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Fishery of *Chelidonichthys lucerna* (Linnaeus, 1758) in portuguese northwest atlantic coast: Exploratory baseline study

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Abstract
Biological parameters (Sex, Total Length, Total Weight, Gonad Weight and Sexual Maturation Phase) and total metal (Cd, Hg and Pb) concentrations were assessed in fish species *Chelidonichthys lucerna* (LINNAEUS, *1758)* from Portuguese northwest (NW) Atlantic coast during January to May 2008. Metals were determined in muscle tissues by Atomic Absorption Spectrometry. The main objectives of this work were: (i) study temporal variations and correlations between biological parameters and metals during fishing season; (ii) classify the ecological quality of Portuguese coast based on metals in this fish species and (iii) assess human health risks associated with their consumption. Biological parameters showed significant temporal variations ($p< 0.05$; Total Length: $F_{(5, 14)}=6.24$; Total Weight: $F_{(5, 14)}=8.42$; Gonad Weight: $F_{(5, 14)}=1.84$), except Sexual Maturation Phase. Metal concentrations (µg.kg^{-1}, dry basis) ranged: (i) Cd: <2.0 – 89.1; (ii) Hg: <20.0 – 1044 and (iii) Pb: <4.8 – 403. Only Cd showed significant temporal variations ($p< 0.05$; *Cd*: $F_{(5, 14)}=29.84$): January = April << February = March = May. No significant ($p > 0.05$) correlations were obtained between any biological parameter and metals of *C. lucerna.* Although high metal concentrations in *C. lucerna* suggested anthropogenic contamination, according European Regulations Nº 1881/2006 and 629/2008, Portuguese coast was classified as “Unpolluted” during January – May 2008. Indeed, health risk assessment study showed that there was no potential human health risks associated with the consumption of *C. lucerna* from Portuguese seawaters.

Keywords: Fishery, metals, ecological quality, anthropogenic contamination, health risk assessment

1. Introduction

*Chelidonichthys lucerna (*Linnaeus, 1758) is a *Triglidea* fish species existing in Portugal, whose commercial designation is “Ruivo”, “Cabra-Cabaço” or “Cabra” [1]. This species has great commercial importance among *Triglidea* or gurnards, as it reach big dimensions in Portuguese coast and high market value [2].

This species is an important ecological marine fish distributed along Atlantic Sea (from Norway to Cape White), Mediterranean Sea and Black Sea [3]. Its global coloration varies from orange to brown [3]. It distinguishes of the remaining gurnards by its colourful pectoral fins (between blue and violet) with white or green spots and red or light-blue margins [3]. Their juveniles colonize Portuguese estuaries as maternity and shelter areas and they were abundantly reported in Aveiro, Mondego and Tagus waters [4-8].

Considering their high commercial and ecological value in Portuguese coast, we selected *C. lucerna* as biomonitor species of metal (Cd, Hg and Pb) contamination in Portuguese Northwest Atlantic Coast in 2008. The purpose of our baseline study is to provide data base against which to monitor and assess fishery’s progress and effectiveness during time. Commonly the data needed for baseline and comparison studies does not exist or it will be incomplete and of poor quality. This is our contribution for reporting scientific valid information about *C. lucerna* fishery in Portugal.

Non-essential elements to human life can produce adverse effects on human health when ingested even in small amounts [9-10]. Although, most of the accumulation of these toxic metals occurs in liver and kidneys, they can also be found in other tissues such as muscles, skin or bones [9-10]. Total elimination of toxic metals from human body can take between 17 to 38 years [10]. Considering the fact that some toxic metals, particularly Cd, Hg and Pb, are neurotoxic to humans with no or very low degradation along aquatic food chains, it is essential to monitor their concentrations in commercial fish species, such as *C. lucerna* [11-28].

Indeed, European Commission Regulation (EC) No 1881/2006 sets maximum levels for Cd, Hg and Pb allowed in fish species, in order to protect public health and keep them at levels which do not cause health concerns [29-30]. These maximum levels for Cd, Hg and Pb must be safe and as low as reasonably achievable based upon good fishery and manufacturing practices [29-30].

The only work regarding metals in *C. lucerna* captured in Portuguese seawaters was performed by Cid *et al*. (2001), which obtained very low concentrations (µg.kg^{-1}, wet basis) in their muscle tissues ranging: (i) Cd: 5.6 – 12.7 and (ii) Pb: 71 – 145 [11]. This work concluded that the Portuguese coastal lagoon "Ria de Aveiro" presented no metal contamination and suggested that *C. lucerna* can be used as bioindicator of metal contamination in future monitoring programmes [11].

