



Effet de l'environnement sur les stratégies comportementales du bar *Dicentrarchus labrax*. Cas d'une pénurie de nourriture et d'une marée noire

Cassandre Aimon

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HAL Id: tel-02957679

<https://theses.hal.science/tel-02957679>

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THESE DE DOCTORAT DE

L'UNIVERSITE
DE BRETAGNE OCCIDENTALE

COMUE UNIVERSITE BRETAGNE LOIRE

ECOLE DOCTORALE N° 598

Sciences de la Mer et du littoral

Spécialité : « *Biologie Marine* »

Par

Cassandra AIMON

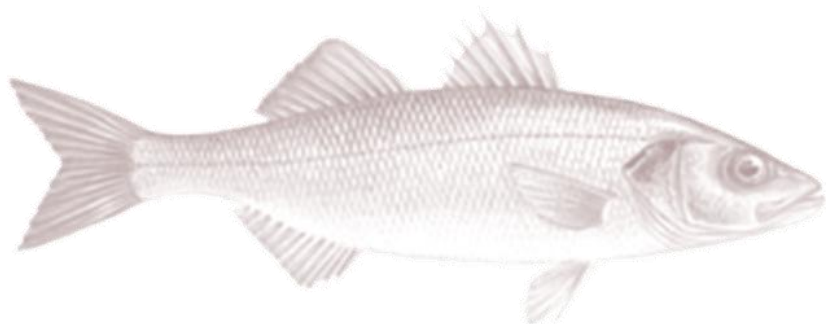
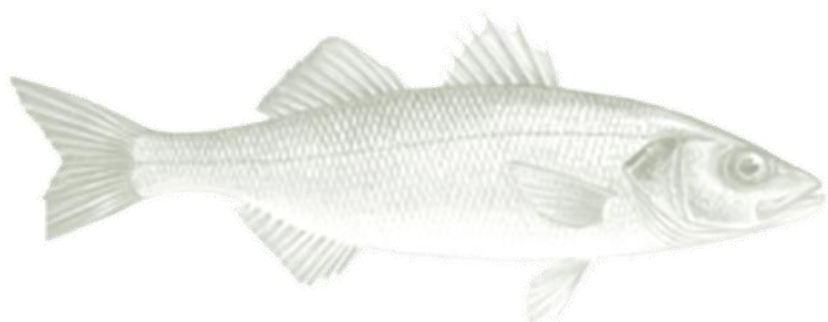
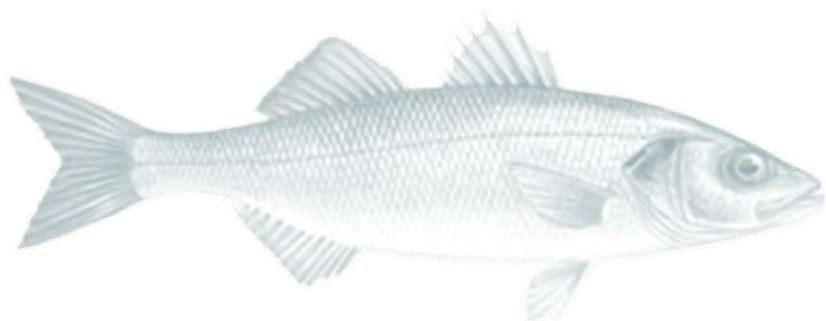
Effet de l'environnement sur les stratégies comportementales du bar
Européen *Dicentrarchus labrax*. Cas d'une pénurie de nourriture et d'une
marée noire

Thèse présentée et soutenue à Brest, le 21 octobre 2019

Unité de recherche : LEMAR (UMR 6539), Université de Bretagne occidentale,
Centre Ifremer de Bretagne, Unité PFOM-ARN
CEDRE, département recherche

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REMERCIEMENTS

Pour commencer, je souhaite remercier l'ensemble des membres du jury pour avoir accepté d'évaluer mon travail de thèse. Merci aux deux rapporteurs de cette thèse, David McKenzie et Rachid Amara, pour m'avoir fourni des commentaires constructifs et positifs sur mon travail; merci aux deux examinatrices, Christel Lefrançois et Carole Di Poï qui ont apporté leur expertise dans ce jury.

Je remercie également Guy Claireaux et Stéphane Le Floch pour m'avoir permis de continuer à travailler, comme je le souhaitais, dans les domaines du comportement animal et de la physiologie, que j'affectionne tant et pour toute l'autonomie que j'ai eu pour mener ma thèse. Merci de m'avoir permis de participer à des congrès internationaux qui m'ont permis d'élargir mon réseau relationnel, indispensable pour mon avenir professionnel.

Merci également aux personnes qui ont pris part à mes comités de thèses, Carole Di Poï, Sabine Roussel et Mark Whittington pour leurs précieux conseils.

Merci au personnel de l'école doctorale, en particulier Elodie Benon et Elisabeth Bondu pour avoir géré la partie administrative et m'avoir toujours aussi bien accueillie. Merci au LEMAR et à l'équipe PANORAMA pour les aides financières et la gestion administrative des différentes missions.

Je remercie le Cedre dans son ensemble, pour le demi-financement de cette thèse, ainsi que l'équipe technique pour son aide dans les analyses toxicologiques des tissus et bien sur Camille Lacroix pour le renfort apporté.

Je tiens à remercier le directeur José Zambonino ainsi que l'ensemble des membres du laboratoire PFOM-ARN pour m'avoir accueillie bien plus longtemps que prévu au sein de leur équipe et de leurs infrastructures. Merci à tout le personnel pour leur sympathie et la bonne humeur aux pauses. Merci de m'avoir toujours permis de continuer mes expérimentations, même s'il a fallu redoubler d'astuces après l'incendie pour que je puisse retrouver un espace où effectuer mes expériences.

Un énorme merci à Nicolas Le Bayon pour son ingéniosité m'ayant permis de toujours trouver une place dans les infrastructures et une alternative quelques fussent les problèmes rencontrés ! Merci de m'avoir aidé à mettre en place toutes mes constructions les plus loufoques et pour la transmission de tes techniques de bricolage, que je ne te laissais jamais faire seul car oui je comptais bien tout savoir faire par moi même, comme tu l'avais deviné ! Ce message est maintenant devenu particulier, mais merci où que tu sois, pour le partage de ta connaissance qui semblait infinie, ton humour et bien sûr pour m'avoir fait découvrir le paddle surf. J'espère que tu es en paix maintenant et j'aurais toujours une pensée pour toi lors d'une sortie en paddle.

Un grand merci à Yvette Lemonnier, pour avoir été mon chaperon lors de mes heures toujours plus tardives au laboratoire. Merci pour tous les bavardages toujours sympathiques avec toi, qui est tellement agréable et gentille. Les pauses cafés étaient aussi bien animées avec ta bonne humeur avant ton départ en retraite. Merci pour avoir gardé contact après ainsi que pour les bons moments et repas partagés.

Merci à Patrick Quazuguel pour ne pas avoir eu trop peur de moi après avoir vu mes premières expériences sur le comportement des poissons et pour avoir autant participé à la bonne humeur des pauses café et des ppvr du vendredi. Merci aussi pour tes invitations après ton départ en retraite, je suis également bien heureuse d'avoir continué à partager de bons moments en ta compagnie.

Je tiens tout particulièrement à adresser un très grand merci à David Mazurais, pour tout son soutien au cours de cette thèse, pour avoir été à mon écoute et m'avoir donné de précieux conseils. Sans compter que mon séjour en Bretagne n'aurait jamais été rempli d'autant d'humour, de joie, de partages et de découvertes sans toi. Les sorties sportives avec des paysages à en couper le souffle, agrémentées de rencontres avec des bébêtes que je ne pensais pas pouvoir voir dans leur milieu naturel, auront fait de ma vie à Brest un réel moment de plaisir et une source de bons souvenirs.

Je remercie aussi Christophe Lebigre pour sa gentillesse et son enthousiasme à chaque rencontre, même si je venais encore et toujours avec des statistiques complexes. Il t'a fallu m'écouter te raconter toutes mes expériences et toutes les analyses que j'avais faites et voulais faire. Pourtant ça ne t'a jamais découragé même si ça t'a parfois bien décoiffé ! Merci pour ton aide bien précieuse et de m'avoir confortée dans toutes ces analyses.

Merci à ma co-bureau Hélène Ollivier, pour ses coups de mains dans la maintenance des poissons, notre climat tropical dans le bureau et pour ne pas avoir pris peur des bruits de Teddy.

Merci à Lauriane pour sa gentillesse et sa bonne humeur et les petits plus partagés à l'extérieur. Merci à Marie pour tes conseils et tes coups de mains en congrès. Merci à Sophie, dernière arrivée mais avec qui il y aura eu quelques sorties sympa. Merci à Karine, Magali, Cricri et Arianna pour leur bonne humeur et leur sympathie. Merci à Dorothee pour les coups de pouce avec notamment le prêt d'un ordinateur. Merci également à Hervé pour ton aide avec les macros sur Excel.

Merci à mes compagnons de thèse avec qui j'ai partagés de bons moments lors des congrès, Laura, Florian, Sarah, Clémence et Louise. Merci aussi à tous les stagiaires de passage et surtout à Luana qui même après avoir effectué son stage avec moi a continué de me côtoyer à l'extérieur !

Un très grand merci à mes trois copains du marché, David, Patrick et Sylvie, pour m'avoir accueillie dans leur clan et pour avoir rendu cette dernière année bien plus plaisante avec ce rendez-vous hebdomadaire toujours plaisant, rempli d'humour et de bons cafés. Ces moments feront partis de mes meilleurs souvenirs.

Je souhaite également remercier Manon qui m'a soutenue et m'a permis de continuer de partager des choses même en étant séparées par 1311km (oui j'ai regardé précisément !).

Un grand merci à toute ma famille et surtout à mes parents qui ont toujours été là pour moi, à me soutenir, m'encourager, m'apporter énormément d'affection et vouloir m'aider même si ce que je faisais devenait de plus en plus incompréhensible. Bien sûr j'ai un mot particulier pour mon père car ça y est je suis la plus diplômée !

Merci à ma petite sœur qui même loin de moi depuis si longtemps est toujours aussi adorable, forte et qui a très bien su se débrouiller toute seule, tout en me faisant participer à ses choix ou à quelques uns de ses devoirs, pour mon plus grand plaisir. Je suis fière de toi et de pouvoir toujours être aussi proche de toi même si je vois combien tu as grandi. Tu es et resteras celle qui réussit à me faire sourire peu importe le moment et même juste en évoquant ton nom.

Merci aussi à Mamie Dédée, si j'y suis arrivée c'est également grâce à toi et au plaisir que j'ai eu à vivre avec toi au cours de mes études. Même si tu n'as pas pu voir ma soutenance, pour que je t'explique ce que je fais de façon concrète, je compte bien continuer à travailler avec les poissons mais je suis désolée car ça ne veut toujours pas dire que je pourrais postuler à la poissonnerie de l'Intermarché d'à côté.

Merci à ma tante, ma cousine et mon père pour avoir partagé leurs apéros du samedi soir et permis de rester proche même à distance.

Je terminerai en remerciant mes deux lapins nains. Oui, même eux ont leur place dans ces remerciements puisqu'ils étaient en direct du déroulement de cette thèse. Grâce à eux j'ai pu vivre dans un environnement bien plus agréable, avec leur câlins mais aussi leurs moments gourmandise.

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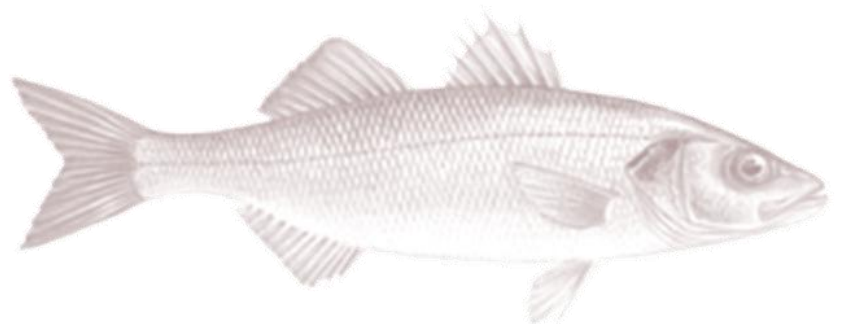
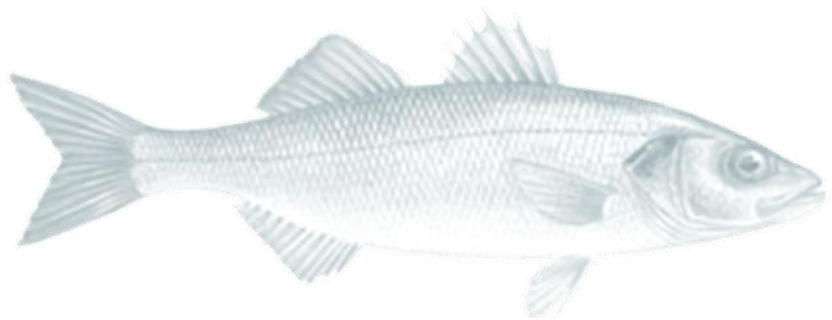
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Cette thèse a été financée par le Centre de documentation, de recherche et d'expérimentation sur les pollutions accidentelles des eaux (Cedre) et l'Université de Bretagne Occidentale (UBO).





INTRODUCTION GENERALE



Un environnement variable

Les écosystèmes marins sont marqués par de fortes variations des caractéristiques physiques (densité, température, courant, lumière) et chimiques (salinité, oxygénation) de l'eau (Wolfe & Kjerfve, 1986; Boeuf, 2016). Ces variations se déroulent sur une gamme d'échelles spatiales allant du millimètre (*e.g.*, micro-habitats) aux milliers de kilomètres (*e.g.*, masses d'eau identifiables, courants océaniques), et temporelle sur des durées allant de la seconde à plusieurs centaines de millions d'années (vague, marée, saison, ère géologique).

De par leur position à l'interface océans-continents, les écosystèmes côtiers sont parmi les écosystèmes marins les plus instables. Ils subissent en effet les influences marines et continentales et ils sont, de ce fait, soumis à des forçages nombreux et complexes par leurs dynamiques et leurs interactions (climats, courants, marées, débit des fleuves, apports en substances organique et minérale; Crossland et al., 2006; Halpern et al., 2008). Les écosystèmes côtiers sont donc marqués par des fluctuations périodiques et stochastiques de leurs conditions abiotiques (température, salinité, oxygénation) mais également biotiques (productions primaires, présence de proies, prédateurs ; Wolfe and Kjerfve, 1986).

Outre la variabilité naturelle, et du fait de l'urbanisation croissante des côtes et des bassins versants des grands fleuves (60 % de la population mondiale vit à moins de 60km du littoral; (Emesellem et al., 2019), les écosystèmes côtiers sont le réceptacle de nombreux forçages d'origine anthropique qui sont de nature à affecter leur structure et leur fonctionnement. Nous pouvons citer pour exemple la pêche, l'aquaculture, les transports ou encore les aménagements de production électrique implantés en mer (UNEP, 2006).

Interactions entre facteurs environnementaux

Les interactions entre variables environnementales rendent très difficile les prévisions quant à leurs effets sur les organismes et leurs écosystèmes (Sih et al., 2004c; Whitehead, 2013). A titre d'exemple, il a été montré que la salinité de l'eau module la solubilité et donc la biodisponibilité des hydrocarbures aromatiques polycycliques (HAP) et des métaux (Levitan & Taylor, 1979; Heugens et al., 2001; Ramachandran et al., 2006). Réciproquement, il a été montré que l'exposition à des hydrocarbures pétroliers peut affecter le fonctionnement de la branchie des poissons et conduire ainsi à une moins bonne tolérance à des contraintes

environnementales telles qu'une fluctuation de la salinité (Baden, 1982; Kennedy & Farrell, 2005; Matsuo et al., 2005; Goanvec et al., 2011) ou une baisse de la disponibilité en O₂ (Davison et al., 1993; Claireaux et al., 2004).

Face aux changements environnementaux : adaptation et régulation

Face aux modifications des conditions de milieux, les organismes marins font appel à deux grandes catégories de réponses qui participent à la préservation de leur valeur sélective (fitness) et assurent ainsi la pérennité de leur espèce : l'évolution par la sélection naturelle (adaptation) et la plasticité phénotypique (régulation).

L'adaptation par la sélection naturelle prend appui sur les différences génétiques entre les individus d'une même population. Ces différences peuvent faire l'objet d'un tri sur la base d'un différentiel de survie. Les organismes possédant le phénotype le mieux adapté aux conditions environnementales sont sélectionnés et au fil des générations leur génotype se répand au sein de la population. L'adaptation par la sélection naturelle est donc un processus à long terme, qui s'étend sur des dizaines voire des centaines de générations.

La plasticité phénotypique, quant à elle, permet un ajustement rapide des performances des individus. La plasticité phénotypique définit la capacité d'un génotype à exprimer différents phénotypes selon les conditions biotiques et abiotiques de son environnement (West-Eberhard, 1989; Grenier et al., 2016). A travers la plasticité phénotypique, certaines caractéristiques du phénotype (*i.e.*, morphologie, biochimie, physiologie, comportement) vont être ajustées et ainsi réduire la pression de sélection exercée par l'environnement (= augmenter la valeur sélective du génotype associé).

Les 4 dimensions de la plasticité phénotypique

Selon les espèces et les individus au sein d'une même espèce, la plasticité peut concerner une ou plusieurs des 4 dimensions d'un phénotype, à savoir :

- la morphologie : *e.g.*, face un prédateur, le poisson globe (*Diodon holocanthus*) peut gonfler son corps jusqu'à tripler son volume initial afin de se défendre (L. Brainerd, 1994).

- la biochimie : *e.g.*, en hiver, la truite arc-en-ciel (*Oncorhynchus mykiss*) montre une hypothyroïdie qui va conduire à l'augmentation de sa fréquence cardiaque et de la taille de son cœur afin de s'acclimater à la chute des températures (Tiitu & Vornanen, 2003; Gamperl & Farrell, 2004).
- la physiologie : *e.g.*, face à une baisse de la disponibilité en O₂ dans l'eau, la carpe (*Cyprinus carpio*) est capable d'augmenter sa surface branchiale et ainsi améliorer les échanges gazeux (Stecyk & Farrell, 2006).
- le comportement : *e.g.*, lorsque la pression de prédation augmente, diverses espèces de poissons privilégient la vie en groupe, afin de bénéficier d'une meilleure détection des prédateurs (vigilance du groupe) et réduire la probabilité d'être capturé (effet de dilution; (Seghers, 1974; Magurran A. E. et al., 1987; Brown & Warburton, 1997).

Evaluation intégrée des effets des changements environnementaux via la plasticité comportementale

Dans le cadre de cette thèse, je m'intéresse plus particulièrement à la plasticité comportementale du poisson en réponse à des stress environnementaux, d'origines naturelles et anthropiques. L'interaction entre facteurs anthropiques et naturels, ou comment les facteurs anthropiques peuvent perturber la réponse comportementale des poissons à une contrainte naturelle, est également abordée. Ces travaux s'inscrivent dans la ligne directrice des nouveaux schémas conceptuels. Le but est d'évaluer les conséquences des perturbations environnementales sur le comportement des animaux avec une approche intégrative permettant d'en estimer les effets à de multiples niveaux d'organisation, de l'individu à l'écosystème. Pour cela ces schémas proposent d'évaluer, à la fois, les répercussions directes de perturbations environnementales au niveau individuel et les conséquences indirectes possibles au niveau populationnel et des communautés.

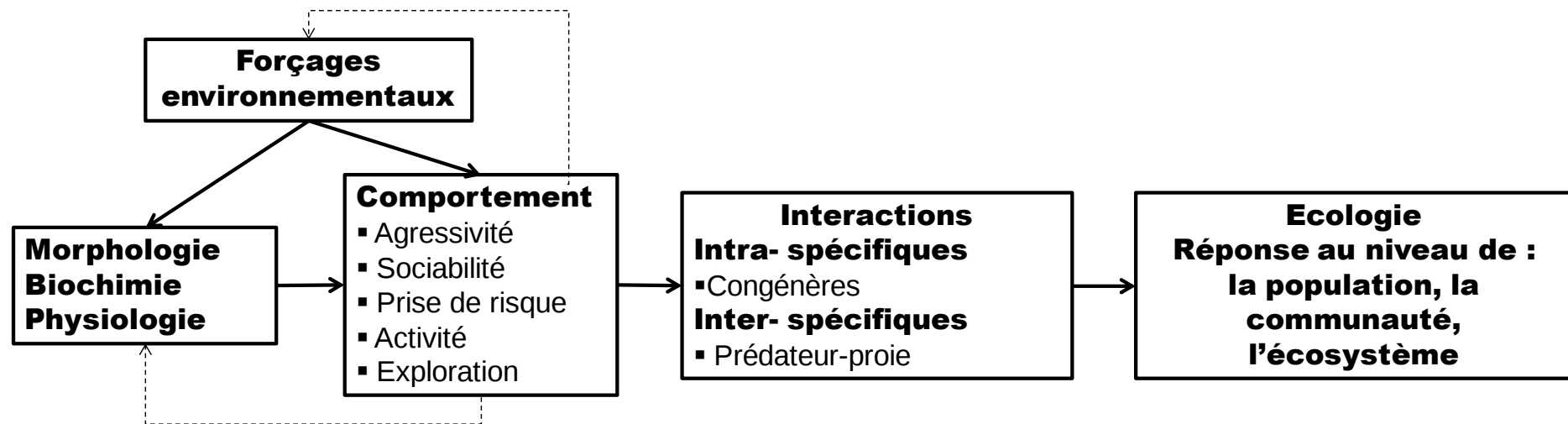


FIGURE I. Schéma conceptuel modélisant les effets directs et indirects d'un changement dans les forçages environnementaux. Les réponses individuelles peuvent perturber des traits essentiels à la valeur sélective d'un individu ou des interactions sociales complexes. Ces altérations au niveau individuel peuvent donc avoir des conséquences écologiques en cascade sur les niveaux organisationnels dans lesquels s'inscrit l'individu, de la population à l'écosystème (adapté de Peterson et al., 2017; Sih et al., 2010).

Ces nouveaux schémas conceptuels ont pour but de promouvoir la pertinence écologique de la recherche comportementale dans les études visant à évaluer les conséquences des changements environnementaux (Sih et al., 2010, 2016; Berger-Tal et al., 2011; Peterson et al., 2017; Saaristo Minna et al., 2018). Pour ce faire, ces schémas mettent en avant deux caractéristiques du comportement, sa valeur intégrative de l'état de santé de l'animal dans son ensemble (processus sub-organismiques) son rôle clef dans les dynamiques écologiques, du fait de son implication dans les interactions entre un individu et les autres organismes qui partagent son environnement (Fig. I).

Le comportement : une dimension intégrative

Le comportement revêt une dimension hautement intégrative (Little et al., 1990a; Sih et al., 2010; Saaristo Minna et al., 2018). Il est souvent considéré comme l'expression visible de l'état de santé de l'individu (Benca et al., 2009; McCarthy, 2010; Sih et al., 2010; Weis, 2014). En effet, le comportement résulte à la fois des conditions environnementales et de l'état physiologique, biochimique et morphologique de l'animal (Sih et al., 2010). En outre, le comportement permet à l'individu d'interagir avec son environnement et ainsi de pouvoir rétroagir sur les forçages environnementaux qui s'exercent sur son système.

Le comportement au cœur de la dynamique des écosystèmes

Le comportement joue un rôle primordial dans les dynamiques écologiques. De part ses interactions, l'individu fait un lien avec les autres niveaux organisationnels du vivant. La réponse comportementale d'un organisme est directement impliquée dans ses interactions intra- et inter-spécifiques (Fleeger et al., 2003; Scott & Sloman, 2004; Peterson et al., 2017; Saaristo Minna et al., 2018). Aussi, les changements dans la réponse comportementale d'un organisme peuvent engendrer des modifications au niveau de la population et des communautés avec lesquelles il interagit, et ainsi perturber la structure et le fonctionnement de l'écosystème (Little et al., 1990a; Dell'Omo, 2002a; Saaristo Minna et al., 2018). En outre, ces changements peuvent même affecter indirectement des espèces résistantes au stress environnemental rencontré. Par exemple, suite à un changement dans l'environnement la, prise de risque de l'espèce A est accrue. Celle-ci peut induire une plus forte pression de prédation sur ses proies (espèce B) et diminuer leur abondance dans l'écosystème (Saaristo Minna et al., 2018). Même si l'espèce B était résistante à la perturbation environnementale, les

changements comportementaux de l'espèce A l'auront affectée de manière indirecte via les relations trophiques. Les effets d'une perturbation environnementale peuvent donc être amplifiés par les interactions intra- et inter- spécifiques. Ce type d'interactions dynamiques illustre l'importance d'interpréter un changement de comportement dans son contexte environnemental global (Peterson et al., 2017; Saaristo Minna et al., 2018).

Des mesures comportementales difficiles à interpréter

Bien qu'il soit clair que la plasticité comportementale est importante pour l'étude des impacts des changements environnementaux, de nombreux modèles conceptuels ne prennent toujours pas en compte le comportement (Gordon 2010). Initialement descriptives, les études comportementales ont longtemps été dépréciées, étant considérées comme non objectives (Geoffroy Saint-Hilaire, 1854). Elles sont devenues progressivement quantitatives avec le développement d'outils technologiques et d'approches statistiques multivariées (Carter et al., 2012). En effet, les avancées depuis la chronophotographie, permettant de détailler les mouvements (Marey, 1894), jusqu'aux techniques d'enregistrement vidéographiques de type numérique d'aujourd'hui, ont permis aux comportementalistes de ré-analyser les séquences comportementales à posteriori et ainsi compléter et rendre plus précises et quantitatives les informations acquises (Candland et al., 1972; Lehner, 1979; Haimoff, 1981; Miltenberger, 2011). Avec l'émergence de l'informatique et la démocratisation de ces outils (ordinateurs, logiciels), des programmes informatiques permettant le codage d'observation comportementale ont fait leur apparition (Crossman et al., 1978; Flowers & Leger, 1982; Kahng & Iwata, 1998; Koch & Zumbach, 2002). C'est surtout depuis les années 90 que les systèmes sont devenus assez élaborés pour coupler à la fois la vidéo et le codage comportemental par des logiciels de plus en plus automatisés. Le développement et l'utilisation de logiciels automatisés de suivi sur vidéo a notamment permis de pallier au manque de méthodes quantitatives et objectives souligné précédemment et permet aujourd'hui un nouvel essor des études comportementales (Carter et al., 2012).

Ces développements ont conduit à faire des avancées substantielles dans les concepts théoriques de l'écologie comportementale. Cependant, il persiste encore des difficultés dans l'interprétation des mesures qui peuvent refreiner leur utilisation (Carter et al., 2013; Roche et al., 2016).

Une approche multivariée pour objectiver l'interprétation des mesures comportementales

Un test comportemental peut évaluer simultanément différents aspects du comportement animal (*e.g.*, activité d'exploration et prise de risque dans un nouvel environnement; Budaev, 1997; Burns, 2008). Afin de distinguer objectivement les différents aspects comportementaux mesurés dans un test comportemental, il a été suggéré d'utiliser des techniques de réduction des données. Les analyses en composantes principales (ACP) peuvent, par exemple, aujourd'hui être utilisées pour éviter une mauvaise interprétation des mesures issues des tests comportementaux (Dingemanse et al., 2010; Carter et al., 2013). Par le biais des techniques de réduction de données, si un test évalue un unique trait comportemental, alors l'analyse n'identifiera qu'une seule composante expliquant un pourcentage substantiel de la variance totale des mesures. En revanche, si plusieurs caractères comportementaux indépendants sont mesurés dans le test, il est présumé que chacun d'entre eux ressortira sur une des composantes de l'ACP, expliquant un pourcentage conséquent de la variance des mesures (Carter et al., 2012, 2013). Même si ces techniques peuvent rendre objective l'identification et l'interprétation des mesures comportementales, elles sont encore aujourd'hui très peu utilisées (Carter et al., 2013).

Les objectifs de la thèse

Dans mes travaux de thèse, j'ai utilisé la réponse comportementale comme indicateur des effets directs de perturbations environnementales sur l'individu, mais aussi comme indicateur indirect des effets au niveau populationnel et des communautés en prenant en compte les aspects de sociabilité, de prise de risque et d'activité d'exploration.

