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Effets d'une exposition alimentaire chronique à un mélange environnemental de PCB et PBDE sur les traits d'histoire de vie, la bioénergétique et la dynamique des populations de poissons

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Résumé

Les polluants organiques persistants (POP) constituent un large ensemble de composés aux propriétés physico-chimiques et de toxicité variables. Parmi ceux-ci, les polychlorobiphényles (PCB) et les polybromodiphényléthers (PBDE) sont deux familles de composés hydrophobes partageant certaines propriétés physico-chimiques similaires, leur conférant des propriétés environnementales proches : persistance, bioaccumulation et toxicité. Des travaux antérieurs ont montré que ces composés pouvaient affecter les traits d'histoire de vie des poissons tels que la survie, la croissance et la reproduction. Il s'avère cependant que les études approchant des situations environnementales sont rares du fait de l'utilisation soit d'un congénère unique, soit d'une famille unique de molécules parmi les POP, soit de concentrations élevées. Les résultats sont alors difficilement transposables aux populations naturelles. Cette thèse porte sur l'évaluation des réponses individuelles et populationnelles des poissons à une exposition chronique par la voie alimentaire à un mélange de PCB et de PBDE représentatif du milieu marin. Elle se divise en trois parties.

La première partie s'est attachée à évaluer les effets d'une exposition expérimentale au mélange de PCB et de PBDE représentatif de l'estuaire de la Seine sur les traits d'histoire de vie du poisson-zèbre pris comme espèce modèle. Des individus ont été exposés par voie alimentaire au mélange de polluants à partir du premier repas et tout au long de leur cycle de vie. Leurs traits d'histoire de vie ont été suivis et comparés à ceux de poissons contrôles et ce pour plusieurs réplicas. Les résultats ont montré un ralentissement de la croissance mais une taille asymptotique plus élevée et une probabilité de ponte retardée chez les individus exposés. De plus, les descendants issus des premiers évènements de ponte des individus exposés avaient une survie larvaire plus faible en situation de jeûne. Ces résultats suggèrent une augmentation de l'allocation d'énergie vers la croissance au détriment de la maturation, la maintenance et la reproduction chez les individus exposés qui correspondrait à un déplacement du compromis énergétique entre ces deux compartiments. **La seconde partie** a cherché à identifier, sur la base d'un modèle bioénergétique, les modes d'action physiologique (PMoA) du mélange de PCB et PBDE pouvant expliquer les résultats expérimentaux. Un modèle de Budget Energétique Dynamique (Dynamic Energy Budget ; DEB) a été calibré pour chaque traitement sur la base des données expérimentales et les paramètres estimés ont été ensuite comparés entre individus exposés et contrôles. Le modèle DEB a permis de révéler deux PMoAs probables : le premier est lié à l'augmentation de la fraction d'énergie allouée à la maintenance et la croissance somatique au détriment de la maturation, sa maintenance et la reproduction et le deuxième est lié à l'augmentation des coûts de production d'un œuf. Ces deux PMoAs concourent à une croissance vers des tailles plus élevées et une fécondité plus faible chez les poissons exposés. **La troisième partie** de la thèse s'est intéressée à évaluer les conséquences des réponses individuelles à l'exposition au mélange de PCB et de PBDE, via les deux PMoAs identifiés, sur la dynamique de population de poissons et la productivité des pêcheries commerciales. Un

modèle de population structurée physiologiquement (PSP) a été couplé (i) au modèle DEB pour décrire la trajectoire ontogénique des traits d'histoire de vie des individus et (ii) à un modèle de ressource dynamique pour inclure la boucle de rétroaction environnementale entre les individus et leur ressource. Le modèle DEB-PSP a prédit une réduction de l'abondance numérique de la population exposée en même temps qu'une augmentation de sa biomasse. L'abondance numérique réduite chez la population exposée est probablement due à la diminution de la fécondité alors que la biomasse plus élevée est liée à l'augmentation de la croissance qui surcompense le déficit numérique. L'étude par bifurcation des effets indépendants des deux PMoAs a montré qu'ils participent conjointement à la diminution de l'abondance et l'augmentation de la biomasse chez la population exposée. Le modèle prédit également que la population exposée s'éteint pour des taux de mortalité plus faibles que pour la population contrôle lorsque la mortalité est indépendante de la taille des individus et plus forts lorsque la mortalité décroit avec la taille, comme pour la mortalité par prédation. Lorsqu'une mortalité par pêche est ajoutée à la mortalité par prédation et que celle-ci augmente avec la taille comme c'est le cas classiquement dans les pêcheries au chalut, la population exposée supporte des taux d'exploitation moindre que la population contrôle. Enfin, la population exposée permettrait un rendement maximum durable plus élevé que la population contrôle mais à un taux d'exploitation moindre, exposant la population contaminée à un plus grand risque de surexploitation.

Mots clés : poisson-zèbre**,** écotoxicologie, polluants organiques persistants, allocation d'énergie, modes d'action, dynamique des populations, populations structurées

Abstract

Persistent organic pollutants (POPs) are a broad group of compounds with varying physicochemical and toxic properties. Among POP, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are two families of hydrophobic compounds sharing some similar environmental properties such as hydrophobicity, persistence, bioaccumulation and toxicity. Previous studies have demonstrated that these compounds could affect fish life-history traits such as survival, growth and reproductive success. However, few studies have focused on environmental situations as most previous works investigated the effect of either single congeners, or single families of molecules among POP, or high concentrations, so that results can hardly be transposed to natural populations. The present thesis focuses on the evaluation of individual and population responses of fish to a chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs. It is divided into three parts.

The first part aimed to evaluate the effects of an experimental exposure to a mixture of PCBs and PBDEs that is representative of the Seine estuary on the life-history traits of zebrafish taken as a model species. Exposure was conducted through diet from the first meal and throughout the life cycle of the fish. Life-history traits of exposed fish were compared to those of control individuals using several replicate populations in each treatment. The results showed a slower growth, but to a larger asymptotic length, and delayed spawning probability in exposed fish. In addition, offspring issued from early spawning events of exposed fish exhibited a lower larval survival under starvation condition. These results suggest an increase in energy allocation towards growth at the expense of maturation, maintenance and reproduction in exposed individuals, which could correspond to a shift in the energy trade-off between these two compartments. **The second part** aimed to identify, on the basis of a bioenergetic model, the physiological modes of action (PMoA) of PCBs and PBDEs that can explain the experimental results. A Dynamic Energy Budget (DEB) model was calibrated for each treatment using experimental data and the estimated model parameters were compared between control and exposed fish. The DEB model revealed two potential PMoAs: the first one was through an increase of the fraction of energy allocated to somatic maintenance and growth at the expense of maturation, its maintenance and reproduction and the second one was through an increase of the cost of production of an egg. **The third part** focused on the population dynamical consequences of the individual life-history effects of the two identified PMoAs of PCBs and PBDEs. A Physiologically Structured Population Model (PSP) was coupled with (i) the DEB model to describe the ontogenetic trajectory of individuals' life-history traits, and (ii) a dynamic resource model to include the feedback loop between individuals and their resource. The DEB-PSP model predicted a lower abundance and a higher biomass in exposed population compared to control population. These results could be explained by a weaker fecundity that leads to a lower abundance of individuals and an increased growth, which results

into a larger biomass by overcompensating the numerical deficit for populations. The bifurcation analysis of the independent effects of the two PMoAs showed that they participate jointly in the decrease of the abundance and the increase of the biomass of the exposed population. The model also predicted that lower intensities of size-independent natural mortality are necessary to drive exposed population to extinction relative to control population, whereas higher intensities are necessary when mortality decreases with size as for predation mortality. When fishing mortality is added to predation mortality and increases with size, as in trawl fisheries, the control population seems to persist at higher exploitation rates than the exposed one. Finally, the exposed population may allow for a larger maximum sustainable yield but at a lower exploitation rate, thus exposing the contaminated population to a higher risk of overexploitation.

Keywords: zebrafish, ecotoxicology, persistent organic pollutants, energy allocation, modes of action, population dynamics, structured populations

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INTRODUCTION GÉNÉRALE

Exposition aux polychlorobiphényles (PCB) et polybromo-diphényléthers (PBDE) en milieu marin côtier

Les écosystèmes côtiers ont une grande valeur économique et écologique car ils fournissent nourriture, habitat et protection pour 80 à 90 % des ressources marines consommées mondialement (Amara, 2011). Parallèlement, les activités humaines affectent fortement les écosystèmes côtiers et estuariens, où les activités industrielles et la densité de population sont particulièrement élevées. De plus, les sédiments de ces écosystèmes constituent souvent un important réservoir pour les substances chimiques produites par l'activité humaine, ce qui amplifie le risque d'exposition de la biocénose. Les zones côtières sont donc des habitats de première importance écologique soumis à d'intenses perturbations d'origine anthropique, notamment chimiques. Parmi ces substances, les polluants organiques persistants (POP) rassemblent un grand nombre de composés résistants à la dégradation naturelle dans l'environnement et partageant certaines propriétés physico-chimiques communes telles que le fait d'être des composés organiques et hydrophobes. Ces propriétés contribuent à leur mobilité, c'est-à-dire leur capacité à être dispersés parfois à longue distance des sources, leur persistance dans l'environnement et leur bioaccumulation dans les organismes. Les polychlorobiphényles (PCB) et les polybromodiphényléthers (PBDE) sont deux familles de POP composées de 209 congénères chacune obtenus par substitution de certains des 10 atomes d'hydrogènes d'une molécule de biphényle par un atome de chlore ou de brome, respectivement. Les 209 congénères correspondent aux différentes combinaisons possibles dans la répartition des 1 à 10 atomes potentiels de chlore ou de brome sur la molécule de biphényle (Fig. 1). Les PCB ont été utilisés depuis les années 1930 à l'échelle mondiale pour divers usages industriels tels que les fluides diélectriques dans les

condensateurs électriques, les transformateurs et les systèmes hydrauliques (United Nations Environment Programme, 1999) et les PBDE sont utilisés comme retardateurs de flammes dans une grande variété de produits, y compris les matières plastiques, les meubles, la tapisserie, les équipements électriques, les appareils électroniques, les textiles et autres produits ménagers (United Nations Environment Programme, 2012).

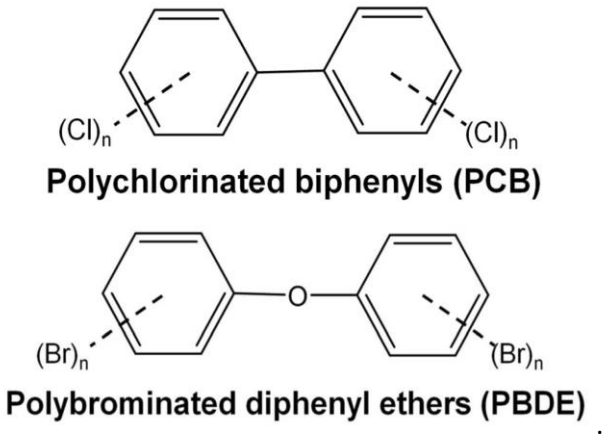

Fig. 1. Structure chimique des PCB et des PBDE (Walter et al., 2017)

Les PCB et les PBDE peuvent pénétrer dans l'environnement par différentes voies telles que les fuites de sources ponctuelles, les effluents de sites d'enfouissement, ou les émissions dans l'atmosphère après combustion pour les PCB ou parce qu'ils ne sont pas chimiquement liés aux produits dans lesquels ils sont utilisés comme retardateurs de flammes pour ce qui concerne les PBDE. En raison de la façon dont ils ont été produits, de la multiplicité des sources, les PCB et les PBDE se retrouvent dans l'environnement sous forme de mélanges complexes de nombreux congénères (Mizukawa et al., 2009). Les mélanges de congénères ainsi libérés pénètrent ensuite dans le milieu aquatique par le lessivage des sols et les retombées des émissions atmosphériques directement sur l'eau ou sur le sol suivi du lessivage. Dans l'environnement aquatique, ils sont alors associés à la phase particulaire et peuvent se retrouver à des concentrations élevées dans le compartiment sédimentaire. Ils peuvent ensuite entrer dans le réseau trophique

benthique par le biais d'une activité de bioturbation ou directement dans le réseau trophique pélagique après avoir été libérés des sédiments soit par des processus naturels, dont les évènements extrêmes tels que les tempêtes et les inondations, soit par des activités humaines comme le chalutage ou le dragage. Une fois dans le réseau trophique, de par leur hydrophobicité et leur métabolisation relativement faible, les PCB et les PBDE vont se bioaccumuler et être bioamplifiés par transfert trophique dans la plupart des compartiments biotiques des écosystèmes terrestres et aquatiques (ex. poissons, phoques, ours polaires, oiseaux, crocodiles; Couderc et al., 2015; Letcher et al., 2009; Wu et al., 2014) ainsi que dans les tissus humains (Dirinck et al., 2011; Rocca et al., 2008).

Les PCB ont été progressivement interdits par divers pays depuis les années 1970 tandis que les PBDE ont été interdits plus récemment dans les années 2000. Ces interdictions ont été approuvées internationalement par la Convention de Stockholm sur les Polluants Organiques Persistants (United Nations Environment Programme, 2001) et ses 5 amendements de 2005 à 2015. Aujourd'hui, seule la production commerciale de Deca-PBDE (BDE-209) est encore autorisée pour certaines applications en raison de sa faible menace pour le biote due à sa forte hydrophobicité et sa grande taille moléculaire (He et al., 2011; Munschy et al., 2011). Malgré ces restrictions et en raison de leur utilisation historique intensive, de leurs propriétés physico-chimiques et de leur interdiction tardive dans le cas des PBDE, les PCB et les PBDE se sont accumulés et restent omniprésents, parfois à des concentrations élevées, dans différents écosystèmes, notamment les écosystèmes aquatiques (Muir et al., 2003; Shaw and Kannan, 2009). Les congénères de PCB et de PBDE sont présents dans les sédiments marins du monde entier (de Boer et al., 2003; Eljarrat et al., 2004; Fernández et al., 1999; La Guardia et al., 2007; Labadie et al., 2010; Luo et al., 2007; Moon et al., 2007a, 2007b; Sapozhnikova et

al., 2004). Comme mentionné précédemment, les PCB et les PBDE présents dans les sédiments sont biodisponibles et peuvent entrer dans la chaîne trophique au niveau de la production primaire. Dans le cas des niveaux trophiques plus élevés, l'exposition alimentaire est la principale voie d'entrée dans l'organisme chez les vertébrés (Muir et al., 2003; Nyman et al., 2002). Au cours des dernières décennies, les concentrations de PBDE ont augmenté de façon exponentielle dans l'environnement marin et le biote associé (plancton, bivalves, poissons, mammifères marins ; Frouin et al., 2013; Johansson et al., 2006; Moon et al., 2007a, 2007b; Ross et al., 2009) alors que, selon certaines études, les concentrations de PCB étaient légèrement en baisse (Gómez-Gutiérrez et al., 2007; Rigét et al., 2016). Cependant, les PCB sont encore détectés à des concentrations élevées dans les estuaires industrialisés et urbanisés comme par exemple le long des côtes françaises (réseau de surveillance ROCCH-France; Fig. 2).

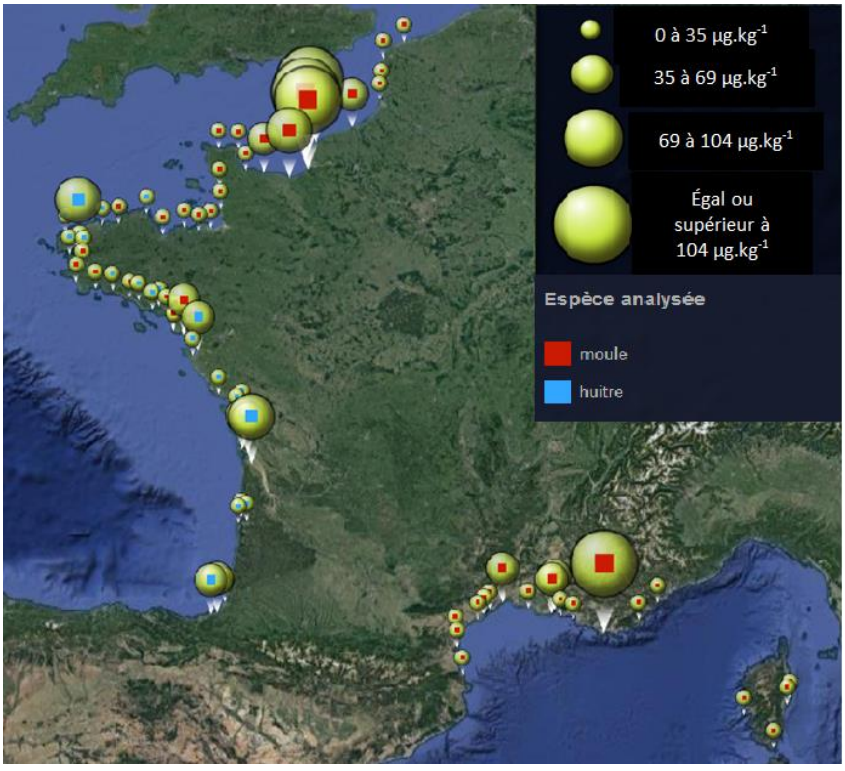

Fig. 2. Niveaux de PCB (CB-153, µg.kg^{-1}) dans les huitres et les moules du littoral français (réseau de surveillance ROCCH-France).

L'un des exemples les plus caractéristiques est celui de l'estuaire de la Seine qui a les plus fortes concentrations de POP de la côte française et parmi les plus fortes des côtes européennes en raison du nombre considérable d'activités industrielles et urbaines dans le bassin versant (Johansson et al., 2006; Munschy et al., 2008; Fig. 3). Dans le même temps, c'est une zone de nourricerie importante pour de nombreuses espèces de poissons, en particulier la sole commune (Riou et al., 2001). Il a été rapporté que la population de Manche Est de cette espèce était en constante diminution potentiellement en lien avec l'exposition à des contaminants en Baie de Seine, qui est l'estuaire le plus étendu de ce bassin maritime (Le Pape et al., 2007; Rochette et al., 2010). En effet, il a été montré que la fonction de nourricerie de cet estuaire était altérée avec des densités d'individus et des performances de croissance plus faibles que dans les autres estuaires (Amara et al., 2007; Gilliers et al., 2006) et une contribution plus faible qu'attendu au renouvellement du stock principalement due à une dégradation de la qualité des eaux (Riou et al., 2001; Rochette et al., 2010). Etant donné les concentrations importantes de POP observées en Baie de Seine, l'altération de la productivité de sa nourricerie pourrait être la conséquence des effets toxiques des POP exercés sur certaines fonctions physiologiques des individus et les traits d'histoire de vie qui en découlent comme la survie des individus exposés et/ou de leur descendance, la croissance ou la reproduction. Cependant, les effets des mélanges environnementaux marins de POP sur les traits d'histoire de vie des poissons ont été très peu étudiés, que ce soit in situ ou par l'expérimentation en milieu contrôlé, de sorte qu'il est difficile de statuer sur leur implication dans la dégradation de la nourricerie de Baie de Seine et plus généralement sur leurs conséquences potentielles sur le renouvellement et la dynamique des populations de poissons.

Fig. 3. Niveaux de PCB (CB-153, µg.kg^{-1}) dans les huitres et les moules de l'estuaire de la Seine (réseau de surveillance ROCCH-France).

Effets des PCB et des PBDE sur la physiologie et les traits d'histoire de vie des individus

Compte tenu de l'exposition potentielle du biote aux POP, il s'avère crucial d'étudier la toxicité de ces composés et leur devenir au cours du cycle de vie des organismes. Ces effets, incluant ceux des PCB et PBDE, ont été étudiés à l'échelle individuelle ou infra-individuelle à partir d'expositions expérimentales, dont des tests de toxicité, qui permettent de contrôler les effets potentiellement confondants dus à d'autres polluants ou facteurs environnementaux (ex. température, nourriture), d'établir des liens entre les polluants et leurs effets sans ambiguïté et de prendre en compte les interactions entre les facteurs biotiques et abiotiques. En revanche, peu d'études ont été réalisées à partir des observations de terrain (Bodiguel et al., 2009; Jenkins et al., 2014; Yu et al., 2015) en raison de la présence de multiples familles de polluants chimiques qui pourrait rendre difficile la détermination des liens entre la présence d'une seule classe de polluant et ses effets sur le biote (Baillon et al., 2016). Les études expérimentales ont été menées chez de nombreux organismes à un ou plusieurs stades de vie, voire sur l'ensemble du cycle

de vie: mollusques (Christensen et al., 2002), poissons (Berg et al., 2011; Daouk et al., 2011; Foekema et al., 2012; Lyche et al., 2010; Muirhead et al., 2006), oiseaux (Bargar et al., 2001) et mammifères (Dickerson et al., 2010).

Ces travaux expérimentaux ont démontré des altérations de plusieurs fonctions physiologiques chez les poissons après exposition à des POP: le comportement, la croissance, les fonctions reproductrices, hépatiques et rénales ainsi que le système immunitaire et endocrinien (Berg et al., 2011; Daouk et al., 2011; Han et al., 2011, Lyche et al., 2010; Muirhead et al., 2006; Péan et al., 2013; Yu et al., 2015). Au-delà des fonctions physiologiques, plusieurs études ont montré que l'exposition à des mélanges de POP pouvait affecter les traits d'histoire de vie de poissons, qui correspondent à l'ensemble des caractères individuels affectant directement la survie et la reproduction des individus ou leur timing au cours du cycle de vie (ex. patron de croissance, âge et taille à la maturité sexuelle, survie en fonction de l'âge ou la taille, fécondité, longévité). Par exemple, il a été rapporté que l'exposition alimentaire chronique du poisson-zèbre à un mélange de congénères de PCB pouvait entraîner une diminution de la taille de ponte et du taux de fécondation (Daouk et al., 2011). Une altération de la croissance chez la même espèce de poisson exposée par voie alimentaire à des mélanges d'hydrocarbures aromatiques polycycliques (HAP) a été observée avec une diminution du poids et de la longueur (Vignet et al., 2014), alors qu'une augmentation du poids a été montrée chez ce même poisson après une exposition à un mélange de PCB et de PBDE (Lyche et al., 2010). Ce dernier résultat a été expliqué par une perturbation endocrinienne. D'autre part, la condition des poissons peut aussi être affectée par les POP, comme montré en réponse à une exposition ponctuelle mais forte à des HAP (Gilliers et al., 2012).

En outre, d'autres études se sont intéressées aux conséquences de l'exposition sur la descendance des poissons exposés. D'une part, il a été montré qu'un transfert maternel

des POP à la progéniture pouvait se faire au travers des œufs à cause de leur propriété lipophile qui leur fournit une voie de transfert en s'associant aux lipides stockés chez la femelle (Daouk et al., 2011; Westerlund et al., 2000). D'autres part, des effets trans-générationnel peuvent également affecter les descendants des poissons exposés, que ces effets soient liés au transfert maternel ou pas. Des altérations comportementales ont notamment été démontrées chez le poisson-zèbre après une exposition aux PCB (Péan et al., 2013). De plus, l'exposition d'œufs de sole à un mélange de POP, incluant des PCB et des PBDE, via l'eau de sorte à mimer l'exposition des embryons due au transfert maternel peut provoquer une mortalité aiguë chez les larves après l'éclosion (Foekema et al., 2012, 2014).

Il faut cependant souligner que peu d'études se sont focalisées sur des situations environnementales. Dans la plupart des cas, les conditions d'exposition sont très différentes des conditions environnementales du fait de l'utilisation soit d'un congénère unique, soit d'une famille unique de molécules parmi les POP (PCB ou PBDE ou HAP par exemple), soit de concentrations élevées. Les résultats expérimentaux sont alors difficilement transposables aux populations naturelles. Ainsi, les effets de l'exposition des poissons à des mélanges réalistes (en termes de composition et de concentration) de PCB et de PBDE au cours de leur cycle de vie sur leur physiologie et leurs traits d'histoire de vie restent largement méconnus.

Modélisation bioénergétique des effets des contaminants: Budget Energétique Dynamique (DEB)

La modélisation est un outil précieux pour interpréter les données expérimentales de toxicité des polluants et plus généralement la réponse de l'individu à différents facteurs comme les contaminants mais aussi la nourriture ou la température par exemple. L'analyse de la réponse des traits d'histoire de vie, qui sont considérés comme un

résultat de l'allocation des ressources énergétiques au sein de l'individu durant son cycle de vie (Partridge et al., 1991; Perrin and Sibly, 1993; Stearns, 1992), peut notamment se faire au travers de modèles bioénergétiques. Plus précisément, au cours du cycle de vie, l'organisme consomme des ressources présentes dans son environnement et utilise ces ressources sous forme d'énergie pour son développement, le maintien et l'entretien de son propre corps et la production de sa progéniture. L'ensemble de ces processus définissent l'allocation de l'énergie acquise à partir de la ressource aux différentes fonctions biologiques. L'allocation d'énergie est conforme aux principes de conservation de l'énergie et de la masse de sorte que toute augmentation de l'allocation des ressources à une fonction doit être corrélée à une diminution de l'allocation aux autres fonctions, un principe connu sous le nom de compromis énergétique (« energetic trade-off » en anglais). Les modèles décrivant ces principes sont généralement appelés modèles bioénergétiques ou modèles d'allocation d'énergie. Une de leurs nombreuses applications potentielles est d'être utilisés pour comprendre les effets de polluants sur l'allocation de l'énergie entre les compartiments énergétiques des individus et de prédire ensuite les conséquences sur leurs traits d'historie de vie.

Plusieurs types de modèles bioénergétiques ont été proposés en écologie. L'un des premiers est le modèle de croissance de von Bertalanffy (von Bertalanffy, 1957; Von Bertalanffy, 1938). Celui-ci décrit par une équation différentielle la croissance de la (bio)masse d'un individu comme la différence entre l'anabolisme (synthèse de masse) et le catabolisme (dégradation de masse) : $dW/dt = aW^{2/3} - cW$ où W représente la masse de l'individu, $aW^{2/3}$ décrit le gain de masse par anabolisme, cW exprime la perte de masse par catabolisme, et a et c sont les coefficients de proportionnalité avec la masse déterminant les flux anabolique et catabolique, respectivement. Contrairement à des modèles bioénergétiques récents et plus complexes (comme ceux décrits ci-

dessous), ce modèle ne prend pas en compte le compartiment de la reproduction ainsi que l'influence des variations de certains facteurs du milieu (ex. température, nourriture) mais parvient à décrire la croissance d'une très large gamme d'espèces, dont les poissons, de manière étonnamment précise et robuste.

Une approche de modélisation bioénergétique plus générique, appelée théorie du Budget Energétique Dynamique (Dynamic Energy Budget, DEB), a été développée par Kooijman (1993, 2000, 2010) et est utilisée pour des applications en écotoxicologie. Cette approche est considérée comme l'un des cadres théoriques de la bioénergétique individuelle les mieux éprouvés et les plus génériques qui permet de relier les traits d'histoire de vie aux paramètres environnementaux et aux facteurs de pressions comme les polluants au travers de l'allocation d'énergie. Elle décrit d'une manière mécanistique les taux auxquels un organisme acquiert de l'énergie au cours de son cycle de vie et l'utilise pour la maintenance, la croissance, le développement et la reproduction. Deux types de formulations de ce modèle, qui diffèrent par les règles d'allocation d'énergie, sont distingués: un modèle dit de « *production nette* » proposé par (Lika and Nisbet, 2000) et un modèle dit de la « règle du kappa » (« *Kooijman's kappa-rule* ») ou DEB standard (Kooijman, 2010). Le modèle de *production nette* considère que l'énergie assimilée est allouée en premier lieu à la maintenance somatique, puis l'énergie restante est allouée aux autres processus métaboliques. En revanche, le modèle de la *règle du kappa* suppose que l'énergie assimilée est d'abord accumulée dans les réserves, qui sont ensuite mobilisées pour alimenter les autres processus métaboliques en suivant la règle du kappa (Fig. 4). Selon cette règle, une fraction κ de l'énergie mobilisée est allouée à la maintenance et la croissance du somatique (structure), avec une priorité à la première ; et la fraction restante d'énergie $1-\kappa$ est allouée à la maintenance de la maturité (maturity) et soit son accroissement qui représente le développement et la maturation

chez les individus non reproducteurs soit les gonades (buffer) et la production des gamètes pour les adultes (Fig. 4). L'inconvénient de l'utilisation du modèle de *production nette* par rapport au modèle de la *règle du kappa* est que si l'énergie assimilée n'est pas suffisante pour couvrir les coûts de la maintenance somatique, l'individu meurt de faim ce qui n'est pas pertinent dans le cas où un individu possède assez de réserves pour couvrir les coûts de ce compartiment et donc survivre.

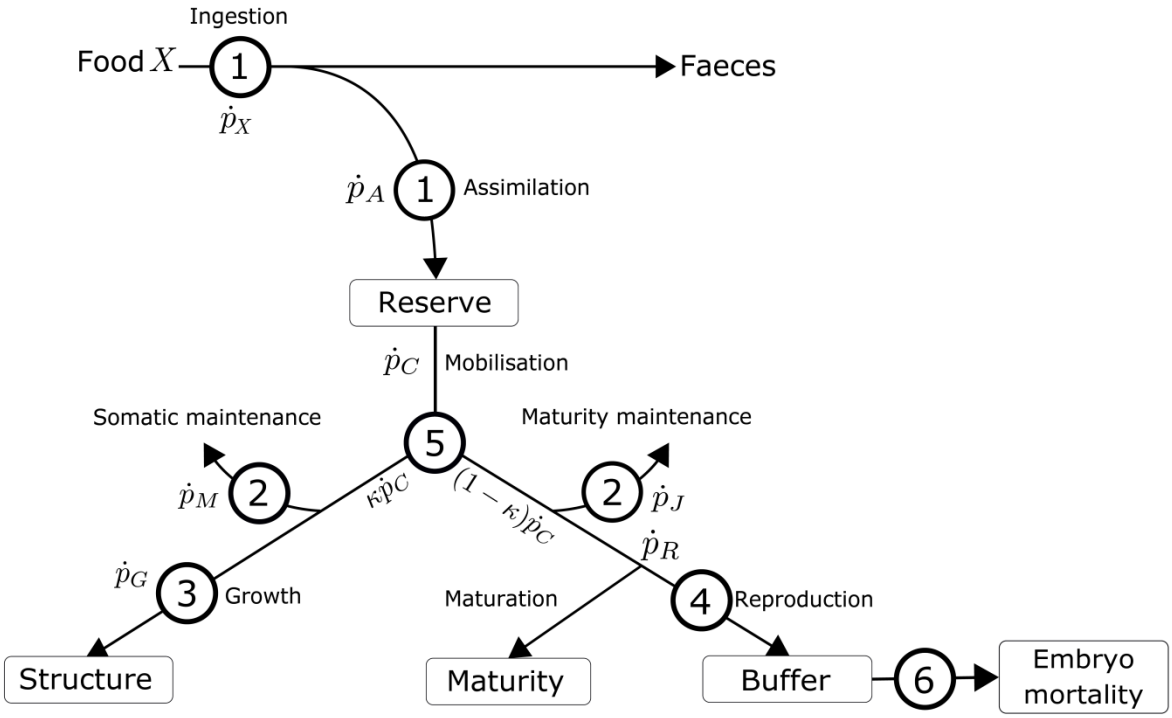

Fig. 4. Représentation schématique du modèle de Budget Energétique Dynamique (DEB) standard. $\dot{p}_X$, $\dot{p}_A$, $\dot{p}_C$, $\dot{p}_M$, $\dot{p}_G$, $\dot{p}_J$, et $\dot{p}_R$ représentent les différents flux d'énergie à travers un organisme : ingestion, assimilation, mobilisation, maintenance somatique, croissance somatique, maintenance de la maturité, accroissement de maturité/des gonades. Ceux-ci vont approvisionner différents compartiments énergétiques (variables d'état du modèle) : les réserves, la structure (ou soma), la maturité (ou stade de développement) et les gonades (buffer). κ est un paramètre considéré constant tout au long du cycle de vie de l'organisme.

La théorie DEB a été largement utilisée pour décrire le cycle d'histoire de vie chez différents animaux. Le nombre d'applications de ce modèle sur les différentes espèces ne cesse d'augmenter (voir la collection publiée en ligne des espèces et leurs propriétés métaboliques et biologiques : Add-my-Pet; http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/index.html). Hormis ces

applications du DEB à des populations non exposées à une pression environnementale, ce modèle a été également appliqué avec succès pour interpréter les effets de substances toxiques seules ou en interaction avec d'autres facteurs de pression (ex. nourriture) chez divers organismes (ex. nématodes, collemboles, vers de terre, bivalves, crustacés, poissons, mammifères ; Álvarez et al., 2006; Augustine et al., 2012; Desforges et al., 2017; Jager and Klok, 2010; Jager et al., 2004; Kooijman and Bedaux, 1996; Kooijman and Metz, 1984; Muller et al., 2010; Pieters et al., 2006). La première formulation du modèle DEB pour interpréter les tests de toxicité, notamment en milieu aquatique, a été proposée par (Kooijman and Metz, 1984). Celle-ci a été ensuite développée et simplifiée au fil du temps au travers de divers hypothèses (notamment taille à la maturité et coûts de production d'un œuf fixes) pour donner une branche spécifique de la théorie DEB appliquée à l'écotoxicologie et appelée « DEBtox » (Billoir et al., 2008; Jager and Zimmer, 2012; Kooijman and Bedaux, 1996). La théorie DEBtox constitue un modèle couplé de toxicocinétique, qui décrit la relation entre la concentration externe d'un polluant et sa concentration à l'intérieur de l'organisme, et de toxicodynamique, qui permet de relier la concentration interne du composé chimique aux effets sur les fonctions biologiques (en allant du site cible, tel que l'enzyme, aux effets sur les paramètres biologiques). Par rapport aux autres modèles couplés de toxicocinétique/toxicodynamique, le DEBtox fournit une approche métabolique de la croissance, la reproduction et la survie en fonction du temps et de la concentration du contaminant. Cette approche est considérée comme une des rares étant basée sur des principes biologiques et mécanistiques (par opposition à statistiques) disponibles actuellement pour étudier les effets sublétaux de composés toxiques sur les traits physiologiques et d'histoire de vie (Jager et al., 2010). En revanche, le DEBtox nécessite pour son application des données de type « dose-réponse » i.e., qui représentent comment les effets du produit toxique sur un ou des

caractères varient en fonction de sa concentration. Dans le cas d'expérimentations ne testant une exposition qu'à un seul niveau de contamination, le DEB standard peut être appliqué sans couplage avec un modèle toxicocinétique, par simple calibration indépendante du modèle en condition non exposée (ou contrôle) et en condition exposée. Jusqu'à présent les applications de la théorie DEB aux données écotoxicologiques ont essentiellement portées sur des espèces d'invertébrés et sur des molécules uniques plutôt que sur des mélanges environnementaux réalistes.

Les mécanismes et modes d'action des PCB et des PBDE

Les fonctions biologiques, telles que la croissance et la reproduction, sont chez les vertébrés sous contrôle endocrinien. Depuis quelques années, les perturbations de l'activité endocrinienne dues à l'exposition à différents polluants sont renseignées dans la littérature sous les termes de 'perturbation endocrinienne'. Parmi les perturbateurs endocriniens, on trouve notamment certains PCB et PBDE, capables d'influencer les systèmes endocriniens des poissons et d'autres vertébrés (Mills and Chichester, 2005). Ils peuvent agir à plusieurs niveaux : synthèse et transport des hormones, métabolisme, ou encore liaison avec les récepteurs, cibles naturelles des hormones. Plusieurs études ont montré que certains PCB et PBDE pouvaient avoir des effets œstrogéniques, anti-œstrogéniques ou androgéniques (He et al., 2011). Pour les PCB, cela serait lié à leur niveau de chloration (Pliskova et al., 2005). D'autre part, les PCB et les PBDE peuvent modifier les niveaux des gonadotrophines sériques (LH et FSH), d'hormones thyroïdiennes (TH) et d'hormones stéroïdes (Han et al., 2011). Au-delà de ces observations, il y a de plus en plus de signes d'une perturbation par les PCB et les PBDE au niveau de l'axe hypothalamo-hypophyso-gonadique (Chan and Chan, 2012; Dickerson et al., 2010; Han et al., 2011) qui appellent une attention supplémentaire.

Toutes ces perturbations au niveau moléculaire peuvent conduire à identifier et aider à la compréhension de ce qu'on appelle les « mécanismes » et les « modes » d'action des PCB et des PBDE qui ne sont pas entièrement explorés à ce jour. Un mécanisme d'action désigne la séquence des événements allant de l'absorption d'un produit chimique jusqu'à la production d'une réponse biologique spécifique par interaction du produit avec son site cible moléculaire. Un mode d'action est une description plus générale de l'action du produit chimique sur et de son interaction avec une fonction biologique. En d'autres termes, le mode d'action d'un produit chimique est défini comme la catégorie des processus biologiques par lesquels il induit une altération particulière des fonctions biologiques d'intérêt telles que la survie, la croissance et la reproduction (Schlosser and Bogdanffy, 1999).

Généralement, les modes d'action des composés chimiques sont identifiés et classés soit en se basant sur leur structure soit en utilisant des tests de toxicité expérimentale. Selon la structure des produits chimiques, les modes d'action sont subdivisés en quatre classes: les narcotiques, les narcotiques polaires, les produits chimiques réactifs et les produits « chimiques agissant spécifiquement » qui présentent une toxicité due à des interactions avec certaines molécules réceptrices (Verhaar et al., 2000). Cette classification est basée sur les groupes fonctionnels des produits chimiques, i.e., les composés chimiquement similaires ont des modes d'action biochimiques similaires. Cette définition n'est pas toujours vraie, notamment dans le cas où le mode d'action peut varier en fonction de l'espèce, du composé chimique et de la fonction biologique étudiée. L'évaluation des modes d'action des contaminants exige des bases de connaissances en toxicocinétique et/ou toxicodynamique qui sont clairement définies à partir des couplages entre tests de toxicité et modélisation telle que ceux utilisant le modèle DEB.

Dans le contexte de la théorie DEB, les modes d'action physiologiques (PMoA) d'un produit chimique sont définis comme des changements dans un ou plusieurs paramètres énergétiques entraînant des changements dans les profils d'allocation d'énergie qui sont révélés par la réponse empirique aux composés chimiques de différents traits d'histoire de vie correspondant aux variables d'état du modèle (croissance somatique, maturation, fécondité). Six grandes catégories de PMoAs potentiels des produits chimiques ont été étudiées ou proposées dans le contexte de la théorie DEB: (1) une diminution de l'ingestion ou l'assimilation d'énergie, (2) une augmentation des coûts de la maintenance somatique ou de la maturité, (3) une augmentation des coûts de la croissance, (4) une augmentation des coûts de la reproduction, (5) une modification de l'allocation énergétique entre la croissance et la reproduction et (6) une augmentation de la mortalité embryonnaire (Fig. 4) (Álvarez et al., 2006; Jager et al., 2006, 2010; Kooijman and Bedaux, 1996; Martin et al., 2013a). Les PMoAs peuvent être déduits des connaissances acquises sur les trajectoires des traits d'histoires de vie lorsque ceux-ci sont affectés par un produit chimique. Par exemple, (Martin et al., 2013a) ont identifiés les PMoAs de la 3,4-dichloroaniline chez la daphnie en comparant les trajectoires de la croissance et de la reproduction prédites pour chaque PMoA possible avec celles observées dans les données expérimentales. En conséquence, l'identification des PMoAs nécessite des données sur une partie significative, si ce n'est l'ensemble du cycle de vie de l'organisme. En pratique, il est difficile d'identifier un mode d'action métabolique unique à partir de données limitées. Il est par exemple compliqué de distinguer entre l'implication d'un seul ou plusieurs PMoAs lorsque seulement des observations sur la reproduction sont utilisées, plusieurs PMoAs pouvant provoquer des effets similaires sur un même trait d'histoire de vie. Des observations sur des traits d'histoire de vie supplémentaires, la croissance par exemple, sont généralement nécessaires (Martin et

al., 2014). Inversement, un seul PMoA peut affecter plusieurs traits d'histoire de vie et ce potentiellement à différents stades de développement. Au-delà de simplement documenter les effets des polluants sur les caractères des individus, l'identification de leurs PMoAs et ses difficultés sont des problématiques à ne pas négliger. En effet, en plus de la compréhension des fonctions physiologiques affectées, l'extrapolation des effets des contaminants de l'échelle individuelle à l'échelle populationnelle dépend de manière critique de leurs PMoAs.

Effet potentiel des PCB et PBDE à l'échelle populationnelle

Les modifications des caractéristiques physiologiques des individus et leurs conséquences en termes de traits d'histoire de vie, en particulier la survie, la croissance, le développement et la reproduction, peuvent avoir des répercussions sur la dynamique des populations de poissons (De Roos et al., 2003; Koons et al., 2016; Stearns, 1992) et, par conséquent, sur la structure des communautés et des écosystèmes marins. En effet, les traits d'histoire de vie déterminent la natalité et la mortalité au sein des populations et vont donc à ce titre déterminer leur démographie. Comme vu précédemment, ces changements de traits d'histoire de vie peuvent être causés par des facteurs de stress environnementaux comme l'exposition aux contaminants. Par exemple le fort potentiel de bioaccumulation des PCB et des PBDE pourrait impacter les communautés animales et la dynamique de leurs population à travers leurs effets à l'échelle individuelle (Vasseur and Cossu-Leguille, 2006). En particulier, certains scientifiques soupçonnent que les produits chimiques ont contribué au déclin de certaines populations de poissons marins sauvages (Barnthouse et al., 1990; Hamilton et al., 2015) et il a été suggéré que la productivité de certains stocks de poissons marins exploités pouvait être réduite en raison de l'altération du recrutement (entrée des jeunes individus dans la partie exploitable de la population) due à la dégradation de la qualité des habitats liée à

l'accumulation des polluants (Gilliers et al., 2006; Riou et al., 2001; Rochette et al., 2010). Étant donné la forte dépendance de la dynamique des populations aux traits d'histoire de vie (De Roos et al., 2003; Stearns, 1992), les effets potentiels des PCB et des PBDE sur les traits d'histoire de vie des poissons pourraient affecter le recrutement et la dynamique des populations de poissons ainsi que la productivité halieutique associée pour les espèces de poissons commerciales.

Ainsi, comprendre les effets des contaminants à l'échelle individuelle est essentiel pour pouvoir les extrapoler et quantifier leurs conséquences au niveau de la population. Les PMoAs peuvent être utilisés comme paramètres de changement des traits d'histoire de vie au niveau individuel comme cela a été montré chez différentes espèces (Jager and Klok, 2010; Kooijman and Metz, 1984; Martin, 2013; Martin et al., 2014). Néanmoins, l'extrapolation des effets individuels à la population exige des approches de modélisation, même si pour les espèces de petite taille à cycle de vie court des études expérimentales de dynamique de population sont envisageables (ex. Martin et al., 2013). Des approches de modélisation couplant modèle bioénergétique et modèle de dynamique de population ont été développées dans cette optique, à savoir *Les modèles d'Euler-Lotka* (Jager et Klok, 2010; Klok et de Roos, 1996, Kooijman et Metz, 1984), *Les modèles matriciels de Leslie* (Klok et de Roos, 1996, Billoir et al., 2007) et *les modèles individu-centrés* (Martin et al., 2013a, 2013b).

Les modèles d'Euler-Lotka en temps continu ne sont pas vraiment utilisés pour étudier la dynamique des populations, mais plutôt leur taux de croissance intrinsèque basé sur l'hypothèse irréaliste d'une population en croissance exponentielle sans densité-dépendance et rétroaction environnementale. *Les modèles matriciels de Leslie* sont plus réalistes et permettent de prendre en compte la densité-dépendance, mais ils ne

permettent pas d'inclure explicitement la boucle de rétroaction environnementale à travers le couplage avec la dynamique de la ressource et plus généralement d'inclure un environnement variable (Caswell, 1989). *Les modèles individu-centrés* (Martin et al., 2013a, 2013b) permettent d'englober tous les détails à l'échelle de l'individu ainsi que les interactions explicites avec l'environnement et sa rétroaction mais ils pourraient être limités par des contraintes de calcul.

D'autres modèles dits de *Population Structurée Physiologiquement* (PSP), qui n'ont jamais été utilisés dans un contexte d'effet des contaminants sur la dynamique des populations, pourraient être un bon outil pour un couplage entre les dynamiques du consommateur et de la ressource avec un temps de calcul relativement court. Ils sont particulièrement bien adaptés pour extrapoler les changements des traits d'histoire de vie sur la base de la bioénergétique des individus, en particulier lorsque les traits d'histoire de vie de l'individu sont décrits en continu (De Roos, 2008), comme dans les modèles DEB. Ils relient les processus d'histoire de vie qui déterminent les caractéristiques d'un individu (ou l'état de l'individu : *i-state*) (ex. âge, longueur, réserves d'énergie) à son environnement (ou l'état de l'environnement : *E-state*) caractérisé par un ensemble de facteurs biotiques et abiotiques (ex. densité de la nourriture, température) et aux processus au niveau de la population (taux de mortalité, taux de naissance) qui déterminent l'état de la population (*p-state*) (ex. abondance, biomasse, structure des tailles ; Fig. 5) (De Roos et al., 1992; Metz and Diekmann, 1986).

Malgré les quelques applications citées précédemment, portant principalement sur des invertébrés, l'étude couplée de la bioénergétique et de la dynamique de population chez des poissons reste très rare (Barnthouse et al., 1990; Goodyear, 1985). Dans le contexte des espèces de poissons exploitées par la pêche, celles-ci seraient potentiellement plus

vulnérables aux contaminants car elles sont soumises à un stress supplémentaire i.e., la mortalité par pêche (Barnthouse et al., 1990; Goodyear, 1985). Au-delà de la simple interaction entre mortalité par pêche et mortalité induite par les polluants, les effets sublétaux de ces derniers sur les traits d'histoire de vie des individus pourraient affecter la capacité des populations de poissons commerciaux à soutenir la pêche. Par exemple, une faible fécondité associée à un stress due aux contaminants pourrait entraîner un déclin de la population de poissons (Goodyear, 1985) au travers d'une réduction de son renouvellement et donc entrainer une diminution de la productivité des pêcheries associées. Si l'on considère le cas de l'estuaire de la Seine, au vue de l'importance économique et écologique de cette zone, les très hauts niveaux de contamination par les POP, dont les PCB et les PBDE en premier lieu, justifient une évaluation du risque d'altération de la dynamique des populations de poissons qui vivent dans cette zone, notamment pour les espèces d'intérêt commercial comme la sole.