Metals had been assessed in gurnard fish species (*C. lucerna* and *Trigla gurnardus*) captured in different aquatic ecosystems (coastal seawaters, estuaries, rivers and bays) of several European Member States (Italy, France and England) during the last decades (1994-2018) [12-15, 18-19]. These works reported metal (Cd, Hg and Pb) concentrations (µg.kg^{-1}) ranging: (i) Cd: 10-250; (ii) Hg: 10 – 2277 and (iii) Pb: <10 – 900 [12-15, 18-19]. These concentrations were much higher than those reported in Ria de Aveiro by Cid *et al* (2001) and proved that gurnard fish species can evidence the different local and regional metal contamination of aquatic ecosystems [11]. Indeed, different gurnard fish species (*C. lucerna, C. Kumu, C. gabonensis, T. gurnardus, T. lyra* and *T. cuculus*) had been used as bionomitor of metal contamination in different worldwide countries (Africa, Australia, New Zealand and Turkey) [16-17, 20-28]. These works also obtained similar or higher metal concentrations than *C. lucerna* from Portugal and those non-European gurnard fish species reported metal (Cd, Hg and Pb) concentrations (µg.kg^{-1}) ranging: (i) Cd: 2-790; (ii) Hg: 100 – 370 and (iii) Pb: 4 – 4270 [16-17, 20-28].

Comparisons with maximum metal (Cd, Hg and Pb) concentrations allowed in fish species for human consumption, defined by European Regulations (EC) No 1881/2006 and 629/2008, revealed that European gurnard fish species represented no risk for human health during 1994 - 2018, except *C. lucerna* from Adriatic Sea (Italian coast) in 2006 and *T. gurnardus* from Bay of Biscay (French coast) during 2001-2010 [11-15, 18-19, 29-30]. However, the high metal concentrations obtained in gurnard fish species (*C lucerna, T. gurnardus, T. lyra* and *T. cuculus*) captured in all Turkish waters (Bay of Iskenderum, Yalova coast and Aegeon Sea) represented significant adverse effects for human health [16-17, 25-30].

Generally, the interaction between Cd and Pb levels and total weight and total length of fish is negative [28]. The size of marine organisms plays an important role in the content of metals in their tissues [31-32]. Although this relationship is important for muscle of *C. lucerna*, it was only consistently demonstrated for Hg [28]. Normally, the amounts of Hg increase according to size and age of marine organisms [33].

Following these previous evidences, our work will study the main biological parameters (Sex, Total Length, Total Weight, Gonad Weight and Sexual Maturation Phase) and toxic metal (Cd, Hg and Pb) concentrations in muscle of the commercial fish species *Chelidonichthys lucerna (*Linnaeus, 1758) from Portuguese Northwest (NW) Atlantic seawaters in order to:

1. Study if their biological parameters and metal concentrations showed significant temporal variations during fishing season;
2. Establish significant correlations between their biological parameters and metal concentrations;
3. Classify the ecological quality of Portuguese NW Atlantic seawaters based on metal concentrations in this fish species;
4. Assess human health risks associated with the consumption of this fish species.

2. Materials and Methods

2.1 Study Area

The biological samples of *C. lucerna* were obtained monthly during January to May 2008 and came from the trawler fleet operating on the Portuguese NW Atlantic coast, particularly between 6 – 100 nautical miles of fish-market of Matosinhos, between Caminha and Figueira da Foz (Figure 1). This sampling was framed in the National Biological Sampling Program (PNAB) of IPMA and supported by the Data Collection Framework (PNAB/EU-DCF).

2.2 Reagents and Materials

All reagents were of pro analysis (p.a.) or superior grade: HNO_3 (Fluka, 35% (w/w), suprapure); Cd, Hg and Pb standard solutions of 1000 mg.L^{-1} (Fluka, p.a.). All working solutions were prepared with ultrapure water (Millipore Milli-Q System, conductivity: 0.054 µS.cm^{-1} at 25 ºC) and materials were previously decontaminated in acid solution (HNO_3: 20% (v/v)) at least during 24 h.

2.3 Sampling strategy and analytical methods

A total of 89 independent individuals of *C. lucerna* were sampled during January and May 2008. For each individual, the main biological parameters were measured, such as: (i) Sex; (ii) Total Length (precision of 1mm); (iii) Total Weight (precision 0.01 g); (iv) Gonad Weight (precision 0.01 g) and (v) Sexual Maturation Phase (MP: Immature; Developing; Spawning and Regressing) [34-35]. Then, each quantitative biological parameter was distributed by sampling month and MP. For each individual, approximately 10 g of fresh muscle tissues were dissected, lyophilized and homogenized and three representative replicates (dry mass: 300 mg; N=3) were acid-digested following Reis and Almeida (2008) method and APHA (1998a,b,c) recommendations [36-39].

Total Cd, Hg and Pb concentrations were determined by Atomic Absorption Spectrometry (SpectrAA 220 FS, Varian) with electrothermal atomization (GTA 110, Varian) and cold vapour generation (Generator VGA 77, Varian) following APHA recommendations [37-39].

Significant matrix effects and suitability of the analytical methods for *C. lucerna* were assessed using standard reference materials of dogfish muscle (NRCC DORM-2) certified for trace metals. This reference material showed acceptable mean recovery for all metals (Mean ± Standard Deviation, N=10): Cd: 99±17%; Hg: 109±36% and Pb: 94±20%.

2.4 Data analyses

For metal (Cd, Hg and Pb) analyses, Varian software SpectrAA (v5.1) was programmed to compile data with precision errors below 10% between replicates. Aqueous standards solutions of metals were used on the external calibration and his limit of detection (LOD) was calculated following APHA recommendations: (i) Cd: 2.0 µg.kg^{-1}; (ii) Hg: 20.0 µg.kg^{-1} and (iii) Pb: 4.8 µg.kg^{-1} [40]. Metal concentrations in blank solutions were always below those LOD values.

