PCB contamination does not alter aerobic metabolism and tolerance to hypoxia of juvenile sole (Solea solea L. 1758)

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Abstract: Coastal habitats play a major role as nurseries for many fish species; however, they are also submitted to pollutants and oxygen fluctuations. Fry's concept of metabolic scope for activity was used to evaluate the effect of polychlorinated biphenyls (PCBs) on the aerobic metabolism in juvenile common sole (0–1 year old). Aerobic metabolic scope (AMS) in control and PCB-contaminated fish via food pathway was determined using respirometry techniques. Furthermore, the hypoxia tolerance in control and PCB-contaminated fish was evaluated by assessing their critical oxygen concentration (O_{2crit}). Our results showed that while PCB-contaminated fish were able to maintain a constant AMS, PCBs tend to affect their aerobic metabolism by acting on standard metabolic rate, maximal oxygen consumption (MO_{2max}) in hypoxia and O_{2crit}. In conclusion, we can hypothesise that a long-term exposure to PCBs may decrease their tolerance to hypoxia and consequently impair the survival and/or development of soles in their natural environment.

Keywords: bioenergetics; Aerobic Metabolic Scope; persistent organic pollutant; flatfish

1. Introduction

Coastal areas play a key role as nurseries for juveniles of many species of teleosts. Such ecosystems are characterised by a high productivity and offer the food sources necessary for the development of growing juveniles (Gibson, 1994; Amara, 2001). However coastal areas are also under the influence of aquatic and terrestrial inputs, and are thereby particularly exposed to fluctuations in physico-chemical factors, such as salinity, temperature, oxygen, carbon dioxide and pH. In addition, during the last decades, human activity brought about changes in water quality principally through the land-associated run-off and waste waters. These excessive inflows of nutrients (i.e. phosphorus and nitrogen) can induce eutrophication episodes often associated with hypoxia. Furthermore, land-associated waste water contains xenobiotics such as metals and persistent organic pollutants (POPs) leading to an increase of their concentrations in different compartments such as water and sediments.

Amongst the POP released in the marine environment, polychlorinated biphenyls (PCBs) are considered as the most toxic and widely distributed class (Abarnou, 2010). In invertebrates seawater constitutes an important exposure pathway (Danis et al., 2003, 2005 a,b) whereas vertebrates accumulate PCBs mainly by the trophic pathways (Boon, 1984; Kelly et al., 2007). Because of their high liposolubility, PCBs easily bioaccumulate in marine organisms and biomagnify along the food chain (Borga et al., 2001; Hoekstra et al., 2003; Nfon et al., 2008). Although their use as a raw material or chemical intermediate has been banned in the EC since 1985 (85/467/EEC, 6^{th} amendment to Directive 76/769/EEC), it is estimated that over 30% of the one million tons of PCBs produced are still present in aquatic and terrestrial ecosystems (Borlakoglu et al., 1991). PCBs provoke several toxicological responses, depending on the number and the position of their chlorine atoms (Ahlborg et al., 1994). For instance, previous studies showed that sub-lethal effects of PCB include: impairment of the

neurosystem activity (e.g. in the Long-Evans rat, *Rattus norvegicus*, Lehmler et al., 2005); impairment of the endocrine system, as shown by the decrease in the level of cortisol circulating in response to stress (e.g. in yellow perch, *Perca flavescens* and northern pike, *Exos lucius*, Hontela et al., 1992; and in women, Mendola et al., 1997); decrease of the offspring number (e.g. in white-footed mice, *Peromyscus leucopus*, Voltura et al., 2007) and also impairment in swimming behaviour (e.g., in young carp, *Cyprinus carpio*, Schmidt et al., 2005). Nevertheless, only few studies to date have focused on the sub-lethal effects of PCBs on fish energetics (e.g. in European eel, *Anguilla anguilla*, van Ginneken et al., 2009) and on the interactions with other environmental stressors such as hypoxia (e.g. in killifish, *Fundulus heteroclitus*, Kramer and Schulte, 2004).

Bioenergetics studies give an essential viewpoint on the interactions between fish and their environment (Fry, 1971; Claireaux and Lefrançois, 2007). Fish activities (e.g., swimming, search for food, digestion, reproduction) are all related to energy-demanding processes that have to be accommodated within the animal aerobic metabolic scope (AMS). AMS was defined by Fry (1971) as the energy potential of an organism and is recognised as a proxy to assess physiological performance and fitness in fish (Priede, 1985; Claireaux et al., 2000; Mallekh and Lagardere, 2002; Claireaux and Lefrancois, 2007, Pörtner and Farrell, 2008; Guderley and Pörtner, 2010). AMS is partly driven by the environmental factors that were divided by Fry (1947, 1971) into five classes depending on their effects on aerobic metabolism: controlling, masking, limiting, lethal and directive factors. In this classification, oxygen and pollutants are considered as limiting factors as they interfere with metabolic processes and can thereby reduce the maximal metabolic rate sustainable by the organism, defined by Fry as the active metabolic rate (AMR). In addition, pollutants are classified as masking factors, since pollutant-related processes of defence and detoxification are likely to induce supplementary energy costs (Sokolova and Lannig, 2008, Niyogi et al., 2006). An

associated elevation of the standard metabolic rate (SMR) measured in resting individuals (Fry, 1971) is therefore expected as observed in juvenile of rainbow trout (*Oncorhynchus mykiss*) exposed to sub-lethal concentrations of aluminium (Wilson et al., 1994).

