The impact of mercury contamination on the population dynamics of Peringia ulvae (Gastropoda) on a temperate coastal lagoon (Ria de Aveiro, Portugal)

Eva Carvalho de Sousa

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia realizada sob a orientação científica do Professor Doutor Miguel Pardal (Universidade de Coimbra) e da Doutora Patrícia Cardoso (Investigadora Auxiliar IMAR-CMA)

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2013

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# INDEX

## RESUMO

Foram avaliados os efeitos da contaminação de mercúrio na estrutura e dinâmica populacional do gastrópode *Peringia ulvae* (também conhecida como *Hydrobia ulvae*) e o seu impacto na cadeia trófica, ao longo de um gradiente de mercúrio, na Ria de Aveiro (Portugal). O gastrópode revelou ser uma espécie tolerante ao contaminante, pois os valores de densidade, biomassa e produção secundária mais elevados foram registados na zona de contaminação intermédia, seguidos da menos contaminada e por fim da zona mais contaminada. *P. ulvae* foi, no entanto, negativamente afectada pela contaminação de mercúrio, pois teve o seu crescimento e tempos de vida reduzidos devido à contaminação. Assim, a área mais contaminada foi caracterizada principalmente pela presença de juvenis e indivíduos jovens. A zona de contaminação intermédia mostrou um maior equilíbrio em termos de proporção de grupos etários, com uma dominância por parte dos adultos. A zona menos contaminada apresentou valores intermédios. Os tempos de vida de *Peringia ulvae* foram mais reduzidos na zona mais contaminada (7 e 8 meses), seguidos da área menos contaminada (10 e 11 meses) e por fim da zona de contaminação intermédia (11 e 14 meses).P. ulvae, apesar de não ser considerada um bom bio-indicador para a contaminação de mercúrio, visto a população não ter sofrido alterações profundas na sua estrutura e funcionamento, poderá ter um papel crucial nos processos de biomagnificação ao longo da cadeia trófica.

## ABSTRACT

The effects of mercury contamination on the population structure and dynamics of the gastropod *Peringia ulvae* (also known as *Hydrobia ulvae*) and its impact on the trophic web were assessed along a mercury gradient in Ria de Aveiro (Portugal). The gastropod was revealed to be a tolerant species to the contaminant, since the highest densities, biomasses and growth productivity values were recorded at the intermediate contaminated area followed by the most contaminated one and finally the least contaminated area. *P. ulvae* was however negatively affected by mercury in terms of growth and life span. So, in the most contaminated area the population was characterised mainly by the presence of juveniles and young individuals. The intermediate contaminated area showed a greater equilibrium in terms of groups' proportion, being the adults the dominant set. The least contaminated area presented intermediate values. *P. ulvae* life spans were shortest in the most contaminated area (7/8 months), followed by the least contaminated area (10/11 months) and finally, the intermediate one (11/14 months). *P. ulvae,* despite being revealed not to be a good bio-indicator of mercury contamination, since it did not suffer profound modifications in its structure and functioning, may be a crucial element in the mercury biomagnification processes throughout the food web.

# CHAPTER 1 - INTRODUCTION

### 1.1 Estuaries: Ecological importance and impacts

Estuaries are semi-enclosed costal bodies of shallow-water connected to the ocean, influenced by tides and where seawater meets freshwater derived from inland (Pritchard, 1967; Kennish, 2000).

They stand among the most productive, heterogeneous, diverse, dynamic and economically important habitats on Earth (Hobbie, 2000; Paerl, 2006; Dauvin, 2007).

They provide unique habitats for wildlife, such as birds and fish and a large range of invertebrates. Many of these species have commercial interest and depend on estuaries for nesting, feeding, breeding and refuge. Estuaries also have other important biological roles such as decomposition, nutrient cycling and flux regulation of water and sediments (Kennish, 2002; Bergstrom et al. 2004; Dolbeth et al., 2007).

Consequently, they are always surrounded by very high densities of population and industrialization (Roberts & Hawkins, 1999). Besides being used for shoreline protection, recreational purposes and navigation routes and harbours, estuaries also provide a great number of natural resources (salt terns, aquaculture, fishing, shellfish, algae, etc.) (Kennish, 2002).

Since the beginning of modern civilization, estuarine and coastal transformation has taken place, but, in recent years, it has dramatically accelerated. The chemical contamination, the replacement of wild life habitats by urbanisation, intensive agriculture and tourism are causing a severe degradation of ecosystems all over the world. The on-growing population will continue to explore estuaries and by the year 2020 more than 75% of the population will occupy these coastal zones around the world (Roberts & Hawkins 1996; Madgwick and Jones, 2002; Molles, 2002; Lotze et al., 2006).

There is a strong urge to assess the impacts of the ecosystems destabilization, in order to be able to explore them sustainably (Dolbeth et al., 2011).

### 1.2 Mercury contamination

Mercury is a "blacklisted" environmental pollutant. It is a major concern in the scope of European Water Framework Directive (WFD) and on the global scale (Boening, 2000; Pereira et al., 2009).

Estuaries become deposits of mercury as they receive contaminated water and sediments from rivers (Bergamaschi et al., 2012). This contaminant adsorbs to fine suspended carbon compounded particles, that are abundant in these ecosystems, and then settles to the bottom. It allows the contaminant to persist in the environment for several decades. Its speciation, solubility, mobility and toxicity in aquatic environments are influenced by the sediment and water's physicochemical properties such as dissolved organic matter or polysulphides content (Kennish, 1996; Patra M. & Sharma A., 2000; Jay et al., 2002; Ravichandran, 2003).

Inorganic mercury is oxidized to $Hg^{2+}$ and converted to methylmercury (CH3Hg+) in most cases by anaerobic sulphate-reducing bacteria but also abiotically or in red blood cells in animal lungs (Silver et al., 2007).

This organic form is the most toxic form of Hg and can be readily available, easily bioaccumulated and biomagnified by biota (Boening, 2000).

Mercury enters the food chain mostly through primary producers (rooted macrophytes, micro and macroalgae) and it can be trapped or transported to the higher trophic levels (Coelho et al., 2005; Cairrão et al., 2007). Moving up the food chain, the methylmercury concentration increases by several orders of magnitude and it finds its way to species of commercial interest (*Biomagnification*) (Clarkson et al., 2003).

Mercury exposure causes several disturbances in biota as, for example, reductions in growth, delayed development time and decrease locomotor activity of insect larvae and decrease of abundance and species richness of macroinvertebrates, (Azevedo-Pereira H. M. V. S., 2010; Nunes

et al., 2008). Besides all this, it has a tendency to accumulate in organisms' tissues throughout the life (*bioaccumulation*) (Pereira et al. 2009).

An estimated 40 to 90% of the mercury occurring in shellfish and more than 90% of that in finfish is methylmercury (Kennish, 1996). This constitutes a severe problem not only for sea life but also for humans who consume it (Kennish, 2000) as evidenced by the effects of Minamata disease which proved fatal for thousands of Japanese and continues to affect people all over the world (Kennish, 1996; Ministry of the Environment of Japan, 2011).

### 1.3 Case study: Ria de Aveiro

The Ria de Aveiro is a Portuguese north-western costal lagoon. Its central area receives the major inputs of freshwater and sediments (Cunha, 2003). Among its four main channels we can find the Mira channel, the Ílhavo channel, the Espinheiro channel and the S. Jacinto-Ovar channel. The latter has other secondary channels and inner bays, like the Murtosa channel, the Laranjo Bay and the Estarreja channel.