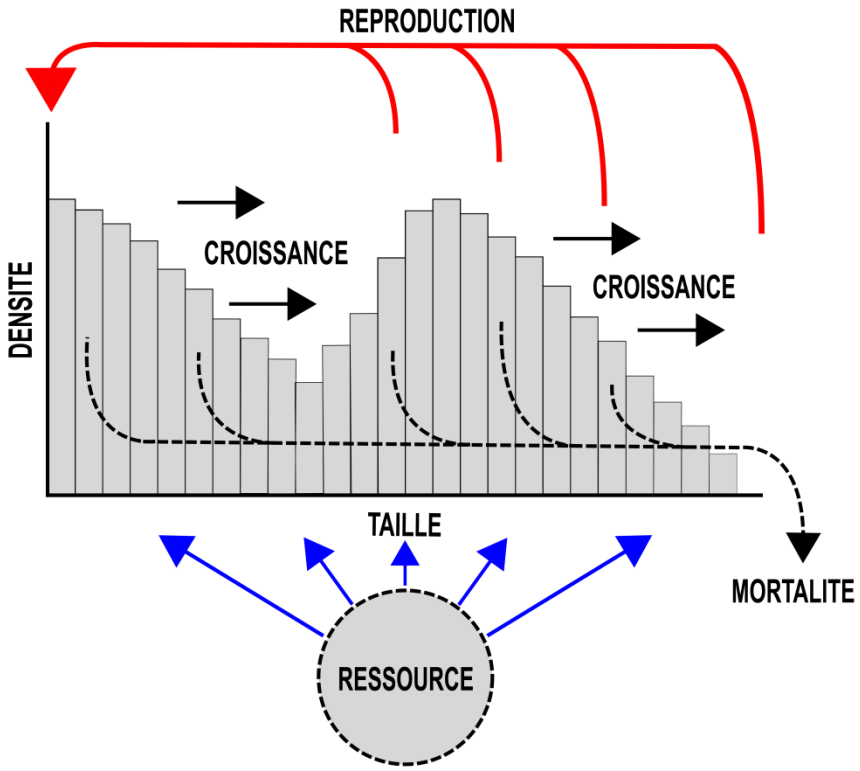

Fig. 5. Modèle de dynamique de population structurée en taille (figure adaptée de De Roos and Persson, 2013). La dynamique des consommateurs est décrite par le changement de leur densité, au cours du temps, due (i) à la croissance en taille (flèches noires) qui dépend de la disponibilité de la ressource (flèches bleues) et (ii) à la mortalité (flèches pointillées). Le renouvellement de la population (i.e. processus de recrutement) se fait à partir de la reproduction des adultes (flèches rouges) qui dépend également de la disponibilité de la ressource.

Objectifs et organisation de la thèse

Une large part des études expérimentales précédentes sur les effets individuels des POP chez les poissons reposent sur des expositions qui ne sont pas représentatives du milieu naturel en termes de profil de congénères et de concentration, de sorte qu'il est délicat d'extrapoler les résultats aux populations sauvages. De plus, très peu de travaux concernant l'identification des modes d'action physiologiques ou bioénergétiques des contaminants portent sur des perturbateurs endocriniens, tels que certains POP qu'ils soient en mélange ou pas, et une majorité utilisent des invertébrés comme espèce modèle. Enfin, les études visant à estimer les conséquences des effets individuels générés par différents PMoAs sur la dynamique des populations ne considèrent pas elles aussi de mélanges réalistes de molécules, notamment de POP, et sont également restreintes à des espèces d'invertébrés, de sorte qu'il est difficile d'extrapoler les résultats à des populations de poissons sauvages en général, voire exploitées en particulier.

L'objectif principal de cette thèse est d'évaluer les réponses individuelles et populationnelles des poissons à une exposition chronique par la voie alimentaire à un mélange de PCB et de PBDE représentatif du milieu marin. Plus précisément, il s'agit (i) d'évaluer les effets du mélange sur les traits d'histoire de vie (survie, croissance et reproduction) des poissons à l'échelle individuelle, (ii) d'en déduire le ou les modes d'actions physiologiques et (iii) d'en inférer les conséquences sur la dynamique de

population de poissons, voire la productivité des pêcheries dans le cas d'espèces commerciales.

Les travaux se sont appuyés sur un mélange de PCB et PBDE représentatif des écosystèmes marins côtiers en termes de profil des congénères et de leur concentration. Pour les PCB, le mélange correspond à celui trouvé dans les moules en estuaire de Seine (Antunes et al., 2007; Bodin et al., 2007; Schnitzler et al., 2011; ROCCH-Réseau d'Observation de la Contamination Chimique), choisi comme un cas typique d'estuaire ayant une fonctionnalité de nourricerie pour les poissons (Gilliers et al., 2006; Riou et al., 2001; Rochette et al., 2010) et étant fortement impacté par les activités industrielles et l'urbanisation, notamment en termes de pollution par les POP (Abarnou et al., 2000). Les invertébrés benthiques tels que les moules sont des proies importantes pour une large part des poissons benthiques et démersaux abrités dans les nourriceries. Concernant les PBDE, étant donné que leur profil de contamination au sein des organismes dépend fortement de leur métabolisation (Christensen et al., 2002; Ma et al., 2013; Stapleton et al., 2004; Voorspoels et al., 2003), le mélange n'a pas été défini à partir de mesures spécifiques mais correspond à un mélange à des concentrations environnementales des congénères les plus représentés dans le biote marin et du principal congénère stocké dans les sédiments et qui ont été identifié pour une action prioritaire par la Convention OSPAR (Law et al., 2006; OSPAR Commission, 2009, 2013).

Les effets individuels de ce mélange ont été évalués pour une espèce modèle, le poisson-zèbre. L'utilisation d'une espèce modèle ici est justifiée par le fait que l'identification des PMoAs des contaminants et l'extrapolation des effets individuels à l'échelle populationnelle nécessitent des données sur une portion significative, voire l'ensemble des stades de vie de l'organisme. Le cycle de vie court du poisson-zèbre permet des

expositions expérimentales et un suivi de leurs conséquences sur l'ensemble des stades de vie des individus et même d'une génération à l'autre. Leur petite taille et leur facilité d'élevage pour ce qui concerne les lignées de laboratoire — notamment reproduction abondante et continue tout au long de l'année, grande tolérance pour les variations physico-chimique de l'eau (température, pH ou dureté) et facilité d'alimentation sur aliment inerte — permettent l'élevage de populations expérimentales de grandes taille dans des espaces de taille raisonnables et facilitent la réplication des expériences. Par ailleurs, les connaissances biologiques, de la génétique à la morphologie en passant par la physiologie, accumulées sur cette espèce permettent une interprétation poussée des effets observés. Le poisson-zèbre est une espèce dulçaquicole. Cependant, de nombreux articles ont rapporté des voies de toxicités similaires chez les espèces de poissons dulçaquicoles et marines pour un certain nombre de fonctions biologiques comme la croissance (Bodiguel et al., 2009; Daouk et al., 2011) le comportement ou la reproduction(Bodiguel et al., 2009; Daouk et al., 2011a; Sun et al., 2015; Vignet et al., 2016).

Les différentes études menées dans cette thèse afin de répondre aux trois sous-objectifs listés ci-dessus sont ici rapportées en autant de chapitres (Fig. 6). Le **Chapitre 1** s'est attaché à évaluer les effets d'une exposition expérimentale au mélange de PCB et de PBDE représentatif de l'estuaire de la Seine sur les traits d'histoire de vie (survie des parents, croissance, reproduction, survie des descendants) du poisson-zèbre pris comme espèce modèle. Les individus ont été exposés par voie alimentaire au mélange de polluants à partir du premier repas et tout au long de leur cycle de vie. Leurs traits d'histoire de vie ont été suivis et comparés à ceux de poissons contrôles, i.e., non exposés, et ce pour plusieurs réplicas.

Le **Chapitre 2** a visé à identifier, sur la base d'un modèle bioénergétique, les modes d'action physiologique du mélange de PCB et de PBDE pouvant expliquer les résultats expérimentaux en termes de réponses des différents traits d'historie de vie. Un modèle de Budget Energétique Dynamique a été calibré pour chaque traitement sur la base des données expérimentales et les paramètres estimés ont été ensuite comparés entre individus exposés et contrôles. Les différents modes d'action physiologiques du mélange de PCB et de PBDE, i.e., les paramètres bioénergétiques altérés, ont été identifiés par cette procédure de calibration, puis validés de façon croisée par comparaison des patrons de changement des traits d'histoire de vie observés et ceux prédits pour chacun des six modes d'actions possibles.

Enfin, le **Chapitre 3** s'est intéressé aux conséquences des effets du mélange de PCB et de PBDE sur la croissance et la reproduction des individus sur la dynamique des populations de poissons et la productivité des pêcheries commerciales dans le cas où ces effets s'avéraient transposables. Pour ce faire, un modèle de population structurée physiologiquement (PSP) a été couplé (i) au modèle DEB pour décrire la trajectoire ontogénique des individus et de leurs traits d'histoire de vie et (ii) à un modèle de ressource dynamique pour inclure la boucle de rétroaction environnementale entre les individus et leur ressource.

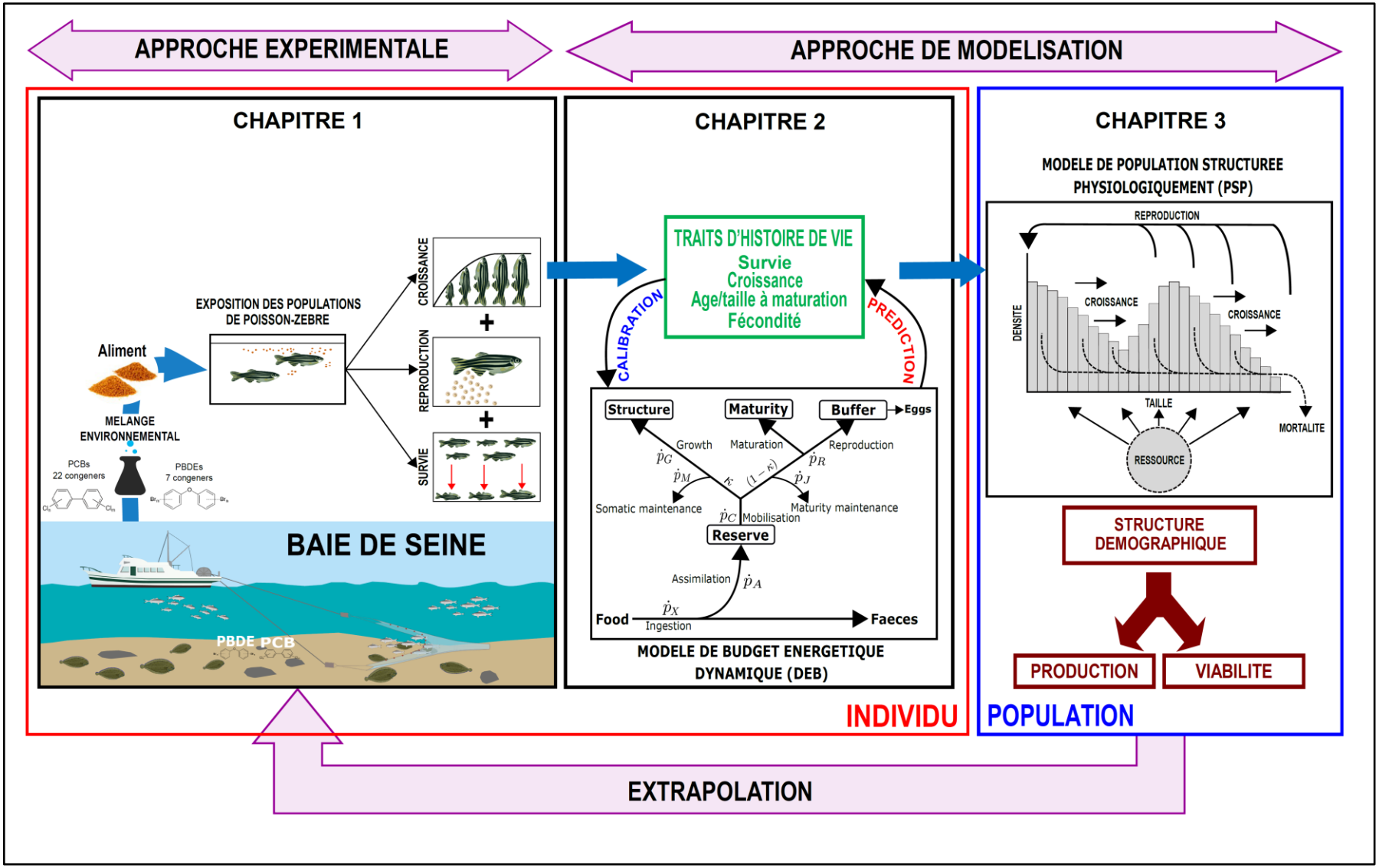

Fig. 6. Représentation schématique des trois chapitres de la thèse (les chapitres sont décrits en détail dans la section « objectifs et organisation de la thèse »)

Contexte scientifique de la thèse : le projet Fish'N'POPs

Les travaux présentés dans ce manuscrit de thèse s'inscrivent dans le projet national Fish'N'POPs financé par l'agence nationale de la recherche (ANR-13-CESA-020). Ce projet a été mis en place afin d'étudier la toxicité d'un mélange réaliste de congénères de PCB et de PBDE chez deux espèces modèles de poissons, la sole commune et le poisson-zèbre, au cours de leur cycle de vie ainsi que le transfert de ces polluants à la descendance et leur devenir au cours du développement et de la croissance.

Le projet Fish'N'POPs est organisé en **7 tâches** reliant plusieurs approches disciplinaires interdépendantes : endocrinologie, biologie moléculaire et cellulaire, histologie, physiologie, chimie et modélisation :

La **tâche 1** « Exposition des poissons » est consacrée à la production d'aliments contaminés et à l'exposition expérimentale chronique par voie alimentaire des poissons;

La **tâche 2** « Statut endocrinien » se concentre sur l'analyse des altérations possibles du système endocrinien chez les poissons contaminés. Cela comprend des études sur les composants clés de l'axe reproducteur (hormones stéroïdiennes et marqueurs moléculaires associés) ;

La **tâche 3** « Mécanismes moléculaires et aspects fonctionnels de la reproduction » examine en détail l'ovogenèse et la spermatogenèse et inclut des études histologiques pour l'évaluation fonctionnelle des gonades ;

La **tâche 4** « Performances reproductrices » examine la quantité et la qualité des gamètes, le taux de fécondation, la fertilité et le succès reproducteur. Le développement embryonnaire de la progéniture issu de géniteurs contaminés est mesuré, en particulier les altérations des processus de morphogenèse ;

La **tâche 5** « Développement des larves et performances physiologiques des juvéniles » est conduite à l'aide du phénotypage classique (survie, croissance) et de challenges physiologiques et comportementaux ;

La **tâche 6** « Devenir des polluants » étudie le devenir des PCB et des PBDE chez les adultes au cours de la maturation des gonades et évalue le transfert de ces POP aux œufs et leur devenir chez les stades de vie précoces ;

Enfin, la **tâche 7** « Modélisation du devenir et des effets des POP à l'échelle de la population » intègre toutes les connaissances acquises durant le projet Fish'N'POPs et les projets antérieurs, dans le but de développer des modèles de dynamiques bioénergétiques, démographiques et évolutifs.

Le présent travail de thèse s'inscrit dans cette dernière tâche tout en bénéficiant également des résultats des autres tâches.

1. CHAPITRE 1: Fish life-history traits are affected after chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs

1.1 Introduction du chapitre

Des travaux antérieurs ont montré que les PCB et les PBDE pouvaient affecter les traits d'histoire de vie des poissons tels que la survie, la croissance et la reproduction. A l'échelle individuelle ou infra-individuelle, leur toxicité a été étudiée à partir d'expositions expérimentales. Cependant, peu d'études expérimentales se sont focalisées sur des situations environnementales. Dans la plupart des cas, les conditions d'exposition sont très différentes des conditions environnementales du fait de l'utilisation soit d'un congénère unique, soit d'une famille unique de molécules parmi les POP, soit de concentrations élevées. Les résultats expérimentaux sont alors difficilement transposables aux populations naturelles. De plus, les effets de l'exposition des poissons à des mélanges réalistes (en termes de composition et de concentration) de PCB et de PBDE au cours de leur cycle de vie sur leur physiologie et leurs traits d'histoire de vie restent largement méconnus.

L'objectif de ce chapitre était d'évaluer les effets d'une exposition expérimentale au mélange de PCB et de PBDE représentatif de l'estuaire de la Seine sur les traits d'histoire de vie (survie des parents, croissance, reproduction, survie des descendants) du poisson-zèbre pris comme espèce modèle.

Dans un premier temps, plusieurs réplicas de populations de poissons-zèbres ont été produits à partir de la lignée sauvage TU de poisson-zèbre. Chaque population a été séparée en groupe contrôle et exposé qui ont été élevés par la suite dans plusieurs bacs contenant le même nombre d'individus. Concernant les groupes exposés, ils ont été contaminés par voie alimentaire par un mélange de congénères de PCB et de PBDE de manière chronique dès le premier repas (5 jours post-fécondation) et tout au long de leur cycle de vie. En parallèle, les groupes contrôles ont été suivis durant la même période et nourris avec de l'aliment ayant été préparé de manière similaire à l'aliment contaminé mais sans contaminants. Les traits de la génération parentale (F0), à savoir la survie, la croissance et la reproduction (incluant la probabilité de ponte, la taille de ponte et le taux de fécondation), ont été suivis dès le début de la contamination. Ensuite,

une expérience de survie chez les descendants (F1) a été réalisée sur des larves en situation de jeûne afin d'évaluer les effets intergénérationnels du mélange de PCB et de PBDE.

Dans un second temps, les effets de l'exposition au mélange de PCB et de PBDE ont été évalués en comparant les traits d'histoire de vie entre populations contrôles et exposées à l'aide d'une approche statistique basée sur les modèles linéaires et non-linéaires à effets mixtes.

Ce chapitre permettrait d'identifier à l'échelle de l'individu les effets sur l'histoire de vie potentiellement transposables aux populations de poissons marins avec des conséquences possibles sur leur dynamique et leur productivité.

Fish life-history traits are affected after chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs

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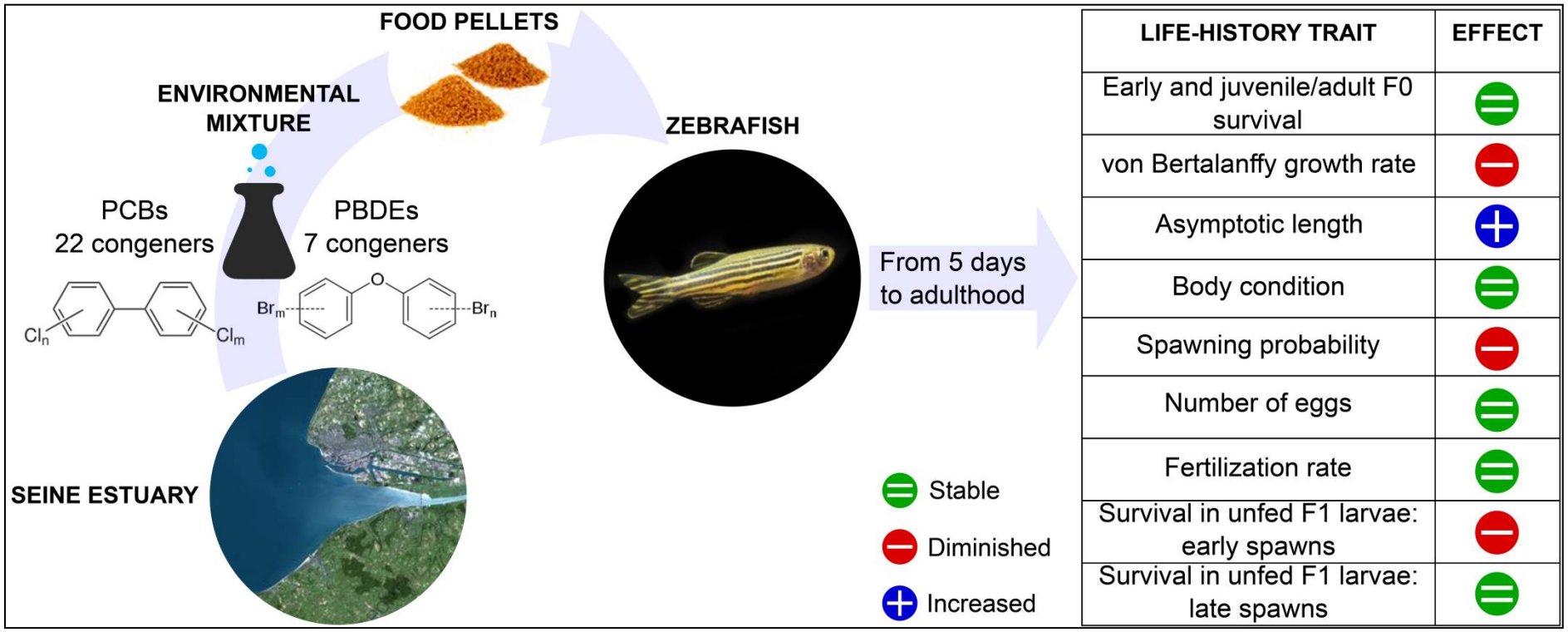
Graphical abstract



LIFE-HISTORY TRAIT	EFFECT
Early and juvenile/adult F0 survival	=
von Bertalanffy growth rate	−
Asymptotic length	+
Body condition	=
Spawning probability	−
Number of eggs	=
Fertilization rate	=
Survival in unfed F1 larvae: early spawns	−
Survival in unfed F1 larvae: late spawns	=

Abstract

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants that have been shown to affect fish life-history traits such as reproductive success, growth and survival. At the individual level, their toxicity and underlying mechanisms of action have been studied through experimental exposure. However, the number of experimental studies approaching marine environmental situations is scarce, i.e., in most cases, individuals are exposed to either single congeners, or single types of molecules, or high concentrations, so that results can hardly be transposed to natural populations. In the present study, we evaluated the effect of chronic dietary exposure to an environmentally realistic marine mixture of PCB and PBDE congeners on zebrafish life-history traits from larval to adult stage. Exposure was conducted through diet from the first meal and throughout the life cycle of the fish. The mixture was composed so as to approach environmentally relevant marine conditions in terms of both congener composition and concentrations. Life-history traits of exposed fish were compared to those of control individuals using several replicate populations in each treatment. We found evidence of slower body growth, but to a larger asymptotic length, and delayed spawning probability in exposed fish. In addition, offspring issued from early spawning events of exposed fish exhibited a lower larval survival under starvation condition. Given their strong dependency on life-history traits, marine fish population dynamics and associated fisheries productivity for commercial species could be affected by such individual-level effects of PCBs and PBDEs on somatic growth, spawning probability and larval survival.

Keywords: contaminants, Body length, Condition, Fertilization rate, Energy allocation, Trade-off.

1.2 Introduction

Persistent organic pollutants (POPs) gather a wide number of chemicals that are of great concern because of their persistence, bioaccumulation and toxicity. In addition, given their propensity for long-range transport, they are globally distributed in various environments worldwide including some far from source areas (Bogdal et al., 2013; Corsolini, 2009; Rigét et al., 2016). Among POPs, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are two families made of 209 congeners differing by the number and position of one to ten substitution by chlorine and bromine, respectively. PCBs have been used since the 1930s for various industrial purposes, such as dielectric fluids in electrical capacitors, transformers and hydraulic systems (United Nations Environment Programme, 1999), while PBDEs have been used since the 1970s as flame retardants in plastics, furniture, upholstery, electrical equipment, electronic devices, textiles and other household products (United Nations Environment Programme, 2012).

PCBs have been progressively banned in various countries since the 1970s whereas PBDEs have been banned or restricted more recently, beginning in the early 2000s. These regulations were endorsed internationally by the Stockholm Convention on POPs (United Nations Environment Programme, 2001). Today, only the commercial production of deca-PBDE is allowed, although with some restrictions in Europe. Although a decrease in their levels has been reported in biota from various locations (Byer et al., 2015; Rigét et al., 2016) and despite these restrictions, PCBs and PBDEs are still present in all environmental compartments worldwide, including aquatic ecosystems. Therefore, they still represent a potential environmental concern.

PCBs and PBDEs are found in the marine environment as complex mixtures of numerous congeners. Due to their long-term persistence and elevated lipophilicity (Mizukawa et al., 2009), they are significantly bioaccumulated and biomagnified through trophic transfer, in most biotic compartments of marine ecosystems (e.g. mollusks, fish, seals; Couderc et al., 2015; Johansson et al., 2006; Letcher et al., 2009). Such bioaccumulation could be a threat for animal communities and their population dynamics through the scaling up of their individual-level effects to the population level (Vasseur and Cossu-Leguille, 2006). Notably, some scientists suspected that chemicals have contributed to the decline of some wild marine fish populations (Hamilton et al., 2015) and it has been suggested that the productivity of some marine fish stocks could be altered due to recruitment impairment caused by nursery habitat degradation in relation to pollutant accumulation (Gilliers et al., 2006; Riou et al., 2001; Rochette et al., 2010). Given the strong dependency of population dynamics on life-history (De Roos et al., 2003; Stearns, 1992), potential individual-level effects of PCBs and PBDEs on fish life-history traits could indeed affect fish population recruitment and dynamics and associated fisheries productivity for commercial species (Vasseur and Cossu-Leguille, 2006).

It is difficult, however, to ascertain the relationship between the presence of one class of chemical and its effects on biota from field observations, because of the accumulation of multiple potential stresses, including many families of chemicals, in natural environments (Baillon et al., 2016). In contrast, the experimental approach allows controlling for potential confounding effects and establishing such links without ambiguity. The effects and the underlying mechanisms of action of PCBs and PBDEs at the individual level have thus been intensively studied through experimental exposure, notably in fish. These studies have demonstrated an alteration of behavior, growth, reproductive, hepatic, and renal functions as well as of the immune and the endocrine

systems in fish (Berg et al., 2011; Daouk et al., 2011; Han et al., 2011, 2013, Lyche et al., 2010, 2011; Muirhead et al., 2006; Péan et al., 2013; Yu et al., 2015 and references therein). In particular, several studies have demonstrated that exposure of fish to either PCB or PBDE congener mixtures can affect fish life-history traits such as reproductive success, growth and survival. For example, it has been reported that long-term dietary exposure of zebrafish to a PCB mixture led to a decrease in the number of eggs per spawn and in their fertilization rate (Daouk et al., 2011). Furthermore, dietary exposure of fathead minnows to a single PBDE congener (BDE-47) was shown to reduce cumulative egg production (Muirhead et al., 2006), and McCarthy et al.(2003) showed that Atlantic croaker larvae originating from parents exposed to PCBs technical mixture (Aroclor 1254) through diet were characterized by diminished growth. In addition to their effects on reproductive success and growth, these compounds have been shown to increase fish early-life-stage mortality. Indeed, it has been reported by Foekema et al. (2014) that exposure of common sole eggs to a mixture of POPs (including PCBs and PBDEs) via the water caused acute mortality in larvae after hatching. However, although experimental studies provide valuable information on the potential effects of PCBs and PBDEs, few have focused on environmental situations (see Berg et al., 2011; Lyche et al., 2010, 2011 for experiments mimicking freshwater lake environmental situations). In most cases, the exposure conditions are indeed quite different from environmental situations because of the use of either single congeners, or single types of molecules (i.e. PCBs, or PBDEs, or PAHs), or high concentrations and results can thus hardly be transposed to natural populations. How fish life-history traits may be affected by lifelong exposure to mixtures of both PCB and PBDE congeners that are realistic for the environment therefore remains largely unknown. More precisely, questions about the

effects of environmentally realistic mixtures on growth, reproduction and survival and their consequences on individual fitness and population dynamics are still pending.

In this study, we used the zebrafish model to explore the life-history effects associated with long-term dietary exposure to a mixture of PCBs and PBDEs. Due to their lipophilicity, these compounds are mostly found associated to organic matter and not in the water, so that dietary exposure is considered the major route of exposure to PCBs and PBDEs for vertebrates (Muir et al., 2003; Nyman et al., 2002). The selected congeners were chosen to approach environmentally representative conditions for marine ecosystems in terms of both concentrations and compositions. For PCBs, the mixture corresponded to the profile and concentrations found in mussels from an estuary highly-impacted by industrial and urban activities, the Seine estuary in France (Abarnou et al., 2000), which is a nursery area for many flatfish species (Riou et al., 2001). Benthic invertebrates such as mussels are indeed an important food source for many exploited fish species, notably flatfish and demersal fish. As PBDEs profiles in living organisms depend upon their metabolism (Christensen et al., 2002; Ma et al., 2013; Stapleton et al., 2004; Voorspoels et al., 2003), the mixture for this type of molecules was not defined based on specific measurements, but corresponded to a mixture at environmental concentration of the most representative congeners in marine biota and the main congener in marine sediments that were identified for priority action by OSPAR (OSPAR Commission, 2009, 2013).

These PCBs and PBDEs mixtures representative of environment situations were used for identifying life-history effects potentially transposable to wild marine fish populations with possible consequences for their population dynamics and productivity. Effects on

early survival, growth, reproduction and larval survival in the progeny were specifically investigated.

1.3 Materials and methods

This study was conducted under the approval of the Animal Care Committee of Poitou-Charentes # 84 COMETHEA (France) under project authorization number CE2012-23.

1.3.1 Fish rearing

Experiments were performed with wild type strain TU zebrafish (ZFIN ID: ZDBGENO-990623-3) from a stock kept at the Fish Ecophysiology Platform (PEP - http://wwz.ifremer.fr/pep_eng) originating from the European Zebrafish Resources Center (EZRC, Karlsruhe, Germany). Fish were maintained in heterosexual groups (n = 28 ± 5 individuals per 10 L-tank) under a 14h day/10h night light cycle. The resulting rearing density fell within the recommended range of 1 to 5 individuals/L (Nüsslein-Volhard and Dahm, 2002; Singleman and Holtzman, 2014). Water used in the rearing system was a mixture of approximately 2/3 reverse osmosis water and 1/3 tap water, both being initially treated by sediment and charcoal filters. Water physicochemical parameters were remained constant during the experiment: temperature 27 ± 1°C, conductivity 300 ± 50 µS cm^{-1} and pH 7.5 ± 0.5.

To produce the F0 generation, eggs were obtained by random pairwise mating of zebrafish placed together in spawning boxes the evening before collection (AquaSchwarz, Germany). Eggs from each clutch with a fertilization rate greater than 80% were collected the next morning in a Petri dish containing 30 mL of isotonic mixture E3 (1 L: 17.2 g NaCl, 0.76 g HCl, 2.9 g $CaCl_2$. 2 H_2O, 4.9 MgSO4. 7 H_2O) and placed at 28°C. Twenty-four hours post-fertilization (hpf), eggs from 5 clutches were mixed in a balanced way (taking the same number of eggs from each clutch) and distributed in 20

Petri dishes at a rate of 60 larvae per Petri dish. The 20 groups of 60 larvae were considered together as one replicate population. At 5 days post fertilization (dpf), the groups of 60 larvae were transferred from their Petri dishes to separate 1-L tanks. At 15 dpf, the groups of larvae were transferred to tubes inserted inside separate 10-L rearing tanks disposed on flow-through racks and were then freed into the tanks at 27 dpf (after Vignet et al., 2014). In the flow-through racks, an hourly automated addition of 150 mL of system water resulted in a daily total renewal of one third of the volume of each tank. Discarded water was collected and treated with activated charcoal before being discharged into sewers. Mesh bags filled with zeolite stones (~30 cL) were also added in each tank to guarantee water quality. Tanks were inspected daily and cleaned by siphoning if necessary. Furthermore, tanks were fully emptied and cleaned, together with zeolite bags, monthly from the first biometry at 2 or 3 months age onwards. With this rearing protocol, concentrations of ammonia, nitrites and nitrates measured were always below critical values for zebrafish (Lawrence, 2007) and even below quantification level in most cases.

Fish were fed three times per day, twice with pellets in the morning and the evening, and once with freshly hatched crustaceans (*Artemia salina*) at noon. Food pellet size was adapted to fish's mouth size using their age as a proxy. From 5 to 60 dpf, fish were fed sequentially with 100, 200 and 300 µm SDS (Special Diet Service; Dietex international, United Kingdom) with 5 to 10 days of overlap; from 60 to 70 dpf, fish were fed with a mixture of 300 µm SDS and Inicio^{+} 500 µm (Biomar, France), and from 70 dpf onwards, they were fed with Inicio^{+} 500 µm only.

1.3.2 Fish exposure

Fish were exposed to contaminants through food pellets spiked with a mixture of PCB and PBDE congeners following the food-pellet size depending on age sequence presented above. The spiking procedure was similar for all pellet sizes except for vessel and solution volumes that were of course adapted to the quantity of food to be spiked. PCB and PBDE congeners used in contaminated diet and their concentrations were chosen in order to represent environmental conditions.

For PCBs, the choice was based on the contamination levels and profiles reported in mussels from the Seine estuary, one of the most contaminated site along the French coastlines (Abarnou et al., 2000). This choice was justified by the fact that many exploited fish species, especially flatfish (e.g. sole, plaice, turbot) and demersal fish (e.g. cod, haddock, seabass), feed largely on benthic invertebrates and/or have nursery grounds in industrialized estuaries. More precisely, contaminated food was spiked with a mixture of 22 PCB congeners, i.e., congeners CB-8, CB-18, CB-28, CB-31, CB-44, CB-49, CB-52, CB-77, CB-101, CB-105, CB-110, CB-118, CB-128, CB-132, CB-138, CB-149, CB-153, CB-156, CB-170, CB-180, CB-187 and CB-194 at targeted concentrations between 28 and 280 ng g^{-1} ww (wet weight) per congener. The 22 congeners used covered a wide range of chlorinated substitutions (2-8) and a range of hydrophobicity (log K_{ow} - octanol/water partition coefficient) from 5.07 to 7.80 (Hawker and Connell, 1988).

For PBDEs, targeted contamination levels and profiles were based on the 6 most representative congeners in marine biota and the main congener in marine sediments that were identified for priority action by OSPAR (OSPAR Commission, 2009, 2013). The rationale for this choice is that PBDE congeners are metabolized more easily and quickly than PCB congeners in low trophic level marine organisms (Grimm et al., 2015; Zhang et

al., 2016). As a result, the PBDE congener profiles found in an organism's tissue will depend on the species considered as PBDE metabolism varies between species. It follows that we did not want to define the target profile of PBDE congeners based on measurements on a particular species. More specifically, contaminated food was also spiked with a mixture of 7 PBDE congeners, i.e., congeners BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-183 for marine biota and congener BDE-209 for marine sediments at targeted concentrations of between 10 and 200 ng g^{-1} ww per congener. The reason why BDE-209 was included is that, despite the fact it is not listed among the main congeners in marine biota in general as it disappears at high trophic levels (Burd et al., 2014) and in pelagic biota (Desforges et al., 2014), it is the main PBDE congener found in marine sediments and thus one of the main in benthic invertebrates (together with BDE-47, BDE-99 and BDE-100; (Burd et al., 2014; Dinn et al., 2012). As explained previously, many exploited fish are benthic or demersal species feeding largely on benthic invertebrates, so that it was sensible to include BDE-209 in the mixture tested. The 7 congeners used covered a wide range of brominated substitutions (3-10) and a range of hydrophobicity (log K_{ow}) from 6.7 to 12.1 (Kelly et al., 2008).

The precise targeted and measured concentrations for each PCB and PBDE congener are available in Table 1.S1. The contaminated diet was prepared after dilution of a stock PCB and PBDE solution in isooctane solvent for incorporation as described in Daouk et al. (2011). The control diet was prepared in the same manner, i.e., with isooctane solvent but without addition of POPs. Control and contaminated diets were prepared with food of all sizes.

Fish were fed from their first meal (5 dpf) with either a control diet or a contaminated diet using the feeding schedule described earlier. A total of 5 replicate populations, each

composed of 9 ± 1 tanks per dietary treatment (46 tanks per treatment in total, see Table 1.S3 for details), were used in this study to increase the significance and confidence level of the experimental results. Hereafter, we will refer to fish exposed to the control and the contaminated diet as SOLV (as solvent) and MIX (as mixture) fish, respectively.

1.3.3 PCB and PBDE analyses

PCBs and PBDEs were analysed in all batches of MIX (n = 12) and SOLV diets (n = 16), and in MIX (PCBs: $n_{females} = n_{males} = 3$; PBDEs: $n_{females} = n_{males} = 3$) and SOLV fish (PCBs: $n_{females} = n_{males} = 1$; PBDEs: $n_{females} = n_{males} = 3$) at 180 dpf using the methods described in Daouk et al. (2011) and Munschy et al. (2011), respectively. Briefly, PCBs were extracted using a hot Soxhlet apparatus (Soxtec), purified sequentially with concentrated sulfuric acid and by adsorption chromatography on Florisil column, and analysed using gas chromatography equipped with an electron capture detector (GC-μECD). PBDEs were analysed using Accelerated Solvent Extraction (ASE, Dionex Corp., USA) followed by gel permeation chromatography, silica and alumina column, and concentrated sulfuric acid treatment prior to quantification by gas chromatography coupled to mass spectrometry (GC-MS) in electron capture negative ionisation mode (ECNI). Analyses on fish were done on entire individuals.

1.3.4 Quality assurance/Quality control

1.3.4.1 PCB analysis

The calibration of the system was performed within a large range using a multi-point (6) calibration curve to define the linearity range of our detector (ECD) for all contaminants. The relative precision of the method was checked for this type of samples by the analysis of 6 aliquots of a homogeneous preparation of fish (laboratory control card).

The results showed coefficients of variation of less than 10 % for all congeners, which indicates a satisfactory reproducibility of the method. Detailed information is provided in Supplementary Text 1.S2.

1.3.4.2 PBDE analysis

Quality Assurance / Quality Control procedures were implemented for each batch of 8 to 10 samples, including procedural blanks, use of recovery surrogates in all samples, analysis of certified reference material and participation to inter-comparison exercises. Detailed information can be found in Munschy et al. (2011) and an update is provided in Supplementary Text 1.S2.

1.3.5 Trait monitoring

The following description covers trait monitoring of all replicates at once while some details (number of tanks, precise date of biometries, etc.) may vary between replicates. Such details are given in supplementary Table 1.S3 and corresponding variation is indicated as standard deviation or minimum and maximum values in the main text below.

1.3.5.1 Survival

Early survival of individuals was followed by counting daily the number of live fish in each tank of each replicate from 5 dpf to the juvenile stage (30 ± 6 dpf, mean ± SD; see date for each replicate in Table 1.S3) for all replicates.

Juvenile/adult survival was monitored monthly from 30 ± 6 dpf to 183±4 dpf for all replicates on the occasion of growth monitoring (see below for growth monitoring and Table 1.S3 for precise dates of biometries). As individuals were sampled from various tanks within each replicate during this period for additional analyses but without

keeping the information of the tanks sampled, data had to be aggregated at the replicate level.

1.3.5.2 Growth

Growth of all fish was monitored monthly from 65 ± 10 dpf to between 181 and 362 dpf depending on the replicate (see dates for each replicate in Table 1.S3). On each occasion, individual standard length (mm) and body weight (g) were measured after anesthesia with benzocaine (Vignet et al., 2014). Sex was determined whenever possible based on morphological clues, generally from around 3 months old onwards. All replicates were used for this monitoring.

1.3.5.3 Reproduction test

After 72 ± 11 dpf, reproduction was monitored for 17 ± 7 days by placing two spawning boxes into each rearing tank (see dates for each replicate in Table 1.S3). Eggs were collected the next morning and sorted to count the total number of eggs as well as the number of fertilized ones. The fertilization rate was then calculated for each spawning event. The total number of spawning events obtained relative to solicitation number (equal to the number of inspected rearing tanks per treatment) was calculated for each treatment and replicate. For each tank, sex-ratio S, mean individual food consumption C, number of individuals N, length of females l_f, and length of males l_m were measured and the age a of fish was recorded. In this test, the length of both males and females from each tank was measured on day 30 ± 18 (depending on the replicate) only to avoid repetitive stress during the test. Reproduction monitoring was very time-consuming and due to manpower limitations only a selection of replicates (1, 2 and 4, 28 tanks in total per treatment) was used for this assessment.

1.3.5.4 Larval survival in the progeny

A survival experiment was performed on unfed F1 larvae of replicate 4. The purpose of this experiment was to determine whether the exposure of parents (F0) to PCB and PBDE congeners could affect the survival of their offspring larvae (F1).

Survival of unfed larvae was monitored on two occasions corresponding to the 1st and 10th spawning events of F0 fish. On each occasion, survival was studied on 3 different clutches from 10 rearing tanks per dietary treatment. Thirty eggs were collected from each clutch and kept in a Petri dish in 30 mL of E3 medium at 28°C until the end of the survival experiment. In total, the survival of 900 larvae per treatment and spawning event (1st and 10th) was monitored (n = 30 eggs × 3 clutches × 10 tanks × 2 treatments × 2 dates = 3600 larvae).

1.3.6 Statistical analyses

1.3.6.1 Modeling approach

Statistical analyses were performed in R version 3.2.2 (R Development Core Team, 2015). All traits were analyzed using mixed-effects models. Random effects were used to account for variability due to rearing tanks and/or replication depending on the trait considered: two nested random effects, namely replicates and tanks nested within replicates (replicate/tank), were included in models describing F0 generation traits, except for juvenile/adult survival for which only replicates were included due to data aggregation, and clutches nested within tanks were included in the model describing unfed F1 larvae survival. Random effects affected either the model intercept only or both model intercept and slopes. For each trait, the fixed part of the model included a dietary treatment effect (T, MIX versus SOLV diet) to assess the effect of fish exposure to the mixture of PCBs and PBDEs plus relevant covariates likely to biologically affect the

considered trait (see description below for each trait as well as Table 1.S4 for details on fixed and random effects).

For each trait, the full model was reduced by backward selection in two steps: the random part of the model was reduced first and the fixed part was selected afterwards (Pinheiro and Bates, 2000; Zuur et al., 2009). Selection was based on significance of the effects at a 5% alpha risk threshold determined by likelihood ratio tests (LRT) between nested models while respecting the marginality of the effects. Such LRTs are supposed to follow a χ^2 distribution under the null hypothesis (type II tests; Fox and Weisberg, 2011). Diagnostics based on residuals were used to assess the adequacy of the reduced model and compliance to the underlying assumptions. Variables were transformed whenever necessary to ensure that the residuals followed the assumed error distribution (Table 1.S4). Finally, the effects of treatment T and other active covariates were estimated from the reduced models and their significance was tested by LRT. When the reduced models did not include the treatment effect T, the test provided for this effect correspond to the LRT test during the selection procedure. Only the fixed part of the selected models will be presented in the Results section. Random effects are mere technical parameters included to account for variation due to tanks and replication and thus to obtain robust estimates and significance tests of fixed effects against such variation. Therefore, detailed information on the random part is given in Table 1.S5 in Supplementary Material.

In the following sections, we describe how the fixed part of the full model was defined for each trait.

1.3.6.2 Survival

Time to death at early and juvenile/adult stages was modeled using survival analysis (Therneau and Grambsch, 2000) as the data analyzed here are right-censored data because some individuals were still alive at intermediate sampling dates (individuals sampled for additional analyses) and at the end of the study. More precisely, two mixed-effects Cox proportional hazards models (COXME) were used to estimate how the hazard rate, i.e., the death rate, was affected by the contamination treatment T as a fixed effect (Table 1.S4) during early and juvenile/adult stages. Cox models allow the death rate to be modeled as being affected proportionally (i.e. multiplicatively) by the explanatory variables. In practice, a logarithmic link function is used to allow for a linear predictor of the death rate. The COXME models were implemented using the *coxme* package in R (Therneau, 2015).

1.3.6.3 Growth

Fish growth was modeled according to the Von Bertalanffy growth (VBG) function using a non-linear mixed-effects model (NLME; Pinheiro and Bates, 2000). The VBG equation describes fish length $l(a)$ (mm) according to individual age a (days) as

$$l(a) = l_\infty - (l_\infty - l_0)e^{-k(a-a_0)}$$

where l_∞ is the asymptotic standard length (mm), l_0 is the initial standard length (mm) at age a_0 (days), a_0 is the age at the first biometric measurement and k is the growth rate coefficient (day^{-1}). To ensure well-behaved residuals of the fitted model, the VBG function to the power of 3 was fitted to length data to the power of 3. This is because the VBG function was initially developed to describe growth in mass and that individual

mass is roughly proportional to individual length to the power of 3 (von Bertalanffy, 1938).

Nested within the VBG function, the three parameters of this model, l_0, l_∞, and k were themselves modeled as depending linearly on fixed effects, namely treatment T only for l_0, and treatment T, individual's sex S and their interaction for l_∞ and k, as well as on random effects (replicate/tank; Table 1.S4). l_0 was not modeled as dependent on sex as no sexual length dimorphism was observed at age a_0. The NLME model was implemented using the *nlme* package in R (Pinheiro et al., 2016).

1.3.6.4 Condition

Fish condition, defined as length-specific weight, was modeled via the length-weight allometry $W = al^b$ linearized by log-transformation (Froese, 2006):

$$\log W = \log a + b \log l$$

where W is wet weight (g), l is standard length (mm), $\log a$ is the regression intercept and b is the regression slope. A linear mixed-effects model (LME) was used to analyze how treatment T, individual's sex S and their interaction taken as fixed effects modified this relationship (Table 1.S4). The LME model was fitted using the *nlme* package in R (Pinheiro et al., 2016).

1.3.6.5 Spawning probability

A generalized linear mixed-effects model (GLMM) with binomial error distribution and logit link function was used to analyze the effect of PCBs and PBDEs exposure on spawning probability based on the number of spawning events relative to solicitation number in reproduction tests (i.e. the number of trials to obtain a spawn). Fixed effects

included treatment T, but also age a to assess how spawning probability increased with age, plus their interaction, as well as the mean length of females $\bar{l}_f$, the mean length of males $\bar{l}_m$, and the mean individual food consumption c in the rearing tank as covariates (Table 1.S4). In this analysis, all continuous explanatory variables were standardized, i.e., centered and scaled to unit variance. The GLMM model was fitted using the *lme4* package in R (Bates et al., 2015).

1.3.6.6 Number of eggs

To test whether PCB and PBDE congener mixtures affect reproductive output, the number of eggs produced by each rearing tank during reproduction tests was modeled using an LME model. The fixed effects included in the full-model were treatment T, age a (as clutch size often increases with age in fish), their interaction, plus the mean individual food consumption c, the number of individuals N, and the sex-ratio s in the rearing tank as covariates (Table 1.S4). A Box-Cox transformation was applied to the response variable (eggs number) to obtain a normal distribution of the residuals and all continuous explanatory variables were standardized. The LME model was fitted using the *nlme* package in R (Pinheiro et al., 2016).

1.3.6.7 Fertilization rate

Fertilization rate, obtained as the number of fertilized eggs relative to the total number of eggs in each rearing tank during reproduction tests was analyzed in the same way as spawning probability (see description above).

1.3.6.8 Larval survival in the progeny

As for early survival of the F0 generation, survival of unfed F1 larvae was analyzed using a COXME model. In this case, data were uncensored data as all individuals were dead at

the end of the experiment. The fixed effects included treatment T, occasion O (1st and 10th F0 spawning event) and their interaction.

1.4 Results

1.4.1 PCB and PBDE concentrations in diet and fish

PCB and PBDE concentrations in MIX and SOLV diets are presented in Table 1.S1. In MIX diet (n=12), the total concentrations, i.e., summed across congeners, were 1932.3 ± 90.4 ng g^{-1} ww for PCBs and 479.8 ± 50.8 ng g^{-1} ww for PBDEs. In SOLV diet (n=16), the total concentrations were circa 245 and 522 times lower, i.e., 7.9 ± 3.5 ng g^{-1} ww for PCBs and 0.92 ± 0.36 ng g^{-1} ww for PBDEs.