In this context, the aim of our study was to investigate the effects of both sub-lethal concentrations of PCBs and hypoxia on the aerobic metabolism of common sole chronically exposed through the food pathway. Our main hypothesis was that chronic exposure to PCBs may constrain aerobic metabolic scope by reducing AMR and/or increasing SMR. Since a PCB-related increase in SMR would raise the oxygen requirement in resting fish, it can be hypothesised that such individuals may sooner experience respiratory distress when facing a progressive hypoxia. The influence of PCB contamination on the sole response to limited oxygen environment was therefore also investigated. This study focus on juvenile sole because 1) common sole is a bottom-dwelling species, which occupies the coastal zone during its juvenile phase; 2) during this phase, soles feed on the invertebrates living in the sediments which are expected to be highly contaminated by PCBs (Cicero, 2000; Chau, 2006) and 3) due to their reduced swimming capacity, soles have to cope with a set of interacting environmental stressors, such as hypoxia.

2. Materials and methods

2.1 Experimental animals

Juveniles (~1 year old) of common sole *Solea solea* (mean ± S.D.; length: 11.4 ± 1.0 cm; mass: 16.0 ± 3.6 g) were obtained from a fish farm (Solea BV, Ijmuiden, Netherlands). Upon arrival at the laboratory, fish were transferred to 500 l indoor rearing tanks with recirculated and filtered seawater (temperature: 20 °C; salinity: 31 psu; fixed photoperiod: 12 h/12 h). Fish were then maintained for a three-week period of acclimation in the laboratory before being

transferred in 60 l glass-aquaria for the experiments. Soles were fed daily with commercial pellets. Feeding was discontinued 24 h before any manipulation of the fish.

2.2 Protocol of food contamination

Sole contamination occurred through the food pathway. Food consisted of commercial pellets (DAN-EX 1362, Danafree ® Horsens, Denmark) contaminated with a mixture of PCBs prepared by a specialised laboratory (IFREMER, Department of Biogeochemistry and Ecotoxicology, Brest, France) following the protocol described in Eichinger et al. (2010). Two concentrations of PCB were used; $[PCB]_{low}$ and $[PCB]_{high}$ which correspond to 670 and 2239 ng PCB g^{-1} of food, respectively. The lowest concentration was adjusted in order to experimentally attain a level of PCB contamination within in the range measured in juvenile soles caught in the Seine estuary (i.e. an average hepatic concentration of 400 ng PCB g^{-1} dry weight, Loizeau, personal communication). This estuary is considered as the most heavily PCB contaminated area along the entire French coast (Abarnou and Simon, 1986). On the other hand, the highest concentration of PCB was ~3.5 times higher than $[PCB]_{low}$ and was used as a positive control, which aimed at inducing PCB-related physiological effects. The PCB mixture employed for contamination contained four congeners selected because of their widespread presence in the environment: CB 105, CB 118, CB 149, and CB 153. The CB 153 (2,2′,4,4′,5,5′- Hexachlorobiphenyl), a di-*ortho*-substituted compound, is one of the most abundant congener in marine organism. Albeit CB 153 has a low cytotoxicity, it is considered as the most recalcitrant congener with respect to the biotransformation and therefore the most persistent in the food web (Kannan et al., 1995). On the other hand, the CB 149 (2,2',3,4',5',6-Hexachlorobiphenyl), a di-*ortho*-substituted compound, can be partially metabolised (Andersson and Förlin, 1992). The CB 105 (2,3,3′,4,4′-Pentachlorobiphenyl) and CB 118 (2,3′,4,4′5-Pentachlorobiphenyl) are mono-*ortho*-substituted which makes them more toxic

than the other congeners. The congeners 118 and 153 are also considered amongst the 'indicator PCBs' as suggested by the International Committee for the Exploration of the Sea (ICES), i.e. these congeners are considered as a representative index of PCB contamination (MacGregor et al., 2010). For each congener, the concentration was measured in the experimentally-contaminated pellets (Table 1). Because of the low solubility of PCB, iso-octane was used as solvent coating to facilitate their incorporation into the food. The concentration of the employed iso-octane was 40 ml kg^{-1} of pellets. After the incorporation of PCB, pellets were evaporated with a nitrogen jet in order to remove the solvent.

2.3 Protocol of sole contamination

Ten days before the beginning of contamination, 75 soles were anaesthetised with clove oil (0.04 ml L^{-1}), weighed, measured and then transferred into 60 L glass-aquaria. A total biomass of 100 g per aquaria (i.e. 6 to 9 fish) was maintained. All the aquaria were placed in a temperature controlled room where temperature was set to 20 °C allowing constant thermal oscillations < 1°C throughout the experiment. Ten days after their transfer into the aquaria, fish were exposed to one of the 4 following treatments: a group was fed with food containing the lowest concentration of PCB and a group with the highest one (i.e. group PCB_{low}, n = 21 and group PCB_{high}, n = 19, respectively); a group was fed with food which contained the iso-octane solvent (i.e. group *Solvent*, n = 21); and a group control was fed with uncontaminated food which contained no solvent (i.e. group *Control*, n = 14). Comparison between the group *Solvent* and the group *Control* was used to assess the effects of the solvent itself. Fish were daily fed with 1% of their total body mass. The food was given to the fish at once. After ~ 6 hours, the remaining pellets (i.e. not-eaten by the fish) were carefully removed. Fish were fed these diets up to 30 or 60 days.