As most coastal lagoons, Ria de Aveiro is extremely rich in biodiversity and constitutes a very important ecological service provider: besides storing carbon, controlling floods and droughts it also contributes for the preservation of the fish stocks and recycling of nutrients. It also serves recreational and industrial purposes as long as aquaculture, production of salt, fishing and shellfish collection (Nunes et al., 2008, Pereira et al., 2009)

For more than 40 years the Ria de Aveiro, has been submitted to waste discharges from the Estarreja industrial complex. The rejects from a chlor-alkali plant, loaded of mercury were directly discharged into the system through the Estarreja channel (Fig. 1) and reached the Laranjo Bay, the most polluted area of the Ria. This bay still holds most of the total 30 tons of mercury deposited in the sediments (Pereira et al., 1998b).

Fortunately, the mercury cycling between water column and pore waters is not significant. Therefore, most of the contamination is confined to this inner bay (Ramalhosa et al., 2006).

The anthropogenic sources of mercury into the aquatic systems have been reduced due to legal restrictive rules (e.g. 50 $\mu L^{-1}$ is the limit value for discharges from chlor-alkali plants, in accordance with the European Union Directive 82/176/EEC). Nevertheless, Hg-contaminated sediments are still a cause for concern due to the potential release of Hg into other environment matrices, such as the overlying water column and biota.

Several studies have been performed to assess the mercury pollution in Ria de Aveiro, as for example, the mercury contamination in urban and agricultural soils (Cachada et al., 2009), the assessment of methylmercury production (Válega et al., 2008), the macroalgae response to mercury contamination gradient (Coelho et al., 2005) and the accumulation of mercury in macroinvertebrates and fishes (Abreu et al., 2000). However, there is a lack of information concerning the impact of mercury on the population dynamics and productivity of key species, like the gastropod *Peringia ulvae*, also known as *Hydrobia ulvae*.

### 1.4 *Peringia ulvae*

*Peringia ulvae* (Pennant, 1777) is a very abundant species in estuaries. It is a deposit feeder that lives in intertidal mudflats and muddy/sandy sediments. It is also found in a wide variety of intertidal substrata, including saltmarshes or macroalgae assemblages when present at sediment or rocky surface (Sola, 1996; Cardoso et al., 2008). Besides that, *P. ulvae* can also be a periphyton grazer (Philippart, 1995).

The gastropod feeds selectively on living components of the sediment, more specifically diatoms and fungal hifas that are decomposers of the structural carbohydrates and other difficult decomposable materials that form the substrate. So, its strong connection with the sediments can represent a risk of contamination for the higher trophic levels which feed on it and also may represent a threat for the functioning and success of the own species. It is a very important primary consumer and it provides food for many species of birds and fishes. Thus, it constitutes an

important link in the estuarine food web (Newell, 1965; Fenchel et al., 1975; Newell, 1979; Riera et al., 2010).

Adding the fact that it is simple to identify and easy to sample, the invertebrate *P. ulva*e is considered to be an important biomonitor. (Amin et al., 2009)

In the literature, there are some studies that assess the metals concentrations in water, sediments and fauna (Mucha et al., 2005; Dauvin et al., 2008) but very few works have been developed to evaluate the impact of the metal contamination on the structure and productivity of coastal fauna (Weis et al., 2004; Rumisha et al. 2012). Therefore, assuming the relevance of this gastropod species as a primary consumer and as a potential vehicle for the transference of mercury, the main goals of this work are to evaluate the impact of mercury contamination on the population dynamics of *Peringia ulvae*, through the analysis of its population structure (density, biomass and proportion of age classes) and functioning (reproduction and secondary production) and to evaluate the mercury bioaccumulation by the species.

# CHAPTER 2 - MATERIALS AND METHODS

## 2.1 Study Area

The Ria de Aveiro is a shallow costal lagoon, adjacent to the Atlantic Ocean, located in the northwest coast of Portugal (40'38'N, 8'44' W). The lagoon has a surface area of about 45 $km^2$ (45 km length and 10 km wide) but the wetland area is of about 83 $km^2$, during high tide, and 66 $km^2$ during low tide. It has a variety of habitats and a very important ecological richness (Pereira et al., 2009; Cunha et al., 2003; Válega et al., 2008).

Its islands and channels form a complex network. There are four main branches: the Mira channel, which runs to the south, Ílhavo, in the southeast direction, Espinheiro to the northeast and finally S.Jacinto-Ovar to the north (Nunes et al., 2008; Pereira et al., 2009). The rivers Vouga and Antuã are responsible for the majority of the freshwater input. The water circulation is dependent on a single connection to the sea.

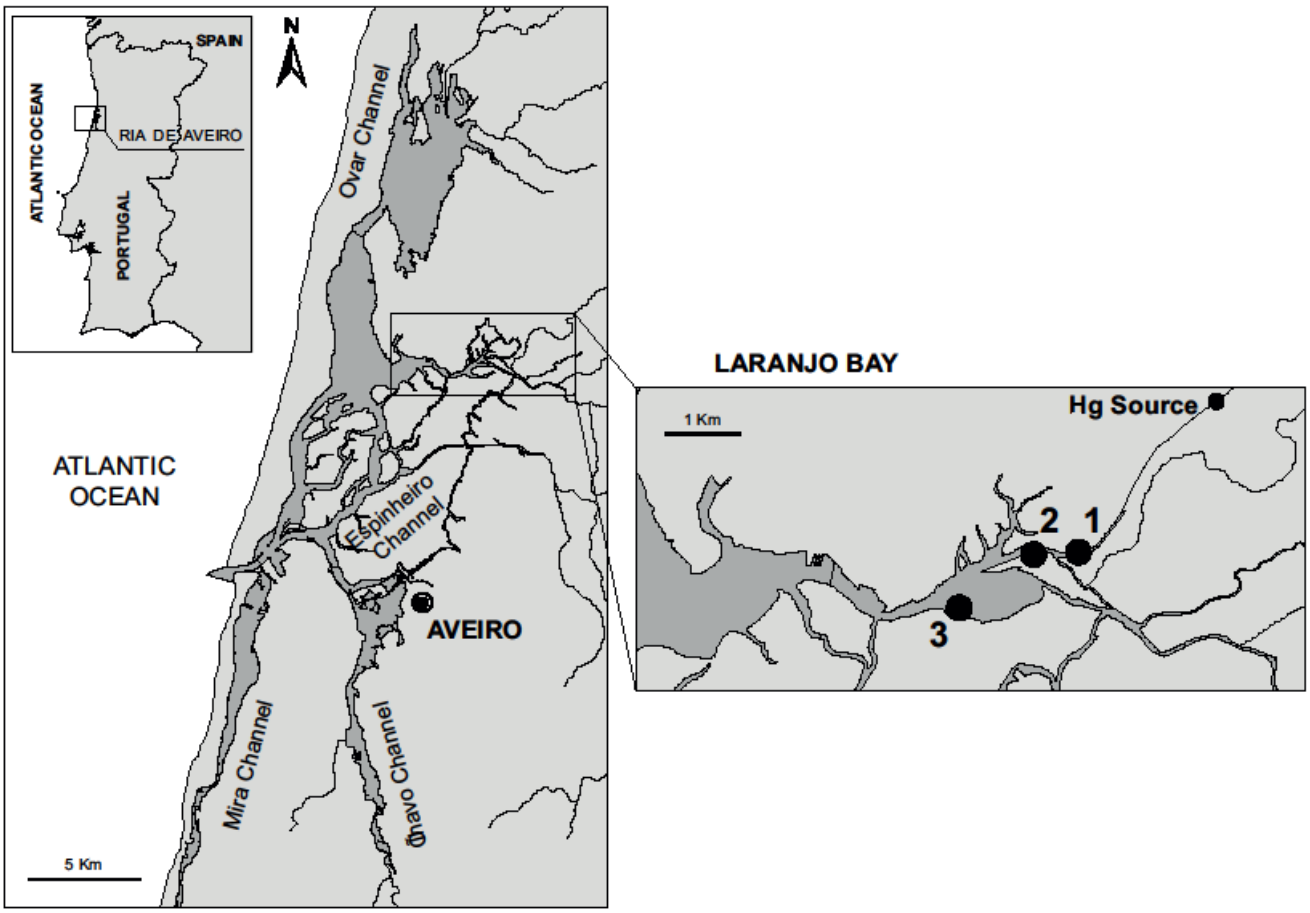


**Figure 1 – The Ria de Aveiro coastal lagoon and the sampling locations.**

There is an inner bay on the lagoon called Laranjo Bay (Pereira et al., 1998), which corresponds to the most contaminated site of the Ria (Ramalhosa et al., 2006). In that location, three sampling sites were chosen, according to a mercury contamination gradient. The most contaminated site (St. **1**), is considered to be at the point source in the lagoon, the intermediate site (St. **2**) is situated 600 m from the point source and, the least contaminated site (St. **3**), at 3000 m distant from the point source.