The level of significance used in all statistical analyses of quantitative biological parameters and metals was 0.05 and a package of sequential statistical tests were carried out [31-32, 41]: (i) normality with Shapiro-Wilk test by SPSS software; (ii) homogeneity of variances with Cochran test by WinGMAv 5 software (EICC, University of Sydney); (iii) significant differences among biological parameters or metals with one-way analyses of variance (ANOVA) by WinGMAv 5 software (EICC, University of Sydney); (iv) clusters of similar biological parameters or metals were established with Student-Newman-Keuls (SNK) test for ANOVAs (post hoc test) by WinGMAv 5 software (EICC, University of Sydney).

Following this sequence of statistical analyses, our work will test several hypotheses:

1. Biological parameters (Sex, Total Length, Total Weight, Gonad Weight and Sexual Maturation Phase) and metal (Cd, Hg and Pb) concentrations in *C. lucerna* will showed significant temporal variations during fishing season;
2. Metal concentrations in *C. lucerna* will be significantly ($p< 0.05$) correlated and dependent of their biological parameters;
3. Human health risks associated with the consumption of this fish species can be assessed by metal concentrations in this fish species.

3. Results and Discussions

3.1 Biological Parameters

Biological parameters (Sex; Total Length; Total Weight; Gonad Weight and Sexual Maturation Phase) measured in *C. lucerna* from Portuguese NW Atlantic seawaters are shown in Table 1 and Figure 2.

Correlations between these biological parameters (Total Length; Total Weight and Gonad Weight) are shown in Figure 3.

All quantitative biological parameters showed significant temporal variations ($p< 0.05$; *Total Length*: $F_{(5, 14)}=6.24$; *Total Weight*: $F_{(5, 14)}=8.42$; *Gonad Weight*: $F_{(5, 14)}=1.84$). Total Length and Total and Gonad Weights showed that this fish species is growing from January to April, until reaching their final spawning phase at April (Table 1). The distribution of biological parameters by their sex also shows that female individuals are always bigger, heavier and with bigger gonads than males, even after spawning (Figure 2).

As previously reported by Canlie and Atli (2003), there are positive increasing correlations between Total Length of *C. lucerna* individuals and their Total and Gonad Weights (Figure 3) [27]. There are also a positive increasing correlation between Total Weight and Gonad Weight. However, only the correlation between Total Length and Total Weight was statically significant ($p< 0.05$; $F_{(5, 14)}=23.21$) (Figure 3). Thus, the Total Length of individuals of *C. lucerna* can be estimated directly by their Total Weight (and vice-versa) with 95% of confidence.

3.2 Metal concentrations in muscle of *C. lucerna*

Total metal (Cd, Hg and Pb) concentrations obtained in muscle tissues of *C. lucerna* from Portuguese NW Atlantic Coast in January - May 2008 are shown in Table 1 and Figure 2. Their mean concentrations ($\mu g.kg^{-1}$, dry basis) ranged: (i) Cd: <2.0 – 89.1; (ii) Hg: <20.0 – 1044; (iii) Pb: <4.8 – 403. The correlations between quantitative biological parameters (Total Length; Total Weight and Gonad Weight) and metals (Cd, Hg and Pb) concentrations in muscle of *C. lucerna* from the Portuguese NW Atlantic Coast are shown in Figure 4.

The mean metal concentrations in muscle of *C.lucerna* were higher than those previously reported to muscle of *C. lucerna* from Ria de Aveiro or Italian Coast, respectively, by Cid *et al*. (2001) and Camusso *et al*. (1998) [11-12]. Thus, the high concentrations of toxic-metals could suggest that Portuguese NW Atlantic Coast is starting to suffer metal anthropogenic contamination. Similar results were also obtained by Reis *et al*. (2012a,b) in this Portuguese region using the crustaceans *Chthamalus montagui* and *Pollicipes pollicipes* during 2009 – 2011 [31-32].

Individually, only Cd concentrations in *C. lucerna* showed significant temporal variations ($p< 0.05$; *Cd*: $F_{(5, 14)}=29.84$) during 2008: January = April << February = March = May (Table 1). Thus, at the end of spawning season, *C. lucerna* individuals are bigger and seem to have active Cd detoxication mechanisms. This phenomenon was already reported for crustacean species by Reis *et al*. (2012a,b) [31-32]. Both Hg and Pb showed no significant ($p > 0.05$; *Hg*: $F_{(5, 14)}=0.55$ and *Pb*: $F_{(5, 14)}=0.16$) temporal variations during 2008 (Table 1).

No significant ($p > 0.05$) correlations were obtained between biological parameters and metal concentrations (Figure 4). However, Cd and Pb showed negative relations with Total Length, Total Weight and Gonad Weight of individuals, proving previous suggestions that bigger and older (advanced sexual maturation phase) individuals of *C. lucerna* can metabolize and regulate Cd and Pb in their muscles. These same results were obtained with *A. cuculus* in Turkey by Canlie and Atli (2003) [27]. On other hand, Hg showed positive relations with Total Length, Total Weight and Gonad Weight of individuals, suggesting that *C. lucerna* has no effective detoxification mechanism of Hg from their bodies. This Hg increasing bioaccumulation was also obtained on *C. lucerna* from Italy [12-15], England [18] and France [19] and in other gurnard fish species, such as *C. kumu*, *C. gabonensis* and *Trigla gurnardus* from Australia, África and France [Table 2; [19, 21-24].