2.4 Respirometry

The experimental set up consisted of 4 respirometers (4.5 l) placed into the same thermo-regulated room as the aquaria. Inside of each respirometer, a flat propeller was set on a central axis and lay on the bottom in order to force the sole to swim when necessary. The axis was connected to a motor, whose speed was regulated by the experimenter between 0 and 67 turns min^{-1}. Respirometers were positioned in buffer tanks (100 cm x 80 cm x 60 cm), which aim at regulating the water temperature. Water supply in each respirometer was provided by flushing pumps placed in the buffer tanks. Each flushing pump was connected to a timer which regulated the flushing period. A pump was added to each respirometer to ensure an adequate mixing of the water inside of the measuring chamber. Oxygen content was measured with a polymer optical fiber (Presens, Germany) positioned on the wall of the respirometer and connected to a multichannel oxygen measuring system (OXY 4 mini, PreSens, Germany). Oxygen data were sampled every 30 seconds and recorded in a computer. Each buffer tank was equipped with a counter-current gas-equilibration column to control oxygen level. A pump supplied the column with the water coming from the buffer tank. An air stone positioned down in the water column bubbled air or nitrogen to set normoxia or hypoxia, respectively.

2.5 Experimental protocol

After 30 days of contamination, aerobic metabolism was assessed by intermittent flow-respirometry in individuals of each of the 4 groups ($Control_{-30}$, n = 8; $Solvent_{-30}$, n = 8; PCB_{low-30}, n = 7 and $PCB_{high-30}$, n = 8). The other soles were maintained in the aquaria for a longer contamination lasting 60 days and constituted the groups $Control_{-60}$ (n = 6), $Solvent_{-60}$ (n = 13), PCB_{low-60} (n = 14) and $PCB_{high-60}$ (n = 11). Fish were tested once and individually. An experiment lasted 3 days, during which one fish of each treatment (i.e. a total of 4 fish)

were concomitantly tested in the 4 respirometers. An experiment consisted in exposing each fish to 3 consecutive steps. First, as soon as transferred into the respirometer, each fish was chased in order to increase its energy-demanding activities and assess active metabolic rate (AMR), defined as the fish maximal metabolic rate in normoxia, i.e. in non-limiting oxygen conditions (Fry 1971). To force fish to swim, the propeller connected to the motor was switched on until the fish reached exhaustion (i.e. when the fish was not able to swim anymore, which occurred within ~10 min). Then, fish oxygen consumption (MO_2) was measured during 30 min. The second step consisted in exposing sole to a progressive hypoxia in order to assess (i) the maximal MO_2 (MO_{2max}) the fish can sustain as a function of limited oxygen levels, as well as (ii) the critical oxygen saturation (O_{2crit}). Fish were therefore successively exposed to four level of water oxygenation: 60%, 40%, 20% and 10%. At each of these levels, fish were chased until exhaustion by activation of the propeller in order to raise its MO_2 to the maximum allowed by the oxygen level tested. After the last measurement, normoxia was restored (within ~15 min) by bubbling oxygen into the counter-current gas-equilibration column. Thirdly, MO_2 was measured in resting animals to estimate standard metabolic rate (SMR). To do so, the propeller was switched off and fish were left undisturbed throughout the following 48 h during which measurements of MO_2 carried out automatically. Each measure of MO_2 lasted 30 minutes and a flushing period of 25 minutes was respected between consecutive measurements. Blank respiration was routinely assessed before and after each experiment by assessing MO_2 in empty respirometers to be afterwards subtracted to the total measure of MO_2.

2.6 Calculation of the oxygen consumption and O_{2crit}

The fish MO_2 ($mgO_2\ kg^{-1}\ h^{-1}$) was calculated with the following equation, commonly employed in such studies (Lefrançois and Claireaux, 2003):

$$MO_2 = \Delta [O_2] \,.\, \Delta t^{-1} \,.\, VOL_{resp} \,.\, m^{-1} \qquad \text{Eq. (1)}$$

where $\Delta [O_2]$ is the decrease in water oxygen concentration ($mgO_2\ l^{-1}$) during the measuring period (Δt), VOL_{resp} is the volume of the respirometer (4.5 l) minus the volume of the fish and m is the mass of the fish. To determine $\Delta [O_2]. \Delta t^{-1}$, $[O_2]$ was plotted as a function of time and a linear regression was adjusted for each MO_2 measurement (R development Core Team 2011, version 2.13.2, script D. Chabot ©). The regression coefficient (R^2), which illustrates the accuracy of the regression, was > 0.98 in all cases.

Since the respiratory metabolism depends on the animal mass, each MO_2 was then standardised for a 0.1 kg fish using the following relationship (Lefrançois and Claireaux, 2003):

$$MO_{2\,cor} = MO_2 \,.\, (m \,.\, m_{cor}^{-1})^{1-A} \qquad \text{Eq. (2)}$$

where $MO_{2\,cor}$ ($mgO_2\ kg^{-1}\ h^{-1}$) is the oxygen consumption of a fish with a corrected mass (i.e., m_{cor} = 0.1 kg in the present case), MO_2 ($mgO_2\ kg^{-1}\ h^{-1}$) is the measured oxygen consumption calculated with Eq. (1) and m (kg) is the mass of the fish experimentally tested. The coefficient A is the allometric exponent describing the relationship between the metabolic rate and the body mass. A value of 0.8 was used as in other teleosts (Steffensen et al., 1994).