### 2.2 Field and sampling procedures

The *P. ulvae* population was monitored monthly, during low tide from September 2010 to September 2011.

In each site, 8-10 replicates were collected randomly, with a 141 $cm^2$ section core to a depth of approximately 20 cm. The biological samples were all washed in the Ria's water through a 500 μm mesh sieve bag and then placed into plastic bottles. All physicochemical parameters were measured in situ (temperature, dissolved oxygen, pH and salinity) and water was collected from the intertidal water pools for determination of cholorophyll a, dissolved mercury and suspended particulate matter (SPM) mercury.

Sediments from each site were also collected for organic matter, total mercury content determination and granulometry.

The sediments were homogenized, a portion was freeze-dried for mercury analysis and the remaining was oven dried at 60 ºC and incinerated at 450 ºC for 8 hours for organic matter quantification. The grain size was analysed and classified according to the following nomenclature: gravel: > 2 mm; 2.0 mm > coarse sand > 0.5 mm; 0.5 mm > medium sand > 0.250 mm; 0.250 mm > fine sand > 0.063 mm; 0.063 mm > silt > 0.039 mm; clay < 0.039 mm (adapted from Gray and Elliott, 2009).

The biological samples were fixed with 4% buffered formalin. Later, the animals were sorted and the *P. ulvae* individuals were identified and kept in 80% alcohol. They were counted and their total shell length (TSL) and maximum width (MW) were measured (Cardoso et al., 2005, Grilo et al., 2012). A conversion equation was used, based on previous works, (TSL = 2.2289*MW - 0.3886, n = 339, r = 0.97; Lillebø et al., 1999) and the individuals were classified in different size classes (Cardoso et al., 2005; Grilo et al., 2012). Organisms for mercury determination were collected, separated by age groups, frozen and posteriorly freeze-dried for later analysis.

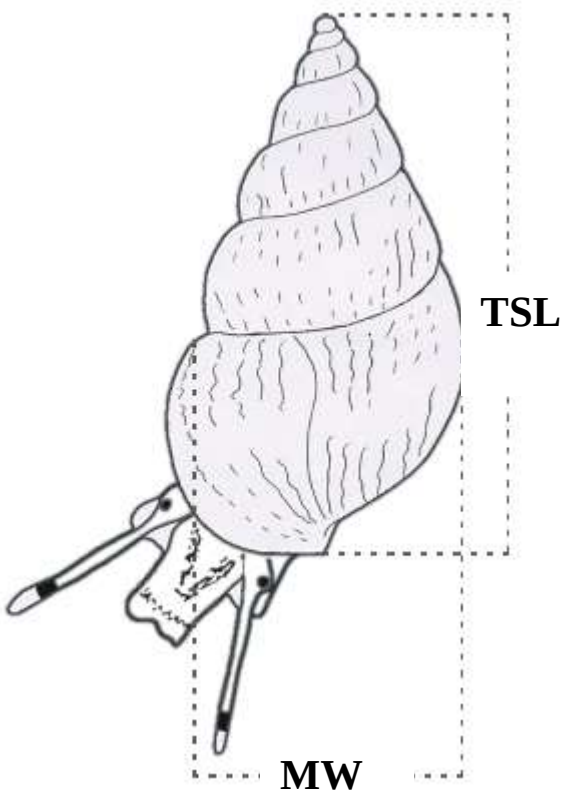


**Figure 2-** ***Peringia ulvae*** **MW (maximum width) and TSL (total shell length)**

To estimate secondary production, length-weight relationships were determined. In order to do so, an ANOVA of length x AFDW relationships was calculated and it showed no significant seasonal differences. An overall regression equation was used (AFDW= 0.0564TSL2.2381, n = 191, r = 0.98; Lillebø et al., 1999). The following conversion equation was also used MW= 0.4369TSL + 0.2091, n = 339, r = 0.97 (Lillebø et al., 1999).

Cohorts were tracked using the FAO ICLARM Stock Assessment Tool package (FISAT II software) (Gayanilo and Pauly, 1997) and size frequency distributions over successive sample dates.

## 2.3 Mercury quantifications

### 2.3.1 Sediments and Organisms

Total mercury content of the sediments and organisms was analyzed by atomic absorption spectrometry with gold amalgamation, using a leco AMA-254 (Advanced Mercury Analyzer) (Costley et al., 2000). The analytical quality control of the total mercury determination was performed using Certified Reference Materials (CRMs), MESS-3 (for less contaminated sediments), PACS-2 (for high contaminated sediments) and TORT-2 (for organisms). The results were corrected according to the daily recovery percentage of the CRM analyses. The values obtained for the whole CRM analysis ranged from 96.8 to 103.3% (at 0.05 significance level) for sediments and 113% for organisms. Analyses of CRMs were always performed in triplicate and coefficient of variation was less than 10%.

All mercury content in sediments values were then compared to two sediment quality guidelines (SQG), the ERL (effects range low) and ERM (effects range median) which define the concentration ranges that are rarely, occasionally or frequently linked to adverse biological effects. (Long et al. 1995).

### 2.3.2 Water

Water samples were filtered with 0.45 μm pore size Millipore filters and acidified with concentrated $HNO_3$ "mercury free" to pH < 2 and maintained in a room at 4ºC. Total mercury analysis in water samples was performed by cold vapour atomic fluorescence spectroscopy (CV-AFS), on a PSA cold vapour generator, model 10.003, associated with a Merlin PSA detector, model 10.023, and using SnCl2 as reducing agent. This analytical methodology is highly sensitive, allowing the measurement of 1 ng $L^{-1}$ of mercury (Mucci et al., 1995)

### 2.3.3 SPM (Suspended Particulate Mercury)

Filters (from the previous process of water filtration) were oven-dried at 60º C and digested with $HNO_3$ 4 mol $L^{-1}$ for determination of the total mercury concentration in the SPM fraction. (as described in Coelho et al. (2007)).

## 2.4 Data analysis

### 2.4.1 Environmental parameters

Some data (i.e. physicochemical variables and density/biomass of P. ulvae) were presented graphically as moving averages (Oct-10 to Sep-11) to minimize the effects of high-frequency variability and visually simplify the data series. Mercury concentrations were presented each two months from Sep-10 to Sep-11.

One-way ANOVAs were carried out for the environmental parameters in order to detect differences between sampling stations. Two-Way ANOVAs were performed for the mercury concentrations in *Peringia ulvae* in order to detect differences between sampling stations and size classes. All data were previously checked for normality using the Kolmogorov-Smirnov test and for homogeneity of variances using the Levene's test (Zar, 1999). Data not meeting these criteria were transformed appropriately (Zar, 1999) and checked again for normality and homogeneity. Whenever data did not meet those criteria were applied non-parametric tests.

