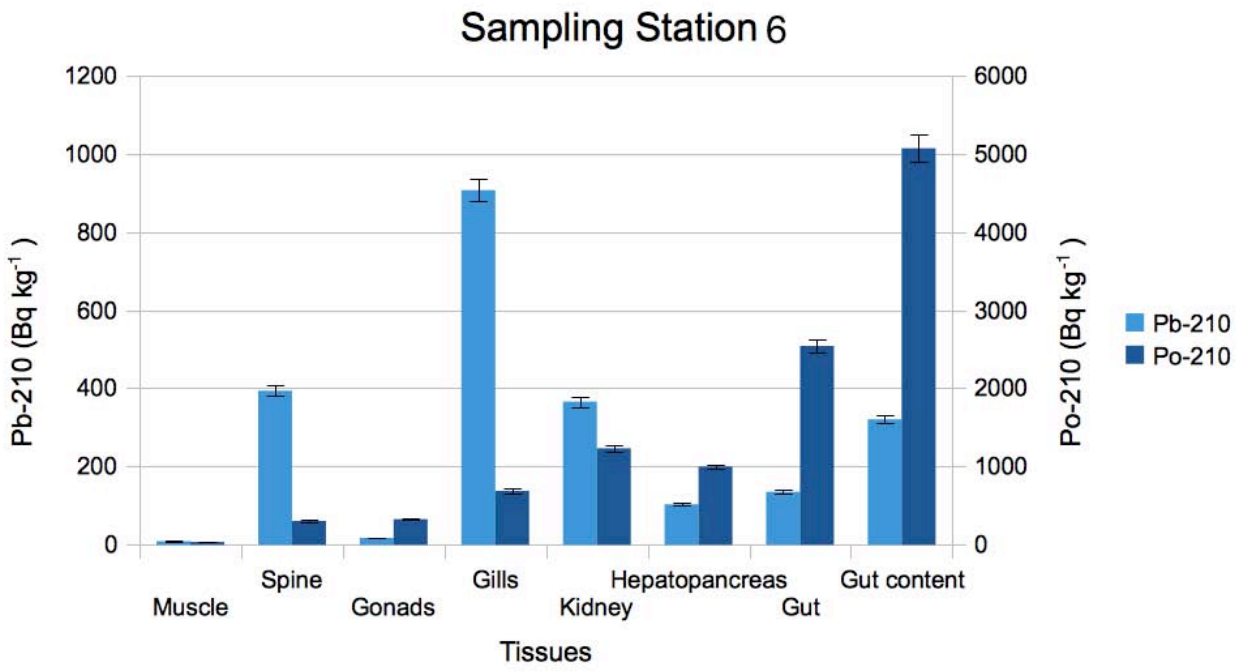

Figure 30. Concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues of *C. labrosus* (n=5) from the sampling station 2. Results are shown in dry weight.

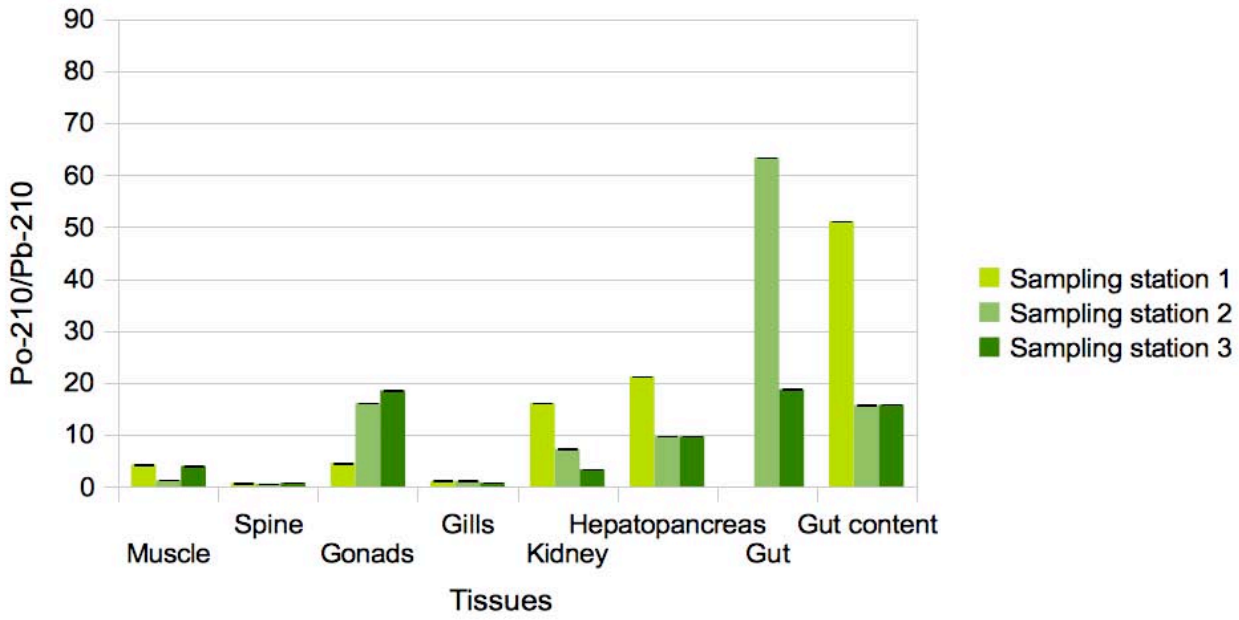

Figure 31. ^{210}Po/^{210}Pb ratio in the tissues of individuals of *C. labrosus* (n=5) from the three sampling stations.

In general terms, ^{210}Pb concentration is higher in fishes from Wst 3 and 6, but accumulation of ^{210}Po in tissues does not point higher in one specific sampling station. Furthermore, *C. labrosus* is a specie that moves within different waters, from sea to marsh water. It would be difficult to establish a relation between water concentrations of ^{210}Pb and ^{210}Po and concentration in tissues of this species.

Regarding fish size, there is no apparent relation between ^{210}Po and ^{210}Pb concentration and weight or length.

7.3. Blank Samples

7.3.1. *Gambusia holbrooki*

Concentrations of ^{210}Po and ^{210}Pb in *Gambusia hobrooki* are shown in Table 9. As can be observed from this table, all the concentrations of ^{210}Po were higher than those of ^{210}Pb. Values of ^{210}Pb ranged from 5±2 to 17±2 Bq kg^{-1} and concentrations of ^{210}Po ranged from 19±5 to 41±8 Bq kg^{-1}.

^{210}Po/^{210}Pb ratios were calculated and they shown that ^{210}Po is accumulated from 1.65 to 7.03 times more than ^{210}Pb.

Table 9. Biological measures and concentrations of ^{210}Po and ^{210}Pb (in Bq kg^{-1} dry weight)in tissues of blank samples of *Gambusia holbrooki* (n=5).