2.7 Assessment of Active Metabolic Rate (AMR), Standard Metabolic Rate (SMR), Aerobic Metabolic Scope (AMS) and critical oxygen saturation ($O_{2\,crit}$)

AMR was assessed as the MO_2 measured in exhausted fish after being chased. In order to assess SMR, a frequency distribution of the MO_2 values measured during the last 48 hours

was plotted, as recommended by Steffensen et al. (1994). In general, it is possible to observe a bimodal frequency distribution, due to the fact that fish may present routine activity. The first peak is considered to reflect the value of the SMR, and the second is considered to be the consequence of the routine activity (Steffensen et al., 1994). Since we did not notice such a difference in the frequency distribution of MO_2, only the data measured during the last day were used. During this period, the fish was supposed to recover from the stress derived from experimental manipulation and from hypoxia exposure (Steffensen et al., 1994). AMS was calculated as the difference between AMR and SMR.

MO_{2max}, and as consequence AMS, decrease with water oxygen content (van den Thillart et al., 1994; Claireaux and Lagardère, 1999; Lefrançois and Claireaux, 2003; Dutil et al., 2007; Chabot and Claireaux, 2008). Critical oxygen saturation (O_{2crit}) was defined as the water oxygen content at which the maximal sustainable MO_{2max} was equal to SMR, i.e. when the AMS was null (Fry, 1948; Steffensen et al., 1994; van den Thillart et al. 1994; Schurmann and Steffensen, 1997; Iversen and McKenzie, 2010). O_{2crit} was evaluated according to the method proposed by Bilberg et al. (2010). Briefly, MO_{2max} was plotted as a function of oxygen water saturation. Hereafter, a curve was fitted using the 'exponential rise to maximum' function [$y=a\ (1-\exp^{-bx})$] (SigmaPlot, Systat Software 11.0). For each fish, O_{2crit} was defined as the intersection between the curve obtained, and its SMR and according to the following equation:

$$O_{2crit} = \left(\frac{-\ln\ (1-\ (SMR/\ a)}{b} \right) \qquad \text{Eq. (3)}$$

where *a* and *b* are the constants provided by the curve fittings software.

AMR, SMR, AMS, as well as O_{2crit} were assessed in each fish.

2.8 PCB analysis

After the dietary exposure period, soles were killed by an overdose of anesthetic (Clove oil: 0.2 ml L^{-1}), the liver was removed and frozen at -80 °C for further analysis of PCB contents. Concentration of CB 153, CB 118, CB 149 and CB 105 were measured in the liver according to the protocol described in Jaouen-Madoulet et al. (2000). Only the data concerning the CB 153 are presented (Table 2) as an indicator of the level of contamination of the sole, since (i) CB153 is poorly metabolised in comparison to the three other congeners and (ii) it has been shown to have a good correlation with the sum of congeners present in the sample (Atuma et al. 1996). Briefly, frozen livers were freeze-dried and then ground to obtain a fine homogeneous powder. Water content was estimated by the determination of the weight lost after freeze-drying. To quantify the PCBs contained in the powdered materials, a weighed amount was solvent-extracted (by a hexane: acetone mixture 80:20, respectively using a hot Soxhlet extraction apparatus (Soxtec). Subsequently, the solvent was completely evaporated using a rotavapor. In order to purify PCB-containing fractions, the extracts were cleaned-up by addition of concentrated sulphuric acid to destroy lipids and co-extractible materials (Jaouen-Madoulet et al. 2000). PCBs were then quantified by gas chromatography, with an electron capture detector on a HP 5890 series II equipped with a CP-Sil19 capillary column following optimised conditions as described in details by Jaouen-Madoulet et al. (2000) and Bilberg et al. (2008). Contamination by the congener CB 153 was quantified using a standard solution. Hepatic concentration of CB 153 (ng g^{-1} of dry weight, hereafter d.w.) was measured after 30 and 60 days of contamination (i.e. n = 6 and n = 7 for PCB_{low} and n = 7 and n = 7 for PCB_{high} at 30 and 60 days, respectively). Limit of detection of CB 153 was 1.2 µg l^{-1}.