### 2.4.2 Secondary production

Secondary production (P) was estimated based on cohort recognition, as described in Dauvin (1986). Total values of P for the population are expressed as:

$$P=\sum_{n=1}^{N} Pcn$$

where Pcn is the growth production of cohort n. $\overline{B}$, the annual mean population biomass, is calculated as:

$$\overline{B} = (1/T)\sum_{n=1}^{N} \overline{B}\,nt$$

where T is the period of study, N is the number of successive cohorts in the period T, $\overline{B}$ n is the mean biomass of cohort n; and t is the duration of the cohort n (Cardoso et al., 2005).

# CHAPTER 3 - RESULTS

### 3.1 Physicochemical parameters of the intertidal water pools.

The results of the environmental parameters for each site at each sampling date are presented in Figure 3.

The temperature, salinity and chlorophyll a values (Fig. 3A, 3B and 3C) presented a seasonal pattern with higher values during summer and lower values during winter. The lowest temperature recorded was 12.3 ºC (in Station **2**, January) and the highest was recorded in June (26.6 ºC, in Station 1). Salinity (Fig. 3B) was lower in February (Station **1**, with 7.7 ºC) and higher in August, Station **2**, with 36.6 ºC. The lowest value for chlorophyll a was 0.3 $mg.m^{-3}$, in October, Station **2**, and the highest was observed in August (10.8 $mg.m^{-3}$), in Station **1**. No significant differences between the three sampling areas was observed (One-way ANOVA, Temperature: $F_2$=0.14, P>0.05; Salinity: $F_2$= 0.18, P>0.05; chlorophyll a: $F_2$= 1.35, P>0.05).

Dissolved oxygen (DO) values (Fig.3D) also varied according to the seasonal pattern, with lower values during spring/summer and higher ones during autumn/winter. The values varied between 7 mg. $L^{-1}$(June, Station **2**) and 10.8 mg. $L^{-1}$ (October, Station **3**) The pH values (Fig. 3E) and the organic matter content (Fig. 3F) did not show any particular variation through the year. No significant differences for the three variables were observed between the three sampling areas (One-way ANOVA, DO: $F_2$= 0.22, P>0.05; pH: $F_2$= 1.27, P>0.05, organic matter: $F_2$ = 2.57, P>0.05).

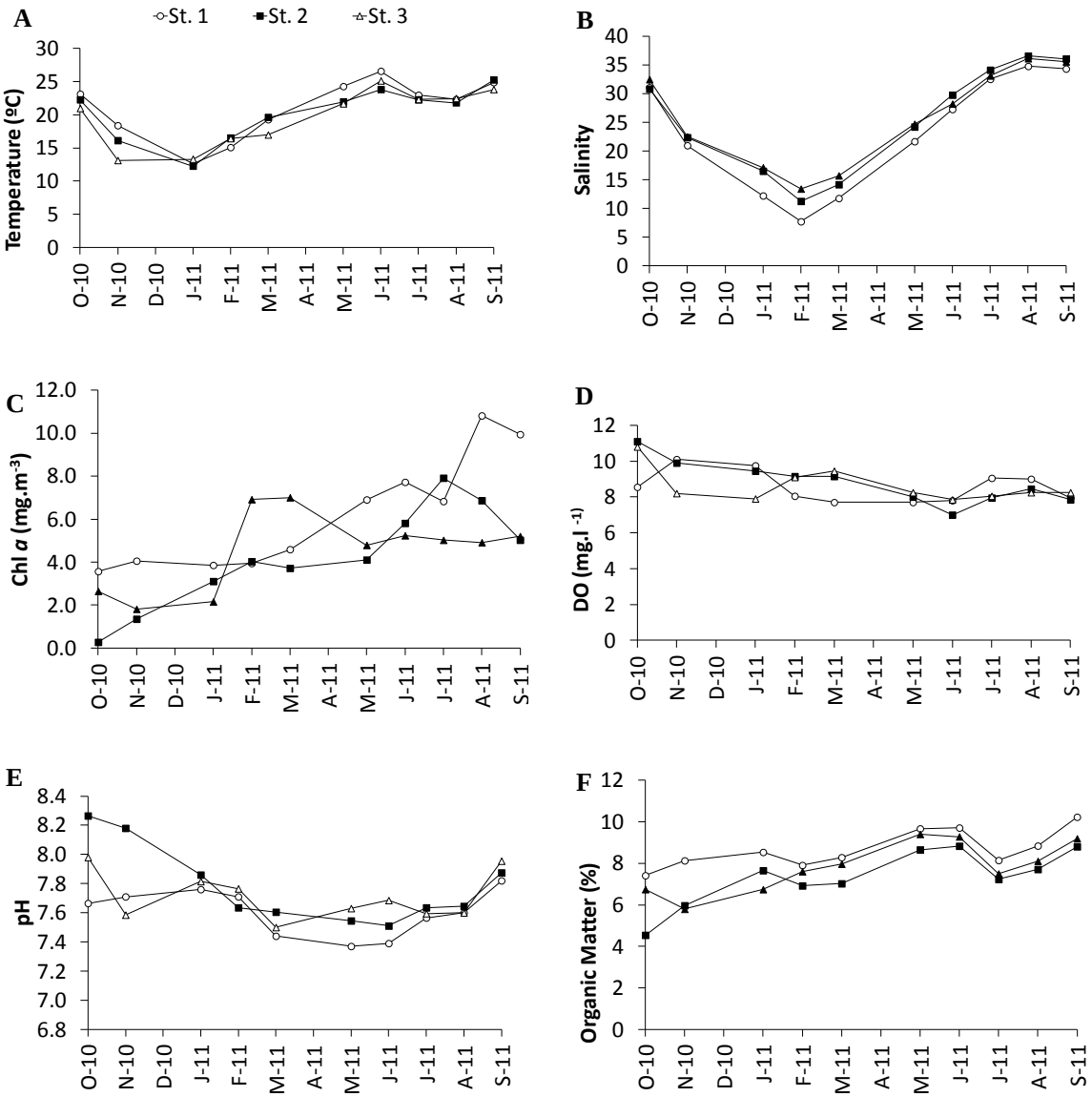


**Figure 3 – Environmental Parameters of the Water. (A) Temperature; (B) Salinity; (C) Chlorophyll a; (D) Dissolved oxygen; (E) pH; (F) Organic matter (%) in the three sampling sites.**

### 3.2 Sediment's Granulometry

Concerning the granulometry, all the stations were dominated by fine sand, ranging from 50% (St. **2**) to 77% (St. **3**). However, no strong oscillations throughout the year were observed (Fig. 4). Regarding most of the sediment fractions (gravel, medium sand and silt) no significant differences were observed between the sampling areas (One-way ANOVA, gravel, $F_2 = 0.18$, $P > 0.05$; medium

sand, $F_2 = 0.83$, $P > 0.05$; silt, $F_2 = 0.39$, $P > 0.05$), except for the coarse and fine sand fractions where significant differences were observed between Stations **2** and **3** (One-way ANOVA, coarse sand, $F_2 = 6.89$, $P < 0.05$; fine sand $F_2 = 5.98$, $P < 0.05$).