Code	Sampling Station	Sex	SL (mm)	Body Weight (g)	Dry:wet weight ratio	^{210}Pb	^{210}Po	^{210}Po/^{210}Pb
50	Blank samples	M	18.01	0.07	0.49	13 ± 4	38 ± 6	3.0 ± 0.3
51		M	16.91	0.09	0.38	8 ± 3	29 ± 5	3.7 ± 0.4
52		F	21.11	0.12	0.41	5 ± 2	36 ± 5	7.0 ± 0.4
53		M	18.60	0.10	0.37	11 ± 3	19 ± 5	1.6 ± 0.4
54		F	16.05	0.10	0.33	17 ± 4	41 ± 8	2.4 ± 0.3

In comparison with results obtained from Peníscola marsh, average concentration of ^{210}Pb in marsh fish samples double the average concentration of ^{210}Pb in blank samples (24±13 and 11±1 Bq kg^{-1}, respectively). Furthermore, concentrations of ^{210}Po in marsh samples (with a mean value of 188±55 Bq kg^{-1}) are 6 times higher than concentrations found in blank samples (with a mean value of 33±1 Bq kg^{-1}). Therefore, it can be concluded that individuals of *G. holbrooki* from the Peníscola marsh show increased levels of concentrations of ^{210}Pb and ^{210}Po in their tissues.

7.3.2. *Carassius auratus*

Concentration values of ^{210}Po and ^{210}Pb in tissues of a blank sample of *Carassius auratus* are shown in Table 10. Highest concentration of ^{210}Pb was shown in hepatopancreas, with an extremely high value (740±187 Bq kg^{-1}). Also spine and kidney showed high concentrations of ^{210}Pb (60±27 and 59±19 Bq kg^{-1} respectively). The rest of the tissues displayed concentrations of ^{210}Pb ranging from 7±3 to 37±8 Bq kg^{-1}, being muscle the tissue with the lowest concentration.

Concentrations of ^{210}Po were generally higher than those of ^{210}Pb (ranging from 49±5 to 1183±55 Bq kg^{-1}), except in the case of hepatopancreas, where concentration of ^{210}Pb was 2.5 times higher. Extremely high concentrations were found on gut content and gut, followed by hepatopancreas. Kidney, gonads, gills and spine showed intermediate values and the lowest concentration of ^{210}Po was found in muscle.

The ^{210}Po/^{210}Pb ratio was calculated and it was found that in gut content, ^{210}Po was accumulated 119 times more than lead. Gut, gills and gonads show similar values. Unlike other species, in this one, ^{210}Po/^{210}Pb ratio for spine was higher than 1, meaning that ^{210}Po accumulation was higher than ^{210}Pb accumulation. Despite of that, ^{210}Po/^{210}Pb ratio in hepatopancreas was lower than 1. The rest of the values of the ratio range from 1.7±0.5 -spine, the lowest, excepting hepatopancreas - to 20.0±0.2 - gut.

Table 10. Biological measures and concentrations of ^{210}Po and ^{210}Pb (in Bq kg^{-1} dry weight) in tissues of a blank sample of *Carassius auratus* (n=1)

C. auratus		Tissue	Dry:wet weight ratio	^{210}Pb	^{210}Po	^{210}Po/^{210}Pb
Sampling station	Blank sample	Muscle	0.21	8 ± 3	49 ± 5	6.1 ± 0.4
		Spine	0.45	60 ± 27	105 ± 28	1.7 ± 0.5
Number of individuals	1	Gonads	0.22	10 ± 4	146 ± 13	14.4 ± 0.4
		Gills	0.13	7 ± 4	109 ± 9	14.7 ± 0.5
TL (cm)	6	Kidney	0.24	59 ± 18	146 ± 25	2.5 ± 0.3
		Hepatopancreas	0.21	740 ± 187	297 ± 164	0.4 ± 0.6
Body weight (g)	4	Gut	0.16	37 ± 8	737 ± 39	20.0 ± 0.2
		Gut content	0.08	10 ± 6	1183 ± 55	119.0 ± 0.6

Despite the sample was considered as a blank in the beginning, results show that this individual does not correspond to a blank sample, due to its high concentrations of ^{210}Pb in hepatopancreas and ^{210}Po in gut content, gut and hepatopancreas. This sample was collected from a little reservoir without any water input or output except rainwater and evaporation. Thus, the water in this little reservoir (and therefore organisms living there) could be affected by the geology of the surrounding area.

In this species, comparison between marsh individuals and blank samples can not be done.

7.3.3. *Cyprinus carpio*

Concentration values of ^{210}Po and ^{210}Pb in tissues of a blank sample of *Cyprinus carpio* are shown in Table 11. The highest concentration of ^{210}Pb was shown in spine (25±9 Bq kg^{-1}), whereas gonads showed the second highest concentration (9±3 Bq kg^{-1}). The rest of the tissues displayed concentrations of ^{210}Pb ranging from 0.8±0.3 to 5±1 Bq kg^{-1}, being muscle and gut the tissues with the lowest concentration.

Concentrations of ^{210}Po were, in general terms, higher than those of ^{210}Pb, except in the case of spine (10±5 Bq kg^{-1} of ^{210}Po in comparison with 25±9 Bq kg^{-1} of ^{210}Pb), where as observed with all other species, concentration of ^{210}Pb was higher. Highest concentrations were found on gut and hepatopancreas (67±4 and 57±5 Bq kg^{-1} respectively), followed by gonads and kidney, each one with more than a third of the activity of the two tissues mentioned before. Finally, the lowest concentration of ^{210}Po was found on muscle (1.8±0.3 Bq kg^{-1}).

The ^{210}Po/^{210}Pb ratio was calculated and it was found that in gut, ^{210}Po was accumulated 37 times more than lead. On the other hand, and as it was observed in spine of other species, accumulation of ^{210}Pb is higher to ^{210}Po, so the ^{210}Po/^{210}Pb ratio was below 1. The rest of the values of the ratio range from 1.89 -gills, the lowest, excepting spine- to 10.50 - hepatopancreas.

Table 11. Biological measures and concentrations of ^{210}Po and ^{210}Pb (in Bq kg^{-1} dry weight) in tissues of a blank sample of *Cyprinus carpio* (n=1).

C. carpio		Tissue	Dry:wet weight ratio	^{210}Pb	^{210}Po	^{210}Po/^{210}Pb
Sampling station	Blank sample	Muscle	0.19	0.8 ± 0,3	1.8 ± 0.3	2.3 ± 0.4
		Spine	0.47	25 ± 9	10 ± 5	0.4 ± 0.6
Number of individuals	1	Gonads	0.25	9 ± 3	23 ± 5	2.6 ± 0.4
		Gills	0.18	5 ± 1	10 ± 1	1.9 ± 0.2
TL (cm)	19	Kidney	0.19	4 ± 1	27 ± 2	7.3 ± 0.3
		Hepatopancreas	0.16	5 ± 2	57 ± 5	10.5 ± 0.4
Body weight (g)	103	Gut	0.16	2 ± 1	67 ± 4	37.1 ± 0.3
		Gut content	0.19	3.3 ± 0,5	15 ± 1	4.7 ± 0.2

In comparison with results obtained from Peníscola marsh, average concentration of ^{210}Pb in marsh samples ranges from 7 to 47 times higher than concentrations in blank sample. However, ^{210}Pb concentration in gonads from marsh sample are only 1.7 times higher than blank samples (16±2 Bq kg^{-1} in marsh sample and 9±3 Bq kg^{-1} in blank sample). Furthermore, concentrations of ^{210}Po in marsh samples (average concentrations range from 28±8 Bq kg^{-1} in spine to 3782±789 Bq kg^{-1} in gut content) are from 8 to 245 (for spine and gut content, respectively) times higher than concentrations found in blank samples (concentrations of ^{210}Po 1.8±0.3 Bq kg^{-1} in muscle and 15±1 Bq kg^{-1} in gut content). Therefore, it can be said that individuals of *C. carpio* from Peníscola marsh show increased levels of concentrations of ^{210}Pb and ^{210}Po in their tissues.