In MIX fish, the total concentrations of PCBs (n=3) and PBDEs (n=3) at 180 dpf in entire individuals were respectively 2188.3 ± 132.26 and 110.9 ± 1.14 ng g^{-1} ww in females, and 2140 ± 73.95 and 96.4 ± 8.65 ng g^{-1} ww in males. In SOLV fish, the total concentrations of PCBs (n=1) and PBDEs (n=3) at 180 dpf were respectively 26.2 and 1.1 ± 0.31 ng g^{-1} ww in females, and 26.7 and 2.7 ± 0.54 ng g^{-1} ww in males.

1.4.2 Survival

Early survival probabilities at the end of the survival experiment (30 ± 6 dpf) ranged from 47% to 89% and from 51% to 87% in MIX and SOLV fish, respectively (Kaplan-Meier curve; Fig. 1.1). Juvenile/adult survival probabilities at 183 ± 4 dpf ranged from 44% to 73% and from 38% to 70% in MIX and SOLV fish, respectively (Kaplan-Meier curve; Fig. 1.2). No significant effect of the treatment on both early and juvenile/adult death rate was detected (T effect Table 1.1; Fig. 1.1; Fig. 1.2).

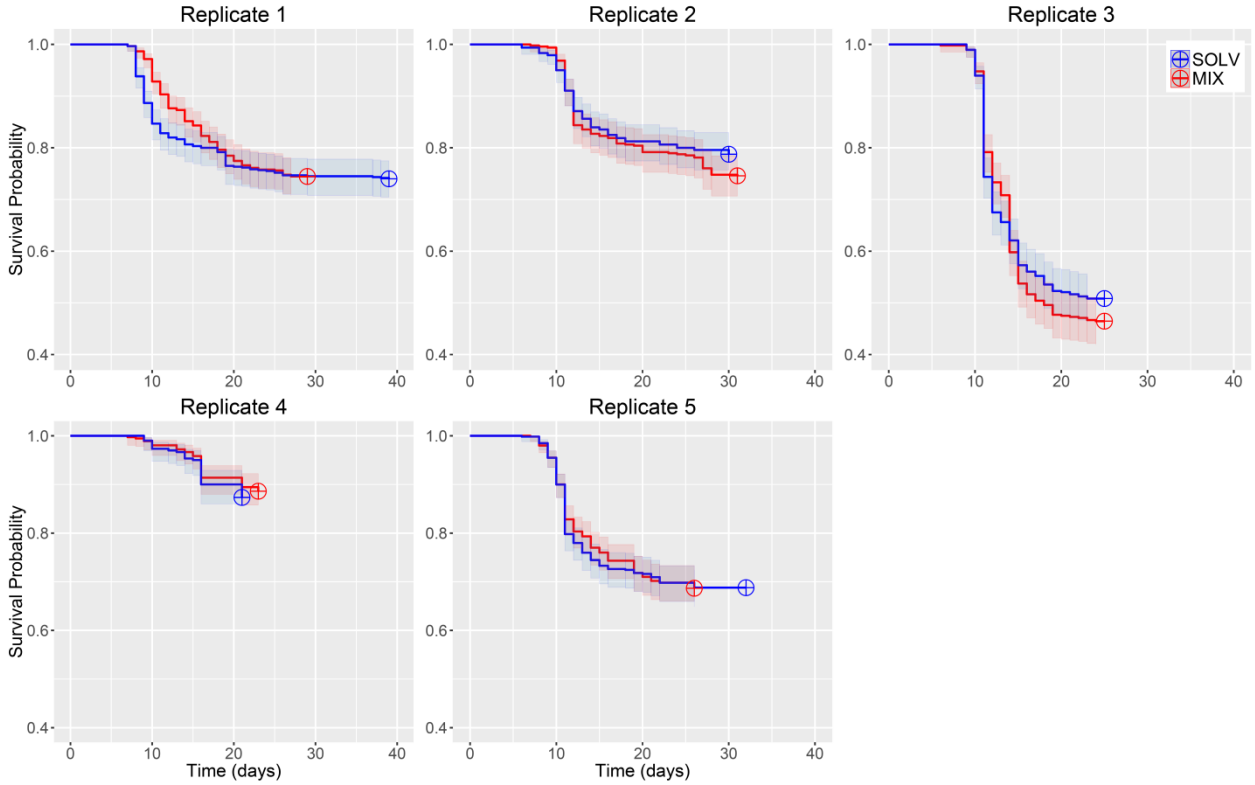


Fig. 1.1. Effect of dietary exposure to a PCB and PBDE congeners mixture on the early death rate of zebrafish. Curves represent the estimated decrease in individuals' survival probability with time by Kaplan-Meier estimator for MIX (red) and SOLV (blue) treatment, respectively, and shaded areas are the corresponding confidence intervals.

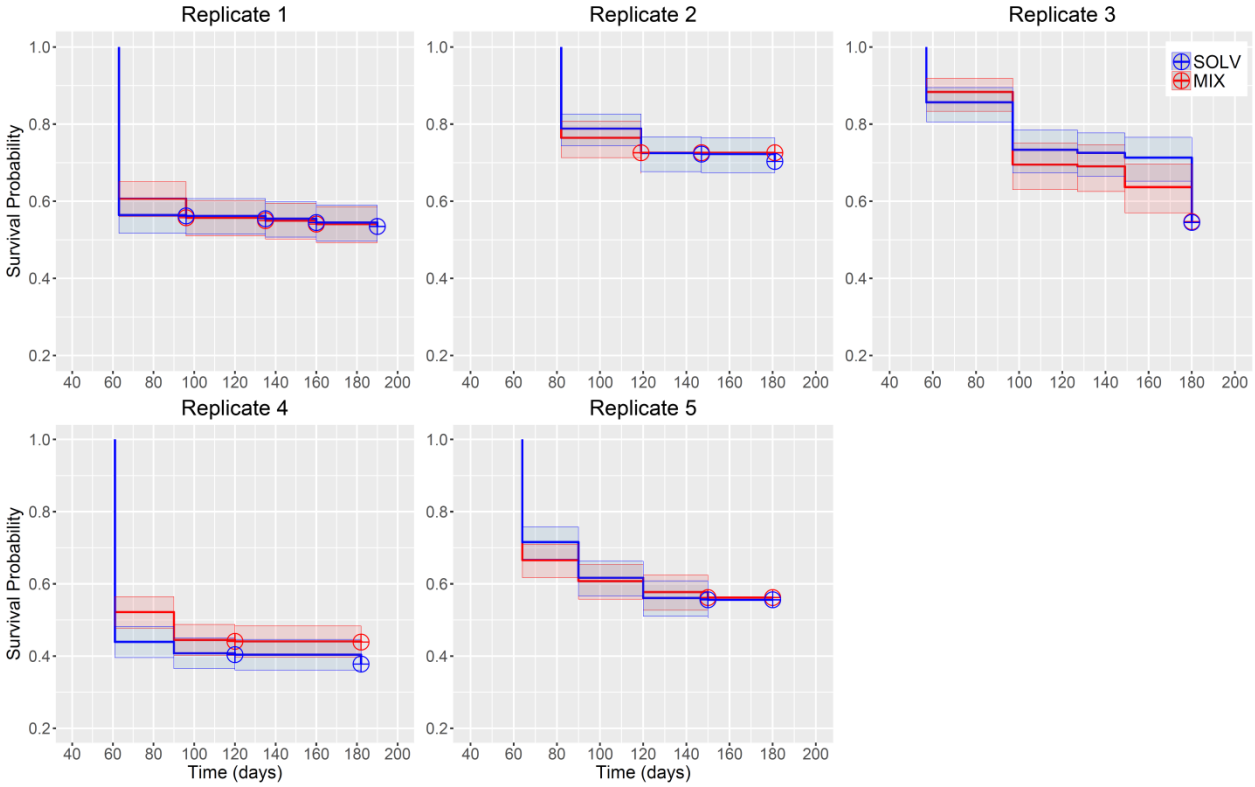


Fig. 1.2. Effect of dietary exposure to a PCB and PBDE congeners mixture on the juvenile and adult death rate of zebrafish. Curves represent the estimated decrease in individuals' survival probability with time by Kaplan-Meier estimator for MIX (red) and SOLV (blue) treatment, respectively, and shaded areas are the corresponding confidence intervals.

Table 1.1. Results of mixed-effects models testing for the effects of dietary treatment (MIX vs. SOLV) and other explanatory variables on the life-history traits of zebrafish. For categorical variables, effects are estimated for one category (indicated between parentheses) relative to the reference one taken as the intercept, namely MIX relative to SOLV for treatment T, and males M relative to females F for sex S.

Function	*Trait*	*Effect*	*Estimate*	*s.e.*	*df*	*χ2*	*p-value*
Survival	Early death rate	baseline hazard (SOLV)	n.a.	n.a.	n.a.	n.a.	n.a.
		T (MIX)	0.034	0.072	1	0.23	0.631
Survival	Juvenile/adult death rate	baseline hazard (SOLV)	n.a.	n.a.	n.a.	n.a.	n.a.
		T (MIX)	-0.087	0.048	1	3.29	0.070
Growth	l_0	intercept (SOLV)	2.946	0.024	n.a.	n.a.	n.a.
		T (MIX)	0.019	0.016	1	1.97	0.160
Growth	k	intercept (F/SOLV)	-4.317	0.119	n.a.	n.a.	n.a.
		S (M)	0.112	0.027	1	19.56	**<0.001**
		T (MIX)	-0.141	0.047	1	6.40	**0.011**
Growth	l_∞	intercept (F/SOLV)	3.527	0.005	n.a.	n.a.	n.a.
		S (M)	-0.067	0.005	1	171.52	**<0.001**
		T (MIX)	0.024	0.006	1	11.35	**<0.001**
Condition	W	Intercept (F/SOLV)	-11.573	0.040	n.a.	n.a.	n.a.
		$\log(l)$	3.247	0.008	1	29057.02	**<0.001**
		S (M)	-0.165	0.003	1	2219.74	**<0.001**
		T (MIX)	-0.010	0.008	1	1.84	0.175
Reproduction	Spawning probability	intercept (SOLV)	0.829	0.238	n.a.	n.a.	n.a.
		a	2.033	0.248	1	127.21	**<0.001**
		$\bar{l}_f$	-0.746	0.228	1	10.70	**0.001**
		$\bar{l}_m$	0.600	0.211	1	8.06	**0.004**
		T (MIX)	-0.464	0.287	1	1.30	0.253
		$T \times a$	-0.924	0.275	1	11.30	**<0.001**
Reproduction	Number of eggs	intercept (SOLV)	10.022	0.294	n.a.	n.a.	n.a.
		a	1.409	0.166	1	72.21	**<0.001**
		N	-0.646	0.193	1	11.29	**<0.001**
		T (MIX)	-0.486	0.403	1	1.46	0.227
Reproduction	Fertilization rate	intercept (SOLV)	-0.175	1.246	n.a.	n.a.	n.a.
		a	0.978	0.478	1	4.19	**<0.041**
		T (MIX)	0.041	0.346	1	0.014	0.906

p-values < 0.05 are in bold.

1.4.3 Growth

Growth data revealed sexual length dimorphism, females being larger than males, and suggested that MIX fish grew to larger lengths than SOLV fish (Fig. 1.3). These observations were confirmed by the VBG model. Males grew at a faster rate k than females but to smaller asymptotic length l_∞ (S effect on both parameters, Table 1.1). More importantly, the VBG model showed that MIX fish grew at slower rate k (-13.00 % for MIX individuals relative to SOLV ones; T effect on parameter k, Table 1.1) but to larger asymptotic length l_∞ than SOLV fish (+2.40 % for MIX individuals relative to SOLV ones; T effect on l_∞, Table 1.1) and that these effects were independent of sex (no significant $T \times S$ interaction was found and it was therefore not kept in the model). In contrast, no significant effect of the treatment was observed on initial length l_0 (T effect, Table 1.1), which is the length at first biometric measurement.

1.4.4 Condition

The length-weight relationship differed significantly across sexes (main S effect, Table 1.1; Fig. 1.4), males being lighter than females at equivalent length (-15.23 % of weight difference at 16, 24 and 32 mm for males). In contrast, there was no significant difference between MIX and SOLV fish, indicating that exposure had no effect on fish condition (main T effect, Table 1.1; Fig. 1.4). Moreover, the absence of interactions between $\log(l)$ and both S and T in the reduced model indicated that the shape of the allometric relationship between length and weight, determined by the exponent $b = 3.247$ ($\log(l)$ effect, Table 1.1), was unaffected by sex or treatment (Fig. 1.4).

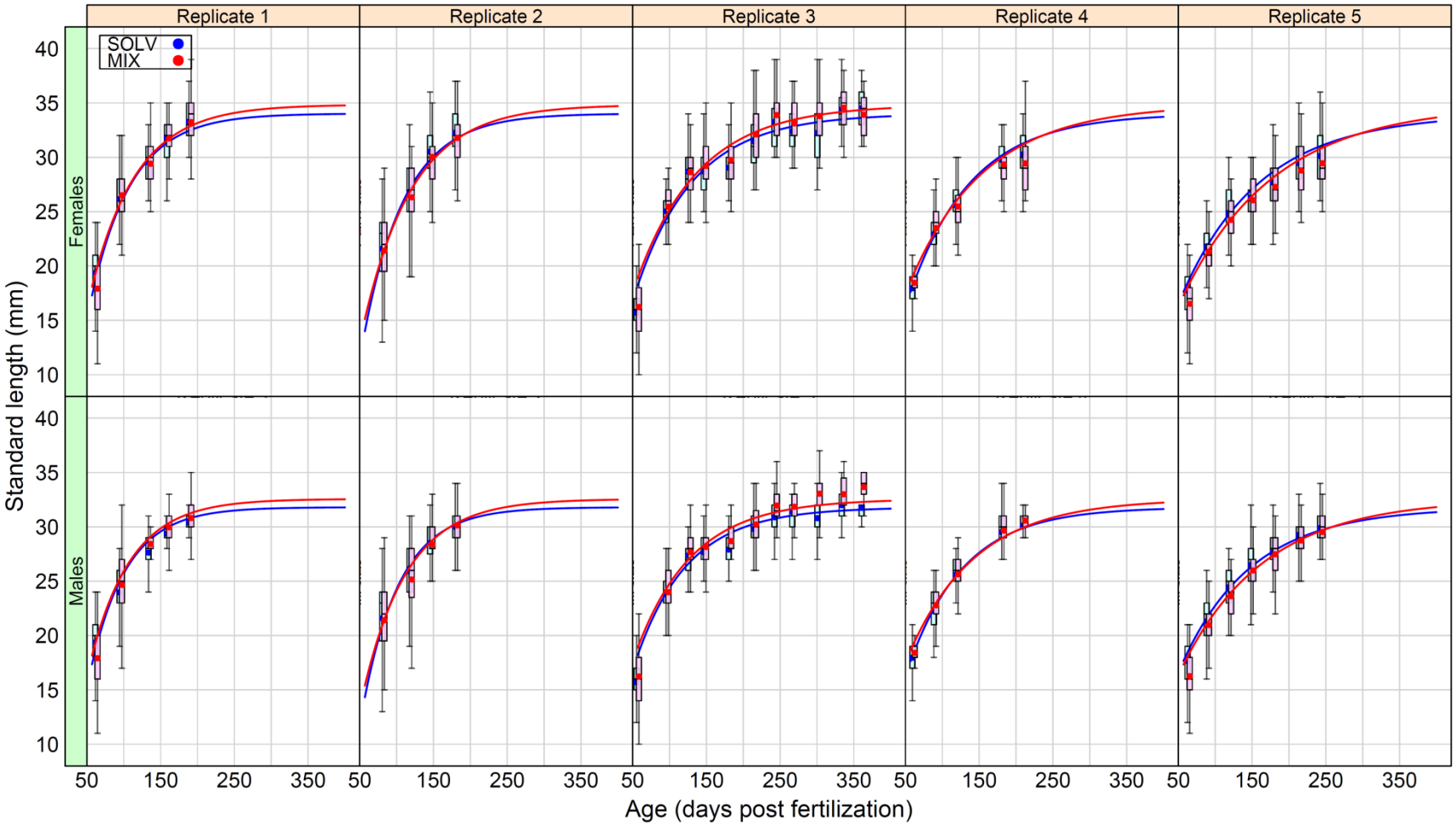


Fig. 1.3. Effect of dietary exposure to a PCB and PBDE congeners mixture on growth (in terms of standard length) in zebrafish. Rows correspond to the sex of fish and columns to the replicates. Boxplots represent observations per treatment (MIX in red and SOLV in blue) and curves represent the fitted model per treatment. For boxplots, the bottom and top of the box are the first and the third quartiles of the data distribution, the horizontal segment and the dot inside the box are the median and the mean, respectively, whiskers represent the most extreme data point within the 1.5 interquartile range.

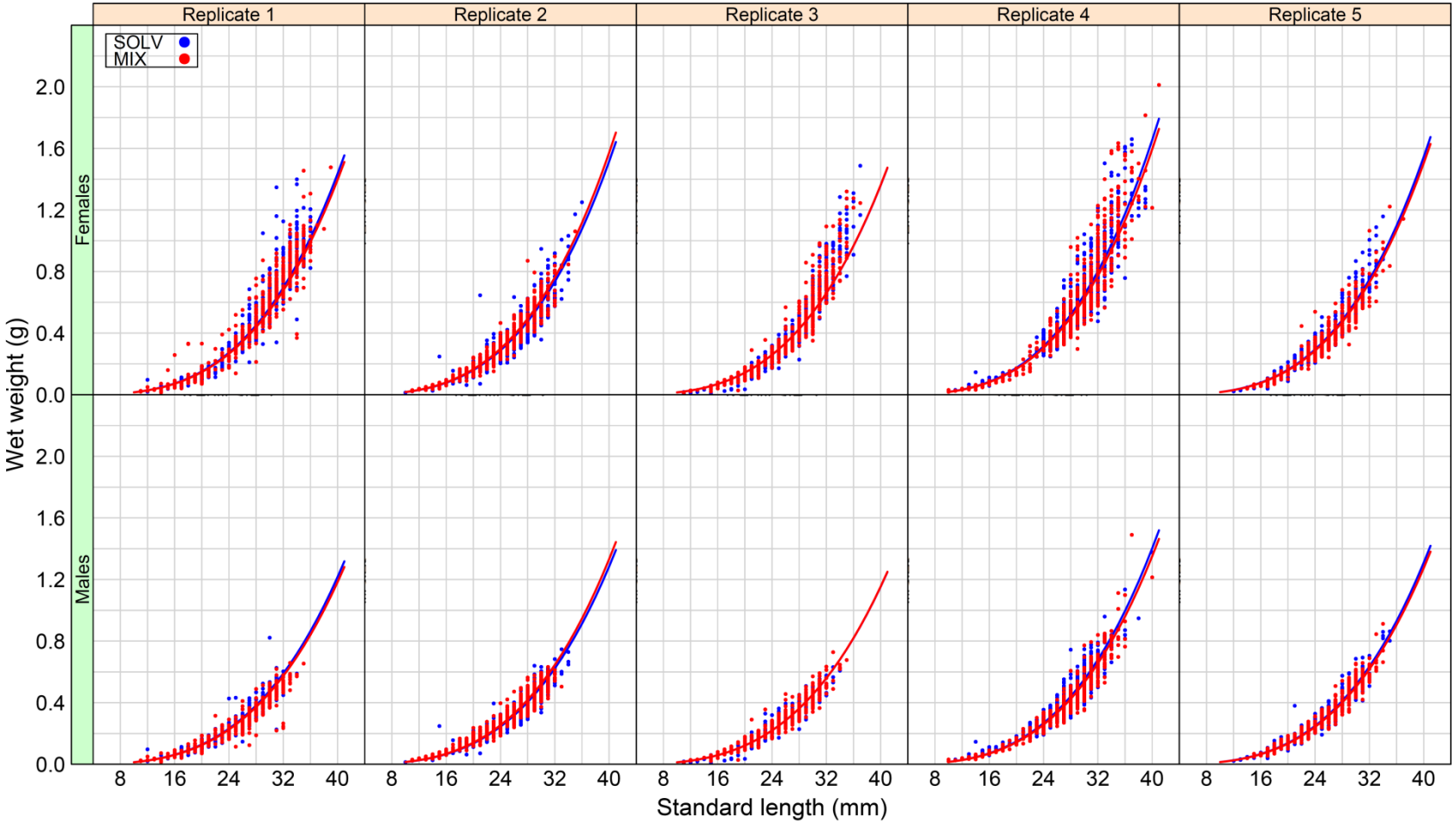


Fig. 1.4. Effect of dietary exposure to a PCB and PBDE congeners mixture on zebrafish body condition. Rows correspond to the sex of fish and columns to the replicates. Dots represent observations per treatment (MIX in red and SOLV in blue) and curves represent the fitted model per treatment.

1.4.5 Spawning probability

Spawning probability increased significantly with age and average length of male and decreased with average length of female (a, $\bar{l}_m$, $\bar{l}_f$, effects, Table 1.1; Fig. 1.5). As mean individual food consumption c had no effect on spawning probability, this covariate was not kept in the reduced model. The overall spawning probability was not affected by exposure to contaminant (no significant T effect, Table 1.1) but there was a significant interaction effect between treatment and age ($T \times a$ effect, Table 1.1) showing that spawning probability in MIX fish increased with age at a slower rate than in SOLV fish (Fig 1.5). Consequently, over the duration of the reproduction test, SOLV fish had a significantly higher likelihood to spawn compared to MIX fish.

1.4.6 Number of eggs

The comparison of the cumulative number of eggs produced by all successful spawning events throughout the reproduction test indicated a similar number of eggs produced by MIX fish (110 ± 82 eggs per female) than by SOLV fish (122 ± 82 eggs per female) (T effect Table 1.1; Fig. 1.5). In contrast, the number of eggs produced increased significantly with age and decreased significantly with the number of individuals in tanks (a and N effects, Table 1.1). Although the number of eggs produced by MIX fish seemed to increase with age faster than for SOLV fish (Fig. 1.5), the interaction between treatment and age was not significant ($T \times a$ effect that was not kept in the model).

1.4.7 Fertilization rate

Fertilization rate increased significantly with age (a effect, Table 1.1; Fig. 1.5) but there was no significant difference between MIX and SOLV fish in terms of both the overall level of fertilization rate and its increase with age (T and $T \times a$ effects, Table 1.1; the latter was not kept in the model; Fig. 1.5).

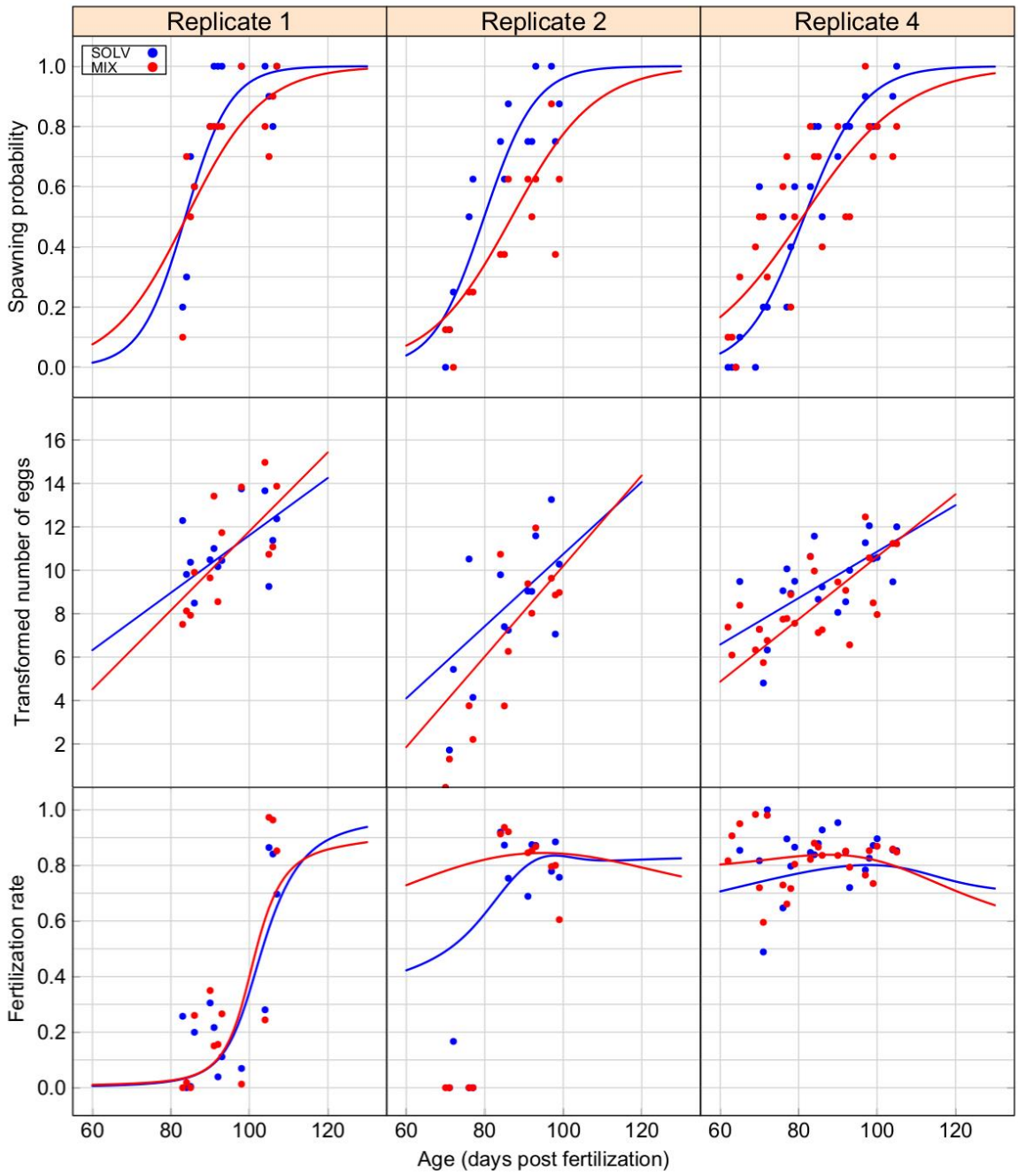


Fig. 1.5. Effect of dietary exposure to a PCB and PBDE congeners mixture on reproduction traits in zebrafish. Rows correspond to the spawning probability, number of eggs (Box-Cox transformed) and fertilization rate, respectively. Traits are represented as functions of age (dpf) in the three replicates (in columns) used for the reproduction test. Dots are for observed reproduction traits in rearing tanks for MIX (in red) and SOLV (in blue) fish and curves represent the fitted model per treatment.

1.4.8 Larval survival in the progeny

The overall level of survival of unfed larvae was unaffected by exposure to contaminants (T effect, Table 1.2; Fig. 1.6), but survival differed significantly according to the

spawning event considered (O effect, Table 1.2) and this difference was dependent on treatment ($T \times O$ interaction effect, Table 1.2). More precisely, survival probability was higher at the 10th F0 spawning event than at the 1st F0 spawning event and this difference was more pronounced for larvae originating from MIX progenitors than from SOLV ones (Table 1.2). This analysis was followed by a post-hoc multiple comparison test (Hothorn et al., 2008) to assess which groups differed from the rest. The test revealed that, on the 1st F0 spawning event, the survival probability of larvae produced by SOLV F0 fish was higher than that of larvae produced by MIX F0 fish (post-hoc test MIX/1st spawn vs. SOLV/1st spawn: Z = -2.55, p-value = 0.021, Fig. 1.6, 1st spawning event) whereas no difference was detected on the 10th spawning event (post-hoc test MIX/10th spawn vs. SOLV/10th spawn: Z = 0.08, p-value = 1, Fig. 1.6, 10th spawning event).

Table 1.2. Analysis of the effects of dietary treatment (MIX vs. SOLV) on zebrafish F1 larval survival in non-feeding conditions by a mixed-effects Cox proportional hazards model. For categorical variables, effects are estimated for one category (indicated between parentheses) relative to the reference one taken as the intercept, namely MIX relative to SOLV for treatment T, and second observation date (10th spawning event) relative to the first one (1st spawning event) for spawning event O.

Trait	***Effect***	***Estimate***	***s.e.***	***df***	***χ2***	***p-value***
Larval survival in progeny	baseline hazard (SOLV/1st spawn)	n.a.	n.a.	n.a.	n.a.	n.a.
	O (10th spawn)	-0.405	0.283	1	30.73	**<0.001**
	T (MIX)	0.986	0.318	1	1.65	0.198
	$T \times O$	-1.053	0.440	1	8.92	**0.003**

p-values < 0.05 are in bold.

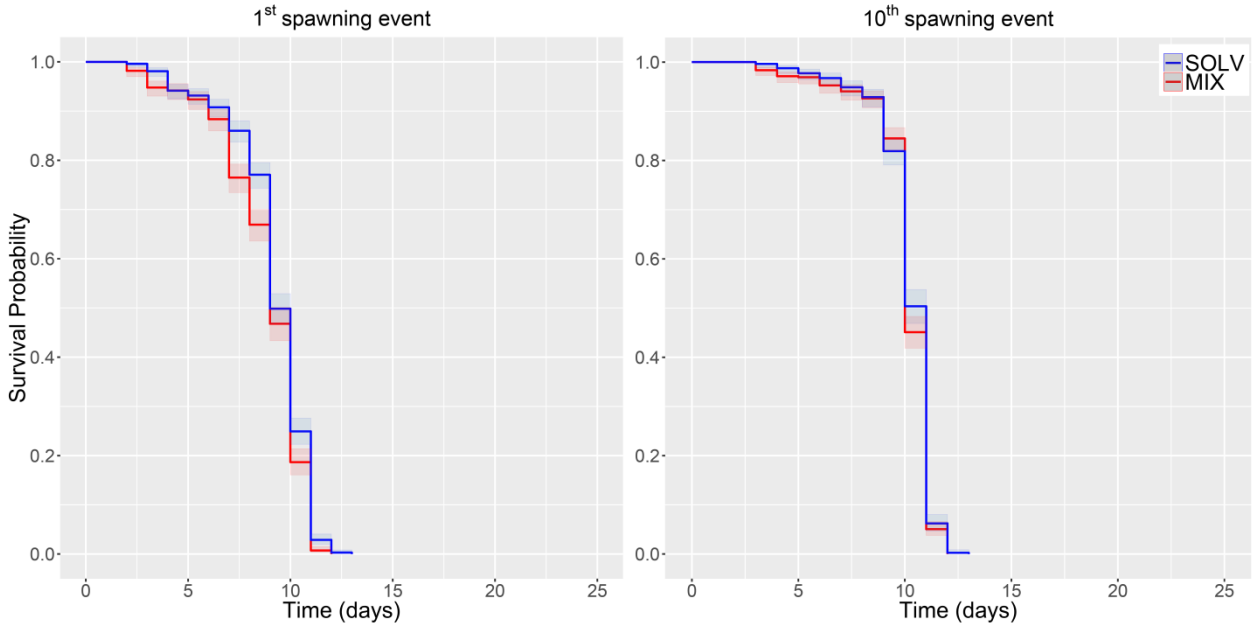


Fig. 1.6. Effect of parental (F0) dietary exposure to a PCB and PBDE congeners mixture on the survival of unfed offspring (F1) larvae in zebrafish. Curves represent the estimated decrease in individuals' survival probability with time by Kaplan-Meier estimator for MIX (red) and SOLV (blue) treatment, respectively, and shaded areas are the corresponding confidence intervals.

1.5 Discussion

1.5.1 PCB and PBDE levels in MIX and SOLV fish

The very low concentration of PCB and PBDE congeners measured in SOLV diet showed that no external or cross-contamination between treatments occurred during diet preparation and exposure experiment. In contrast, MIX diet was actually contaminated with spiking efficiencies ranging from 86 to 130% of targeted concentrations (Table 1.S1). As a result, concentrations of PCB and PBDE congeners measured in both females and males were clearly higher in MIX fish than in SOLV ones (80-85 times and 35-100 times, respectively). PBDE levels in fish at the end of the experiment were in the range of those reported in fish from European industrialized estuaries (Law et al., 2006). PCB levels at the end of the experiment were also in the range of those encountered in the environment (Robinson et al., 2017). These results show the ability of these contaminants to accumulate in fish tissues through trophic contamination and allow us

to assign the observed differences between MIX and SOLV fish life-history traits to the effects of the trophic transfer of the PCB/PBDE mixture.

1.5.2 Effects on survival of exposed fish

No difference in early and juvenile/adult survival was observed between SOLV and MIX fish. Our result on early survival is in accordance with a study describing the effects of exposure to a single congener of PBDE (BDE-47) in zebrafish on roughly the same part of the life-cycle (Chou et al., 2010). PCBs and PBDEs have been shown to accumulate over time in fish tissues in experimental conditions (Daouk et al., 2011; Nyholm et al., 2009). However, as food was spiked at environmental doses, the contamination levels in tissues was relatively low during the entire life cycle, which may explain the absence of acute mortality in MIX fish at any stage. The second point that might explain the absence of a difference in survival between MIX and SOLV fish, specifically at early stage, is that the exposure began at the larval stage from mouth opening (at 5 dpf) and not at the embryonic stage, which has been described as the most critical life-stage (Belanger et al., 2010). Indeed, previous studies in sole showed that exposure to POPs (among which PCBs and PBDEs) at the embryonic stage, i.e. eggs, via the water can lead to a delayed effect on survival during larval stage from mouth opening until metamorphosis (Foekema et al., 2012). Furthermore, Lema et al. (2007) also observed a decrease in zebrafish larval survival after exposure to high concentrations of BDE-47 from embryonic to larval stages. These studies therefore suggest that exposure to POPs at early embryonic stages, as for example through maternal transfer of accumulated pollutants into eggs, may affect larval survival. In the present study, such potential effects were not tested through the analysis of exposed fish early survival, but through the analysis of the larval survival of their progeny (see subsection “Larval survival of exposed fish progeny” below).

1.5.3 Effects on growth of exposed fish

In both sexes, MIX fish grew to larger lengths (asymptotic length L_∞) than SOLV fish but at a slower pace (growth rate coefficient k). This result contrasts with other studies in which long term exposure to POP mixtures were performed. Dietary exposure of zebrafish to mixtures of polycyclic aromatic hydrocarbons (PAHs) produced a decrease in both weight and length (Vignet et al., 2014), the amplitude of which depended on PAHs concentrations and fish's sex. Such reduction in length growth as well as reduction in body condition was also observed after a short exposure via the water of sole juveniles to a PAH mixture at high concentration mimicking an oil spill (Gilliers et al., 2012). In another experiment, the dietary exposure of zebrafish to environmentally realistic freshwater mixtures of POPs (including PCBs and PBDEs as part of the major contaminants identified) produced a significant increase in weight without changes in fish length, i.e., a significant increase in fish condition (Nourizadeh-Lillabadi et al., 2009). Analyses of gene expression indicated disruption of endogenous hormone function, including genes involved in growth regulation (Berg et al., 2011; Lyche et al., 2010, 2011; Nourizadeh-Lillabadi et al., 2009). Taken together, these reports suggest that POP mixtures can increase or decrease growth in length and/or in weight depending on their chemical composition, which is indicative of the triggering of different mechanisms. However, in the above mentioned studies, the ratio of weight to length is either unchanged as in the present study (Vignet et al., 2014), increased (Nourizadeh-Lillabadi et al., 2009) or decreased (Gilliers et al., 2012). Regarding fish condition, our results showed no effect of an environmentally realistic marine mixture of PCBs and PBDEs on fish condition or length-weight relationship, suggesting that weight was affected by exposure only according to its allometric relationship with length. In zebrafish, early growth rate is very important in juveniles and slows down at the time of sexual

maturation (Gómez-Requeni et al., 2010). The higher growth rate in MIX fish may therefore be related to changes in the sexual maturation process, which may be delayed in MIX fish.

1.5.4 Effects on reproduction of exposed fish

Three different aspects of reproduction were evaluated, namely the spawning probability, the number of eggs and the fertilization rate. Spawning probability increased with age faster in SOLV fish than in MIX fish, thus revealing a delay in reproduction in the latter. This result is contradictory with the earlier onset of puberty observed after exposure of zebrafish to a freshwater POP mixture (Nourizadeh-Lillabadi et al., 2009). However, in this earlier study and as previously stated, exposure produced an increase in female condition, which is one important positive driver of maturation and thus spawning probability in fish (Grift et al., 2007; Mollet et al., 2007; Uusi-Heikkilä et al., 2011; Wright, 2007). Numerous studies have demonstrated that POPs may act as endocrine disrupters by disrupting hormone pathways that regulate reproductive functions, leading to a decrease in reproductive success components such as egg production and fertilization rate in fish (reviewed in Mills and Chichester, 2005; Yu et al., 2015). For instance, Muirhead et al. (2006) showed that exposure to BDE-47 caused a significant reduction in mature sperm in fathead minnows, suggesting that PBDEs can affect male reproductive function and reduce male fertility. In the present study, the delayed increase in reproductive output observed in MIX fish could also be explained by such disruption caused by PCBs and PBDEs producing a delay in follicle maturation as this has been shown after exposure to PCBs (Daouk et al., 2011) or PAHs (Vignet et al., 2016).

In the present study, MIX fish produced on average similar eggs per female than SOLV fish. Previous works have shown that PCBs and PBDEs reduce clutch size in zebrafish (Kuiper et al., 2008; Muirhead et al., 2006; Örn et al., 1998). Note however that contaminants were administered at very high doses in these studies unlike in the present study during which the mixture of PCBs and PBDEs was administered at doses representative of the marine environment. Hence, PCBs and PBDEs might have not reached a sufficient concentration in fish tissue to alter clutch size in the present study. For example, Kuiper et al. (2008) observed no effect of BDE-71 (commercial pentabromodiphenylether mixture) on zebrafish egg production at environmentally relevant exposure, but they suspected a decrease in egg production when fish were exposed to higher levels of BDE-71. However, this decrease in egg production was not statistically significant, likewise in the present study. In addition to the number of eggs, several other reproduction traits can be used in order to give more information on realized fecundity or reproductive output. Indeed, realized fecundity can be seen as the combination of spawning probability and clutch size. In the present study, the combination of delayed spawning probability and an unaltered increase in clutch size with age can thus be interpreted as a diminution of realized fecundity at young ages for MIX treatment. Besides fecundity, fertilization rate did not differ between MIX and SOLV fish. This contrasts with previous studies in zebrafish where exposure to environmentally relevant concentrations of PCB congeners via diet (Daouk et al., 2011) or of BDE-71 via water (Han et al., 2013) could reduce fertilization success. However, the effect of a mixture of PCBs and PBDEs may differ from those assessed for a single type of POPs (Daouk et al., 2011) or a single congener (Han et al., 2013) because of potentially differing mechanisms of action between PCBs and PBDEs as well as synergistic and antagonistic effects. In addition, fertilization can be highly variable

within one treatment, which makes mean fertilization rate a poor predictor (Vignet et al., 2016).

Beyond exposure to PCBs and PBDEs, spawning probability and the number of eggs produced could also depend on other factors. Previous studies have shown that the probability of spawning in zebrafish increases with length of female (Paull et al., 2008; Spence and Smith, 2006; Uusi-Heikkilä et al., 2010) and male (Pyron, 2003). The latter is in agreement with our results that showed an increase in spawning probability with average male length that could be explained by female mating preferences for larger males (Pyron, 2003). In contrast, the observed decrease in spawning probability with average female length has never been reported before and seems rather counter-intuitive. However, another aspect that should be taken into account is the fact that fish used for reproduction tests were young adults. In zebrafish, female reach sexual maturity earlier than males (Gonzales, 2012), which is associated to larger weight and condition (Cousin et al., 2012; Gómez-Requeni et al., 2010). Therefore mature females are larger than males of the same age, which was confirmed by our results on growth and condition. In turn, if the preference of females for larger males implies that males should be larger than females themselves, then such choosiness could favor reproduction of the smaller females as they could find larger males more easily, especially in experimental populations where all individuals have the same age.

1.5.5 Larval survival of exposed fish progeny

Maternal transfer of contaminants in general – i.e., heavy metals, organochlorine pesticides, PAHs, PCBs – to offspring has been well documented in many species, including birds (Ackerman et al., 2016; Bargar et al., 2001), amphibians (Metts et al., 2013), and reptiles (Rauschenberger et al., 2007). In fish, several studies have focused

more specifically on the maternal transfer of POPs to eggs, particularly PCBs and PBDEs, because of their high concentrations and widespread occurrence in aquatic environments (e.g. Daouk et al., 2011; Miller, 1993; Niimi, 1983; Nyholm et al., 2008; Yu et al., 2011; Zhang et al., 2010). These compounds have lipophilic properties that provide a route for transfer from a female's stored lipids to offspring through eggs. In other words, PCBs and PBDEs present in a female's body fat are transported to its oocytes via egg yolk lipoproteins derived from vitellogenin (Nyholm et al., 2008; Russell et al., 1999; Ungerer and Thomas, 1996; Zhang et al., 2010). Given such maternal transfer and the documented detrimental effect on larval performances of exposure to POPs at embryonic stage (Foekema et al., 2012, 2014; Grimes et al., 2008; Yu et al., 2011), the elevated mortality observed in unfed larvae descending from exposed fish in the present study was expected.

However, survival probability in unfed larvae descending from MIX fish increased from the 1st to the 10th spawning event so as to become similar to that of larvae originating from SOLV fish. This increase may be related to a gain in egg quality as females age and thus between early and later spawns, which is a common phenomenon in teleost fish (Brooks et al., 1997; Brunel, 2010; Nasiadka and Clark, 2012; Trippel et al., 1997). More precisely, one may hypothesizes that there was a gain in egg quality between the 1st and the 10th spawning event that compensated for the detrimental effect of PCBs and PBDEs transferred from mothers to their eggs. These results suggest that the negative effect of a realistic marine mixture of PCBs and PBDEs on larval survival may be mostly expressed in young females' progeny.

1.5.6 Energy-allocation interpretation and life-history implications

In terms of bioenergetics, life-history traits are considered as resulting from the allocation of limited energy resources acquired through feeding to three main compartments: maintenance, growth and reproduction (Partridge et al., 1991; Perrin and Sibly, 1993; Stearns, 1992). These compartments are supposed to be linked by energy-based trade-offs, so that any increase in resource allocation to one trait should be correlated with a decrease in allocation to the others. This is for example suggested by the slowing-down of growth at the beginning of sexual maturation (Gómez-Requeni et al., 2010). In our study, the increase in individual growth and the decrease in the reproductive output observed in exposed fish can be interpreted in terms of the energetic trade-off between growth and reproduction. In other words, it suggests that the mixture of PCBs and PBDEs altered the allocation of energy between these two compartments, with fish diverting more energy towards somatic growth at the expense of the reproductive function, and hence, MIX fish grew larger than SOLV fish but reproduced later. These results are consistent with the fact that PCBs and PBDEs are known endocrine disruptors favoring obesity (Berg et al., 2011; Lyche et al., 2010, 2011; Nourizadeh-Lillabadi et al., 2009) and impairing reproduction (Mills and Chichester, 2005; Yu et al., 2015), which can be seen as favoring energy investment towards soma against of gonads. These direct bioenergetic effects linked to the suspected endocrine activity of the PCB/PBDE mixture are additional to and independent from the indirect bioenergetics effect on basal metabolism and energy dedicated to maintenance due to detoxification that is often observed in contaminated individuals (Jørgensen et al., 2016; Kumaraguru and Beamish, 1983; Newman and Clements, 2008). The latter is supposed to be relatively weak in our case given that the PCB/PBDE mixture was administered at

low environmental doses and that lethal effects were only observed at larval stage in the progeny.

In contrast to their suspected effect on the energetic trade-off between somatic growth and reproduction, a purely obesogenic effect of the PCB and PBDE mixture seems unlikely. Despite the fact that a variety of environmental endocrine disrupting chemicals such as POPs can cause changes in fat mass and subsequent obesity as it has been observed in fish (Berg et al., 2011; Lyche et al., 2010, 2011; Nourizadeh-Lillabadi et al., 2009), humans (Dirinck et al., 2011; Grün and Blumberg, 2009) and rats (Grün and Blumberg, 2009), the present study shows indeed no difference in condition between MIX and SOLV fish. Still, the present results do not allow rejecting the possibility that these compounds could cause simultaneous but independent changes in body growth and the reproductive function.

Another question is the net effect of these changes in growth and reproduction on individuals' reproductive output throughout the whole life-cycle, and thus their fitness. Given that fecundity is known to increase with length in teleost fish species (Kamler, 2012), it remains to be investigated whether the potential gain in fecundity due to the increase in body growth could (over-)compensate for delayed reproduction and potentially smaller clutch size at a given age. A study based on bioenergetics modeling may help to answer this question and to confirm the alteration of the energetic trade-off between growth and reproduction. Bioenergetic models at the individual level are often used to describe the effects of chemical stressors and their physiological modes of action (e.g. feeding, maintenance, reproduction, growth) (Álvarez et al., 2006; Augustine et al., 2012; Martin et al., 2013). They are also especially well adapted for extrapolating individual effects of stressors to populations (Beaudouin et al., 2015; Jager and Klok,

2010; Kooijman and Metz, 1984; Martin et al., 2013). One of the best-tested and most extensive bioenergetic approaches is the Dynamic Energy Budget theory (DEB) (Kooijman, 2010; Sousa et al., 2010), which describes the rates at which an individual organism acquires energy and utilizes it for three energetic compartments, namely maintenance, growth and reproduction or maturity. Developing such a model to investigate the effects of an environmentally realistic marine mixture of PCBs and PBDEs would allow assessing (i) whether changes in growth and reproduction can be independent or not by testing theoretically different physiological mode of actions and (ii) whether they could compensate each other in terms of consequences on lifetime reproductive output and fitness.

1.5.7 Potential consequences of life-history effects of environmental mixtures of PCBs and PBDEs on fish population dynamics

Contaminant exposure may have serious implications for population dynamics and, consequently, on the structure of ecosystems as shown in many species (Barnthouse et al., 1990; Klok and de Roos, 1996; Munns et al., 1997). However, the effects of POPs on the population dynamics of fish, especially on exploited marine fish, are still poorly investigated. This study shows that chronic dietary exposure to an environmentally realistic marine mixture of POPs can delay reproduction and decrease progeny larval survival under starvation conditions while increasing body growth in zebrafish. We acknowledge that the results reported here using a freshwater species may not fully apply to marine fish, especially when considering the additional metabolic costs due to osmotic pressure maintenance in an hyperosmotic environment. There are however a large number of articles reporting similar toxicity pathways in marine and freshwater fish species for a number of biological functions such as growth (Bodiguel et al., 2009; Daouk et al., 2011), behavior (Gravato and Guilhermino, 2009; Oliveira et al., 2012;

Vignet et al., 2017) and reproduction (Sun et al., 2015; Vignet et al., 2016). These studies therefore support the hypothesis that the present results on a freshwater fish species are indicative of what could happen in marine fish. Under the assumption that these life-history effects can be transposed to marine teleost fish in the wild, they may have detrimental consequences on their population dynamics.