3.3 Ecological quality of seawaters

As mentioned before, our total metal (Cd, Hg and Pb) concentrations obtained in muscle tissues of *C. lucerna* from Portuguese NW Atlantic Coast, in January - May 2008, were higher than those from Ria de Aveiro Table 2; [11]. Comparisons with worldwide works concerning metals in gurnard fish species showed that our metal concentrations were also similar or higher than those reported in Italy [12-14; 16], England [18], New Zealand [20], Australia [21-23], Africa [24] or Turkey [25-27] coasts (Table 2). However, metal concentrations in gurnard fish species from Gulf of Tigullio (Italian coast), Bay of Biscay (French coast) and Mediterranean Sea (Turkish coast) were much higher than our metal concentrations in *C. lucerna* [Table 2; 15; 19; 28]. These results suggested that Portuguese Northwest Atlantic Coast was starting to suffer metal anthropogenic contamination.

European Community Commission (Regulations No 1881/2006 and 629/2008) established maximum Cd, Hg and Pb concentrations ($\mu g.kg^{-1}$, wet basis) allowed in foodstuff for consumer health protection, including on fish species: Cd < 50; Hg < 500; Pb < 300 [29-30]. These maximum allowable concentrations in fresh basis were converted into dry basis concentrations (µg.kg^{-1}, dry basis) considering that fresh fish species contained 80% of water: Cd < 250; Hg < 2500; Pb < 1500 [31-32, 41] Regarding these guideline values and our metal concentrations in *C. lucerna*, the seawater in Portuguese NW Atlantic Coast could be always classified as "Unpolluted" during January – May 2008 (Table 1).

Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) also established maximum concentrations of contaminants (Cd, Pb and Hg) that a person can be exposure per week over a lifetime without unacceptable risks to their health and defined "Provisional Tolerable Weekly Intake (PTWI)" values (µg.kg body weight^{-1}.week^{-1}) of Cd: 7, Pb: 25 and Hg: 0.0049 [42]. Regarding the different types of health injuries and diseases that these metals may produce in human, United States - Environmental Protection Agency (US-EPA)/FAO/WHO also defined "Daily Reference Dose" (RfD; µg.kg body weight^{-1}.day^{-1}) values for Health Risk Assessment studies: Cd: 1, Pb: 3.6 and Hg: 0.3 [42-46].

Applying the sequential step-by-step EPA/FAO/WHO method (detailed description can be found in FAO/WHO, 2011), our "Estimated Daily Intake (EDI)" and "Hazard Quotient" (HQ) values were calculated (Table 3) and our final HQ values were always below 1, which means that there were no potential human health risks associated with the consumption of *C. lucerna* from Portuguese seawaters during 2008 [42].