2.9. Statistical analysis

AMR, SMR, AMS and O_{2crit} measures were examined for normality and homogeneity of variance. Two-way ANOVA was used to determine the effect of PCB exposure on the variables AMR, SMR, AMS and O_{2crit}. ANOVAs were set with the different treatments (i.e. PCB_{low}, $PCB_{high,}$ and *Solvent* vs. *Control*) and both contamination periods (i.e. 30 vs. 60 days) as main factors. Two-way ANOVA with treatments (PCB_{low}, PCB_{high}) and contamination periods (30 and 60 days) as main factors was used to compare the hepatic concentration of CB 153 between the fish from the groups PCB_{low} and PCB_{high}. In addition, a Pearson test was employed to evaluate the correlation between the hepatic concentration of CB 153 of the PCB-contaminated groups and the variables AMR, SMR and O_{2crit}. Because it has been already showed that in sole MO_{2max} declines as oxygen availability decreases (van den Thillart et al., 1994; Claireaux and Lagardère, 1999; Lefrançois and Claireaux, 2003; Dutil et al., 2007; Chabot and Claireaux, 2008), statistical analysis were focused on the effects of PCB on MO_{2max}. To do that, at each levels of water air saturation (i.e. at 100%, 60%, 40%, 20% and 10% in air saturation, hereafter a.s.) a two-way ANOVA was employed with contamination periods (30 versus 60 days) and treatment (*Solvent*, PCB_{low}, PCB_{high} vs. *Control*) as main factors. When ANOVA revealed a significant effect, Tukey's post hoc test was employed to assess the statistical significance of specific differences in the statistical analysis design, and a planned comparison was used to compare the treatment *Control* to each of the three others treatments when a significant interaction between the factors tested was found, (Underwood, 1981). The level of statistical significance was set to $P < 0.05$. Statistical analyses were conducted using STATISTICA 8.0 (StatSoft, Inc., USA). Data are presented as mean ± S.D.

3. Results

Hepatic CB 153 concentrations in individuals from the groups *Control* and *Solvent* were negligible, i.e. 36 and 29 ng PCB g^{-1} of food respectively, contrary to the concentrations measured in the contaminated soles (Table 2). CB 153 concentrations showed a significant effect of the treatments ($F_{1:24} = 13$, $P < 0.01$) which differed with the exposure period to the pollutant ($F_{1:24} = 7.6$, $P < 0.05$ for the interaction term). Planned comparison showed that CB 153 concentration between the groups $PCB_{low\text{-}30}$ and $PCB_{high\text{-}30}$ was not significantly different ($P > 0.05$). On the contrary in individuals contaminated during 60 days, CB 153 concentration was approximately two-fold higher in soles belonging to the group $PCB_{high\text{-}60}$ than those of the group $PCB_{low\text{-}60}$ ($P < 0.05$). Independent of the exposure period and PCB treatments, no significant correlation was observed between the hepatic CB 153 concentration and AMR ($0.02 < r^2 < 0.16$, $P > 0.05$ in all cases) as well as for the hepatic CB 153 concentration and O_{2crit} ($0.14 < r^2 < 0.49$ and $P > 0.05$ in all cases). For SMR, a similar pattern was observed for the individuals belonging to the groups $PCB_{low\text{-}30}$ and $PCB_{high\text{-}30}$ and for $PCB_{low\text{-}60}$ ($0.03 < r^2 < 0.6$ and $P > 0.05$ in all cases), except for the individuals exposed to $PCB_{high\text{-}60}$, for which a positive correlation was found ($r_{PEARSON} = 0.85$; $P < 0.001$, Fig. 1).

No significant effect of treatments was found on SMR, AMR, AMS and O_{2crit}, (treatment $P > 0.05$ in all cases; Table 3). The exposure period had a significant effect exclusively on O_{2crit} ($F_{1:41} = 5.4$, $P < 0.05$). A significant interaction between the two factors tested (exposure period and treatment) was observed only on SMR ($F_{3:67} = 2.8$, $P < 0.05$). A posteriori planned comparison showed that this pattern was due to the soles exposed to the highest PCB concentration, which showed a SMR significantly higher after 60 days of contamination than after 30 days ($P < 0.05$).

Regardless of the treatment and the exposure period to PCBs, a general decrease in MO_{2max} with hypoxia was observed (Fig. 2). In severe hypoxia (< 20% a.s.), MO_{2max} did not show any significant difference among the four treatments ($P > 0.05$) neither between the 30 and 60 days of exposure ($P > 0.05$, for both exposure period and interaction term in all cases). Only few isolated significant differences were observed at 60% and 40% a.s. (Fig 2). At 60% a.s., a significant effect of both treatments ($F_{3:67} = 3.9$, $P < 0.05$) and duration of contamination ($F_{1:67} = 6.2$, $P < 0.05$) was found (Fig.2), while no interaction was detected ($P > 0.05$). Tukey's post-hoc test revealed a significant difference between the *Solvent* and the PCB_{low} treatments. At 40% a.s., only a significant effect of duration of contamination was found ($F_{1:67} = 9.1$, $P < 0.01$; Fig.2).

4. Discussion

The results suggested that under our experimental conditions, PCB exposure did not affect AMR, AMS or O_{2crit}. The unique variable influenced was the SMR, but only at the highest PCB concentration between 30 and 60 days of exposure.