7.3.4. *Chelon labrosus*

Concentration values of ^{210}Po and ^{210}Pb in tissues of a blank sample of *Chelon labrosus* are shown in Table 12. Highest concentration of ^{210}Pb was shown in spine (24±10 Bq kg^{-1}), followed by gut (19±2 Bq kg^{-1}). The rest of the tissues displayed concentrations of ^{210}Pb ranging from 0.3±0.2 to 9±2 Bq kg^{-1}, being muscle the tissues with the lowest concentration.

Concentrations of ^{210}Po were generally higher than those of ^{210}Pb, except in the case of spine, where concentration of ^{210}Pb was higher (^{210}Po concentration was 11±7 Bq kg^{-1}) and also ^{210}Pb concentration in gonads was higher than this from ^{210}Po (7±3 and 5±3 Bq kg^{-1} respectively), but values were very similar. Highest concentrations were found on gut and hepatopancreas (82±5 and 46±3 Bq kg^{-1} respectively), followed by gut content (40±2 Bq kg^{-1}). Kidney, gills and spine showed intermediate values and the lowest concentration of ^{210}Po was found on muscle (2.4±0.3 Bq kg^{-1}).

The ^{210}Po/^{210}Pb ratio was calculated and it was found that in gut content, ^{210}Po was accumulated 16.7 times more than lead, and in kidney, 13 times more. On the other hand, and as it happened the same in other species, in spine, accumulation of ^{210}Pb is higher to ^{210}Po, so the ^{210}Po/^{210}Pb ratio was below 1. In this species, accumulation of ^{210}Po in gonads was also lower than accumulation of ^{210}Pb (0.7±0.8). The rest of the values of the ratio range from 2.2±0.2 -gills, the lowest, excepting spine and gonads- to 9.0±0.6 -muscle.

Table 12. Biological measures and concentrations of ^{210}Po and ^{210}Pb (in Bq kg^{-1} dry weight) in tissues of a blank sample of *Chelon labrosus* (n=1).

C. labrosus		Tissue	Dry:wet weight ratio	^{210}Pb	^{210}Po	^{210}Po/^{210}Pb
Sampling station	Blank sample	Muscle	0.23	0.2 ± 0.2	2.4 ± 0.3	9.0 ± 0.6
		Spine	0.63	24 ± 10	11 ± 7	0.4 ± 0.8
Number of individuals	1	Gonads	0.70	7 ± 3	5 ± 3	0.7 ± 0.8
		Gills	0.30	9 ± 2	20 ± 2	2.2 ± 0.2
TL (cm)	16	Kidney	0.25	2 ± 1	28 ± 2	13.1 ± 0.3
		Hepatopancreas	0.31	7 ± 1	46 ± 3	6.9 ± 0.2
Body weight (g)	36	Gut	0.27	19 ± 2	82 ± 5	4.4 ± 0.1
		Gut content	0.23	2 ± 1	40 ± 2	16.7 ± 0.2

In comparison with results obtained from Peníscola marsh, average concentration of ^{210}Pb in marsh samples ranges from 4 to 201 times higher than concentrations in blank sample (average concentrations range from 26±8 Bq kg^{-1} in gonads to 706±311 Bq kg^{-1} in gills in individuals from Peníscola marsh). Furthermore, concentrations of ^{210}Po in marsh samples (average concentrations range from 61±54 Bq kg^{-1} in muscle to 8558±6378 Bq kg^{-1} in gut content) are from 24 to 216 (for muscle and gut content, respectively) times higher than concentrations found in blank samples (concentrations of ^{210}Po 2.4±0.3 Bq kg^{-1} in muscle and 40±2 Bq kg^{-1} in gut content). Therefore, can be said that individuals of *C. labrosus* from Peníscola marsh show increased levels of concentrations of ^{210}Pb and ^{210}Po in their tissues.

7.4. Bioaccumulation Factors (BAF)

Although it is generally recognized that accumulation of radionuclides by aquatic organisms is a dynamic process, many bioaccumulation models assume that the aquatic organisms are in equilibrium with reference media, such as water or sediments, in their surrounding environment. As a result, radionuclide accumulation in aquatic biota is often represented by simplified ratios that relate radionuclide concentrations in biotic tissues to concentrations in the reference media (IAEA, 2009)

The steady state models can be subdivided into two categories on the basis of the chemical behavior of a given radionuclide and its associated transfer processes to biotic tissues. These categories are:

- Models that are based on simple radionuclide partitioning between organisms and reference phases (such as surface water or sediments).
- Specific activity models which assess partitioning of radionuclides relative to stable analogues in the body (IAEA, 2009)

Depending on the radionuclide uptake pathway considered, several representations of partitioning can be defined:

- the concentration ratio (CR), which is the ratio of the radionuclide concentration in biota (C_b) from all exposure pathways (including water, sediment and ingestion/ dietary pathways) on a per unit tissue fresh weight basis to that in water (C_w)

- the biota sediment concentration ratio ($C_{s,b}$), which is the ratio of the concentration of a radionuclide in an organism (C_b) on a fresh weight basis to the radionuclide concentration (fresh weight) measured in the sediment (C_{sed}). C_{sed} derived from studies in which only the sediment shows high levels of radioactivity, where the contribution of sediment associated radionuclides can be of particular importance with respect to radionuclide uptake by benthic species.

(IAEA, 2010)

Most contaminant transfer factors in the literature do not distinguish between uptake pathways, and therefore represent CR values, also called the bioaccumulation factor, BAF (IAEA, 2010). Therefore, in order to determine the level of transference of radionuclides from water to biota, BAF were calculated.

7.4.1. *Gambusia holbrooki*

In this species, BAF for ^{210}Pb had the same order of magnitude for all the individuals (10^2), independently of the sampling station where they were taken. On the other hand, BAF for ^{210}Po ranged from 10^3 to 10^4 depending on the sampling station. Individuals from sampling station 1 showed higher BAF (one order of magnitude above samples from St 6) and individuals from St 3 showed variations on the BAF, having values which differ up to two orders of magnitude.

Table 13. Bioaccumulation factors (BAF) of ^{210}Pb and ^{210}Po of *G. holbrooki*.

Individuals	Sampling Station	BAF ^{210}Pb	BAF ^{210}Po
1	2	$4.48 \cdot 10^2$	$1.57 \cdot 10^4$
2		$1.82 \cdot 10^2$	$1.33 \cdot 10^4$
3		$2.18 \cdot 10^2$	$1.16 \cdot 10^4$
4		$2.02 \cdot 10^2$	$1.16 \cdot 10^4$
5		$2.76 \cdot 10^2$	$1.69 \cdot 10^4$
6	3	$2.08 \cdot 10^2$	$1.02 \cdot 10^4$
7		$3.20 \cdot 10^2$	$7.93 \cdot 10^3$
8		$3.34 \cdot 10^2$	$1.73 \cdot 10^4$
9		$7.24 \cdot 10^2$	$1.32 \cdot 10^4$
10		$4.08 \cdot 10^2$	$8.73 \cdot 10^3$
11	6	$3.70 \cdot 10^2$	$7.84 \cdot 10^3$
12		$2.12 \cdot 10^2$	$4.10 \cdot 10^3$
13		$2.19 \cdot 10^2$	$3.65 \cdot 10^3$
14		$2.43 \cdot 10^2$	$5.29 \cdot 10^3$
15		$2.40 \cdot 10^2$	$7.83 \cdot 10^3$

7.4.2. *Carassius auratus*

BAF factors for *C. auratus* showed high differences depending on the tissue and the sampling station where they were taken. BAF for ^{210}Pb displayed values ranging from 10^1 to 10^3, with differences in two orders of magnitude, and so did BAF for ^{210}Po, where ranged from 10^3 to 10^5.