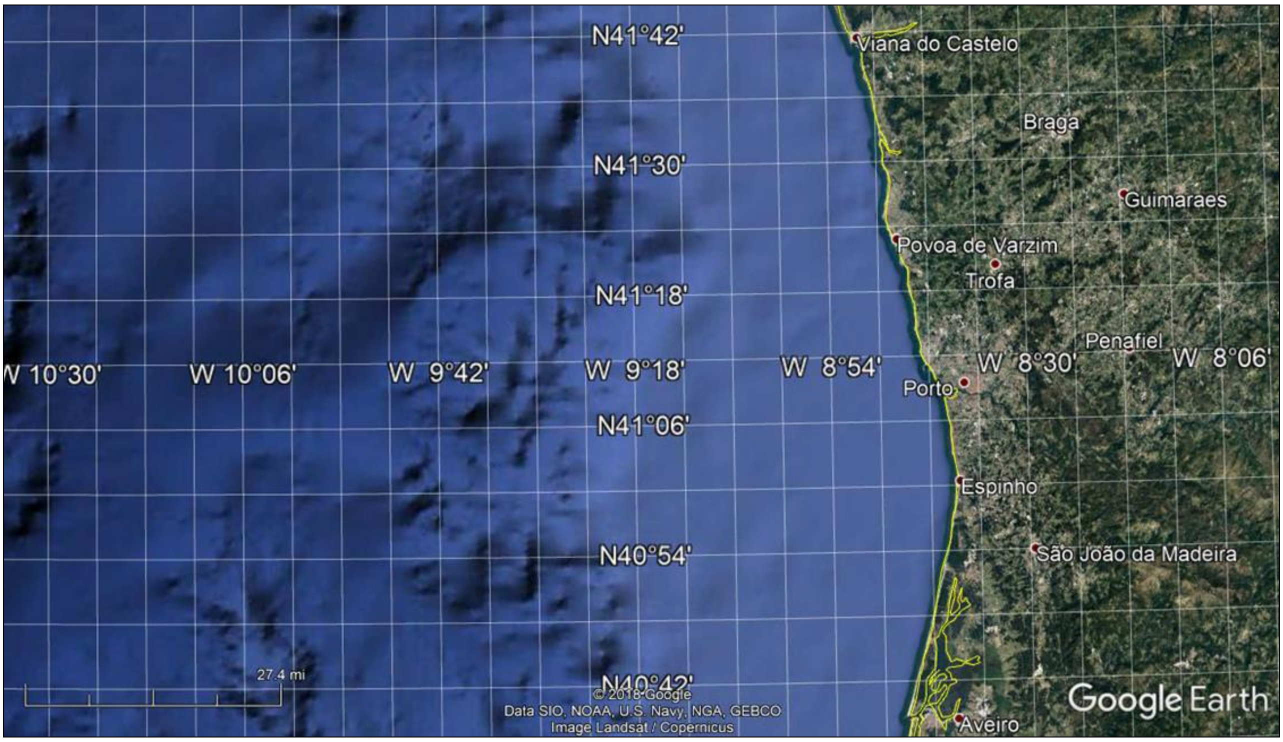
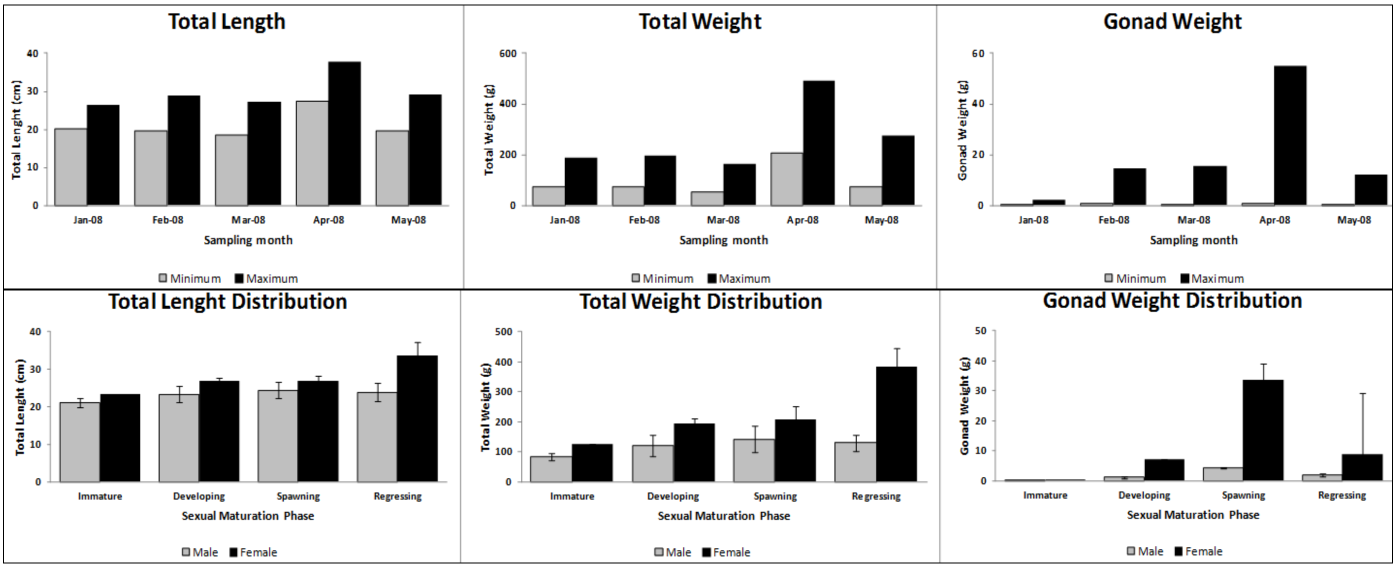


Fig 1: Identification of fishing area (demarked area) of trawler fleet operating on the Portuguese NW Atlantic Coast located between 6 – 50 nautical miles of Matosinhos



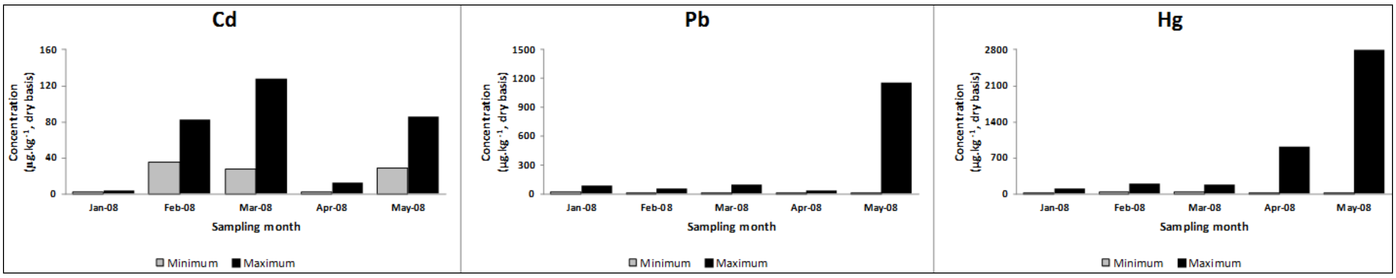


Fig 2: Biological parameters (Total Length (cm), Total Weight (g) and Gonad Weight (g)) and their distribution by Sexual Maturation Phase of *C. lucerna* from Portuguese NW Atlantic Coast captured during January - May 2008. Total metal (Cd, Hg and Pb) concentrations (µg.kg^{-1}, dry basis) obtained in muscle of *C. lucerna*: Minimum and Maximum concentrations. Each column represents: Mean ± Standard Deviation (Error bar) (N>14)