In the current study, the average AMR measured in sole ranged between 129.5 ± 22.2 and 173.2 ± 73.9 $mgO_2\ kg^{-1}\ h^{-1}$. These data are in agreement with previous studies carried out on the same species at 20 °C [i.e. 152.2 $mgO_2\ kg^{-1}\ h^{-1}$ in van den Thillart et al. (1994) and 159.2 $mgO_2\ kg^{-1}\ h^{-1}$ in Lefrançois and Claireaux (2003)]. Neither AMR nor AMS were influenced by the PCB contamination experimentally-induced in the present study (Tables 2 and 3). This suggests that the sub-lethal concentrations of PCB we tested did not prevent the soles from meeting their maximal oxygen requirements. Therefore PCB-exposed soles should not present any reduction in oxygen-demanding activities such as locomotion, digestion or growth (Claireaux and Lefrançois, 2007). The growth rate was not assessed in this study. However,

our results are consistent with a recent study carried out on juvenile of common soles exposed to the same protocol of contamination, and which showed that PCB-exposure did not affect growth (Eichinger et al.; 2010). In addition, the PCB-treatments did not influence the maximal metabolic rate the sole can sustain (MO_{2max}) in hypoxic conditions. Indeed, contaminated sole never showed significant difference in MO_{2max} when compared to *Control* individuals (Figure 2); while MO_{2max} strongly declined with the oxygen as observed in the same species by Lefrançois and Claireaux (2003), as well as in other fish species by various authors (Priede, 1985; Schurmann and Steffensen, 1997; Chabot and Claireaux, 2008; Iversen and McKenzie, 2010). This suggests that PCB contamination does not impair the organs and cellular mechanisms involved in the oxygen transport contrary to other type of pollutants. For instance, tributyltin chloride contained in the antifouling paints for boats, was found to affect red cell function in rainbow trout (*Oncorhynchus mykiss*, Virkki and Nikinmaa, 1993). Oil-treated soles exposed to normoxic and hypoxic conditions showed reduced MO_{2max} associated to weakened cardiovascular performance (Davoodi and Claireaux, 2007; Claireaux and Davoodi, 2010). PCBs and hypoxia response induce both the activation of Ah-receptor, by using the same transcriptional pathways. As a consequence, PCB-hypoxia interaction may be hypothesised, which could affect the findings (Nie et al. 2001; Nguyen and Bradfield, 2008; Fleming et al. 2009). The lack of information regarding the specific effects of PCBs on such functions limits further discussion. Moreover, it cannot be excluded that the solvent employed to fix PCB on the experimental-contaminated pellets (i.e. iso-octane) participated to the regulation in MO_{2max} of soles while coping with mild hypoxia (Fig 2).

Energy balance in an organism also depends on the part of energy obligatorily allocated to its maintenance activities. SMR measured in the uncontaminated soles (Table 3) are in agreement with the SMR assessed in 20 °C-acclimated sole by van den Thillart et al. (1994; 41 $mgO_2\ kg^{-1}\ h^{-1}$) and Lefrançois and Claireaux (2003; 49 $mgO_2\ kg^{-1}\ h^{-1}$). SMR only differed

between the soles exposed to the highest concentration of PCB during 30 and 60 days (i.e. $PCB_{high\text{-}30}$ *versus* $PCB_{high\text{-}60}$; Table 3). In the particular $PCB_{high\text{-}60}$ treatment, SMR tends to increase with CB 153 concentration, even if the type of relationships cannot be established (Fig. 1). Data do not allow assessing if SMR may increase proportionally with PCB or only above a determined threshold. Nevertheless, the most relevant observation is that no significant difference was detected between the *Control* and the contaminated sole. This suggests that maintenance activities do not tend to be significantly influenced by the PCB contamination experimentally-induced in the present study. This is contrary to our initial hypothesis, which stated that an increase in SMR was expected because of supplementary energy costs induced by PCB-related processes of defence and detoxification. For instance, Lannig et al., (2006) showed a 40–86% increase of SMR in cadmium-exposed oyster, *Crassostrea virginica*. They concluded that this pattern was mostly due to the elevated costs of protein synthesis involved in detoxification or protective mechanism such as glutathione expression, antioxidant enzyme, cellular repair mechanism, as well as expression of stress proteins.

Critical oxygen saturation is defined as the minimal level in water oxygen content needed to sustain SMR (Fry, 1948; Steffensen et al., 1994; van den Thillart et al. 1994; Schurmann and Steffensen, 1997; Iversen and McKenzie, 2010). Facing a progressive hypoxia, individuals with an elevated O_{2crit} would sooner be unable to extract enough oxygen to meet their SMR, and have thus to cope with physiological distress. Absence of difference in O_{2crit} in healthy soles when compared to those contaminated to PCB is consistent with the absence of PCB effects observed for SMR. It can thus be hypothesised that the PCB concentrations tested would not affect the tolerance to hypoxia in soles by influencing their aerobic metabolism. However, it is worth noting that in the killifish *Fundulus heteroclitus* exposed to hypoxia, glycolytic enzymes were down-regulated in presence of PCB, which thereby limits the

efficiency of the anaerobic pathway (Kraemer and Schulte, 2004). Compensation of the hypoxia-related reduced supply in ATP, as well as maintenance of the energy-demanding activities, may be therefore limited by PCB.