Dans ce contexte, ma thèse a pour but de:

- 1) Utiliser des tests comportementaux permettant une analyse intégrative des effets directs de perturbations environnementales sur des comportements (*e.g.*, activité d'exploration, prise de risque) et des effets indirects de ces changements sur les interactions intra- et inter- populationnelles (*e.g.*, sociabilité, réaction au prédateur).
- 2) Mettre en application ces analyses comportementales au travers de deux cas d'étude courants en milieu côtier : un stress naturel (une pénurie de nourriture) et un stress

anthropique (une marée noire). Ces résultats seront intégrés dans le schéma conceptuel sur lequel se base cette thèse.

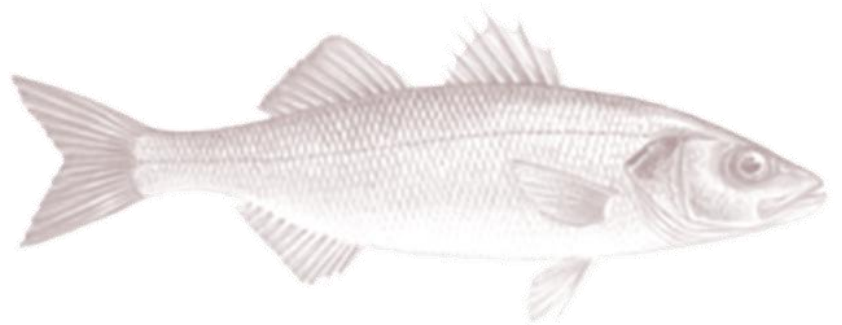
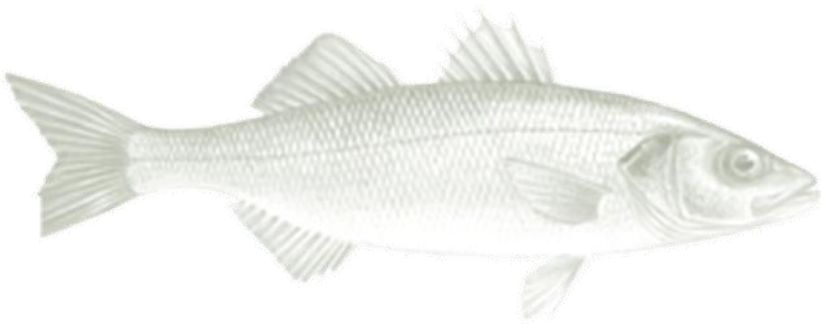
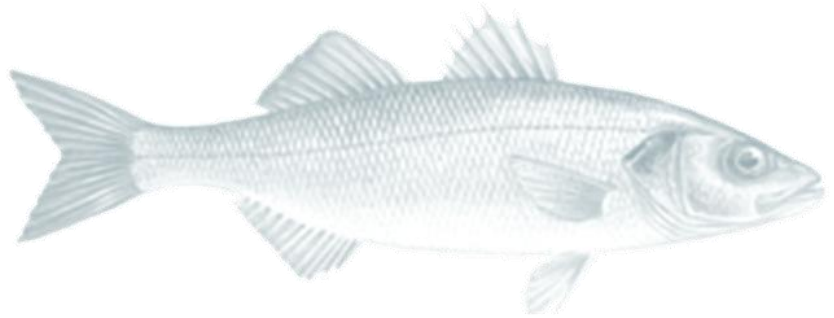
- 3) Evaluer la capacité de récupération post-stress des animaux pour avoir une estimation des impacts plus pertinente.
- 4) Adapter des outils statistiques en composantes principales et multivariées pour objectiver les mesures et l'interprétation des analyses comportementales.

Structure du manuscrit

Cette thèse est structurée en trois chapitres. Dans le premier (chapitre 1) j'analyse les effets directs de la privation de nourriture sur les réponses comportementales (*i.e.*, prise de risque, activité exploratoire et sociabilité) des juvéniles de bar Européens (*Dicentrarchus labrax*). Dans ce chapitre, la difficulté d'interpréter et de définir contextuellement les réponses comportementales est abordée. Dans les chapitres 2 et 3 sont évalués les effets d'une exposition à une perturbation anthropique, une marée noire. Dans le chapitre 2, les conséquences d'une exposition à des hydrocarbures pétroliers sont évaluées sur le comportement d'individus isolés (*i.e.*, activité exploratoire et évitement d'une zone ouverte non familière). La capacité de récupération des individus est également regardée sur une période de deux semaines post-exposition pour avoir une estimation plus pertinente des impacts. Dans le troisième chapitre (chapitre 3), ces analyses ont été poursuivies en analysant les conséquences d'une exposition aux produits pétroliers dans un cadre plus large que la réponse individuelle, avec des mesures effectuées en groupe. L'analyse d'un groupe d'individus permet d'estimer des conséquences indirectes au niveau de la population (sociabilité dans le groupe). De plus, dans ce chapitre, la réponse anti-prédateur est évaluée et nous permet d'interpréter les effets indirects de l'exposition aux hydrocarbures pétroliers sur l'interaction proie-prédateur au sein de la communauté. Enfin, la dernière partie sera consacrée à la discussion générale des analyses comportementales, leur interprétation et intégration dans le schéma conceptuel de cette thèse. Pour finir, quelques orientations pour les travaux futurs seront suggérées.



CHAPITRE 1



Au cours de ce premier chapitre j'étudie, chez des juvéniles de bar Européen, les effets directs de la privation de nourriture sur trois réponses comportementales essentielles à la survie, à savoir l'activité d'exploration, la sociabilité et la prise de risque.

La privation de nourriture est un stress environnemental fréquent pour les poissons (Gingerich et al., 2010). Dans ces conditions, leur fitness est d'autant plus dépendante de leur capacité à maximiser la fréquence de rencontre de nourriture tout en minimisant le risque de prédation (Lima & Dill, 1990).

Les trois comportements évalués dans cette étude sont primordiaux d'une part dans les challenges (*e.g.*, prédation, recherche de nourriture) auxquels les animaux doivent faire face en permanence et d'autre part car ils apportent des informations importantes quant à leur état de santé (*i.e.*, état des fonctions physiologiques et morphologiques; Houston et al., 1993; Lima and Dill, 1990).

L'exploration, permet à l'individu d'acquérir de l'information sur son environnement et de se créer une carte mentale des habitats potentiels (Little & Finger, 1990). Les connaissances acquises peuvent ensuite être utilisées dans la recherche de ressources, telles que la nourriture, ou pour éviter les situations à risque. Il a également été proposé que la capacité d'un individu à collecter les informations sur son environnement reflète ses performances cognitives (Archer & Birke, 1983; Renner, 1990; Laland & Reader, 1999; Griffin & Guez, 2014; Reader, 2015; Jacquin et al., 2017).

En ce qui concerne la sociabilité, elle résulte de la propension d'un individu à exprimer de l'intérêt pour les interactions sociales avec ses congénères. Le comportement grégaire est un aspect de la sociabilité qui résulte du choix de certains individus de se joindre à leurs congénères pour former un groupe. La vie en groupe est une caractéristique fondamentale du cycle biologique de nombreux poissons, avec vingt-cinq pour cent de toutes les espèces formant des groupes ou des bancs au cours de leur vie, et cinquante pour cent aux stades larvaire et juvénile (Radakov & Williams, 1974; Pavlov & Kasumyan, 2000; Kane et al., 2005). La grégarité permet de maximiser l'acquisition de nourriture, d'améliorer l'évitement des prédateurs et de diminuer les risques d'être capturé grâce aux avantages que confère le nombre. Le nombre permet en effet une augmentation du taux de détection des ressources,

une meilleure vigilance et des effets de dilution et de confusion pour les prédateurs (Pulliam & Caraco, 1984; Clark & Mangel, 1986; Godin, 1986; Pitcher & K. Parrish, 1993b; Krause et al., 2000; Krause & Ruxton, 2002). En plus, le fait de mettre moins d'énergie dans la vigilance leur permet d'en allouer davantage à d'autres fonctions.

Enfin, la prise de risque est une réponse comportementale consistant à accepter un certain niveau de danger en contre partie de récompenses potentielles (Réale et al., 2007; Bell et al., 2009; Laskowski & Bell, 2014; Jolles et al., 2015). Ce comportement peut jouer un rôle important dans la dynamique et le fonctionnement des groupes d'animaux (Laskowski & Bell, 2014; Jolles et al., 2015). En effet, la prise de risque des individus peut influencer l'organisation sociale, les interactions au sein du groupe et la dominance (Ward et al., 2004; Pike et al., 2008; Harcourt et al., 2009; Nakayama et al., 2012). De plus, la prise de risque a été corrélée positivement avec des indices fondamentaux de la fitness tel que, la croissance, la reproduction et le taux de consommation alimentaire (Biro & Stamps, 2008; Burton et al., 2011; Conrad et al., 2011; Careau & Garland, 2012).

D'un point de vue analytique, ce chapitre propose une méthode d'analyse des données en composante principale (ACP) qui permet d'atténuer la redondance d'information qui peut nuire à l'interprétation des réponses comportementales. Les corrélations entre mesures comportementales seront également analysées. En outre, j'expose certaines limites quant à l'interprétation de ces données comportementales dues au fait que la répétabilité de nos mesures n'ait pas été testée.

RESEARCH ARTICLE

Food deprivation reduces social interest in the European sea bass *Dicentrarchus labrax*

Cassandre Aimon^{1,2,*}, Nicolas Le Bayon³, Stéphane Le Floch² and Guy Claireaux¹

ABSTRACT

Periods of food deprivation of several months are common events for fishes and in such conditions, fitness will be determined by their capacity to maximize food encounters while minimizing predation risk. In this context, the propensity to take risks and the willingness to associate with conspecifics are particularly important as they contribute to alleviating the trade-off between predation avoidance and foraging efficiency. This study examined to what extent food deprivation modulates fish risk-taking and social behaviours, as well as the relationship between them. To address these issues, juvenile European sea bass were either fed daily with a maintenance ration or food deprived for a period of 3 weeks. Risk taking and sociability were assessed through measurements of fish willingness to explore a novel environment, and to interact with a novel object or a conspecific. Multivariate analysis allowed the identification of three behaviours: risk taking, exploratory activity and solitariness. Food-deprived fish interacted less with conspecifics than control fish; however, no difference in terms of risk taking and exploratory patterns was observed. Finally, the relationship between risk taking and solitariness was influenced by feeding status. When food-deprived, fish with a higher propensity to take risk displayed increased solitariness, while when fed normally, they interacted more with conspecifics.

KEY WORDS: Behaviour, Teleost fish, Exploratory activity, Sociability, Risk taking, Behavioural interaction

INTRODUCTION

Food availability and accessibility are key determinants of fitness (Patrick et al., 2017). In aquatic ecosystems, the finding and capture of prey are not guaranteed and periods of starvation of several months are not unusual (Gingerich et al., 2010). In such conditions, animals preserve their fitness through behavioural responses that maximize food encounter and capture rates while minimizing predation risk (Lima and Dill, 1990). When food is scarce, increasing the duration and extent of food searching, further away from a shelter, for instance, is a risk-prone behaviour which favours food encounter. The drawback of such behaviour is increased predator encounter rate and, therefore, increased mortality risk (Biro and Stamps, 2010; Lima and Dill, 1990). In contrast, risk-averse behaviours, such as staying in a protective area, benefit the individual through energy saving and reduced predation risk. The

downside of this strategy is obviously a reduction in feeding opportunities (Krause and Ruxton, 2002).

Risk taking has major consequences for an individual's fitness as it has been shown to correlate with growth, energy metabolism, dispersal, breeding success, offspring nourishment and social dominance (Ariyomo and Watt, 2012; Bell and Sih, 2007; Brown et al., 2005; Cote et al., 2010; Dingemanse et al., 2004; Greenberg and Mettke-Hofmann, 2001; Huntingford et al., 2010; Jolles et al., 2015; Mutzel et al., 2013; Rudin and Briffa, 2012). Risk-prone individuals accept higher risk in return for gaining information and possibly increased reward. Risk-averse individuals, in contrast, tend to avoid potentially risky situations, accepting reduced gains in return for lower risk. However, the willingness of an individual to take risk is context dependent (Coleman and Wilson, 1998). For instance, Galhardo et al. (2012) showed that social context influences risk taking of male cichlid fish (*Oreochromis mossambicus*), with fish in the presence of familiar conspecifics being more prone to accept risk than fish in the presence of unfamiliar conspecifics or in social isolation. This context dependency of risk taking is the cause of disagreement among authors regarding the most appropriate methodology to assess it (Carter et al., 2013; Conrad et al., 2011; Réale et al., 2007; Wilson et al., 1993). While some authors restrict the measures of risk acceptance to the context of predation (Réale et al., 2007), others extend the relevance of the notion to any context where an animal has to make a decision towards unfamiliar and potentially dangerous stimuli (Frost et al., 2013; Leblond and Reeb, 2006; Nakayama et al., 2012; Toms et al., 2010; Wilson et al., 1993). In the present study, risk taking was considered in its broader sense, with exploratory tendency and neophilia been recognized as components of an individual's willingness to take risk.

Sociability is an important modulator of fish behavioural responses to the environmental context and, particularly, of the trade-off between maximizing foraging opportunities and minimizing predator encounter. Sociability is classically defined as one animal's reaction to conspecifics, excluding aggressive interactions (Conrad et al., 2011). Fish express sociability in a species-specific manner, one example of which is the willingness to shoal. Shoaling behaviour is considered as a flexible strategy by which individuals increase food detection while lowering predation risk through additive vigilance, numerical risk dilution and predator confusion effect (Clark and Mangel, 1986; Godin, 1986; Krause and Ruxton, 2002; Krause et al., 2000a; Pitcher and Parrish, 1993; Pulliam and Caraco, 1984). However, shoaling is also associated with increased competition within the group (Pitcher, 1986; Pulliam and Caraco, 1984). Shoal formation relies on the decision of individuals to remain together and it has been proposed that food restriction influences individual decisions regarding self-assembly with conspecifics (Frommen et al., 2007; Raubenheimer et al., 2012). For instance, Krause (1993), Arber et al. (1995) and Hensor et al. (2003) reported that food-deprived fish tended to spend more time alone than well-fed individuals. However, at least one study

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Received 23 August 2018; Accepted 21 December 2018

described the reverse response (Killen et al., 2016). Sociability characterizes an animal's inclination for social interaction such as shoaling. In the present study, solitariness was used as the reciprocal of sociability, with high solitariness reflecting an individual's unwillingness to group with conspecifics and low solitariness indicating an individual's inclination for social interaction.

Analysing behaviours is a key component in understanding how fish populations respond to seasonal and stochastic variation in environmental conditions. Equally important is the interaction between those behaviours. The relationship between risk taking and sociability has been examined in fish, and whereas a relationship was found in some species (*Symphodus ocellatus*: Budaev, 1997; *Gasterosteus aculeatus*: Ward et al., 2004), it was not observed in others (*Danio rerio*: Moretz et al., 2007). Moreover, when found, this relationship displayed different forms depending on the species considered. In most species examined, a negative correlation between risk taking and sociability has been reported (*S. ocellatus*: Budaev, 1997; *G. aculeatus*: Ward et al., 2004; Harcourt et al., 2009a,b; *Lepomis gibbosus*: Wilson et al., 1993). In the mosquitofish (*Gambusia affinis*: Cote et al., 2010) as well as in two terrestrial ectotherms, red ants (*Myrmica* spp.: Chapman et al., 2011) and the European green lizard (*Lacerta vivipara*: Cote and Clobert, 2007), the reverse was observed, with a positive correlation between risk taking and sociability. It has been proposed that correlations among behaviours might be adaptive as they contribute to individual fitness (Bell, 2005; Bell and Sih, 2007; Bell and Stamps, 2004). In the three-spined stickleback, for example, no interaction between risk taking (swimming activity after a predator attack) and aggressiveness (towards conspecifics) was observed under low predation pressure. Under high predation pressure, however, a positive correlation between these behaviours was observed (Bell, 2005). Although disappointing, these conflicting results may not be all that surprising as there is no reason why relationships between behaviours should all have the same form across vastly different experimental systems using species with different ecologies and behavioural repertoires. Thus, to better assess the true adaptive value of fish behavioural strategies, future studies should examine the relationships between behavioural traits, with particular consideration for the strength and form of these relationships.

Using juvenile European sea bass, *Dicentrarchus labrax*, as a model species, the present study investigated to what extent food deprivation modulates fish risk-taking and social behaviours, as well as the relationship between them. At the juvenile stage, European sea bass form shoals that occupy shallow coastal habitats where spatial and seasonal fluctuations in prey availability are common, exposing them to periods of starvation (Claireaux et al., 2013; Dupont-Prinet et al., 2010). Four commonly used behavioural tests were implemented, with the initial anticipation that they would allow assessment of risk taking and sociability through measuring the willingness of individuals to explore a novel environment, to interact with a novel object or a conspecific and to use a shelter. Behavioural responses of 3 week food-deprived fish were compared with those of control individuals fed daily with a maintenance ration. Three hypotheses were tested: (1) starvation favours solitariness to avoid competition for food, (2) starvation increases risk taking and exploratory activity to enhance food encounters and (3) food deprivation modulates the relationships between solitariness, exploratory activity and risk taking.

MATERIALS AND METHODS

Study animals

Fifty-six juvenile European sea bass, *Dicentrarchus labrax* (Linnaeus 1758) (age 0⁺, mass 14.6±0.08 g, mean±s.e.m.), were obtained from a local fish farm (Aquastream, Lorient, France).

Upon arrival at the laboratory (Ifremer, Brest, France), fish were anaesthetized (MS-222; 20 mg l⁻¹), measured for total length and mass, and implanted subcutaneously with an identification tag (RFID; Biolog-id, Bernay, France). Fish were then placed in a 2000 l indoor tank supplied with open-flow, thermoregulated (20°C) and fully aerated seawater (salinity 32 ppt). Artificial lighting followed local photoperiod. Fish were fed daily with a maintenance ration (1% body mass) using commercial feed (Neo Start Coul 2, Le Gouessant, Lamballe, France). The experiments were approved by the French Ethics Committee in charge of Animal Experimentation no. 74 (permit number: APAFIS#3814-2016012715396101 v2) and were in accordance with institutional guidelines. The experimental procedures were non-invasive.

Experimental protocol

Fish were acclimated to the laboratory conditions for 2 months before the following procedure was implemented. On alternate weeks a set of eight fish was randomly selected from the holding tank, anaesthetized (MS-222; 20 mg l⁻¹), measured for total length and mass and transferred to one of two treatment tanks (50 l). These treatment tanks were situated in the experimental room and received the same water and light conditions as the original holding tank. The two experimental treatments were a control treatment, in which fish were fed normally, and a food-deprived (FD) treatment, in which fish were not fed during a 3 week period.

Following the 3 weeks of either control or FD treatment, a set of eight fish from one experimental treatment tank was subjected to the following protocol (Fig. S1). Fish were gently placed (without emersion) into one of eight individual confinement chambers. These chambers consisted of an opaque PVC tube (13 cm×5 cm length×diameter) closed at both ends with plastic meshing to allow water renewal inside the chamber. These chambers were then placed side by side on the bottom of a recovery tank and fish were left undisturbed for an additional 24 h. The eight fish were then successively subjected to a sequence of four consecutive trials over a period of 4 days. On each testing day, the running order of the eight fish was randomized. Each chamber was smoothly moved from the recovery tank to the experimental arena using a 2 l plastic container filled with water. After 3 min to allow the fish to recover from potential disturbance arising from the transfer, one end of the tube was opened from a distance and the fish were allowed 1 min to exit the chamber. In most cases (49/56), the tube had to be gently lifted to encourage the fish to swim out. Following their entrance in the arena, nearly all fish displayed a period of agitation which typically lasted less than a minute. To avoid including this 'flight response' in our analysis, the first minute following fish entry in the testing arena was not taken into consideration during video analysis.

The testing arena consisted of a white rectangular shallow tank (156 cm×99 cm×14 cm, length×width×height, respectively). A curtain placed around and over the arena screened fish from visual disturbance. The arena was filled with the same water as the rearing and treatment tanks and was homogeneously lit (30 lx) using neon lamps.

A camera (Logitech webcam C930e) situated 1 m above the water surface allowed the recording (15 frames s⁻¹) of fish movements during the 30 min that followed fish entry into the arena. Following the 30 min trial, the tested fish was returned to its PVC tube, which was then placed back into a new recovery tank until the next day. This recovery tank allowed separation of the already tested fish from those waiting to be tested, preventing the transfer of chemical cues between fishes. Water conditions in the recovery tanks were identical to those in the treatment tank. Once a fish had been removed, the arena was emptied, refilled and the next fish brought in.

Experimental sets of fish were successively subjected to the following sequence. On day 1, fish were subjected to a novel environment test which consisted of exposing fish to a bare tank. On day 2, a novel object was placed at the centre of the arena prior the introduction of the fish. The object consisted of a white, opaque polypropylene jar (13 cm height, 10.5 cm in diameter) with holes (1.5 mm) all around. The object was placed in such a way that no shadow was created in the arena. On day 3, a conspecific was placed in the opaque jar and 20 min later the tested fish was transferred in the arena. The perforations in the jar allowed the transfer of chemical cues and pressure waves related to movement but its opacity prevented visual contact with the focal fish and the establishment of dominant–subordinate relationships. Finally, on day 4, a refuge, which consisted of a flat piece of black plastic (19 cm×19 cm, length×width), was placed on the bottom in one corner of the arena, opposite the fish release site (Nelson and Claireaux, 2005). This fixed temporal order of the four assays allowed the potential carry-over effects between trials carried out on successive days to be kept the same. In addition, this order was established to familiarize the fish to one condition before testing the following one, introducing only one change in each test. The day 1 assay allowed the fish to familiarize itself with the arena, which was then no longer considered as a novelty on day 2. On day 2, the novel object was added as the only source of novelty in the familiar arena. On day 3, the fish was already familiar with this object, allowing evaluation of the fish's interest in a conspecific through the addition of a non-focal fish inside the jar. This individual was used as a social stimulus for the whole day (8 trials). It was taken from the holding tank and was placed inside the polypropylene jar 20 min prior to the beginning of the experiment. Finally, on day 4, refuging behaviour was tested by evaluating the fish's capacity to localize and use a refuge in an already well-explored arena. At the end of the week, the eight fish were anaesthetized (MS-222; 20 mg l⁻¹) and their total length and body mass measured to estimate specific growth rate. They were then returned to their original holding tank.

Behavioural analyses

On day 1, four indices of activity were measured during the first 5 min of the test (i.e. the total time spent swimming, T_{swim} ; the number of sections crossed, N_{SC} ; the time spent in the central section of the arena, T_{central} ; and the number of entries into the central section, $N_{\text{ent,cent}}$). To score N_{SC} , a grid was placed over the screen of the computer used for video analysis; this grid divided the arena in 16 sections of equal dimension (39 cm×24.75 cm). To score T_{central} , the central section (78 cm×49.5 cm) was distinguished from the periphery of the arena. Analysis on day 2 consisted of measuring, during the first 5 min period of the test, four new indices of activity (i.e. the latency to the first approach to the novel object, $L_{\text{app,NO}}$; the time spent close to the novel object, T_{NO} ; the number of approaches to the novel object, $N_{\text{app,NO}}$; and the number of revolutions around the novel object, $N_{\text{rev,NO}}$). In this test, the fish was considered to be close to the conspecific when it was less than a body length away from it. During day 3, the variables of interaction with the conspecific were measured during the last 5 min of the 30 min test to ensure that the focal fish detected the conspecific. During this test, the same four variables as during day 2 were measured with respect to the conspecific (i.e. the latency to approach the conspecific, $L_{\text{app,con}}$; the time spent close to it, T_{con} ; the number of approaches to the conspecific, $N_{\text{app,con}}$; and the number of revolutions around the conspecific, $N_{\text{rev,con}}$). Again, the fish was considered to be close to the object containing the conspecific when it was less than a body length away from it. Finally, analysis on day

4 consisted of recording the following three indices over the 30 min of the test (the latency to enter the shelter, L_{shelter} ; the time spent in the shelter, T_{shelter} ; and the number of exits from the shelter, $N_{\text{exit,shelter}}$). Note that the same observer scored the different behaviours by visual observation from the videos.

Statistical analysis

Two fish were excluded from the analyses because video recordings failed (total of 54 fish).

Owing to our experimental design, one possible option was to analyse each test separately, as in Dingemans et al. (2010), for example. We opted for a more integrative approach using a principal components analysis (PCA) that regrouped, on the same axes, the variables that assessed a common behaviour. Three principal components (PCs) were chosen according to Kaiser's criterion (Kaiser, 1961), retaining only factors with eigenvalues greater than 1. Linear combination of the variables was used to label the three PCs: risk taking (PC1), exploratory activity (PC2) and solitariness (PC3). Behavioural scores of FD and control fish were compared using parametric multivariate analysis of variance (MANOVA), followed by ANOVA tests to generate univariate statistics. A linear model was fitted to compare the relationship between risk taking and sociability in control and FD fish. In this model, feeding treatment, risk taking (PC1) and exploratory activity (PC2) including the interactions treatment×(PC1+PC2), were used as explanatory factors influencing the sociability (PC3), which was used as the response variable. A backward stepwise reduction of the full model was then applied to exclude non-significant interactions.

All statistical analyses were performed using R version 3.3.1 (<http://www.R-project.org/>) with the package FactoMineR (function PCA), with all variables scaled, and the package stats (functions manova, summary.aov and lm). Model diagnostics were evaluated using graphical procedure (Q–Q plot). Statistical significance was accepted at $P<0.05$.

RESULTS

Data were collected from $N=30$ control and $N=23$ FD fish. Mean initial mass was 14.6 ± 0.10 g and 14.5 ± 0.16 g for the control and FD fish, respectively. Mean specific growth rate measured over the 3 week treatment period was $0.9\pm0.07\%$ day⁻¹ and $-0.3\pm0.03\%$ day⁻¹ for the control and FD fish, respectively.

Reduction and structure of the variables

The impact of starvation on the behaviour of juvenile sea bass was analysed using two successive PCA. The first PCA included the 15 variables extracted from the novel environment (day 1), novel object (day 2), conspecific (day 3) and shelter (day 4) experimental trials. The variables that made a lower contribution to the construction of the PCA axes than the mean contribution of the 15 variables initially tested were then removed and a second PCA was conducted (Cibois, 1986, 1997). In this process, the three variables measured during the shelter trial (day 4) were excluded. Moreover, one individual with a contribution to the structure of the axes that was 20 times higher than the median contribution of all the individuals was considered as an outlier and removed from the analysis. In this second PCA, three PCs were selected (eigenvalue>1) which represented 75.16% of the total variance in our dataset (Table 1). The three principal components were respectively interpreted as indicators of risk taking, exploratory activity and solitariness. This labelling resulted from the specific linear combination of variables on each axis. PCs are described in the following paragraphs.

Table 1. Eigenvalues of the axes of the principal components analysis (PCA)

PC	Eigenvalue	% Variance	Cumulative % variance
1	4.157	34.639	34.639
2	3.049	25.404	60.043
3	1.814	15.114	75.157
4	0.912	7.604	82.761
5	0.625	5.211	87.972

The PCA was performed with the 12 behavioural variables extracted from the novel environment (day 1), novel object (day 2) and conspecific (day 3) trials.

PC1 explained 34.64% of the variability. This axis was labelled 'risk taking' as it incorporated eight variables measuring a fish's propensity to take risk when exposed to novelty. These included the three variables measuring the interaction with the novel object (day 2: T_{NO} , $N_{app,NO}$, $N_{rev,NO}$) and the three variables measuring the interaction with the newly introduced conspecific within the familiar object (day 3: T_{con} , $N_{app,con}$, $N_{rev,con}$). Also included here are the two variables measuring the latency before the first approach to the novel object/conspecific ($L_{app,NO}$ and $L_{app,con}$, respectively). The first six variables correlated positively with PC1 while the last two correlated negatively with PC1 (Table 2). For PC1, positive scores indicated risk-prone individuals, while negative scores indicated risk-averse individuals.

PC2 explained 25.40% of the total variance. This axis was labelled 'exploratory activity' as it was defined by four variables measuring fish activity during exploration of the novel environment on day 1 (i.e. T_{swim} , N_{SC} , $T_{central}$, $N_{ent,cent}$). These four variables correlated positively for this axis (Table 2). For PC2, individuals with a higher exploratory tendency had positive scores, while those with a lower tendency to explore had negative scores.