Marine fish population recruitment is known to depend strongly on food availability, especially during the critical period corresponding to the transition from endogenous to exogenous feeding (Hjort, 1914), and on the match or mismatch between larvae emergence timing and that of their prey (Cushing, 1990), which can partly depend on hydrodynamic factors (Cury and Roy, 1989; Lasker, 1978). Starvation episodes during larval stages are common and generally lead to lower recruitment and smaller year classes (Cushing, 1990; Leaf and Friedland, 2014; Lusseau et al., 2014; Pritt et al., 2014). The results of this study on progeny larval survival suggest that exposure to PCBs and PBDEs in the marine environment could amplify the detrimental consequences of starvation on larval survival and the resulting decrease in recruitment level due to maternal transfer. This is in line with the hypothesis that the recruitment of some exploited marine fish species could be impaired due to nursery degradation partly related to the accumulation of PCBs and PBDEs (Gilliers et al., 2006; Riou et al., 2001; Rochette et al., 2010, 2012). Moreover, lower reproductive output because of delayed reproduction may also decrease recruitment although this effect may be partly compensated for by the observed increase in body growth and the associated increase in fecundity due to its dependency on length.

Finally, these life-history effects may also render commercial fish populations more vulnerable to exploitation. Increased growth could increase fishing mortality at young

ages as fisheries select most often larger individuals (Garcia et al., 2012) while delayed reproduction could diminish spawning stock biomass (Enberg et al., 2010; Fiorentino et al., 2008) and lower larval survival could decrease its reproductive output. Moreover, the typical diminution in the average age of the spawning stock due to fishing (Berkeley et al., 2004; Brunel, 2010; Ottersen et al., 2006) could favor the expression of the decrease in larval survival due to the exposure to PCBs and PBDEs documented in the present study, and thus amplify the potential negative effect on total reproductive output. As a result, population growth rates of exploited fish could diminish under exposure to POPs so that they may sustain lower exploitation levels and produce lower fishing yields.

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1.8 Supplementary material

Table 1.S1. Diet composition. List of PCB and PBDE congeners used in this work along with the chlorine and bromine numbers. Targeted and measured congeners concentrations (ng g-1 ww) in MIX (n = 12) diet are indicated (mean ± SD), as well as the spiking efficiency. In the SOLV diet (n=16), the concentration of each congener is also indicated. n.a.: not available. LOD: limit of detection.

Congener	Br/Cl	MIX diet					SOLV diet			
		Targeted concentration (ng g^{-1})	Measured concentration (ng g^{-1})			Spiking efficiency (%)	Measured concentration (ng g^{-1})			Occurrence (n)
CB-8	2	28	27.20	±	1.21	97.1	0.20	±	0.05	3
CB-18	3	38	32.70	±	1.37	86.1	0.60	±	0.28	4
CB-28	3	75	69.00	±	3.35	92	0.30	±	0.07	5
CB-31	3	42	47.40	±	1.74	112.9	0.30	±	0.05	4
CB-44	4	65	66.50	±	1.83	102.3	0.40	±	0.23	4
CB-49	4	54	53.20	±	4.67	98.5	n.a.		n.a.	n.a.
CB-52	4	65	64.10	±	2.76	98.6	0.20	±	0.04	6
CB-77	4	30	31.80	±	1.54	106	n.a.		n.a.	n.a.
CB-101	5	150	151.90	±	6.47	101.3	0.90	±	0.48	8
CB-105	5	76	78.90	±	5.43	103.8	0.40	±	0.18	8
CB-110	5	166	170.60	±	7.03	102.8	0.60	±	0.19	8
CB-118	5	110	107.30	±	3.05	97.5	0.60	±	0.16	8
CB-128	6	37	32.60	±	1.30	88.1	0.20	±	0.07	6
CB-132	6	71	68.70	±	6.46	96.8	0.20	±	0.11	5
CB-138	6	207	199.60	±	4.94	96.4	0.60	±	0.30	8
CB-149	6	158	161.70	±	2.47	102.3	0.80	±	0.24	8
CB-153	6	280	270.40	±	14.24	96.6	0.60	±	0.42	8
CB-156	6	38	34.10	±	3.71	89.7	0.10	±	0.01	7
CB-170	7	71	69.10	±	4.76	97.3	<LOD		-	8
CB-180	7	130	129.30	±	5.55	99.4	0.30	±	0.12	8
CB-187	7	35	32.60	±	2.91	93.1	0.60	±	0.45	4
CB-194	8	35	33.60	±	3.56	96	<LOD		-	4
Sum PCBs		1991	1932.30	±	90.35	97.05	7.90		3.45	
BDE-28	3	9.95	10.78	±	0.60	108.3	0.03	±	0.01	5
BDE-47	4	49.97	49.93	±	2.91	99.9	0.35	±	0.13	16
BDE-100	5	14.94	15.95	±	1.19	106.8	0.09	±	0.04	16
BDE-99	5	30.25	30.25	±	1.63	100.0	0.11	±	0.07	16
BDE-153	6	9.74	10.18	±	0.56	104.5	0.03	±	0.004	6
BDE-183	7	100.48	130.20	±	12.65	129.6	0.14		-	1
BDE-209	10	195.73	232.53	±	31.27	118.8	0.17	±	0.11	16
Sum PBDEs		411.1	479.82	±	50.81	116.7	0.92	±	0.36	

1.S2 text

Quality assurance/Quality control

PCB analysis

The calibration of the system was performed within quite a range using a multi-point (6) calibration curve to define the linearity range of our detector (ECD) for all contaminants, for example from 4 to 2000 $pg.\mu l^{-1}$ for CB-153. The relative precision of the method was checked for this type of samples by the analysis of 6 aliquots of a homogeneous preparation of fish (laboratory control card). The results showed coefficients of variation of less than 10 % for all congeners, which indicates a satisfactory reproducibility of the method. During the analysis of the real samples, analytical blanks were systematically measured every 10 samples. The blank were about 0.2 $pg.\mu l^{-1}$, 20 times less than the concentrations of the lowest standard of CB-153 (i.e. <0.1 $ng.g^{-1}$), and much less for other determinants. Surrogate recoveries were 86 ± 6% for CB-30, 97 ± 8% for CB-198 and 102 ± 9% for CB-209 (mean ± SD calculated on n = 93 analyses) and no surrogate correction was applied for the reported concentrations.

Finally, 6 replicates of a Standard Reference Material, SRM2977 (mussel tissue) were analysed in order to determine the accuracy and precision of the method. PCB recoveries varied between 77 and 115%. The concentrations of the real samples were not corrected for recoveries. Moreover, the RSD values ranged from 3.8 to 19.6%, with a mean of 9.7% for all PCBs. All these results were in agreement with certified reference.

The limit of quantification (LOQ) was estimated taking into account a signal to noise ratio of 3, the injection volume (1µl), the volume of the concentrated extract before injection (250 µl) and the extracted sample mass. Average value varied between 2 to 15 pg depending to congener.

PBDE analysis

Quality Assurance / Quality Control procedures were implemented for each batch of 8 to 10 samples, including procedural blanks, use of recovery surrogates in all samples, analysis of certified reference material and participation to inter-comparison exercises. Detailed information can be found in Munschy et al. (2011) and an update is given below.

Procedural blank samples were analysed in every batch of 8 to 10 samples using glass powder, extracted and processed in the same manner as the fish samples. Blanks were contamination-free, with the exception of BDE-209, which was found at 0.5 ± 0.3 pg injected (average value calculated on 14 blanks). BDE-209 concentrations were corrected from blank values in every sample. The values obtained in each blank were used to correct the values in samples analysed in the same batch.

The limit of quantification (LOQ) was calculated for each sample taking into account a signal to noise ratio of 3, the injection volume, the volume of the concentrated extract before injection and the extracted sample mass. Average value was 0.09 ± 0.05 ng g^{-1} ww (n = 93).

Surrogate recoveries were 88 ± 8% for BDE-139 and 77 ± 17% for ^{13}C BDE-209 (mean ± SD calculated on n = 93 analyses) and no surrogate correction was applied for the reported concentrations.

Certified reference material (WMF-01, supplied by Wellington laboratories Inc., Ontario, Canada) was routinely included in each sequence of samples and the results were within ± 2 times the standard deviations of the means of the certified values. WMF-01 consists of a freeze-dried fish sample for which certified or indicative values are given for 7 congeners (namely, BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183) at concentrations of between 0.53 ± 0.40 ng g^{-1} dry weight (dw) for BDE-183 and 123.2 ± 24.8 ng g^{-1} dw for BDE-47.

The laboratory regularly takes part in Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) inter-comparison exercises for PBDEs in biota and our Z-scores are satisfactory, i.e., between -2 and +2 (for example, they were between -0.28 and -0.77 in 2015 and between -0.83 and + 0.32 in 2016).

Table 1.S3. Trait monitoring protocol for each replicate.

				Survival monitoring				Growth monitoring		Reproduction monitoring		
Replicates	Birth date	Number of tanks		Early		Juvenile/adult		Number of biometries	Ages (dpf) at biometry	Starting date (age in dpf)	End date	Duration (days)
		SOLV	MIX	Starting date (5 dpf)	End date (age in dpf)	Starting date (age in dpf)	End date (age in dpf)					
R1	19/02/2014	10	10	24/02/2014	30/03/2014 (39)	30/03/2014 (39)	28/08/2014 (190)	5	63, 96, 135, 160, 190	13/05/2014 (83)	06/06/2014	13
R2	12/03/2014	8	8	17/03/2014	12/04/2014 (31)	12/04/2014 (31)	09/09/2014 (181)	4	82, 119, 147, 181	21/05/2014 (70)	19/06/2014	14
R3	01/10/2014	9	8	06/10/2014	26/10/2014 (25)	26/10/2014 (25)	30/03/2015 (180)	11	57, 97, 127, 149, 180, 210, 240, 268, 302, 335, 362	-	-	-
R4	26/11/2014	10	10	01/12/2014	19/12/2014 (23)	19/12/2014 (23)	27/05/2015 (182)	5	61, 90, 120, 182, 211	27/01/2015 (62)	11/03/2015	25
R5	01/04/2015	10	10	06/04/2015	03/05/2015 (32)	03/05/2015 (32)	28/09/2015 (180)	7	64, 90, 120, 150, 180, 215, 244	-	-	-

Table 1.S4. Statistical analyses and models used in this study. COXME: mixed-effects Cox proportional hazards model; NLME: non-linear mixed-effects model; LME: linear mixed-effects model; GLMM: generalized linear mixed-effects model. The following symbols are used in formulae for fixed effects : T dietary treatment, S individual's sex, l individual's length, a individual's age, $\bar{l}_f$ average female length in the tank, $\bar{l}_m$ average male length in the tank, C mean individual food consumption in the tank, N number of individuals in the tank, S sex-ratio in the tank, O number of the spawning event.

Generation	Function	Trait	Replicate	Model	Distribution	Link function	Variable transformation	Formula (fixed-effects)	Random effects	Random effects sources
F0	Survival	Early death rate	All	COXME	Semi-parametric	Log	Identity	T	Intercept	replicate/tank
F0	Survival	Juvenile/adult death rate	All	COXME	Semi-parametric	Log	Identity	T	Intercept	replicate
F0	Growth	Initial length l_0	All	NLME	Gaussian	Identity	Identity	T	Intercept	replicate/tank
F0	Growth	Growth rate k	All	NLME	Gaussian	Identity	Identity	$S+T+S\times T$	Intercept	replicate/tank
F0	Growth	Asymptotic length l_∞	All	NLME	Gaussian	Identity	Identity	$S+T+S\times T$	Intercept	replicate/tank
F0	Condition	Weight	All	LME	Gaussian	Identity	Log	$S+T+S\times T+\log(l)+\log(l)\times T+\log(l)\times S$	Intercept	replicate/tank
F0	Reproduction	Spawning probability	R1, R2, R4	GLMM	Binomial	Logit	Identity	$a+\bar{l}_f+\bar{l}_m+c+T+T\times a$	Intercept and slope ($a+T$)	replicate/tank
F0	Reproduction	Number of eggs	R1, R2, R4	LME	Gaussian	Identity	Box-Cox (λ=0.3)	$a+c+N+s+T+T\times a$	Intercept and slope ($a+T$)	replicate/tank
F0	Reproduction	Fertilization rate	R1, R2, R4	GLMM	Binomial	Logit	Identity	$a+\bar{l}_f+\bar{l}_m+c+T+T\times a$	Intercept and slope ($a+T$)	replicate/tank
F1	Larval survival	Larval death rate	R4	COXME	Semi-parametric	Log	Identity	$O+T+T\times O$	Intercept	tank/clutch

Table 1.S5. Random effects testing using Likelihood Ratio Test (LRT).

Generation	*Function*	*Trait*	**Random effects**	**Random effects sources**	*df*	*χ2*	*p-value*
F0	Survival	Early death rate	Intercept	Replicate	1	82.53	**<0.001**
				Replicate/tank	1	21.11	**<0.001**
	Survival	Juvenile/adult death rate	Intercept	Replicate	1	259.79	**<0.001**
F0	Growth	l_0	Intercept	Replicate	1	37.57	**<0.001**
				Replicate/tank	1	107.68	**<0.001**
	Growth	k	Intercept	Replicate	1	99.02	**<0.001**
				Replicate/tank	1	8.76	**0.003**
	Growth	l_∞	Intercept	Replicate	1	4.54	**0.033**
				Replicate/tank	1	60.28	**<0.001**
F0	Condition	W	Intercept	Replicate	1	100.24	**<0.001**
				Replicate/tank	1	243.18	**<0.001**
F0	Reproduction	Spawning probability	Intercept and slope ($a + T$)	Replicate	6	1.06	0.983
				Replicate/tank	6	32.65	**<0.001**
F0	Reproduction	Number of eggs	Intercept and slope ($a + T$)	Replicate	6	3.51	0.742
				Replicate/tank	6	16.27	**0.012**
F0	Reproduction	Fertilization rate	Intercept and slope ($a + T$)	Replicate	6	66.39	**<0.001**
				Replicate/tank	6	6150.70	**<0.001**
F1	Larval survival	Larval death rate	Intercept	Tank	1	334.48	**<0.001**
				Tank/clutch	1	1007.20	**<0.001**

p-values < 0.05 are in bold.

2. CHAPITRE 2 : Chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs affects the energetic trade-off between growth and reproduction as well as reproduction costs in fish

2.1 Introduction du chapitre

Les approches de modélisation, en particulier les modèles bioénergétiques, sont souvent utilisées pour relier les traits d'histoire de vie aux paramètres environnementaux et aux facteurs de pressions comme les polluants. L'un des cadres théoriques de la bioénergétique individuelle les mieux éprouvés et les plus génériques est la théorie du Budget Energétique Dynamique (Dynamic Energy Budget, DEB).

L'objectif de ce chapitre était d'identifier, sur la base d'un modèle bioénergétique, les modes d'action physiologique du mélange de PCB et de PBDE pouvant expliquer les résultats expérimentaux observés dans le chapitre 1 en termes de réponses des différents traits d'historie de vie.

Dans un premier temps, un modèle DEB a été calibré pour chaque traitement sur la base des données expérimentales (reproduction et croissance) et les paramètres estimés ont été ensuite comparés entre individus exposés et contrôles. Ensuite, les différents modes d'action physiologiques du mélange de PCB et de PBDE, c'est-à-dire, les paramètres bioénergétiques altérés, ont été identifiés par cette procédure de calibration, puis validés de façon croisée par comparaison des patrons de changement des traits d'histoire de vie observés et ceux prédits pour chacun des modes d'actions possibles. Enfin, des mesures des hormones stéroïdes sexuelles ont été réalisées afin d'approfondir notre interprétation des résultats de la modélisation bioénergétique.

Chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs affects the energetic trade-off between growth and reproduction as well as reproduction costs in fish

Functional Ecology, in preparation

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Abstract

Persistent organic pollutants (POPs) gather a wide number of chemicals which are of great concern because of their persistence, bioaccumulation and toxicity. Among POPs, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were shown to affect fish physiological traits. Their toxicity and underlying physiological modes of action (PMoA), at the individual level, have been studied through experimental exposure. However, in most cases, single congener and/or high concentrations are used while studies approaching environmental situations are scarce. Modeling approaches, in particular bioenergetic models are often used to infer links between the effects of chemical stressors and their PMoA at the individual level and their consequences at the population level. One of the best-tested and most extensive bioenergetic approaches is the Dynamic Energy Budget theory (DEB). In the present study, we used DEB theory to study the PMoA of an environmentally relevant mixture of PCB and PBDE congeners using data on growth and reproductive life-history traits in experimental control and exposed zebrafish populations. Exposure was conducted through diet from the first meal and throughout the life cycle of the fish. A DEB model was calibrated for each treatment. Then, estimated parameters of the DEB models were compared between control and exposed fish and various PMoAs were tested by pattern comparison. We found that the PMoAs of the PCB and PBDE mixture were through an increase of the fraction κ of energy allocated to somatic maintenance and growth at the expense of that dedicated to maturation and reproduction and an increase of the cost of an egg E_0. The shift of the energetic trade-off between growth and reproductive function due to the fraction κ was confirmed by the observed increase in growth (benefits) and a decrease in reproduction output (costs), which are related to this parameter. No confounding effect was observed on the other DEB parameters in exposed fish. Given the strong dependency of population dynamics on life-history traits, such individual-level effects of PCBs and PBDEs could affect fish population recruitment and dynamics and associated fisheries productivity for commercial species.

Keywords: zebrafish, physiological modes of action, Persistent Organic Pollutants, ecotoxicology, energetic trade-off.

2.2 Introduction

Ecological risk assessment of exposure to contaminants has become an important topic as a result of the potential environmental hazard of contaminants on natural populations (Barnthouse et al., 2008; Forbes and Calow, 2002). Over the last decades, persistent organic pollutants (POP), especially polychlorinated biphenyls (PCB) and polybrominated diphenyl ethers (PBDE), have received increasing attention because of their toxic effects in wildlife, their long-term persistence and elevated lipophilicity (Mizukawa et al., 2009). Their long-term persistence and elevated lipophilicity promote their bioaccumulation and biomagnification through trophic transfer in most biotic compartments, notably in marine ecosystems (e.g. mollusks, fish, seals; Couderc et al., 2015; Johansson et al., 2006; Letcher et al., 2009). This can pose a risk to animals' fitness but also to their population dynamics through the scaling up of PCBs' and PBDEs' individual-level effects to the population-level (Vasseur and Cossu-Leguille, 2006). The decline of some estuarine and coastal marine fish populations has been partly attributed to sublethal doses of chemicals (Matthiessen and Law, 2002; Rochette et al., 2010) although it is always difficult to disentangle the contribution of pollutants relative to the numerous other abiotic and biotic factors affecting fish population dynamics (Hamilton et al., 2015).

Assessing and/or predicting the effect of contaminants on demographic processes at the population-level requires to know how individual-level physiological effects of chemicals can be translate in terms of demographic or life-history traits such as survival at various life-stages, growth, age and size at sexual maturity, fecundity, or gamete and embryo quality (Hamilton et al., 2015). In most cases, these individual-level physiological and life-history responses are documented through experiments in controlled conditions which have the advantage of removing confounding factors.

Experimental exposure of fish to PCBs and PBDEs has notably been shown to alter the immune system, the hepatic and renal functions, the behavior and some life history traits such as growth and reproduction (Berg et al., 2011; Daouk et al., 2011; Han et al., 2011, 2013, Lyche et al., 2010, 2011; Muirhead et al., 2006; Yu et al., 2015). Some of these effects may be due to their endocrine disruptor properties, indeed there are some evidences, that PCBs and/or PBDEs are able to affect synthesis, transport and excretion of hormones (Legler and Brouwer, 2003; Safe et al., 2001; Yang et al., 2005). Nevertheless, experiments use rarely chronic exposure to environmentally realistic chemical mixtures and most often focus on acute exposure to a single molecule or a single type of molecules, which renders extrapolation to natural situations difficult. However, in a recent paper, Horri et al. (2018) showed that chronic dietary exposure of zebrafish to a mixture of PCB and PBDE that is mimicking the marine environment in terms of congener profile and concentrations favored growth to a larger size, decreased fecundity via a delay in spawning probability and reduced larval survival in starved offspring issued from early spawning events.

Ecological modeling can be a powerful tool for predicting how individual-level effects of contaminants on life-history traits can be translate in terms of population dynamics (e.g. Beaudouin et al., 2015; Kooijman and Metz, 1984; Martin et al., 2013). Extrapolation to the population-level however depends critically on a mechanistic understanding and modelling of the effect of contaminants on individual life-history traits. In general terms, the mode of action of a chemical is defined as the category of biological processes by which it induces a particular alteration in the biological endpoints of interest such as reproduction, growth or survival (Schlosser and Bogdanffy, 1999). Bioenergetic models at the individual level, that describe individuals' mass and energy acquisition and utilization while respecting conservation laws, provide an efficient way to identify

physiological modes of action (PMoAs) of chemical stressors, i.e., their effects on various energy fluxes related to different physiological processes (e.g. feeding, maintenance, reproduction, growth), using toxicity data on various endpoints such as life-history traits (Álvarez et al., 2006; Augustine et al., 2012; Jager et al., 2004, 2007; Martin et al., 2013). Besides their explanatory power, bioenergetic models, once calibrated, can be used to predict the effect of toxicants on life-history traits and are thus especially well adapted for extrapolating individual effects of chemical stressors to populations (Beaudouin et al., 2015; Jager and Klok, 2010; Jager et al., 2014; Kooijman and Metz, 1984; Martin et al., 2013).

One of the best-tested and most extensively applied bioenergetic approaches is the Dynamic Energy Budget theory (DEB) (Kooijman, 2010; Sousa et al., 2010), which describes the rates at which an individual organism acquires energy throughout its life history and utilizes it for maintenance, growth, development and reproduction (Fig. 2.1). This framework allows relating life-history traits (survival, growth, age and size at life-history transitions, and reproduction) to environmental parameters (e.g. food availability, temperature) and stressors (e.g. contaminants) through energy allocation. In the context of bioenergetic models and DEB theory, chemical's PMoAs are defined as changes in one or several energetic parameters resulting in the changes in energy allocation patterns revealed by the response of different endpoints (Álvarez et al., 2006). Six broad categories of potential PMoAs of chemicals have been studied or proposed in the context of DEB theory (Fig. 2.1): a decrease in energy acquisition from food either through a reduction of feeding (1) or assimilation capability (1'), an increase in maintenance costs of soma (2) or maturity (2'), an increase in growth costs (3), an increase in reproduction costs (4), a change in the allocation of energy between growth and reproduction (5) and an increase in embryo mortality (6) (Álvarez et al., 2006; Jager

et al., 2006; Kooijman and Bedaux, 1996; Martin et al., 2013). Depending on the PMoA, one (PMoAs 2, 2', 3, 4 and 6) or several (PMoAs 1, 1' and 5) energetic compartments and corresponding life-history traits may be affected (Fig. 2.1) and the patterns of change in life-history traits under exposure to a chemical can thus be used to infer back the PMoA at play.

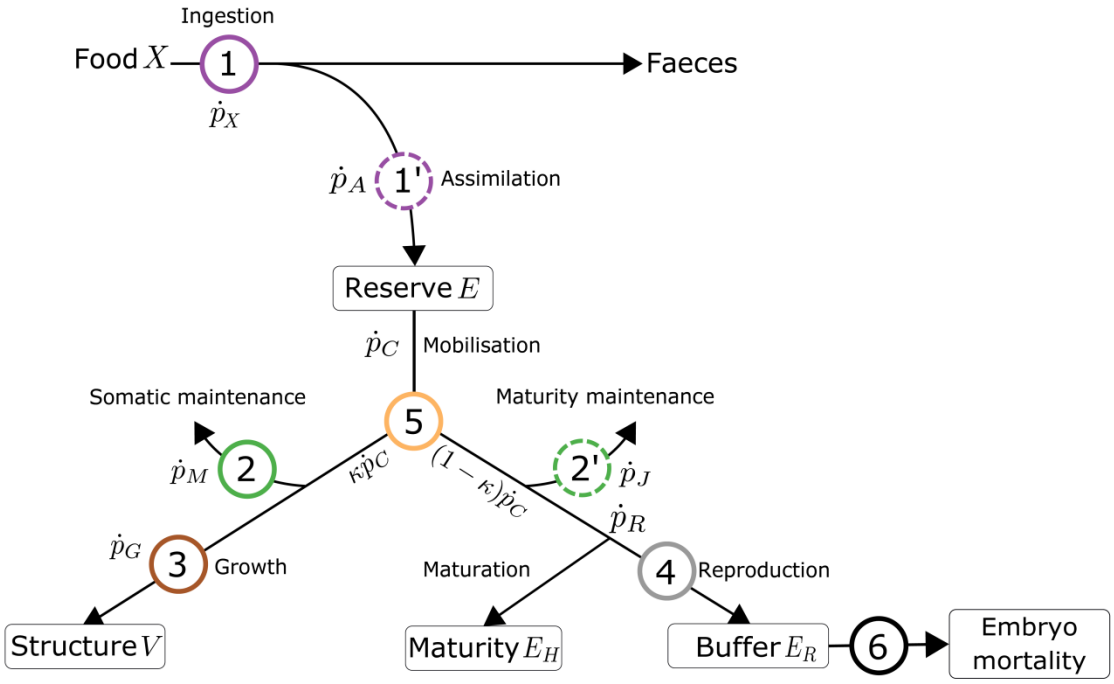

Fig. 2.1. Schematic representation of individual-level bioenergetics and of potential chemicals' PMoAs according to the standard DEB model. The standard DEB model describes how part of the food X is ingested (at rate $\dot{p}_X$) and then assimilated (at rate $\dot{p}_A$) for storage into reserves E while the remainder is rejected as faeces. Reserves are then mobilized (at rate $\dot{p}_C$) and allocated according to a fixed fraction (i) κ to growth (at rate $\dot{p}_G$) of structure V and its maintenance (at rate $\dot{p}_M$) and (ii) $1-\kappa$ to increase (at rate $\dot{p}_R$) in maturity E_H before puberty or in reproductive buffer E_R after and maturity maintenance (at rate $\dot{p}_J$). Energy contained in the reproductive buffer is then converted into eggs. PMoAs are represented with numbers located on the energy flow they affect: 1. Feeding, 1'. Assimilation, 2. Somatic maintenance, 2'. Maturity maintenance, 3. Growth costs, 4. Reproduction costs, 5. Fraction of energy allocated to growth and to reproduction, 6. Embryo mortality.

Most PMoAs have been documented for various contaminants so far (Jager et al., 2006; Kooijman and Bedaux, 1996), except the share of energy or energetic trade-off between growth and reproduction (PMoA 5; Fig. 2.1). It has however been suggested that it could be related to the effects of endocrine disrupters (Kooijman, 2010). PMoAs of PCB and

PBDE have not been studied in a bioenergetic context so far but they are good candidates as chemicals altering the energy allocation between growth and reproduction since some of them are known endocrine-disruptors altering reproduction (Mills and Chichester, 2005; Yu et al., 2015) and favoring obesity (Berg et al., 2011; Lyche et al., 2010, 2011). Moreover, a recent study in zebrafish showed that chronic dietary exposure to an environmentally-realistic marine mixture of PCBs and PBDE induced growth to larger sizes while delaying spawning probability which suggested modification of the energetic allocation between growth and reproduction (Horri et al., 2018).

In the present study, we used DEB theory to study the PMoAs of the marine mixture of PCB and PBDE congeners studied in Horri et al. (2018) using data on growth and reproductive (spawning effort) life-history traits in experimental control and exposed zebrafish populations. Fish exposure to the PCB and PBDE mixture was conducted through diet from the first meal and throughout the life cycle of the fish. A DEB model was calibrated for each treatment (control vs. exposed). Then, the estimated parameters of the DEB models were compared between control and exposed fish to identify the PMoAs of the PCB/PBDE mixture. In order to insure that potential alternative PMoAs were not overlooked through the calibration procedure, the various PMoAs identified in the literature were systematically tested. This was done by comparing the observed growth and reproduction patterns and those predicted by the DEB model when allowing only the parameter corresponding to the PMoA considered to vary between control and exposed fish. Finally, to further deepen our interpretation of modeling results, the functionality of reproduction and the underlying hormonal control were monitored.

2.3 Materials and methods

This study was conducted under the approval of the Animal Care Committee of Poitou-Charentes # 84 COMETHEA (France) under project authorization number CE2012-23.

2.3.1 Experimental data

Details of the experiments can be found in Supplementary Text 2.S1 and Horri et al. (2018). A summarized version is given here.

2.3.1.1 Fish production and rearing

Experiments were performed with wild type strain TU zebrafish (ZFIN ID: ZDBGENO-990623-3) from a stock kept at the Fish Ecophysiology Platform (PEP - http://wwz.ifremer.fr/pep_eng). Adult fish were maintained in heterosexual groups (n = 28 ± 5 individuals) in 10-L tanks in a recirculation system at a 14:10 light:dark photoperiod and a temperature of 27 ± 1°C. Fertilized eggs were obtained by random pairwise mating of zebrafish placed together in spawning boxes (AquaSchwarz, Germany) and collected in a Petri dishes containing 30 mL of isotonic mixture E3 (1 L: 17.2 g NaCl, 0.76 g HCl, 2.9 g CaCl2. 2 H2O, 4.9 MgSO4. 7 H2O) at 28°C. At one day post-fertilization (dpf), eggs from 5 clutches were mixed in a balanced way and distributed in Petri dishes at a rate of 60 larvae per Petri dish. After larval rearing in semi-static conditions in 1-L tanks, offspring fish were freed into 10-L tanks of the recirculation system at 27 dpf (after Vignet et al., 2014). Fish were fed three times per day, twice with pellets in the morning and the evening, and once with freshly hatched crustaceans (*Artemia salina*) at noon, food pellet size being adjusted to fish's developmental stage SDS 100 µm, 200 µm, and 300 µm (Special Diet Service; Dietex international) and Inicio^{+} 500 µm (Biomar, France).

2.3.1.2 Fish exposure

Fish were exposed to contaminants through food pellets spiked with a mixture of PCB and PBDE congeners chosen in order to represent environmental conditions. For PCBs, the choice was based on the contamination levels and profiles reported in mussels from the Seine estuary, one of the most contaminated site along the French coastlines (Abarnou et al., 2000). For PBDEs, targeted contamination levels and profiles were based on the 7 most representative congeners in marine biota and the main congener in marine sediments that were identified for priority action by OSPAR (OSPAR Commission, 2009, 2013). The precise targeted and measured concentrations for each PCB and PBDE congener are available in Table 1.S1. A contaminated diet was prepared after dilution of a stock PCB and PBDE solution in isooctane solvent for incorporation as described in Daouk et al. (2011). A control diet was prepared in the same manner, i.e., with isooctane solvent but without contaminants. Control and contaminated food pellets of all sizes (100, 200, 300 and 500 µm) were prepared such that fish were fed from their first meal at 5 dpf onwards. This study is part of a larger experiment where a total of 5 replicate populations, each composed of 9 ± 1 tanks per dietary treatment were produced (46 tanks per treatment in total, see Horri et al., 2018 Table 1.S3 for details). In the present study, we used Replicate R3 (composed of 9 and 8 replicated tanks of control and contaminated treatment, respectively) for trait monitoring and fish from another replicate population reared in parallel for hormonal assays. Hereafter, we will refer to fish exposed to the control and the contaminated diet as SOLV (as solvent) and MIX (as mixture) fish, respectively.

2.3.1.3 Trait monitoring

Growth of all fish per treatment and tank was monitored roughly monthly from 2 months to one year of age (measurements at 57, 97, 127, 149, 180, 210, 240, 268, 302,

335, and 362 dpf). On each occasion, individual standard length L_w (cm) and body weight W_w (g) were measured after anesthesia with benzocaine (Vignet et al., 2014). Sex was determined whenever possible based on morphological clues, generally from around 3 months old onwards.

Reproduction was monitored once a week during 10 consecutive weeks starting at 4 months of age (117, 124, 131, 138, 145, 152, 159, 166, 173 and 180 dpf) for each treatment. On each date and for each tank, mating pairs were made by randomly selecting a male and a female and placing them in a spawning box in the evening and the resulting eggs were collected in the next morning, this is later called solicitation, whatever the success of reproduction. In total 123 SOLV and 119 MIX mating pairs were settled. The total number of eggs (fertilized plus unfertilized) N was averaged across mating pairs originating from the same tank on each date and was used as a measure of reproductive output in the DEB model.

All data except reproduction data were published and statistically analysed in Horri et al. (2018). Statistical analyses of reproduction data are provided in Supplementary Text 2.S2.

2.3.1.4 Measuring sex steroid hormones through time

Sex steroid hormones (17beta-estradiol (E2) and 11keto-testosterone (11KT)) were measured in grinded whole body samples either in pools of 2 juveniles at 30 dpf (N = 15 pools per treatment, for E2 only) or in individual (E2 and 11KT) at 60 dpf (n = 18 per treatment); hormone levels were normalised by the measure of total protein content (Bicinchoninic Acid Protein Assay, Sigma Aldrich, Saint Louis, MO, USA) and expressed in pg mg^{-1} protein. At later stages, blood samples (20 µl) were taken and hormone levels are expressed in ng ml^{-1} or pg ml^{-1} with 15 individuals sampled per sex and treatment at

90 dpf and 180 dpf. For E2, IBL 17beta-Estradiol Saliva ELISA Kit (ref. #52601, IBL, Hamburg, Germany) were used and for 11KT, Cayman 11-keto Testosterone EIA Kit, (ref. #582751, Cayman Chemical Company, Ann Harbor, MI, USA) were used. In all cases, samples were treated according to kit's instructions and fluorescence was determined using a Synergy HT multi-mode microplate reader (Biotek).

2.3.2 Modeling approach

2.3.2.1 Dynamic Energy Budget model

The standard DEB model was applied to study the effect of the mixture of PCB and PBDE congeners on metabolism and their physiological modes of action (PMoA) in zebrafish. The model describes mechanistically the rates at which an individual organism ingests and assimilates energy throughout its lifespan, stores it into reserves E and then allocates it to growth of structural volume V and maintenance on the one hand and to maturity E_H (before puberty) or reproduction buffer E_R (after puberty) and maturity maintenance on the other hand (see Fig. 2.1 for more details) (Kooijman, 2010; van der Meer, 2006; Sousa et al., 2010). Maturity represents organism's developmental stage in terms of complexity until reaching adulthood. Zebrafish has four life stages: embryonic, larval, juvenile and adult stage (Nüsslein-Volhard and Dahm, 2002). The life-history transitions from one stage to another occur when maturity E_H reaches the corresponding maturity thresholds, i.e., E_H^b for birth (b), E_H^j for metamorphosis (j) and E_H^p for puberty (p). The increase in maturity E_H stops at puberty because individuals start allocating energy into reproduction E_R instead.

The simplest DEB model assumes that, under constant food density X, individuals follow a von Bertalanffy growth curve throughout their whole lifespan. However, zebrafish most often follow a sigmoid growth curve which, according to Augustine et al. (2011),

could be related to metabolism acceleration between birth and metamorphosis, i.e., during larval stage ($E_H^b \leq E_H < E_H^j$). Therefore, we use the version of the standard DEB model with metabolic acceleration of assimilation and mobilisation at larval stage (Kooijman, 2014; Lika et al., 2014); see a detailed description in Supplementary Text S3). Physical length L_W being related to structural length $L = V^{1/3}$ via a shape coefficient δ through the relationship $L = \delta L_W$, the switch between accelerated at larval stage and non-accelerated metabolism at juveniles and adult stage requires that two different shape coefficients are considered in the present DEB model, one for larvae (δ_J) and another for juveniles and adults (δ_M).

In the following, only the equations at juvenile and adult stage, i.e., after metabolic acceleration, and describing the dynamics of the compartments related to traits monitored, namely structural length L and reproduction buffer E_R, are presented. For a detailed description of the DEB model at larval, juvenile and adult stages, the reader is referred to Supplementary Text S3.

After the metabolic acceleration, individuals' growth in structural length L follows a von Bertalanffy-like growth curve at constant food density, which is described by:

$$\frac{dL}{dt} = \dot{r}_B(L_\infty - L) \quad (1)$$

with $L_\infty = f\frac{L_j}{L_b}\frac{\kappa\{\dot{p}_{Am}\}}{[\dot{p}_M]}$ the ultimate structural length (cm)

and $\dot{r}_B = \frac{1}{3}\frac{[\dot{p}_M]}{\kappa\{\dot{p}_{Am}\}/\dot{v}+[E_G]}$ the von Bertalanffy growth rate (d^{-1})

where $f = \frac{X}{K_X+X}$ is a Holling type II functional response that describes how the increase in an individual's intake rate with food density X decelerates according to the half-saturation constant K_X, i.e., the food density level at which intake rate reaches half of its

maximum value, L_b and L_j length at birth and metamorphosis (cm), respectively, $[\dot{p}_M]$ the volume-specific somatic maintenance costs (J.d^{-1}.cm^{-3}), κ the fraction of energy allocated to somatic (i.e. structure) maintenance and growth, $\{\dot{p}_{Am}\}$ the maximum surface-area-specific assimilation rate (J.d^{-1}.cm^{-2}), $\dot{v}$ the energy conductance (cm.d^{-1}), and $[E_G]$ the cost of synthesis of a unit of structure (J.cm^{-3}).

The dynamic of maturity E_H before puberty $E_H < E_H^p$ are described by

$$\frac{dE_H}{dt} = (1-\kappa)\dot{p}_C - \dot{k}_J E_H \quad (2)$$

and those of reproduction buffer E_R after puberty $E_H = E_H^p$ by:

$$\frac{dE_R}{dt} = (1-\kappa)\dot{p}_C - \dot{k}_J E_H^p \quad (3)$$

where $\dot{p}_C$ is the mobilization rate (J.d^{-1}), $\dot{k}_J$ is the maturity maintenance rate coefficient (d^{-1}) and E_H^p is the maturity threshold at puberty. Notice that after puberty, maturity no longer increases and stays fixed at $E_H = E_H^p$.

The conversion of the reproduction buffer E_R into a number of eggs is given by:

$$N = \frac{\kappa_R}{E_0} E_R \quad (4)$$

where κ_R is the reproduction efficiency that accounts for overheads paid to convert the reproduction buffer energy into the energy reserves of the eggs and E_0 is the initial amount of energy in an egg (J). The factor E_0/κ_R represents then the total cost of producing an egg.

As in ectotherms, physiological rates depend on environmental temperature (Kooijman, 2010), some parameters used in the DEB model were corrected using a temperature

correction factor based on the Arrhenius relationship (see Supplementary Text S3 for more detail).

2.3.2.2 Model calibration

Excluding the parameters of the Arrhenius relationship, the standard DEB model with metabolic acceleration relies on 14 parameters (Table 2.1). The covariation method was applied to estimate these parameters using DEBtool software package (Lika et al., 2011a, 2011b). The method links the parameters to observed data at different life-history stages, i.e., experimental data in this study. The method distinguishes between two types of data: (i) *zero-variate* data are 0-dimensional data or single-data points (e.g. size and age at puberty, maximum size) that can include *real data,* i.e., a set of real observations (e.g. the set of sizes and ages at life-history transitions), and *pseudo-data*, i.e., a set of parameters for a generalized (i.e. hypothetical) animal determined from parameter estimates of a wide range of species, and (ii) *uni-variate* data that are one-dimensional data or vectors of repeated observations (e.g. lengths and weights at various ages). The observed zero-variate data used in the present study were a mixture of experimental data coming from the present study as well as taken from the literature (Table 2.2). Uni-variate data all came from the experiments of the present study, namely standard length at age $L_w(a)$, weight at age $W_w(a)$, the cumulative egg number at age $\int_0^a N(x)dx$ and the length-weight relationship $L_w(W_w)$.

The simultaneous estimation of all parameters is based on the minimization of the loss function $F = \sum_{i=1}^{n} \sum_{j=1}^{n_i} w_{ij} \frac{(d_{ij}-p_{ij})^2}{d_i^2+p_i^2}$ (Augustine et al., 2017) where d_{ij} is the observed value j in dataset i, p_{ij} is the corresponding predicted values, w_{ij} is the partial weight of this data point, $d_i = \frac{1}{n_i}\sum_{j=1}^{n_i} d_{ij}$ is the mean value for dataset i and $p_i = \frac{1}{n_i}\sum_{j=1}^{n_i} p_{ij}$ is the

mean predicted value for the same dataset. The total weight coefficient for each observation given by $1/n_i$ is inversely proportional to the number of points in dataset i and proportional to partial weight w_{ij}, which is set so as to reflect accuracy and/or certainty in the data (Table 2.S1). Minimization is achieved using a numerical optimization algorithm specifically designed for multidimensional functions, namely the Nelder-Mead method or downhill simplex method.

All initial parameter values were chosen according to the generalized animal (Kooijman, 2010) or the literature on zebrafish (Augustine et al., 2011) and are available in Table 2.S2. Two DEB models per treatment, one for SOLV fish and another for MIX fish, were calibrated in the same manner except for reproduction efficiency κ_R and maturity thresholds at birth E_H^b, metamorphosis E_H^j, and puberty E_H^p of MIX fish that were fixed at the same values as those estimated for SOLV fish (Table 2.1, fitted column). This choice relies on two assumptions: first, the assumption that direct effects of contaminants on reproduction through energetic costs are multiplicative (Kooijman and Bedaux, 1996) and thus comes through the total cost of producing an egg E_0/κ_R, so that letting the numerator E_0 vary while fixing the denominator κ_R is equivalent to letting the ratio of the two vary and can be interpreted as such and second, maturity thresholds, which reflect the degree of development at which life-history transitions occur, cannot be affected by contaminants within one generation, i.e., can be affected evolutionarily only (i.e. over generation), and thus that only the age and size at which such developmental thresholds are reached can be affected by contaminants.

The estimates of each parameter θ_k ($k \epsilon \{1; 2; \dots; 10\}$, i.e., 14 DEB parameters minus 4 fixed) were compared between treatments (SOLV, MIX) on the basis of a percentage change $\%C$ calculated as follows:

$$\%C_k = 100\frac{\theta_{k,\mathrm{MIX}} - \theta_{k,\mathrm{SOLV}}}{\theta_{k,\mathrm{SOLV}}}$$

where $\theta_{k,\mathrm{SOLV}}$ and $\theta_{k,\mathrm{MIX}}$ are the parameter values for SOLV and MIX individuals, respectively. The contribution of each parameter θ_k to the fitting to MIX data $\mathbf{d}_{\mathrm{MIX}}$, and thus its potential for being a PMoA route for the tested PCB/PBDE mixture, was quantified by computing the loss function $F(\theta_{k,\mathrm{MIX}}, \theta_{l\neq k,\mathrm{SOLV}}|\mathbf{d}_{\mathrm{MIX}})$ obtained with MIX data $\mathbf{d}_{\mathrm{MIX}}$ when fixing the parameter at the value obtained for MIX data $\theta_{k,\mathrm{MIX}}$ while all others were fixed at the values obtained for SOLV data $\theta_{l\neq k,\mathrm{SOLV}}$. The more the parameter contributes to fitting MIX data, the lower the loss function $F(\theta_{k,\mathrm{MIX}}, \theta_{l\neq k,\mathrm{SOLV}}|\mathbf{d}_{\mathrm{MIX}})$ should be.

2.3.2.3 Physiological modes of action simulations

In addition to the independent calibration of models for the two diet treatments, the potential PMoAs were tested one by one, independently, on the basis of growth and reproduction pattern change when comparing between observations and predictions (Martin et al. 2014). More precisely, for each PMoA, we estimated the corresponding DEB parameter value (e.g. $\{\dot{p}_{Am}\}$ maximum area-specific assimilation rate for PMoA 1'; Fig. 2.1) using MIX data but while fixing all other parameters at the same values as those estimated for SOLV fish. Two values were estimated for each of these DEB parameters, one using MIX growth data and the other using MIX reproduction data, and were then used to predict the cumulated number of eggs at age and standard length at age, respectively. The predicted changes in growth and reproduction obtained for the different PMoAs were then compared with those observed experimentally between SOLV and MIX fish. The PMoAs that provided the same qualitative patterns of change as

those observed for both growth and reproduction were considered as the most likely PMoAs of the tested PCB and PBDE mixture.

2.4 Results

2.4.1 Effect of PCB and PBDE congeners mixture on DEB parameters

Four parameters appeared to differ for more than a few percent between SOLV and MIX diet (Table 2.1, %C column). All of them increased in the MIX treatment relative to the SOLV one: the pre-metamorphic shape coefficient δ_J (+9.79%) suggesting an increase in body condition, the energy conductance $\dot{v}$ (+7.06%) suggesting an increase in energy mobilization rate, the fraction of energy allocated to somatic maintenance and growth κ versus the expense of maturity maintenance and maturity/reproduction (+8.56%) suggesting growth prioritization at the expense of reproduction, and the total cost of an egg E_0 (+19.13%) suggesting reproduction impairment. Among these four parameters, only two reduced the loss function F significantly, namely κ and E_0, suggesting that they were the two main contributing parameters to fitting the observation for MIX individuals.

Table 2.1. DEB model's primary parameters for fish exposed to SOLV and MIX diet. SOLV: value estimated for SOLV fish; MIX: value estimated for MIX fish; %C: percentage change between SOLV and MIX values; F: loss function obtained with MIX data when fixing the parameters to the values obtained for MIX data while all others are fixed at the values obtained for SOV data. The lower F, the more the parameter contributes to the fitting to MIX data and thus is a potential PMoA for the tested PCB/PBDE mixture. The loss function F could not be computed for the shape parameter at larval stage δ_j (hence the NA value) as it influences physical length L_w predictions at larval stage only whereas experimental length data were available for juvenile and adult stages only.

Symbol	SOLV	MIX	%C	F	Units	Description	Fitted
f	0.93	0.89	-3.990	0.068	-	Scaled functional response for 1-var data	Fitted
δ_J	0.130	0.142	9.790	NA	-	Pre-metamorphic shape coefficient	Fitted
δ_M	0.252	0.256	1.550	0.230	-	Post-metamorphic shape coefficient	Fitted
$\{\dot{p}_{Am}\}$	85.343	85.450	0.125	0.236	$J.d^{-1}.cm^{-2}$	Maximum area specific assimilation rate	Fitted
$\dot{v}$	0.025	0.026	7.060	0.343	$cm.d^{-1}$	Energy conductance	Fitted
κ	0.65	0.71	8.560	0.014	-	Fraction of energy allocated to somatic maintenance and growth	Fitted
κ_R	0.95	0.95	-	-	-	Reproduction efficiency	Fixed
$\dot{k}_J$	0.002	0.002	-1.150	0.230	d^{-1}	Maturity maintenance rate coefficient	Fitted
$[\dot{p}_M]$	165.3	167.5	1.330	0.210	$J.d^{-1}.cm^{-3}$	Volume specific somatic maintenance costs	Fitted
$[E_G]$	5224	5229	0.095	0.227	$J.cm^{-3}$	Cost of synthesis of a unit of structure	Fitted
E_0	0.674	0.803	19.134	0.044	J	Initial amount of energy in an egg	Fitted
E_H^b	0.166	0.166	-	-	J	Energy invested in maturity at birth	Fixed
E_H^j	4.343	4.343	-	-	J	Energy invested in maturity at metamorphosis	Fixed
E_H^p	504.2	504.2	-	-	J	Energy invested in maturity at puberty	Fixed

2.4.2 Effect of PCB and PBDE congeners mixture on life-history traits

2.4.2.1 Body length

A good fit of growth data was achieved for both SOLV and MIX fish with a relative error (RE) of 0.036 and 0.031 for SOLV and MIX fish, respectively. Growth curves showed that MIX fish had a slightly higher growth in terms of both body length (Fig. 2.2) and weight (Fig. 2.S1) than SOLV fish. The growth increase in the MIX treatment is consistent with the increase in the fraction of energy κ allocated to structure growth and maintenance detected by DEB model calibration.