Even if a large range of PCB concentration was tested in the present study (i.e. the highest concentration tested was four-fold higher than the levels measured in wild juvenile soles from the Bay of Seine, Loizeau, personal communication), no clear pattern was revealed. Furthermore, comparisons with other studies suffer from a lack of available data, mainly because of differences in the type of pollutants tested and/or in the experimental approach employed for contamination. For instance, effect of PCB was investigated in the European eel (*Anguilla anguilla*) by van Ginneken et al. (2009) who showed that PCB-contaminated eels were characterised by lower aerobic metabolism than healthy individuals. However, adult eels were injected intraperitoneally with a mixture of PCB consisting of CB 153, 126 and 77 at very high concentrations (i.e. 5mg kg^{-1}, 7µg kg^{-1} and 50µg kg^{-1} of body weight, respectively). Furthermore, aerobic metabolism was measured in individuals swimming at a low speed (i.e. 0.5 body length s^{-1} or 0.4 $m.s^{-1}$) and was therefore representative of the eel routine metabolic rate rather than the maximal, or active, level. Another study (Voltura et al., 2007) carried out on the white-footed mouse (*Peromyscus leucopus*) showed no effect of PCB on energy metabolism and growth. The authors suggested that this finding may be due to a large dose-dependent decrease in thyroid hormone concentrations. To date, it appears that comparative assessment of the effects of PCB exposure on aerobic metabolism is limited and that scattered information prevents a general pattern from emerging. Effects of metal pollution were on the contrary extensively studied (e.g. Sibly and Calow, 1989; Calow and Forbes, 1998; Sherwood et al., 2000). However, despite these numerous studies, effects of metals on aerobic metabolism are still controversial (see the review by Sokolova and Lannig, 2008). Effects appear to be dependent of a large set of factors such as the metal studied, its concentration, the

pathway of metal uptake, as well as other natural factors such as temperature. It is likely that the effects of PCB and those of POP in general follow the same pattern.

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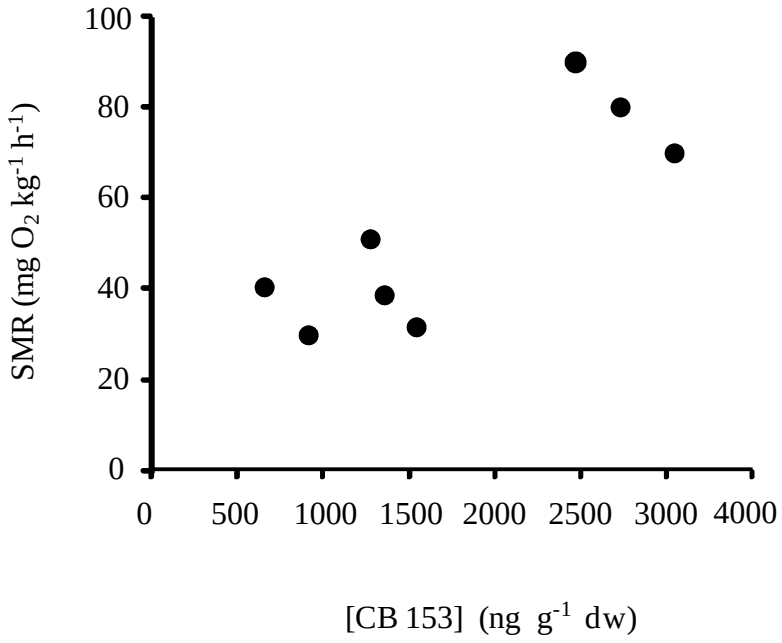

Fig.1. Correlation between the concentrations of CB 153 (ng g^{-1} dw) and standard metabolic rate (SMR) in the high PCB-contaminated group during 60 days (Pearson correlation: $r_{PEARSON}$= 0.85, P < 0.001).