Lowest values of ^{210}Pb BAF were shown generally in muscle, excepting the values at St 2, where the lowest values were shown on spine and hepatopancreas. Nevertheless, the highest values of ^{210}Pb BAF were gut contents from all the sampling stations.

Moreover, ^{210}Po BAF showed similar patterns, with lowest BAF in muscle and the highest in gut content. Within the other tissues, kidney showed higher values than the other tissues, followed by spine at St 2 and St 3 and by hepatopancreas at St 2 and St 6.

Table 14. Bioaccumulation factors (BAF) of ^{210}Pb and ^{210}Po of *C. auratus* (n=7).

C. auratus		**Tissue**	**BAF ^{210}Pb**	**BAF ^{210}Po**
Sampling station	2	Muscle	$3.80 \cdot 10^{2}$	$5.51 \cdot 10^{3}$
		Spine	$2.28 \cdot 10^{2}$	$5.78 \cdot 10^{4}$
Number of individuals	3	Gonads	$3.09 \cdot 10^{3}$	$3.28 \cdot 10^{4}$
		Gills	$4.01 \cdot 10^{3}$	$1.24 \cdot 10^{4}$
Water station	2	Kidney	$1.09 \cdot 10^{3}$	$9.44 \cdot 10^{4}$
		Hepatopancreas	$2.48 \cdot 10^{2}$	$4.20 \cdot 10^{4}$
		Gut	$3.52 \cdot 10^{2}$	$1.06 \cdot 10^{5}$
		Gut content	$3.21 \cdot 10^{3}$	$1.23 \cdot 10^{5}$
Sampling station	3	Muscle	$7.23 \cdot 10^{1}$	$3.21 \cdot 10^{3}$
		Spine	$5.95 \cdot 10^{2}$	$4.80 \cdot 10^{4}$
Number of individuals	2	Gonads	$1.62 \cdot 10^{2}$	$2.62 \cdot 10^{4}$
		Gills	$2.07 \cdot 10^{3}$	$3.19 \cdot 10^{3}$
Water station	3	Kidney	$8.07 \cdot 10^{2}$	$6.53 \cdot 10^{4}$
		Hepatopancreas	$2.88 \cdot 10^{2}$	$1.57 \cdot 10^{4}$
		Gut	$4.30 \cdot 10^{2}$	$3.46 \cdot 10^{4}$
		Gut content	$4.68 \cdot 10^{3}$	$1.15 \cdot 10^{5}$
Sampling station	6	Muscle	$6.38 \cdot 10^{1}$	$1.08 \cdot 10^{4}$
		Spine	$3.88 \cdot 10^{2}$	$9.93 \cdot 10^{4}$
Number of individuals	2	Gonads	$1.89 \cdot 10^{3}$	$2.87 \cdot 10^{4}$
		Gills	$1.49 \cdot 10^{3}$	$3.82 \cdot 10^{3}$
Water station	6	Kidney	$5.96 \cdot 10^{2}$	$1.07 \cdot 10^{5}$
		Hepatopancreas	$2.76 \cdot 10^{2}$	$6.71 \cdot 10^{4}$
		Gut	$4.08 \cdot 10^{2}$	$9.37 \cdot 10^{4}$
		Gut content	$3.06 \cdot 10^{3}$	$2.25 \cdot 10^{5}$

7.4.3. *Cyprinus carpio*

In this species, BAF for ^{210}Pb displayed also differences of three levels of magnitude (from 10^{1} to 10^{3}). Once again, the lowest values for BAF were found in muscle. Highest BAF values were shown in spine, followed by kidney, hepatopancreas and gills in different order depending on the sampling station.

BAF for ^{210}Po had also differences of three levels of magnitude. Lowest concentrations were found again in muscle, and the highest in gut content, followed by gut and kidney in all the sampling stations.

Table 15. Bioaccumulation factors (BAF) of ^{210}Pb and ^{210}Po of *C. carpio* (n=4)

C. carpio		Tissue	BAF ^{210}Pb	BAF ^{210}Po
Sampling station	2	Muscle	$1.69 \cdot 10^{2}$	$1.10 \cdot 10^{3}$
		Spine	$6.73 \cdot 10^{3}$	$1.26 \cdot 10^{4}$
Number of individuals	1	Gonads	$1.66 \cdot 10^{2}$	$4.95 \cdot 10^{3}$
		Gills	$1.75 \cdot 10^{3}$	$1.11 \cdot 10^{4}$
Water station	2	Kidney	$1.96 \cdot 10^{3}$	$8.70 \cdot 10^{4}$
		Hepatopancreas	$1.35 \cdot 10^{3}$	$3.26 \cdot 10^{4}$
		Gut	$2.81 \cdot 10^{2}$	$6.36 \cdot 10^{4}$
		Gut content	$2.21 \cdot 10^{3}$	$2.20 \cdot 10^{5}$
Sampling station	3	Muscle	$4.54 \cdot 10^{1}$	$1.19 \cdot 10^{3}$
		Spine	$8.83 \cdot 10^{2}$	$1.59 \cdot 10^{3}$
Number of individuals	2	Gonads	$1.63 \cdot 10^{2}$	$3.38 \cdot 10^{4}$
		Gills	$6.48 \cdot 10^{2}$	$1.07 \cdot 10^{4}$
Water station	3	Kidney	$5.10 \cdot 10^{2}$	$7.49 \cdot 10^{4}$
		Hepatopancreas	$2.85 \cdot 10^{2}$	$2.91 \cdot 10^{4}$
		Gut	$2.33 \cdot 10^{2}$	$6.83 \cdot 10^{4}$
		Gut content	$4.34 \cdot 10^{2}$	$1.12 \cdot 10^{5}$
Sampling station	6	Muscle	$5.76 \cdot 10^{1}$	$8.50 \cdot 10^{2}$
		Spine	$2.75 \cdot 10^{3}$	$4.81 \cdot 10^{3}$
Number of individuals	1	Gonads	$1.69 \cdot 10^{2}$	$1.13 \cdot 10^{4}$
		Gills	$5.32 \cdot 10^{2}$	$4.50 \cdot 10^{3}$
Water station	6	Kidney	$1.96 \cdot 10^{2}$	$2.53 \cdot 10^{4}$
		Hepatopancreas	$2.58 \cdot 10^{2}$	$7.92 \cdot 10^{3}$
		Gut	$1.15 \cdot 10^{2}$	$2.89 \cdot 10^{4}$
		Gut content	$1.74 \cdot 10^{3}$	$8.84 \cdot 10^{4}$

7.4.4. *Chelon labrosus*

In this species, BAF for ^{210}Pb had differences of 4 orders of magnitude. Again, lowest BAF were found on muscle, but in sampling stations 2 and 3, lowest BAF were found also in gonads and hepatopancreas - for the St 3 - and gonads for St 6. For this radionuclide, highest concentrations were found on spine in all the sampling stations and in gills at St 2 and St 6 and in gut content at St 3.