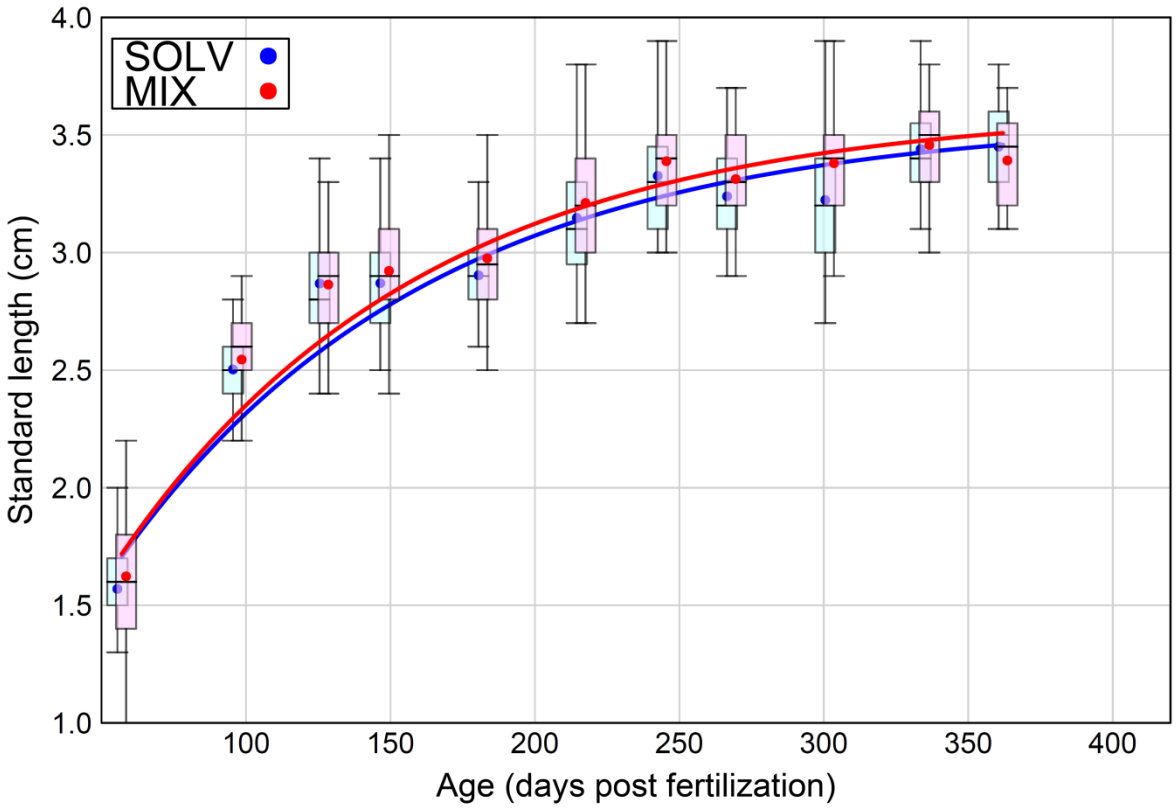

Fig. 2.2. Effect of chronic dietary exposure to a PCB and PBDE congener mixture on growth in terms of standard length in zebrafish. Boxplots represent observations per treatment (SOLV in grey and MIX in white) and curves represent the DEB model predictions per treatment (SOLV in solid line and MIX in dashed line). For boxplots, the bottom and top of the box are the first and the third quartiles of the data distribution, the horizontal segment and the dot inside the box are the median and the mean, respectively, and whiskers represent the most extreme data point within the 1.5 interquartile range.

2.4.2.2 Cumulative reproductive outputs

As for growth data, the DEB model fitted well reproduction data over time for both SOLV and MIX fish with a RE of 0.065 and 0.024 for SOLV and MIX fish, respectively. The comparison of the cumulative number of eggs produced by female indicated a clear difference between MIX fish and SOLV fish with the latter producing more eggs than the former (Fig. 2.3A). The decrease of reproductive output in the MIX treatment is consistent with the decrease in the fraction of energy $1-\kappa$ allocated to maturity maintenance and maturity/reproduction as well as the increase in the total costs of an egg E_0/κ_R detected by DEB model calibration. The pure effect of change in energy allocation fraction κ can be appreciated by considering change in energy accumulated in the reproductive buffer E_R (Fig. 2.3B), the effect of the total costs of an egg E_0/κ_R coming in only when turning the reproduction buffer into a number of eggs $N = {\kappa_R}/{E_0}\, E_R$.

Globally, DEB predictions showed that reproduction is more sensitive (+ 33.64 % of the predicted mean number of eggs calculated over the whole time of prediction for SOLV fish relative to MIX fish, Fig. 2.2) to PCBs and PBDEs exposure than growth (- 2.92 % of difference of the mean predicted length calculated over the whole time of prediction for SOLV fish relative to MIX fish, Fig. 2.3A), with change in the reproduction buffer representing a large part of change in reproduction output (+ 21.39 % of difference for SOLV fish relative to MIX fish).

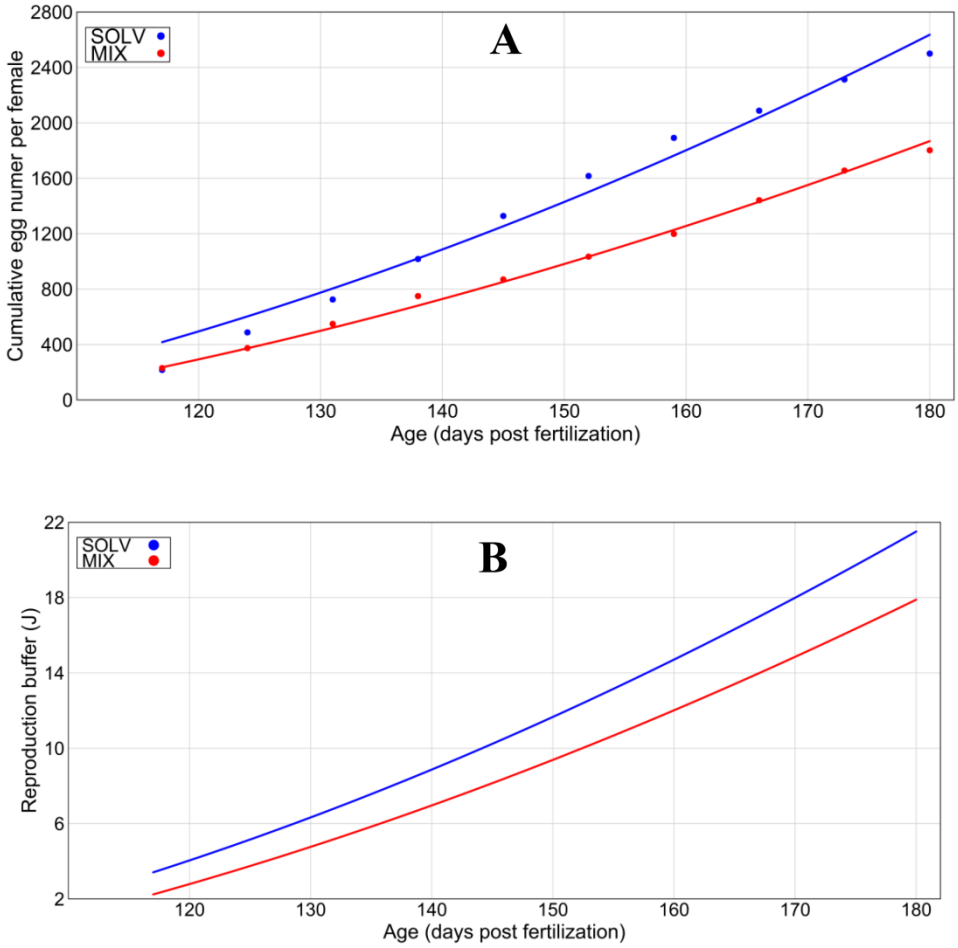

Fig. 2.3. Effect of chronic dietary exposure to a PCB and PBDE congener mixture on reproductive output in zebrafish in terms of A. cumulative egg number per female as a function of age (dpf) and B. cumulated energy in reproduction buffer. Dots are for observed cumulative number of eggs per female and curves represent the DEB model predictions per treatment.

2.4.2.3 Ages and lengths at life-history transitions

Ages and lengths at life-history transitions were also well predicted by the DEB model (Table 2.2). More specifically, length at puberty L_w^p of MIX fish was predicted to be larger than that of SOLV fish (+ 6.88 %; Table 2.2). In addition and as expected from their increased growth, MIX fish were predicted to reach larger ultimate length L_w^∞ and weight W_w^∞ than SOLV ones (+ 5.62 % in L_w^∞ and + 19.54 % in W_w^∞; Table 2.2).

Table 2.2. Zero-variate data used for the estimation of the DEB model parameters in SOLV and MIX fish. All values are given for an experimental temperature of 299.15 K and a scaled functional response of 1. Lengths are presented as physical lengths. Obs: Observed value. Pred: Value predicted by the DEB model. R.E: Relative error. Source: source of the observed value.

	SOLV			MIX					
Symbol	**Obs**	**Pred**	**R.E**	**Obs**	**Pred**	**R.E**	**Units**	**Description**	**Source**
a_b	5	5.104	0.021	5	5.147	0.029	d	Age at birth	This study
a_j	21	20.13	0.042	21	20.26	0.035	d	Age at metamorphosis	This study
a_p	72	79.58	0.105	73	80.17	0.098	d	Age at puberty	This study
a_m	1643	1643	0.0003	1643	1643	0.000	d	Life span	Augustine et al. (2011)
L_w^b	0.312	0.295	0.055	0.312	0.293	0.062	cm	Standard length at birth	Augustine et al. (2011)
L_w^j	0.8	0.850	0.063	0.8	0.843	0.054	cm	Standard length at metamorphosis	Augustine et al. (2011)
L_w^p	1.92	1.86	0.031	1.99	1.988	0.001	cm	Standard length at puberty	This study
L_w^∞	3.26	3.862	0.185	3.36	4.079	0.214	cm	Ultimate standard length	Horri et al. (2018)
W_w^∞	1.66	1.612	0.029	2	1.927	0.036	g	Ultimate wet weight	This study

2.4.3 PMoA of PCB and PBDE congeners mixture

Among the 7 tested PMoA, only the fraction of energy κ predicted the pattern of change observed in growth and reproduction between SOLV and MIX fish, i.e. growth to larger lengths and lower reproductive outputs (Fig. 2.4). This suggests that the most likely PMoA for the effect of the tested PCB and PBDE congener mixture is an increase of the fraction of energy allocated to somatic maintenance and growth at the expense of the fraction dedicated to maturation and reproduction resulting in an increase in growth and a decrease in reproductive outputs. For all the other PMoAs, the predicted change in the trait (growth or reproduction) that was not used for estimating the PMoA parameter was in an opposite direction to the one observed except for maturity maintenance costs and reproduction costs. Maturity maintenance costs were predicted to have no effect on reproduction. Reproduction costs did not affect growth when estimated using reproduction data, which was expected given that the cost of an egg E_0 affects the production of eggs from the reproduction buffer only, and they also did not affect reproduction when estimated using growth data as reciprocally any change in growth does not lead to a change in E_0 estimate.

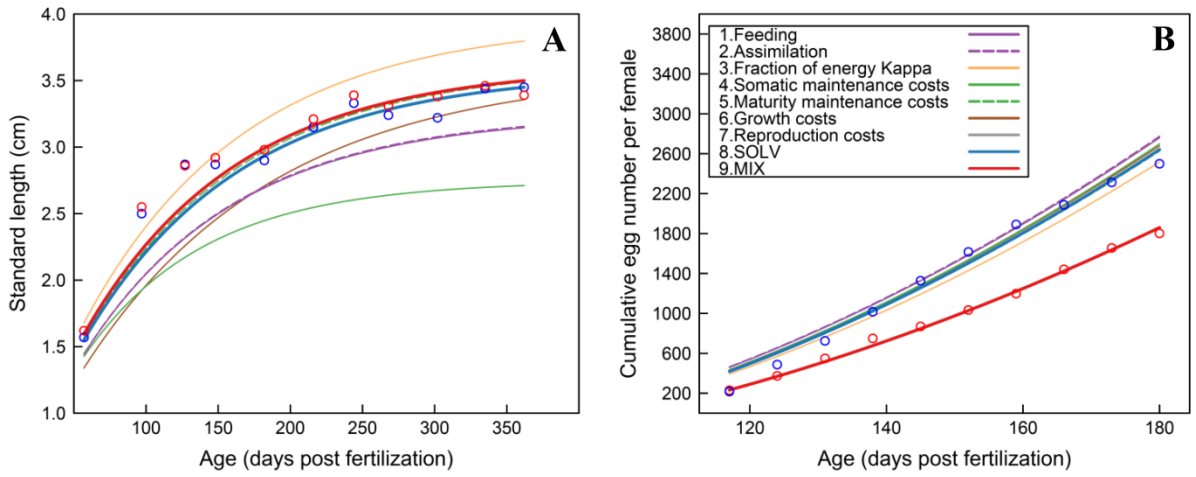

Fig. 2.4. Test of the PMoAs of the mixture of PCB and PBDE congeners. A. Growth curves predicted for each PMoA when estimating the PMoA parameter value using MIX reproduction data while setting all other parameters at their value estimated with SOLV data. B. Cumulative

energy in reproduction buffer predicted for each PMoA when estimating the PMoA parameter value using MIX growth data while setting all other parameters at their value estimated with SOLV data. Lines are predictions and open circles are observations (color legend is given in B panel).

2.4.4 Endocrine disruption

Level of sex steroid hormones (17beta-estradiol (E2) and 11keto-testosterone (11KT)) was monitored monthly over fish maturation and in adults (Fig. 2.5). No difference in E2 has been observed in 30 dpf juveniles (MW-U = 101.5, p = 0.661). At 60 dpf, MIX juveniles have a lower E2 (MW-U = 34.5, p < 0.0001) and a higher 11KT (MW-U = 86, p = 0.015) when compared to SOLV juveniles. Starting at 90 dpf, it was possible to sex fish according to morphological cues and hormone levels were analyzed separately for each sex. MIX females have lower E2 (MW-U = 8, p < 0.0001) and 11KT (MW-U = 27, p = 0.0001) compared to SOLV females. Similarly, MIX males have lower E2 (MW-U = 1, p < 0.0001) and 11KT (MW-U=9, p<0.0001) compared to SOLV males. Reproducing MIX females have a slightly lower level of E2 (MW-U = 51, p = 0.0178) compared to SOLV females while no difference in level of 11KT has been observed (MW-U = 100, p = 0.616). Male MIX have a higher level of E2 compared to SOLV (MW-U=43, p=0.0032) and a lower level of 11KT (MW-U=54, p=0.0141).

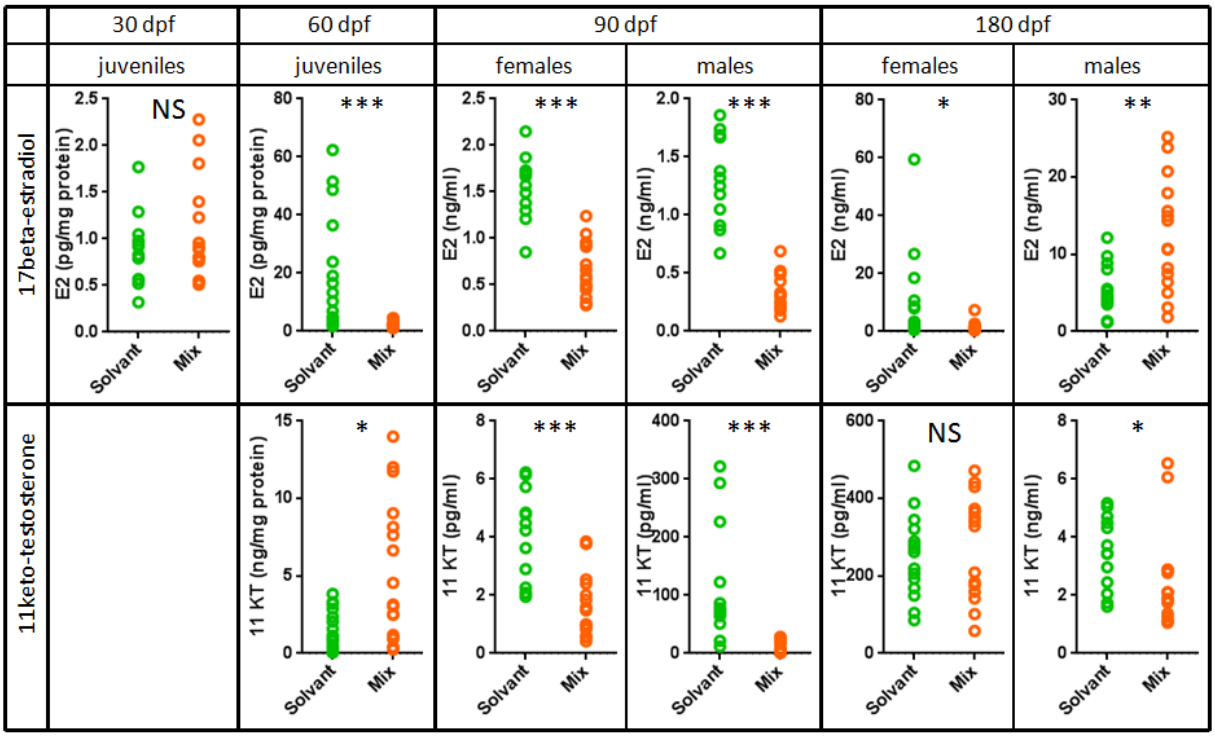

Fig. 2.5. Effect of chronic dietary exposure to a PCB and PBDE congeners mixture on sex steroid hormones level over maturation and in reproducing adults. Concentrations of 17beta-estradiol (E2; top row) and 11keto-testosterone (11KT; bottom row) have been measured in whole organisms (30 and 60 dpf) or in blood (90 and 180 dpf). When morphological sex was identifiable (starting at 90 dpf), levels are indicated for females and males. In all cases, statistical significances (Mann-Whitney test) are indicated as: NS, not significant, *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

2.5 Discussion

In this study, two DEB models were developed using experimental data on zebrafish growth and reproduction, one for fish exposed to an environmentally realistic marine mixture of PCBs and PBDEs (MIX fish) and another for control fish (SOLV fish). Then, the estimated parameters of the two DEB model were compared to identify the different PMoAs of this POP mixture. PMoA identification was cross-validated by comparing observed patterns of change of life-history traits between SOLV and MIX fish with that predicted for the 6 potential PMoAs and their biological underpinning was explored by monitoring sex steroid hormones.

2.5.1 DEB fitting and prediction of life-history traits in control and exposed fish

DEB model predictions reproduced correctly that MIX fish had a slightly higher growth than SOLV fish, in terms of both length and weight. In a previous study using the same data, this difference in growth was shown to be statistically significant (Horri et al., 2018). However, this effect contrasts with other studies in which exposure of zebrafish to POP mixtures caused either no change in fish length while increasing weight (mixture of PCBs, PBDEs and DDT: Nourizadeh-Lillabadi et al., 2009) or a decrease in length (polycyclic aromatic hydrocarbons; PAHs: Hooftman et al., 1993; Vignet et al., 2014).As to the reproduction traits, DEB model predictions also reproduced that SOLV fish produced more eggs per female than MIX fish, which is consistent with previous studies that have demonstrated that PCBs and PBDEs reduce egg production in zebrafish (Daouk et al., 2011; Kuiper et al., 2008; Muirhead et al., 2006; Örn et al., 1998).

Only DEB parameters estimated for control fish are comparable with those given in the literature as our study is the first to calibrate a DEB model for fish exposed to a mixture of PCBs and PBDEs. Our estimated parameters for SOLV fish differ from those obtained by Augustine et al. (2011) on the same species. For example, in our study, the estimated fraction of energy κ is about 0.65 indicating that fish allocated more energy to growth plus somatic maintenance than to maturity maintenance plus reproduction. In contrast, a κ value of 0.43 has been estimated by Augustine et al. (2011) indicating the opposite, i.e., more energy allocated to maturity maintenance and reproduction than to growth and somatic maintenance. The κ reported here is close to the one estimated for the generalized animal ($\kappa = 0.8$; see the Add-my-pet collection). This difference in DEB parameters might be explained by variations in laboratory conditions as well as the difference of fish lines and the rearing protocol (e.g. rearing temperature, food availability).

2.5.2 Identification of the physiological modes of action of an environmentally realistic marine mixture of PCBs and PBDEs

After comparing the parameters estimated for SOLV and MIX fish, four of them seemed to be affected by PCBs and PBDEs mixture (Table 2.1): the pre-metamorphic shape coefficient δ_J , the energy conductance $\dot{v}$, the fraction of energy allocated to growth κ (and reciprocally to maturity/reproduction $1 - \kappa$) and the total cost of an egg E_0 were all increased in MIX fish relative to SOLV ones. The two first ones had little effect on the degree of fitting of DEB life-history traits predictions to the data, as shown by the relative high associated values of the loss function F. In contrast, the fraction of energy κ and the total cost of an egg E_0 reduced markedly the loss function F, indicating that variation in both parameters improved the degree of fitting of DEB life-history trait predictions to the data. This is partly in agreement with a sensitivity analysis by Beaudouin et al. (2015) on a DEB model applied to zebrafish, that showed that the contribution of energy conductance $\dot{v}$ to variations in both growth and reproduction DEB predictions is negligible whereas the fraction of energy κ was found to be among the main contributors to variations in DEB predictions. This suggests that the observed difference in κ between SOLV and MIX individuals has significant consequences for growth and reproduction. In contrast, Beaudouin et al. (2015) found that the costs of reproduction E_0 had little effect on DEB output, which is contradictory with our results. Comparison with Beaudouin et al. (2015) for pre-metamorphic shape coefficient δ_J is not possible as they did not use a model with metabolic acceleration. The fraction of energy κ and the costs of reproduction E_0 appear as the two best candidates as PMoAs of the PCB and PBDE mixture to which MIX fish were exposed.

This conclusion is also supported by comparing the patterns of change in life-history traits triggered by all potential PMoAs with that observed in the data (Fig. 2.4). The

potential PMoAs of contaminants are well documented, especially in the DEB context (Álvarez et al., 2006; Jager et al., 2007, 2014; Kooijman and Bedaux, 1996; Martin et al., 2013; Muller et al., 2010). In this paper, we have considered the five main broad PMoAs: feeding/assimilation (1-1'), somatic and maturity maintenance costs (2-2'), ,growth costs (3), reproduction costs (4) and the fraction of energy allocated to somatic maintenance and growth (5) (Fig. 2.1). Among these, the fraction of energy κ , which represents the energetic trade-off between growth and reproduction, is the only PMoA affecting growth and reproduction in opposite directions, i.e. leading to an increase in growth and a reduced reproductive output, as observed in MIX fish. All other PMoAs are either affecting growth and reproduction in the same direction (feeding/assimilation, growth costs, maintenance costs) or reproduction only (reproduction costs). For instance, in a study simulating growth and reproduction predicted by different physiological modes of action (feeding, maintenance costs, growth costs and reproduction costs) of 3,4-dichloroaniline in *Daphnia magna*, it has been found that growth was reduced or unchanged and reproduction was always reduced under stress whatever the mode of action but in no case growth has increased (Martin et al., 2013). Based on this and on our simulations, increase in the fraction of energy allocated to growth κ appears to be the only factor producing these trajectories of growth and reproduction.

Fecundity appears to be diminished jointly by the two combined PMoAs, i.e., (i) through a direct effect due to the increase in the energy costs per egg E_0, which acts on the number of eggs produced through the conversion of energy stored in the reproductive buffer into gametes, and (ii) through an indirect effect related to the decrease in the fraction of energy $1-\kappa$ allocated to reproduction, thus resulting in a decrease in the reproduction buffer itself. The amplitude of change in $1-\kappa$, however, is constrained by

the reciprocal effect on somatic growth. As a result, it is not sufficient to reduce fecundity as much as observed in the experimental data. Hence, the increase in reproduction costs is necessary to decrease fecundity further.

To our knowledge, the PMoA corresponding to the energetic trade-off between growth and reproduction has never been observed whatever the contaminant. Yet, it has been discussed before as a potential mode of action of chemicals in the case of endocrine disrupters such as PCBs and PBDEs (Jager et al., 2010; Kooijman, 2010). This PMoA is in agreement with the fact that PCBs and PBDEs have a obesogenic effect (Berg et al., 2011; Lyche et al., 2010, 2011; Nourizadeh-Lillabadi et al., 2009) and can alter reproduction (Mills and Chichester, 2005; Yu et al., 2015). In contrast, the PMoA "costs of reproduction" E_0 was already identified for a number of contaminants in invertebrates (e.g. Pentachlorobenzene in *Caenorhabditis elegans*: Álvarez et al., 2006; Ashauer and Jager, 2018; Jager et al., 2004, 2006, 2010; cadmium in Daphnia: Kooijman and Bedaux, 1996; 3,4-dichloraniline in Daphnia: Martin et al., 2013; Muller et al., 2010; aldicarb in *Caenorhabditis elegans*: Wren et al., 2011). However, as far as we are aware, effects on reproduction costs have never been observed in fish and for a mixture of PCB and PBDE.

2.5.3 Biological underpinning of the physiological modes of action of the PCB and PBDE congeners mixture

Although it is always difficult to interpret metabolic changes and PMoAs from a physiological point of view, endocrine disruption could provide an explanation for the two PMoAs identified here. Several studies have demonstrated that POPs may act as endocrine disrupters affecting hormone pathways that regulate growth and reproductive functions resulting in a decrease in reproductive success components such as egg production and fertilization rate (reviewed in Mills and Chichester, 2005; Yu et al., 2015), an increase in weight (Berg et al., 2011; Lyche et al., 2010, 2011; Nourizadeh-

Lillabadi et al., 2009) or a decrease in weight and length (Vignet et al., 2014) in fish. The observed effect on the fraction of energy κ in this study could be interpreted by an over-expression of growth hormone (GH) due to endocrine disruption by PCBs and PBDEs. Studies on transgenic growth hormone (GH-transgenic) in tilapia have indeed demonstrated that an over-expression of this hormone decreases sperm production and reduces gonadosomatic index (Rahman et al., 1998, 2001). Rahman et al. (2001) suggested that the decrease in reproductive effort is probably due to the increase in energy dedicated to somatic growth promoted by the over-expression of GH.

Alternatively to growth hormone, the driver actually may be the endocrine disruption of sex steroid hormones. 17beta-estradiol (E2) and 11keto-testosterone (11KT) production was altered in MIX fish, the concentration of these hormones being significantly lower in MIX individuals during part of the life-cycle corresponding to gonad maturation. The PMoA related to the energetic trade-off between growth and reproduction κ is likely to be resulting from this perturbation. Indeed, disruption of E2 and/or 11KT levels has already been shown to impair reproduction but, in addition, could also interfere with growth. Regarding reproduction, ovaries differentiation and maturation is associated to an increase in E2 (Clelland and Peng, 2009), which is exemplified by the 20-fold increase in E2 concentration in SOLV fish between 30 and 60 dpf (even if fish sex could be determined at these ages). Over the same time period, E2 level only increased 2-fold in MIX fish. This lower increase could cause a delay in or even impair correct ovarian maturation, thus explaining a lower fecundity in exposed fish. Regarding growth, a recent study has demonstrated that exposure of fish to sex steroid hormones could modify expression of *ghrelin* hormone and *nucb2* genes (that encodes the neuropeptide Nesfatin-1) in a variety of tissues (Bertucci et al., 2016). Ghrelin has been shown to be involved in several regulations of several physiological functions in

fish, notably to stimulate GH release and food intake (Rainbow trout: Jönsson et al., 2007; tilapia: Kaiya et al., 2003; zebrafish: Li et al., 2009; goldfish: Matsuda et al., 2006). Nesfatin-1 has been shown to reduce food intake in goldfish (Gonzalez et al., 2010; Kerbel and Unniappan, 2012) for which its circulating level is significantly decreased after exposure to testosterone (Bertucci et al., 2016). Based on these observations, it is plausible to hypothesize that food intake/growth regulation by sex steroids as observed in mammals is similar in fish. Hence, the disruption of sex steroids by PCBs and PBDEs observed in our study could also be related to increased growth of MIX fish.

As to the PMoA "reproduction costs" resulting from an increase in egg costs E_0, hormone and gene expression disruptions could also be involved. According to Williams (2005), egg formation in birds requires a substantial increase in the weight or metabolic activity of organs such as the liver (that produces vitellus precursors) or reproductive organs such as the ovary. The increase in the weight of the organ could cause, in turn, an additional energy cost for its maintenance which could translate in term of DEB bioenergetic parameters into an increase in the cost of producing an egg. In fish, POPs have been shown to disrupt liver and ovarian activity by altering gene transcription (Lyche et al., 2010, 2011). Moreover, it is known that energy stores accumulated during the year before the spawning season and used for egg production are stored in the liver in numerous fish (e.g. Northern pike: Diana and Mackay, 1979; cod: Kjesbu et al., 1991). This is typical in capital breeders that store their energy in the liver, muscles or mesenteric fat before the spawning season to produce their eggs later, by opposition to income breeders that use energy as it is acquired during the spawning season to produce eggs (McBride et al., 2015). Under the assumption that such energy storage induces an increase in maintenance of the corresponding organ, the increase in egg costs observed in our study could be seen as a perturbation of the activity this organ such as

the vitellogenin synthesis activity of the liver. Additional measures, such as the metabolic (energetic) activity of the liver and/or ovary in control and exposed fish, could clarify this hypothesis.

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2.8 Supplementary material

Text 2.S1: Detailed experimental protocol

Fish rearing

Experiments were performed with wild type strain TU zebrafish (ZFIN ID: ZDBGENO-990623-3) from a stock kept at the Fish Ecophysiology Platform (PEP - http://wwz.ifremer.fr/pep_eng) originating from the European Zebrafish Resources Center (EZRC, Karlsruhe, Germany). Fish were maintained in heterosexual groups (n = 28 ± 5 individuals per 10 L-tank) under a 14h day/10h night light cycle. The resulting rearing density fell within the recommended range of 1 to 5 individuals/L (Nüsslein-Volhard and Dahm, 2002; Singleman and Holtzman, 2014). Water used in the rearing system was a mixture of approximately 2/3 reverse osmosis water and 1/3 tap water, both being initially treated by sediment and charcoal filters. Water physicochemical parameters were remained constant during the experiment: temperature 27 ± 1°C, conductivity 300 ± 50 µS cm-1 and pH 7.5 ± 0.5.

Eggs were obtained by random pairwise mating of zebrafish placed together in spawning boxes the evening before collection (AquaSchwarz, Germany). Eggs from each clutch with a fertilization rate greater than 80% were collected the next morning in a Petri dish containing 30 mL of isotonic mixture E3 (1 L: 17.2 g NaCl, 0.76 g HCl, 2.9 g CaCl2. 2 H2O, 4.9 MgSO4. 7 H2O) and placed at 28°C. Twenty four hours post-fertilization (hpf), eggs from 5 clutches were mixed in a balanced way (taking the same number of eggs from each clutch) and distributed in 17 Petri dishes at a rate of 60 larvae per Petri dish. At 5 days post fertilization (dpf), the groups of 60 larvae were transferred from their Petri dishes to separate 1-L tanks. At 15 dpf, the groups of larvae were transferred to tubes inserted inside separate 10-L rearing tanks disposed on flow-through racks and were then freed into the tanks at 27 dpf (after Vignet et al., 2014). In

the flow-through racks, an hourly automated addition of 150 mL of system water resulted in a daily total renewal of one third of the volume of each tank. Discarded water was collected and treated with activated charcoal before being discharged into sewers. Mesh bags filled with zeolite stones (~30 cL) were also added in each tank to guarantee water quality. Tanks were inspected daily and cleaned by siphoning if necessary. Furthermore, tanks were fully emptied and cleaned, together with zeolite bags, monthly from the first biometry at 2 or 3 months age onwards. With this rearing protocol, concentrations of ammonia, nitrites and nitrates measured were always below critical values for zebrafish (Lawrence, 2007) and even below quantification level in most cases.

Fish were fed three times per day, twice with pellets in the morning and the evening, and once with freshly hatched crustaceans (Artemia salina) at noon. Food pellet size was adapted to fish's mouth size using their age as a proxy. From 5 to 60 dpf, fish were fed sequentially with 100, 200 and 300 µm SDS (Special Diet Service; Dietex international, United Kingdom) with 5 to 10 days of overlap; from 60 to 70 dpf, fish were fed with a mixture of 300 µm SDS and Inicio+ 500 µm (Biomar, France), and from 70 dpf onwards, they were fed with Inicio+ 500 µm only.

Fish exposure

Fish were exposed to contaminants through food pellets spiked with a mixture of PCB and PBDE congeners following the food-pellet size depending on age sequence presented above. The spiking procedure was similar for all pellet sizes except for vessel and solution volumes that were of course adapted to the quantity of food to be spiked. PCB and PBDE congeners used in contaminated diet and their concentrations were chosen in order to represent environmental conditions.

For PCBs, the choice was based on the contamination levels and profiles reported in mussels from the Seine estuary, one of the most contaminated site along the French coastlines (Abarnou et al., 2000). This choice was justified by the fact that many exploited fish species, especially flatfish (e.g. sole, plaice, turbot) and demersal fish (e.g. cod, haddock, seabass), feed largely on benthic invertebrates and/or have nursery grounds in industrialized estuaries. More precisely, contaminated food was spiked with a mixture of 22 PCB congeners, i.e., congeners CB-8, CB-18, CB-28, CB-31, CB-44, CB-49, CB-52, CB-77, CB-101, CB-105, CB-110, CB-118, CB-128, CB-132, CB-138, CB-149, CB-153, CB-156, CB-170, CB-180, CB-187 and CB-194 at targeted concentrations between 28 and 280 ng g-1 ww (wet weight) per congener. The 22 congeners used covered a wide range of chlorinated substitutions (2-8) and a range of hydrophobicity (log Kow - octanol/water partition coefficient) from 5.07 to 7.80 (Hawker and Connell, 1988).

For PBDEs, targeted contamination levels and profiles were based on the 6 most representative congeners in marine biota and the main congener in marine sediments that were identified for priority action by OSPAR (OSPAR Commission, 2009, 2013). The rationale for this choice is that PBDE congeners are metabolized more easily and quickly than PCB congeners in low trophic level marine organisms (Grimm et al., 2015; Zhang et al., 2016). As a result, the PBDE congener profiles found in an organism's tissue will depend on the species considered as PBDE metabolism varies between species. It follows that we did not want to define the target profile of PBDE congeners based on measurements on a particular species. More specifically, contaminated food was also spiked with a mixture of 7 PBDE congeners, i.e., congeners BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-183 for marine biota and congener BDE-209 for marine sediments at targeted concentrations of between 10 and 200 ng g-1 ww per congener. The reason why BDE-209 was included is that, despite the fact it is not listed among the

main congeners in marine biota in general as it disappears at high trophic levels (Burd et al., 2014) and in pelagic biota (Desforges et al., 2014), it is the main PBDE congener found in marine sediments and thus one of the main in benthic invertebrates (together with BDE-47, BDE-99 and BDE-100; (Burd et al., 2014; Dinn et al., 2012). As explained previously, many exploited fish are benthic or demersal species feeding largely on benthic invertebrates, so that it was sensible to include BDE-209 in the mixture tested. The 7 congeners used covered a wide range of brominated substitutions (3-10) and a range of hydrophobicity (log Kow) from 6.7 to 12.1 (Kelly et al., 2008).

The precise targeted and measured concentrations for each PCB and PBDE congener are available in Table S1. The contaminated diet was prepared after dilution of a stock PCB and PBDE solution in isooctane solvent for incorporation as described in Daouk et al. (2011). The control diet was prepared in the same manner, i.e., with isooctane solvent but without addition of POPs. Control and contaminated diets were prepared with food of all sizes.

Fish were fed from their first meal (5 dpf) with either a control diet (9 replicate rearing tanks) or a contaminated diet (8 replicate rearing tanks) using the feeding schedule described earlier. Replication was meant to increase the significance and confidence level of the experimental results. Hereafter, we will refer to fish exposed to the control and the contaminated diet as SOLV (for solvent) and MIX (for mixture) fish, respectively.

Text 2.S2: Statistical analysis of cumulative reproductive output data

Total number of spawns obtained relative to solicitation number was calculated for each treatment and compared using Permutational two sample t-test. The number of eggs (fertilized or not) and the fertilization rate were measured for each spawning event. Fertilization rates were partitioned in five classes: from 0 to 20, 20-40, 40-60, 60-80 and

80-100 % and fertilization rate distribution between diets tested using a homogeneity Chi^2 test.

SOLV couples spawned 67 times on 123 solicitations (54%) versus 53 on 119 solicitations (45%) for MIX couples. Proportions of obtained spawns per solicitation were not different between treatments (Homogeneity Chi^2 test, $\chi^2 = 2.00$, df = 1, p = 0.157). Taken globally, a significant difference was found for spawn size between treatments SOLV females produced more eggs (259 ± 21 eggs) than MIX females (186 ± 19 eggs ; Permutational two sample t-test, t = -2.478, p = 0.021). Average fertilization rate was 79.94 ± 2.83 % for spawns from SOLV fish versus 73.68 ± 4.05 % for spawns from MIX fish. Classification of spawns according to their fertilization rates revealed a slightly weaker rate for MIX fish compared to SOLV fish (Homogeneity Chi^2 test, $\chi^2 = 9.63$, df = 4, p = 0.047).

Text 2.S3: Dynamic Energy Budget

The standard DEB model describes the dynamics of 3 state variables: energy reserves E (J), structural length L (cm) (or volume $V = L^3$) and maturity E_H (J) that becomes the reproduction buffer E_R (J) after puberty. The mass of the individual is then a combination of contributions from energy reserves, structural volume and reproduction buffer. Maturity represents an organism's developmental stage in terms of complexity and thus has no mass or energy and is only quantified as energy investment. Once reaching puberty, the energy allocated to maturity starts being used reproduction.

The life-cycle starts at age 0 with structural length and maturity equal to 0 and energy reserves such that reserve density E/V of the embryo equals that of the mother at egg formation. For zebrafish, we consider 4 life stages: embryonic, larval, juvenile and adult stage. Transitions between life stages occur when maturity E_H reaches maturity

thresholds E_H^b for birth b, E_H^j for metamorphosis j, and E_H^p for puberty p. Notice that birth is defined as the start of exogenous nutrition in DEB theory, i.e., mouth opening for fish larvae.

Experimental data show that zebrafish follow a sigmoid growth curve, which suggest that they undergo metabolic acceleration during larval stage, i.e., between birth b and metamorphosis j ($E_H^b \leq E_H < E_H^j$) (Augustine et al., 2011). Therefore, we use the version of the standard DEB model with metabolic acceleration of assimilation and mobilisation at larval stage (Kooijman, 2014; Lika et al., 2014). This metabolic acceleration corresponds to the concomitant increase of surface-area specific assimilation rate and energy conductance during the larval period (Kooijman, 2014). The simplest (i.e. without metabolic acceleration) standard DEB model postulates that individuals' growth is isomorphic, i.e., that their shape stays constant during growth, so that their surface area is proportional to structural volume to the power 2/3. In such case, the resulting growth curve is of von Bertalanffy type. Metabolic acceleration, and the resulting sigmoid growth curves, is obtained by assuming that during the acceleration period (larval stage), individuals follow an anisomophic growth, i.e., their shape changes with size as they increase their surface area proportional to structural volume, whereas they behave as isomorphs as embryos and after metamorphosing into juveniles.

Physical length L_W (cm) relates to structural length L via a shape coefficient δ through the relationship $L = \delta L_W$. To accommodate metabolic acceleration, two different shape coefficients need to be considered, one for larvae δ_j and another one for juveniles and adults δ_M.

In the following, rates (in units t^{-1}) are written with a single dot diacritic, e.g. $\dot{p}_A$; surface-area specific quantities (in units cm^{-2}), i.e., scaled by structural area L^2, are written in curly brackets, e.g. $\{\dot{p}_{Xm}\}$; and volume-specific quantities (in units cm^{-3}), i.e., scaled by structural volume $V = L^3$, are written in square brackets, e.g. $[\dot{p}_M]$.

Ingestion and assimilation

The feeding or ingestion rate $\dot{p}_x$ ($J.d^{-1}$) of an organism depends on its interaction with the environment through its surface area L^2 and is related to food density X (by volume) through a Holling type II functional response f (Kooijman, 2010). It is given as

$$\dot{p}_x = \{\dot{p}_{Xm}\} f s_M L^2 \tag{A.1}$$

$$\text{with } f = \begin{cases} 0 & \text{if } E_H < E_H^b, \text{ i.e., before mouth opening} \\ \frac{X}{K_X + X} & \text{otherwise} \end{cases}$$

$$\text{and } s_M = \max\left(L_b, \min\left(L, L_j\right)\right)/L_b$$

where $\{\dot{p}_{Xm}\}$ ($J.d^{-1}.cm^{-2}$) is the maximum surface-area specific ingestion rate, K_X the half-saturation constant, i.e., the food density level at which ingestion is half of its maximum value, L_b is structural length at birth and L_j is structural length at metamorphosis, so that s_M allows describing the metabolic acceleration at larval stage.

Only a fraction κ_X of ingested food energy is fixed into reserves due to faeces and digestion losses so that maximum surface-area-specific assimilation rate $\{\dot{p}_{Am}\} = \kappa_X\{\dot{p}_{Xm}\}$ ($J.d^{-1}.cm^{-2}$) and assimilation rate $\dot{p}_A$ ($J.d^{-1}$) is given as

$$\dot{p}_A = \{\dot{p}_{Am}\} f s_M L^2 \tag{A.2}$$

Reserves dynamics

Energy enters the reserve compartment at rate $\dot{p}_A$ and is mobilized at rate $\dot{p}_C$ (J.d^{-1}) to cover metabolic expenses of the organism. The dynamics of the energy reserves E are thus given by:

$$\frac{dE}{dt} = \dot{p}_A - \dot{p}_C \tag{A.3}$$

with $\dot{p}_C = E\frac{\dot{v}[E_G]L^2 s_M + \dot{p}_M}{\kappa E + [E_G]L^3}$

where $\dot{v}$ is energy conductance (cm·d^{-1}), $[E_G]$ is the volume-specific costs of synthesis of a unit structure (J.cm^{-3}), κ is the fraction of energy allocated to growth and maintenance of structure L^3, and $\dot{p}_M$ (J.d^{-1}) are the structure maintenance costs described below.

Growth dynamics

A fraction κ of mobilized energy $\dot{p}_C$ is allocated to somatic maintenance and growth. Somatic or structure maintenance costs are proportional to structural volume L^3 and are given as

$$\dot{p}_M = [\dot{p}_M]L^3 \tag{A.5}$$

where $[\dot{p}_M]$ are the volume-specific maintenance costs (J·d^{-1}·cm^{-3}). The rate of energy $\dot{p}_G$ (J.d^{-1}) invested into somatic growth is obtained after paying maintenance costs as

$$\dot{p}_G = \kappa\dot{p}_C - \dot{p}_M \tag{A.6}$$

and somatic growth is then described by

$$\frac{dL^3}{dt} = \dot{p}_G/[E_G] \tag{A.7}$$

The resulting growth in structural length L is then given by

$$\frac{dL}{dt} = \frac{1}{3}\frac{1}{\kappa[\mathrm{E}] + [E_G]}(\kappa\dot{v}s_M[E] - [\dot{p}_M]L) \tag{A.8}$$

where $[E] = E/V$ is energy reserve density (J.cm^{-3}).

Under the assumption of constant food, energy reserve density is at equilibrium and equal to $[E] = f[E_m]$ where $[E_m] = \{\dot{p}_{Am}\}/\dot{v}$ (J.cm^{-3}) is maximum energy density. Equation A8 can then be rewritten as

$$\frac{dL}{dt} = \frac{1}{3}\frac{[\dot{p}_M]}{\kappa f[E_m] + [E_G]}(f s_M L_m - L) \quad \text{(A.9)}$$

where maximum structural lentgh $L_m = \kappa\{\dot{p}_{Am}\}/[\dot{p}_M]$ results from the balance between food assimilation, which is surface-specific, and structure maintenance, which is volume-specific.

At larval stage $s_M = L/L_b$, so that growth in structural length is exponential as shown by replacing s_M and rearranging equation (A.9)

$$\frac{dL}{dt} = \dot{r}L \quad \text{(A.10)}$$

with $\dot{r} = \frac{1}{3}\frac{[\dot{p}_M]}{\kappa f[E_M]+[E_G]}\frac{(fL_m - L_b)}{L_b}$ (d^{-1})

From juvenile stage onward $s_M = L_j/L_b$, so that growth in structural length is of von Bertalanffy type as shown again by replacing s_M and rearranging equation (A.9)

$$\frac{dL}{dt} = \dot{r}_B(L_\infty - L) \quad \text{(A.11)}$$

with $\dot{r}_B = \frac{1}{3}\frac{[\dot{p}_M]}{\kappa f[E_M]+[E_G]}$ (d^{-1})

and $L_\infty = f\frac{L_j}{L_b}L_m$.

Actual asymptotic or ultimate structural length L_∞ is thus the maximum length L_m modulated by the functional response f and the acceleration factor s_M.

Maturation and reproduction dynamics

The remaining fraction $1-\kappa$ of mobilized energy $\dot{p}_C$ is allocated first to maturity maintenance and second to increase in maturity E_H before puberty $E_H < E_H^p$ or in the reproduction buffer E_R after puberty $E_H = E_H^p$. Maturity maintenance costs are proportional to the level of maturity E_H according to

$$\dot{p}_J = \dot{k}_J E_H \tag{A.12}$$

where $\dot{k}_J$ is the maturity maintenance rate coefficient (d^{-1}). The rate of energy $\dot{p}_R$ ($J.d^{-1}$) invested into maturity ($E_H < E_H^p$) or reproduction ($E_H > E_H^p$) is obtained after paying maintenance costs as

$$\dot{p}_R = (1-\kappa)\dot{p}_C - \dot{p}_J \tag{A.13}$$

so that the dynamics of maturity before puberty $E_H < E_H^p$ are given by

$$\frac{dE_H}{dt} = \dot{p}_R \tag{A.14a}$$

and those of the reproduction buffer after puberty $E_H = E_H^p$ by

$$\frac{dE_R}{dt} = \dot{p}_R \tag{A.14b}$$

Notice that after puberty, maturity no longer increases and stays fixed at $E_H = E_H^p$. During adulthood, the mean reproduction rate $\dot{R}$, i.e., the average number of eggs produced per unit of time is given by

$$\dot{R} = \frac{\kappa_R}{E_0}\dot{p}_R \tag{A15}$$

where κ_R is reproduction efficiency that accounts for overheads paid for reproduction and E_0 is the initial amount of energy into an egg or the cost of an egg (J). The number of

eggs N produced from the reproduction buffer at each reproductive event is then given by

$$N = \frac{\kappa_R}{E_0} E_R = \frac{\kappa_R}{E_0} \int_t^{t+\Delta t} \dot{p}_R(x) dx \tag{A.16}$$

where Δt is the time period between 2 reproduction events.