PC3 explained 15.11% of the variability. This axis was labelled 'solitariness' as it was defined by two sets of variables that distinguished interaction with the empty object (day 2) from interaction with the object in the presence of a conspecific within that object (day 3). The first set of variables included T_{NO} , $N_{app,NO}$ and $N_{rev,NO}$ and it correlated positively with PC3. The second set

Table 2. Coefficients of correlation of the three first principal components (PCs) for each variable

Variable	PC1 risk taking	PC2 exploratory activity	PC3 solitariness
N_{SC}	0.108	0.878	0.207
T_{swim}	-0.044	0.744	0.351
$T_{central}$	0.012	0.748	0.171
$N_{ent,cent}$	0.035	0.894	0.178
$L_{app,NO}$	-0.744	0.075	-0.147
$N_{app,NO}$	0.748	-0.223	0.521
T_{NO}	0.689	-0.257	0.575
$N_{rev,NO}$	0.682	-0.302	0.448
$L_{app,con}$	-0.761	-0.146	0.290
$N_{app,con}$	0.693	0.297	-0.476
T_{con}	0.759	0.066	-0.392
$N_{rev,con}$	0.674	0.202	-0.546

N_{SC} , number of sections crossed; T_{swim} , total time spent swimming; $T_{central}$, time spent in the central section of the arena; $N_{ent,cent}$, number of entries into the central section of the arena; $L_{app,NO}$, latency to the first approach to the novel object; $N_{app,NO}$, number of approaches to the novel object; T_{NO} , time spent close to the novel object; $N_{rev,NO}$, number of revolutions around the novel object; $L_{app,con}$, latency to approach the conspecific; $N_{app,con}$, number of approaches to the conspecific; T_{con} , time spent close to the conspecific; $N_{rev,con}$, number of revolutions around the conspecific. These three PCs represent 75.16% of the variance in the data. The variables used for the delineation of each component are in bold.

included T_{con} , $N_{app,con}$ and $N_{rev,con}$ and it correlated negatively with PC3 (Table 2). For PC3, solitary individuals had positive scores, while individuals with more social interest displayed negative scores. It is important to point out, however, that these variables also loaded heavily on PC1. PC3 should therefore be interpreted with caution. Nevertheless, it allowed us to explore fish social behaviour by differentiating interaction with the empty object from interaction with a conspecific inside this object.

To summarize, when transferred to the experimental arena, fish that scored highly on PC1 took less time to make the first approach to the object in the centre (day 2 and day 3; low $L_{app,NO}$ and $L_{app,con}$) and spent more time close to it (day 2 and day 3; elevated T_{NO} , $N_{app,NO}$, $N_{rev,NO}$, T_{con} , $N_{app,con}$ and $N_{rev,con}$) (Fig. 1A–D). In contrast, fish that scored highly on PC2 spent less time motionless (elevated T_{swim}), moved greater distances (elevated N_{SC}) and explored the central zone of the arena more actively (elevated $T_{central}$ and $N_{ent,cent}$) (Fig. 1A,B,E,F). Finally, fish that scored highly on PC3 were more in contact with the object when it represented a novelty (day 2; elevated T_{NO} , $N_{app,NO}$ and $N_{rev,NO}$) but had minimal interaction with the central object when it contained a conspecific (day 3; low T_{con} , $N_{app,con}$ and $N_{rev,con}$) (Fig. 1C–F).

Effects of the feeding regime

MANOVA showed that, overall, the effect of the feeding regimes on fish behaviour was significant (Pillai's trace=0.242, $F_{1,49}=5.202$, $P=0.003$). Specifically, fish from the two feeding regimes showed no statistically different scores on PC1 (Fig. 1B,D; risk taking, $F_{1,51}=0.678$, $P=0.414$). However, they showed nearly statistically different scores on PC2, the significance threshold being almost attained (Fig. 1B,F; exploratory activity, $F_{1,51}=3.784$, $P=0.057$). Finally, the two feeding treatments yielded statistically different scores on PC3 (Fig. 1D,F; solitariness, $F_{1,51}=9.670$, $P=0.003$), with FD fish displaying higher scores than control fish, meaning that FD fish interacted less with the conspecific than did well-fed fish.

Factors influencing solitariness

Individual degree of risk taking was expected to influence social behaviour. This relationship was investigated using a linear modelling approach (Table 3). The resulting model indicated that PC3 (solitariness) was influenced by PC1 (risk taking) differently according to the feeding treatment ($F_{4,48}=5.423$; treatment $\times$ PC1 risk taking interaction, $P=0.004$; Table 3). Fig. 2 illustrates the different correlations between scores on PC1 and PC3 according to the feeding treatment. These scores correlated positively for FD fish ($P=0.022$), while they displayed a nearly significant negative correlation in control fish ($P=0.064$). Despite these differences between feeding treatment groups, it should be noted that at the left end of PC1 (risk-averse individuals), fish were indistinguishable from one another with regard to their social interest (PC3). However, the solitariness level of individuals progressively differentiated according to their feeding regime as their scores on PC1 increased (Fig. 2).

DISCUSSION

The aim of the present study was to examine to what extent sea bass nutritional status modulates risk taking and sociability. Three hypotheses were tested: (1) starvation favours solitariness to reduce competition for food, (2) starvation increases exploratory activity and risk taking to enhance food encounters and (3) food deprivation modulates the relationships between solitariness, exploratory activity and risk taking. In accordance with our first hypothesis, food deprivation modulated the interaction with a conspecific, with FD fish interacting less with conspecifics than did control fish.

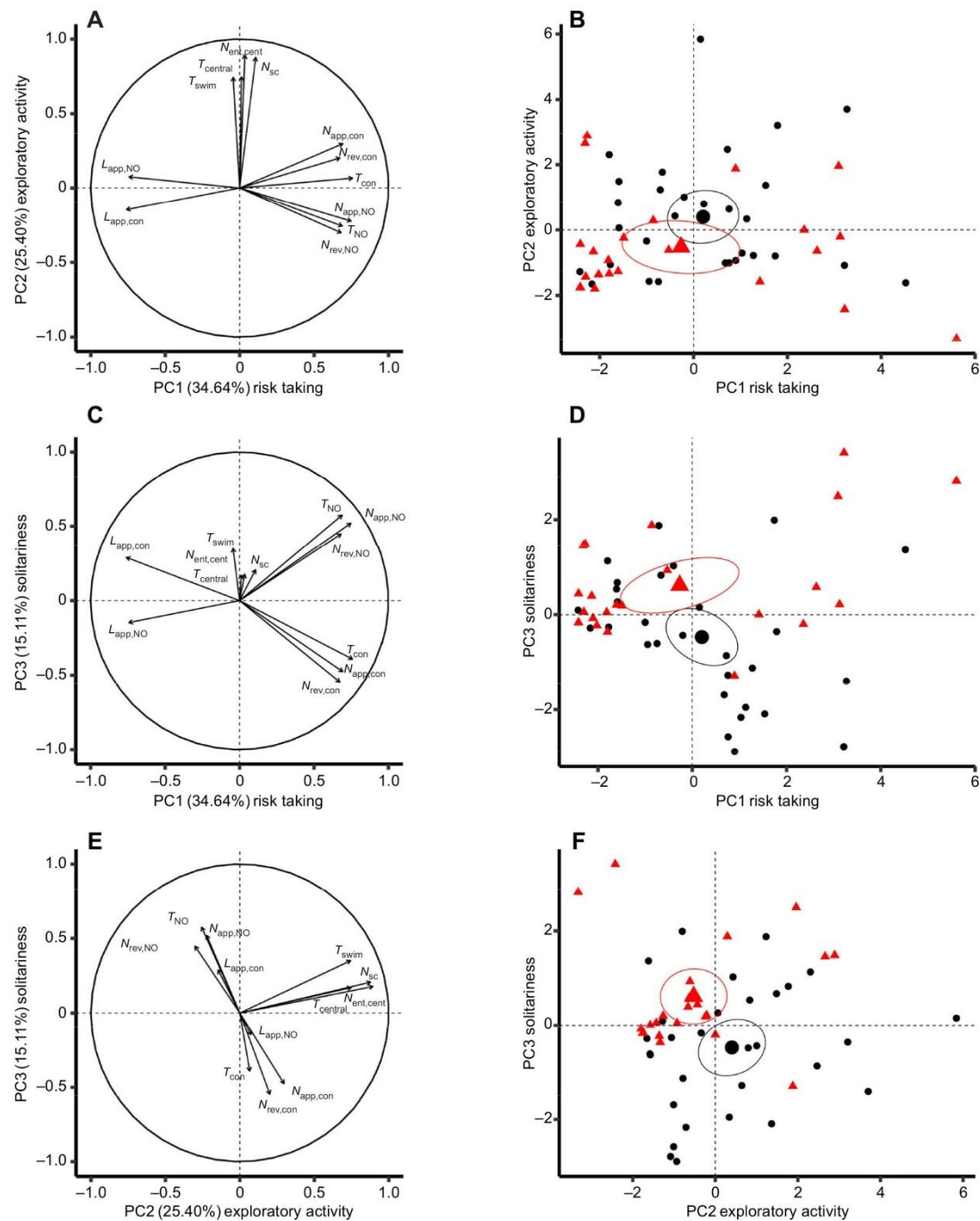


Fig. 1. Position of individuals along the risk-taking, exploratory activity and solitariness axes of the PCA. (A,C,E) Relationships between behavioural variables and their contribution to the principal component (PC). (B,D,F) Factor maps for individual fish. Top row: exploratory activity versus risk taking; middle row: solitariness versus risk taking; bottom row: solitariness versus exploratory activity. Red triangles: food-deprived fish ($N=23$); black circles: control fish ($N=30$). Confidence ellipses are plotted around group mean points.

Table 3. Linear model describing the interaction between feeding regime, risk taking and solitariness

Included variables	Excluded variables	Estimate	s.e.	t-value	P-value
(Intercept)		-0.416	0.217	-1.915	0.061
Treatment		1.088	0.329	3.305	0.002**
PC1 risk taking		-0.270	0.129	-2.097	0.041*
Treatment×PC1 risk taking		0.490	0.164	2.981	0.004**
	PC2 exploratory activity	0.117	0.096	1.212	0.232
	Treatment×PC2 exploratory activity	-0.090	0.202	-0.446	0.657

Estimates, s.e. (standard error), *t*-value and *P*-value were generated by the linear models fitting procedure. Included variables refer to those that made a significant contribution to the model; excluded variables are those that made a non-significant contribution to the model. Significant effect of the variables on PC3 (solitariness): **P*<0.05, ***P*<0.01.

However, contrary to our second hypothesis, control and FD fish did not differ in terms of risk taking and exploratory patterns. In agreement with our third hypothesis, we observed that feeding status influenced the relationship between risk taking and solitariness, with risk-prone fish displaying increased solitariness when food restricted and reduced solitariness when fed normally.

Behaviours

Using PCA, the impact of feeding status on the fish's response to novelty was explored. Through this approach, the 15 measured variables were combined into smaller sets of interpretable linear combinations (components). Three components were identified that explained approximately 75% of the variability of fish behavioural patterns. The first component was considered to indicate risk taking as it included variables usually attributed to the propensity to take risk when faced with novelty. The second component of our PCA was termed exploratory activity as it informed about fish swimming activity level and pattern while exploring a novel environment. The third component was considered to specify fish level of solitariness as it was defined by measures of an individual's unwillingness to group with a conspecific.

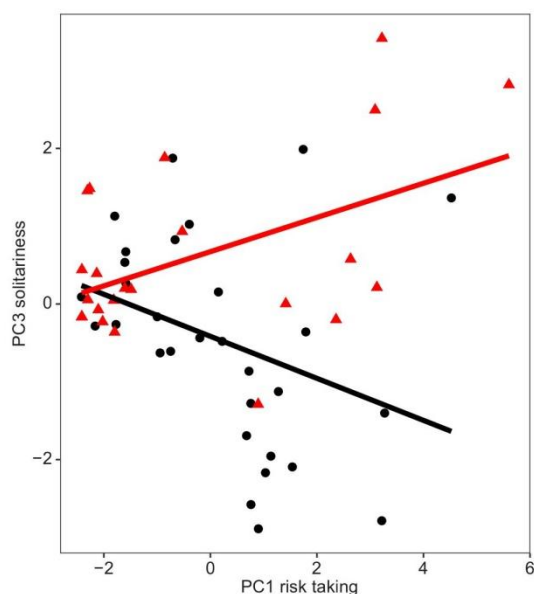


Fig. 2. Relationships between PC1 risk taking and PC3 solitariness according to the feeding treatment. Red triangles and lines: food-deprived fish (*N*=23); black circles and lines: control fish (*N*=30).

Because of terminological and methodological inconsistencies, the delineation of behaviours can be somewhat subjective, potentially resulting in conflicting interpretations (Carter et al., 2013; Roche et al., 2016; Toms et al., 2010). The current dataset provides a new illustration of the difficulty of contextually interpreting and labelling behavioural responses. The exploration of the central zone in a novel environment test as well as the response to a novel object during the novel object test are classically used to assess an individual's degree of risk taking in response to novelty (Boulton et al., 2014; Budaev, 1997; Burns, 2008; Frost et al., 2013; Sneddon, 2003; Wilson et al., 1993; Wright et al., 2003). In the present study, however, variables from these two tests did not correlate with each other, indicating that they may not evaluate the same behaviour. Whereas variables from the novel object test correlated on PC1, the number of entries into the central zone and the duration of stays in this area correlated with the total time spent swimming and the number of sections crossed in the novel environment test on PC2. This linear combination on PC2 supports the hypothesis that the number of entries and the time spent in the central zone may simply be proportional to an individual's activity level, with more-active fish passing more often and spending more time in the centre of the experimental arena than less-active fish. We suggest that those four variables are indicators of exploratory activity level rather than indicators of risk taking.

One major requirement to define an animal's personality trait is that the inter-individual differences in the corresponding behavioural measure must be consistent. However, demonstrating consistency requires that the measure is repeated several times in exactly the same context. In the present experiment, this was clearly not the case, as fish behaviours were measured only once. Consistent with the fact that the repeatability of fish responses was not evaluated, we considered that the tests used in the present study evaluated the impact of starvation on behavioural tendencies (risk taking, exploratory activity and solitariness) and not on personality traits (boldness–shyness, exploration–avoidance, sociable–solitary).

Effect of food deprivation

The effect of a 3 week food deprivation period on solitariness, risk taking and exploratory activity was evaluated. In line with our first hypothesis, starvation favoured solitariness in sea bass. Conflicting with our second hypothesis, however, food deprivation had no impact on risk taking and exploratory level.

It is important to emphasize that the third component of our PCA, solitariness, explained only 15% of variance in our dataset, suggesting that it should be interpreted with caution. Moreover, because of the methodological approach that we followed, two issues must be outlined regarding how solitariness was assessed in the present work.

First, during the conspecific test, the social stimulus was not visible to the focal fish as it was placed in an opaque jar situated at the centre of the arena. This absence of visual contact between the focal fish and

its conspecific allowed us to interpret the interaction as reflecting true social interest without any establishment of dominant–subordinate relationships. Whether the results would have been the same if the conspecific had been visible or was presented to the focal fish in a different manner (e.g. transparent partition or at a different location in the arena) is open to question. It must be noted, however, that, although not visible, the conspecific inside the jar could still interact with the focal fish through holes that allowed chemical cues and pressure waves to disseminate into the arena.

Second, the possibility exists of a carry-over effect of the risk response to the jar alone (day 2) on that to the presence of a conspecific inside the jar on day 3. For example, fish with reduced interactions with the novel object on day 2 were considered as risk averse and these individuals might have continued to show aversion to this object on day 3, despite the presence of a conspecific inside the jar. Although risk taking was evaluated in the first 5 min of the novel object test (day 2), fish actually remained in the arena for a total of 30 min, familiarizing themselves with the object. It was therefore considered that by day 3, the jar was no longer a novel object, allowing us to consider that interactions with the jar were then fully attributable to the presence of the conspecific inside.

With regard to our first hypothesis, control fish interacted more with conspecifics than did FD fish. Juvenile European sea bass form shoals, a social behaviour that is believed to rely on the fish's voluntary decision to remain together (Réale et al., 2007). Grouping behaviour is increasingly considered as a flexible response by which individuals tune the trade-off between predator avoidance and resource acquisition (Krause et al., 2000b; Lima and Dill, 1990). Shoaling indeed contributes to maximizing food acquisition through increased detection rate and, at the same time, enhances predator avoidance through greater vigilance in addition to dilution and confusion effects. However, grouping behaviour is also associated with stronger competition between conspecifics and easier detection to predators, shoals being more visually conspicuous than solitary individuals (Clifton and Robertson, 1993). The observation that FD fish displayed reduced interactions with the conspecific suggests that when food is scarce, individuals may benefit from solitariness as it reduces competition and aggressiveness prompted by hunger (Webster and Hart, 2006).

Conflicting with our second hypothesis, the experimental results revealed that food deprivation had no impact on risk taking and exploratory activity in sea bass. Individuals from the FD group were initially expected to be risk prone and to display a higher activity level than fish from the control group. It has indeed been hypothesized that a higher level of activity and risk taking favours habitat exploration and consequently enhances food encounter rates (Killen et al., 2011; McFarlane et al., 2004; van Dijk et al., 2002). Moreover, increased activity and risk taking has been reported following starvation (Godin and Crossman, 1994; Killen et al., 2011; McFarlane et al., 2004). To explain this inconsistency between the present results and the literature, differences in experimental design must be considered. While in the present study activity and risk taking were assessed from the viewpoint of novelty, using an unfamiliar environment or a novel object, the studies mentioned above measured spontaneous activity in a familiar environment and assessed risk taking in response to attractive and/or repulsive stimuli. Because of these among-studies discrepancies in experimental design, swimming activity and risk taking were actually evaluated in different contexts in which fish may have expressed a different behavioural response. In the present study, the fact that exploratory activity and risk taking did not change in starved sea bass suggests that after 3 weeks of food deprivation, the trade-off between fulfilling a nutritional requirement and risk taking

in response to novelty was unchanged. It remains to be tested, however, whether this trade-off would have been resolved differently if the fish's energetic status had been aggravated further, by a longer starvation period, for instance.

It should be noted that exploratory activity was nearly statistically different between control and FD fish ($P=0.057$). This suggests that compared with control fish, the exploratory inclination of starved individuals tended to be reduced (Fig. 1B,F). This result is in line with other studies in which decreased activity after food deprivation has been reported (Binner et al., 2008; van Dijk et al., 2002). Swimming is energetically costly and it would make sense that fish with a reduced energy reserve lowered the expression of energetically demanding activities such as swimming. However, lowering exploratory activity also implies reduced chances of finding food. In the present study, no food was made available to the fish, possibly rendering increased swimming inappropriate. Yet, the strategy could have been different if food had been made available, augmenting the potential reward of increased exploratory activity.

Correlation among behaviours

The observed trade-off between risk taking and solitariness in risk-prone individuals validated our third hypothesis. After 3 weeks of food restriction, we indeed demonstrated that risk-prone FD fish interacted less with conspecifics (increased solitariness) while risk-prone control fish interacted more with conspecifics. As discussed above, food restriction does not affect risk taking. This suggests, therefore, that the origin of the risk taking $\times$ solitariness interaction in risk-prone fish lies in the modulating effect of fish nutritional status upon solitariness.

Under the control feeding condition, individuals that interacted the most with the novel object also exhibited the highest interest for conspecifics. This result is in accordance with observations reported by Cote and Clobert (2007) on *Lacerta vivipara*, but it contradicts studies in which risk-prone fish were reported to ignore conspecifics and to engage in fewer interactions than risk-averse individuals (Budaev, 1997; Harcourt et al., 2009a; Wilson et al., 1993). Juvenile sea bass are gregarious fish that form shoals (Barnabé, 1980) and the sharing of food is not always equal within a shoal (Bumann and Krause, 1993; Krause, 1993; Ward et al., 2004). It can be hypothesized that fish with a high propensity to take risks also benefit from solitariness to find and secure food without competition. Similarly, Harcourt et al. (2009b) reported that hunger modulated shoaling behaviour differently in shy and bold three-spined sticklebacks. They showed that under normal feeding conditions, shy and bold individuals both preferred to shoal with bold conspecifics. In contrast, under restrictive feeding conditions, bold sticklebacks continued to prefer shoaling with bold fish while shy individuals displayed a preference for shy conspecifics. Harcourt et al. (2009b) hypothesized that the change in shoaling preference observed in the starved shy individuals suggests that these fish perceived more acutely the potential intraspecific competition by the bold fish and responded by shoaling with shy and potentially less competitive conspecifics. This modulation of social behaviour by the interaction between feeding status and risk-taking behaviour is along the same lines as our own results, which indicate that in periods of food shortage, the potential reward associated with taking more risks and being less sociable may overcome the cost resulting from increased predation risk related to solitariness.

To conclude, the present findings document how hunger may affect sociability in juvenile sea bass and contribute to increase current knowledge regarding the interaction between feeding status and risk taking and how it can drive social behaviour.

RESEARCH ARTICLE

Journal of Experimental Biology (2019) 222, jeb190553. doi:10.1242/jeb.190553

Acknowledgements

We would like to thank the members of the PFOM-ARN laboratory (Ifremer) for their technical assistance. We are also grateful to C. Lebigre for helpful advice on statistical analyses. We also thank the anonymous referees for their helpful comments on the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.A., G.C.; Methodology: C.A., N.L.B.; Formal analysis: C.A.; Investigation: C.A.; Writing - original draft: C.A.; Writing - review & editing: C.A., G.C.; Visualization: C.A., G.C.; Supervision: S.L.F., G.C.

Funding

C.A. was supported by a joint CEDRE-Région Bretagne doctoral grant.

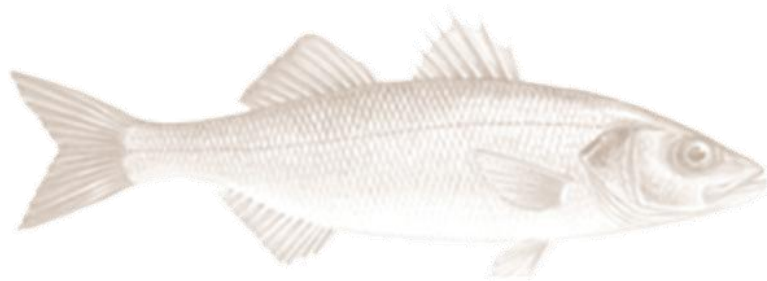
Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.190553.supplemental

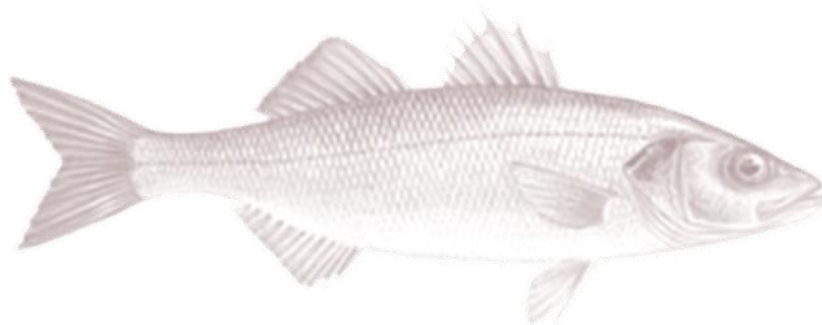
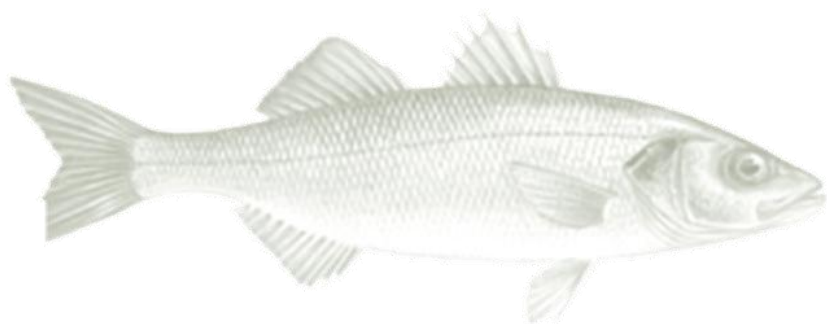
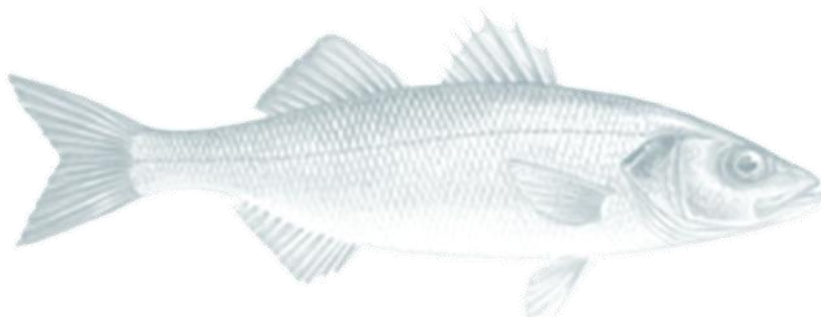
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CHAPITRE 2



Dans ce deuxième chapitre j'évalue les effets d'une perturbation anthropique, le cas d'une marée noire, sur les comportements d'exploration et de prise de risque (*i.e.*, occupation d'une zone ouverte) chez les juvéniles de bar Européen en utilisant un test d'exposition à un nouvel environnement.

Le déversement accidentel de pétrole à la mer est un enjeu économique et écologique très important (Chang et al., 2014). Les résultats des recherches conduites sur les effets d'une telle perturbation environnementale sur le comportement des individus sont pourtant difficilement comparables entre eux et ce pour deux raisons principales. Premièrement les études ont été préférentiellement axées sur les premiers stades de développement ou sur des espèces à faible valeur économique (Incardona et al., 2014a; Le Bihanic et al., 2014; Vignet et al., 2014a; Perrichon et al., 2016; Jacquin et al., 2017). Deuxièmement, les études ayant été principalement conduites sur des composés isolés un manque d'information persiste quant aux effets des mélanges de composés pétrolés sur le comportement des poissons. En somme, malgré les résultats notables obtenus dans les études précédentes sur les effets des hydrocarbures pétroliers sur le comportement des poissons, il reste des limites importantes à l'utilisation de ces données pour les intégrer aux multiples facteurs influençant la dynamique des populations exploitées et ainsi qu'elles contribuent à l'amélioration des politiques de gestions des stocks.

Le test du nouvel environnement a été choisi pour deux raisons principales. Premièrement, l'exploration est fondamentalement basée sur l'activité locomotrice. La locomotion est reconnue comme l'un des indices comportementaux les plus sensibles à l'exposition aux substances toxiques. Des altérations ont, en effet, été détectées à des concentrations bien inférieures à celles pouvant causer une baisse de la croissance ou la mort des individus (Woodward et al., 1987; Little & Finger, 1990). Deuxièmement, la mise en place du test du nouvel environnement est simple, rapide et ne nécessite pas de période d'acclimatation à l'arène expérimentale puisque la mesure de l'exploration débute dès que l'animal entre dans l'arène. Ce test permet également d'évaluer un aspect de prise de risque dans le phénomène d'exploration à travers la mesure du temps passé dans la zone centrale, la plus exposée de l'arène. Au cours des expériences réalisées dans le chapitre 1, il a pu être mis en évidence que les juvéniles de bar Européen présentaient une activité d'exploration stéréotypée, caractérisée

par une augmentation graduelle de l'activité locomotrice pendant les 20 premières minutes du test dans le nouvel environnement. Il m'a donc semblé plus pertinent dans cette nouvelle étude de prendre en considération cette dynamique d'exploration pour évaluer l'activité exploratoire et déterminer si l'exposition aux produits pétroliers pouvait affecter ce comportement stéréotypé.

En terme d'objectifs scientifiques, cette étude a pour but d'évaluer, à la fois, les effets directs de l'exposition aux hydrocarbures pétroliers sur l'activité d'exploration et la prise de risque et de déterminer si les réponses comportementales sont modifiées de façon dose-dépendantes. Pour ce faire trois doses d'hydrocarbures pétroliers ont été utilisées.

Enfin, l'expérience a été conduite sur deux semaines post-exposition afin d'évaluer la capacité de récupération des individus ; ceci afin d'avoir une évaluation plus pertinente des impacts en estimant la durée des altérations.