^{210}Po BAF showed differences of 3 levels of magnitude, being muscle once again the tissue with the lowest BAF. The highest BAF were found on gut content, followed by kidney. At St 2 high values were observed for hepatopancreas and at St 6 BAF was also high in gut.

Table 16. Bioaccumulation factors (BAF) of ^{210}Pb and ^{210}Po of *C. labrosus* (n=5)

C. labrosus		**Tissue**	**BAF ^{210}Pb**	**BAF ^{210}Po**
Sampling station	2	Muscle	$7.20 \cdot 10^{1}$	$1.37 \cdot 10^{3}$
		Spine	$1.60 \cdot 10^{4}$	$4.95 \cdot 10^{4}$
Number of individuals	1	Gonads	$1.07 \cdot 10^{3}$	$2.19 \cdot 10^{4}$
		Gills	$4.23 \cdot 10^{3}$	$2.03 \cdot 10^{4}$
Water station	2	Kidney	$9.41 \cdot 10^{2}$	$6.71 \cdot 10^{4}$
		Hepatopancreas	$6.80 \cdot 10^{2}$	$6.37 \cdot 10^{4}$
		Gut	-	-
		Gut content	$9.75 \cdot 10^{2}$	$2.21 \cdot 10^{5}$
Sampling station	3	Muscle	$8.53 \cdot 10^{2}$	$4.90 \cdot 10^{3}$
		Spine	$1.45 \cdot 10^{4}$	$3.94 \cdot 10^{4}$
Number of individuals	2	Gonads	$2.76 \cdot 10^{2}$	$2.05 \cdot 10^{4}$
		Gills	$7.41 \cdot 10^{3}$	$3.89 \cdot 10^{4}$
Water station	3	Kidney	$2.55 \cdot 10^{3}$	$8.75 \cdot 10^{4}$
		Hepatopancreas	$8.66 \cdot 10^{2}$	$3.87 \cdot 10^{4}$
		Gut	$4.09 \cdot 10^{2}$	$1.20 \cdot 10^{5}$
		Gut content	$1.16 \cdot 10^{4}$	$8.44 \cdot 10^{5}$
Sampling station	6	Muscle	$1.15 \cdot 10^{2}$	$1.41 \cdot 10^{3}$
		Spine	$1.01 \cdot 10^{4}$	$2.38 \cdot 10^{4}$
Number of individuals	2	Gonads	$1.69 \cdot 10^{2}$	$9.52 \cdot 10^{3}$
		Gills	$1.03 \cdot 10^{4}$	$2.39 \cdot 10^{4}$
Water station	6	Kidney	$5.69 \cdot 10^{3}$	$5.83 \cdot 10^{4}$
		Hepatopancreas	$1.55 \cdot 10^{3}$	$4.55 \cdot 10^{4}$
		Gut	$1.25 \cdot 10^{3}$	$7.15 \cdot 10^{4}$
		Gut content	$4.69 \cdot 10^{3}$	$2.26 \cdot 10^{5}$

7.5. Comparison between species

As *G. holbrooki* differs totally from the other species in terms of metabolism, physiology, behavior, size, etc. This species can not be compared with other species, although it should be taken into consideration that *G. holbrooki*, despite its small size, it greater accumulates ^{210}Pb and ^{210}Po, in comparison with other species.

Table 17. Average concentrations of ^{210}Pb and ^{210}Po (in Bq kg^{-1} dry weight) in tissues from *C. carpio* (n=4), *C. labrosus* (n=5) and *C. auratus* (n=7).

Species	Tissue	^{210}Pb	^{210}Po
Cyprinus carpio	Muscle	8 ± 4	28 ± 8
	Spine	169 ± 50	76 ± 16
	Gonads	16 ± 2	398 ± 310
	Gills	112 ± 49	261 ± 73
	Kidney	90 ± 82	1641 ± 569
	Hepatopancreas	65 ± 56	596 ± 266
	Gut	28 ± 8	1745 ± 261
	Gut content	155 ± 83	3782 ± 789
Chelon labrosus	Muscle	38 ± 53	61 ± 54
	Spine	461 ± 140	310 ± 55
	Gonads	26 ± 8	332 ± 207
	Gills	706 ± 311	682 ± 303
	Kidney	248 ± 154	1544 ± 573
	Hepatopancreas	93 ± 36	1103 ± 95
	Gut	94 ± 59	2927 ± 543
	Gut content	475 ± 481	8558 ± 6378
Carassius auratus	Muscle	12 ± 8	164 ± 155
	Spine	253 ± 85	167 ± 77
	Gonads	14 ± 8	635 ± 481
	Gills	194 ± 23	634 ± 183
	Kidney	88 ± 21	2488 ± 1141
	Hepatopancreas	30 ± 9	1144 ± 758
	Gut	65 ± 21	3261 ± 2159
	Gut content	384 ± 193	3833 ± 1453

Generally speaking, the three species (*C. carpio, C. labrosus* and *C. auratus*) have high concentration levels of ^{210}Pb and ^{210}Po in their tissues. However, there are several differences. *C. labrosus* showed extremely high values of ^{210}Po concentration in gut content (8558±6378 Bq kg^{-1}) and also high levels of ^{210}Pb in spine and gills (461±140 and 706±311 Bq kg^{-1}, respectively) in comparison to the other species. Both, *C. labrosus* and *C. auratus* show high levels of ^{210}Pb in gut content (475±481 and 384±193 Bq kg^{-1}) in comparison with *C. carpio*, whose levels are lower (155±83 Bq kg^{-1}). The same pattern