Temperature correction of physiological rates

In ectotherms, physiological rates will depend on environmental temperature as no control on internal temperature is exerted. This effect of temperature is captured in the DEB model by the Arrhenius relationship according to which a physiological rate at temperature T (K) within the species tolerance range is obtained as

$$\dot{k}(T) = \dot{k}_R \exp\left(\frac{T_A}{T_R} - \frac{T_A}{T}\right) \tag{A.17}$$

where $T_A = 3000$ K is the Arrhenius temperature that gives the sensitivity of physiological rates to temperature and $\dot{k}_R$ is the known value of the physiological rate at a reference temperature T_R taken here to be $T_R = 293.15$ K (Augustine et al., 2011).

Table 2.S1: Data weight in calibration procedure

Symbol	w_{ij}
a_b	1
a_j	1
a_p	1
a_m	1
L_w^b	1
L_w^j	1
L_w^p	1
L_w^∞	1
W_w^∞	1
Length at age	0.4545
Length vs wet weight	0.9091
Wet weight at age	0.4545
Cumulative number of eggs at age	0.50

Table 2.S2: DEB parameter initial values

Symbol	*Initial value*
f	1
δ_J	0.105
δ_M	0.133
$\dot{v}$	0.028
κ	0.437
$\dot{k}_J$	0.017
$[\dot{p}_M]$	500.9
$[E_G]$	5652
E_H^b	0.5402
E_H^j	19.66
E_H^p	2062

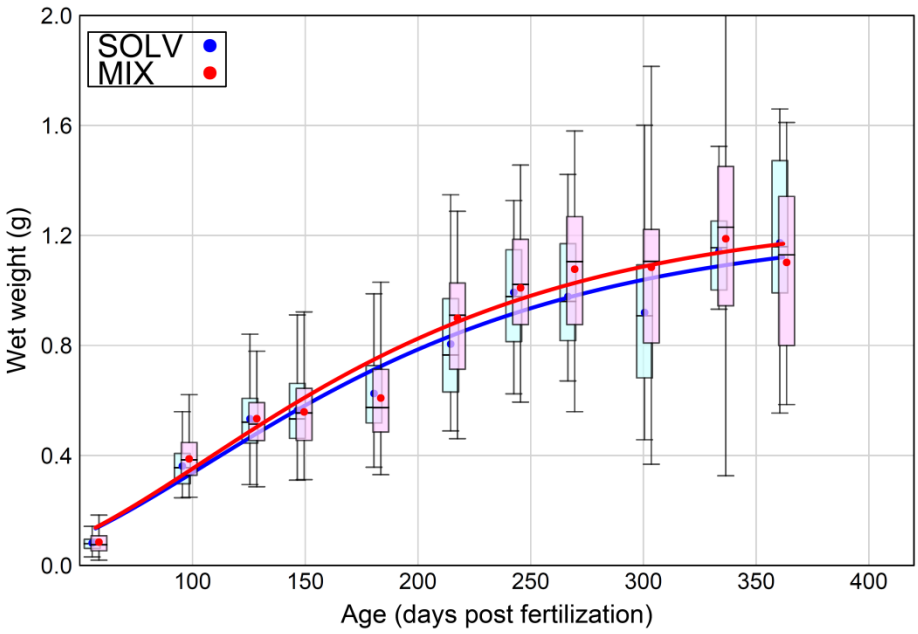

Fig. 2.S1. Effect of chornic dietary exposure to a PCB and PBDE congener mixture on growth in terms of weight in zebrafish. Columns correspond to the sex of fish. Boxplots represent observations (grey for SOLV and white for MIX in white) and curves represent the DEB model predictions (solid line for SOLV and dashed line for MIX). For boxplots, the bottom and top of the box are the first and the third quartiles of the data distribution, the horizontal segment and the dot inside the box are the median and the mean, respectively, and whiskers represent the most extreme data point within the 1.5 interquartile range.

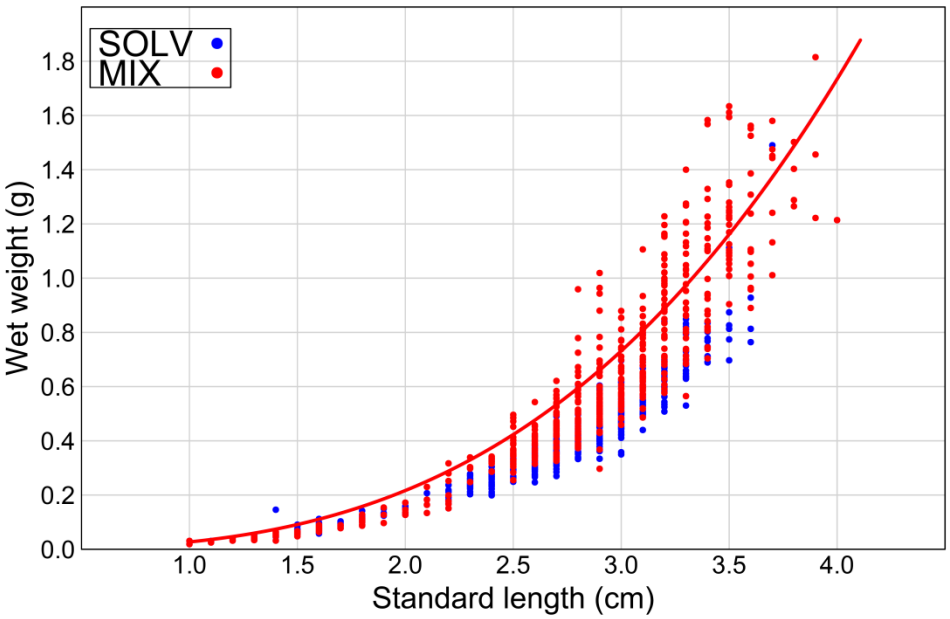

Fig. 2.S2. Effect of chronic dietary exposure to a PCB and PBDE congener mixture on the length-weight relationship in zebrafish. Dots represent observations and curves represent the DEB model predictions.

3. CHAPITRE 3 : Fish population dynamics are affected by individual-level effects of chronic dietary exposure to an environmental mixture of PCBs and PBDEs

3.1 Introduction du chapitre

Les modifications des caractéristiques physiologiques des individus et leurs conséquences en termes de traits d'histoire de vie, en particulier la survie, la croissance, le développement et la reproduction, peuvent avoir des répercussions sur la dynamique des populations de poissons. Ces changements de traits d'histoire de vie peuvent être causés par des facteurs de stress environnementaux comme l'exposition aux contaminants.

L'objectif de ce chapitre était d'évaluer les conséquences des réponses individuelles à l'exposition au mélange de PCB et de PBDE, via les deux PMoAs identifiés à partir du modèle DEB (décrit dans le chapitre 2), sur la dynamique de population de poissons et la productivité des pêcheries commerciales.

Un modèle de population structurée physiologiquement (PSP) a été couplé (i) au modèle DEB pour décrire la trajectoire ontogénique des traits d'histoire de vie des individus et (ii) à un modèle de ressource dynamique pour inclure la boucle de rétroaction environnementale entre les individus et leur ressource. Le couplage DEB-PSP a permis, dans un premier temps, d'inférer les conséquences potentielles des contaminants chez les populations de poissons naturels mais aussi chez les populations de poissons exploités en incluant au modèle DEB-PSP, dans un second temps, un patron d'exploitation en fonction de la taille correspondant à celui du stock de sole commune de la Manche Est mis à l'échelle, en termes de taille et de longévité, pour la population de poisson-zèbre. Pour ce dernier point, le raisonnement était de considérer le stock de sole commune de Manche Est en lien avec l'estuaire de Seine comme un cas d'étude typique de pêcherie associée à un bassin versant et un estuaire fortement pollué.

Fish population dynamics are affected by individual-level effects of chronic dietary exposure to an environmental mixture of PCBs and PBDEs

In preparation

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Abstract

Changes in individuals' life-history traits, notably growth, development, reproduction and mortality can have serious implications for fish population dynamics and, consequently, on the structure of ecosystems. These changes can result from several processes, such as phynotypic plasticity and evolutionary responses, and be associated to many environmental factors, including stressors such as contaminants. In the case of contaminants, physiological modes of action (PMoA) can be used as parameters of change of life-history traits at the individual level to study the consequences at the population level. In this study, we evaluated the population dynamical consequences of the individual life-history effects of two PMoAs, the sharing of energy between growth and reproduction and reproduction costs, associated with long-term dietary exposure to a marine environmental mixture of PCBs and PBDEs in a model fish species, namely zebrafish (Horri et al., 2018b). A Physiologically Structured Population Model (PSP) was coupled with (i) a Dynamic Energy Budget (DEB) model (described in Horri et al., 2018b) to describe the ontogenetic trajectory of individuals' life-history traits, and (ii) a dynamic resource model to include the feedback loop between individuals and their resource. The DEB-PSP model predicted a lower abundance and a higher biomass in exposed population compared to control population. These results could be explained by a weaker fecundity that leads to a lower abundance of individuals and an increased growth, which results into a larger biomass by overcompensating the numerical deficit for populations. The bifurcation analysis of the independent effects of the two PMoAs showed that they participate jointly in the decrease of the abundance and the increase of the biomass of the exposed population. The model also predicted that lower intensities of size-independent natural mortality are necessary to drive exposed population to extinction relative to control population, whereas higher intensities are necessary when mortality decreases with size as for predation mortality. When fishing mortality is added to predation mortality and increases with size, as in trawl fisheries, the control population seems to persist at higher exploitation rates than the exposed one. Finally, the exposed population may allow for a larger maximum sustainable yield but at a lower exploitation rate, thus exposing the contaminated population to a higher risk of overexploitation.

Keywords: population dynamics, contaminants, energy allocation, exploitation, Endocrine-disrupting chemicals

3.2 Introduction

Endocrine-disrupting chemicals (EDCs) are a family of chemicals that exert an effect on organisms by mimicking endogenous hormones leading to their impaired functioning (Mills and Chichester, 2005). Among them are persistent organic pollutants (POPs) (Geyer et al., 2000) among which two families, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), are characterized by their persistence. This explain why, despite an almost complete ban (for several decades in the case of PCBs) PCBs and PBDEs are still present, sometimes at high level, in some compartments of aquatic environment (Muir et al., 2003; Shaw and Kannan, 2009). Because of their elevated lipophilicity, PCBs and PBDEs are significantly bioaccumulated while being biomagnified through trophic transfer in most biotic compartments, notably in aquatic ecosystems (e.g. mollusks, fish, seals; Couderc et al., 2015; Johansson et al., 2006; Letcher et al., 2009; Mizukawa et al., 2009).

Due to their characteristics, these compounds are known to have a high potential to affect life-history traits related to reproduction, growth and survival in fish (Daouk et al., 2011; Horri et al., 2018a; Lyche et al., 2010, 2011). Such alterations of life-history traits can be associated with the so-called physiological modes of actions (PMoAs) of the chemicals incriminated. PMoAs are defined as the sets of pathways or energy fluxes related to different physiological processes such as maintenance, reproduction or growth through which contaminants affect biological endpoints. In the specific context of bioenergetics models such as Dynamic Energy Budget (DEB) theory (Kooijman, 2010), the PMoAs can be understood as changes in energetic parameters, such as an increase in reproduction costs or a decrease in assimilation energy, leading to changes in different life-history endpoints (Álvarez et al., 2006). Such bioenergetic approaches are very helpful to study the PMoAs of contaminants because they allow accounting for the interactions between several endpoints,

provide precise information on the altered bioenergetic parameters and permit disentangling potential confounding effects affecting the association between PMoAs and endpoints' alteration. For example, as life-history endpoints are connected by the process of energy allocation (e.g. the energetic trade-off between growth and reproduction), in the case of a simple observation, we cannot know whether the endpoint (e.g. reproduction) is directly affected by the contaminant (e.g. costs of egg production; Kooijman and Bedaux, 1996) or indirectly affected through an effect on another endpoint (e.g. assimilation thus affecting energy available for reproduction; Kooijman and Bedaux, 1996). Beyond PCBs and PBDEs, generally the PMoAs of contaminants acting as EDCs have been studied in a few species through approaches combining experiments and bioenergetic modelling, especially DEB models (nematodes: Álvarez et al., 2006; zebrafish: Augustine et al., 2012; Horri et al., 2018; *Daphnia magna*: Kooijman and Bedaux, 1996; Martin et al., 2013a). Studies were limited to the effect of single molecules except for Horri et al., (2018b) who showed that chronic dietary exposure of zebrafish to a mixture of PCBs and PBDEs that is mimicking the marine environment triggered two PMoAs: an increase in the allocation of energy towards growth at the expense of reproduction and an increase in the energy allocated to produce an egg or in the cost of an egg.

Changes in individuals' life-history traits, notably growth, development, reproduction and mortality can have serious implications for fish population dynamics and, consequently, on the structure of ecosystems (De Roos et al., 2003). These changes can result from several processes, phenotypic plasticity and/or evolutionary responses (Stearns, 1992), and be related to many environmental factors, including stressors (e.g. food availability, temperature, contaminants). In the case of contaminants, PMoAs can be used as parameters of change of life-history traits at the individual level to study the consequences at the population level as it has been shown in several previous studies (Jager and Klok, 2010; Klok

and de Roos, 1996; Kooijman and Metz, 1984; Martin et al., 2014, 2013a). Although experimental population dynamics studies can be carried out on small-sized and short-lived model species such as *Daphnia magna* (e.g. Martin et al., 2013), the scaling-up of individual-level effects of PMoAs to the population level often requires modelling approaches, especially for longer-lived vertebrates such as fish. For this purpose, coupled approaches combining an individual-level bioenergetics model and a population model are often used. Notably, there has been a tradition since the 1980's of coupling DEB models to population models of increasing realism to study population-level effects of contaminants: continuous time Euler-Lotka integral equation (Jager and Klok, 2010; Jager et al., 2007; Klok and de Roos, 1996; Kooijman and Metz, 1984), Leslie matrix models (Billoir et al., 2007; Klok and de Roos, 1996) and individual based models (Martin et al., 2013a, 2013b). However, to our knowledge none of these studies dealt with vertebrates, and thus fish, and all of them focused on the effect of singles molecules whereas individuals are generally exposed to mixtures of contaminants in the environment.

In the context of fisheries ecology, exploited fish species are expected to be more specifically vulnerable to contaminants because they are subjected to additional pressure, i.e., fishing mortality, which can be size selective (Barnthouse et al., 1990; Goodyear, 1985). Beyond simple interaction between contaminant-induced and fishing mortality, sublethal effects on life-history traits could also affect a commercial fish population's capability of withstanding fishing pressure. For instance, a low fecundity associated with a contaminant stress could also induce a decline of fish population (Goodyear, 1985) by affecting fish population recruitment and thus a decrease in associated fisheries productivity for commercial species (Vasseur and Cossu-Leguille, 2006). More generally, some scientists suspected that contaminants have contributed to the decline of some wild marine fish populations (Barnthouse et al., 1990; Hamilton et al., 2015) and it has been suggested that the

productivity of some marine fish stocks could be altered due to recruitment impairment caused by nursery habitat degradation in relation to pollutant accumulation (Gilliers et al., 2006; Riou et al., 2001; Rochette et al., 2010). However, the effects of contaminants on the population dynamics of fish, especially of exploited marine fish, are still poorly investigated. In the specific case of PCBs and PBDEs, Horri et al. (2018a) showed that exposure to a marine environmental mixture caused (i) individual zebrafish to grow to larger sizes, which, if the effect can be transposed to exploited marine fish species, would expose individuals to higher fishing mortality, and (ii) delayed spawning probability, which could decrease spawning stock biomass and potentially affect recruitment.

In the present study, we try to overcome some of the limitations previously identified and develop a modelling study of the population dynamical consequences of the individual life-history effects of two PMoAs, the sharing of energy between growth and reproduction and reproduction costs, associated with long-term dietary exposure to a marine environmental mixture of PCBs and PBDEs in a model fish species, namely zebrafish (Horri et al., 2018b). The extrapolation of the effects of the two PMoAs from the individual to the population level was performed by coupling a DEB model describing zebrafish bioenergetics when exposed to the PCB/PBDE mixture (described in Horri et al., 2018b) and a physiologically structured population model (PSP; De Roos, 1997; Metz and Diekmann, 1986). Resulting population dynamics are considered in the context of natural fish populations but also in the context of exploited fish populations by including in a second step size-dependent fishing mortality. Fishing size selectivity observed for the North Sea sole (WGNSSK 2017; ICES, 2017) was rescaled to zebrafish range size and applied in the model. The coupled DEB-PSP model was then used (i) to compare non-exposed and exposed populations, (ii) to investigate the consequences of varying intensities of the two PMoAs triggered by the PCB/PBDE mixture

and (iii) to explore the difference in non-exposed and exposed population dynamical responses to varying strength of natural and fishing mortalities.

3.3 Materials and methods

This study was conducted under the approval of the Animal Care Committee of Poitou-Charentes # 84 COMETHEA (France) under project authorization number CE2012-23.

3.3.1 Model formulation

The DEB-PSP model coupling individual's bioenergetics to population dynamics was applied to experimental zebrafish (consumer) populations: some non-exposed (control populations) and some exposed to a marine environmental mixture of PCBs and PBDEs through diet (see Horri et al. (2018a) for a detailed description of the experimental design). The model relates the life-history processes that will determine an individual's characteristics or state (*i*-state: e.g. age, length, energy reserves) to its environment characterized by a collection of influential biotic and abiotic factors or environmental state (*E*-state: e.g. food density, temperature) and to the population-level processes (death rate, birth rate) that will determine the population's state (*p*-state: e.g. number density, biomass density, size-structure) (De Roos et al., 1992; Metz and Diekmann, 1986).

3.3.1.1 Individual-level processes

The individual-level processes of feeding, growth and reproduction are modeled using the DEB model developed for control and exposed populations in Horri et al. (2018b). In short, the DEB model describes the rates at which an individual acquires energy throughout its life history by feeding and utilizes it between four state variables, namely reserve E, structural volume V or length $L = V^{1/3}$ and maturity E_H (for larvae and juveniles) or reproduction buffer E_R (for adults) (see Horri et al., 2018b Fig. 2.1) (Kooijman, 2010; van der Meer, 2006; Sousa et al., 2010). The energy ingested at rate $\dot{p}_X$ from the resource R is assimilated at rate

$\dot{p}_A$ and enters the reserve compartment E. Then, it can be mobilized at rate $\dot{p}_C$ and allocated to structural length L and maturity/reproduction buffer E_H/E_R following the so-called kappa rule where a fraction κ of mobilized energy $\dot{p}_C$ goes to growth of structural length L at rate $\dot{p}_G$ plus its maintenance at rate $\dot{p}_M$ and a fraction $(1-\kappa)$ goes to increase in maturity E_H (for larvae and juveniles) or in reproduction buffer E_R (for adults) at rate $\dot{p}_R$ plus maturity maintenance at rate $\dot{p}_J$. Maturity represents the individual's developmental stage in terms of complexity until reaching adulthood. We represent three life stages: larval, juvenile and adult stage. The life-history transitions from one stage to another occur when maturity E_H reaches the corresponding maturity thresholds, i.e., E_H^j for metamorphosis (j) and E_H^p for puberty (p). The increase in maturity E_H stops at puberty because individuals start allocating energy into reproduction E_R instead. We neglect the embryonic phase, so that all individuals within each cohort are assumed to be born at the same time with the same structural length L_b and the same initial maturity energy of E_H^b. In the following the main elements of individual-level processes necessary for the DEB-PSP coupling are described. A detailed description of the DEB model can be found in Horri et al. (2018b).

Assimilation rate is proportional to the individual's surface area L^2 according to a Holling type II functional response

$$\dot{p}_A(L,R) = \kappa_X\{\dot{p}_{Xm}\}f(R)s_M(L)L^2 \quad (1)$$

with κ_X the assimilation efficiency, $\{\dot{p}_{Xm}\}$ (J.d^{-1}.cm^{-2}) the maximum surface-area specific ingestion rate, $f(R) = \frac{R}{K+R}$ the functional response, R food density (J.L^{-1}), K the half-saturation coefficient (J.L^{-1}), and $s_M(L)$ the metabolic acceleration factor (see below).

Zebrafish growth curves along the whole life-cycle are sigmoid, which suggests that zebrafish metabolism accelerates after birth until metamorphosis. Therefore, we use the

version of the standard DEB model with metabolic acceleration of assimilation and mobilization at larval stage (Kooijman, 2014; Lika et al., 2014). This is accounted for by using the metabolic acceleration factor

$$s_M(L) = \max\left(L_b, \min\left(L, L_j\right)\right)/L_b) \quad (2)$$

where L_b and L_j are structural length at birth and metamorphosis, respectively. The growth rate in structural length is then described as

$$\frac{dL}{dt} = g(L,R) = \frac{1}{3}\frac{[\dot{p}_M]\dot{v}}{\kappa\kappa_X\{\dot{p}_{Xm}\}f(R) + [E_G]\dot{v}}(f(R)s_M(L)L_m - L) \quad (3)$$

where $[\dot{p}_M]$ is the volume specific somatic maintenance costs (J.d^{-1}.cm^{-3}), $\dot{v}$ is the energy conductance (cm.d^{-1}), $L_m = \kappa\kappa_X\{\dot{p}_{Xm}\}/[\dot{p}_M]$ is the maximum structural length (cm), κ is the fraction of energy allocated to somatic maintenance and growth, and $[E_G]$ is the cost of synthesis of a unit of structure (J.cm^{-3}). Replacing the acceleration factor $s_M(L)$ by its value according to life-history stage, growth in structural length is then exponential during larval stage ($L_b \leq L < L_j$ so that $s_M(L) = L/L_b$) and of Von Bertalanffy type after metamorphosis, i.e., during juvenile and adult stages ($L_b < L_j \leq L$ so that $s_M(L) = L_j/L_b$) (see Horri et al., 2018b for more details).

The increase in maturity energy was modeled by considering maturity energy scaled by maximum assimilation rate $U_H = E_H/(\kappa_X\{\dot{p}_{Xm}\})$ for better tractability:

$$\frac{dU_H}{dt} = h(L, U_H, R) = \begin{cases} \dfrac{(1-\kappa)}{1 + g/f(R)} L^2 \left(g s_M(L) + \dfrac{L}{L_m}\right) - \dot{k}_J U_H & \text{if } U_H < U_H^p \\ 0 & \text{otherwise} \end{cases} \quad (4)$$

where $g = \frac{[E_G]\dot{v}}{\kappa\kappa_X\{\dot{p}_{Xm}\}}$ is the energy investment ratio, $\dot{k}_J$ is the maturity maintenance rate coefficient (d^{-1}).

We assume continuous reproduction that is represented by a size-dependent birth rate modelled as:

$$b(L, U_H, R) = \begin{cases} 0 & \text{if } U_H < U_H^p \\ \dfrac{\kappa_R \, h(L, U_H^P, t, R)}{e_0} & \text{otherwise} \end{cases} \quad (5)$$

where κ_R is the reproduction efficiency and e_0 is the scaled energy invested in an egg (d.cm^2). As explained before, the energy rate h that was invested into maturity during larval and juvenile stages (Eq. 4) is then invested into reproduction at adult stage. Notice that maturity level no longer increases during adult stage, so that rate h depends on the puberty maturity threshold U_H^p.

Eq. 3 to 5 are based on the assumption that energy reserves E dynamics are fast compared to structure and maturity/reproduction buffer dynamics and more generally population dynamics, so that reserve density $[E] = E/V$, i.e., energy reserve per unit volume, is always at equilibrium (Maury and Poggiale, 2013). It can be shown that this equilibrium value is equal to $[E]^* = f(R)\frac{\kappa_X\{\dot{p}_{Xm}\}}{\dot{v}}$, so that scaled energy density is fully determined by the functional response and resource abundances $e^* = \frac{\dot{v}[E]^*}{\kappa_X\{\dot{p}_{Xm}\}} = f(R)$.

Given that in ectotherms, physiological rates depend on environmental temperature, a temperature correction factor was defined according to the Arrhenius relationship $\dot{p}(T) = \dot{p}(T_R)\exp(\frac{T_A}{T_R} - \frac{T_A}{T})$, where $\dot{p}$ is the physiological rate, $T_A = 3000$ K is the Arrhenius temperature, T_R is the reference temperature at which the physiological rate $\dot{p}(T_R)$ is supposed to be known and which was taken to be 293.15 K, and T is the environmental temperature actually experienced (K). This temperature-dependent correction factor was applied to primary metabolic rates, i.e., ingestion rate $\{\dot{p}_{Xm}\}$ and maintenance rate $[\dot{p}_M]$.

We assume four types of mortality for consumers throughout lifetime. First, there is a constant egg/embryo mortality μ_0 that reflects the high background and predation mortality at these stages. Then, after birth we assume a size-dependent natural mortality rate $\mu(L)$ that decreases with weight or length as expected from predation. In case of exploited population, a size-dependent fishing mortality $F(L)$, that increases with length, also occurs. Total death rate after birth was then obtained as $d(L) = \mu(L) + F(L)$. Finally, all individuals die from ageing after reaching a maximum age, a_{m}.

The size-dependent natural mortality was adapted from Beaudouin et al., (2015) and calibrated for our data :

$$\mu(L) = M\, W(L)^{\pi} \qquad (6)$$

M is the weight-specific natural mortality or natural mortality at unit weight ($d^{-1}.g^{-\pi}$), π is the allometric scaling exponent of mortality with weight, $W(L)$ is the weight of the individual (g) that is converted from structural length L according to the length-weight relationship $W(L) = (\delta L)^3$. The shape coefficient δ is used to convert physical length (L_W) to structural length (L) through the relationship $L = \delta L_W$. To accommodate metabolic acceleration, two different shape coefficients need to be considered, one for larvae δ_j and another one for juveniles and adults δ_M.

Size-dependent fishing mortality was modelled as increasing with individual's physical length $L_W = L/\delta$ according to a sigmoid selectivity curve:

$$F(L) = \frac{F_{max}}{1 + e^{F_1(L/\delta - L_{W,50})}} \qquad (7)$$

where F_{max} is the maximum fishing mortality (d^{-1}), F_1 is the slope of the sigmoid fishing size selectivity (cm^{-1}), and $L_{W,50}$ is the physical length (cm) at $F(L_{W,50}) = 0.5$. The sigmoid shape of the size-dependent fishing mortality is typical of the exploitation pattern of trawlers that

are operating most industrial marine demersal fisheries. Here, we took the size selectivity pattern observed for North Sea sole (ICES, 2017) and rescaled the parameters (F_{max}, F_1 and $L_{W,50}$) relative to asymptotic size and to a daily time unit.

The individual-level rates of assimilation $\dot{p}_A(L,R)$, growth $g(L,R)$, maturation $h(L,U_H,R)$, birth, $b(L,U_H,R)$, and death $d(L)$ depend entirely on structural length L and scaled maturity U_H that are thus the *i*-states while resource density R is the *e*-state necessary to define them. Age a was considered as an additional *i*-state in order to be able to represent the change of the other *i*-states or individual characteristics with age.

3.3.1.2 Resource dynamics

Consumers rely on a shared unstructured resource that follows, in the absence of consumers, semi-chemostat dynamics, which is considered to have a stabilizing influence on the population dynamics compared to logistic growth dynamics (Persson et al., 1998; Roos et al., 1990). Typical semi-chemostat dynamics are complemented by a term representing consumption by the consumer population:

$$\frac{dR}{dt} = \rho(R_{max} - R) - \int_{a_b}^{a_{max}} \int_{L_b}^{+\infty} \int_{U_H^b}^{U_H^p} \{\dot{p}_{Xm}\} f(R) s_M(L) L^2 n(a,L,U_H,t) da\, dL\, dU_H \qquad (8)$$

where R_{max} is the maximum resource density that is reached in the absence of consumers and ρ is the turn-over rate. The interaction between the consumer and the resource constitutes an indirect density-dependence representing the environmental feedback loop (De Roos, 1997). Semi-chemostat resource dynamics are particularly adapted when the resource has a physical refuge, or includes small size classes escaping predation that grow into vulnerable size classes, as for zooplankton preyed upon by planktivorous fish (Persson

et al., 1998), or to represent an abundant resource at large spatial scale preyed upon locally by consumers.

3.3.1.3 Population-level processes

At the population level, we assumed an age-length-maturity structured population for consumers on the basis of the modeled individual processes $\dot{p}_A(L,R)$, $g(L,R)$, $h(L,U_H,R)$, $b(L,U_H,R)$ and $d(L)$ and the *i*-states determining them. The population is represented by a density function $n(a,L,U_H,t)$ describing the distribution of individuals across *i*-states a, L, and U_H at any time t. The dynamics equations formulated for both consumers and resource are given in Table 3.1.

Table 3.1. Dynamics equations of the age-length-maturity structured consumer population and the unstructured resource.

Consumer dynamics
$$\frac{\partial n(a,L,U_H,t)}{\partial t}+\frac{\partial n(a,L,U_H,t)}{\partial a}+\frac{\partial g(L,R)n(a,L,U_H,t)}{\partial L}+\frac{\partial h(L,U_H,R)n(a,L,U_H,t)}{\partial U_H}=-d(L)n(a,L,U_H,t)$$
Boundary condition
$$g(L_b,R)h(L_b,U_H,R)n(a_b,L_b,U_H^b,t)=\int_{a_p}^{a_{max}}\int_{L_p}^{L_\infty} b(L,U_H^p,R)n(a,L,U_H^p,t)\,da\,dL$$
Resource dynamics
$$\frac{dR}{dt}=\rho(K-R)-\int_{a_b}^{a_{max}}\int_{L_b}^{+\infty}\int_{U_H^b}^{U_H^p}\{\dot{p}_{Xm}\}f(R)s_M(L)L^2n(a,L,U_H,t)da\,dL\,dU_H$$
Initial conditions
$$n(0,a,L,H)=\Psi(a,L,H)$$ $$R(0)=R_0$$

Consumer dynamics describe the change in the consumer density $n(a,L,U_H,t)$, over time t, due to individual aging $\frac{\partial n(a,L,U_H,t)}{\partial t}$, growth $\frac{\partial g(L,R)n(a,L,U_H,t)}{\partial t}$, development in terms of maturity

$\frac{\partial h(L,U_H,R)n(a,L,U_H,t)}{\partial t}$ and mortality $-d(L)n(a,L,U_H,t)$, respectively. The boundary condition accounts for the consumer population renewal due to reproduction. The left side of the boundary condition represents the rate at which newborns with length L_b and maturity level U_H^b integrates the population through reproduction. The right side of the boundary condition represents how this rate depends on the product between adult's fecundity and their density, $b\left(L,U_H^p,R\right)n\left(a,L,U_H^p,t\right)$, over their age $\left[a_p,a_{max}\right]$ and length $\left[L_p,L_\infty\right]$ ranges. Notice that there is no summation other the scaled maturity level as all reproducing adults have the same scaled maturity, which is equal to the puberty maturity threshold U_H^p. Resources dynamics are described as in Eq. 8 and initial conditions represent the initial, arbitrarily chosen, states of the structured consumer population and the unstructured resource, respectively, at $t = 0$.

3.3.2 Model calibration and simulations

3.3.2.1 Calibration

To investigate the consequences of the individual-level effects of the marine environmental mixture of PCB and PBDE on fish population dynamics, the coupled DEB-PSP model was separately calibrated for control and exposed zebrafish populations (Horri et al., 2018a,b). For each population, we formulated two versions of the model, one without fishing mortality and another with fishing mortality. The idea here was to determine whether the inclusion of size-dependent fishing mortality could cause a change in exposed population dynamics relative to control ones due to the interaction of fishing size-selectivity with the increase in size-at-age due to chronic exposure to the PCB/PBDE mixture that has been shown in Horri et al. (2018a,b). Moreover, in a context of extrapolation to commercially exploited species such as sole, these results would give an idea of whether exposure to these contaminants affects the degree of exploitation a population can withstand.

Parameters values of the DEB part of the consumer model were estimated in Horri et al. (2018b). The other parameters of the consumer dynamics model were either calibrated so as to obtain a realistic consumer biomass (M) or scaled from literature values (F_{max}, F_1 and $L_{W,50}$) or taken as is from the literature (π and μ_0; Beaudouin et al., 2015). The parameters of the resource dynamics model, ρ and K, were also adjusted to obtain a realistic consumer biomass. All parameters values are given in Table 3.2. Numerical integration of the DEB-PSP model was performed using the Escalator Boxcar Train (EBT) method (Metz and Diekmann, 1986; de Roos, 1988) that is implemented in the EBTtool package (https://staff.fnwi.uva.nl/a.m.deroos/EBT/Software/index.html).

Hereafter, we will refer to control and exposed population as SOLV and MIX population, respectively

Table 3.2. Description of constant parameters, their numerical values for SOLV and MIX zebrafish populations and state variables used in the DEB-PSP model.

Symbol	SOLV value	MIX value	Unit	Description	Source
i-state					
a	–	–	d	Age	–
L	–	–	cm	Structural length	–
U_H	–	–	J	Scaled maturity	–
p-state					
$n(a, L, U_H, t)$	–	–	L^{-1}	Population distribution across age, length and maturity at time t	–
e-state					
R	–	–	$J.L^{-1}$	Resource density	–
Parameters					
L_b	0.038	0.042	cm	Structural length at birth	
κ_X	1.250	1.250	–	Assimilation efficiency	a
$\{\dot{p}_{xm}\}$	106.679	106.810	$J.d^{-1}.cm^{-2}$	Maximum ingestion rate	a
K	16.412	16.432	$J.L^{-1}$	Half-saturation coefficient	a
$\dot{v}$	0.025	0.026	$cm.d^{-1}$	Energy conductance	a
$[\dot{p}_M]$	165.3	167.5	$J.day^{-1}.cm^{-3}$	Volume specific somatic maintenance costs	a
κ	0.653	0.709	–	Fraction of energy allocated to somatic maintenance and growth	a
$[E_G]$	5224	5229	$J.cm^{-3}$	Cost of synthesis of a unit of structure	a
U_H^b	0.002	0.002	$d.cm^2$	Scaled cumulated energy invested in maturity at birth	a
U_H^j	0.051	0.051	$d.cm^2$	Scaled cumulated energy invested in maturity at metamorphosis	a
U_H^p	5.908	5.908	$d.cm^2$	Scaled cumulated energy invested in maturity at puberty	a
$\dot{k}_J$	0.002	0.002	d^{-1}	Maturity maintenance rate coefficient	a
κ_R	0.95	0.95	–	Reproduction efficiency	a
e_0	0.008	0.009	$d.cm^2$	Scaled energy invested in an egg	a
T_A	3000	3000	K-	Arrhenius temperature	a
T_R	293.15	293.15	K	Reference temperature	a
T	299.15	299.15	K	Actual temperature	a
μ_0	0.025	0.025	d^{-1}	Egg/embryo mortality	b
M	0.175	0.175	$d^{-1}.g^{-\pi}$	Weight-specific natural mortality coefficient	c
π	-0.382	-0.382	–	Allometric scaling exponent of natural mortality with weight	b
F_{max}	1.369×10^{-3}	1.369×10^{-3}	d^{-1}	Maximum fishing mortality	d
F_1	-11.602	-11.602	dcm^{-1}	Slope of the sigmoïd fishing size-selectivity	d
$L_{W,50}$	1.696	1.696	cm	Physical length at $F(L_{W,50}) = 0.5$	d
δ_j	0.13	0.14	–	Larvae shape parameter	a
δ_M	0.25	0.26	–	Juvenile/.adult shape parameter	a
a_m	1643	1643	d	Maximum age	c
ρ	0.18	0.18	d^{-1}	Resource growth rate or tunr-over rate	c
R_{max}	10	10	$J.L^{-1}$	Maximum resource density or carrying capacity	c

a: Horri et al. (2018b), b: Beaudouin et al. (2015), c: this study, d: ICES (2017)

3.3.2.2 Comparison of SOLV and MIX populations

In a first step, SOLV and MIX populations were simply compared according to several characteristics: *i*-state and life-history trait ontogenetic trajectory (i.e. according to age), density of individuals (number of individuals per unit volume or number density, $\#/L^{-1}$) and of their biomass (biomass density, $g.L^{-1}$) either in the total population or per life-stage, and the average *i*-state per life-stage.

3.3.2.3 Effect of PCB and PBDE physiological modes of action (PMoAs) on population dynamics

In order to study more generally the effect of the previously identified PCB and PBDE PMoAs, the allocation of energy towards growth κ and the cost of an egg e_0 (Horri et al., 2018b), on population dynamics, we performed a bifurcation analysis by varying these two parameters, called here bifurcation parameters, at given intervals to identify regions of potential alternative dynamical attractors of the population as well as bifurcation parameter values at which the population can persist or go extinct. This bifurcation analysis of PMoAs was performed by setting all other parameters to their SOLV value, the idea being here to understand how the population behaves if the amplitude of the effect of contaminants through these modes of action changes, as a result of varying levels of contamination for instance. The bifurcation range of κ and e_0 were chosen arbitrarily as ± 50 % around their SOLV value (Table 3.3). The principle of the bifurcation analysis is to increase progressively the bifurcation parameter at regular intervals until the exploration of the whole bifurcation range. The initial state of the population for a particular value of the bifurcation parameter is the final state that was reached for the previous value of that parameter (https://staff.fnwi.uva.nl/a.m.deroos/EBT/Software/index.html).

3.3.2.4 Differential response of SOLV and MIX populations to varying natural and fishing mortalities

Differential responses of SOLV and MIX populations to varying natural and fishing mortalities were also investigated using bifurcation analysis on mortality parameters but while having other all parameters set to either their SOLV value or their MIX value. Several scenarios, taking into account mortalities interactions, were considered to quantify the proportion of each parameter in the change of the population dynamics. The various bifurcation scenarios were the following: (1) a simple size-independent mortality μ, the value of which was bifurcated; (2) the size-dependent natural mortality $\mu(L)$ alone, the weight-specific natural mortality coefficient M being then bifurcated; (3) the size-dependent natural mortality $\mu(L)$ together with size-dependent fishing mortality $F(L)$, the coefficient M being bifurcated again; and (4) $\mu(L)$ and $F(L)$ together again but while bifurcation maximum fishing mortality F_{max} this time. All bifurcation parameters and their bifurcation range and step size are given in Table 3.3.

Table 3.3. Range and step size considered for bifurcation parameters.

Population	Parameter	Range	Step size
SOLV	κ	[0.33, 0.98]	0.01
SOLV	e_O	[0.004, 0.012]	0.0001
SOLV and MIX	μ	[0.01, 0.08]	0.005
SOLV and MIX	M without $F(L)$	[0.015, 0.05]	0.001
SOLV and MIX	M with $F(L)$	[0.015, 0.05]	0.001
SOLV and MIX	F_{max}	[0.01, 0.1]	0.001

3.4 Results

3.4.1 Comparison of SOLV and MIX populations

3.4.1.1 Consumer life-history traits

As expected, our DEB-PSP model predicts the same patterns of life-history (growth, maturity and reproduction) variation between SOLV and MIX populations as predicted by the individual-level DEB model in Horri et al. (2018b). Overall, MIX fish grow to larger length than SOLV fish (Fig. 3.1E) but mature later (Fig. 3.1A,B) and produce fewer eggs (Fig. 3.1D). No effect of fishing on life history traits is observed.

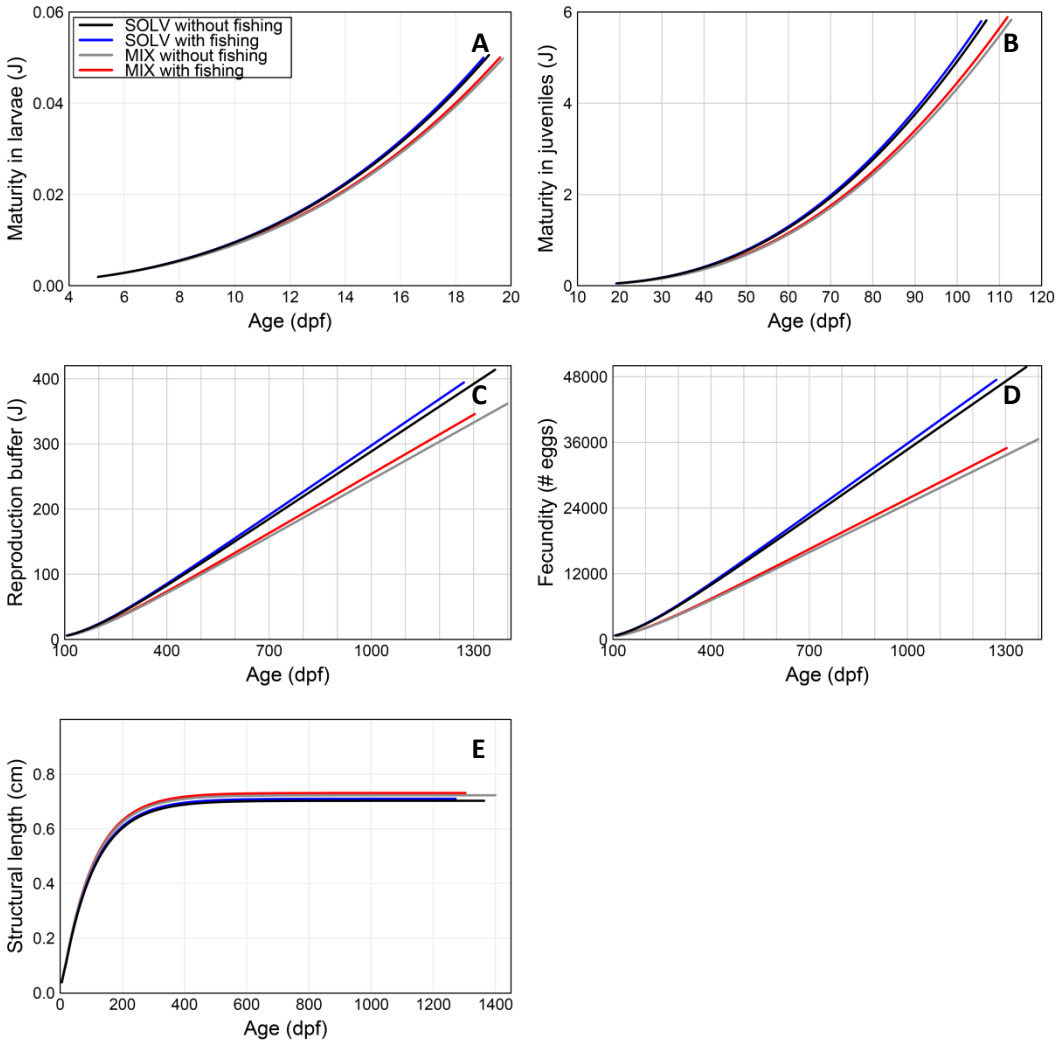

Fig. 3.1. The consumer life-history traits, i.e., maturity (A-B), reproduction buffer (C), cumulative fecundity (D) and structural length (E) as functions of age, predicted by the DEB-PSP model.

3.4.1.2 Total number and biomass density

The DEB-PSP model predicted a number density lower in MIX than in SOLV populations (Fig. 3.2A), because of their lower fecundity (Fig. 3.1D), but a higher total biomass density (Fig. 3.2B) because of their larger size (Fig. 3.1E) that overcompensates the numerical deficit. These differences are almost the same without fishing and with fishing (Fig. 3.2). Fishing induced a slight decrease of the total number and biomass density in both SOLV and MIX populations (-2.83 % and -1.27 % in total number and biomass, respectively, in SOLV populations and -3.11 % and -1.35 % in total number and biomass, respectively, in MIX populations). Despite small differences in population size reduction due to fishing, when we compare the catches in terms of biomass, we observe that MIX individuals experience more fishing pressure (0.64 $g.L^{-1}.year^{-1}$) than SOLV individuals (0.57 $g.L^{-1}.year^{-1}$) with a difference of about -13 % in SOLV individuals. This is probably due to the larger size of MIX individuals that favors their catch because of fishing selectivity increasing with size.

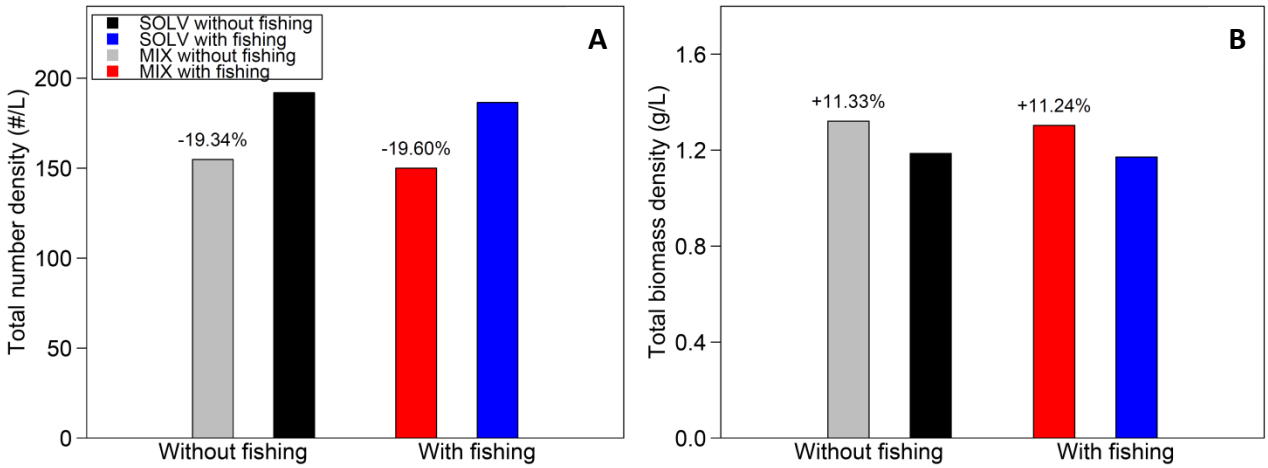

Fig. 3.2. The predicted total number density (#/L) (A) and biomass density (g/L) (B) for SOLV and MIX populations without and with fishing. The percentages above the bars represent the relative differences between SOLV and MIX population taking SOLV population as reference.

3.4.1.3 Number and biomass density per life stage

The same qualitative results are observed for number and biomass density at each life stage (larvae, juveniles and adults), i.e., a higher number density and a lower biomass density in SOLV populations compared to MIX ones, be it without or with fishing (Fig. 3.3). However, the amplitude of the difference between SOLV and MIX populations differs from one life stage to another. More interestingly, the difference in the number density decreases from larval to adult stage (roughly -21 % at larval stage, Fig. 3.3A, to -9 % at juvenile stage, Fig. 3.3B, and between -7 % and -4 % at adult stage, Fig. 3.3C). In contrast, difference in biomass density at larval stage is negligible (Fig. 3.3D) and then becomes maximal at juvenile stage (roughly +14 %, Fig. 3.3E) and smaller but still substantial at adult stage (between +8 % and +9 %, Fig. 3.3F). This is explained by the fact that the difference in size between SLV and MIX individuals at larval stage is still rather small whereas large numbers of SOLV larvae are produced due to differential fecundity. In contrast, as individual grow, size difference becomes larger while at the same time difference in number density fades away, potentially because of a size advantage of MIX individuals in terms of natural mortality (which decreases with increasing size). This results in an overcompensation of the numerical deficit in MIX populations by their size advantage, so that their biomass density is larger than that of SOLV populations at juvenile and adult stages.