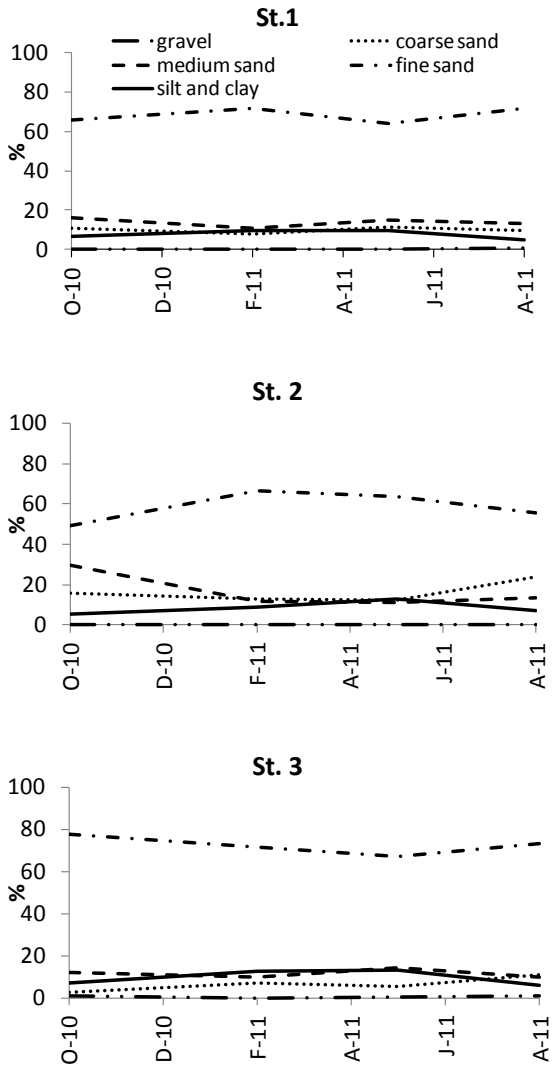


**Figure 4 - Variation in the sediment's granulometry in the 3 stations all over the study period.**

### 3.3 Mercury in sediments, water and SPM.

Concerning total mercury in sediments it was clearly visible a spatial gradient from St. **1** to St. **3** (Fig. 5A). St. **1** always presented the highest values ranging from 82-206 µgHg.g$^{-1}$. St. **2** showed intermediate values, with little variation, from 11 to 24 µgHg.g$^{-1}$, and finally St. **3** showed residual, ranging from 1.3 to 2.0 µgHg.g$^{-1}$. Significant differences were observed between the three sampling areas (1-way ANOVA, $F_2 = 338.03$, $P < 0.05$).

Regarding the total dissolved mercury (Fig. 5B) it is possible to observe that mercury concentrations at St. **1** and **2** were similar and higher than at St. **3**. Generally, it was evident an increase in mercury concentrations throughout the year, especially during the summer. No significant differences were observed between the three areas (One-way ANOVA, $F_2 = 0.63$, $P > 0.05$).

For the SPM mercury concentrations (Fig. 5C) it was possible to observe, once again, St. **1** and **2** with similar and high values (3.43-8.33 µgHg.g$^{-1}$), than St. **3** (1.03-2.70 µgHg.g$^{-1}$). No significant differences were observed between St. **1** and **2** (Wilcoxon two-sample test, $W = 59$, $P > 0.05$), but significant differences were observed between the St. **1** and **3** (Wilcoxon two-sample test, $W = 77$, $P < 0.05$) and between the St. **2** and **3** (Wilcoxon two-sample test, $W = 77$, $P < 0.05$).

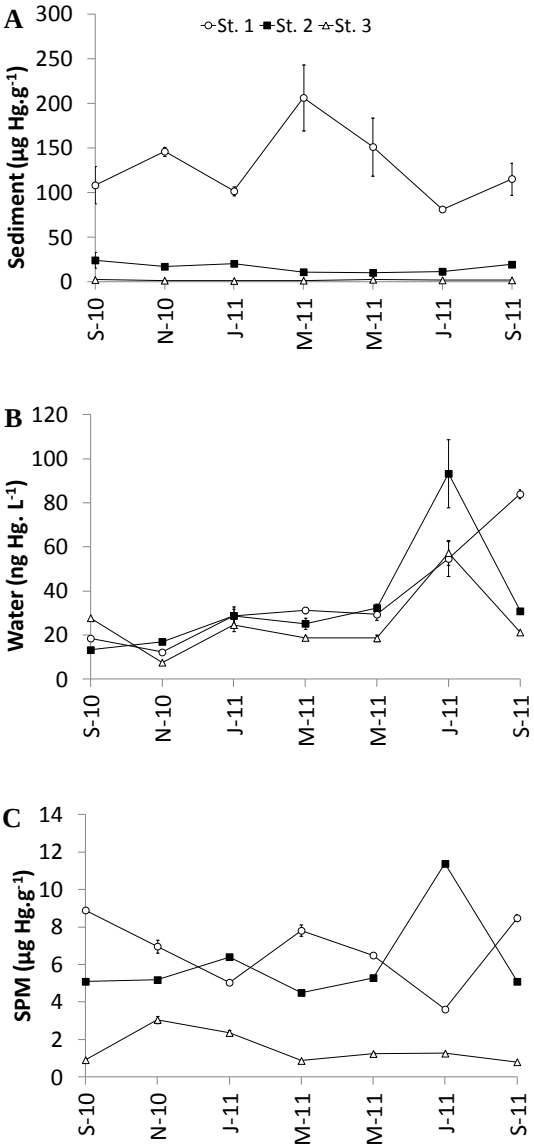


**Figure 5 - Mercury concentrations in sediments (A), water (B) and SPM (C). The error bars represent standard deviations.**

### 3.4 *Peringia ulvae* density and biomass.

Concerning the density pattern, all the stations presented a similar trend all over the time with higher values during winter/spring and lower during summer (Fig. 6 A). It is clear that St. **2**

showed the highest values, reaching the maximum in February with 9882.98 ind. $m^{-2}$. St. **1** and St. **3** presented similar values with minimum values recorded at St. **3** (648.94 ind. $m^{-2}$, in June).

The biomass pattern was relatively constant all over the time for the three areas. St. **2** recorded the highest values (3-5 g AFDW.$m^{-2}$) followed by St. **1** (1-3 g AFDW.$m^{-2}$) and finally St. **3** with the lowest values (0.1-0.6 g AFDW. $m^{-2}$) (Fig. 6B).

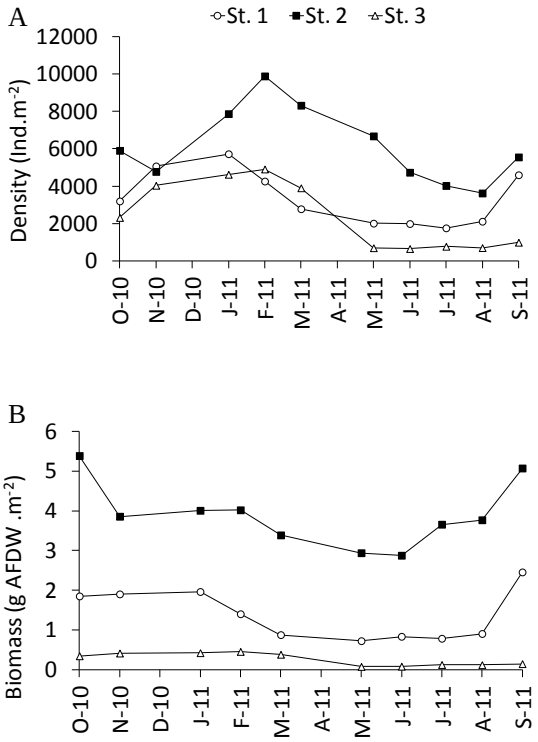


**Figure 6 - Total density (6A) and biomass (6B) of *Peringia ulvae* in the 3 sampling sites.**

### 3.5 Population structure and growth.

Size-frequency distributions were analysed for recognizable cohorts for each sampling station (Fig. 7). Marked differences in the population structure were observed at the three sites. At St. **2** several modes were apparent in the population representing individuals of all age classes (juveniles

< 2 mm TSL; young individuals 2-3 mm TSL; adults > 3 mm TSL (Cardoso et al., 2005). In contrast, St. **1** and **3**, mainly St. **1** were dominated by small individuals of *P. ulvae*, with few adults (> 3 mm) present (Fig. 7).

Regarding the percentage of the different age groups, St. **2** was dominated by adults (38%), contrarily to the other stations. St. **1** and St. **3** presented very similar proportions of size class individuals. Approximately 35% of individuals were juveniles (< 2 mm TSL), 45% were young individuals (2-3 mm TSL) and only 20% were adults (< 3 mm TSL) (Fig. 8).