is observed with gut, where in *C. carpio* the average is 28±8 Bq kg^{-1} while in *C. labrosus* and *C. auratus* the value is tripled and doubled, respectively. Regarding to hepatopancreas, *C. labrosus* and *C. auratus* show similar concentrations for ^{210}Po (1103±95 and 1144±758 Bq kg^{-1}, respectively) while in *C. carpio* concentration is a half. ^{210}Pb concentration in hepatopancreas from individuals of *C. auratus* is lower than those from *C. labrosus* and *C. carpio* (30±9, 93±36 and 65±56 Bq kg^{-1}, respectively).^{210}Po concentration in kidney from the three species show similar values, ranging from 1544±573 Bq kg^{-1} the lowest, *C. labrosus*, to 2488±1141 Bq kg^{-1} the highest, *C. auratus*. ^{210}Pb concentration in kidney in *C. labrosus* tripes the values from the other species, with values of 248±154 Bq kg^{-1} in *C. labrosus* and 90±82 and 88±21 Bq kg^{-1} in *C. carpio* and *C. auratus,* respectively. ^{210}Po accumulation in gills was low (in comparison with other tissues) in the three species, with values ranging from 682±303 Bq $kg^{-1}$the highest (*C. labrosus*) to 261±73 Bq kg^{-1} the lowest (*C. carpio*). Levels of ^{210}Pb and ^{210}Po in gonads are lower than in gills in the three species, being ^{210}Pb concentration always lower than ^{210}Po. Levels of ^{210}Po in gonads ranged from 332±207 Bq kg^{-1} the lowest (*C. labrosus*) to 635±481 Bq kg^{-1} the highest (*C. auratus*). Levels of ^{210}Pb in gonads ranged from 26±8 Bq kg^{-1} the highest (*C. labrosus*) to 14±8 Bq kg^{-1} the lowest (*C. auratus*). Regarding to spine, there were high differences between the ^{210}Pb content in the three species. The highest value, as said before, was 461±140 Bq kg^{-1} from *C. labrosus*. The lowest value was for *C. carpio*, whose spines had an average concentration of ^{210}Pb of 169±50 Bq kg^{-1}, almost 4 times lower. ^{210}Po concentrations in spines were low, ranging from 76±16 Bq kg^{-1} the lowest (*C. carpio*) to 310±55 Bq kg^{-1} the highest (*C. labrosus*). Finally, refering to muscle, concentrations of ^{210}Pb and ^{210}Po were so low, ranging from 8±4 Bq kg^{-1} (^{210}Pb) and 28±8 Bq kg^{-1} (^{210}Po) the lowest (both in *C. carpio*) to 38±53 Bq kg^{-1} (^{210}Pb) and 164±155 Bq kg^{-1} (^{210}Po) for *C. labrosus* and *C. auratus* respectively.

The BAF values for organisms living in the same habitat vary by orders of magnitude (especially with ^{210}Pb BAF) and demonstrate that ^{210}Po and ^{210}Pb bioaccumulation is not from simple radionuclide absorption from water, underscoring the role of feeding as the cause for ^{210}Pb and ^{210}Po accumulation in fishes. Therefore, *C. carpio,* who is an species characterized by being benthic omnivorous and uprooting macrophites when feeds, did not show the highest values of ^{210}Po nor ^{210}Pb in gut content. As this behaviour increases turbidity in water, because the sediment is removed, it was expected that this specie would have shown the highest values, levels which *C. labrosus* shown. However, as in waters where *C. carpio* lives, the level of particulate matter in water increases and when feeding, species ingest more particulate matter (the fraction where polonium is binded), the highest concentrations of ^{210}Pb and ^{210}Po in gut content could be explained.

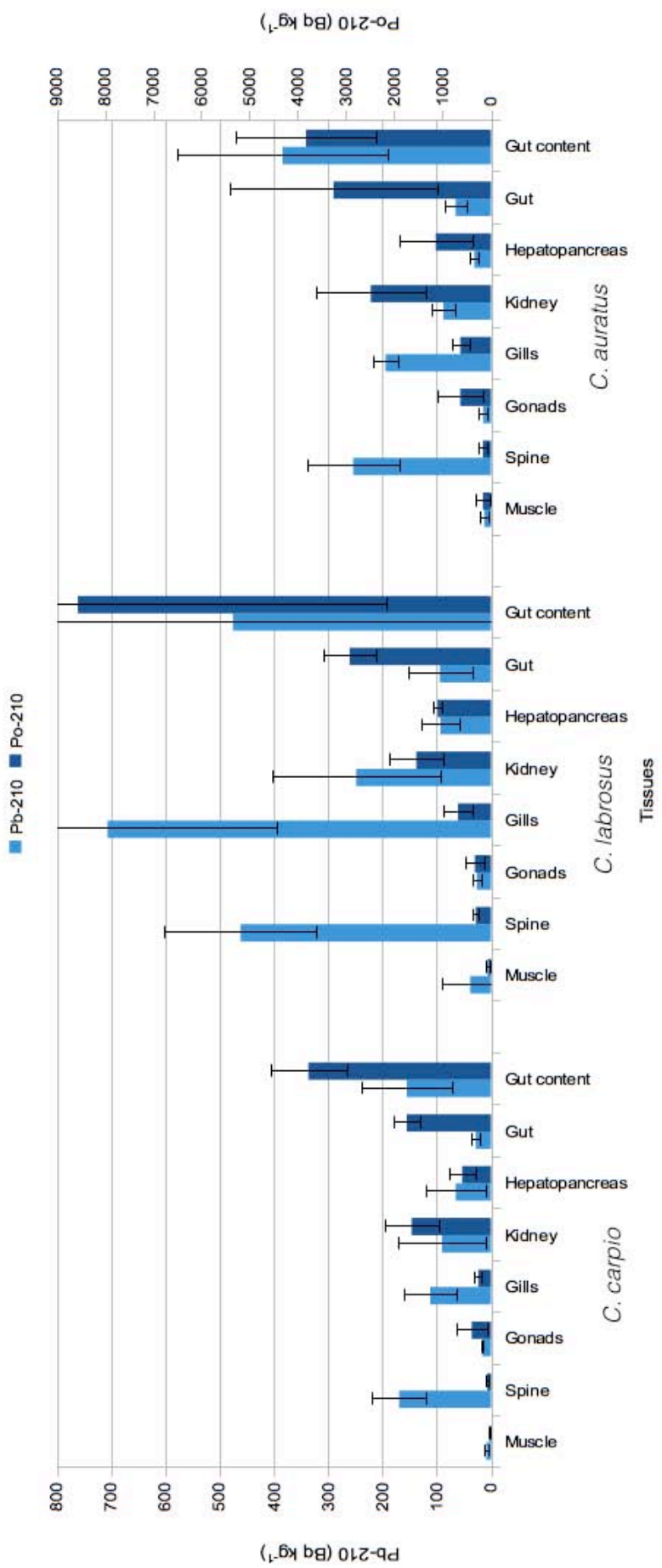

Figure 32. Average concentrations of ^{210}Pb and ^{210}Po (In Bq kg^{-1} dry weight) in tissues from *C. carpio* (n=4), *C. labrosus* (n=5) and *C. auratus* (n=7).

7.6. Comparison with bibliographical data

In general terms it can be concluded that almost all the values shown in all the species analyzed in this study are higher in comparison with the existing data. Results from this research agree with the existing data regarding ^{210}Pb and ^{210}Po accumulation in fish tissues.

According to Skwarzek (1988) the highest ^{210}Po concentrations occur in organs involved in digestion and metabolism, such as intestine, stomach, spleen and pyloric caecal of fish. Yet in our research, the highest ^{210}Po concentrations are found in organs involved in digestion and metabolism, such as gut, kidney and hepatopancreas.

We also observed that the concentration of ^{210}Po in wet weight in the muscles of fish was always higher than in the spine, according with results observed by Iyengar *et al.* (1980). Lower concentration of ^{210}Po in that tissue would be of high relevance since humans predominantly consume this part of the fish, and therefore account for low radiation dose.

Dahlgaard (1996) found a large individual variation of the ^{210}Po concentration in fish meat.

Levels of ^{210}Po found in fish digestive organs tend to correlate with the degree of stomach repletion and thus decrease if food is scarce (Skwarzec, 1988). The residence time of ^{210}Po within the digestive system of fish is short, resulting in a rapid decrease in ^{210}Po content in the liver and intestine when the stomach is empty (Lazorenko *et al.*, 2002). However, once ^{210}Po is uptaken by the organism is subsequently distributed in fish in the following order: entrails ≥ liver > skeleton > muscles (Lazorenko *et al.*, 2002). As observed in our study, the distribution pattern agree with the data found in bibliography. Furthermore, results from the accumulation of ^{210}Po in liver are higher than those from spines, and in spines, were also higher than ^{210}Po accumulation in muscles.