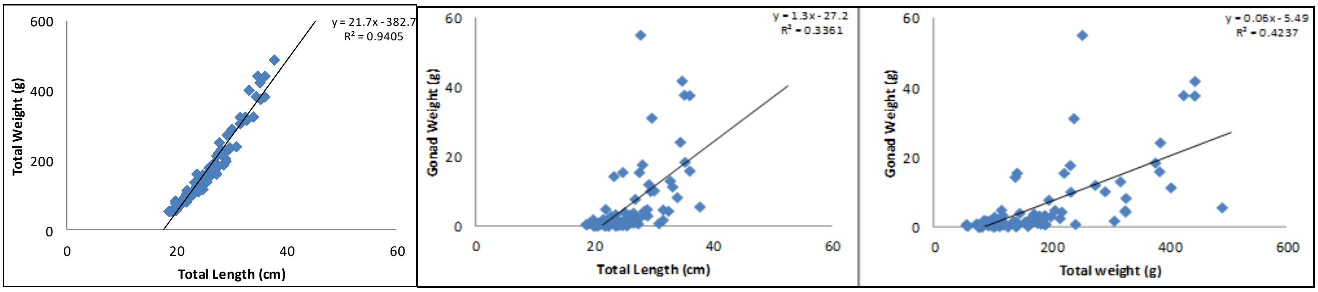


Fig 3: Correlations between biological parameters (Total Length (cm), Total Weight (g) and Gonad Weight (g)) of C. *lucerna* from Portuguese NW Atlantic Coast captured during January - May 2008. *Significant correlation (p< 0.05) between biological parameters.