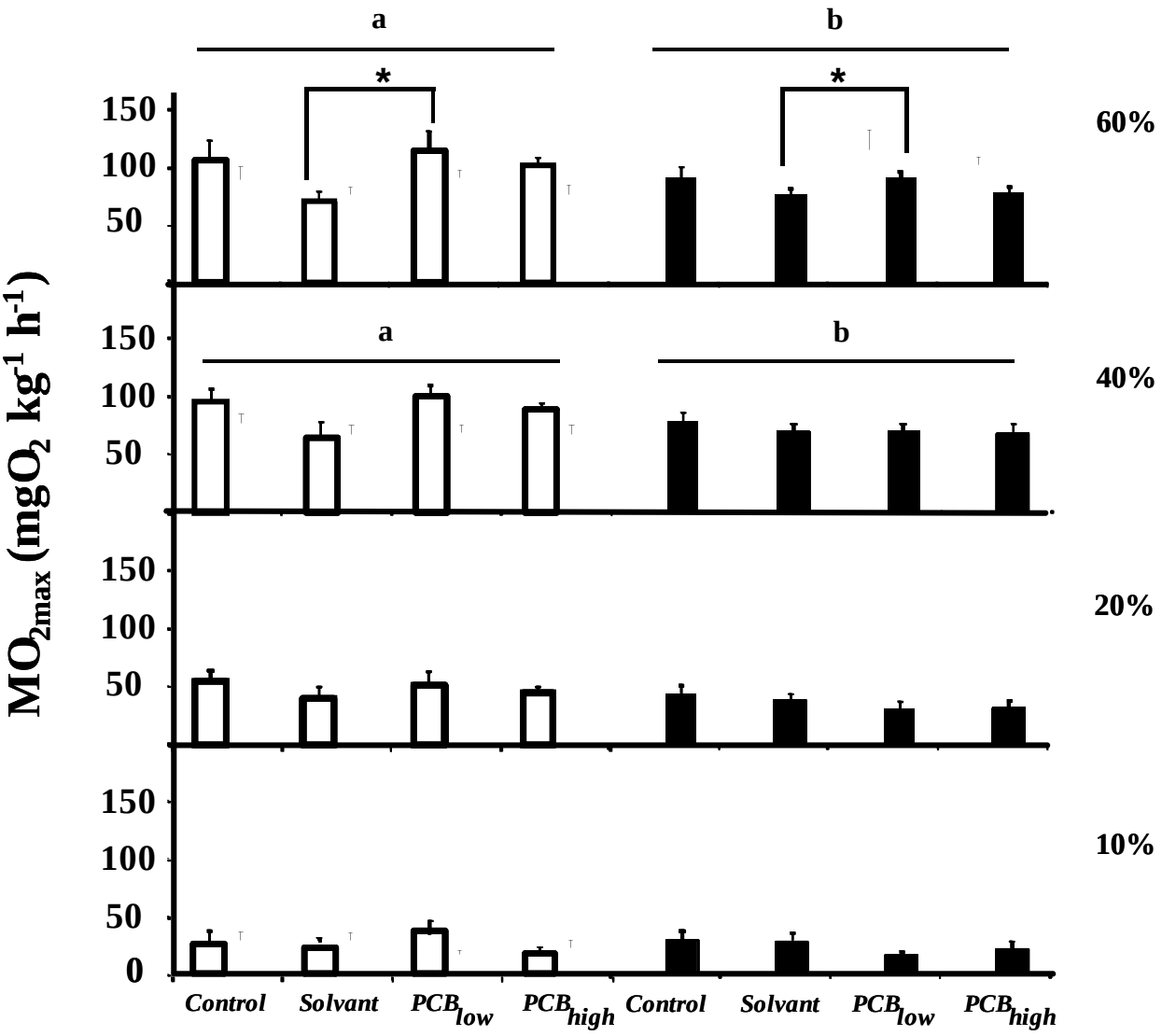

Fig.2. Average maximum metabolic rate (MO_{2max}) measured in tested groups after 30 (*Control*, n = 8; *Solvent*, n = 8; PCB_{low}, n = 7; PCB_{high}, n = 8) and 60 days of contamination (*Control*, n = 6; *Solvent*, n = 13; PCB_{low}, n = 14; PCB_{high}, n = 11). *Open bars*: 30 days of contamination. *Filled bars*: 60 days of contamination. At 60% a.s., * indicates a significant difference between the treatments ($P < 0.05$) independently of duration of contamination. For a given level of O_2 saturation, treatments not sharing a common superscript are significantly different between 30 and 60 days of contamination ($P < 0.05$). All the values are means ± S.D.

Table 1. Concentration (ng g^{-1} dw) of the four PCB congeners used for the contamination of commercial pellets. PCB_{low} and PCB_{high} correspond to a total concentration of 670 and 2239 ng PCB g^{-1} of food, respectively.

CB composition	PCB_{low}	PCB_{high}
CB 153	314	1019
CB 149	134	456
CB 118	150	503
CB 105	72	261

Table 2. Concentration (Mean ± SD, ng g^{-1} dw) of the congener CB153 in the liver of PCB-contaminated soles after 30 and 60 days of contamination. Soles from the group PCB_{low} and PCB_{high} were exposed to a total of PCB concentration of 670 and 2239 ng PCBs g^{-1} of food, respectively. For a given treatment (PCB_{low} or PCB_{high}), values not sharing a common superscript were significantly different ($P < 0.05$)

Exposure period	PCB_{low}	PCB_{high}
30 days	584 ± 567^{a}	763 ± 386^{b}
60 days	401 ± 172^{a}	1783 ± 934^{c}

Table 3. Average (± S.D.) of the Active Metabolic Rate (AMR), Standard Metabolic Rate (SMR), Aerobic Metabolic Scope (AMS) and critical oxygen saturation (O_{2crit}) in the four experimental groups of sole: the PCB-exposed individuals (PCB_{low} and PCB_{high}) and the uncontaminated individuals (*Control* and *Solvent*). For each treatment, * indicates values that are significantly different between 60 and 30 days ($P < 0.05$). Independently of the treatment, italics values indicate a significant effect of the exposure period (i.e. $O_{2\ crit}$ was found to be significantly higher in the soles tested after 60 days).

	Control		*Solvent*		PCB_{low}		PCB_{high}	
	30 days (n=8)	60 days (n=6)	30 days (n=8)	60 days (n=13)	30 days (n=7)	60 days (n=14)	30 days (n=8)	60 days (n=11)
AMR ($mgO_2\ kg^{-1}\ h^{-1}$)	151.9±41.2	155.8±24.7	129.5±22.2	143.9±43.3	159.7±28.3	137.1±23.5	153.7±59.2	173.2±73.9
SMR ($mgO_2\ kg^{-1}\ h^{-1}$)	46.9±9.6	45.8±18	42.3±10.2	53.8±12.1	52.9±14.7	44.3±16.5	38.7±8.4	52.7±18.4*
AMS ($mgO_2\ kg^{-1}\ h^{-1}$)	105±39.6	110±27.4	87.2±24.8	90.1±37.6	106.9±21.7	92.8±31.3	114.9±60.7	120.5±71
$O_{2crit\ (\%\ a.s.)}$	15.5±6.4	*20.9±12.4*	22.5±2.3	*30.5±12.2*	16.5±4.3	*25.7±14.4*	16.1±5.1	*30.2±18.8*