Solea solea and *Sparus* sp. are species that were investigated in other studies and results showed that low activities of ^{210}Po were found in the gills, skins, bones and muscle (the latter one presenting the lowest activity) and the highest activities were observed in the livers and intestine (Connan *et al.,* 2007). However, results varied from the species due to their lifestyle (Connan *et al.,* 2007). Results obtained by Connan *et al.* (2007) agree with results found on the present study as ^{210}Po concentration in tissues obtained herein match with the resulting pattern of their study.

Other studies focused on the ^{210}Po and ^{210}Pb concentration in organisms and their transfer in marine food chains (Carvalho, 2011). This study reported that common intertidal fishes such as those from the Blenniidae family displayed low ^{210}Po concentrations in muscle tissue (1.5-2.8 Bq kg$^{-1)}$, and high concentrations in other internal organs. Yet in their study, ^{210}Po/^{210}Pb ratios were always above 1. In coastal fishes, the general ^{210}Po and ^{210}Pb distribution pattern was: lower concentrations in muscle tissue and higher concentrations in the gut, liver, gonad and spine. Carvalho (2011) also reported high inter-individual variations and often, apparent seasonal variations of ^{210}Po activity concentrations.

^{210}Pb concentrations in muscle tissue reported by Carvalho ranged from 0.15±0.07 to 2.1±1.3 Bq kg^{-1} wet weight (w.w.). These concentrations are low in comparison with the concentrations found in our research, which ranged from 1.1±0.1 to 21.3±0.8 Bq kg^{-1} w.w.. ^{210}Po concentrations in muscle in the study of Carvalho (2011) ranged from 0.52±0.01 to 66±2 Bq kg^{-1}w.w.. Results of ^{210}Po concentration in muscle from this

research found that values ranged from 4.4±0.6 to 76.7±2.0 Bq kg^{-1}w.w.. The lowest value of the range is higher in this research, in comparison with the lowest value in the range of results from Carvalho (2011). Regarding the values obtained in spine, ^{210}Pb concentrations reported by Carvalho ranged from 0.65±9.1 to 31±1 Bq kg^{-1} w.w. while results from this report ranged from 4.0±1.5 to 362.5±12.0 Bq kg^{-1}w.w.. Both values, the lowest and the highest from the range are higher than the results reported by Carvalho (2011). ^{210}Po concentrations in spine from Carvalho ranged from 5.9±0.2 to 197±16 Bq kg^{-1} w.w.. In this research, ^{210}Po concentrations in spines ranged from 8.6±1.0 to 705.7±20.1 Bq kg^{-1} w.w.. Highest values of this radionuclide concentration from fishes of Peníscola marsh are 3.5 times higher than values reported by Carvalho (2011). ^{210}Pb concentration in gonads reported by Carvalho (2011) ranged from 0.20±0.01 to 22±2 Bq kg^{-1} w.w.. Results from this research are higher, reporting values from 2.9±0.2 to 54.7±44.1 Bq kg^{-1} w.w.. ^{210}Po concentrations in gonads from Carvalho (2011) showed concentrations between 4.5±0.2 and 275±9 Bq kg^{-1} w.w. while in the present study, values that range between 19.8±1.4 to 183.0±6.8 Bq kg^{-1} w.w. are reported. In this case, the highest concentrations reported by Carvalho were higher than the highest concentrations found in the present study. Carvalho (2011) did neither report kidney ^{210}Pb and ^{210}Po accumulation nor gills. Regarding to hepatopancreas, ^{210}Pb concentrations found by Carvalho (2011) ranged from 0.29±0.02 to 134±12 Ba kg^{-1}w.w.. ^{210}Pb concentrations found by this research ranged from 4.4±0.6 to 33.4±0.9 Bq kg^{-1}w.w.. In this case, concentrations found by Carvalho (2011) were generally higher. ^{210}Po concentration in hepatopancreas reported by Carvalho (2011) ranged from 5.36±0.42 to 2140±60 Bq kg^{-1} w.w.. Concentrations found by this research were in the range between 56.3±7.1 to 476.9±11.4 Bq kg^{-1} w.w. In this case, concentrations reported by Carvalho were higher. Finally, Carvalho (2011) analyzed gut from fishes, reporting ^{210}Pb concentration that ranged from 0.29±0.01 to 100±8 Bq kg^{-1} w.w.. Results of ^{210}Pb found in this research ranged from 2.5±0.2 to 27.1±0.9 Bq kg^{-1} w.w.. Concentration of ^{210}Pb in gut from Carvalho (2011) is higher than concentrations found by this research. ^{210}Po concentrations in gut reported from Carvalho (2011) ranged from 9.86±0.3 to 28000±2000 Bq kg^{-1} w.w.. Concentrations of ^{210}Po in gut from this research ranged from 187.3±5.2 to 666.0±19.0 Bw kg^{-1} w.w.. In this case, concentrations reported by Carvalho (2011) are higher. Although, most of the highest values reported by Carvalho (2011) came from a specific species, *Sardina pilchardus*, which in his research, Carvalho (2011) found that it accumulates extremely high values of ^{210}Po from the environment. In the present study, higher ^{210}Pb and ^{210}Po concentrations were reported. Thus would mean that higher ^{210}Pb and ^{210}Po concentrations in water may lead to higher ^{210}Pb and ^{210}Po concentrations in tissues.

In comparison to NKS (2009), which studied ^{210}Pb and ^{210}Po concentrations in fishes from a terrestrial and freshwater environment, results provided in this present research are higher. They analyzed edible parts (i.e. muscle) and other parts from fish separately. They found concentrations of ^{210}Pb that ranged from 0.014±0.003 to 0.13±0,02 Bq kg^{-1} w.w. for edible parts and from 0.123±0.021 to 1.507±0.256 Bq kg^{-1} w.w. for other parts. On the other hand, they found concentrations of ^{210}Po that ranged from 0.079±0.018 to 1.863±0.35 Bq kg^{-1} w.w. for edible parts and ranges from 1.492±0.269 to 8.950±1.611 Bq kg^{-1} w.w.. Concentration of both, ^{210}Pb and ^{210}Po reported by this article are extremely low in comparison with results reported from this research, which the concentration of ^{210}Pb and ^{210}Po in the tissue equivalent to edible parts (muscle) ranged from 1.1±0.1 to 21.3±0.8 Bq kg^{-1} w.w. for ^{210}Pb and from 4.4±0.6 to 76.7±2.0 Bq kg^{-1}w.w.for ^{210}Po.

With regards to ^{210}Pb and ^{210}Po concentrations in freshwater, NKS (2009) reported values that oscillate around 3.2±0.5 Bq m^{-3} for ^{210}Pb and 1.9±0.3 Bq m^{-3} for ^{210}Po. These values

are lower than the values reported for this present research, which ranged from 17.7±0.7 to 25.0±0.9 Bq m^{-3} for ^{210}Pb and from 4.0±0.3 to 7.1±0.5 Bq m^{-3} for ^{210}Po.