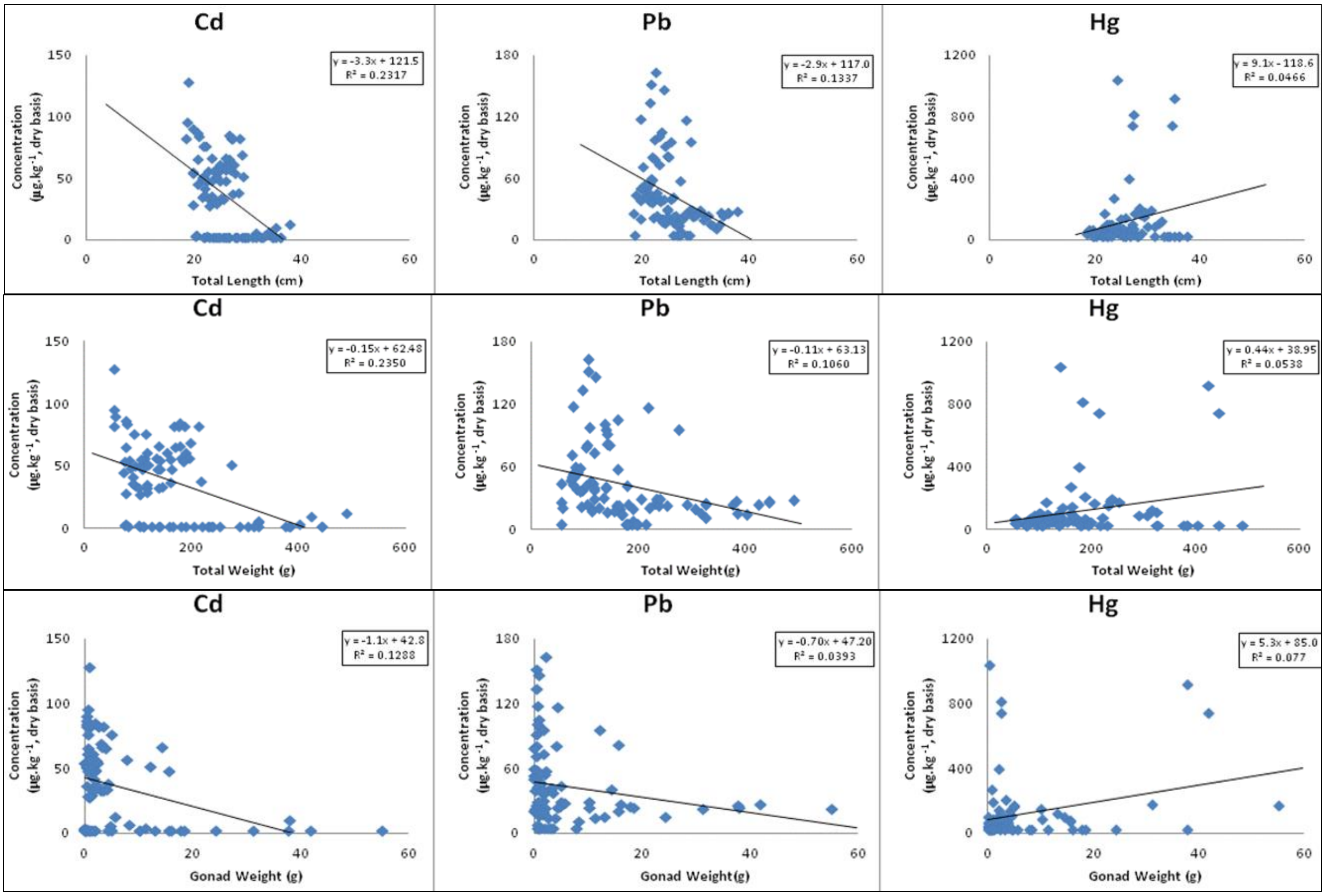


Fig 4: Correlations between biological parameters (Total Length (cm), Total Weight (g) and Gonad Weight (g)) and metal (Cd, Hg and Pb) concentrations (µg.kg^{-1}, dry basis) in muscle of *C. lucerna* from Portuguese NW Atlantic Coast captured during January - May 2008

Table 1: Biological parameters (Number of individuals, Sex, Total length, Total Weight, Gonad Weight and Sexual Maturation Phase) and metal (Cd, Pb and Hg) concentrations (µg.kg^{-1}, dry basis) obtained in muscle tissues of *C. lucerna* from Portuguese NW coastal seawaters during January - May 2008. Ecological quality classification of Portuguese Northwest seawaters based on maximum Cd, Pb and Hg concentrations (µg.kg^{-1}, dry basis) allowed in seafood by the European Community Commission - Regulations No 1881/2006 and 629/2008: Cd: 250; Hg: 2500; Pb: 1500 [29-30]. Legend: ♂ - Male; ♀- Female; a) Mean ± Standard Deviation. Different numbers shows significant (p< 0.05) differences: increasing order of values is 1<2.

Biological Parameters of *C. lucerna* from Portuguese NW coastal seawaters					
	January 2008	**February 2008**	**March 2008**	**April 2008**	**May 2008**
Number of individuals	14	19	17	21	18
Genre	9♂ + 5♀	16♂ + 3♀	16♂ + 1♀	3♂ + 18♀	13♂ + 5♀
Total Length (cm) a)	23.6 ± 2.1 ^{1}	24.3 ± 2.9 ^{1}	22.4 ± 2.5 ^{1}	32.2 ± 3.0 ^{2}	24.4 ± 2.9 ^{1}
Total Weight (g) a)	125.5 ± 37.8 ^{1}	131.8 ± 42.8 ^{1}	101.2 ± 34.5 ^{1}	326.5 ± 85.2 ^{2}	152.4 ± 54.5 ^{1}
Gonad Weight (g) a)	0.62 ± 0.61 ^{1}	2.45 ± 3.05 ^{1}	2.51 ± 3.65 ^{1}	17.7 ± 1.50 ^{2}	2.44 ± 3.06 ^{1}
Sexual Maturation Phase	Developing	Spawning	Spawning	Spawning - Regressing	Regressing
Metal concentrations (µg.kg^{-1}, dry basis) in muscle tissues of *C. lucerna* from Portuguese NW coastal seawaters					
	January 2008	**February 2008**	**March 2008**	**April 2008**	**May 2008**
Cd a)	2 ± 1 ^{1}	61 ± 14 ^{2}	58 ± 31 ^{2}	3 ± 3 ^{1}	55 ± 13 ^{2}
Pb a)	41 ± 24 ^{1}	25 ± 15 ^{1}	49 ± 24 ^{1}	23 ± 5 ^{1}	144 ± 259 ^{1}
Hg a)	51 ± 21 ^{1}	76 ± 40 ^{1}	73 ± 38 ^{1}	151 ± 236 ^{1}	354 ± 690 ^{1}
***Ecological Classification – Portuguese* NW *Coastal Seawaters* [29-30]**					
	January 2008	**February 2008**	**March 2008**	**April 2008**	**May 2008**
2008	Unpolluted	Unpolluted	Unpolluted	Unpolluted	Unpolluted

Table 2: Metal (Cd, Hg and Pb) concentrations (µg.kg^{-1}, dry basis) obtained in muscle tissues of gurnard fish species from Portuguese NW coastal seawaters (our study) and other similar worldwide works. a) Concentrations expressed as (µg.kg^{-1}, wet basis).