Fishing effect on number and biomass density is weak at all stages and for both SOLV and MIX populations. However, it is in a direction opposite to expectation for juveniles: their number density is almost unchanged (+0.23 % and -0.71 % in SOLV and MIX populations, respectively) and their biomass density increases slightly (+1.20 % and +1.68 % in SOLV and MIX populations, respectively). Fishing effect is negative for both number and biomass density in larvae (-3.35 % and -3.11 % in number and biomass

density, respectively, for SOLV populations and -3.56 % and -2.54 % in number and biomass density, respectively, for MIX populations) and in adults (-3.48 % and -4.07 % in number and biomass density, respectively, for SOLV populations and -5.43 % and -5.12 % in number and biomass density, respectively, for MIX populations).

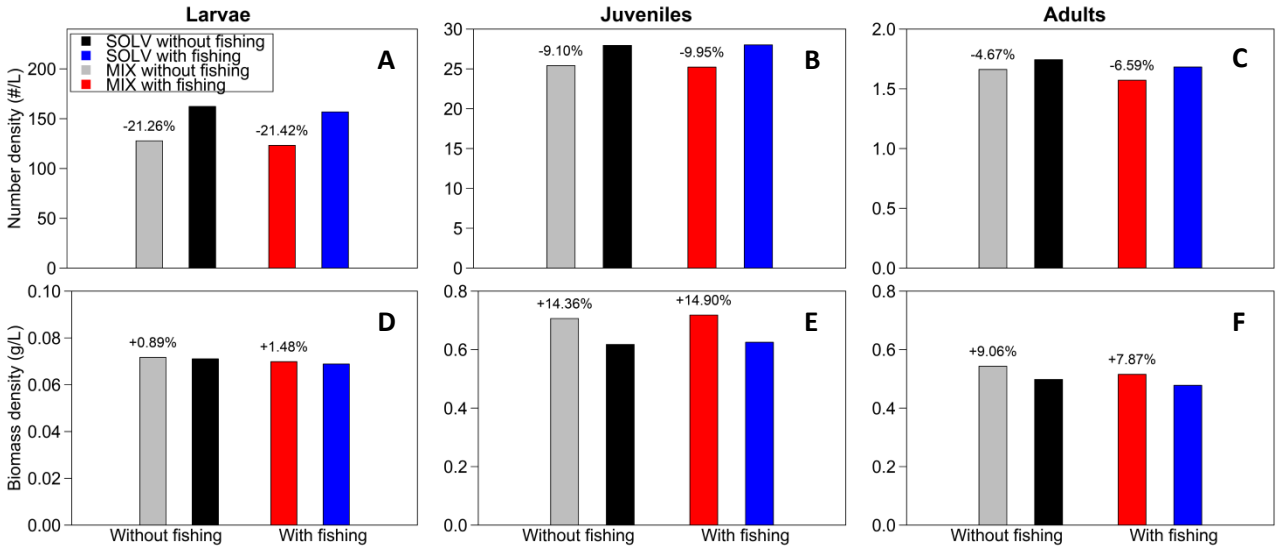

Fig. 3.3. The predicted number density (#/L) (top panels: A-C) and biomass density (g/L) (bottom panels: D-F) for SOLV and MIX populations without and with fishing for each life stage (in columns: larvae A,D; juveniles: B,E; adults: C,F). The percentages above the bars represent the relative differences between SOLV and MIX population taking SOLV population as reference.

3.4.1.4 Mean i-states per life stage

Mean age, structural length and maturity energy are larger in MIX populations than in SOLV ones at all life stages but average fecundity at adult stage is lower (Fig. 3.4). This means that MIX individuals are on average older than SOLV ones for the same life stage. This is linked to the fact that they have a lower maturity at age (Fig. 3.1A, B) so that they reach the maturity thresholds of life history transitions (U_H^j and U_H^p for metamorphosis into juveniles and maturation to adults, respectively) older. Despite this, mean maturity is larger in MIX populations at both larval and juvenile stage, which is seemingly contradictory. This is however likely related to different distribution of maturity levels within life stage, with more individuals with high maturity in MIX populations, related to their lower natural mortality because of their size advantage. There are more old individuals in MIX populations within each life stage and thus more individuals with

high maturity level despite their maturity deficit relative to SOLV individuals of the same age. The larger mean structural length in MIX populations whatever the life stage is as expected from their higher fraction of energy κ allocated to somatic maintenance and growth at the expense of the fraction $1-\kappa$ allocated to maturity and reproduction. The latter combined with the higher costs of egg production e_0 explains their lower mean fecundity at adult stage. No impact of fishing is observed on mean age, structural length and maturity for all life stages. In contrast, mean cumulative fecundity decreases in both SOLV and MIX populations when they are fished (-4.54 % and -3.52 % in SOLV and MIX populations, respectively; Fig. 3.4).

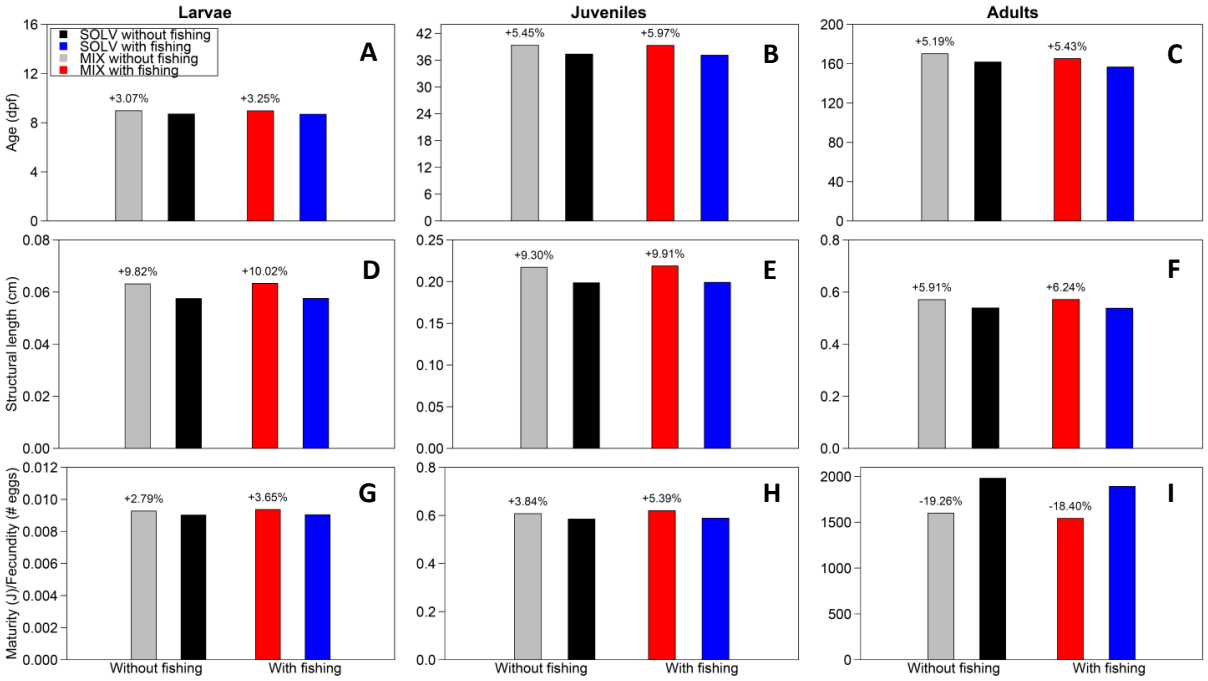

Fig. 3.4. The predicted mean *i-states* of the consumer for SOLV and MIX populations without and with fishing at each life stage (in columns; larvae: A,D,G; juveniles: B, E, H; adults: C, F, I). The percentages above the bars represent the relative differences between SOLV and MIX population taking SOLV population as reference. The top panels (A-C) show the mean age (in days post fertilization) of the consumer population per life stage. The middle panels (D-F) show the mean structural length (cm) of the consumer population per life stage. The bottom panels (G-I) show the mean maturity energy (J) for larvae and juveniles (G and H, respectively) and the mean fecundity (number of eggs) for adults (I).

3.4.2 Effect of PCB and PBDE PMoAs on population dynamics

Figure 3.5 represents the bifurcation diagram of the consumer population dynamics as a function of the 2 parameters κ and e_0 corresponding to PCBs and PBDEs PMoAs. Stable

dynamics are obtained over the entire range of the bifurcation parameters. The results show that the total number density decreases with increasing values of κ (Fig. 3.5A) and e_0 (Fig. 3.5B), be it without or with fishing. For κ, the population goes extinct at a value of 0.97, whereas for e_0 the population never reaches extinction over the bifurcation range but it may still reach it at higher values (although there seem to be an exponential and thus leveling off decay).

In contrast, the total consumer biomass density increases with κ until reaching a maximum at $\kappa = 0.91$ and 0.89 without and with fishing, respectively, from which it begins to collapse (Fig. 3.5C). Again, note that increasing κ decreases fecundity and increases individual growth. Before reaching the maximum biomass density, the decrease in fecundity due to increasing κ is overcompensated by the increase of individuals' size that may be reinforced by the size advantage in terms of natural mortality. After reaching maximum biomass density, the size advantage however becomes insufficient to compensate for the loss of fecundity that tends towards a very low value until it provokes the extinction of the population at $\kappa = 0.97$. Total consumer biomass density also increases with increasing e_0 but without extinction of the population after reaching some maximum value (Fig. 3.5D). This is probably due to an increase in resource density resulting from the decrease of fecundity and thus of the number of foragers, which allows individuals to grow to larger sizes. The feedback loop between consumer and resource density generates the same effect as allocating more to growth at the expense of reproduction through resource competition relaxation: the increase in reproduction costs leads to a decrease in the number of recruits that relaxes resource competition and thus increases growth.

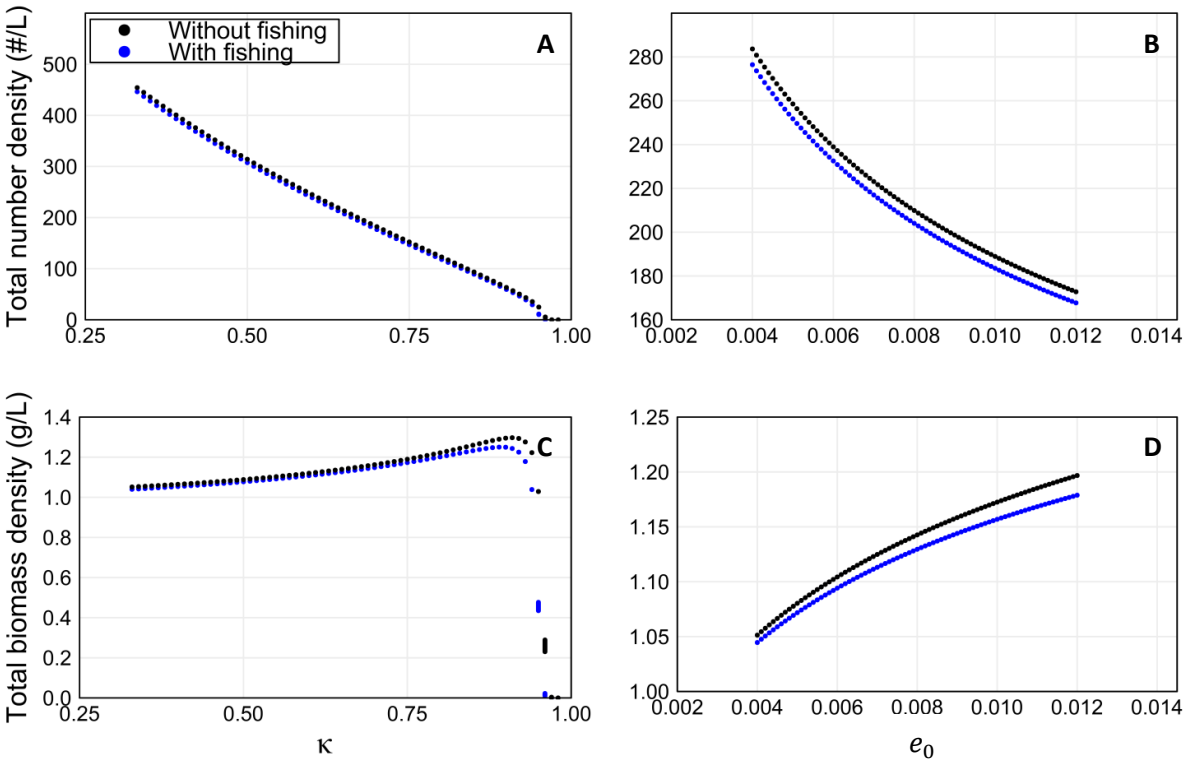

Fig. 3.5. Bifurcation diagrams of the consumer population without and with fishing for the two PMoA parameters κ (left panels: A, C) and e_0 (right panels: B, D). Top (A-B) and bottom (C-D) panels show the total consumer number and biomass density, respectively.

3.4.3 Response of SOLV and MIX populations to varying natural and fishing mortalities

Among the various mortality parameters bifurcated here, only change in the size-independent mortality μ leads to two types of attractors for the consumer population dynamics, periodic oscillations and steady state, in a similar way for both SOLV and MIX populations (Fig. 3.6A,E). In contrast, bifurcation of other mortality parameters, i.e., M without (Fig. 3.6B,F) or with fixed size-dependent fishing mortality (Fig. 3.6C,G) and F_{max} with fixed size-dependent natural mortality (Fig. 3.6D,H), lead only to steady state over the whole bifurcation range.

Periodic oscillations are observed for $0.01 < \mu < 0.049$ and $0.01 < \mu < 0.052$ for MIX and SOLV populations, respectively (Fig. 3.6A,E). These oscillations decrease progressively in amplitude with increasing μ until stabilizing completely at steady state

from $\mu = 0.052$ and $\mu = 0.049$ onward for SOLV and MIX populations, respectively. These periodic cycles for lower μ values are so-called *juvenile-driven cycles* (De Roos and Persson, 2013; De Roos and Persson, 2003). To illustrate this phenomenon, we investigate the consumer population dynamics in the oscillation region at $\mu = 0.01$ for each life stage (Fig. 3.7). These dynamics are characterized by juvenile consumer abundance (Fig. 3.7, brown curves) varying almost inversely (or out of phase) to that of larvae and adult consumers (Fig. 3.7, blue and violet curves, respectively) because of competition for the resource. Juveniles are more competitive than adults because they have a higher total surface area (summing all juveniles) due to a higher surface to volume (and thus biomass) ratio. Hence, as their density increases because of the arrival of a new cohort, they reduce adults' intake so much, due to resource depletion, that these grow very little and thus produce only a few larvae. After reaching a maximum, the density of juveniles and their biomass decrease again as (i) fewer larvae enter the juvenile stage, (ii) resource get depleted and (iii) juveniles enter the adult stage. This relaxes resource depletion and competitive pressure on adults that start increasing in density, growing larger and produce more larvae that will create a new juvenile cohort. Density of adults and larvae and their biomass reach a maximum before decreasing again when the new juvenile cohort takes over. When the level of background mortality μ is increased for all individuals, the cycles disappear because the density of individuals, especially juveniles, is decreased enough to relax competition for resource.

Both SOLV and MIX populations eventually go extinct as size-independent mortality μ increases but at a lower value for MIX $\mu = 0.079$ than for SOLV $\mu = 0.082$ populations (Fig. 3.6A,E). This indicates that SOLV populations are able to withstand higher size-independent mortality than MIX ones. Since in this scenario mortality is size-independent, only the fecundity advantage of SOLV individuals enters into play.

For the scenarios where the intensity of size-dependent natural mortality M is bifurcated, without and with fixed size-dependent fishing mortality $F(L)$, we also observe an extinction of the populations as mortality intensity reached high values (Fig. 3.6B,C,F,G). Extinction mortality values are the same without and with fishing, but this time they are lower for SOLV populations than for MIX ones ($M = 0.044$ and 0.046 for SOLV and MIX population, respectively). In constrat, when the intensity of size-dependent fishing mortality F_{max} is bifurcated (Fig. 3.6D,H), while leaving in the model a fixed size-dependent natural mortality $\mu(L)$, extinction occurs for lower fishing intensity in MIX populations than in SOLV ones ($F_{max} = 0.07$ and 0.065 for SOLV and MIX population, respectively). To summarize, whether MIX or SOLV populations become first extinct as natural or fishing mortality intensity increases depends on the balance between the fecundity gain plus the lower fishing mortality because of smaller size in SOLV population and the survival gain due to lower natural mortality in MIX population because of larger size.

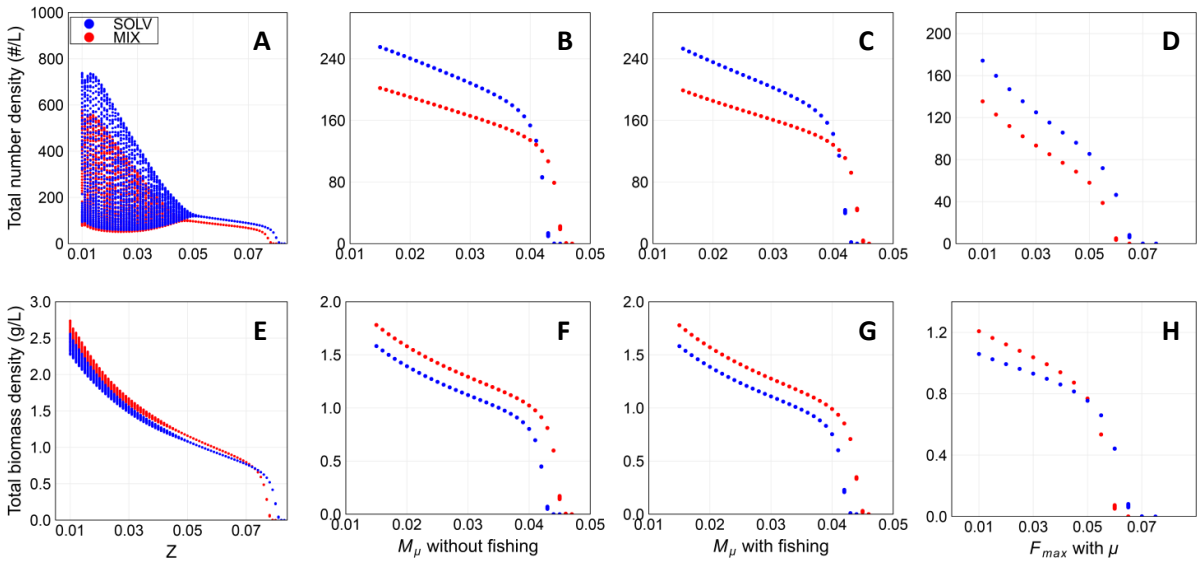

Fig. 3.6. Bifurcation diagrams of SOLV and MIX consumer populations as a function of mortality parameters: size-independent background mortality μ (A, E), size-dependent mortality coefficient M without fishing (B, F), M with fixed size-dependent fishing $F(L)$ (C, G) and

maximum fishing mortality F_{max} with fixed size-dependent natural mortality $\mu(L)$ (D, H). Top (A-D) and bottom (F-H) panels show the total consumer number and biomass density, respectively.

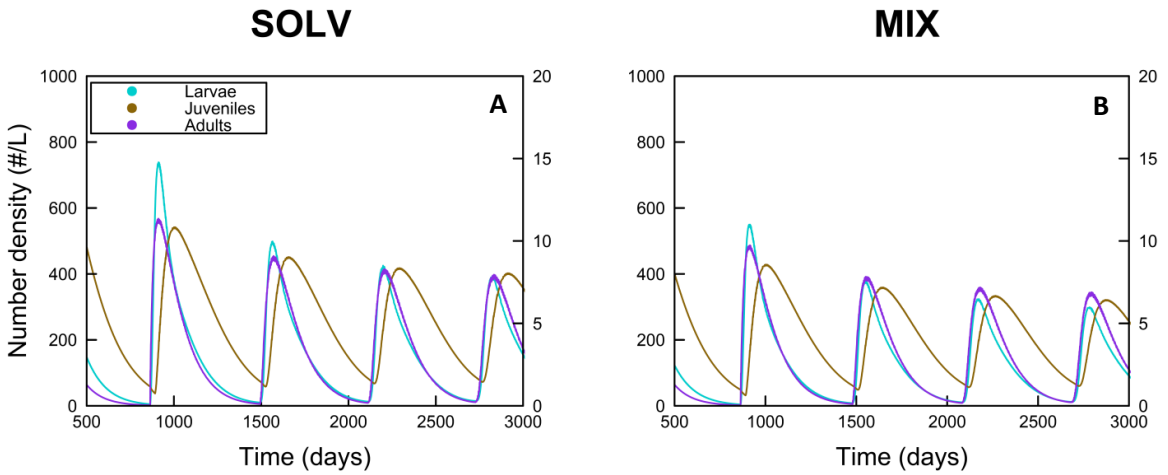

Fig. 3.7. Juvenile-driven cycles dynamics in SOLV and MIX populations at a constant background mortality $\mu = 0.01$. Densities of larvae, juveniles (primary y axis) and adults (secondary y axis) are represented as functions of time (days) for SOLV (A) and MIX (B) populations.

Finally, to determine the effect of the PCB/PBDE mixture on fisheries productivity in case of exploited populations, we investigate how catch biomass or yield varies as function of the maximum fishing mortality F_{max} (Fig. 3.8). The maximum of such a curve identifies the maximum sustainable yield (MSY), i.e., the maximum catch that can be extracted from the population on the long-term, i.e., without impairing population viability. The fishing intensity associated, F_{MSY}, defines the level of exploitation ensuring this MSY and is often used in fisheries management as a reference point or threshold below which fishing intensity should stay. The results show that SOLV populations produce lower fishing yields than the MIX population, notably lower MSY, but that it can sustain higher exploitation levels (MSY = 4.47 $g.L^{-1}.year^{-1}$, F_{MSY} = 18.25 $year^{-1}$; Fig. 3.8) than MIX populations (MSY = 5.59 $g.L^{-1}.year^{-1}$, F_{MSY} = 16.43 $year^{-1}$; Fig. 3.8).

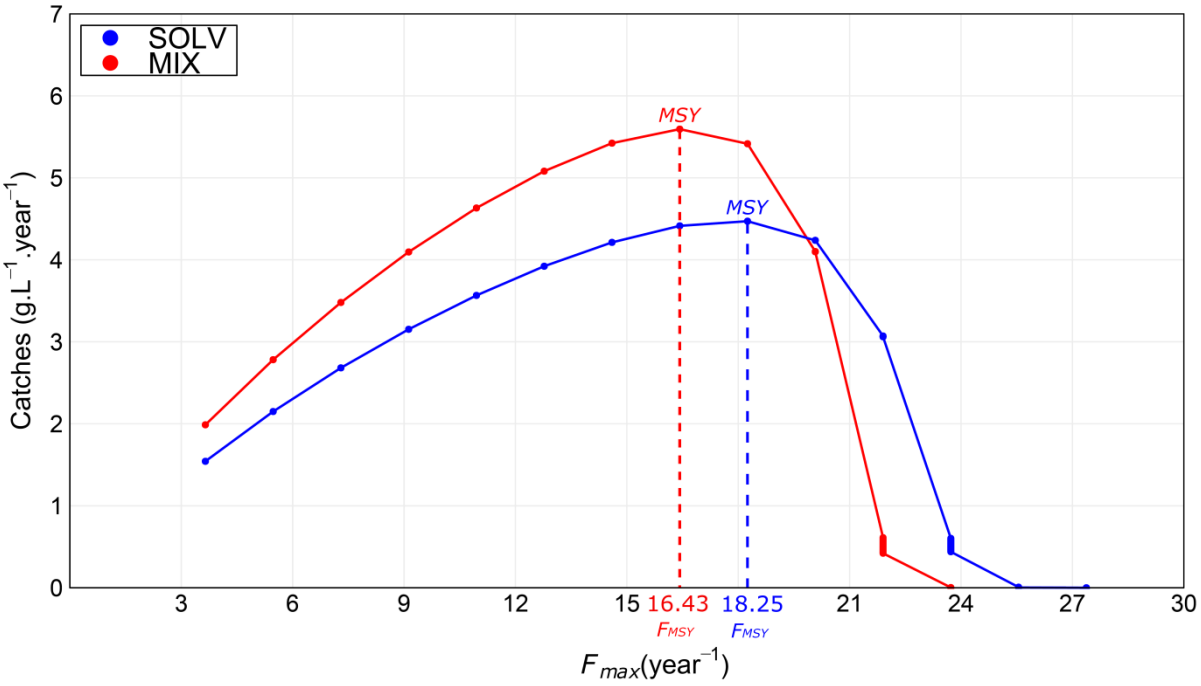

Fig. 3.8. Yield as a function of fishing intensity in exploited SOLV and MIX populations.

3.5 Discussion

In this study, we have investigated the consequences of the individual effects of the two physiological modes of action of a realistic marine environmental mixture of PCBs and PBDEs on fish population dynamics. The consequences for the productivity of commercial fisheries in case of exploited populations have also been considered, under the assumption that the individual-level life-history effects documented on zebrafish can be transposed to marine teleost fish in the wild.

3.5.1 Modelling approaches for extrapolating individual-level effects of contaminants to population dynamics

Extrapolation of toxicants effects from the individual to the population level has been documented in several species, including laboratory and field population species, using coupled modeling approaches combining individual-level models of contaminant effects with population models. Mechanistic models of individual-level contaminant effects, such as DEB models, coupled to population models are specifically useful to identify the

demographic consequences of specific physiological modes of action. They allow generalizing the results to all contaminants triggering the same PMoAs for their individual-level effects. Such generalization is not warranted when simply basing the population-level analysis on the effect of contaminants on individual life-history traits as similar life-history trait changes can be triggered by different PMoAs that may have different additional, but potentially undocumented, life-history effects leading to different demographic consequences. Several studies have been based on coupling DEB and population models. Three main types of population models can be distinguished in these: continuous time Euler-Lotka integral equation to analyze the population-level effects through consequences on population growth rate as a proxy (Jager and Klok, 2010; Jager et al., 2007; Klok and de Roos, 1996; Kooijman and Metz, 1984), matrix population models (Billoir et al., 2007; Jager and Klok, 2010; Klok and de Roos, 1996) and individual based model (Martin et al., 2013a, 2013b). However, 4 broad types of limitations can be noticed in previous studies coupling DEB and population models for considering contaminant effects on population dynamics.

First, Euler-Lotka models were not really used to investigate populations' dynamics, but rather their intrinsic growth rate, which is based on the unrealistic assumption of an exponentially growing population without any density-dependent or environmental feedback. Matrix models are more realistic and allow considering density-dependence but they are not suitable for including explicitly the environmental feedback loop through the coupling with resource dynamics and more generally for including varying environment (Caswell, 1989). Individual-based models allow encompassing all individual-level details as well as explicit interactions with the environment and the resulting feedback but may be limited by computational constrains. PSP models represent a good compromise between the explicit inclusion of individual-level

processes together with the environmental feedback loop via coupling between consumer and resource dynamics and relatively short computational time. They are also known to be particularly well adapted to extrapolate changes in life-history on the basis of individual bioenergetics, especially when the individual-level processes are described on a continuous time basis (De Roos, 2008), such as in DEB models. However, to our knowledge, in the context of the effect of contaminants on population dynamics, no study has used a coupled DEB-PSP approach, although it was applied to other purposes (De Roos et al., 1990; Roos and Persson, 2002).

Second, no such coupled modeling study treated the population dynamical effects of contaminants in fish. Actually, to our knowledge, all previous studies dealt with invertebrates. Beaudouin et al. (2015) devised an individual-based model coupled with DEB for exploring contaminants' toxicological effects on zebrafish populations. However, they did not apply their framework to a specific case so far. Other studies on fish where either based on a very simplified bioenergetic model coupled to IBM (Jaworska et al., 1997) or on coupling directly observed individual-level toxicity data on life-history and survival to matrix population model (Barnthouse et al., 1990; Munns et al., 1997). Very few studies have focused on commercial fish species (Barnthouse et al., 1990; Goodyear, 1985). In this study, we propose one of the first treatments of the consequences of contaminants PMoAs on a fish species' population dynamics.

Third, not all PMoAs were explored in terms of population level effects, notably changes in the share of energy between growth and reproduction κ that is one of the potential PMoAs of EDCs (Kooijman, 2010) and that has been experimentally documented in zebrafish (Horri et al., 2018b). The possible population-level effects of other PMoAs (e.g. increase in hazard, increase in growth costs, increase in reproduction costs, increase in

maintenance or decrease in assimilation energy) have been systematically explored in a few studies only (Kooijman and Metz, 1984; Billoir et al., 2007; Martin et al., 2014, 2013a). Here, we specifically targeted the share of energy between growth and reproduction κ and the cost of an egg e_0 that are the 2 PMoAs triggered by the marine environmental mixture of PCBs and PBDEs we have been testing (Horri et al. 2018a,b) but that are also extremely likely to be the 2 main PMoAs of EDCs in general. In that sense, our results give an idea of potential population dynamical effects of EDCs in fish.

Fourth, most previous studies focused on the effect of single molecules whereas individuals are generally exposed to mixtures of contaminants in the environment.

3.5.2 Effects the environmental mixture of PBCS and PBDEs on population abundance

We have shown that the combined effects of the two modes of action of PCBs and PBDEs, the allocation of energy towards growth κ and the cost of an egg e_0, on individuals' life-history lead to a change in population dynamics resulting in lower total density of individuals and higher total density of biomass in exposed (MIX) populations compared to control (SOLV) ones. These results are the same in the presence or the absence of fishing and they reflect exactly life-history changes at the individual-level, i.e., a weaker fecundity that leads to a lower density of individuals for MIX populations and an increased growth, which results into a larger biomass by overcompensating the numerical deficit for MIX populations. Although the biomass of MIX populations is larger than that of SOLV ones, their extinction risk might be higher as they consist of less individuals, the fecundity of which is reduced. This higher propensity of extinction was further tested through bifurcation of the two PCBs and PBDEs PMoAs.

Given that our study is the first one to test for the population dynamical effects of the share of energy between growth and reproduction κ as a contaminant PMoA, the results related to this PMoA are difficult to compare to previous findings in the literature. Notably, an increase in biomass in a contaminated population has never been observed before and can clearly only be related to a positive effect of contaminants on the energy inflow towards growth. We believe that this result can be extrapolated to EDCs as these are known to affect the energy balance between growth and reproduction. The exact amplitude of the effect on population abundance and biomass will then depend on the degree of change of κ, which will in turn depend on the contaminants themselves and their concentration level.

In contrast, several studies investigated the population dynamical effect of reproduction costs e_0 that are the second PMoA tested here. For example, Martin et *al.* (2013a) used a DEB-IBM model to assess separately the effects of two PMoAs that affect directly reproduction, embryonic hazard and reproduction costs, and that are triggered by 3,4-dichloroaniline (3,4-DCA) in experimental populations of *Daphnia magna*. Their results at the population level revealed that for the same effect on individual's fecundity, the two PMoAs lead to the same population abundance. Using only the embryonic hazard PMoA for further analyses, they detected a reduction in population abundance at very high concentrations only, reducing fecundity by 40% or more. Despite the negative effect of contaminant observed on fecundity, no serious consequences were observed at the population level when concentration were such that the decrease in fecundity was comparable to our study (between 16 % and 26 % according to age, Fig. 3.1D) contrary to our study. The difference between Martin et al's results and ours could be related to the fact that a single PMoA affecting a single endpoint, reproductive outputs, was involved in their study while in our case, two combined PMoAs affected two endpoints,

growth and reproductive outputs. Another possible explanation could be the differences between species bioenergetics on which population dynamic is highly dependent (De Roos and Persson, 2013), so that populations dynamics of species with different bioenergetics could respond differently to similar PMoAs.

Kooijman and Metz (1984) showed that reduced reproductive outputs lead to reduced population growth rate in daphnia populations exposed to unspecified contaminants. Assuming that the reduced population growth rate could be interpreted as resulting in a decline of population abundance, the results of this study are in better agreement with ours as reduction in reproduction tested varied between 8 % and 35 %. However, their model did not include the environmental feedback between the consumer population dynamics and resource dynamics, so that it is difficult to be conclusive.

One point that must be emphasized is that across studies, including ours, similar modes of action affect in the same qualitative way population abundance or growth rate whatever the type of contaminant and the studied species. For instance, decreased reproductive outputs always lead to a decrease in abundance or population growth rate (Jaworska et al., 1997; Kooijman and Metz, 1984; Martin et al., 2014). In contrast, what can be changed is the intensity of the impact because of varying amplitude of the effects of different contaminants at different concentrations at the individual level. This confirms that the extrapolation of the consequences of a given mode of action, such as reproduction costs, on population dynamics to other contaminants trigerring the same PMoA and to other species is possible. This clearly demonstrates the interest of identifying the PMoAs of the individual-level effect of contaminants and of studying the population dynamical effect of PMoAs.

3.5.3 General effects of the 2 PMoAs triggered by the mixture of PBCS and PBDEs

We showed that increasing the fraction of energy allocated to growth κ (while decreasing that allocated to reproduction) and increasing reproduction costs e_0 decreases numerical abundance (until extinction of the population for the former) but also leads to increased biomass (up to the point where the population collapses and goes extinct for high values of κ). If we put aside the extinction observed for extremely high values of κ for which individuals hardly reproduce, the 2 PMoAs have the same qualitative effects of population number and biomass density but through very different routes. For κ, the decrease in number density and the increase in biomass density are directly resulting from the individual-level effects of this PMoA, i.e., an increase in somatic growth that overcompensates in terms of biomass the numerical deficit resulting from the decrease in fecundity. For e_0, in contrast, this effect emerges from the environmental feedback loop due to the coupling between consumer and resource dynamics: as fecundity decreases, so does recruitment which relaxes competition for food and allows individuals to grow larger, which overcompensates numerical loss and leads to biomass increase. Note that the environmental feedback loop may also play a role in what is observed for κ, so that for this PMoA changes in number and biomass density might actually be due to a combination of direct and indirect mechanisms.

To our knowledge, no such increase in biomass related to contaminants has been observed before. As already pointed above, given that κ was not considered as a PMoA before, there is no comparison possible. There have been however studies looking at the effect of PMoAs reducing reproduction and which can thus be compared to our results on e_0. Notably Martin et *al*. (2014) used a DEB-IBM model to test the effect of several potential PMoAs (growth costs, feeding, maintenance and reproduction costs via embryonic hazard) that all reduced reproduction in interaction with variation in

maximum resource density (also modeled as a semi-chemostat) on both biomass and abundance in Daphnia populations. They observed that for all PMoAs, stress reduced population biomass all the more as maximum resource density was low, except for reproduction costs for which they observed a slight biomass increase (less than 5%) for very strong individual-level effects (above 50 % reduction in reproduction) for high maximum resource densities. This increase in biomass for reproduction costs is much smaller in terms of amplitude than what we observed (Fig. 3.5) but it highlights its potential general scope and the facts that PMoAs affecting directly reproduction, i.e., reproduction costs or embryonic hazard, have different population dynamical effects than those affecting it indirectly (e.g. maintenance costs) and that these are mediated through the environmental feedback loop.

3.5.4 Interaction of sublethal effects on life-history traits with natural mortality

Population dynamics is known to depend strongly on mortality characteristics, namely its intensity but also its potential relationship with *i*-sates such as size-dependency (De Roos and Persson, 2013; Roos and Persson, 2002; van Kooten et al., 2007; van de Wolfshaar et al., 2008). We have seen that lower intensities of size-dependent natural mortality are necessary to drive SOLV populations to extinction relative to MIX populations (Fig. 3.6B,F,C,G). This is due to the difference in growth between individuals of the two types of populations, larger MIX individuals enjoying a reduction in natural mortality that overcompensates the higher fecundity that characterizes SOLV individuals so that MIX populations can withstand higher natural mortality intensities. The fecundity advantage of SOLV populations is observed when all individuals suffer from the same constant mortality: in this case we observed that MIX populations went extinct for lower values of mortality than SOLV populations. This suggests that whether

SOLV or MIX populations can withstand higher natural mortality values will depend on the amplitude of the decay of mortality with size.

When we apply a low constant mortality to individuals, *juveniles-driven cycles* emerge (Fig. 3.6A,E, Fig. 3.7). This finding has already been observed in de Roos and Persson, (2003) and van der Meer (2016). It has been explained by the pressure exerted by juveniles on the food resource because of their higher competitivity (here a higher surface to mass ratio), which can reduce strongly food intake of adults and thus their growth and fecundity. As shown when bifurcating the constant mortality intensity, juvenile-driven cycles are not observed for the same range of mortality inetsities for SOV and MIX populations, which highlights the possibility that besides affecting number and biomass density and extinction boundaries, contaminants may even change the nature of population dynamical attractors.

3.5.5 Interaction of sublethal effects on life-history traits with fishing

In order to extrapolate the effects of PCBs and PBDEs to populations of commercial species, we included the effect of exploitation in our study. This was done by transposing the fishing selectivity parameters in a marine fish species of high commercial value, the common sole. The choice of this species was motivated by the fact that the marine environmental mixture of PCBs and PBDEs tested in the experimental study used to calibrate the DEB model applied in this paper (Horri et al. 2018a,b) was representative of environmental conditions in the Bay of the Seine in France. This Bay presents high concentrations of PCBs and PBDEs (Abarnou et al., 2000; OSPAR Commission, 2009, 2013) and is at the same time a nursery area for a number of flatfish species, including sole. It is thus likely that sole stock of the eastern English Channel and the related fisheries are affected by the population-level consequences of these contaminants.

Results on the effect of fishing mortality on population dynamics show that, in contrast to natural mortality, MIX populations suffer more from this type of mortality than SOLV populations, thus leading to their extinction for lower fishing pressure (Fig. 3.6D,H). Again, this difference is related to the interaction between the size-dependency of mortality, that here increases with size, and the difference in growth between SOLV and MIX individuals. The larger MIX individuals incur additional fishing mortality than SOLV ones and thus withstand lower total fishing mortality intensity despite their advantage in terms of natural mortality (that decreases with size). More generally, the fact that MIX or SOLV populations will first become extinct will depend on the balance between the fecundity gain plus the survival gain due to lower of fishing mortality in SOLV population and the survival gain due to a decreased natural mortality in MIX population. Interestingly, although SOLV populations seem to persist at higher exploitation rates, they produce lower fishing yields than MIX populations in general and give a lower MSY but at a larger F_{MSY} (Fig. 3.8). This suggests that, counterintuitively, contaminated populations may be more productive for fisheries but that there is a higher risk that sustainable exploitation levels are overshooted and collapse occurs.

3.6 Acknowledgements

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DISCUSSION GÉNÉRALE

L'objectif de cette thèse était d'évaluer les effets d'un mélange de PCB et de PBDE représentatif de l'environnement marin côtier sur les traits d'histoire de vie, la bioénergétique et la dynamique des populations de poissons. Afin d'atteindre celui-ci, les travaux ont mis en œuvre une combinaison d'approche expérimentale à l'échelle individuelle et de modélisation pour extrapoler les effets de l'échelle de l'individu à celle de la population afin d'inférer leurs répercussions sur le recrutement et la démographie des populations en milieu naturel. Le mélange testé était représentatif de l'estuaire de Seine, l'estuaire français le plus contaminé en termes de polluants organiques persistants. Une espèce modèle, le poisson-zèbre, a été utilisée pour l'approche expérimentale et la paramétrisation/calibration des modèles. Dans l'hypothèse où les effets observés seraient transposables à des populations marines exploitées, leurs conséquences sur la productivité et la durabilité des stocks halieutiques ont été également considérés en s'appuyant sur le stock de sole commune de Manche Est en lien avec l'estuaire de Seine pris comme scénario caricatural de pêcherie associée à un bassin versant et un estuaire fortement pollué.

Dans un premier temps, les effets de l'exposition par voie alimentaire au mélange de PCB et de PBDE ont été évalués sur les traits de vie de poisson-zèbres en conditions expérimentales à l'aide d'une approche statistique basée sur les modèles linéaires et non-linéaires à effets mixtes (Chapitre 1). Plus précisément, la survie, la croissance et la reproduction (incluant la probabilité de ponte, la taille de ponte et le taux de fécondation) chez la génération parentale et la survie larvaire chez les descendants ont été suivies et comparées entre populations contrôles et exposées. Ensuite, les effets de ce mélange sur les performances bioénergétiques des individus ont été caractérisés à

travers l'identification de leurs modes d'action physiologiques sur la base d'un modèle de Budget Energétique Dynamique (DEB) calibré à partir des données expérimentales (Chapitre 2). Enfin, le changement d'échelle des effets, i.e., de la bioénergétique de l'individu à la dynamique de la population, ainsi que la transposition de ceux-ci de la population expérimentale de poisson-zèbres à une population exploitée, au travers de l'application du patron d'exploitation du stock de sole commune de Manche Est, ont été effectués à l'aide du couplage entre le modèle DEB et un modèle de population structurée physiologiquement (PSP).

Dans les parties qui suivent, les principaux résultats sont synthétisés et discutés à commencer par la vérification de l'exposition et la contamination effectives et réalistes des individus sur lesquelles repose la validité de l'ensemble des résultats, puis les effets du mélange de PCB et PBDE sur les traits d'histoire vie, puis leurs modes d'action physiologique, pour finir par leurs conséquences potentielles pour la dynamique de population et la productivité des pêcheries.

Réalité et réalisme de l'exposition et la contamination des poissons

Avant de caractériser les effets du mélange de PCB et de PBDE sur les traits d'histoire de vie, il a dans un premier temps été nécessaire de confirmer 1) l'exposition effective des individus aux contaminants et aux doses réalistes ciblées via l'aliment contaminé et l'absence ou quasi-absence de ces contaminants dans l'aliment contrôle et 2) la contamination à des niveaux réalistes des poissons exposés, ainsi que l'absence de contamination significative des poissons contrôles. Plusieurs études précédentes ont montré qu'aucun effet de l'isooctane utilisé comme solvant des PCB et PBDE mais également dans la fabrication de l'aliment contrôle n'était détectable que ce soit sur les traits d'histoire de vie (Daouk et al. 2011) ou le comportement (Péan et al. 2013).

Les mesures des niveaux de PCB et de PBDE dans l'aliment contaminé (MIX) montrent que les concentrations des différents congénères oscillaient entre 86 et 113% de la concentration cible pour une moyenne de 97% en ce qui concerne les congénères de PCB et entre 100 et 130% pour une moyenne de 117% pour les congénères de PBDE (MIX ; Table 1.S1, Chapitre 1). A l'inverse, l'aliment contrôle (SOLV) ne montrait pas de présence significative de ces composés avec des concentrations 245 et 522 plus faible en PCB et PBDE, respectivement, que dans l'aliment contaminé. Ceci indique que les animaux du traitement MIX ont bien été exposés à un mélange représentatif de l'estuaire de Seine en termes de profil de congénères et de concentrations *via* leur alimentation et qu'aucune contamination externe ou croisée entre les deux traitements ne s'est produite pendant la préparation de l'aliment de sorte que les animaux du traitement SOLV peuvent effectivement être considérés comme des contrôles non exposés.

L'exposition chronique des poissons par la voie alimentaire a conduit à une bioaccumulation remarquable de PCB et de PBDE dans leurs tissus avec des concentrations qui se situaient en fin d'expérience (180 jours post-fécondation, jpf) dans la gamme de celles rencontrées dans l'environnement, notamment les estuaires industrialisés, à savoir 2188.3 ± 132.26 et 110.9 ± 1.14 $ng.g^{-1}$ (poids frais) pour les PCB et les PBDE, respectivement, chez les femelles et 2140 ± 73.95 et 96.4 ±8.65 $ng.g^{-1}$ (poids frais) chez les mâles. (Law et al., 2006; Robinson et al., 2017). A l'inverse, les concentrations observées dans les tissus des poissons contrôles étaient négligeables (80 à 85 fois plus faibles que les poissons exposés pour les PCB et 30 à 100 fois plus faible pour les PBDE). Comme déjà montré par d'autres études (Daouk et al., 2011; Nyholm et al., 2009), ces résultats confirment le potentiel de ces composés à s'accumuler dans les tissus des poissons après l'exposition par voie alimentaire. Plus important encore, ils montrent que l'exposition expérimentale à bien conduit à une contamination réaliste des

poissons exposés en termes de congénères accumulés et de leur concentration dans les tissus.

Les différences observées entre poissons exposés MIX et contrôles SOV peuvent donc être attribuées à l'exposition aux contaminants d'autant que l'exposition expérimentale a été répliquée.

Effets des PCB et des PBDE sur les traits d'histoire de vie

Les principaux résultats de cette partie sont résumés dans la figure 7.

Survie de la génération parentale

Les résultats obtenus concernant les effets du mélange de PCB et de PBDE sur la survie des divers stades de vie (larvaire, juvénile et adulte) chez la génération parentale (F0) n'ont mis en évidence aucune différence significative entre les poissons SOLV et MIX (Chapitre 1). Les effets conjoints des PCB et des PBDE sur la survie adulte n'ayant jamais été caractérisés jusque-là, les résultats obtenus sur ce point ne sont pas comparables avec les données de la littérature. Concernant la survie précoce (larvaire), des résultats similaires ont été observés par (Chou et al., 2010) chez le poisson-zèbre exposé à un seul congénère de PBDE (BDE-47). Ces résultats pourraient s'expliquer par les faibles niveaux de contaminants accumulés à ce stade en raison de la courte durée d'exposition. En effet, les PCB et les PBDE ont tendance à s'accumuler avec le temps, ce qui entraîne l'augmentation des concentrations dans les tissus (Daouk et al., 2011; Nyholm et al., 2009).

L'absence d'effet au stade larvaire peut également s'expliquer par le fait que l'exposition dans la présente étude a commencé au stade larvaire au moment de l'ouverture de la bouche et non pas au stade embryonnaire qui a été décrit comme le stade de vie le plus

vulnérable (Belanger et al., 2010). En effet, des études précédentes ont montré que l'exposition par balnéation des œufs de sole aux POP, incluant les PCB et les PBDE, de sorte à mimer l'exposition des embryons aux polluants due au transfert maternel pouvait par la suite diminuer fortement la survie des individus au stade larvaire (Foekema et al., 2012, 2014). Chez le poisson-zèbre, (Lema et al., 2007) ont également observé une diminution de la survie des larves après l'exposition à de fortes concentrations de BDE-47 au cours du stade embryonnaire par balnéation des œufs. Cependant, les concentrations observées dans les tissus pour des effets significatifs étaient très élevées. Ces études suggèrent que l'exposition à des fortes concentrations de POP aux premiers stades embryonnaires, par exemple suite à un transfert maternel de ces composés aux œufs, peut affecter la survie des larves alors que les résultats de cette thèse suggèrent que l'exposition des larves par voie alimentaire à un mélange environnemental n'aurait pas d'effet. Le transfert maternel vers les œufs a déjà été observé pour les PCB dans une expérience similaire (Daouk et al., 2011). Par ailleurs, une altération de la survie des larves pourrait être due à une moindre qualité des œufs et/ou à des effets épigénétiques (Baker et al., 2014). Une expérience sur la survie larvaire des descendants a été réalisée pour évaluer ces effets potentiels (voir la sous-partie « *Survie larvaire des descendants*»).