Effects of dispersant treated oil upon exploratory tendency in juvenile European sea bass (*Dicentrarchus labrax*)

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ABSTRACT

Despite a significant decrease in the number of large oil spills over the last 48 years, accidental spills have still substantial economic and ecological consequences. Although resorting to chemical dispersant is one of the most implemented strategy in response to oil spills, it results in an increase in the bio-availability of oil compounds. While these compounds have been recognized as a significant threat to marine organisms, holistic understanding of their impacts on individual health status, and hence populations and ecosystem functioning is challenging, requiring the assessment of sublethal effects following oil exposure. Polycyclic aromatic hydrocarbons, a major component of oil, can disturb the neurosensory capacities of fish and are, therefore, liable to affect their behavioural repertoire, causing improper use of their habitat. This integrator aspect of an oil spill exposure is, however, poorly studied. Using juvenile European seabass as a model species, we evaluated, in a dose-response manner, the influence of a 62h-dispersant treated oil exposure on two indices of fish exploratory tendency *i.e.*, exploratory activity and avoidance of unfamiliar open areas, during an open field test. Three different chemically dispersed oil concentrations were tested and compared to a control condition. The low and medium conditions were chosen to bracket the range of situations that fish are more likely liable to encounter following an oil spill and the high dose represented a more severe condition. Fish behavioural performances were tested during 2 weeks following oil-exposure in order to quantify their recovery capacities. Results indicated that exploratory activity and time spent in the central

zone of the arena were affected in a dose-dependent manner; the lowest dose had no effect on sea bass behavioural response to a novel environment while medium and high doses altered fish typical avoidance of unfamiliar open areas. Moreover, fish exposed to the highest dose also displayed a reduced exploratory activity. These effects may result from a narcosis effect of oil compounds, causing anaesthetic-like sedative behaviours. Only the high dose treatment altered juvenile sea bass exploratory tendency for at least more than two weeks. Conversely, fish exposed to lower concentrations, more likely to occur after an oil spill, were not affected or recovered within the two weeks post-exposure. Altogether, present study shows that juveniles seabass exposed to oil spill exhibit transient behavioural impairments that may still have major population-level consequences given the strong predation pressure on juveniles.

INTRODUCTION

Today's global economy heavily relies on the availability of oil reserves (Birol, 2015) and as the geographical distance between production sites and refineries' location is large, oil shipping is a continuously growing business. Oil transport therefore doubled over the last 40 years, and improved security measures led an overall decline in the number of large oil spills (>700 tons) over that period (International Tanker Owners Pollution Federation, 2017). Although less likely to occur, accidental spills remain, however, a highly relevant economic and ecological issue (Chang et al., 2014).

The typical response to accidental oil spills is to apply chemical dispersants to dissolve oil slicks by breaking them up into small droplets. The major drawback of such treatments of an oil slick is that it increases the bio-availability of oil compounds, especially polycyclic aromatic hydrocarbons (PAH), which are particularly toxic for marine organisms (Ramachandran et al., 2004; Adams et al., 2014).

The use of integrative and sensitive indicators of animal disturbances such as behaviour, that bridge physiology and ecology, is essential to enable a better assessment of environmental toxicants effects (Scott & Sloman, 2004; Weis, 2014). Individual's normal behaviour is the result of specific physiological sequences that are activated by external stimuli acting via the central nervous system (Scott & Sloman, 2004). For instance, upon detection of alarm substances by the olfactory system, sensory information is integrated centrally and depending

on their physiological characteristics (e.g., metabolism and hormones), fish will exhibit stereotypical avoidance, displaying behaviours that minimize risk, such as reduced feeding and swimming activities and increased shoaling. However, detrimental behavioural alterations might also occur if these sequences are disrupted. For example, alteration of sensory systems, central processing or physiological abilities, would likely lead to improper behavioural response to the threat and decreased survival rates due to increased predation risks. Furthermore, it has been suggested that behavioural analyses are among the most sensitive to highlight environmental disturbances. For instance, behavioural alterations often occurs at concentrations well below those causing significant changes in growth or inducing mortality (Woodward et al., 1987; Little et al., 1990a; Gerhardt, 2007).

Behavioural indices are often used to examine sub-lethal effects of toxicants or pharmacological treatment. Specific procedures such as the open field test (OFT) (Prut & Belzung, 2003; Sousa et al., 2006; Ahmad & Richardson, 2013; Vignet et al., 2017) have been implemented to evaluate the exploratory tendencies of an animal in a novel environment with measures of locomotor activity and centre avoidance (thigmotaxis; Sousa et al., 2006; Brown & Nemes, 2008). Fish displaying elevated exploratory behaviour are considered to be more prone to take risk and less averse to novelty, by displaying higher locomotor activity and spending more time in the central part of the open field for instance. Conversely, fish showing lower propensity to explore an unknown environment are generally less active and tend to prefer the periphery of the experimental arena (Maximino et al., 2010; Dahlbom et al., 2011).

Locomotor activity is one of the most sensitive behavioural indices to toxicants exposure, with alterations being detected at concentration well below those inducing growth reduction or individuals' death (Little et al., 1990; Woodward et al., 1987). Therefore, individuals' locomotor activity is the most commonly used sub-lethal index in emphasizing a behavioural modification following contaminant exposures (Woodward et al., 1987; Little & Finger, 1990; Calfee et al., 2016). The assessment of locomotor activity consists in measuring variables such as the frequency and duration of movements, individuals' velocity and distance travelled as well as their change over the duration of the test. The assessed locomotor pattern can reflect either general locomotor activity or exploration depending of the context in which it

has been measured (*i.e.*, respectively familiar or novel environment). These two aspects of animal behaviour are important as they contribute to the gathering of information about the environment and mapping of the habitat which can then be used in relation with foraging and sheltering (Little & Finger, 1990).

In spite of their remarkable achievements, past studies investigating the effect of oil spills on individuals' behaviour and physiology have major limitations. Firstly, most of the studies looking at the effects of dispersant treated oil on animal behaviour focused on early developmental stages (Incardona et al., 2014a) or species of low economical values (Le Bihanic et al., 2014; Vignet et al., 2014a; Perrichon et al., 2016; Jacquin et al., 2017). However, the variation in individuals' resilience capacity can differ substantially among species and increases with age/life stages (Hawkins et al., 2002) along with their physiological and behavioural characteristics. Therefore, information arising from these works is hardly used and comparable between studies. Secondly, studies investigating the effects of PAH on fish behaviour suggested altered general activity and exploratory tendency (Schwarzenbach et al., 2006; Correia et al., 2007; Vignet et al., 2014a). While hypoactivity has been reported by some authors (Westlake et al., 1983; Drummond et al., 1986; Correia et al., 2007; Gonçalves et al., 2008; Vignet et al., 2014b), others described hyperactivity (Walker et al., 1998; Vignet et al., 2014a). Individual tendency to explore have also been suggested to be reduced after chronic exposure to PAH in zebrafish (Vignet et al., 2014a; Jacquin et al., 2017). Yet, all these studies focused on the effects of specific PAH compounds, making it difficult to transpose their results to an oil spill situation where numerous families of compounds are involved.

Dose-response effects following oil exposure have received little attention and remain largely unknown. Yet, some studies have suggested that behavioural and biochemical alterations following oil compounds exposure display a dose-response pattern (Correia et al., 2007; Gonçalves et al., 2008). For instance, antioxidant activity has been shown to respond in a dose response manner to PAH exposure (Correia et al., 2007). Concerning higher organizational-level biomarker, Gonçalves et al. (2008) recorded less swimming activities at increasing exposure concentrations to PAH compounds.

To our knowledge, very few studies have investigated fish recovery capacity when returned in clean sea-water. Mauduit et al. (2016) reported transient reduction in swimming performance and hypoxia tolerance one month following fish exposure to dispersant treated oil, with no residual effect 10 months later. However, no detail was given on the time frame of the recovery process as no measurements were done between 1 and 10 months post-exposure. Solangi et al. (1982) showed histological alterations of gills, olfactory organs and pancreas of *M. beryllina* after exposure to whole crude oil or water soluble fraction for 20 days. However, partial to complete recovery was observed after 17 days in oil-free sea water. A study on *E. coioides* exposed to foodborne benzo[a]pyrene during 4 weeks, also reported a recovery within 1 to 4 weeks of intestinal morphofunctional changes i.e. cell proliferation, hyperplasia of the epithelial mucosa, number of *de novo* crypts (Yuen et al., 2007). There is therefore a critical need of studies that investigate the effects of exposure to multiple compounds mixture on physiological and behavioural processes, and accurately quantify individuals' recovery.

The main objective of this study was therefore to address these limitations. First, we assessed the sub-lethal toxicity of dispersant-treated oil on fish behaviour that is an integrative indicator of animal disturbances. Second, we evaluated dose-response effects of the exposure to an oil spill accident using three environmentally realistic concentrations of oil and dispersant mixtures. Third, we investigated the recovery capacities of individuals' behavioural performances after such exposures. More specifically, the present study aims at evaluating in a dose-response manner, the effect of a 62h exposure to dispersant treated oil on fish exploratory tendency when exposed to a novel environment. Levels and patterns of locomotor activity and time spent in the open area were measured over two weeks post-exposure in order to assess recovery capacities of these behavioural performances. Three hypotheses were tested; (1) exposure to dispersant-treated oil alters fish behaviour, reducing exploratory activity and increasing exposure to risk, (2) the larger the dose, the greater will be the behavioural alterations, (3) recovery of behavioural performances occurs within the first weeks after the exposure.

MATERIALS AND METHODS

Study animals

Juvenile European sea bass *Dicentrarchus labrax* Linnaeus, 1758 were obtained from a local fish farm (Aquastream, Lorient, France). Fish came from a brood stock of wild fish caught off the coast of Morbihan, France. Animals were transported to the Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer) rearing facility in Brest. They were maintained in a 500 l indoor tank supplied with open-flow, thermoregulated (15°C) and fully aerated sea water (salinity of 32). Artificial lighting followed local photoperiod. Fish were fed 3 times a week ad libitum using commercial feed (Neo Start Coul 2, Le Gouessant, France). For this study, we used a total of 378 fish, aged 1⁺ and that have an average mass, before the experiment, of 12.28 g (± 0.33 SE)). Experiments were non-invasive and were APPROVED BY THE FRENCH ETHICS COMMITTEE IN CHARGE OF ANIMAL EXPERIMENTATION N°74 (permit number: APAFIS#7783-2016112515512418 v3) and were in accordance with institutional guidelines.

Fish transport

Fish exposure to oil and dispersant mixtures were conducted at the Centre de documentation, de recherche et d'expérimentation sur les pollutions accidentelles des eaux (Cedre, Brest, France). Fish transportation to and from Cedre (travel time 30 min) was realised using airtight plastic containers (50 L) filled with 40 L of water containing a light dose of anaesthetic (MS-222; 20 mg L⁻¹). The free volume above the water was filled with oxygen. Upon arrival at Cedre, fish were transferred to a polyethylene tank (300 L). Water temperature, salinity and photoperiod at Cedre were similar than in the original rearing tank.

Experimental exposure

Table 1. Experimental exposure.

	Date 2017-01-27 to 2017-01-30		Date 2017-03-17 to 2017-03-20		Date 2017-04-21 to 2017-04-24	
Label	C _H	E _H	C _M	E _M	C _L	E _L
CAL (g L ⁻¹)	0	0.8	0	0.4	0	0.2
Finasol (g L ⁻¹)	0	0.01	0	0.005	0	0.0025
Number of replicates	3	3	3	3	3	3
Number of fish	21	21	21	21	21	21

per replicate

As shown in Table 1, experiments spanned over 3 months. Three doses of dispersant-treated oil were successively tested. The highest dose (E_H) consisted in mixing 0.8 g L^{-1} of weathered crude Arabian light (CAL) with 0.01 g L^{-1} of chemical dispersant (Finasol © OSR 52, Total Fluides, Paris France), the medium dose (E_M) consisted of 0.4 g L^{-1} of CAL added with 0.005 g L^{-1} of dispersant and the lowest dose (E_L) consisted of 0.2 g L^{-1} of CAL mixed with 0.0025 g L^{-1} of dispersant. Each experiment involved 63 control fish (labelled C; $n=21$ per triplicate) and 63 exposed fish (labelled E; $n=21$ per triplicate). Six hours following their arrival at Cedre, fish were distributed among six polyethylene tanks (300 L), three of which housed the control fish while the other three received the fish to be exposed to the experimental mixture.

Crude Arabian light and dispersant were mixed in a bottle in accordance with the manufacturer's recommendation (Dispersant/oil ratio of 4%). The mixture was then directly poured in the tanks and weathered by bubbling air during 5 hours prior the introduction of the fish (Nordvik, 1995). Tanks were equipped with a custom-made device that mimicked oil mechanical dispersion by waves at sea while maintaining homogenous exposure conditions throughout the tanks. This device consisted of a funnel located at the surface and connected to a 12 V submersible bilge pump (L450-500GPH; Johnson) placed on the bottom of the tank. Oil floating at the surface was sucked through the device, homogenized with water and released at the bottom of the tank (Milinkovitch et al., 2011). Following the 62 h exposure period, control and exposed animals were bathed in clean sea water (1 h) and transported back to Ifremer facilities following the same transportation procedure as described above. At Ifremer, C and E fish were placed in two different tanks which meant mixing treatment replicates.

Behavioural assay to assess fish exploratory tendency

Behavioural assays started the day following fish return to Ifremer facilities (24h post exposure). Fish exploratory tendency was examined using an open field test (OFT). To monitor the kinetics of post-exposure recovery, these tests were repeated over two weeks (5 naïve fish per day) and data were combined such that the first week (W1) and the second week post exposure (W2) were distinguished during the analyses. Given the long time span of

the experiment (3 months; end of January to April 2017), each experimental treatment group (E_L , E_M , E_H) was paired with its corresponding control group (C_L , C_M , C_H).

The day prior to the experimental test, fish were randomly selected in the rearing tanks and gently placed (without emersion) into individual confinement chambers. Confinement chambers consisted of an opaque PVC tube (13 cm×5 cm length, diameter respectively) closed at both end with plastic meshing to allow water renewal inside the chamber (Aimon et al., 2019). Fish were left undisturbed 15 h in these chambers, placed side by side on the bottom of a tank that received the same water than the rearing tank. On the testing day, chambers were successively placed in an immersed plastic container (2 L) and then gently moved to the experimental arena. Fish were given 3 minutes to recover from potential disturbance before one end of the chamber was opened from a distance. Fish were allowed 1 min to exit the chamber or the opposite end from the exit was slowly lifted, also from a distance, to encourage the fish to swim out. This encouragement to leave the chamber was applied in 146 cases out of 156. In order to standardize trials, the first minute following fish entrance in the arena was not used in our analysis.

The experimental arena consisted in a white rectangular shallow tank (156 cm×99 cm×14 cm, length, width, depth, respectively). To limit visual disturbances that might influence fish behaviour, a curtain was placed around and over the experimental arena. Neon lamps were placed on each side of the arena to provide an homogenous light (30 lux), and a video camera was placed 1m above the water surface (Logitech webcam C930e, 15 frames s⁻¹). The bottom of experimental tank was covered with a light retro-reflective adhesive foil (Loligosystem, Inc) to improve contrast with the fish.

The open field test consisted in exposing fish to a bare tank and to record its movements during the following 20 minutes. Three indices of exploratory activity were measured in 5-minutes increments: total time spent swimming (labelled *Tswim*), total distance moved (labelled *Dmoved*) and the swimming speed (labelled *Velocity*) and one measure of open area avoidance: time spent in the central zone of the arena (labelled *TZoneC*). The central section of the experimental arena (78 cm×49.5 cm) corresponded to the area situated at two body

lengths from the walls. Videos were analysed using the video tracking software Lolitrack Version 4.2.0 (Loligosystem, Inc).

Chemical analyses

To characterize exposure conditions, total petroleum hydrocarbon concentration ([TPH]) was measured in triplicate in each exposure tank, with seawater samples being taken immediately before and 4, 24 and 48 h after fish introduction into the tanks. Seawater samples were extracted three times with 10 mL of dichloromethane Pestipur quality (SDS, Carlo Erba Reagent, France). The combined extracts were dried by filtering through anhydrous sodium sulfate and then analysed using a spectrophotometer (Evolution 600 UV-VIS; Thermo Fisher Scientific) at 390 nm, as described by Fusey and Oudot (1976).

To assess fish contamination, two individuals per tank were euthanized at the end of the exposure phase and polycyclic aromatic hydrocarbons (PAH) concentrations in the bile and liver were measured. The presence of PAH metabolites in the bile was determined semi-quantitatively using a fluorospectrophotometer (Aas et al., 2000). A 5 nm slit width was used to measure the PAH metabolites on emission and excitation channels (Jasco FP-6200, Tokyo, Japan). Analyses were conducted using three excitation-emission wavelengths i.e., 295–335 nm (naphthalene-type metabolites); 343–383 nm (four-ringed compounds including pyrene-type metabolites) and 380–430 nm (benzo[a]pyrene-type metabolites; Krahn et al., 1987; Lin et al., 1996; Aas et al., 2000). Liver concentrations of 21 polycyclic aromatic hydrocarbons (including the components listed by US-EPA) were measured by GC–MS as described by Lacroix et al. (2014). Briefly, liver PAH were extracted using an alkaline digestion combined with stir bar and were evaluated with sorptive extraction-thermal desorption-gas chromatography-mass spectrometry (SBSE-GC-MS). For the validation of this analytical method, quantification limit of each PAH was estimated. This measure allowed us to identify the lowest concentration of PAH in a liver sample that can be determined with acceptable precision and accuracy under the condition of the test described previously.

Statistical analysis

For each variable, the values obtained in the three control groups (C_H , C_M and C_L) were averaged into one control group (C). The difference between the control condition of each

treatment and the average control group was then calculated and used to take into account the temporal variability resulting from the fact that the three tests were conducted several months apart. This correction was applied to the control and treatment groups. Data were standardized and normalized before the subsequent analyses. As the three indices of exploratory activity (*Tswim*, *Dmoved* and *Velocity*) were highly correlated with one another (Figure S1), principal component analysis (PCA) was used to reduce the number of variables to one principal component (PC) to represent the exploratory activity of the fish. This PC was chosen according to Kaiser's criterion (Kaiser, 1961), retaining only factors with eigenvalues greater than 1. The impact of dispersant-treated oil on juvenile sea bass behaviours was examined using linear mixed effects models to evaluate the relationships between, behavioural trend over time in the OFT, the time post-exposure (*i.e.*, week 1 or week 2) and the treatment. A backward stepwise reduction of the full model was applied to exclude non-significant effects.

The principal component analysis was conducted using FactoMineR package and models were run using the nlme package in R version 3.5.1 (R Core Team, 2018). Model diagnostics were evaluated by visually inspecting the residuals. The normality of the data was calculated using Shapiro-Wilk test. An ANOVA was applied to determine whether there were differences in water concentration in total petroleum hydrocarbons and liver concentration in 21 polycyclic aromatic hydrocarbon compounds among treatment groups. A Tukey test was then carried out to assess significant differences between treatments. Statistical significance was accepted at $P < 0.05$.

RESULTS

Exposure conditions

At the three doses tested, water concentration in total petroleum hydrocarbons ([TPH]) remained stable throughout the exposure periods (Fig.1). Experimental treatments corresponded to TPH of $0.902 \pm 0.031 \text{ g L}^{-1}$ for E_H , $0.243 \pm 0.012 \text{ g L}^{-1}$ for E_M and $0.048 \pm 0.007 \text{ g L}^{-1}$ for E_L . In the control groups (C_H , C_M , C_L), TPH concentrations were not statistically different and were averaged ($0.002 \pm 0.0004 \text{ g L}^{-1}$).

Mean liver concentration in 21 polycyclic aromatic hydrocarbon compounds (Liver Σ PAH) measured one day post-exposure in fish from the C condition was below the quantification limit, while concentrations measured in fish from the E_L , E_M and E_H conditions were

respectively 1.4, 2.4 and 9.8 times the quantification limit (Fig. 2). Only ten (pyrene, fluorene, phenanthrene, naphthalene, benzothiophene, biphenyl, acenaphthene, dibenzothiophene, anthracene, chrysene) of the 21 PAH measured were at concentrations above the quantification limit (Supplementary data, Table S1). Tricyclic PAH represented nearly 70% of total liver PAH in E_L exposed fish and 80% of total liver PAH in the E_M and E_H exposed fish (Table S1).

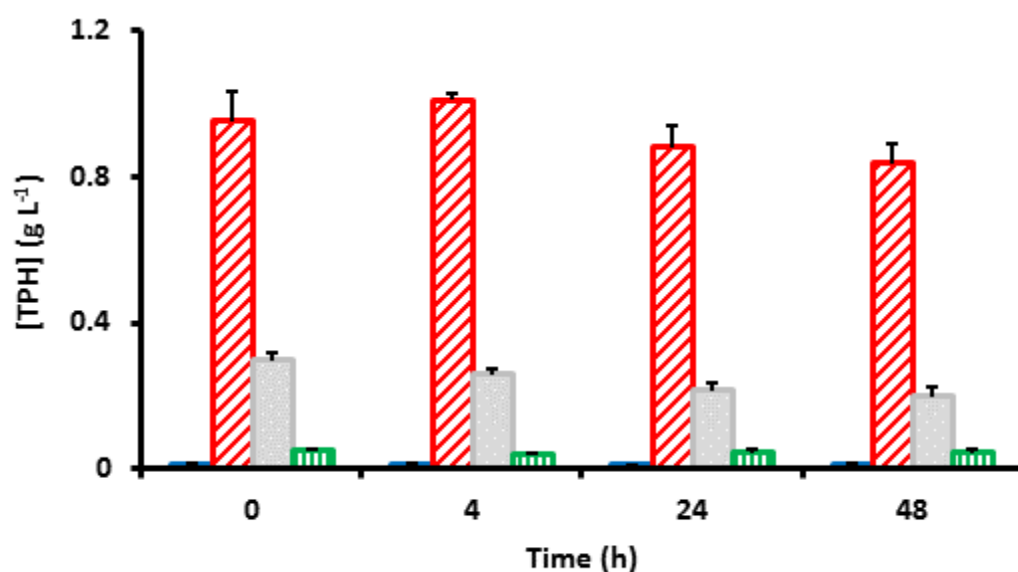


Fig. 1. Water total petroleum concentration ($g L^{-1}$) measured throughout the 62h-exposure period in tanks from the control (C; blue filled bar), high (E_H ; red hatched bar), mild (E_M ; grey pointed bar) and low (E_L ; green vertical hatched bar) treatments exposure to chemically dispersed oil. $N = 9$ (3 samples $\times$ 3 replicate tanks). The error bars indicate the calculated SEM.

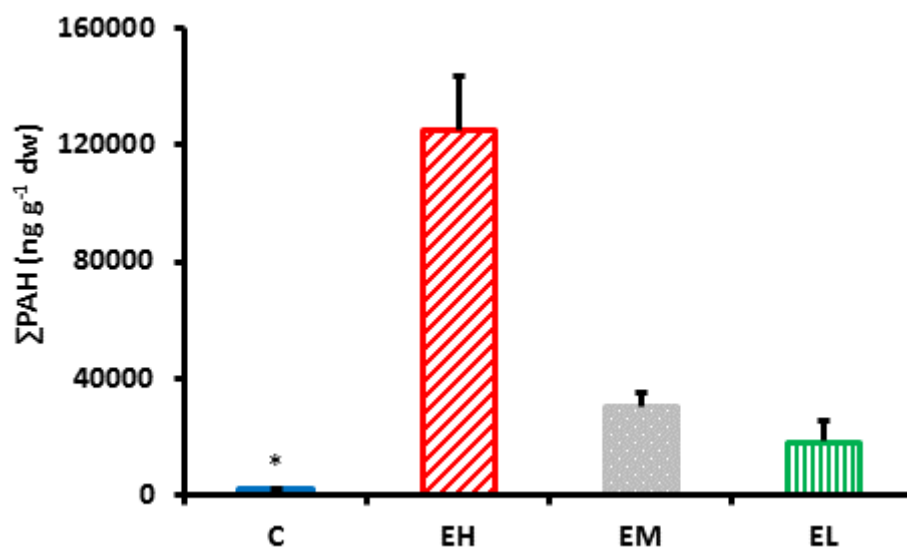
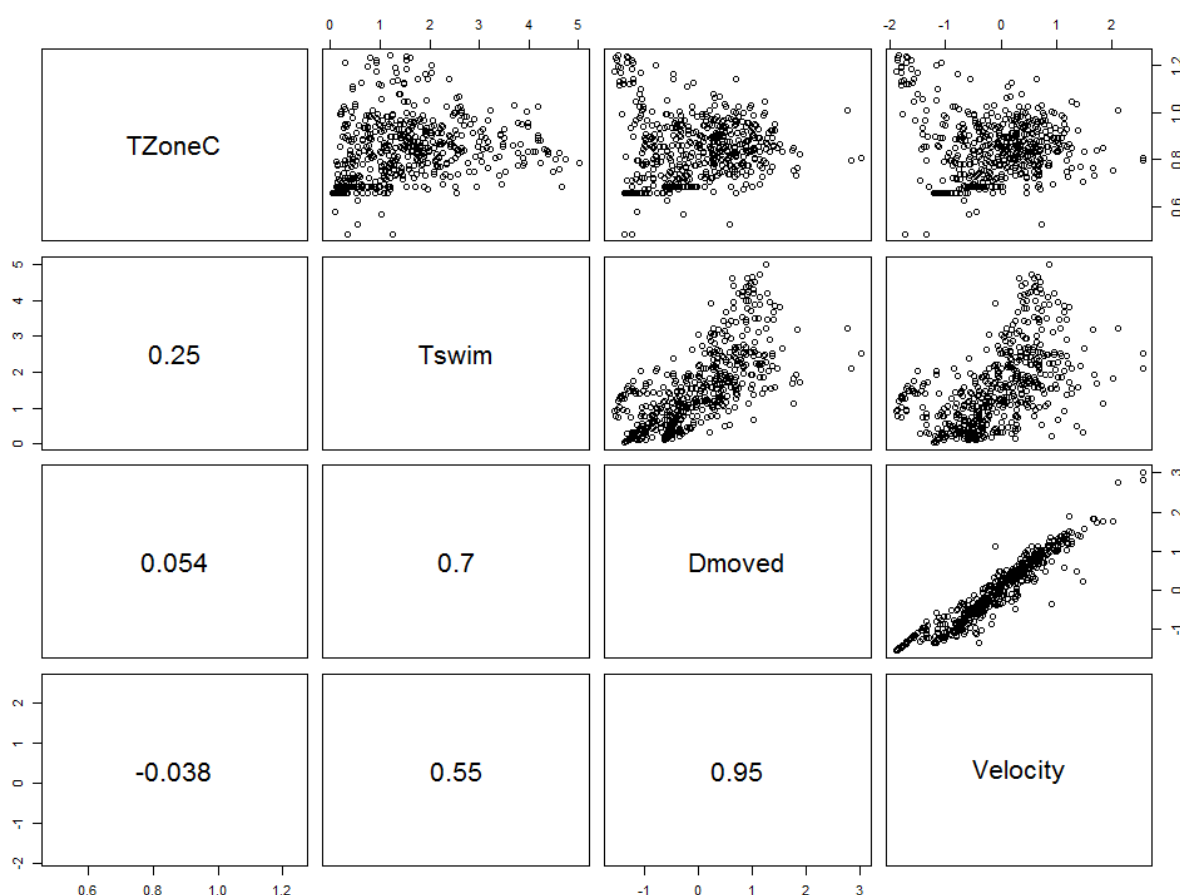


Fig. 2. Liver concentration in 21 PAH (ng g⁻¹ dry weight) measured one day post-exposure in fish from the control (C; blue filled bar), high (E_H; red hatched bar), mild (E_M; grey pointed bar) and low (E_L; green vertical hatched bar) treatments exposure to chemically dispersed oil. N = 3 (1 samples × 3 replicate tanks). The error bars indicate the calculated SEM. * Represents significant difference (p<0.05).

Survival

Due to a lack of information regarding the lethal effect of oil, and in order to account for possible mortality, 63 fish were exposed to each experimental treatment although our objective was to analyse only 30 of them. No mortality was observed in the three controls groups and in the low exposure group (E_L). In the mild exposure (E_M) group, on the other hand, one fish (1.6%) died during the post-exposure 1h cleaning period. In the severe exposure treatment (E_H), there was a 71 % mortality rate, death occurring both during and after the exposure period. Due to this high mortality rate, 18 fish instead of 30 were analysed in this treatment group. For the other treatment groups, 30 fish in total (N=15 fish per week post-exposure) were analyzed for behavioural response in the open field test (OFT).

Fig. S1. Pearson correlation between variables measured in the open field test. Pearson's coefficient of correlation between variables are indicated.