	Ranges of mean metal (Cd, Hg and Pb) concentrations (µg.kg^{-1}, dry basis) muscle tissues of gurnard fish species from Portuguese NW coastal seawaters and other similar worldwide works					
Fish Species	**Location**	**Cd**	**Hg**	**Pb**	**Year**	**Reference**
Chelidonichthys lucerna (“Tub gurnard”)	Portugal – NW Coastal Seawaters	<2.0 – 89.1	<20.0 - 1044	<4.8 - 403	2008	Our Study
	Portugal – Ria de Aveiro a)	5.6 – 12.7	-	71 - 145	1999-2000	[11]
	Italy – River Po Delta	10	110	<100	1994	[12]
	Italy – Tuscany Coast	-	49-534	-	2001-2001	[13]
	Italy – Adriatic Sea a)	10-30	10-530	<10-40	2006	[14]
	Italy – Gulf of Tigullio a)	250	130	900	2016-2018	[15]
	Turkey – Bay of Iskenderun a)	40-70	-	120-420	2003-2004	[16]
	Turkey – Bay of Iskenderun a)	10	-	140	2006	[17]
	England – Bay of Liverpool a)	<50	300	<460	1992-1993	[18]
	France – Bay of Biscay	-	964-1411	-	2001-2010	[19]
Chelidonichthys cuculus (“Red gurnard”)	France – Bay of Biscay	-	354-627	-	2001-2010	[19]
	Turkey – Mediterranean Sea	790	-	4270	2003	[20]
Chelidonichthysgabonensis (“Gabon gurnard”)	Africa – Senegal Coast	-	370	-	2014	[21]
Chelidonichthys kumu (“Bluefin gurnard”)	East Coast of North Island – New Zealand a)	8-24	-	130-400	1974	[22]
	Australia – Sydney Coast a)	3	260	40	1985-1990	[23]
	Australia – Sydney Coast a)	3	230	10	1989	[24]
	Australia – Sydney Coast a)	2	100	4	1990-1994	[25]
Eutrigla gurnardus (“Grey gurnard”)	France - Bay of Biscay	-	301-2277	-	2001-2010	[19]
	Turkey – Yalova Coast a)	20	-	330	2005	[26]
Trigla lyra (“Piper gurnard”)	Turkey – Aegeon Sea a)	40-50	-	140-310	2004-2005	[27-28]
	Turkey – Bay of Iskenderum a)	130	-	420	2004-2005	[27-28]

Table 3: Health Risk Assessment associated with the consumption of Portuguese *C. lucerna* captured during January – May 2008 following FAO/WHO/EPA Method [42-46].

***Health Risk Assessment by FAO/WHO/EPA Method* [42-46]**
Step 1. Hazard Identification Toxic Contaminants: Cd, Pb and Hg (Cancer potential)
Step 2: Dose-Response Assessment Maximum Allowable Reference Dose (RfD, µg.kg^{-1}.day^{-1}): Cd – 1.0 Pb - 3.6 Hg - 0.3
Step 3: Estimated “Average Daily Dose for Intake Processes” (ADDpot, µg.kg^{-1}.day^{-1}) ADDpot = $\frac{Ci \bullet IR \bullet ED}{BW \bullet AT}$ - Ci: Concentration of Metal (µg.kg^{-1} of fish) - IR: Ingestion Rate (0.02923 kg of fish.person^{-1} day^{-1}) [42] - ED: Exposure Duration (1 day) [42] - BW: Adult Body Weight (60 kg.person^{-1}) [42]

- AT: Averaging Time (1 day) [42]

ADDpot ($\mu g.kg^{-1}.day^{-1}$)	January 2008	February 2008	March 2008	April 2008	May 2008
Cd	0.001	0.030	0.028	0.002	0.0027
Pb	0.020	0.012	0.024	0.011	0.070
Hg	0.025	0.037	0.036	0.074	0.172

Step 4. Mean Hazard Quotient (HQ)

$$HQ = \frac{ADDpot}{RfD}$$

If HQ< 1: No potential human health risk
If HQ > 1: Potential human health risk

HQ	January 2008	February 2008	March 2008	April 2008	May 2008
Cd	0.001	0.030	0.028	0.002	0.0027
Pb	0.006	0.003	0.007	0.003	0.019
Hg	0.083	0.123	0.119	0.245	0.574

4. Conclusions

This exploratory baseline study concluded that:

1. the quantitative biological parameters of *C. lucerna* from Portuguese NW Atlantic Coast in January - May 2008 showed significant temporal variations ($p < 0.05$; Total Length: $F_{(5, 14)}=6.24$; Total Weight: $F_{(5, 14)}=8.42$; Gonad Weight: $F_{(5, 14)}=1.84$), except Sexual Maturation Phase;
2. total Length and Total and Gonad Weights suggested that this fish species is growing from January to April, until reaching their final spawning phase;
3. total metal (Cd, Hg and Pb) concentrations ($\mu g.kg^{-1}$, dry basis) obtained in muscle tissues of *C. lucerna* ranged: (i) Cd: <2.0 – 89.1; (ii) Hg: <20.0 – 1044; (iii) Pb: <4.8 – 403. Only Cd showed significant temporal variations ($p < 0.05$; *Cd*: $F_{(5, 14)}=29.84$): January = April << February = March = May;
4. no significant ($p > 0.05$) correlations were obtained between biological parameters and metals of *C. lucerna*. However, Cd and Pb showed negative relations with biological parameters, suggesting that bigger and older individuals of *C. lucerna* can metabolize and regulate them in their muscles. Hg showed positive relations with those biological parameters, suggesting that *C. lucerna* has no effective detoxification mechanism of Hg from their bodies;
5. Considering maximum metal concentrations allowed in fish species defined by European Community Commission (Regulations Nº 1881/2006 and 629/2008) and our metal concentrations in *C. lucerna*, the seawater in Portuguese NW Atlantic Coast could be always classified as "Unpolluted" during January – May 2008. Indeed, our health risk assessment study, performed according US-EPA/FAO/WHO demands, showed that there were no potential human health risks associated with the consumption of *C. lucerna* from Portuguese seawaters.

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6. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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