Croissance

Les résultats obtenus ont montré un effet significatif de l'exposition au mélange de PCB et de PBDE sur la croissance décrite par un modèle de von Bertalanffy, indiquant une diminution du taux de croissance et une augmentation de la longueur asymptotique chez les deux sexes de poissons MIX (Chapitre 1). Ceci entraîne des tailles aux âges élevés plus grandes chez les poissons exposés. D'après la littérature consultée, il semblerait que ces résultats soient les premiers à décrire un effet positif des POP sur la croissance en

longueur des individus. En effet, jusque-là les études s'intéressant aux effets des POP sur la croissance des organismes indiquaient plutôt des effets négatifs ou absents. Par exemple, (Vignet et al., 2014) ont montré une diminution du poids et de la longueur chez le poisson-zèbre après une exposition par voie alimentaire à des mélanges d'hydrocarbures aromatiques polycycliques (HAP). En revanche, une augmentation significative du poids, mais sans modification de la longueur, a été observée chez le poisson-zèbre exposé à des mélanges de POP représentatifs du milieu d'eau douce ((Nourizadeh-Lillabadi et al., 2009). Ce résultat a été expliqué par une perturbation endocrinienne et une modification de l'expression des gènes impliqués dans la régulation de la croissance (Berg et al., 2011; Lyche et al., 2010, 2011; Nourizadeh-Lillabadi et al., 2009). Il est en cohérence avec le caractère obésogène connu des perturbateurs endocriniens dont les POP (Dirinck et al., 2011; Grün and Blumberg, 2009).

En ce qui concerne la condition des poissons, aucun effet sur ce trait n'a été observé dans cette étude (Chapitre 1). Ce résultat indique que le poids est affecté par l'exposition aux contaminants selon sa relation allométrique avec la longueur. Malgré le caractère obésogène reconnu des POP, les résultats concernant leurs effets sur la condition chez les poissons sont très variables. En effet, quelques études, incluant certaines citées ci-dessus, ont montré une augmentation de la condition chez des poissons exposés aux POP (ex. poisson-zèbre exposé à un mélange de PCB, PBDE et DDT , (Nourizadeh-Lillabadi et al., 2009; Vignet et al., 2014) et d'autres une diminution (ex. juvéniles de sole commune exposés à des HAP (Gilliers et al., 2012). Ces différences peuvent être expliquées par les différents mélanges de polluants utilisés dans ces études (ex. la composition et la concentration), leurs différents modes d'action et les différentes espèces exposées.

Reproduction

Trois traits liés à la reproduction ont été analysés dans le présent travail : la probabilité de ponte, la taille de ponte et le taux de fécondation. La probabilité de ponte s'est montrée affectée par l'exposition au mélange de PCB et de PBDE, augmentant moins rapidement avec l'âge chez les poissons MIX que chez les poissons SOLV ce qui traduit un retard de maturité sexuelle et de l'activité de reproduction (Chapitre 1). Ce résultat est en contradiction avec celui de (Nourizadeh-Lillabadi et al., 2009) qui ont constaté une maturité précoce chez le poisson-zèbre après une exposition à un mélange de POP contenant PCB, PBDE et DDT. Cependant, comme indiqué précédemment, l'exposition a provoqué dans leur étude une augmentation de la condition des femelles, qui est un facteur important favorisant la maturation et donc la probabilité de ponte chez les poissons (Grift et al., 2007; Mollet et al., 2007; Uusi-Heikkilä et al., 2011; Wright, 2007). A l'inverse, d'autres études ont également révélé un retard de maturation chez le poisson-zèbre exposé aux PCB (Daouk et al., 2011) et aux HAP (Vignet et al., 2016). De plus, il a été montré que certains congénères de PCB et de PBDE agissent comme perturbateurs endocriniens qui altèrent les voies hormonales de régulation des fonctions reproductrices, entraînant une diminution des facteurs de succès reproducteur tels que la production d'œufs et le taux de fécondation chez les poissons et d'autres vertébrés (Mills and Chichester, 2005; Yu et al., 2015). Dans la présente étude, le taux de 17beta-estradiol (E2) mesuré (voir la partie « *résultats* » du Chapitre 2, cf. §2.4) présente une diminution chez les individus MIX qui est a priori à relier à une perturbation endocrinienne. Etant donné qu'une corrélation entre le niveau de E2 et la maturation ovarienne a déjà été montrée chez le poisson-zèbre ((Clelland and Peng, 2009), cette perturbation endocrinienne pourrait expliquer le retard de ponte observé dans la présente étude.

Concernant la taille de ponte, bien que le nombre journalier d'œufs produit lors des tests de reproduction de masse dans les bacs soit en moyenne plus faible pour les poissons MIX que pour les poisons SOLV, aucune différence statistique significative n'a été détectée (Chapitre 1). Cependant, la taille de ponte mesurée sur la base de couples constitués au hasard était significativement réduite chez les poissons MIX (186 ± 19 œufs par ponte) par rapport aux poissons SOLV (259 ± 21 œufs par ponte) (Chapitre 2). Cette différence de significativité des effets peut être liée aux variations de protocole entre les deux suivis de reproduction. Dans le premier cas, la production d'œuf a été suivie à l'échelle des bacs, impliquant donc des interactions sociales dans la reproduction, sur une période courte (13 à 25 jours selon le réplica) à une fréquence journalière et sur des animaux étant relativement jeune (62 à 83 jpf selon le réplica). Dans le second, le suivi s'est fait à l'échelle du couple (constitué au hasard à chaque évènement de reproduction), sur une période longue (10 semaines) à une fréquence hebdomadaire et avec des animaux plus âgés (à partir de 117 jpf). Il est probable que le protocole du Chapitre 1 moins précis (bac versus couple), sur une période beaucoup plus courte et à des âges moindres n'ait pas permis de détecter le signal statistiquement. D'autant qu'une réduction de la taille de ponte est conforme avec des travaux antérieurs chez le poisson-zèbre qui ont montré que les PCB et les PBDE réduisaient la production d'œufs (Kuiper et al., 2008; Muirhead et al., 2006; Örn et al., 1998). Ce caractère présente cependant une sensibilité dose dépendante : (Kuiper et al., 2008) n'ont observé aucun effet sur la production d'œufs chez le poisson-zèbre après une exposition au BDE-71 administré à des doses environnementales, mais ils ont soupçonné une diminution de la production d'œufs lorsque les poissons étaient exposés à des niveaux plus élevés de BDE-71.

Enfin, les résultats concernant le taux de fécondation dépendent eux aussi du protocole considéré. Aucune différence n'a été détectée dans le Chapitre 1 alors qu'une légère diminution chez les poissons MIX (SOLV : 79.94 ± 2.83 %, MIX : 73.68 ± 4.05 %) était significative dans le Chapitre 2. Une réduction du taux de fécondation est en accord avec des études précédentes qui ont montré une réduction du taux de fécondation chez le poisson-zèbre exposé à un mélange de PCB (Daouk et al., 2011; Han et al., 2013). Il convient de noter encore une fois que l'impact d'un polluant à l'autre peut différer selon la concentration, le type de molécule et le mécanisme d'action.

Survie larvaire des descendants

L'effet du mélange de PCB et de PBDE sur la survie larvaire des descendants (génération F1) a été évalué à travers une expérience de survie réalisée sur des larves en situation de jeûne. L'hypothèse était que l'exposition aux polluants pourrait provoquer une mortalité additionnelle chez la progéniture par plusieurs mécanismes potentiels, non mutuellement exclusifs : (i) l'accumulation dans les œufs à cause d'un transfert maternel conduisant à une exposition embryonnaire, (ii) la production d'œufs et de larves de moins bonne qualité (quantité et qualité du vitellus par exemple) car issus de poissons sous stress chimique et physiologique forts, ou (iii) l'induction d'effets intergénérationnels via des mécanismes épigénétiques notamment. Le transfert maternel d'un grand nombre de contaminants a été bien documenté chez de nombreuses espèces (Ackerman et al., 2016; Metts et al., 2013; Rauschenberger et al., 2007). Chez les poissons, une attention particulière a été donnée au transfert des POP aux œufs, en particulier les PCB et les PBDE, en raison de leurs propriétés lipophiles qui fournissent une voie de transfert facile au travers de l'association aux lipides stockés chez la femelle (Chen et al., 2012; Daouk et al., 2011; Yu et al., 2011).

Dans la présente étude, la survie des larves en situation de jeûne a été affectée significativement chez les larves issues de pontes précoces (1res pontes) par rapport à celles issues de pontes plus tardives (10es pontes), indiquant une amélioration de la survie au fur et à mesure des évènements de ponte. Ce résultat est probablement lié à une amélioration de la qualité des œufs (taille et richesse du vitellus) avec l'âge de la femelle, ce qui a pourrait compenser à la fois l'effet négatif des PCB et des PBDE transférés aux œufs et l'état de stress physiologique de la femelle. Cette amélioration de la qualité des œufs avec l'âge et la taille des femelles est un phénomène commun chez les poissons (Brooks et al., 1997; Nasiadka and Clark, 2012; Trippel et al., 1997).

Dans le milieu marin, le recrutement des individus d'une nouvelle classe d'âge dans la population dépend fortement de la disponibilité de la nourriture, en particulier durant la phase critique au stade larvaire. Les épisodes de famine durant ce stade sont fréquents et entraînent généralement un recrutement plus faible et des classes d'âge plus petites (Cushing, 1990; Leaf and Friedland, 2014; Pritt et al., 2014). Les résultats observés ici sur la survie larvaire des descendants suggèrent que l'exposition aux PCB et aux PBDE dans le milieu marin pourrait amplifier les conséquences néfastes de la famine sur la survie des larves et donc la diminution du niveau de recrutement qui en résulte.

Modes d'action physiologiques des PCB et des PBDE

Les principaux résultats de cette partie sont résumés dans la figure 8.

Après avoir évalué les effets du mélange de PCB et de PBDE sur les traits d'histoire de vie d'un point de vue empirique, le développement d'un modèle de Budget Energétique Dynamique (DEB) s'est avéré être un outil utile pour inférer les effets bioénergétiques du mélange à partir des patrons de variations des traits d'historie de vie et émettre des hypothèses sur ses modes d'action physiologiques (PMoAs).

Identification des modes d'action physiologiques

La calibration indépendante du modèle DEB sur les données des poissons contrôles et exposés a permis l'identification des PMoAs du mélange de PCB et PBDE à partir de la variation des valeurs des paramètres bioénergétiques et de leur impact sur l'ajustement aux données. Cette identification a ensuite été validée de façon croisée par comparaison des patrons de changement des traits d'histoire de vie observés et ceux prédits pour chacun des six modes d'actions possibles. Cette approche a permis de révéler deux PMoAs probables (Chapitre 2). Le premier PMoA est lié à l'augmentation de la fraction d'énergie κ allouée à la maintenance et la croissance somatique et réciproquement à la diminution de celle $1-\kappa$ allouée à la maturité, sa maintenance et la reproduction. Le deuxième PMoA est lié à l'augmentation des coûts énergétiques E_0 pour produire un œuf ou autrement dit les coûts de reproduction. Ces deux paramètres sont ceux présentant à la fois une variation nette entre individus non exposés et exposés (+ 8.5% pour κ et + 19% pour E_0) et une influence forte sur l'ajustement aux données (degré d'ajustement 12 et 4 fois meilleur pour κ et E_0, respectivement, que le degré d'ajustement moyen obtenu pour l'ensemble des PMoAs).

Le paramètre κ représente le compromis énergétique entre la croissance et la reproduction. Il est le seul PMoA ayant des effets dans des directions opposées sur la croissance et la reproduction, i.e., conduisant à la fois à une croissance plus forte et une reproduction plus faible en termes de production d'œuf, comme observé chez les poissons MIX. Tous les autres PMoAs affectent soit la croissance et la reproduction dans la même direction (ingestion-assimilation, coûts de croissance, maintenance) soit seulement la reproduction (coûts de la reproduction et mortalité embryonnaire). Les coûts de reproduction E_0 est un PMoA qui agit sur le nombre d'œufs produits au travers de la conversion des réserves d'énergie gonadique en gamètes. Dans le modèle DEB, le

ratio E_0/κ_R, où le paramètre κ_R représente l'efficacité de reproduction i.e., de la conversion de l'énergie en gamète, est le coût réel de production d'un œuf. Cependant, les deux paramètres ne peuvent être distingués sans des données énergétiques sur les œufs indisponibles dans cette thèse. Une valeur fixe a donc été attribuée au paramètre κ_R lors de la calibration, de sorte que les variations du paramètre E_0 représentent celle du ratio E_0/κ_R et peuvent donc être interprétées en tant que telles.

Dans le contexte de la théorie DEB, le PMoA correspondant au compromis énergétique κ n'a jamais été démontré quel que soit le polluant considéré. Il a cependant été suggéré par (Jager et al., 2010) et (Kooijman, 2010) que la fraction d'énergie κ pourrait être affectée notamment dans le cas d'une exposition à des perturbateurs endocriniens tels que les PCB et les PBDE. Ce PMoA est en effet cohérent avec le fait que les PCB et PBDE ont à la fois un effet obésogène reconnu (Berg et al., 2011; Lyche et al., 2010, 2011; Nourizadeh-Lillabadi et al., 2009) et peuvent altérer la reproduction (Mills and Chichester, 2005; Yu et al., 2015). A l'inverse, les coûts de reproduction E_0 sont un PMoA qui a été mis en évidence pour de nombreux polluants(ex. Kooijman and Bedaux, 1996; Martin et al., 2013). Dans le contexte des PCB et des PBDE, ils peuvent être interprétés comme des coûts énergétiques supplémentaires pour la production des œufs dus à l'altération de la fonction de reproduction.

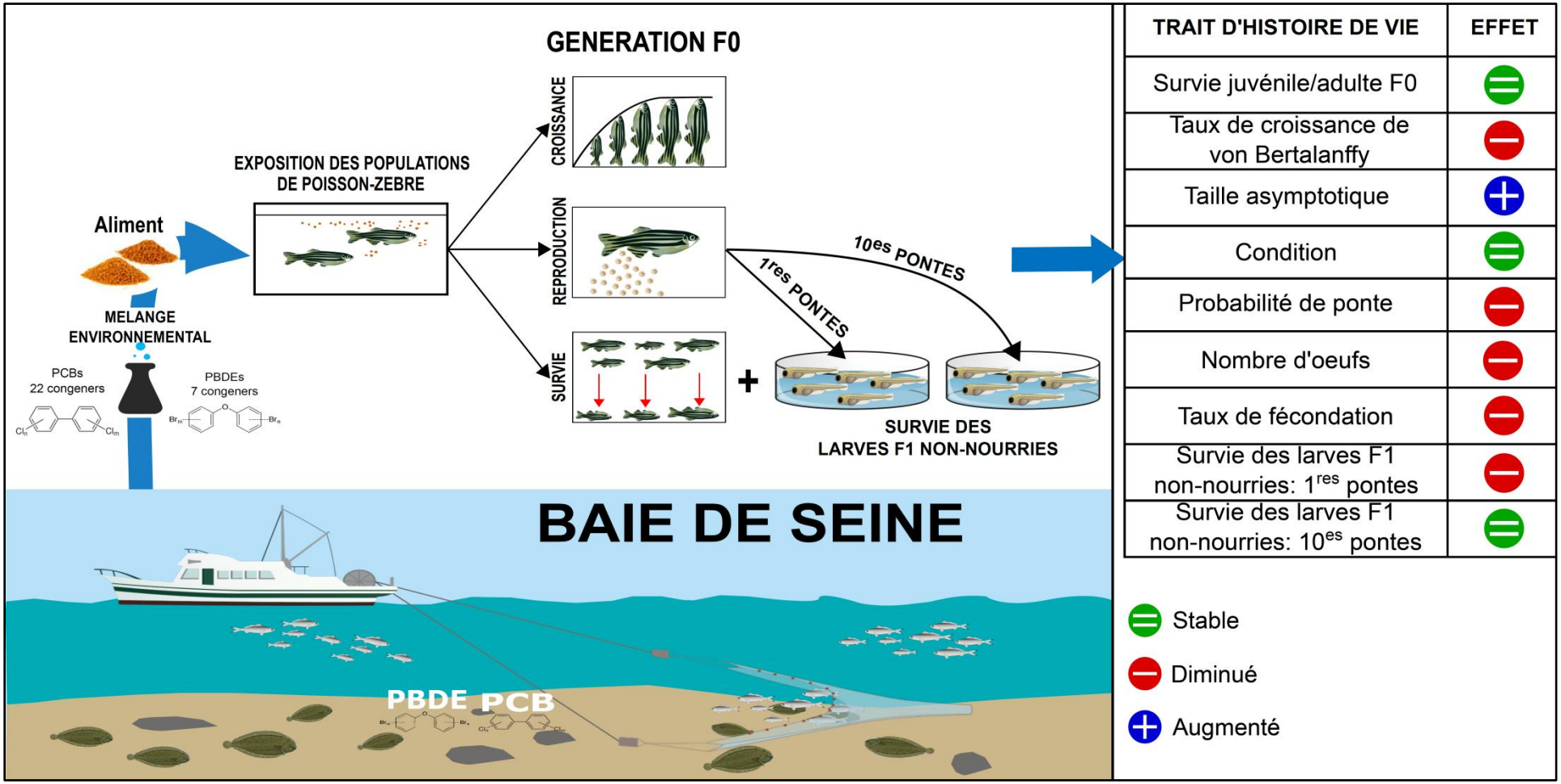

Fig. 7. Schéma synthétique des résultats obtenus sur les effets du mélange des PCB et des PBDE sur les traits d'histoire de vie chez les poissons.

Interprétation biologique des modes d'action physiologiques

La piste d'une perturbation endocrinienne pourrait expliquer nos résultats sur les deux PMoAs identifiés. En effet, des mesures de deux hormones stéroïdiennes sexuelles, la 17beta-estradiol (E2) et la 11keto-testosterone (11KT), ont montré une forte perturbation endocrinienne chez les poissons MIX avec des concentrations de ces deux hormones significativement plus faibles pour ceux-ci pendant la partie du cycle de vie correspondant à la maturation des gonades (Chapitre 2). On peut émettre l'hypothèse que l'effet du mélange de PCB et de PBDE sur le compromis énergétique κ serait lié à cette perturbation endocrinienne des hormones stéroïdes sexuelles. En effet, il a été montré qu'une perturbation des niveaux de 17beta-estradiol (E2) et/ou de 11keto-testosterone (11KT) était capable d'altérer la fonction de reproduction et potentiellement d'affecter également la croissance. Concernant la reproduction, la différentiation et la maturation des ovaires sont par exemple associés à un accroissement de E2 chez le poisson-zèbre (Clelland and Peng, 2009). Cette augmentation a bien été observée dans les populations expérimentales du Chapitre 2 entre 30 et 60 jfp mais l'augmentation des individus exposés était inférieure d'un ordre de grandeur à celle des individus contrôles. Cette augmentation moindre pourrait causer un retard ou une mauvaise maturation de la gonade femelle expliquant la production d'œufs moindre. Concernant la croissance, une étude récente a montré que l'exposition de poissons à des stéroïdes sexuels pouvait modifier l'expression des gènes codant pour la *ghréline* et la *Nesfatin-1* dans divers tissus (Bertucci et al., 2016). Or, la *ghréline* est impliquée dans la régulation de plusieurs fonctions physiologiques chez les poissons, notamment la croissance (via la libération de l'hormone de croissance, GH) et l'ingestion (Truite arc-en-ciel: Jönsson et al., 2007; tilapia: Kaiya et al., 2003; poisson-zèbre: Li et al., 2009; poisson rouge: Matsuda et al., 2006). La *Nesfatin-1* a été décrite comme impliqué

dans la réduction de l'ingestion chez le poisson rouge (Gonzalez et al., 2010; Kerbel and Unniappan, 2012) chez qui son taux est considérablement diminué par une exposition à la testostérone (Bertucci et al., 2016). D'après ces observations, il est plausible d'émettre l'hypothèse que la régulation de l'ingestion et de la croissance par les stéroïdes sexuels telle qu'observée chez les mammifères est similaire chez les poissons. Ainsi la perturbation des stéroïdes sexuels par les PCB et les PBDE observée dans notre étude pourrait également être à relier à l'augmentation de la croissance des poissons MIX.

Concernant l'augmentation des coûts de production d'un œuf E_0 induite par le mélange de PCB et PBDE, les perturbations hormonales pourraient également en être la cause. Selon (Williams, 2005), la formation des œufs chez les oiseaux nécessite une augmentation substantielle du poids ou de l'activité métabolique d'organes tels que le foie (qui produit des précurseurs du vitellus) ou les organes reproducteurs tels que l'ovaire. Cette augmentation du poids de l'organe, à son tour, pourrait provoquer un coût énergétique supplémentaire pour sa maintenance qui pourrait se traduire en termes de paramètre bioénergétique au sein du modèle DEB par une augmentation des coûts de reproduction. Chez les poissons, il a notamment été démontré que les POP perturbent l'activité du foie et de l'ovaire en altérant la transcription des gènes au niveau de ces organes (Lyche et al., 2010, 2011). De plus, il est connu de que les réserves énergétiques accumulée durant la partie non reproductive de l'année et utilisées pour la croissance gonadique et la maturation des gamètes proviennent du foie chez de nombreuses espèces de poisson (Diana and Mackay, 1979; Kjesbu et al., 1991). Ceci caractérise plus spécifiquement les reproducteurs sur capital (capital breeders), qui stockent de l'énergie dans le foie, les muscles ou la graisse mésentérique avant la saison de ponte pour produire plus tard leurs œufs, par opposition aux reproducteurs sur revenu (income breeders), qui utilise les entrées d'énergie durant la saison de ponte pour

produire des œufs (McBride et al., 2015). Sous l'hypothèse que ce stockage induise une maintenance accrue, l'augmentation des coûts de reproduction d'œuf observée dans le présent travail pourrait être liée à une perturbation du fonctionnement de l'organe de stockage telle que la synthèse de la vitellogénine dans le foie. Des mesures supplémentaires, par exemple de l'activité métabolique (énergétique) du foie chez les poissons contrôles et exposés, pourraient apporter une clarification à ce résultat.

Au-delà de la compréhension des effets bioénergétiques du mélange de PCB et PBDE, l'identification des PMoAs est essentielle pour une meilleure prédiction des conséquences démographiques des effets individuels du mélange comme décrit dans la partie suivante.

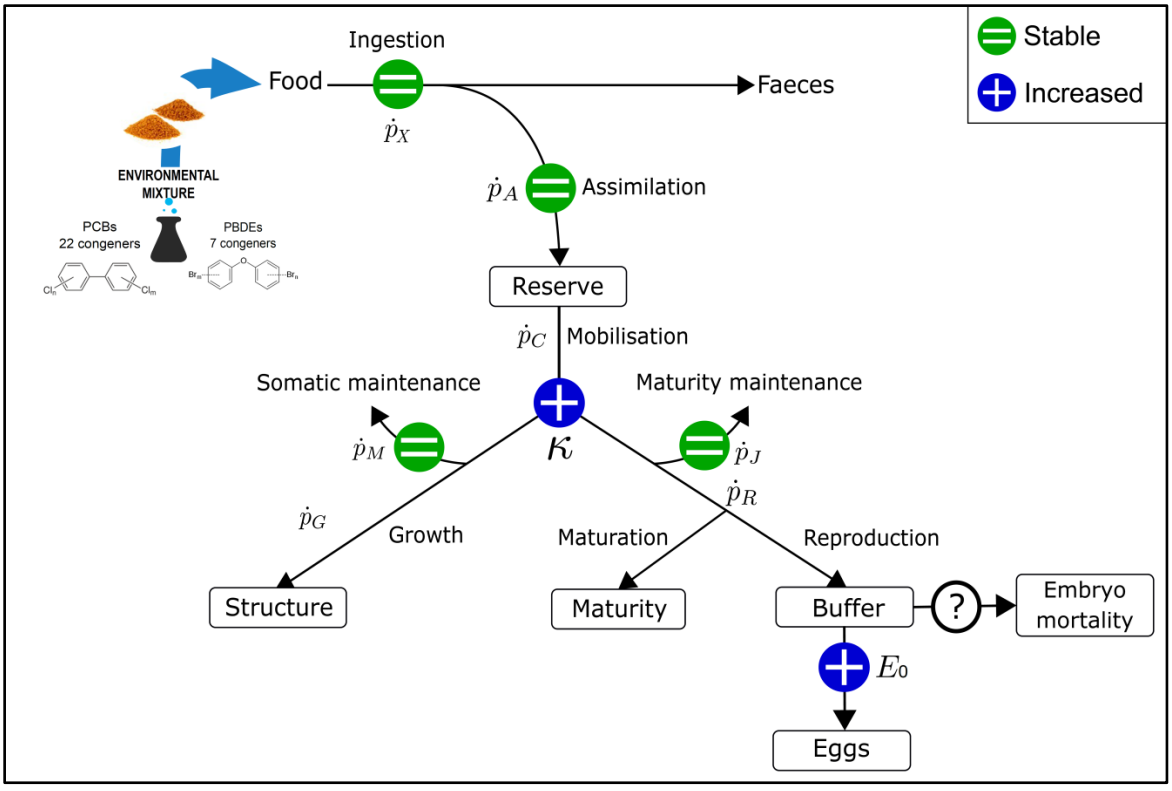

Fig. 8. Schéma synthétique des résultats obtenus sur les effets du mélange de PCB et de PBDE sur les performances bioénergétiques chez les poissons.

Effets des PCB et des PBDE à l'échelle populationnelle

La dernière partie de la thèse a consisté à évaluer les conséquences des réponses individuelles à l'exposition à un mélange de PCB et de PBDE via les deux PMoAs décrits précédemment sur la dynamique de population à l'aide d'un couplage du modèle DEB avec un modèle PSP. Un objectif était également d'inférer les conséquences potentielles pour la productivité des pêcheries commerciales sous l'hypothèse que les effets observés soient transposable aux populations de poissons téléostéens marins. Ceci a été réalisé en intégrant au modèle couplé DEB-PSP un patron d'exploitation en fonction de la taille correspondant à celui du stock de sole commune de la Manche Est mis à l'échelle, en termes de taille et de longévité, pour la population de poisson-zèbre. Comme déjà indiqué, le raisonnement ici était de considérer le stock de sole commune de Manche Est en lien avec l'estuaire de Seine comme un cas d'étude typique de pêcherie associée à un bassin versant et un estuaire fortement pollué.

Les principaux résultats de cette partie sont résumés dans la figure 9.

Couplage entre modèle bioénergétique individuel et modèle de population

Le couplage entre un modèle bioénergétique à l'échelle individuelle et un modèle populationnel permet d'évaluer les conséquences démographiques des contaminants à travers les répercussions de leur PMoA. Ceci présente deux avantages. Tout d'abord, cela permet de prendre en compte en même temps les différentes voies d'altérations physiologiques pouvant avoir des effets directs et indirects sur les traits d'histoire de vie à travers lesquels l'exposition aux polluants peut avoir un impact sur la dynamique de population. Ensuite, les conséquences démographiques des PMoAs peuvent être généralisées à l'ensemble des contaminants qui les partagent. Une telle généralisation en peut se faire en couplant un modèle phénoménologique des effets des contaminants sur

les traits d'histoire de vie avec un modèle populationnel car des effets similaires sur l'histoire de vie peuvent émerger de PMoAs différents qui risqueraient avoir des effets additionnels ayant des conséquences démographiques différentes.

Ce type de couplage entre modèle DEB et modèle populationnel pour étudier les conséquences démographiques des contaminants a déjà été réalisé dans d'autres études (Billoir et al., 2007; Jager and Klok, 2010; Jager et al., 2007; Klok and de Roos, 1996; Kooijman and Metz, 1984; Martin et al., 2013a, 2013b). Cependant aucune de celles-ci ne s'est intéressée à des vertébrés, notamment des poissons, et à des mélanges réalistes de molécules tel que celui testé dans cette thèse. De plus, aucune étude n'a exploré les conséquences démographiques du PMoA « compromis énergétique » associé au paramètre κ dans le modèle DEB alors que celles des autres l'ont été (Billoir et al., 2007; Kooijman and Metz, 1984; Martin et al., 2014, 2013a). Etant donné le lien entre ce PMoA et la perturbation endocrinienne générée par le mélange de PCB et PBDE testé ici, les résultats en termes de conséquences démographiques sont potentiellement généralisables à d'autres perturbateurs endocriniens.

Effets des PCB et des PBDE et de leur PMoA sur l'abondance de la population

Les projections du modèle DEB-PSP montre que l'exposition au mélange de PCB et PBDE entraine une réduction de l'abondance numérique de la population en même temps qu'une augmentation de sa biomasse (Chapitre 3). Ces deux effets sont similaires en présence et en absence de d'une mortalité par pêche et reflètent relativement directement les effets du mélange observés sur les traits d'histoire de vie. L'abondance numérique réduite chez la population exposée est probablement due à l'altération de la fécondité élevée alors que la biomasse élevée est liée à l'augmentation de la croissance qui surcompense le déficit numérique. Bien que la biomasse de la population exposée

soit plus élevée que celle de la population non exposée, son risque d'extinction du fait de la stochasticité environnementale ou démographique est potentiellement plus élevé car elle présente une plus faible abondance (Boyce, 1992; Lande, 1993).

L'étude par bifurcation de l'effet indépendant des deux PMoAs du mélange de PCB et de PBDE identifiés à partir du modèle DEB, à savoir l'augmentation de la fraction d'énergie κ et des coûts de production d'un œuf E_0, montre qu'ils ont participé conjointement aux effets sur l'abondance numérique et la biomasse, i.e., les deux PMoAs provoquent indépendamment une diminution de l'abondance numérique dans la population et une augmentation de sa biomasse (avec la différence qu'au-delà d'une certaine valeur de $\kappa = 0.97$, les femelles ne produisent plus d'œufs de sorte que la population s'effondre). Pour le compromis énergétique κ, ces effets résultent directement des processus bioénergétiques à l'échelle individuelle, i.e., l'augmentation de l'énergie allouée au soma, notamment sa croissance et une diminution de celle allouée à la maturation et la reproduction, dont la croissance gonadique. Pour les coûts de la reproduction E_0, les mêmes effets résultent du relâchement de la compétition trophique via la boucle de rétroaction environnementale, i.e., l'interaction entre la dynamique de la population des poissons consommateurs et celle de leur ressource. En effet, la diminution de fécondité liée à l'augmentation de ces coûts entraîne une diminution du recrutement, ce qui diminue la compétition pour la nourriture notamment des juvéniles, de sorte que les individus ont une croissance accrue qui va surcompenser les pertes numériques et aboutir à une augmentation de biomasse. Il convient de noter ici, que l'effet direct de κ est certainement combiné à un effet indirect via la boucle de rétroaction environnementale selon le même principe de sorte que l'augmentation de κ génère des effets à la foi directs et indirects synergiques.

Les résultats liés au compromis énergétique κ ne peuvent être comparés à la littérature étant donné que cette étude est la première à considérer ce PMoA. A partir de la littérature consultée, il semble que la projection d'une augmentation de biomasse dans une population contaminée soit très rare. L'amplitude exacte de cet effet démographique dépend de celle de l'effet positif sur le flux énergétique vers la croissance qui elle-même dépend de la variation de κ qui est une fonction des contaminants et de leur concentration. Cependant, il est vraisemblable que l'effet qualitatif du compromis énergétique sur l'abondance numérique et la biomasse soit généralisable aux perturbateurs endocriniens ayant le même PMoA.

Concernant les coûts de reproduction E_0, plusieurs études de modèles couplés en ont déjà exploré les conséquences démographiques. Cependant, les résultats ne sont pas toujours facilement comparables. Kooijman et Metz (1984) par exemple ont montré qu'une réduction de la fécondité de 35% due à un contaminant entrainait une diminution du taux de croissance intrinsèque de la population chez la Daphnie. Cependant, leur modèle n'incluait pas de boucle de rétroaction environnementale de sorte que les résultats ne sont pas réellement comparables. Martin et al. (2013a) ont également testé les conséquences de la réduction de la fécondité chez la Daphnie due à une exposition à la 3,4-dichloroaniline dans un modèle intégrant la boucle de rétroaction environnementale mais ils n'ont détecté des effets sur l'abondance de la population que pour des réductions de fécondité supérieures à 40% alors nos résultats reposent sur une réduction de fécondité de 16 à 25% en fonction de l'âge considéré. Les différences de résultats ici pourraient être dues au fait que dans notre cas la réduction de fécondité résulte de l'augmentation de κ en plus de E_0 et/ou les différences bioénergétiques entre espèces, la dynamique des populations étant fortement dépendante de la bioénergétique des individus (De Roos and Persson, 2013). En utilisant

le même modèle, Martin et al. (2014) ont cependant trouvé que quel que soit le PMoA, les contaminants généraient toujours une diminution de biomasse exceptée pour les coûts de reproduction pour lesquels ils ont observé une légère augmentation de la biomasse (moins de 5%) pour un effet individuel fort (réduction de fécondité de 50%) et une forte valeur de la densité maximale de ressource. L'amplitude de cet effet est bien plus faible que celui observé dans cette thèse. Cependant, il souligne son caractère potentiellement générique et le fait que les PMoAs ayant un effet direct sur la production d'œufs ont des effets différents sur la dynamique de population que ceux ayant un effet indirect (excepté κ) et que ceux-ci reposent sur la boucle de rétroaction environnementale.

Effets des PCB et PBDE sur la viabilité des populations et la productivité des pêcheries

L'étude par bifurcation de la viabilité des populations face à la mortalité a montré que la population MIX s'éteint pour des taux de mortalité plus faibles que pour la population SOLV lorsque la mortalité est indépendante de la taille des individus (Chapitre 3), comme attendu du fait de la réduction de fécondité des poissons exposés. Cependant, lorsque la mortalité diminue avec la taille des individus, du fait de la prédation taille-dépendante notamment (Lorenzen, 1996; McGurk, 1986; Peterson and Wroblewski, 1984), la réduction de fécondité est surcompensée par réduction de la mortalité due à taille plus grande des individus exposés, de sorte que la population MIX résiste dans ce cas à des taux de mortalité maximale plus élevés que la population SOLV (Chapitre 3). Le fait que l'avantage en termes de mortalité dû à une taille plus grande surcompense ou pas la réduction de fécondité va alors dépendre de la vitesse à laquelle la mortalité naturelle décroit avec la taille (et donc de l'exposant de la fonction d'allométrie reliant mortalité et longueur des individus).

En outre, lorsque qu'une mortalité par pêche est ajoutée à la mortalité par prédation et que celle-ci augmente avec la taille, comme c'est le cas classiquement dans les pêcheries au chalut et plus spécifiquement de la mortalité par pêche de la sole de Manche Est, la population MIX supporte des taux d'exploitation moindres que la population SOLV. De nouveau, cette différence de viabilité est liée à l'interaction entre mortalité taille-dépendante, ici la mortalité par pêche, et la différence de croissance entre individus non exposés et exposés. Les individus MIX plus grands subissent une mortalité plus importante et résistent à des taux de mortalité par pêche moins grands que les individus SOLV malgré leur avantage en termes de mortalité naturelle. Cette fois-ci, le fait que l'une ou l'autre des populations résiste mieux à l'exploitation va dépendre du ratio coût-bénéfice entre la perte de fécondité, la réduction de la mortalité naturelle et l'augmentation de la mortalité par pêche, qui toutes deux vont également dépendre de la vitesse à laquelle la mortalité naturelle et par pêche varient avec la taille des individus. Ces résultats mettent en évidence que l'extinction des populations de poissons peut être accélérée si ces populations subissent, en plus d'un stress de contamination, une pression d'exploitation forte et réciproquement (Barnthouse et al., 1990; Goodyear, 1985).

L'analyse des captures en fonction de la mortalité par pêche montre enfin que, de façon assez contre-intuitive dans l'absolu mais en ligne avec les résultats précédents, la population MIX permettrait un rendement maximum durable, i.e., l'extraction durable d'une biomasse, plus élevé que la population SOLV (Chapitre 3). Ceci se ferait cependant à un taux d'exploitation moindre, exposant ainsi la population MIX à un plus grand risque de surexploitation. Pour conclure, dans l'hypothèse où ces résultats seraient transposables à des espèces de poissons marins exploités, les travaux de cette étude pourraient constituer une première étape vers des outils d'aide à une gestion durable

des stocks halieutiques tels que la sole de Manche Est, qui prennent en compte les effets de multiples contaminations qui se surajoutent à la pression de pêche.

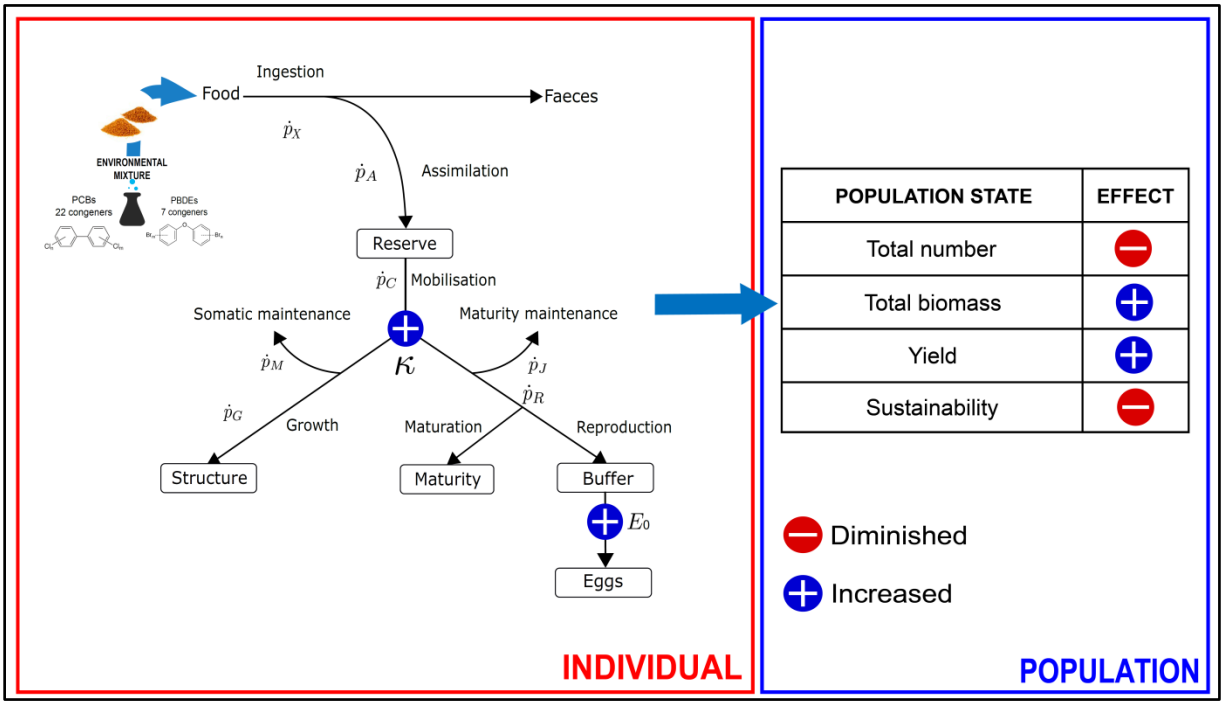

Fig. 9. Schéma synthétique des résultats obtenus sur les effets du mélange de PCB et de PBDE à l'échelle populationnelle.

Perspectives

Les résultats obtenus dans le cadre de cette thèse ouvrent de nombreuses perspectives de recherche qui pourraient être développées pour mieux comprendre les effets des mélanges environnementaux de PCB et PBDE, voire plus généralement des contaminants, sur les populations de poissons.

Une première question est de savoir si les effets individuels observés dans cette thèse sur la génération parentale du poisson-zèbre seront transmis aux générations suivantes. Dans cette hypothèse, une question complémentaire serait de savoir si ces effets sont amplifiés ou atténués. En milieu naturel, une amplification, c'est-à-dire une croissance qui augmente et une reproduction qui diminue d'une génération à l'autre, est peut-être

plus vraisemblable dans le sens où les effets transmis s'ajouteraient aux effets liés à l'exposition directe de la génération suivante. Il apparait donc crucial de réaliser une étude pour explorer les effets transgénérationnels d'un mélange environnemental marin de PCB et de PBDE et leurs conséquences démographiques. La mise en place de cette étude devrait aussi viser à distinguer entre les effets liés à un transfert maternel et ceux liés à des phénomènes épigénétiques transmis d'une génération à l'autre. Ces derniers pourraient être établis à partir d'une évaluation des changements de méthylation de l'ADN par exemple (Bhandari et al., 2015; Kamstra et al., 2015). Des travaux menés dans une thèse parallèle à celle-ci par Sébastien Alfonso (Unité MARBEC, Ifremer) et portant justement sur les effets transgénérationnels montrent que l'expression de gènes impliqués dans la régulation de la méthylation de l'ADN est modifiée chez des larves issues des poissons exposés au même mélange de PCB et PBDE. Ceci suggère donc des effets épigénétiques qui pourraient être transmissibles.

Un deuxième pan en termes de perspectives concerne les bases biologiques des modes d'action physiologique. Lors de cette étude, le développement du modèle DEB sur des populations exposées à un mélange réaliste de PCB et de PBDE a permis d'identifier deux modes d'action physiologiques potentiels associés à ces contaminants. Cependant, le lien entre ces modes d'action, en particulier l'augmentation de la fraction d'énergie κ, et les processus moléculaires et hormonaux reste à préciser. Une étude plus approfondie pourrait être réalisée sur la cinétique d'accumulation de ces contaminants dans les différents tissus des individus et celle de leurs interactions avec certaines cibles moléculaires pour apporter des informations complémentaires. Notamment, au-delà de la perturbation de la sécrétion des hormones stréoïdiennes sexuelles démontrée dans cette thèse, l'interaction de ces contaminants avec d'autres composantes du système endocrinien comme la voie de l'hormone de croissance ou la voie des hormones

thyroïdiennes devrait être étudiée. En effet, des perturbations de ces deux voies par les PCB et/ou les PBDE ont déjà été montrées chez les poissons (hormone de croissance : cf.§ 2.5 ; hormones thyroïdiennes : Blake et al., 2011; Brar et al., 2010; Buckman et al., 2007; Nakayama et al., 2008; Richardson et al., 2008; Talsness et al., 2008; Yu et al., 2011). Les données de cinétique serait utiles dans un second temps pour intégrer au modèle DEB un modèle de cinétique d'accumulation lié à un modèle d'effet dépendant de la concentration interne qui permettrait une meilleure interprétation et extrapolation de l'évolution des effets d'un mélange de PCB et PBDE sur la bioénergétique et les traits d'histoire de vie avec l'âge.

Le troisième volet de questions concerne les conséquences du mélange de PCB et PBDE au niveau de la population. Les résultats présentés dans cette thèse dépendent pour une part importante de la boucle de rétroaction environnementale liée à l'interaction entre dynamique des poissons (les consommateurs) et celle de leur ressource. D'autres hypothèses quant à la configuration de cette boucle pourraient être testées, en particulier une ressource différente pour chaque stade de vie, ou au moins entre stade larvaire et stades ultérieurs, pourrait être considérée contrairement à l'étude présentée en Chapitre 3 qui ne comportait qu'une seule ressource partagée entre les stades de vie. Alternativement, la représentation de la ressource pourrait être structurée en taille et les relations proie-prédateur dépendre de la taille de sorte que les poissons ne consomment pas la même ressource lorsqu'ils diffèrent en taille. De manière plus générale autour des relations proie-prédateur, au-delà de l'évaluation des conséquences des effets individuels générés par différents PMoA sur la dynamique des populations, il semblerait intéressant de développer des travaux, de modélisation a priori, sur les répercussions sur la dynamique des communautés de poissons et leur structure. En effet, au vu des interactions trophiques (prédation et compétition) entres les espèces de

poissons et la dépendance des unes aux autres qui en découle, une approche à l'échelle communautaire apparait nécessaire. Dans ce contexte d'extrapolation des effets démographiques à la communauté, des simulations supplémentaires explorant d'autres PMoAs potentiels (ex. assimilation, maintenance) pourraient être envisagées pour tenir compte d'éventuelles différences de mode d'action entre espèces ou plus probablement de différences de sensibilité.

Toujours en lien avec le niveau populationnel, cette thèse a jeté les premières bases en termes d'étude de l'interaction entre effet des contaminants et exploitation par la pêche des populations de poissons. Une limitation importante cependant est que le modèle utilisé au niveau expérimental mais également pour la modélisation est le poisson-zèbre. Des données sur les effets des PCB et PBDE sont disponibles pour la sole au travers de travaux développés en parallèle durant le projet Fish'N'POPs et plusieurs projets précédents. Celles-ci pourraient être utilisées pour développer une approche de modélisation couplée DEB-PSP appliquée à la sole telle que celle proposée dans cette thèse. Alternativement, il serait envisageable d'appliquer directement le modèle développé dans cette thèse sur d'autres poissons marins représentant des similarités physiologiques et de traits d'histoire de vie que le poisson-zèbre, tels que certains petits pélagiques comme l'anchois, simplement en extrapolant les PMoA observés chez le poisson-zèbre aux paramètres bioénergétiques de ces espèces.

Enfin, d'un point de vue évolutif, il est connu que la toxicité du milieu peut entrainer la sélection de génotypes résistants aux contaminants (Forbes, 1998). Cependant, ces génotypes peuvent avoir des caractéristiques phénotypiques contre sélectives en termes d'adaptation aux autres facteurs environnementaux, plus « naturels », ce qui peut affecter notamment leur aptitude au recrutement. Il serait donc intéressant d'améliorer

notre compréhension des conséquences évolutives intergénérationnelles de l'exposition aux PCB et de PBDE. La mise en place d'une expérience de micro-évolution résultant de la sélection par exposition au mélange de PCB et PBDE sur plusieurs générations pourrait être envisageable afin de :

- Comprendre comment la sélection phénotypique générée par le mélange de PCB et de PBDE pourrait se traduire à l'échelle intergénérationnelle en termes de dynamique évolutive des traits d'histoire de vie au sein des populations exposées ;
- Comprendre les effets supplémentaires sur la dynamique des populations qu'impliqueraient les modifications intergénérationnelles des traits d'histoire de vie ;
- Et tester si le changement des paramètres DEB (ex. κ, E_0) peut également résulter d'un processus évolutif (Kooijman, 2010) entrainé par la sélection d'un phénotype donné générée par les contaminants.

Il y a toutefois une limitation majeure à ces approches de micro-évolution expérimentale liée à la faible variabilité génétique des lignées de poisson-zèbre dites "sauvages" mais cependant de laboratoire. Dans le cadre de cette thèse, une approche de micro-évolution utilisant des poissons-zèbres réellement sauvages issus de populations naturelles était prévue. Malheureusement, nous avons été confrontés à des difficultés pour obtenir un nombre suffisant d'adultes reproducteurs nécessaire au maintien d'une variabilité génétique suffisante pour la réalisation de cette expérience. Si cette limitation était dépassée, le poisson-zèbre pourrait être un modèle prometteur pour la réalisation d'une telle expérience de micro-évolution en raison de son temps de génération très court, de sa facilité d'élevage et des connaissances biologiques, physiologiques et génétiques

accumulées sur cette espèce. Cette étude expérimentale pourrait être complétée par le développement d'un modèle éco-génétique (Dunlop et al., 2009) décrivant les interactions entre processus évolutifs, écologiques et démographiques, calibré sur la base des résultats de l'expérience de sélection. Celui-ci permettrait de prédire les conséquences à long terme, des gradients de sélection imposés par l'exposition au mélange de PCB et de PBDE, sur l'évolution des traits d'histoire de vie des poissons.

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