Reduction and structuration of the variables

Four variables were recorded in the OFT. Three of these variables assessed exploratory activity (total time spent swimming, labelled *Tswim*; distance moved, labelled *Dmoved*; swimming speed, labelled *Velocity*) while the fourth measured the time spent in the central zone (*TZoneC*). With the exception of *TZoneC*, variables were highly correlated to each other (coefficient of correlation >0.55 ; Figure S1). Thus, a principal component analysis (PCA) was conducted to combine *Tswim*, *Dmoved* and *Velocity* into one principal component (eigenvalue >1 ; Table 2). This principal component (PC1) was termed ‘exploratory activity’ and accounted for 79% of total variability. The two other principal components (PC2 and PC3) with eigenvalues <1 and explaining substantially less variance were ignored (Table 2).

Table 2. Axes description of the principal component (PC) analysis.

Variables	PC1	PC2	PC3
	Exploratory		

		activity		
Eigenvalue		1.92	0.46	0.06
Percentage of variance		78.86	18.85	2.30
Cumulative percentage of variance		78.86	97.71	100.00
Loading	<i>Tswim</i>	0.91		
	<i>Dmoved</i>	0.90		
	<i>Velocity</i>	0.83		

Effect of treatment exposure

Linear mixed effect model on PC1 scores revealed a significant interaction between the treatment group and the factor "Time", indicating that there are major changes in fish exploratory activity over the 20 minutes of the OFT ($F_{3,467}=4.73$, $P<0.01$; Table 3; Figure 3). No effect of the weeks post-exposure upon exploratory activity was observed ($F_{1,152}=0.28$, $P=0.60$; Table 3). Over the 20-minute test-time, individuals from the control treatment (C) as well as fish exposed to the E_L and E_M treatments, displayed increasing exploratory activity while fish from the E_H group had no change in exploratory activity over time. In addition, E_H fish showed lower overall exploratory activity in the experimental arena than individuals from the three other conditions (Fig. 3)

Table 3. Backward stepwise reduction of the full model evaluating the relationship between, the behavioural trend over time, the treatment condition and the time post-exposure (week 1 or week 2) upon exploratory activity.

Model	Dropped term	Retained term	F-value	p-value
Treatment:Week:Time	Treatment:Week:Time		2.07	0.10
Treatment+Time +Week +			0.71	0.40
Treatment:Time + Treatment:Week Week:Time + Week:Time				
Treatment+Time +Week +	Treatment:Week		2.18	0.09
Treatment:Time +Treatment:Week				
Treatment+Time +Week +	Week		0.28	0.60
Treatment:Time				

Treatment+Time + Treatment:Time	Treatment	5.42	<0.01	*
	Time	155.09	<0.01	*
	Treatment:Time	4.73	<0.01	*

Lines of the table represent the different steps of the backward stepwise reduction of the full model excluding non-significant interactions until the most parsimonious model is reached on the last line. * Represents significant effects ($p < 0.05$).

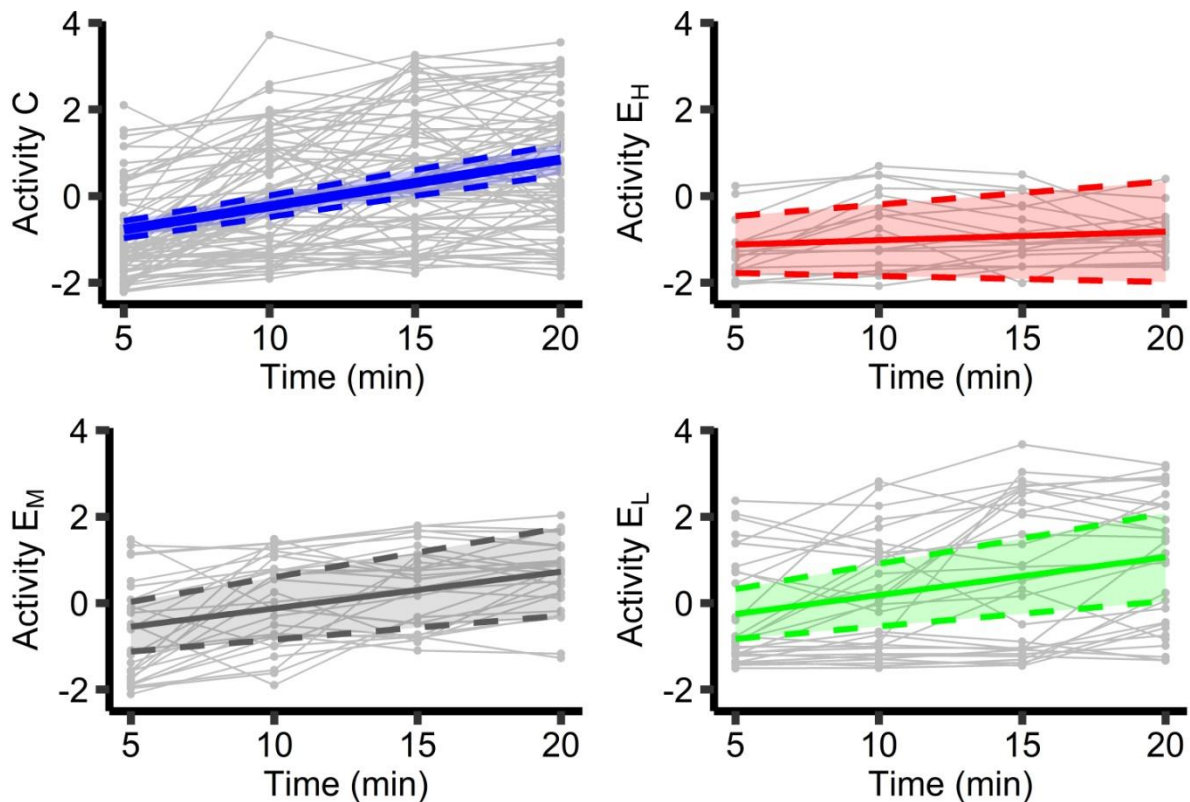


Fig. 3. Exploratory activity pattern over time in the open field test according to the treatment exposure. Solid line: mean; dashed line: SEM; blue : control; red: high dose; grey: medium dose; green: low dose.

Linear mixed effect model revealed that the time response of $TZoneC$ over the 20-minute testing period depended on the treatment group and on the week post-exposure considered ($F_{3,424}=3.68$, $P=0.01$; Table 4). The use of the central zone remained constant over time for fish from the C and E_L treatments (Fig. 4). Moreover, these fish tended to spend less time in the central zone than individuals from the other treatment groups. $TZoneC$ measured in fish from E_M treatment displayed a temporal pattern which was intermediate between that observed in fish from E_L and E_H conditions. During the first week post-exposure, fish from E_M group spent more time in the central zone than fish from the C and E_L treatments but less

than individuals from the E_H treatment. Moreover, the first week post-exposure $TZoneC$ measured in fish from the E_M and E_H groups showed a downward trend over the 20 min of the OFT. However, individuals from the E_M group returned to control level during the second week post-exposure, which was not the case for the E_H group. Furthermore, irrespective of the week post-exposure, E_H group exhibited the highest inter-individual variability in $TZoneC$. Additionally, fish from the E_H treatment presented the highest $TZoneC$ during the first week post-exposure, while presenting the lowest value the second week post-exposure.

Table 4. Linear mixed model describing the effect of the interaction between treatment, week period post-exposure and time trend upon $TZoneC$.

Model	Dropped term	Retained term	F-value	p-value
Treatment×Week×Time		Treatment	8.96	<0.01
		Week	5.94	0.02
		Time	10.18	<0.01
		Treatment:Week	21.77	<0.01
		Treatment:Time	6.57	<0.01
		Week:Time	0.02	0.88
		Treatment:Week:Time	3.68	0.01

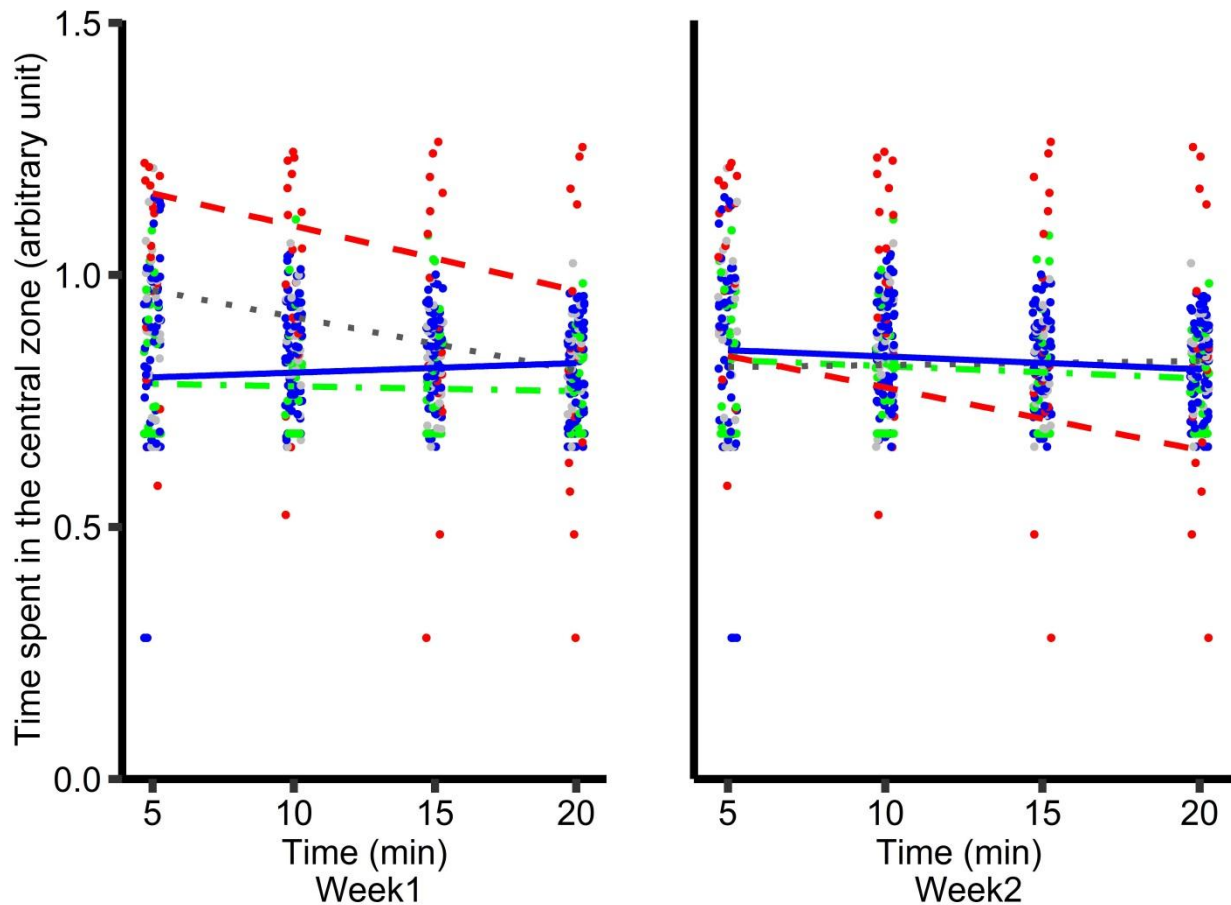


Fig. 4. Time spent in the central zone (TZoneC) over the 20 minutes of the open field test according to the treatment exposure in either the first or the second week post-exposure. Blue: control fish; green: fish exposed to the low dose treatment (E_L); grey: fish exposed to the medium dose (E_M) treatment; red: fish exposed to the high dose (E_H) treatment. Solid line: control; dashed line: E_L ; dotted line: E_M ; twodash line: E_H .

DISCUSSION

Using an open field test (OFT), this study examined whether, and to what extent, exposure to dispersant treated oil affects behavioural performances in juvenile sea bass. Three hypotheses were tested ; (1) exposure to dispersant-treated oil alters fish behaviour, reducing exploratory activity and increasing exposure to risk, (2) the larger the dose, the greater will be the behavioural alterations, (3) recovery of behavioural performances occurs within the first weeks after the exposure. We found that exploratory activity and exposure to risk (time in the central zone of the OFT) were affected in a dose-response manner. Although, no difference with the control group was observed at the lowest dose (0.2 g L^{-1} oil + 0.0025 g L^{-1} dispersant), fish exposed to the medium (0.4 g L^{-1} oil + 0.005 g L^{-1} dispersant) and highest

doses (0.8 g L^{-1} oil + 0.01 g L^{-1} dispersant) spent more time in the central, most exposed, zone of the open field. Within two weeks, however, a full recovery was observed for fish exposed to the medium dose but not for the fish exposed to the highest dose that also had higher occurrence in the central zone and altered exploratory activity (combination of the time spent swimming, the distance moved and the swimming speed). In these fish, no recovery was observed within the time frame of the experiment.

Exposure condition and liver [PAH]

Liver polycyclic aromatic hydrocarbon concentrations (PAH) measured in fish from E_L , E_M and E_H conditions were 1.4, 2.4 and 9.8 times higher than in fish from C group (135.5 ng g^{-1} dw) respectively. The compounds found in the sea bass liver consisted primarily in tricyclic PAH (fluorene, phenanthrene, dibenzothiophene) representing 70% to 80% of the total PAH. This larger fraction of high molecular weight molecules is most likely related to the weathering process which enhanced the evaporation of low-molecular-weight PAH (Short & Heintz, 1997; Carls et al., 1999; Heintz et al., 1999).

To characterize exposure conditions, water concentration in total petroleum hydrocarbon ([TPH]) was monitored in all tanks and throughout fish exposure period. As expected, low (E_L : 0.048 g L^{-1}) and mild (E_M : 0.243 g L^{-1}) exposure conditions bracketed the range of situations that fish are liable to encounter following an oil spill and its treatment with dispersant (0.001 to 0.260 g L^{-1} ; Kim et al., 2010; Sammarco et al., 2013; Spooner, 1970). In contrast, the severe exposure condition (E_H) resulted in [TPH] in the order of three times those classically found in such instances (0.940 g L^{-1}), although, concentrations up to 11.4 g L^{-1} have been reported (Sammarco et al., 2013). There were two reasons for testing the E_H condition i.e., validating the use of OFT in the context of oil exposure and fully assess sea bass tolerance to dispersed oil. Although the OFT is one of the most implemented methodology in toxicology assessment (Little & Finger, 1990; Weber et al., 2014), to our knowledge the present study is the first attempt to use this approach to assess the impact of oil compounds exposure. Hence, testing the E_H condition allowed ascertaining that an absence of response following exposure to E_L and E_M conditions actually meant an absence of effect and not just a lack of sensitivity of the test itself. The behavioural changes observed in fish from the E_H group allowed us to consider that the absence of effect in the other groups was a true negative. The second reason for

testing E_H condition was that it allowed us to fully assess sea bass capacity to withstand, and potentially recover from, particularly severe oil exposure conditions.

After 62 hours of exposure, a 71% mortality rate was recorded in the E_H condition (0.8g L⁻¹ of oil mixed with 0.01g L⁻¹ of chemical dispersant) while no or very few mortality was observed in control and other hydrocarbon exposure conditions. To our knowledge only one study has determined the LC50 for similar pollutant mixture in European sea bass (fish mass: 1g; about 0.1g L⁻¹ after 96h of exposure; Dussauze et al., 2013). In 17g sea bass it was shown that a 48h exposure to a mixture similar to ours did not induced any mortality, although affecting fish performance (Anttila et al., 2017; Zhang et al., 2017). The observed mortality in the E_H group was therefore unexpected and may be related to the lower body mass of the fish used in our study that were 29% lighter than those used in Anttila et al. (2017) and Zhang et al. (2017). This result is also consistent with the exponential relationship between pollutants toxicity and body mass that has already been observed in fish (Hedtke et al., 1982). Moreover, the duration of the exposure was increased by nearly 30% in the present case compare to Anttila et al. (2017) and Zhang et al. (2017), which may have contributed to the increased even further the toxicity of the E_H condition.

Behavioural analyses

In the OFT we evaluated two aspects of fish exploratory tendency i.e., fish exploratory activity, which quantified locomotor activity, and avoidance for open areas (thigmotaxis), which measured the time spent in the central zone (Ahmad & Richardson, 2013; Baker et al., 2018). These two aspects of exploratory tendency displayed a poor coefficient of correlation with each other ($r < 0.3$) and were therefore evaluated separately.

Exploratory activity

Fish exploratory activity was characterized by combining the time spent swimming, the distance moved and the swimming speed over the 20 min of the OFT (Baker et al., 2018). Fish from the control group as well as those exposed to E_L and E_M conditions displayed a continuous increase in exploratory activity (+129%) over the duration of the OFT. In contrast, fish from the E_H condition exhibited a distinctive exploratory pattern which was characterised by an overall lower activity, associated with a much less pronounced increase in exploratory

activity (+40%) over the same time. A progressively increasing locomotor activity following transfer into a novel environment has been suggested to reflect a gradual decrease in anxiety (Lister, 1990), fish being then able to gather information about their novel environment and particularly about associated resources and risks (Archer & Birke, 1983; Renner, 1990; Laland & Reader, 1999; Champagne et al., 2010; Griffin & Guez, 2014; Reader, 2015; Jacquin et al., 2017). Observed modification of the exploratory pattern of fish from the E_H group is in agreement with other studies that also reported alteration of fish exploratory behaviour following exposure to oil compounds (Vignet et al., 2014a; Jacquin et al., 2017). For instance, studies investigating the effect of an exposure to pyrene, fluorene and phenanthrene, which are among the PAH present in our mixture (Table S1), reported a decrease in exploratory tendency *i.e.*, reduced number of zone explored, less success in reaching the farthest area of a maze, less time spent in the upper water layer of a tank and less time spent swimming (Vignet et al., 2014a; Jacquin et al., 2017).

The toxicity of PAH is generally attributed to nonpolar narcosis (van Brummelen et al., 1998). Narcosis is considered as a reversible anaesthetic effect that results from a non-specific mode of toxicity. Mechanistically, it is caused by toxicant partitioning in the lipid bilayer of biological membranes thus interfering with membrane fluidity and affecting essential bound processes such as osmoregulation and neurotransmission. Narcosis especially affects neuronal cells and disrupts central nervous system function and signalling pathways (van Wezel & Opperhuizen, 1995; van Brummelen et al., 1998; Barron et al., 2004; Hsieh et al., 2006; Gonçalves et al., 2008). In humans, toxicity through narcosis is generally characterized by central nervous system depression, loss of consciousness, sleepiness, disorientation, drowsiness, dizziness and even death if exposure persists (Gerlai et al., 2000; Barron et al., 2004; Vignet et al., 2014a). In fish, a narcosis state is likely to alter the capacity to acquire and process information from the surrounding environment possibly resulting in inappropriate or lack of behavioural responses. Furthermore, oil-exposed fish were found to swim in an anaesthetic-like sedative behaviour *i.e.*, slowness, with head-up swimming posture (C. Aimon, pers. obs.). Subsequent disruption of the locomotor activity resulted in this study in fish being more likely drifting across the experimental arena rather than exploring it or taking risk in the open area. Such behavioural impairment can have major consequences on individual's fitness by increasing, for instance, the risk of predator-induced mortality.

One may wonder if the reduced exploratory activity observed in the E_H group could result from fish trading-off an energetically costly activity *i.e.*, swimming, for the metabolic requirement of detoxification. It has indeed been suggested that detoxification processes may mobilize sufficient metabolic resources that it could induce a switch in the metabolic profile with for consequence compensatory mechanisms to deal with the altered metabolism (Reddy & Bhagyalakshmi, 1994; Sørensen et al., 1997; Correia et al., 2007). This hypothesis seems very unlikely in the present case. For instance, respirometry measurements conducted on the European common sole (*Solea solea*) within days following a 48h severe oil exposure revealed that fish standard metabolic rate (SMR) was not significantly increased during the detoxification phase (Davoodi & Claireaux, 2007). This result suggests that, at least in that species, costs for detoxification are minimal or can be handled through reallocation of energy fluxes among life sustaining activities. Additionally, averaged swimming speed measured during the OFT was about 6 cm/s. The metabolic cost of swimming at that velocity is 31mgO₂ kg⁻¹ h⁻¹, representing 0.83 % of the animal aerobic metabolic scope.

Time spent in the central zone

Over the 20 min of the OFT, time spent in the central zone (*TZoneC*) was used to the time spent in the most exposed and potentially risky area of the arena. Present study showed that the temporal pattern of *TZoneC* was significantly different among treatment groups. As illustrated on figure 4, *TZoneC* remain stable over time in control fish. Moreover, risk taking of control fish was characterized by a relatively small amount of time spent in the central zone (22.4 ± 2.07 s over the 20 min of the test). Fish from the E_L group did not differ from the control fish (21.8 ± 4.13 s). In contrast, a gradual decrease in *TZoneC* was observed in both E_M and E_H group. In addition, during the first week post-exposure, fish from E_M and E_H conditions spent respectively 10 % and 32 % more time in the risky zone of the arena than C individuals. Because of their orientation, all exiting from the confinement chambers ended up in the central zone of the arena. When exploring a novel environment, fish typically display avoidance for open areas, for fear of potential dangers (Maximino et al., 2010; Dahlbom et al., 2011). Control individuals perceived the risk of the situation and rapidly swam to a corner or to a side of the arena and then avoided this potentially risky zone (thigmotaxis). In contrast, the altered use of the central zone displayed by fish from the E_M and E_H conditions is in

agreement with the study of Rowsey et al (2019) where oil-exposed larvae of *S. ocellatus* also displayed an increase in time spent in the exposed area of the arena. However, in Rowsey's study this increase was associated to higher percentage of area explored and therefore interpreted as an increase in risk taking. In our study fish exploratory activity is reduced, meaning that the increase in *TZoneC* from the E_M and E_H groups was considered to unlikely indicate inclination for risk-taking. In contrast, present observations suggest that the higher occurrence in the central zone more likely results from neurosensorial dysfunction due to the narcosis induced by the oil compounds. Further, as evoked earlier, oil-exposed fish were found to swim in an anaesthetic-like sedative behaviour i.e., slowness, with head-up swimming posture (C. Aimon, pers. obs.). All reported impairments can be related to the exposure to narcotic compounds which are known to reduce consciousness and induce general slowness, sleepiness, disorientation and impaired coordination and swimming in fish (Gerlai et al., 2000; Barron et al., 2004; Vignet et al., 2014a). This reinforced our idea that fish presenting behavioural disruptions were more drifting across the experimental arena rather than displaying conscious and voluntary activities related to exploration.

Analyses of the second week post-exposure outlined a dose-response effect upon recovery capacities of fish behavioural performances. Individuals exposed to the medium dose displayed similar pattern of *TZoneC* than C fish the second week post-exposure. It could be hypothesised that the recovery of E_M is linked to tissues detoxification. Unfortunately, liver concentration in toxicants was only measured one day post-exposure and therefore we have no information about the time course of the detoxification process. In a future work, it would be interesting to investigate simultaneously behavioural recovery and tissues detoxification. In contrast, the second week post-exposure, fish from the E_H group showed decreasing *TZoneC* over the duration of the test and spent 10% less time in the risky zone of the arena than C individuals. This persistent alteration in the use of the central zone over two weeks post-exposure in individuals exposed to the E_H dose, suggests that under such harsh condition fish may need more time to recover. Even if the second week post-exposure, fish from the E_H treatment are less present in the central and potentially risky zone, they are still suffering from an altered behaviour as suggested by the highest variability in *TZoneC* and their decreasing pattern in *TZoneC* over the 20 min of the OFT, that other fish are not displaying. This longer

period of altered behaviour exhibited by fish from the E_H group reflect sub-optimal capacities that may lead to higher risk of death.

Exploration is an important aspect of animal behaviour, allowing the acquisition of information about the environment and the mapping of potential habitats. Knowledge outcome from exploratory behaviour can be used in the search of resources such as food or to avoid risky situations. In addition, information gathering has been linked to problem solving capacity, learning and behavioural innovation, all predictive of animal cognitive performance (Archer & Birke, 1983; Renner, 1990; Laland & Reader, 1999; Griffin & Guez, 2014; Reader, 2015; Jacquin et al., 2017). Reduced exploratory activity and increased time exposure in the open area of the novel environment suggest impairments of fish cognitive and mechanistic performances that can particularly be linked to foraging and predator avoidance. Observed alteration of exploratory behaviour following oil exposure could, therefore, affect fish environmental use and have consequences on their ecological performance. However, only the harshest condition altered juvenile sea bass exploratory tendency for more than two weeks post-exposure. In contrast, fish exposed to lower concentrations, more susceptible to occur after an oil spill, were not affected or recovered normal responses to the OFT within two weeks post-exposure.

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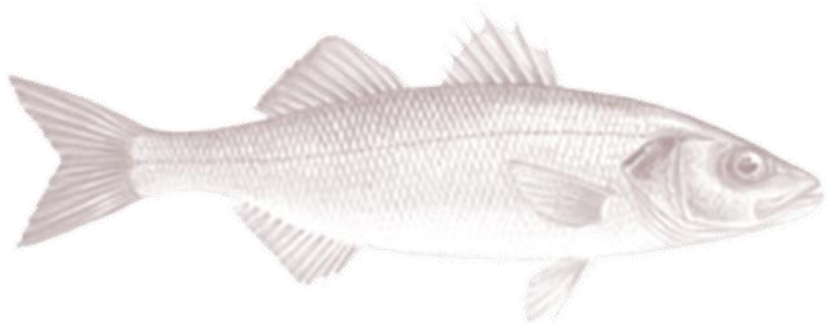
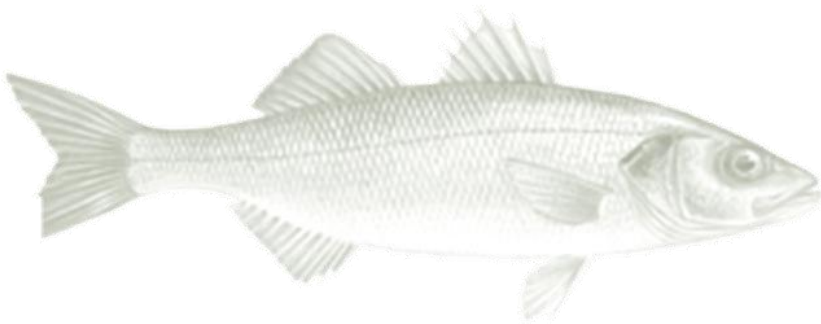
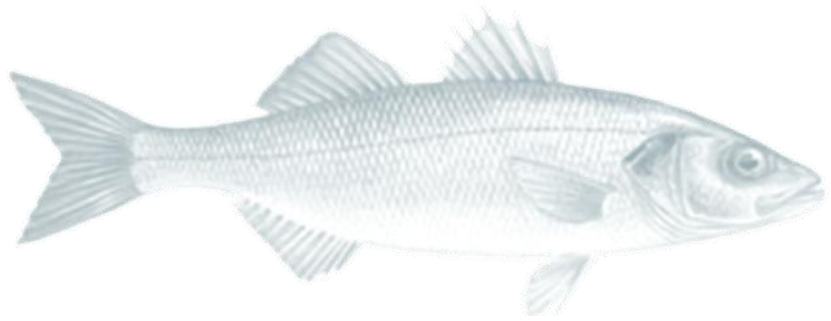
SUPPLEMENTARY DATA

Table S1

Ring number	PAHs	Detection limit (ng g ⁻¹ dw)	Quantification limit (ng g ⁻¹ dw)	Concentration ± sem (ng g ⁻¹ dw) at 1 day post-exposure			
				Control	EL	EM	EH
2	Naphthalene	90.1	270.3	59.0	1667.9	3781.5	29702.0
2	Benzothiophene	45.0	135.1	<LOD	15.4	39.0	403.7
2	Biphenyl	90.1	270.3	<LOD	771.2	1475.1	5609.7
3	Acenaphthylene	45.0	135.1	<LOD	<LOD	<LOD	<LOD
3	Acenaphthene	45.0	135.1	<LOD	357.9	606.1	1920.2
3	Fluorene	45.0	135.1	26.9	2004.6	3099.2	11251.4
3	Dibenzothiophene	45.0	135.1	20.2	11330.0	17745.4	58928.7
3	Phenanthrene	45.0	135.1	26.4	2268.7	3582.9	16608.2
3	Anthracene	4.5	13.5	<LOD	9.3	12.9	106.8
4	Fluoranthene	450.5	1351.4	<LOD	<LOD	<LOD	<LOD
4	Pyrene	9.0	27.0	<LOD	<LOD	<LOD	241.5
4	Benzo(a)anthracene	90.1	270.3	<LOD	<LOD	<LOD	<LOD
4	Chrysene	90.1	270.3	3.0	<LOD	<LOD	491.8
>4	Benzo(b+k)fluoranthene	450.5	1351.4	<LOD	<LOD	<LOD	<LOD
>4	Benzo(e)pyrene	450.5	1351.4	<LOD	<LOD	<LOD	<LOD
>4	Benzo(a)pyrene	450.5	1351.4	<LOD	<LOD	<LOD	<LOD
>4	Perylene	450.5	1351.4	<LOD	<LOD	<LOD	<LOD
>4	Indeno(1,2,3-cd)pyrene	450.5	1351.4	<LOD	<LOD	<LOD	<LOD
>4	Dibenzo(a,h)anthracene	450.5	1351.4	<LOD	<LOD	<LOD	<LOD
>4	Benzo(g,h,i)perylene	450.5	1351.4	<LOD	<LOD	<LOD	<LOD
	Mean of individual's [21 PAH]	4247.7	12743.2	135.5	18425.1	30342.2	125264.1



CHAPITRE 3



Les résultats du chapitre 2 indiquent qu'une exposition à des hydrocarbures pétroliers induit chez le bar Européen une réduction de l'activité exploratoire et une augmentation du temps de présence dans la zone ouverte (« à risque ») de l'environnement nouveau. Ces résultats suggèrent des altérations des performances cognitives des poissons. L'altération de ces performances peut avoir des répercussions négatives sur la recherche de nourriture et l'évitement des prédateurs. Aussi, un des objectifs de ce troisième chapitre est d'évaluer les possibles répercussions d'une exposition à des hydrocarbures pétroliers dispersés chimiquement sur la réponse anti-prédateur du bar Européen.