With regards to bioaccumulation factors (BAF), NKS (2009) reported BAF for edible parts ranging from $5.0 \cdot 10^{1}$ to $1.2 \cdot 10^{3}$ for ^{210}Po and from $9.6 \cdot 10^{0}$ to $2.4 \cdot 10^{2}$ for ^{210}Pb. In this present research, BAF calculated for muscle ranged from $8.5 \cdot 10^{2}$ to $1.1 \cdot 10^{4}$ for ^{210}Po and from $4.5 \cdot 10^{1}$ to $8.5 \cdot 10^{2}$ for ^{210}Pb. As it can be observed from the results, BAF in fishes from Peníscola marsh show, for each radionuclide, always an order of magnitude higher than BAF calculated for terrestrial and freshwater environments. Thus, in relation to ^{210}Pb and ^{210}Po concentrations in water, ^{210}Po and ^{210}Pb concentrations in fish tissues collected in Peníscola are higher than those from freshwater ecosystems. This result reflects that highest ^{210}Po and ^{210}Pb concentration in water is not only the reason why this research has found higher bioaccumulations in fish tissues, and highlights that the absorption with ingested food (gut transfer) as the main route of radionuclide uptake.

8. Conclusions and further perspectives

8.1. Conclusions

The main objective of this project was to determine the bioaccumulation in ^{210}Pb and ^{210}Po, in both, different fish species and fish tissues from samples collected in a Mediterranean coastal wetland characterized to be highly enriched in ^{226}Ra and ^{222}Rn: the Peníscola wetland (Castelló, Spain).

Tissues with the highest accumulation of ^{210}Po were gut, kidney and hepatopancreas ranging from 1745±261 to 3261±2159 Bq kg^{-1} d.w. in gut, from 1544±573 to 2488±1411 Bq kg^{-1} d.w. in kidney and from 596±266 to 1144±758 Bq kg-1 d.w. in hepatopancreas. High concentrations in gut can be justified by the fact that gut content resulted in enhanced concentrations of ^{210}Po ranging from 782±789 to 8558±6378 Bq·kg^{-1} d.w. On the contrary, lowest concentrations of ^{210}Po were found in muscle, spine, gonads and gills, with values ranging from 28±8 to 164±155 Bq kg^{-1}d.w. in muscle, from 76±16 to 310±55 Bq kg^{-1}d.w. in spine, from 332±207 to 635±481 Bq kg^{-1}d.w. in gonads and from 261±73 to 682±303 Bq kg^{-1}d.w. in gills.

Regarding ^{210}Pb accumulation, results showed that the highest concentrations were found in gut content, gills, spine and kidney, with values ranging from 155±83 to 475±481 Bq kg^{-1} d.w. in gut content, from 112±49 to 706±311 Bq kg^{-1} d.w. in gills, from 169±50 to 461±140 Bq kg^{-1} d.w. in spine and from 88±21 to 248±154 Bq kg^{-1} d.w. in kidney. Lowest ^{210}Pb concentrations found by this research were in muscle, gonads, hepatopancreas and gut, with values ranging from 8±4 to 38±53 Bq kg^{-1} d.w. in muscle, from 16±2 to 26±8 Bq kg^{-1} d.w. in gonads, from 30±9 to 93±36 Bq kg^{-1} d.w. in hepatopancreas and finally, from 28±8 to 94±59 Bq kg^{-1} d.w. in gut.

The results showed that the distributions of both radionuclides are consistent with other studies and with the chemical properties of ^{210}Pb and ^{210}Po.

The major input route of ^{210}Pb and ^{210}Po into the fish body seemed to be ingestion, due to the high levels of ^{210}Pb and ^{210}Po found in gut content as well as in the organs involved in digestion and metabolism (i.e. gut, kidney and hepatopancreas). This statement agrees with the literature regarding marine species of fish and invertebrates. On the other hand, breathing organs such as gills, although they could be an entry route of ^{210}Pb, they are not for ^{210}Po.

Bioaccumulation factors (BAF) of different tissues within the same individual showed differences that reached up to two and three orders of magnitude. Lowest BAF were generally found in muscle and the highest in gut and kidney. It was found that the BAF values for organisms living in the same habitat varied by orders of magnitude (especially with ^{210}Pb BAF) and demonstrate that ^{210}Po and ^{210}Pb bioaccumulation is not from simple radionuclide absorption from water, underscoring the role of feeding as the cause for ^{210}Pb and ^{210}Po accumulation in fishes.

It can also be concluded that there is no apparent relationship between ^{210}Pb and ^{210}Po accumulation and fish size or weight within the same species. Generally speaking, highest values of ^{210}Pb and ^{210}Po concentration in tissues were found on *Chelon labrosus* and *Carassius auratus*, being *Cyprinus carpio* the species with the lowest average values of accumulation. This result was not expected, because of the feeding habits of *C. carpio*,

which uproots macrophites and increases turbidity when feeding, it was expected that this species could show highest values of ^{210}Pb and ^{210}Po concentration in gut content. Hence, it is confirmed that the level to which a radionuclide is accumulated in an organism depends on a wide range of factors: its chemical characteristics and speciation in water or sediment, biological processes, including rates of uptake from water or diet, excretion, and metabolic transformation. These in turn, may be influenced directly by the physiology of the organism which is, of course, affected by diverse biological, physical and chemical factors, such as habitat, feeding behavior and species.

Both humans and other species live in a world with natural radioactivity. It is necessary to know our environment in order to understand the processes that are occurring around us. For this reason it is evident that expanding the present database on ^{210}Pb and ^{210}Po concentrations in different species and their tissues will greatly aid in refining estimates of dose and eventual assessments of the effects of ionizing radiation on biota.

8.2. Further perspectives

In order to allow comparisons of bioaccumulation factors between blank fish samples and Peníscola fish samples, water samples from the sites where blank fishes were collected should have been taken. This would have provided comparisons of ^{210}Pb and ^{210}Po concentration in water, not only from data, but also from the environment where blank fishes analyzed in this study lived.

Knowing the high levels of radioactivity found in this naturally enhanced area, this environment should be taken in advantage with further studies on radionuclide bioaccumulation and transference between different ecosystems. Furthermore, it would be of a great interest to extend the type of review carried out in this research to other living organisms.

In addition, as the area where water from the marsh discharges is an important fishing area, studies on concentration levels of ^{210}Po in commercial fishes from the area should be done, in order to know if bioaccumulation of ^{210}Pb and ^{210}Po in fish tissues from coastal areas follow the same pattern as in fish tissues from Peníscola wetland.

9. References

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Legislation

Ley 11/1994, de 27 de diciembre, de Espacios Naturales Protegidos de la Comunidad Valenciana.

Decreto 213/2009, de 20 de noviembre, del Consell, por el que se aprueban medidas para el control de especies exóticas invasoras en la Comunitat Valenciana.

On-line sources:

Banco de datos de biodiversidad de la Comunidad Valenciana: http://bdb.cma.gva.es

Catalogo de zonas húmedas de la Comunidad Valenciana: http://www.cma.gva.es/web/indice.aspx?nodo=922&idioma=C

Enciclopedia Virtual de los Vertebrados Españoles: http://www.vertebradosibericos.org/

Encyclopedia of Life: www.eol.org

Gulf & South Atlantic Regional Panel On Aquatic Invasive Species: http://www.gsarp.org/

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