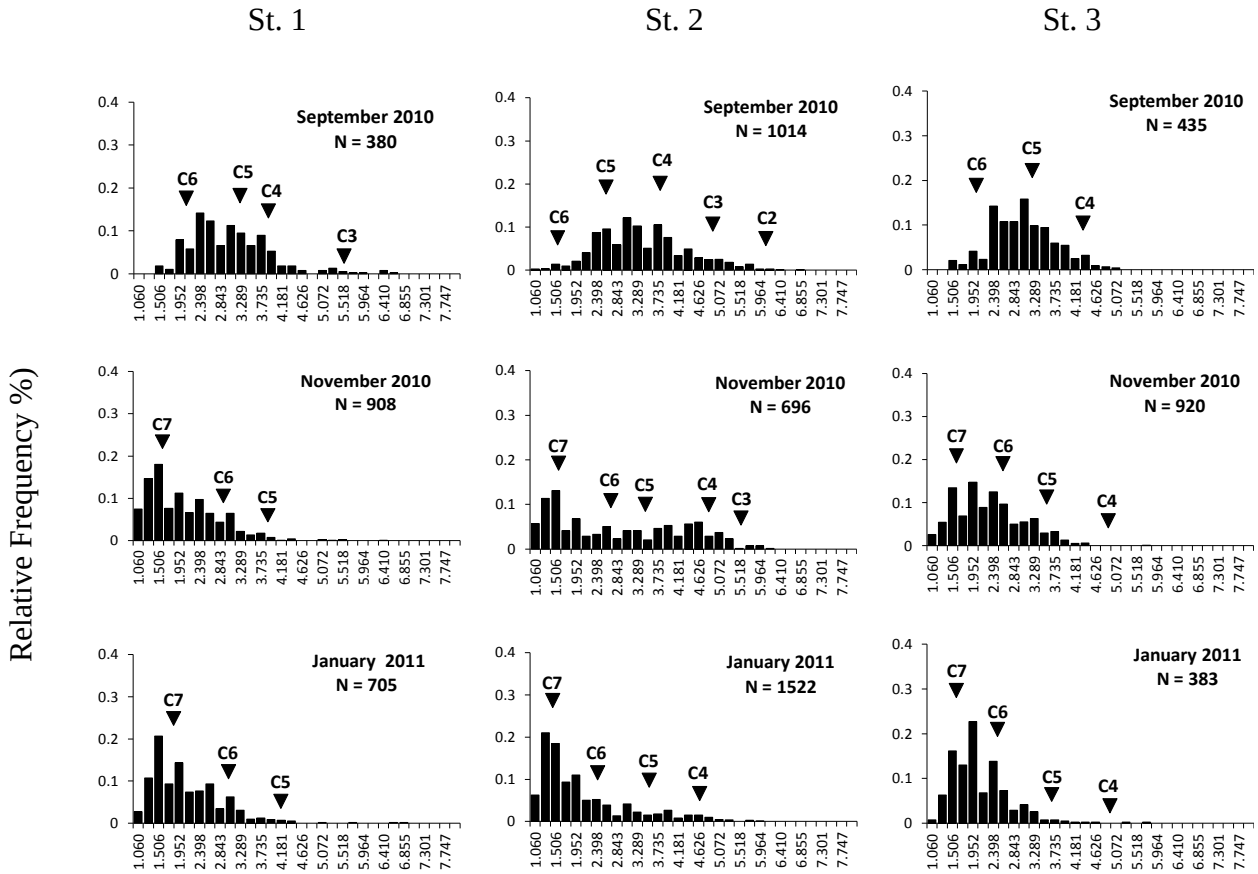


**Figure 7 – *Peringia ulvae*. Size-frequency polymodal distribution at the 4 sampling sites. The cohorts (C) and the number of individuals (N) are represented in the figure.**