Par ailleurs, les résultats du chapitre 1 indiquent que le comportement de prise de risque et la sociabilité sont corrélés positivement en condition contrôle mais que cette relation pouvait être inversée par une perturbation environnementale telle qu'une pénurie de nourriture. Aussi, comme les résultats du chapitre 2 révèlent une augmentation du temps passé dans la zone à risque de l'environnement nouveau suite à une exposition aux hydrocarbures pétroliers, il m'a semblé pertinent d'évaluer si la sociabilité était également affectée par une exposition à ces mêmes polluants.

Dans cette nouvelle étude, seule la dose intermédiaire d'hydrocarbure, montrant des effets sur le comportement sans causer de mortalité (chapitre 2), a été utilisée.

Les expériences reportées dans ce troisième chapitre sont menées sur des groupes d'individus sur lesquels la mesure de la distance inter-individuelle est utilisée pour estimer la sociabilité des membres au sein du groupe. Cette mesure est en effet couramment utilisée pour apprécier le comportement grégaire chez le poisson (Colchen et al., 2017; Bierbach et al., 2018). En outre, le comportement grégaire est particulièrement relié à la réponse anti-prédateur. En effet, comme évoqué dans le chapitre 1, la vie en groupe permet notamment de réduire le risque de prédation (Pulliam & Caraco, 1984; Clark & Mangel, 1986; Godin, 1986; Pitcher & K. Parrish, 1993b; Krause et al., 2000; Krause & Ruxton, 2002). Aussi, les effets d'une exposition aux produits pétroliers ont été évalués sur ces deux réponses comportementales.

Cette étude vise à donner une autre dimension à l'analyse comportementale, en intégrant des conséquences plus larges que les effets à l'échelle individuelle. L'analyse de la cohésion du groupe permet d'évaluer les conséquences d'une exposition à des produits pétroliers sur les

relations intra-spécifiques. En outre, l'impact de l'exposition aux hydrocarbures pétroliers sur l'interaction proie-prédateur est également évalué pour interpréter les effets indirects de ce stress au niveau de la communauté. Par ailleurs, la réponse comportementale du groupe face à la simulation d'une attaque de prédateur permet d'estimer les répercussions au niveau de la fitness mais également des relations inter-spécifiques au sein du réseau trophique et considérer les effets observés au niveau de la communauté.

Effects of dispersant treated oil upon group cohesion and anti-predator response in juvenile European sea bass (*Dicentrarchus labrax*).

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ABSTRACT

The impact of exposure to chemically dispersed oil on ecologically and economically important species of fish is of strong interest. Among sublethal impairments that have been investigated, the effects upon integrated indicators of whole-animal disturbances, such as behaviours, have been understudied. Another knowledge gap remains in the question upon the possible recovery capacities of fish following their exposure. Predation is the primary cause of mortality of juvenile sea bass. The modulation of their locomotor activity and their shoaling behaviour is a flexible antipredatory strategy response.

INTRODUCTION

Over the last 50 years, despite the doubling of sea borne oil trade, the number of oil spills has being reduced by 92% (International Tanker Owners Pollution Federation, 2017). Although less likely to occur, oil transport still resulted in 65 medium (>7 tonnes) and 20 large (>700 tonnes) marine spills over the last decade, releasing a total of 67 000 tonnes of crude oil into the environment (International Tanker Owners Pollution Federation, 2017).

The use of chemical dispersants is one of the most deployed techniques in response to an oil spill. This response aims at breaking up slicks into small droplets to enhance its natural dispersion and dilution at sea (Dispersants: surface application, 2015). A major drawback of this technique is, however, that it increases the bio-availability of oil compounds and especially PAH (Blaxter & Hallers-Tjabbes, 1992; Ramachandran et al., 2004; Milinkovitch et al., 2011; Adams et al., 2014).

Coastal ecosystems are particularly exposed to oil spills and the potential for acute and lasting impacts of oil exposure is therefore of strong interest to environmental managers and decision makers. Coastal areas have important ecological functions for marine biota as a place of nurseries, settlement and habitats for a large variety of species. Many fish species of economic value live in coastal habitats during at least the first years of their life cycle. Oil spills are therefore recognized as posing a significant threat to marine resources and associated services.

Studies have shown a wide diversity of consequences for oil exposed animals, from elevated mortality to stunted growth rates and a variety of sublethal effects. In the last decades, studies have revealed histological and morphological alterations, as well as changes in biochemical pathways such as, for instance, antioxidant enzyme activity (Solangi & Overstreet, 1982; Correia et al., 2007; Yuen et al., 2007), developmental disruptions (Hawkes & Stehr, 1982; Carls et al., 1999; Irie et al., 2011; Kawaguchi et al., 2012; Le Bihanic et al., 2014; Perrichon et al., 2016; Alves et al., 2017; Xu et al., 2017; Magnuson et al., 2018; Philibert et al., 2019), cardiac defects (Claireaux & Davoodi, 2010; Incardona et al., 2014b; Esbaugh et al., 2016; Brette et al., 2017; Khursigara et al., 2017; Nelson et al., 2017), as well as alterations in physiological performance traits such as hypoxia and thermal tolerance, swimming capacity, maximum metabolic rate and aerobic metabolic scope (Kennedy & Farrell, 2006; Davoodi & Claireaux, 2007; Mager et al., 2014; Mauduit et al., 2016; Stieglitz et al., 2016; Johansen & Esbaugh, 2017; Zhang et al., 2017; Pan et al., 2018). Studies of fish behaviour also revealed reductions in fish general activity, exploratory tendency, risk taking, prey-capture ability and social dominance (Aimon et al., in press; Woodward et al., 1987; Correia et al., 2007; Gonçalves et al., 2008; Vignet et al., 2014a; Jacquin et al., 2017; Johansen et al., 2017; Khursigara et al., 2018; Rowsey et al., 2019).

Sociability is commonly defined as one animal's response to conspecifics, excluding aggressive attitudes (Conrad et al., 2011). One form of sociability is animals' tendency to form groups. Many species of fish exhibit collective behaviour, especially at young stages with fifty percent forming schools or shoals during larval and juvenile periods (Radakov & Williams, 1974; Pavlov & Kasumyan, 2000). It is important, however, to distinguish two aspects of grouping behaviour, shoaling and schooling. While a shoal is a social group formed

by individuals that choose to join and stay with their fellows, with no implication for structure or function, schools are synchronized and polarized swimming groups staying together. Schooling is therefore a specific case of shoaling behaviour (Pitcher & K. Parrish, 1993a). Shoaling is a voluntary strategy that fish display in certain circumstances and that can be of particular importance for their survival. This flexible strategy allows individuals to draw on a full range of trade-offs to maximize and lower predation risk by being isolated or by sticking to the group (Pulliam & Caraco, 1984; Clark & Mangel, 1986; Godin, 1986; Pitcher & K. Parrish, 1993b; Krause et al., 2000; Krause & Ruxton, 2002). In the presence of a threatening stimulus, fish tend to minimize movements and to shoal as shoaling provides fish with additive vigilance, as well as numerical dilution and confusion effect for predator (Pulliam & Caraco, 1984; Clark & Mangel, 1986; Godin, 1986; Pitcher & K. Parrish, 1993b; Krause et al., 2000; Krause & Ruxton, 2002). When the source of threat diminishes fish recover higher level of swimming activity and the shoal loses cohesion (Millot et al., 2009a).

Shoaling behaviour therefore heavily relies on fish ability to perceive external stimuli and to integrate this information via the central nervous system which will then activate specific physiological sequences, resulting in the most appropriate response (Scott & Sloman, 2004; Weis, 2014). There remains however a crucial knowledge gap with respect to the potential effects of environmental factors, including toxicants, on this sequence and subsequent responsiveness and capacity for behavioural adjustments. Regarding indicators of fish physiological state, that might be involved in the internal sequences leading to the behavioural response, studies reported reduced aerobic scope, altered hypoxia tolerance and swimming performance in fish exposed to oil compounds (Kennedy & Farrell, 2006; Davoodi & Claireaux, 2007; Mager et al., 2014; Mauduit et al., 2016; Stieglitz et al., 2016; Johansen & Esbaugh, 2017; Zhang et al., 2017). To our knowledge, only two studies investigated the effects of oil exposure on fish response to a threat (Johansen et al., 2017; Milinkovitch et al., 2019). These authors reported that fish responsiveness was unaffected, however, the displayed responsiveness was affected, with altered speed, directionality and manoeuvrability of the escape response. Alteration of spontaneous exploration, ability to capture prey and hierarchic rank have also been reported following oil exposure (Aimon et al., in press; Woodward et al., 1987; Gonçalves et al., 2008; Vignet et al., 2014b; Brown et al., 2016; Khursigara et al., 2018). To our knowledge only one study investigated the effect upon the shoaling cohesion.

This study using the Trinidadian guppies found no effect of oil exposure (Jacquin et al., 2017).

Very few studies have investigated fish recovery capacities following an oil-exposure. Recovery of sub-organismic indices (transcriptomic changes, cell proliferation, hyperplasia of the intestine epithelial mucosa) have been reported to occur between 1 and 4 weeks post-exposure (Yuen et al., 2007; Hook et al., 2018). At higher organisational level, two kinds of results can be found in the litterature. At embryo stage on the one hand, studies did not report any recovery of reduced critical swimming speed and locomotor activity (Mager et al., 2014; Vignet et al., 2014b). At juvenile stage on the other hand, full recovery of hypoxia tolerance, swimming performance and exploratory activity was observed within 2 weeks to 10 months post-exposure (Aimon et al., in press; Mauduit et al., 2016).

To examine the potential effects of oil exposure and recovery dynamics on fish behavioural repertoire, we investigated their responsiveness and capacity to display appropriate behavioural adjustments in response to threatening stimulus, during the two weeks that followed a 62h exposure to a mixture of 0.4 g L⁻¹ of oil with 0.005 g L⁻¹ of dispersant. Using, juvenile sea bass, two sets of experiments were conducted. The first set consisted, using respirometry, in evaluating fish responsiveness with measure of metabolic rate. The second set of experiment consisted in assessing behavioural adjustment of a free-ranging group in an experimental arena. We hypothesized that exposure to dispersant-treated oil ; (1) reduces resting metabolic rate, (2) decreases fish metabolic responsiveness to a stimulus (light), (3) reduces group cohesion and activity, (4) reverses the expected shoal behavioural adjustment to a simulated aerial attack.

MATERIALS AND METHODS

Animals

Juvenile European sea bass *Dicentrarchus labrax* (Linneaus 1758) ($N=352$, age 1⁺; mass=57.65±1.11 g, mean±s.e.m) were obtained from a fish farm (Les poissons du Soleil, Balaruc les bains, France) and maintained in a 500 L indoor tank supplied with open-flow, thermoregulated (15°C) and fully aerated sea water (salinity 32 ppt). Artificial lighting reproduced seasonal variation in local photoperiod. Fish were fed 3 times a week *ad libitum* using commercial feed (Neo Start Coul 2, Le Gouessant, France). Experiments were non-

invasive and were approved by the french ethics committee in charge of animal experimentation n°74 (permit number: APAFIS#13738-20 8022216252268 v4).

Fish transport

Exposures to dispersant-treated oil were conducted at the Centre de documentation, de recherche et d'expérimentation sur les pollutions accidentelles des eaux (Cedre, Brest, France) approximately 12km from Ifremer laboratory. Fish were transported to and from Cedre in airtight plastic containers (50 L) filled with water (40 L) containing a light dose of anaesthetic (MS-222; 20 mg L⁻¹). The volume above the water surface was filled with O₂ gaz. Upon arrival at Cedre, fish were placed in a polyethylene tanks (300 L) in which water temperature, salinity and photoperiod were similar to those in their original rearing tank.

Experimental exposure

Six hours following their arrival at Cedre, fish were randomly assigned to either control (C) or exposed (E) conditions. Fish from the control treatment were maintained in clean water during the exposition phase while fish allocated to the dispersant-treated oil treatment were exposed during 62h to 0.4 g L⁻¹ of weathered crude Arabian light (CAL) added with 0.005 g L⁻¹ of chemical dispersant (Finasol © OSR 52, Total Fluides, Paris France). Each exposition trial involved 14 fish per treatment condition. For experimental constraints, four exposition trial were successively spread over 3 months.

The mixture of CAL and dispersant was made in a glass bottle following the manufacturer's recommendation (dispersant/oil ratio of 4%), and was poured and weathered in the exposition tank. The weathering process consisted in bubbling air during 5 hours to mimic ageing of an oil slick at sea (Nordvik, 1995). At that time, fish were introduced in the tanks. Both, the control and the exposition tanks were equipped with a custom-made device that comprised a funnel placed on the water surface and was connected to a 12V submersible bilge pump (L450-500GPH; Johnson) placed at the bottom of the tank. In the exposition tank, this device enabled us to mimic the mechanical dispersion of oil by waves at sea, while maintaining the exposure condition homogenous throughout the tank. Surface water and floating oil were sucked into the funnel, homogenized and delivered to the bottom of the tank (Milinkovitch et al., 2011). Tanks were continuously bubbled with air to maintain the oxygenation above 90% air saturation during the 62h exposure period.

Following the exposure period, fish were bathed in clean sea water (1h) before their transfer back to Ifremer facilities (transportation procedure similar to the one described above).

Respirometry

Respirometry trials spread over 7-day periods, with 4 fish being tested simultaneously (2 fish from the C and 2 fish from the E group). To this end, four intermittent-flow respirometers (2 L) were submerged in a thermoregulated (15.0 ± 0.5 °C; Teco, Seachill TR20) and aerated (>90% air saturation) water tank (200 cm × 60 cm × 40 cm). Flush pumps (Compact 600, EHEIM, Germany) were used to create water recirculation to each respirometry chamber. These pumps were computer-controlled using AquaResp software (University of Copenhagen, Helsingør, Denmark). Each respirometer had its own circulation loop to which an optical oxygen probe was connected (Robust Oxygen Probe OXROB3, Pyroscience, Germany or Dipping probe oxygen minisensor, PreSens, Germany). This probe was used to continuously measure the dissolved oxygen (DO) concentration inside the chamber. Oxygen probes were calibrated twice i.e. prior to place the fish in the respirometers and then at day 4 of the trials.

Fish were introduced in the respirometers 5 hours upon their return from Cedre (7h post-exposure). Oxygen consumption measurements cycles consisted in two periods. The first period lasted 7min and corresponded to the flushing of the chamber with fully aerated water from the surrounding tank. During the second period (13min), the flushing pumps were turned off and the decrease in DO due to fish oxygen consumption was followed. The first minute of this sealed period was not taken into account to calculate fish MO_2 , as it corresponded to the time needed to obtain reliable steady state between the decrease in water DO and fish MO_2 . Water DO was always kept >85% sat.

Fish oxygen consumption was monitored over 7 days. At days 1, 4, 5, 6 and 7 of each trial, a light stimulus was applied at 8:30am, which consisted in turning on the room light for a few second while the rest of the days these lights were turned off to a relative darkness in the room.

Shoaling behaviour

To reveal the kinetic of post-exposure recovery, behavioural tests were conducted on days 1, 4, 6 and 8 post-exposure. On each of these days ten novel naïve groups were tested.

The experimental arena consisted of a shallow rectangular tank ($156 \times 99 \times 14$ cm, length, width, depth, respectively). Water characteristics in the arena were the same as those of the rearing tanks. However, the shallow water depth prevented fish vertical motion in the water column. A slanting rope ran diagonally over the experimental arena, to simulate the glide path of a predator bird. The experimental arena was screened from visual disturbance with a curtain placed around and over it and it was homogenously lit with neon lamps placed on each side. A retro-reflective adhesive foil (Loligosystem, Inc) was placed on the bottom of the testing tank to enhance the contrast between the fish and the arena. A video camera (Logitech webcam C930e, 15 frames s^{-1}) situated 1m above the water surface was used to record fish movement. The arena was emptied and refilled between each test.

For each trial, 4 fish were randomly selected in the rearing tank and transferred without emersion into the testing arena. Fish were then left undisturbed during 1h to allow them to familiarize with this environment. The last minute of this acclimation period was used as a control to evaluate the shoaling cohesion and swimming activity of the tested group. One hour after fish introduction into the arena, an aerial predator attack was simulated. For the simulation, a life-sized polystyrene model of a generalized bird ($73 \text{ cm} \times 30 \text{ cm}$) was released and run over the experimental arena. Over its path, this visual stimulus gets closer to the water surface and it was no visible to the fish before being released.

Chemical analyses

Exposure conditions were characterized by measuring total petroleum hydrocarbon concentration ([TPH]) in triplicate in each exposure tank. Seawater samples were taken immediately before fish introduction into the tank and after 4, 24 and 48h. These samples were extracted three times with 10 mL of dichloromethane Pestipur quality (SDS, Carlo Erba Reagent, France) before being dried by filtering through anhydrous sodium sulfate. The combined extracts were then analysed using a spectrophotometer (Evolution 600 UV-VIS; Thermo Fisher Scientific) at 390 nm, as described by Fusey and Oudot (1976).

To document fish contamination and the detoxification process, liver concentration in 20 polycyclic aromatic hydrocarbons (PAH) were measured at days 0, 1, 4 and 7 post-exposure. To measure the level of contamination, two fish per treatment were euthanized at the end of the exposure phase, at day 0, after behavioural trials at days 1 and 4 post-exposure and at the end of the respirometry experiment, day 7 post-exposure. Liver PAH concentration (including the components listed by US-EPA) were assessed by GC–MS as described in Lacroix et al. (2014). Briefly, liver PAH were extracted using alkaline digestion combined with stir bar sorptive extraction and thermal-desorption–gas chromatography mass spectrometry (SBSE–TD–GC–MS). Analytical method was validated by determining quantification limit of each PAH. This measure estimated the lowest concentration of PAH in a liver sample that can be measured with acceptable precision and accuracy under the stated conditions of the test.

Data analysis and statistics

Respirometry

$\dot{M}O_2$ was determined for each measurement cycle by calculating the slope of declining DO in the respirometry chamber using a linear regression. MO_2 values were corrected for background bacterial MO_2 (typically <5% of fish MO_2). Night and day metabolic rates were determined using a quantile method ($q = 0.2$) by applying an R script (Chabot et al., 2016) to the continuous $\dot{M}O_2$ measurements obtained during nights (11pm to 5am) or days (7am to 1pm). The reaction to the light stimulus was evaluated by noting if the fish showed an increased in MO_2 after light stimulation. For fish that showed an increase in MO_2 in response to light stimulus, the ratio between the peak of MO_2 and the MR (measured between 7am and 1pm) was calculated to estimate the intensity of this reaction. Furthermore, recovery capacity was also assessed by noting the time to return to previous MR after the MO_2 peak occurred. At the end of the last trial, day 7, fish were removed from the chamber and background MO_2 was measured (30min). The entire system was then disinfected using household bleach.

The effects of treatment and day's post-exposure on metabolic rate and behavioural measurements were again examined using linear mixed effects models. Generalized linear mixed-effects model was used to test for the effects of treatment, day post-exposure and their interaction on the presence of a response to the lightening stimulus, with fish mentioned as random effect. A stepwise backward reduction of the full models was applied to exclude non-significant effects and identify the most parsimonious model.

Shoaling behaviours

Principal component analyses (PCA) were used to combine the indices of activity (*Tswim*, *Dmoved* and *Velocity*) into principal components (PCs). PCA's were applied to two datasets: (i) over the behavioural data recorded during the minute before the frightening stimulus (t-1; individuals' baseline behavioural characteristics); (ii) over the behavioural data collected during the entire experiment (from t-1 to t+20). For the measurements conducted over the 20 minutes following the stimulus, measures were made in 1-min increments, during the first five minutes following the stimulus (t+1,t+2,t+3,t+4,t+5) and at 10 (t+10) and 20 (t+10) minutes post-stimulus. We used Kaiser's criterion to select the number of PCs (Kaiser, 1961). Linear mixed effect models were used to evaluate the main effects of treatment and day post-exposure on fish activity and inter-individual distance within the group. Fish were introduced as a random effect. Again a stepwise backward reduction of the full models was applied to exclude non-significant effects.

In the exposed group, student tests were carried out to determine whether water concentration in total petroleum hydrocarbons was different from zero and liver concentration in 20 polycyclic aromatic hydrocarbon compounds was above the quantification limit.

Group cohesion/disintegration was evaluated by the measure of the mean inter-individual distance within the group. The swimming activity was assessed through the analysis of the principal component combining the total time spent swimming (labelled *Tswim*), the total distance moved (labelled *Dmoved*) and the swimming speed (labelled *Velocity*).

Fish response to the model predator was measured using the parameters of inter-individual distance and swimming activity described previously. The intensity of this response and the time to return to previous level of shoaling and activity were monitored by measuring differences between these variables one minute before the stimulus (t-1) and during the first five minutes following the stimulus and at 10 and 20 minutes post-stimulus (at t+1, t+2, t+3, t+4, t+5, t+10 and t+20). Negative values therefore indicate a reduction in activity or inter-individual distance compared to the behavioural level expressed at t-1. The video tracking software Lolitrack Version 4.2.0 (Loligosystem, Inc) was used to analyze the videos and to

calculate the following behavioural parameters : Activity (*Tswim*), Distance moved, Velocity and inter-individual distance between each individual and its neighbours.

All statistical analyses were conducted on R version 3.5.1 (R Core Team, 2018). The principal component analyses were carried out using FactoMineR package, ANOVA analyses were carried out using the 'stat' package and mixed models were implemented using the 'nlme' package (Le & Husson; R Core Team, 2013; Pinheiro et al., 2019). Model diagnostics were evaluated by visually inspecting the residuals. Statistical significance was assigned at $P < 0.05$.

RESULTS

Exposure condition and bioaccumulation of contaminants

Water concentration in total petroleum hydrocarbons ([TPH]) in the control treatment (C) was not significantly different from 0, whereas in the exposed tank (E) an average [TPH] of $0.096 \pm 0.011 \text{ g L}^{-1}$ was found over the exposure period.

As expected, mean liver concentration in 20 polycyclic aromatic hydrocarbons ([PAH]) compounds measured in fish from the C condition were below the quantification limit and were therefore not taken into account. In oil exposed fish, on the other hand, elevated concentrations were observed at day 0 and day 1 followed by a recovery at day 4 and 7 when mean liver PAH concentrations dropped below the quantification limit (QL; respectively : $t_7 = -1.62$, $P = 0.15$; : $t_7 = -0.10$, $P = 0.92$; Fig.1).

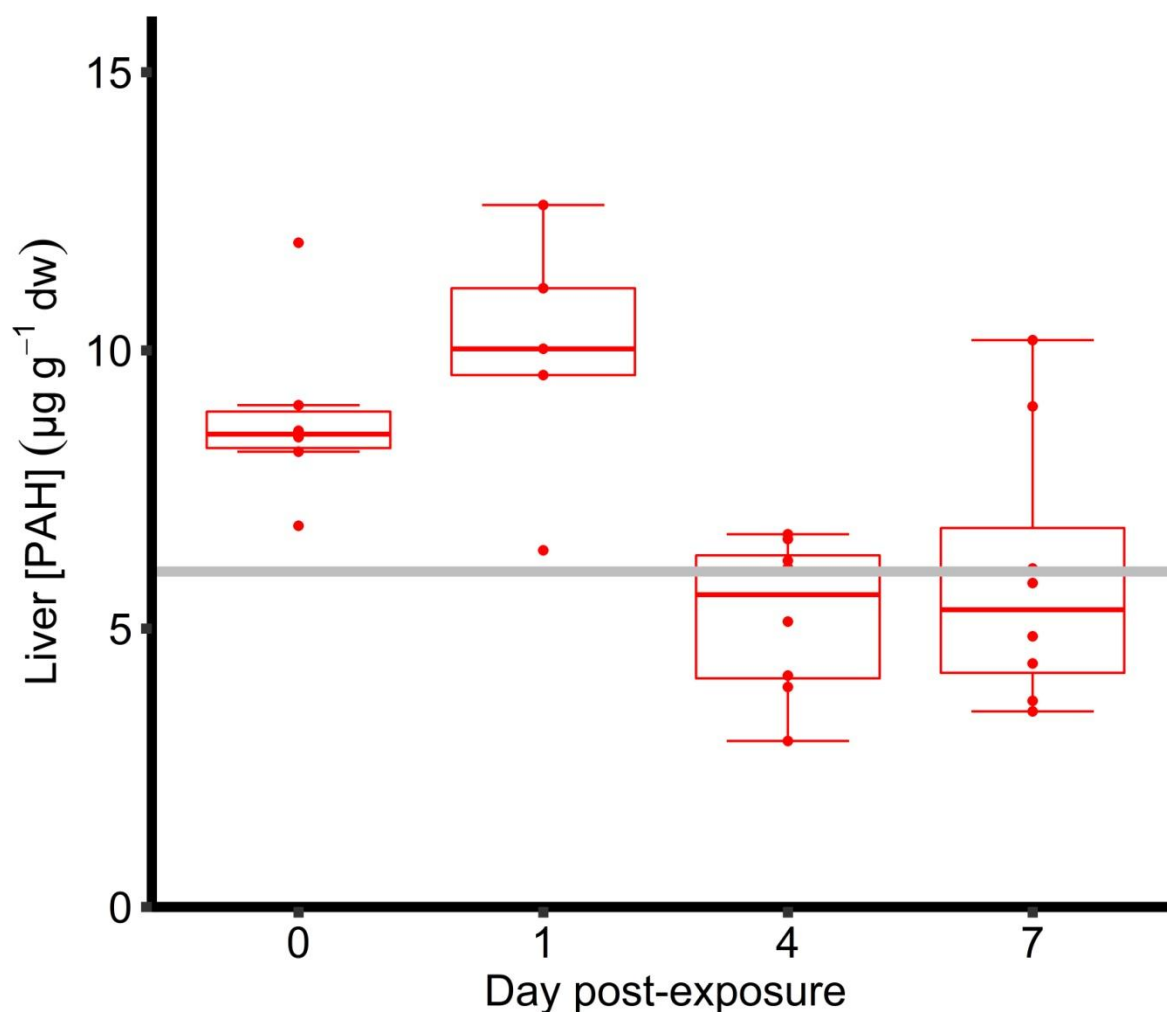


Fig. 1. Liver concentration in 20 PAH ($\mu\text{g g}^{-1}$ dry weight) measured in fish exposed treatment to chemically dispersed oil. Sampling was performed directly at the end of the exposure phase: day 0 ($N=8$); one day later ($N=7$), four days post-exposure ($N=8$) and 7 days post-exposure at the end of the respirometry experiment ($N=8$). The grey solid line indicates the quantification limit (QL) that is the lowest concentration of PAH in a liver sample that is measured with acceptable precision and accuracy under the stated conditions of the test. Below QL, PAH concentration can be considered as zero.

Respirometry

Linear mixed effect model revealed that the variables "Treatment" (treatment group) and "Day" (time post-exposure) interacted to decrease fish MO_2 ($F_{1,94}=13.162$, $P<0.001$; Table S1). Figure 2 illustrates the between-treatment difference in the time course of MO_2 over the week that fish spent in the respirometers. Over that period, MO_2 in C individuals decreased over time and reached SMR within 3-4 days. In E fish on the other hand, MO_2 remained above SMR over the 7 days of experiment.