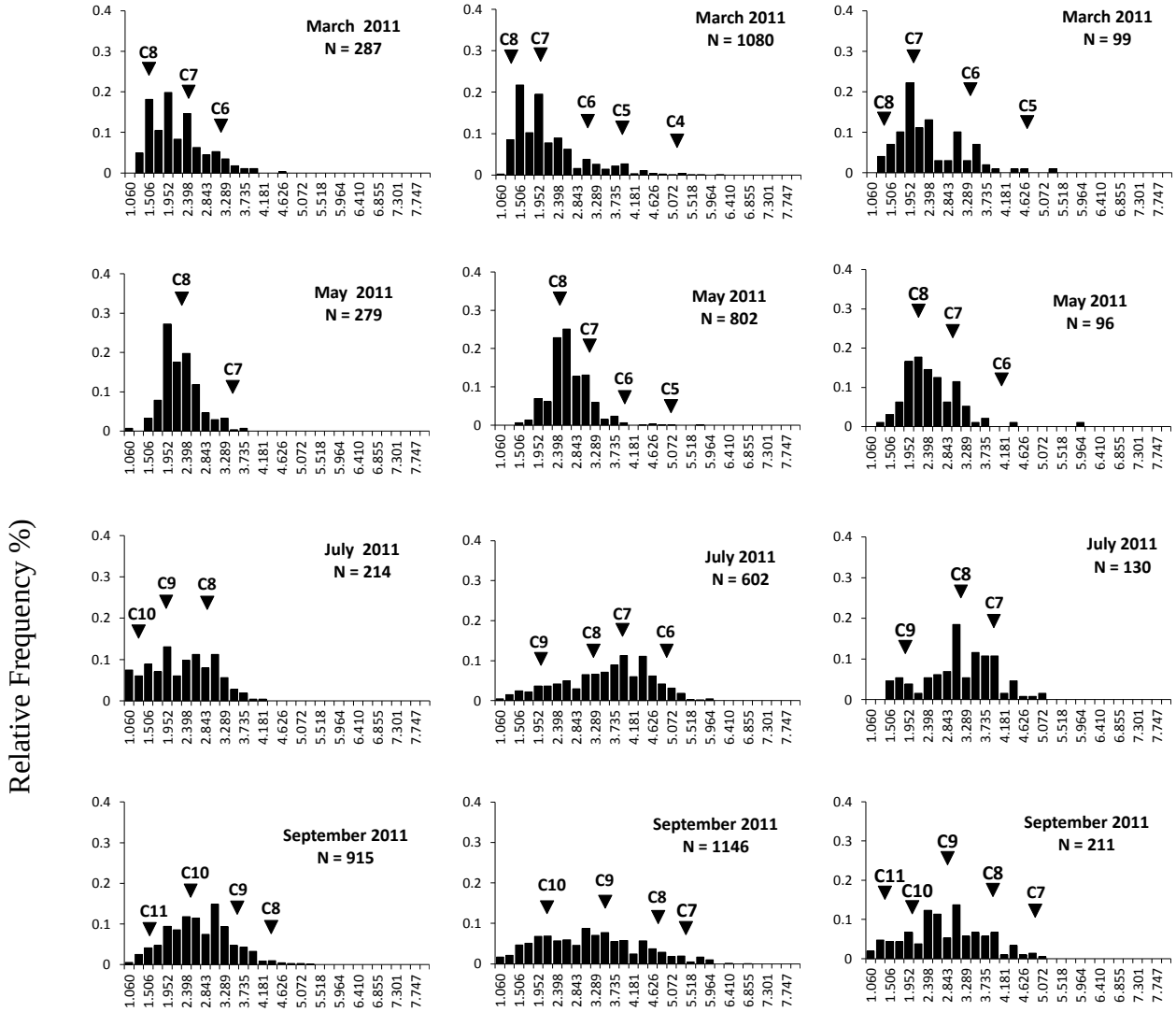


**Figure 7 (continued)**

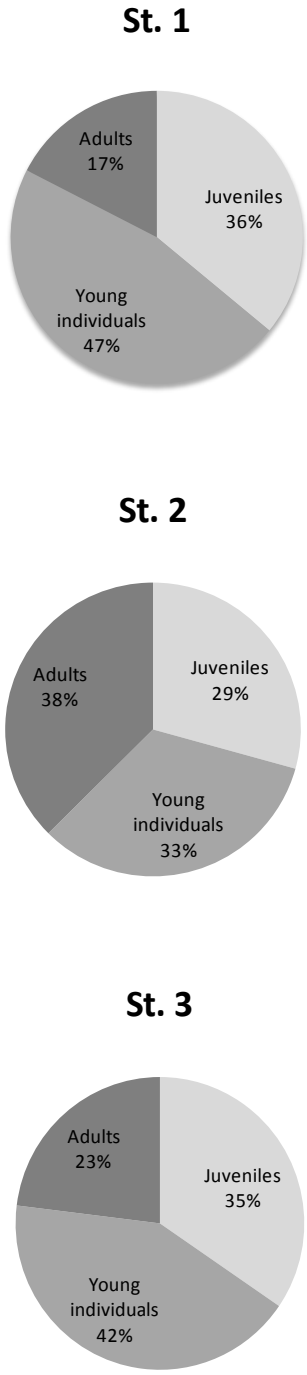


**Figure 8 - Size/Age Class proportion of individuals of *P. ulvae* in the three areas.**

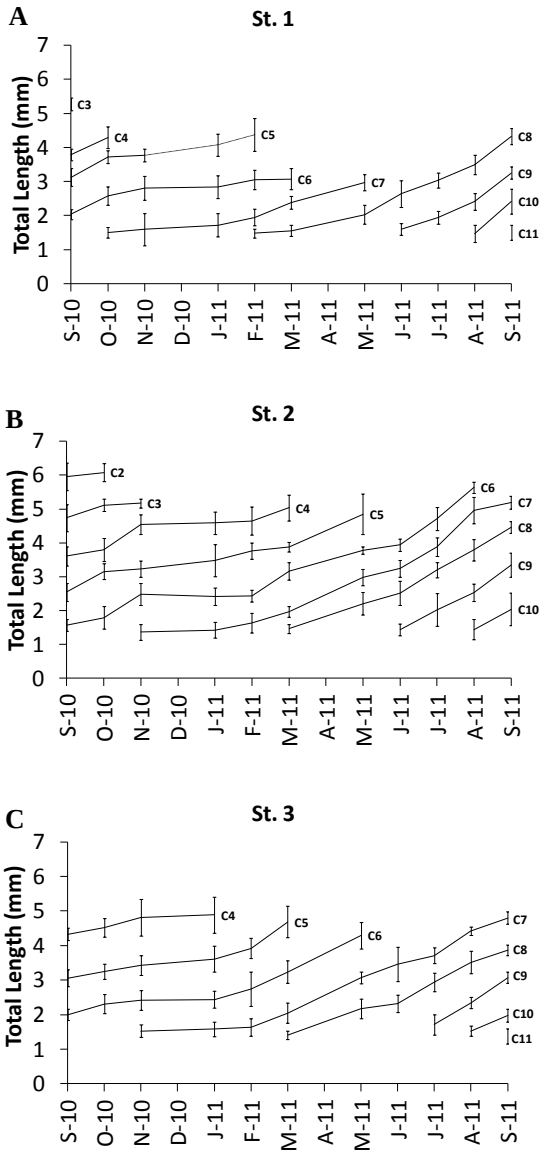


**Figure 9 - Estimated growth of *Peringia ulvae* cohorts (average growth ± standard deviation). (A) St.1, (B) St. 2, (C) St. 3**

### 3.6 Life Span and growth productivity

Analysing figure 9, it is apparent that *P. ulvae* presents 4 cohorts per year (1 spring cohort – February/March, 2 summer cohorts – June and July and 1 autumn cohort – September/October) at all stations. Generally, the autumn and spring cohorts live longer than the summer ones. At St. **2** the

individuals lived longer (mean: 13 months) than at St. **3** (mean: 10 months) and lastly St. **1** (mean: 8 months) (table 1).

Growth production (P) and mean biomass ($\overline{B}$) were considerably higher at St. **2** than at St. **1** and finally St. **3**. The P/$\overline{B}$ ratios were slightly higher at St. **1** and **2** than at St. **3** (table 2).

**Table 1 – Life span estimations for *P. ulvae* in the 3 sampling stations in the different cohorts.**

| | Spring Cohort (months) | Summer Cohort (months) | Summer Cohort (months) | Autumn Cohort (months) |
|---|---|---|---|---|
| St1 | 7-8 | 8 | 7-8 | 8 |
| St2 | 12-13 | 11 | 12 | 14 |
| St3 | 10-11 | 9 | 10 | - |

**Table 2 – Growth production estimations for *P. ulvae* for the 3 sampling stations.**

| | P (g AFDW*$m^{-2}$*$y^{-1}$) | $\overline{B}$ (g AFDW* $m^{-2}$) | P/$\overline{B}$ |
|---|---|---|---|
| St1 | 4.35 | 1.23 | 3.54 |
| St2 | 11.58 | 3.58 | 3.23 |
| St3 | 2.72 | 1.17 | 2.34 |

### 3.7 Mercury accumulation in biota.

Total mercury levels in P. ulvae were generally higher in the most contaminated area, St. **1** (ranging from 0.050-0.250 µg Hg $g^{-1}$), followed by St. **2** with intermediate values (0.050-0.070 µg Hg $g^{-1}$) and finally St. **3** with the lowest values (0.030-0.050 µg Hg $g^{-1}$) (Fig. 10). This bioaccumulation pattern was consistent with the contamination gradient of the sediments.

Significant differences between individuals of St. **1** and **2** and St. **1** and **3** were observed (Two-way ANOVA, $F_2 = 34.4$, $P < 0.05$) however, no significant differences were observed between individuals of St. **2** and **3** (Two-way ANOVA, $P > 0.05$). On the other hand, significant differences between juveniles and adults and between young individuals and adults were observed (Two-way ANOVA, $F_2 = 13.83$, $P < 0.05$). Despite these differences between age classes, no metal accumulation through life was observed (Fig. 10). At St. **1**, a great variation in mercury concentration between age classes was observed.

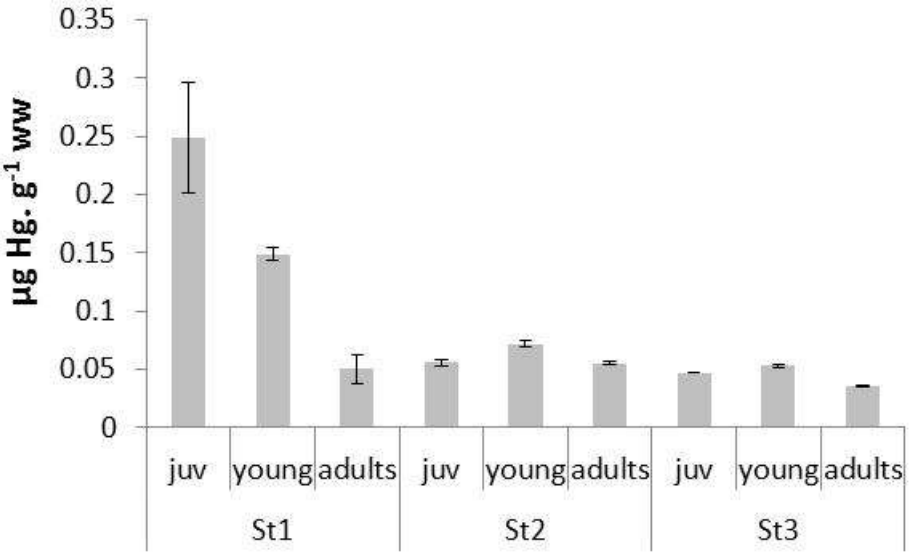


**Figure 10 - Mercury accumulation in *P. ulvae* age classes in the 3 stations. Error bars correspond to standard errors.**