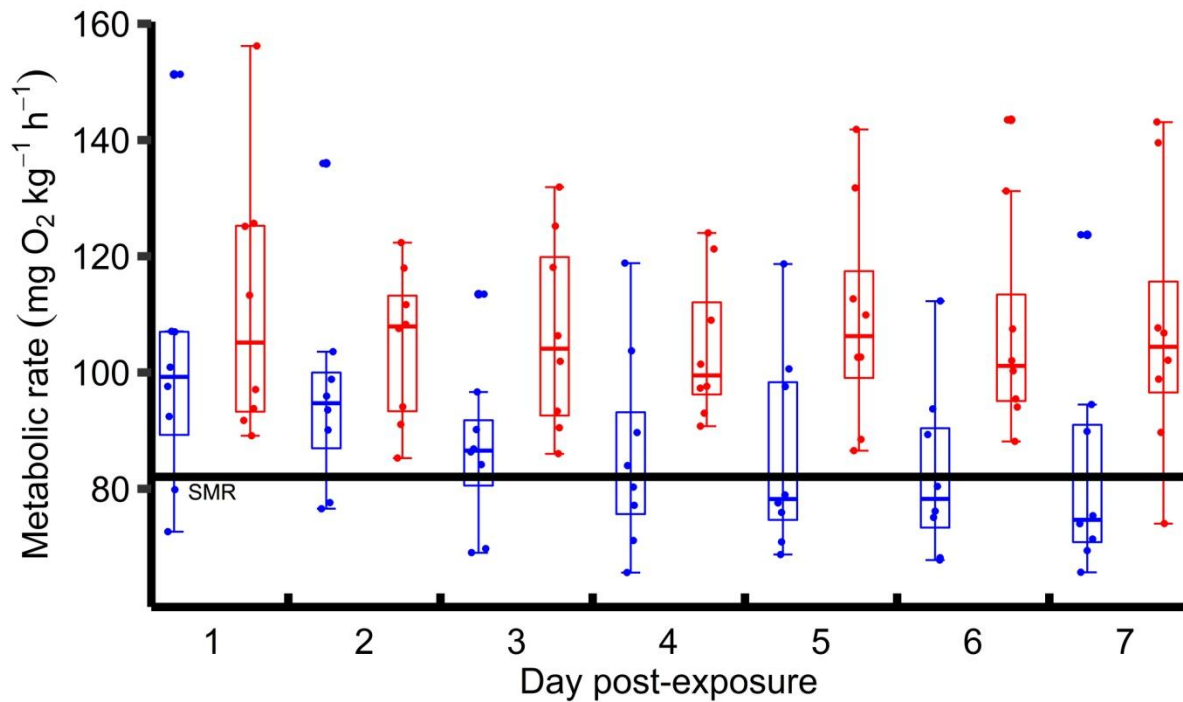


Fig. 2. Evolution of the relationship between night metabolic rate and treatment condition over the 7 days post-exposure. Blue: control fish (N=8); red: exposed fish (N=8); black solid line : SMR.

Generalized linear mixed model revealed that there was no significant difference among treatment groups ($Z = 1.135$, $P = 0.257$) or days post-exposure ($Z = -1.374$, $P = 0.169$) with regards to the number of fish that showed a respiratory response to the light stimulus (Table S2, Fig. 3). Among those individuals that responded (showing a peak; Fig. 4) we evaluated the intensity of the response by calculating the ratio between the peak of MO_2 and the pre-stimulation MR. C fish showed a more intense response to the light stimulus with a bigger ratio between their MO_2 peak and pre-stimulation MR than E fish, especially the first day of the week (Fig. 5). While, linear mixed effect model analysis showed that C fish displayed a reduction in the height of the post-stimulation peak in MO_2 over the 7 days post-exposure, no such change was observed in the E fish ($F_{1,32} = 5.274$, $P = 0.028$; Fig. 5, Table S3). At day 1 in the respirometry experiment, C fish responded to the lightening stimulus with a peak of MO_2 2.3 times higher than their pre-stimulation MR. In contrast, day 7 C fish showed a lower increase in MO_2 with a peak corresponding to 1.6 times the pre-stimulus level (Fig. 5). It has to be noticed, however, that on the 7th day post-exposure, the value of this ratio was similar in E and C fish (Fig. 5). Furthermore, C individuals presented a reduction in intra-group variability over the week spent in the respirometer (Fig. 5).

Concerning the time taken to return to a MO_2 similar to the pre-stimulation MR, it depended on the treatment condition and the day post-exposure ($F_{1,20}=6.641$, $P=0.018$, Table S4). Over the experimental week, both treatment showed a reduction in the time taken to return to the pre-stimulation MR after the peak (Fig.6).

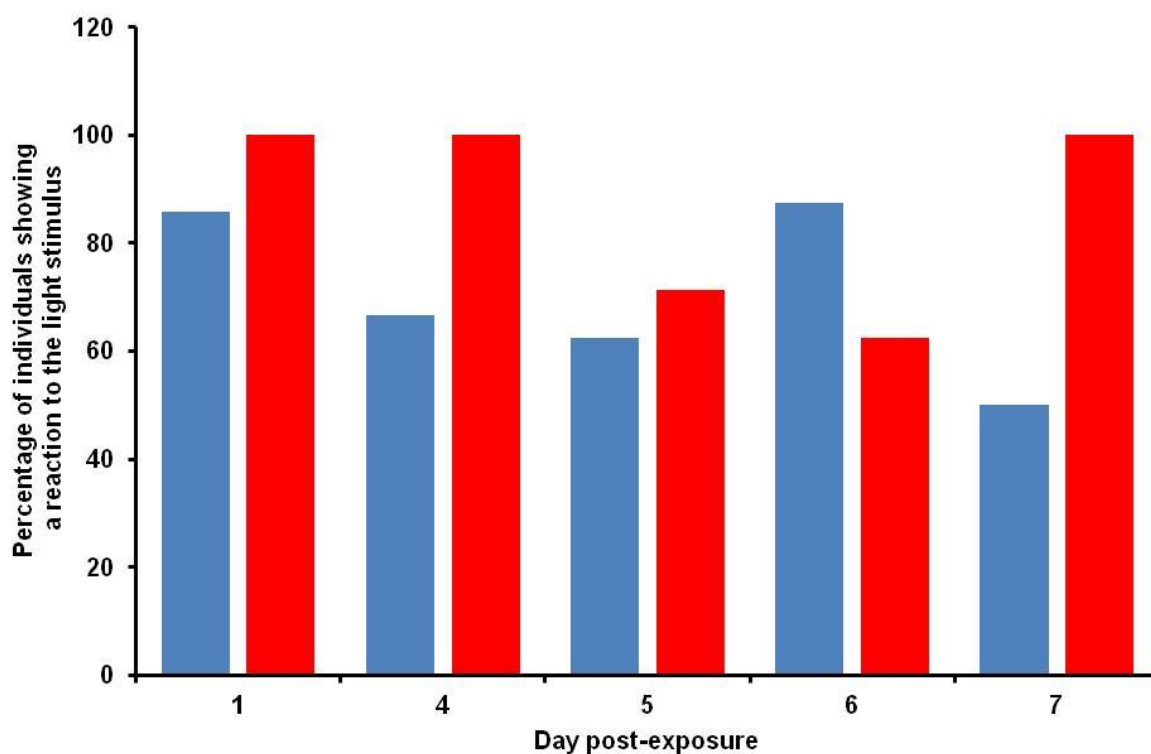


Fig. 3. Percentage of individuals displaying an increased metabolic rate following the lightening stimulus over the 7 days post-exposure. Blue: control fish; red: exposed fish.

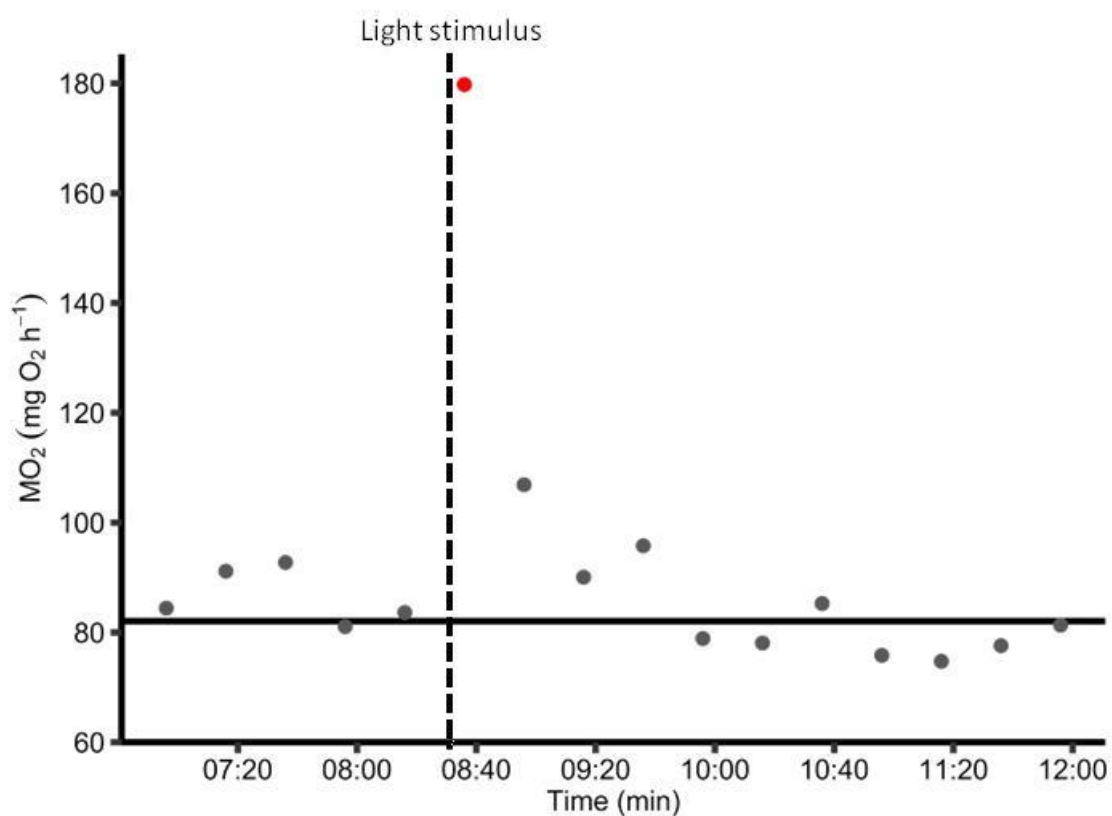


Fig. 4. MO_2 measured in one control fish over a morning and showing a reaction to the light stimulus occurring at 8:30am. Red: peak of MO_2 measured following the light stimulus; black line: SMR.

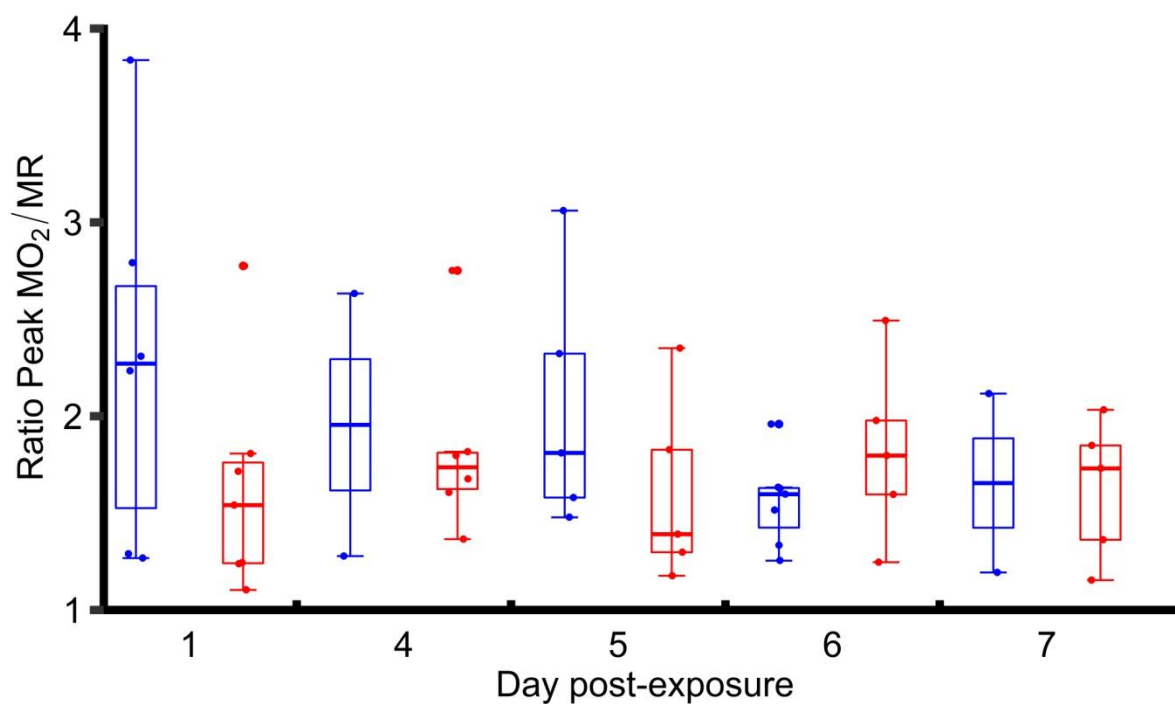


Fig. 5. Relationship between the treatment condition and the ratio (peak of MO_2 /MR) over the 7 days post-exposure. Only individuals showing a response to the light stimulus are represented. Effectives are therefore depending on the number of individuals that displayed an increase MO_2 following the light stimulus. Blue: control fish; red: exposed fish.

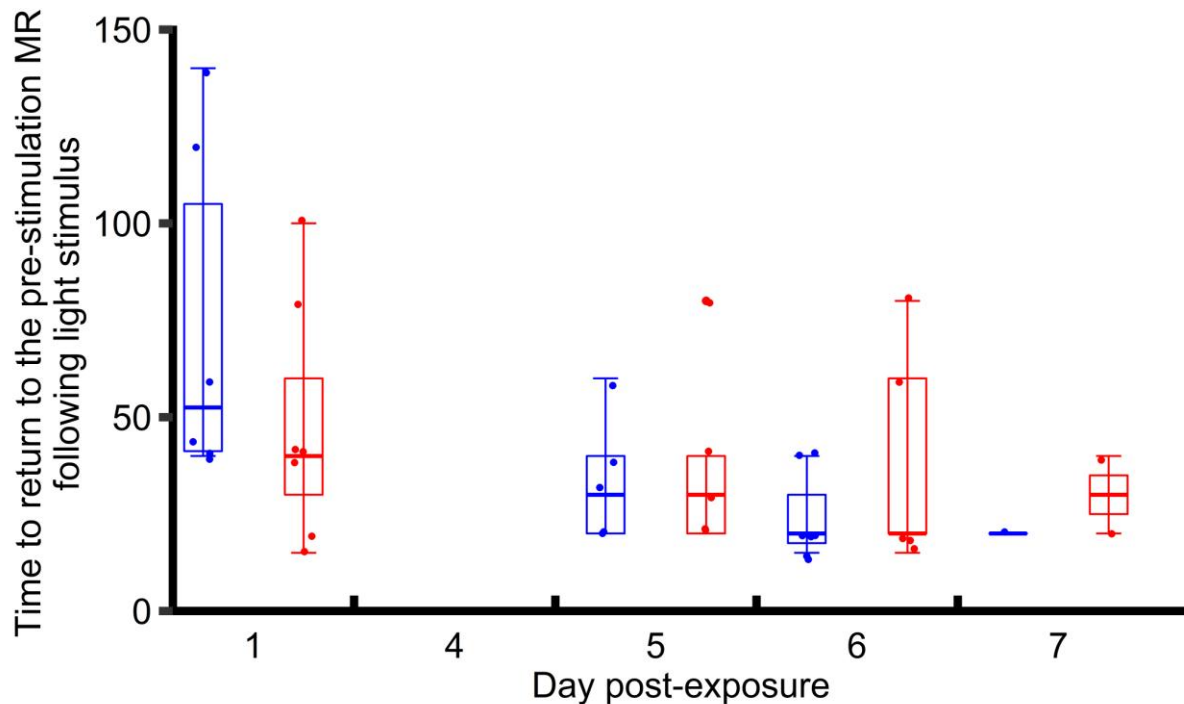


Fig. 6. Relationship between the treatment condition and the time to return to the pre-stimulation MR following the peak of MO_2 in response to the lightening stimulus over the 7 days post-exposure. Only individuals showing a response to the light stimulus are represented. Effectives are therefore depending on the number of individuals that displayed an increase in MO_2 following the light stimulus. Blue: control fish; red: exposed fish.

Shoaling behaviour

Reduction and structuration of behavioural variables

Four variables were recorded during behavioural tests. Three of these variables allowed evaluating fish swimming activity (total time spent swimming, labelled *Tswim*; distance moved, labelled *Dmoved*; swimming speed, labelled *Velocity*) and one measured group cohesion (mean inter-individual distance within the group, labelled *Inter-individual distance*). The principal component analysis (PCA) loaded with the variables *Tswim*, *Dmoved* and *Velocity* showed that only one principal component had an eigenvalue greater than 1 (Table 1). This PC termed Activity explained 82% of the total variance in behaviour measured in the minute before the stimulus, and 79% of the whole dataset (Table 1).

Table 1. Description of the principal components (PC) analyses.

	Variables	Minute	All data
		Pre-stimulus	1 min pre-stimulus + 20 minutes post-stimulus
		PC1	PC1
		Activity	Activity
Eigenvalue		2.449	2.382
Percentage of variance		81.643	79.394
Loading	<i>Tswim</i>	0.785	0.776
	<i>Dmoved</i>	0.982	0.985
	<i>Velocity</i>	0.932	0.899

Effect of treatment

Pre-stimulus shoaling behaviour

Before being exposed to the model predator (t-1) fish from the C treatment displayed significantly higher activity level than those of the E treatment ($F_{1,78}=7.622$, $P=0.007$; Table S1; Fig.7). There was, however, no difference between days post-exposure ($F_{3,75}=0.860$, $P=0.466$; Fig. S5) suggesting no recovery tendency over the 8 days of the experiment. There was no statistically significant difference in the inter-individual distance between treatment groups ($F_{1,75}=0.002$, $P=0.961$; Fig.8, Table S6). Consistent with fish activity, there was no detectable effect of the days post-exposure on inter-individual distances within groups ($F_{3,76}=0.849$, $P=0.472$; Fig. S6).

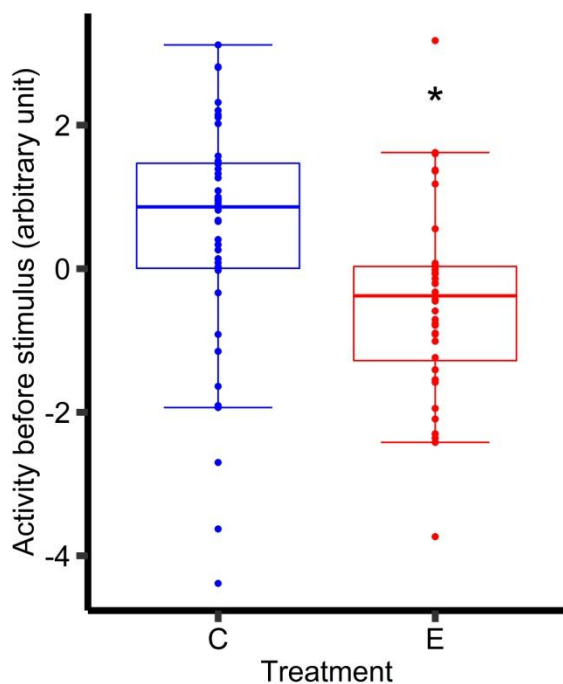


Fig. 7. Effect of Activity and treatment condition before stimulus (t-1). Blue: scores of control fish (N=40); red: scores of exposed fish (N=40).

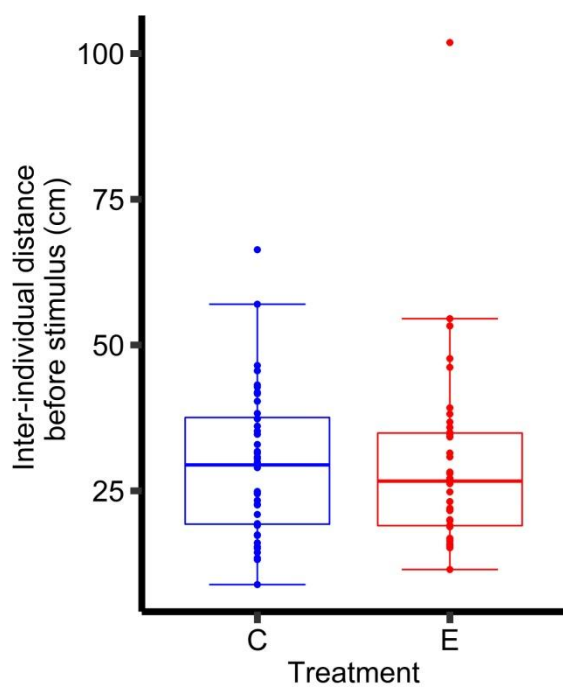


Fig. 8. Effect of treatment on mean inter-individual distance within the group before stimulus (t-1). Blue: control fish (N=40); red: exposed fish (N=40).

Response to the model predator

We found that there were significant changes in fish activity over the 20 minutes following the stimulus and that these changes depended on the treatment (Linear mixed model:

$F_{1,470}=34.305$, $P<0.001$; Fig. 9 and Table S7). Fish from the exposed treatment, which presented already lower activity level than C fish at t-1, showed a lower reduction in activity following the stimulus. Nevertheless, E fish had also an increased activity over the 20 minutes post-stimulus, leading them to draw closer to their level of activity recorded at t-1. Compared to E individuals, fish of the C group had a greater increase in their activity levels over the 20 minutes following the frightening stimulus. In addition, for both treatment groups, fish go back to their initial activity levels between 5 to 10 minutes after the stimulus. Consistent with our analyses at t-1, at the end of the 20 minutes C fish displayed higher activity level than E individuals. Furthermore, no effect of the day post-exposure upon fish activity was observed ($F_{3,74}=1.584$, $P=0.200$; Table S7), suggesting no recovery over the week post-exposure.

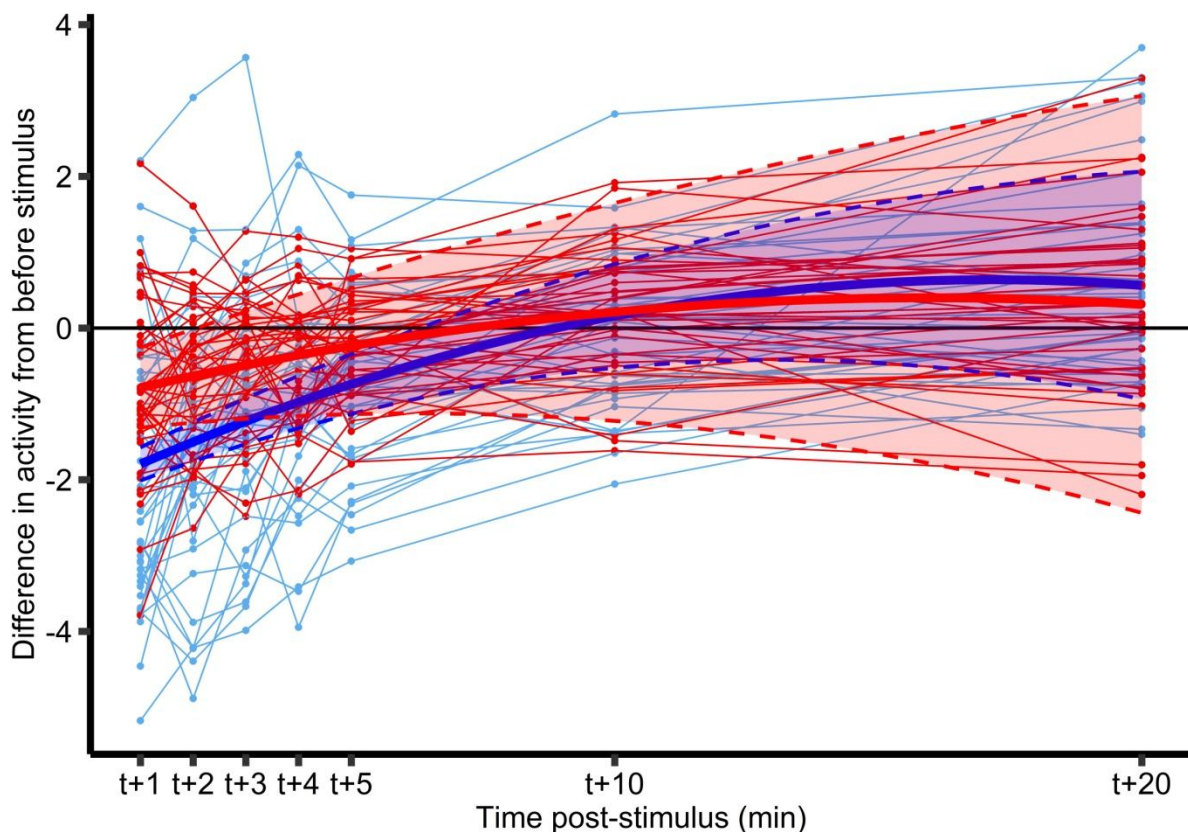


Fig. 9. Evolution of the relationship between Activity and treatment condition over the 20 minutes following the stimulus that occurred at t+0. Blue: control fish (N=40); red: exposed fish (N=40), black horizontal line: represent the relative activity level before stimulus at t-1.

Inter-individual distances changed significantly over the 20 minutes following the stimulus (Linear mixed model: $F_{1,470}=34.669$, $P<0.001$; slope 'Time'=0.401 ; Fig. 10 and Table S8). There was no effect of the treatment or the day post-exposure upon fish inter-individual

distance (Treatment: $F_{1,74}=1.551$, $P=0.217$; Day: $F_{3,75}=1.831$, $P=0.149$; Table S8). For both treatments, there was a rapid decrease in the inter-individual distance within groups in the first minute following the stimulus (negative values in Fig. 10). Then, over the next 20 minutes post-stimulus inter-individual distance progressively returned to pre-stimulation level; recovery being made at *ca.* 10 minutes post-stimulus.

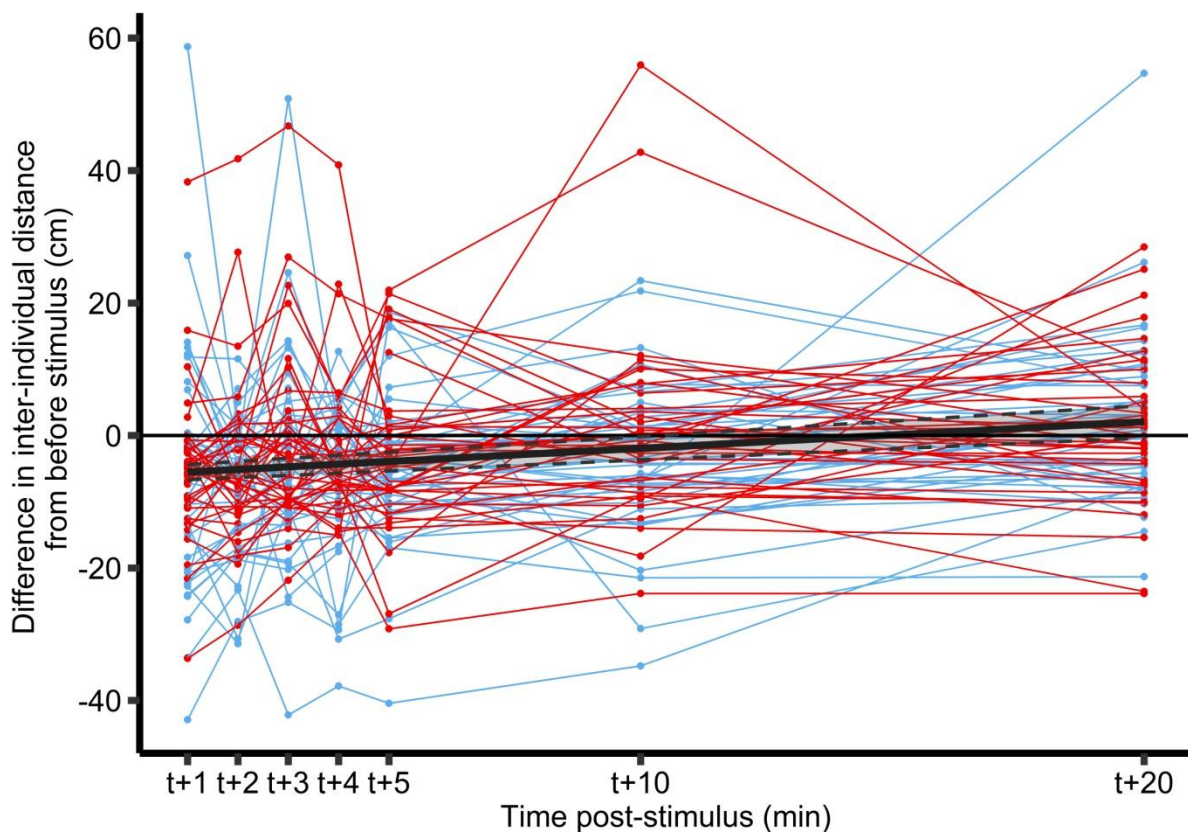


Fig. 10. Evolution of inter-individual distance within group over the 20 minutes following the stimulus. Points represent for each individuals, the difference between the measures taken at time post-stimulus and $t-1$. Black horizontal line: represents the relative inter-individual distance before stimulus ($t-1$). Solid grey line: illustrates the model of the evolution in inter-individual distance over the 20 minutes post-stimulus irrespective of the treatment group. Blue points: control fish ($N=40$); red points: exposed fish ($N=40$).