# CHAPTER 4 - DISCUSSION

### 4.1 Environmental parameters and mercury

Macrobenthic invertebrates are often used to study the impact of pollutants because they are an important link in estuarine food web. Even though they are usually good indicators, for their relative sedentary lifestyle and variety of responses to stress, they can also respond paradoxically (Bilyard, 1987; Weisberg, 1997). All this makes the assessment of pollutants impacts very delicate and it is imperative to be sure that the physico-chemical parameters are not responsible for the differences found between our study sites. (Dauvin et al., 2008)

In the present study, the variations of environmental parameters, such as temperature, salinity, chlorophyll *a* and dissolved oxygen, were mostly due to climatic seasonality. This is probably a result of the changes in freshwater inputs caused by lower precipitation, higher evaporation rates and a larger amount of estuary penetration of seawater. The dissolved oxygen values were within the expected range according to other studies in the Ria (Nunes et al., 2008). Chlorophyll *a* values were in accordance with the typical higher microbial fotossintetic activity caused by the higher solar radiation. (Ramalhosa et al., 2002; Lillebø et al., 2005; Lopes et al., 2007)

pH values, organic matter content (OM) and granulometry showed no important variations throughout the year (Pereira et al., 2009). None of the environmental parameters showed significant differences between the 3 sampling sites.

The mercury content analysis (sediments and water) showed a spatial gradient with higher values at St. **1** and **2** and minimum values at station 3, in accordance with previous studies (Nunes et al., 2088; Pereira et al., 2009). The sediments presented the highest mercury concentrations, throughout the study, especially at St. **1**, suggesting that mercury is strongly associated with sediment (Pereira et al., 2009). The values for Ecotoxicological Assessment Criteria (EACs) for mercury in sediments and water measured by the OSPAR Commission in 2004 revealed that these values ranged from 0.05 mg $Kg^{-1}$ for the sediments and from $5ng.L^{-1}$ for the water (OSPAR, 2004).

In the present work, total mercury concentrations in sediments were all above the EACs threshold, despite at St. **3** the Hg concentration were close to the acceptable values. However, at St.

**1** and **2**, the levels of mercury were much higher than the upper-EAC, contributing to long-term biological effects, such as mortality (OSPAR, 2004).

Regarding total dissolved mercury, there were no significant differences between the three sampling sites which is consistent with other studies in the area that conclude that there are no mercury cycling between sediments and the water column (Ramalhosa et al., 2006). However, the values obtained for St. **1** and **2** were close to the upper limit during most of the study period and in summer/autumn much higher values than the upper EAC limit were observed. On the other hand, at St. **3**, the values were generally lower than the upper EACs threshold.

Concerning the SPM fraction, significant differences in Hg concentrations between the most contaminated areas (St. **1** and St. **2**) and the least contaminated one were observed.

### 4.2 *Peringia ulvae* population dynamics

The present work revealed clear differences in the dynamics and structure of *P. ulvae* population along the mercury contamination gradient. Higher abundances and biomasses, as well as secondary production values were observed at the intermediate contaminated area (St. **2**) than the most contaminated area (St. **1**) and least contaminated (St. **3**) areas. This pattern was quite surprising according to the literature. It would be expected that that abundance and biomass of macrobenthic species should be lower at most polluted sites, according to the findings of Mucha et al. (2005), Calabretta and Oviatt (2008) and Amin et al. (2009). Also, the findings of Araújo et al. (2012) say that *Peringia ulvae* tends to avoid contaminated sites.

However, our findings are in accordance with Dauvin et al. (2008) regarding the macrobenthic *Abra alba-Pectinaria koreni*, found in very high abundance in very contaminated areas. This result also corroborates the findings of MsLusky et al. (1986) which pointed out that in general, molluscs are one of the taxonomic groups least sensitive to metallic pollution compared to annelids and crustaceans.

The *P. ulvae* response to Hg contamination could be explained by the hormesis phenomenon. Hormesis is a contradictory effect of a very toxic chemical that can be beneficial in low doses. This is a common biological effect of metal contamination (Calabrese and Blain, 2004). For example, Heinz et al. (2011) observed that the hatching success of eggs injected with low doses of mercury was significantly greater (93.3%) than that of controls (72.6%). However, by increasing the doses of mercury, the hatching success decreased. Usually, the dose needed to produce a hormetic effect is between 30-60% superior to the control one (Calabrese, 2008).

Although these findings were only tested at an experimental level, in our case we can consider that St. **3** is our control, St. **2** is our low dose and St. **1** is the high dose.

In terms of population structure and growth, *P. ulvae* was negatively affected by the high values of mercury pollution. At St. **1**, a much debilitated population mainly constituted by juveniles and young individuals was observed. At St. **2**, an opposite pattern was observed, characterized by a well structured population, in which all age classes were represented. It was also possible to observe that individuals from St. **2** lived longer than the individuals from the other stations.

Once again, the hormesis phenomenon may explain the success of *P. ulvae* at St. **2**.

According to the SQG (Sediment Quality Guidelines), values much higher than ERM (effects range median) for mercury were observed at St. **1**, which may be responsible for the adverse biological effects observed in the p. ulvae population. The incidence of adverse biological effects caused by mercury is of about 42 % when the concentration of mercury in sediments is higher than 0.71 ppm (ERM) (Long et al., 1995).

Regarding population growth production, there were no effects of mercury contamination. St. **1** presented higher production values than St. **3**, which is consistent with the biomass values.

Apparently, mercury contamination only affected *P. ulvae* to a certain extent (density, biomass, population structure and life span).

Generally, polluted areas are colonized by species with greater ability to resist to adverse conditions. When able to tolerate the pollutant, they proliferate and dominate the ecosystem,

competing with the other species and may reach very high densities for long periods of time (Grizzle et al, 1984; Gaston et al., 1998). That is why, in most cases, abundance and biomass alone are not sufficient to understand the ecosystem functioning (Dolbeth et al., 2011). We also needed to assess population structures and growth as well as secondary production.

As mentioned before, estuaries are environments that usually have a history of anthropogenic stress and instability and so, the species have a natural tendency to resist to future disturbances, and our results may show that *P. ulvae* may be developing or already has a tolerance to mercury contamination (Madwick and Jones, 2002; Whomersley, 2011).

### 4.3 Mercury bioaccumulation.

Finally, regarding the mercury bioaccumulation, it was found that at St. **1** (most contaminated area) there were more differences in Hg bioaccumulation between age groups, than in other stations. However, as far as possible from the discharge point, the variations in mercury concentrations due to size were minimized. The same conclusions were observed by Elliott and Griffiths (1986) for the bivalve *Mytillus edulis*. The younger individuals had higher mercury concentrations than adults. These results show that there is no mercury accumulation trough lifespan, contrarily to, for example, *Scrobicularia plana* (Coelho et al., 2006). This is probably due to the great efficiency of assimilation rates in younger individuals and also the high excretion rates in adults. Another explanation could be based on the growth dilution phenomenon. It is possible that *P. ulvae* grows faster then it absorbs mercury and therefore a growth dilution phenomenon occurs. This phenomenon is also observed in some fish species like *Liza aurata* (Tavares et al., 2011) and the Atlantic salmon *Salmo salar* (Ward et al., 2010).

It is very important to assess the mercury accumulation on such relevant species since it gives us an idea about its possible role in the biomagnification process. *P. ulvae* is a common food resource for several waders (Cabral et al., 1999) and fish species, some of them with economic importance

like *Platichthys flesus* (Aarnio and Mattila, 2000). So, *P. ulvae* may be considered as an important vehicle for mercury transfer through the trophic web.

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