DISCUSSION

The present study investigated the effects of an acute exposure to dispersant-treated oil on indices of responsiveness and behavioural adjustment to environmental threat. Two situations were tested; in the first situation we investigated fish responsiveness to a stimulus (light) through the monitoring of individual MO_2 . In the second situation, we examined whether exposure to dispersant-treated oil affected the behavioural response to a model predator by

measuring a shoal activity and cohesion. Exposure to dispersant-treated oil was hypothesized to : (1) reduce fish resting metabolic rate, (2) decrease fish metabolic responsiveness to a stimulus (light), (3) reduce group cohesion and activity, (4) reverse the expected shoal behavioural adjustment to a simulated aerial attack.

Our data indicated that oiled-fish displayed higher metabolic rate than control group, which is contrary to our first hypothesis. While our experiment indicated that the number of individuals responding to the light stimulus (responsiveness) was unaffected following oil exposure, the intensity of the metabolic response was significantly lowered in fish exposed to dispersant-treated oil compared to control fish. This data supports our second hypothesis. In contrast to our third and fourth hypotheses, we could not find any effect of oil exposure on the cohesion of the group. But in response to the stimulus of a model predator, behavioural analyses indicated an inappropriate increase in shoal activity as suggested in our fourth hypothesis. Though, in the first 10 minutes following the simulated aerial attack shoal activity was significantly higher in fish exposed to dispersant-treated oil compared to control fish

Exposure condition and bioaccumulation of contaminants

To characterize exposure conditions, water concentration in total petroleum hydrocarbon ([TPH]) was monitored throughout fish exposure period. As expected, E condition (TPH= 0.096g L⁻¹) was in the range of situations that fish are liable to encounter following an oil spill and its treatment with dispersant (0.001 to 0.260g L⁻¹; Kim et al., 2010; Sammarco et al., 2013; Spooner, 1970).

Fish from the control treatment displayed background liver polycyclic aromatic hydrocarbon (PAH) concentrations ($0.523 \pm 0.007 \mu\text{g g}^{-1} \text{ dw}$), well below the quantification limit (QL: $6.027 \mu\text{g g}^{-1} \text{ dw}$). In E fish, liver PAH concentration measured at days 0 (*i.e.*, sampled immediately at the end of the exposure period) and at day 1 post-exposure were respectively 1.3 and 1.7 times higher than the QL. In teleost fish the liver is the main site where PAH are metabolized (Stein et al., 2010). In E individuals, the observed increase in liver PAH concentration from day 0 to 1 post-exposure is surprising as fish are known for readily metabolizing PAH. This delayed increase in liver PAH indicates metabolization of residuals PAH accumulated in the tissues. At 4 and 7 days post-exposure, however, mean liver PAH

concentration was back below the QL confirming the efficiency of fish detoxification mechanisms.

Respirometry

Standard metabolic rate

As expected, C individuals showed a decrease in MR over the days post-introduction into the respirometers. Interestingly, C fish returned to values similar to the estimated SMR from the literature within 3-4 days following their transfer into the respirometry chambers. This reinforces methodological recommendations, present in the literature, regarding the measurement of the SMR (Clark et al., 2013; Chabot et al., 2016).

Current results indicate that E individuals displayed higher MR than C fish all over the week, with no recovery observed within 7 days post-exposure. This result is in accordance with previous study showing higher oxygen consumption in Australian Bass (*Macquaria novemaculeata*) 4 days following exposure to dispersant treated oil (Cohen et al., 2001). This higher resting MR observed in E fish could result from an increase metabolic requirement in relation with the detoxification process (Reddy & Bhagyalakshmi, 1994; Sørensen et al., 1997; Correia et al., 2007). Davoodi & Claireaux (2007b) reported that following a 48h period of severe oil exposure SMR of the European common sole (*Solea solea*) increased from 31.6 to 39.2 mgO₂ kg⁻¹ h⁻¹ but this increase was not found statistically significant. The increase in resting oxygen demand of E versus C (107.45±5.19 vs 82.89±4.17 mgO₂ kg⁻¹ h⁻¹) largely stays within the range of seabass aerobic capacities at 15°C (82–244 mgO₂ kg⁻¹ h⁻¹; estimated from Claireaux & Lagardère, 1999).

Responsiveness to light stimulus

Fish from both treatments responded equally in number (responsiveness) to the light stimulus by showing an abrupt increase in oxygen demand. However, E individuals displayed less marked increase in MO₂ (1.6-fold) following the light stimulus than C fish (2.3-fold). In the literature little is available about the effects of dispersant-treated oil on fish behaviour. To our knowledge, only two studies have investigated the effects of oil exposure on fish responsiveness to a threatening stimulus (Johansen et al., 2017; Milinkovitch et al., 2019). Similarly to our results, these studies showed that the percentage of fish responding was

unaffected. However, the displayed locomotor response was altered, with changes in fish swimming speed and directionality.

In E fish, the amplitude of the peak in MO_2 observed following light stimulation was lower than in C fish. This difference can have two origins which are not mutually exclusives *i.e.*, limitation of metabolic pathways and difference in the perception of the stimulus (threat).

The limitation of the metabolic pathways can be hypothesised because our results suggest a decrease in the aerobic capacities of E fish. Following the light stimulus, E fish displayed higher resting MR and a lower amplitude of the peak in MO_2 than C fish. The light stimulus used in the present study has been shown to induce a rapid increase in MO_2 to level corresponding to the maximum metabolic rate (MMR) measured after individual chasing (Claireaux, pers com.). The lower amplitude of the peak in MO_2 showed by E fish might, therefore, suggest a reduction in their MMR. Those, the difference between minimum and maximum in MO_2 , that determines the aerobic scope of an individual, is suggested to be reduced in E fish compared to C individuals.

The hypothesis of a difference in the perception of a threatening stimulus could occur as exposure to oil compounds might induce a narcosis state in fish (van Wezel & Opperhuizen, 1995; van Brummelen et al., 1998; Barron et al., 2004; Hsieh et al., 2006; Gonçalves et al., 2008). The toxicity of PAH is generally attributed to nonpolar narcosis that can affects neuronal cells and disrupts central nervous system functions and signalling pathways. In fish, narcosis state is, therefore, likely to alter the capacity to acquire and process information from the surrounding environment possibly resulting in inappropriate behavioural responses.

Post-stimulation recovery

Day 1 post-exposure, E fish returned faster to their MR (48 ± 12 min) than C fish (74 ± 18 min). This faster return to pre-stimulation MR may indicate lower sensitivity to the stressor and suggest inappropriate responsiveness that could be detrimental if the situation was dangerous.

Time to return to pre-stimulation level displayed a strong habituation component in the control fish but also a treatment component. The habituation component is illustrated by the decrease over time of the peak amplitude and of the time to return to the pre-stimulus MR.

This decrease is observed in both treatments, suggesting that E fish may have conserve some learning abilities after exposure to dispersant treated oil to be able to habituate to the light stimulus over the week. The treatment effect is illustrated by the difference in peak height between C and E.

Recovery of responsiveness over the week post-stimulus

Despite observations of a lower peak amplitude in MO_2 following the light stimulus and a faster return to pre-stimulation MR, present study suggests recovery within one week post-exposure of those parameters. Results showed that at day 7 post-exposure, E and C fish expressed similar respiratory response to the stimulus with equal increase in MO_2 (1.6-fold the pre-stimulation MR). Recovery capacities have been understudied in the literature. To our knowledge this study is the first to report recovery of the responsiveness to threatening stimulus following oil exposure. According to the studies working also on the recovery capacities at the individual level, recovery of hypoxia tolerance was suggested to occur between 5 to 10 months post-exposure (Mauduit et al., 2016; Zhang et al., 2017). In contrast, recovery of exploratory behaviour was suggested to occur within two-weeks following the exposure to similar concentration of dispersant treated oil (Aimon et al., in press). Present results are in agreement with the suggested recovery of behavioural performances within a period of 1 to 2 weeks post-exposure to dispersant treated oil, in sea bass.

Shoaling behaviour

Fish swimming activity in the experimental arena was characterized by combining the time spent swimming, the distance moved and the swimming speed (Little & Finger, 1990; Baker et al., 2018). Measures of group activity level conducted one hour after their introduction into the experimental arena were considered to reflect fish standard activity level in undisturbed familiar condition. As expected in such conditions, E fish displayed an activity level 2 times lower than C fish. This reduction in activity level in oil-exposed fish is in agreement with other studies that also reported reduced swimming activity following exposure to petroleum hydrocarbons (Woodward et al., 1987; Little & Finger, 1990; Gonçalves et al., 2008). For instance, Gonçalves et al. (2008) reported an increase in the percentage of juvenile gilthead seabream (*Sparus aurata*) showing non-locomotor activity following exposure to PAH for 4 days. Hypoactivity can have major ecological consequences through the disruption of crucial behaviours such as foraging efficiency, predation avoidance or reproduction. For instance,

lower swimming activity can interfere with prey capturing ability, lessening the surface of searching area and reducing the chance to encounter prey. Altered foraging success may also decrease the amount of energy available for growth. Further, the suboptimal activity level displayed by E fish has been shown, in this study, to also have consequences upon the behavioural response to a stimulus.

The primary cause of mortality in juveniles is the predation (Almany & Webster, 2006). Antipredatory behaviours are therefore of critical importance regarding their survival. Usually, once under attack from a predator, fish tend to minimize movements, especially away from shelter and to shoal in order to benefit from numerical dilution and confusion effect for the predator, in addition of additive vigilance (Pulliam & Caraco, 1984; Clark & Mangel, 1986; Godin, 1986; Pitcher & K. Parrish, 1993b; Krause et al., 2000; Krause & Ruxton, 2002). In our study, once the threat is believed to be eliminated, fish showed a return within 10 minutes to their standard behaviours displayed in undisturbed condition (Fig. 9 & 10). Visual stimulation used in the present study *i.e.*, model predator passing over the arena, was thought to mimic a predatory attack. It was designed to evaluate fish behavioural response to the presence of a potential danger. After the stimulus fish from both treatment conditions displayed the typical reduction in activity and increased group cohesion, as illustrated by a reduction in the inter-individual distance within groups. However, E fish showed a less marked reduction in activity after the stimulus than C individuals leading them to display higher and inappropriate activity level in a potentially dangerous situation.

Similarly to C individuals, E fish displayed an increasing activity over time post-stimulus to return to background level expressed at t-1. Compared to C fish, however, E individuals showed a higher activity level and return slightly earlier to background activity level, between 5 to 10 minutes post-stimulus. These impairments with inappropriate activity and faster return to standard behavioural level can be critical for individual and population survival by increasing the risk of predator-induced mortality. For instance, if E fish express standard activity level while the environment is still unsecure, they will be more exposed at predation risk than usual, like indicated by C fish. Accordingly, previous studies looking at the effects of petroleum hydrocarbons compounds on fish behaviour, showed reduced sheltering and shoaling behaviours, increased risk taking and altered antipredator behaviours, such as escape response (Gonçalves et al., 2008; Johansen et al., 2017; Milinkovitch et al., 2019). Moreover,

exposure to PAH has been shown to increase predator-induced mortality in six species of Pomacentridae and Lethrinidae families, presenting such types of behavioural alterations (Johansen et al., 2017). All these results suggest that exposure to petroleum hydrocarbons may affect negatively the fitness of individuals and their ability to face predation pressure.

In the present study, exposure to dispersant treated oil affected shoal activity but not its cohesion. However, this results is not surprising as it has been recognized that swimming activity is the most sensitive behavioural indicator of animal disturbance (Little et al., 1990a). Our results are in agreement with the current literature, showing impairment in activity not only before mortality occurs, but also before reduction in growth is detected.

Recovery of behavioural responses

To assess the recovery capacities of exposed individuals, we monitored all the previously cited behavioural parameters over one week post-exposure. Concerning the altered swimming activity displayed in undisturbed condition at t-1 or after the model predator stimulus, no recovery was shown over the 8 days of the experiment. These behavioural impairments might be detrimental for sea bass. It is critically important that individuals recover from sublethal impairments as early as possible after exposure to dispersant treated oil, because with suboptimal behavioural and physiological performances, fish are more prone to predation and less likely to survive to adulthood.

Conclusion

The results of this study suggest that exposure to dispersant treated oil does not inhibit the classical antipredatory response, with the conservation of a reduction in activity and inter-individual distance and an increase in MO_2 following a threat. Despite, the presence of responsiveness to the threatening stimuli, there was evidence that exposed individuals displayed an altered response compared to control fish. Shoaling cohesion was not affected, but we observed lowered metabolic responsiveness to light stimulus as well as reduced fish locomotor activity and altered anti-predator response to a simulated aerial attack. Such results suggest consequences for organism health, hence population fitness. Such inappropriate responses may be the result of altered neuronal network integrity as suggested in earlier studies (Barron et al., 2004; Gonçalves et al., 2008). The narcotic mode of toxicity of oil compounds may have altered fish capacity to acquire and process information from external

stimuli. Although, recovery was observed for the physiological response to the lightening stimulus within 7 days post-exposure, that indicates the transient nature of these impairments. Future studies should investigate recovery capacities of fish behavioural and physiological performances over a longer period post-exposure.

Supplementary data

Table S1. Linear mixed effect model evaluating the relationship between the treatment condition and the time post-exposure upon night resting metabolic rate.

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day		(Intercept)	2.07	<0.01*
		Treatment	4.84	0.04*
		Day	14.82	<0.01*
		Treatment:Day	13.16	<0.01*

* Represents significant effects ($p < 0.05$).

Table S2. Backward stepwise reduction of the full model evaluating the relationship between the treatment condition and the time post-exposure upon the percentage of fish showing a metabolic response to the light stimulus.

Model	Dropped term	Retained term	z-value	p-value
Treatment:Day	Treatment:Day		-0.60	0.55
Treatment+Day	Treatment		1.14	0.26
Day	Day		-1.37	0.17

Table S3. Linear mixed effect model evaluating the relationship between the treatment condition and the day post-exposure upon the ratio (Peak of MO_2 /pre-stimulation MR).

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day		(Intercept)	225.35	<0.01*
		Treatment	0.93	0.35
		Day	4.85	0.04*
		Treatment:Day	5.27	0.03*

* Represents significant effects ($p < 0.05$).

Table S4. Linear mixed effect model evaluating the relationship between the treatment condition and the day post-exposure upon the time to return to the pre-stimulation MO_2 after the light stimulus.

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day		(Intercept)	50.12	<0.01*
		Treatment	0.09	0.77
		Day	22.56	<0.01*
		Treatment:Day	6.64	0.02*

* Represents significant effects ($p < 0.05$).

Table S5. Backward stepwise reduction of the full model evaluating the relationship between the treatment condition and the day post-exposure upon Activity at t-1.

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day	Treatment:Day		0.60	0.62
Treatment+Day	Day		0.86	0.47
Treatment		Treatment	7.62	0.01 *

* Represents significant effects ($p < 0.05$).

Table S6. Backward stepwise reduction of the full model evaluating the relationship between, the treatment condition and the day post-exposure upon inter-individual distance within the group, at t-1.

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day	Treatment:Day		1.46	0.23
Treatment+Day	Treatment		<0.01	0.96
Day		Day	0.85	0.47

* Represents significant effects ($p < 0.05$).

Table S7. Backward stepwise reduction of the full model evaluating the relationship between, the behavioural trend over time (20min post-stimulus), the treatment condition and the day post-exposure upon Activity.

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day:(Time+I(Time ²))	Treatment:Day:I(Time ²)		0.89	0.45
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ²) +Day:I(Time ²) +Treatment:Day:Time	Treatment:Day:Time		1.17	0.32
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ²) +Day:I(Time ²)	Day:I(Time ²)		0.44	0.72
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ²)	Treatment:Day		1.06	0.37
Treatment:Time+Day:Time +Treatment:I(Time ²)	Day:Time		1.65	0.18
Day+Treatment:Time +Treatment:I(Time ²)	Day		1.58	0.20
Treatment:Time +Treatment:I(Time ²)		Treatment	5.56	0.02*
		Time	257.52	<0.01*
		Treatment:Time	34.31	<0.01*
		Treatment:I(Time ²)	22.96	<0.01*

* Represents significant effects ($p < 0.05$).

Table S8. Backward stepwise reduction of the full model evaluating the relationship between, the behavioural trend over time (20min post-stimulus), the treatment condition and the day post-exposure upon inter-individual distance within the group.

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day:(Time+I(Time ²))	Treatment:Day:Time		0.02	1.00
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ²) +Day:I(Time ²)+Treatment:Day: I(Time ²)	Treatment:Day:I(Time ²)		0.14	0.94
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ²) +Day:I(Time ²)	Treatment:I(Time ²)		1.01	0.37
Treatment:Time+Treatment:Day +Day:Time+Day:I(Time ²)	Treatment:Day		1.29	0.28
Treatment:Time+Day:Time + Day :I(Time ²)	Treatment:Time		1.67	0.20
Treatment+ Day:Time +Day:I(Time ²)	Day:Time		1.70	0.17
Treatment+ Time+Day:I(Time ²)	Day: I(Time ²)		1.08	0.40
Treatment+ Time+Day	Treatment		1.55	0.22
Time+Day	Day		1.83	0.15
Time		Time	34.67	<0.01*

* Represents significant effects ($p < 0.05$).

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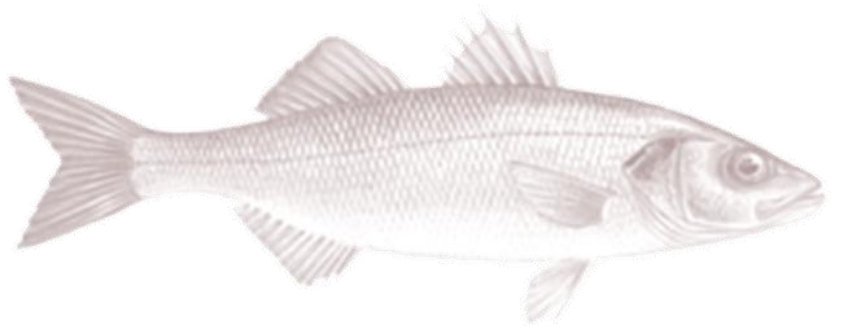
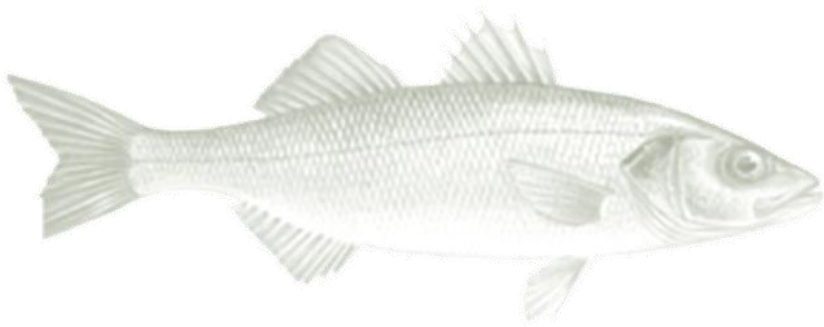
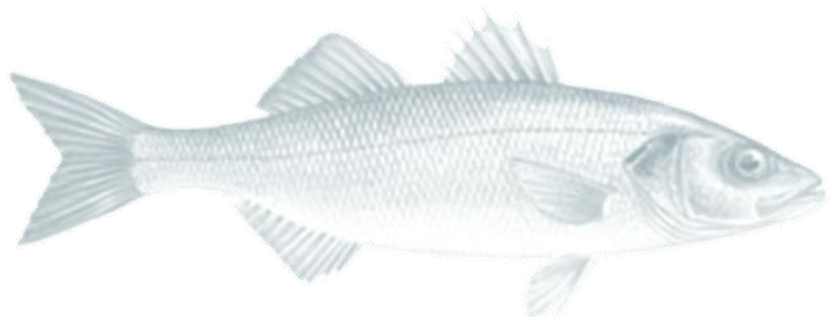
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DISCUSSION GENERALE



La discussion générale ne reprendra pas point par point les questions présentées dans chaque chapitre mais abordera les 4 objectifs de ma thèse, à savoir :

1) Utiliser des tests comportementaux permettant une analyse intégrative des effets directs de perturbations environnementales sur des comportements (*e.g.*, activité d'exploration, prise de risque) et des effets indirects de ces changements sur les interactions intra- et inter- populationnelles (*e.g.*, sociabilité, réaction au prédateur).

2) Mettre en application ces analyses comportementales au travers de deux perturbations environnementales courantes en milieu côtier : un stress naturel (une pénurie de nourriture) et un stress anthropique (une marée noire). Ces résultats seront intégrés dans le schéma conceptuel (voir introduction, figure I) sur lequel se base cette thèse.

3) Evaluer la capacité de récupération post-stress des animaux pour avoir une estimation des impacts plus pertinente.

4) Adapter des outils statistiques en composantes principales et multivariées pour objectiver les mesures et l'interprétation des analyses comportementales.

Certains aspects qu'il semble intéressant de souligner et qui font l'originalité de ce travail seront également abordés :

- La répétabilité de nos données n'ayant pas été testée, leur interprétation éthologique requiert quelques précautions.
- La généralisation de nos données à d'autres espèces reste à valider.
- La prise en compte du comportement stéréotypé observé chez le bar exposé à un environnement nouveau permet de mieux analyser la dynamique exploratoire.

La discussion aboutira enfin à la présentation de quelques perspectives.

1) Utiliser des tests comportementaux permettant une analyse intégrative des effets directs de perturbations environnementales sur des comportements (*e.g.*, activité d'exploration, prise de risque) et des effets indirects de ces changements sur les interactions intra- et inter- populationnelles (*e.g.*, sociabilité, réaction au prédateur).

Cette thèse s'inscrit dans la volonté récente de créer un cadre unificateur des domaines jusqu'ici séparés comme la toxicologie, la conservation, l'écologie comportementale ou encore la physiologie. L'objectif est en effet de fournir des explications concernant les effets des changements environnementaux sur la fitness des individus et sur leurs répercussions au niveau de la dynamique des populations. Du point de vue du comportementaliste, cette réflexion autour d'un schéma conceptuel unifié vise notamment à replacer l'analyse du comportement dans son contexte environnemental (Saaristo Minna et al., 2018). Pour cela, les conséquences sont interprétées de façon directe en terme d'effet sur la fitness de l'individu mais aussi de façon indirecte en prenant en compte les répercussions sur les interactions intra- et inter- spécifiques, telles que la reproduction ou les relations prédateur-proie.

Le schéma conceptuel (Fig. I; Adapté de: Sih et al., 2010; Peterson et al., 2017) sur lequel se base cette thèse, permet d'aller au-delà de la simple observation de changements comportementaux en corrélation avec des modifications des conditions du milieu. Il s'agit, en effet, ici d'en tirer des conséquences en termes de dynamique des communautés et de fonctionnement global de l'écosystème.

Le comportement offre une possibilité de relier l'état de l'animal à la dynamique écologique de l'écosystème dans lequel il évolue (Scherer, 1992; Scott & Sloman, 2004). En effet, le comportement est souvent considéré comme l'expression visible de l'état de santé d'un animal puisqu'il intègre les processus morphologiques, biochimiques et physiologiques (Benca et al., 2009; McCarthy, 2010; Sih et al., 2010; Weis, 2014). Cette réponse hautement intégrative est également directement impliquée dans les interactions intra- et inter- spécifiques, ce qui lui procure un rôle primordial dans la dynamique de l'écosystème (Little et al., 1990b; Dell'Omo, 2002b; Saaristo Minna et al., 2018).

Au cours de cette thèse, j'ai cherché à développer une vision intégrée des conséquences directes et indirectes des changements comportementaux en réponse aux fluctuations environnementales, en utilisant le bar Européen comme espèce modèle. Pour ce faire, j'ai mené plusieurs investigations pour évaluer comment les juvéniles de bar Européens, une espèce commerciale de nos zones côtières, régulent trois des comportements essentiels à leur survie (activité d'exploration, sociabilité, prise de risque) en réponse à différents types de perturbations environnementales, naturelle ou anthropique. Les conséquences des

perturbations sur les interactions intra- et inter- spécifiques dans lesquelles l'individu est impliqué ont été intégrées afin d'estimer les répercussions aux échelles de la population et de la communauté. En montrant que le jeûne induisait une baisse de la sociabilité (interaction sociale ; chapitre 1) et que l'exposition à des hydrocarbures pétroliers engendrait des altérations dans la réponse à la simulation d'une attaque de prédateur (interaction prédateur-proie ; chapitre 2 et 3), j'ai illustré le fait que l'on pouvait interpréter les régulations comportementales observées au niveau individuel dans un contexte intégrateur prenant en compte les effets indirects au niveau de la population et au niveau de la communauté. Ces travaux de recherche illustrent combien la modification de comportement permet de relier les effets des perturbations environnementales à de multiples niveaux d'organisation, de l'individu à l'écosystème. Pour chacun des cas d'étude utilisés, nous pouvons reporter nos résultats dans le schéma conceptuel sur lequel je base mon travail de thèse.

2) Mettre en application ces analyses comportementales au travers de deux perturbations environnementales courantes en milieu côtier : un stress naturel (une pénurie de nourriture) et un stress anthropique (une marée noire). Ces résultats seront intégrés dans le schéma conceptuel (voir introduction, figure I) sur lequel se base cette thèse.

Mon travail de thèse prend appui sur un schéma conceptuel présentant une vision intégrative de l'étude du comportement dans son contexte environnemental. Les changements de comportements induits par la privation de nourriture, mis en évidence dans le chapitre 1 de mon étude, peuvent être reportés sur ce schéma conceptuel comme suit :

La sociabilité, contrairement à l'activité d'exploration et la prise de risque, est réduite en période de jeûne (chapitre 1; *boxes "Comportement" et "Interactions"*, Fig. II). L'animal semble changer de compromis en délaissant les avantages de la vie en groupe, tels qu'une vigilance accrue, un effet de dilution face aux prédateurs ou encore une fréquence plus élevée de détection de la nourriture, au profit de l'isolement associé à une diminution de la compétition pour les ressources (*box "Interactions"*, Fig. II). Cet isolement par rapport au groupe peut déboucher sur une augmentation du risque de prédation et diminuer les chances de survie des individus ne bénéficiant plus de la protection des autres membres (*box "Interactions"*, Fig. II). La réduction de la sociabilité a donc possiblement des conséquences sur la fitness de l'individu.

Mon étude a également permis de mettre en évidence une régulation de la relation entre la sociabilité et la prise de risque en fonction du statut nutritionnel de l'individu (chapitre 1; *box "Interactions"*, Fig. II). Cette régulation suggère que le coût résultant du risque accru de prédation lié à la solitude peut être compensé par les possibles récompenses associées à une plus forte probabilité de rencontre des ressources alimentaires sans compétition avec les congénères.

L'ensemble de ces résultats permet de compléter la dernière boîte du schéma conceptuel de base (*box "Ecologie"*, Fig. II). Un changement de comportement au niveau de l'individu peut modifier des interactions intra-spécifiques et de façon indirecte des interactions inter-spécifiques (*e.g.*, prédateur-proie) et avoir des conséquences au sein de la communauté.

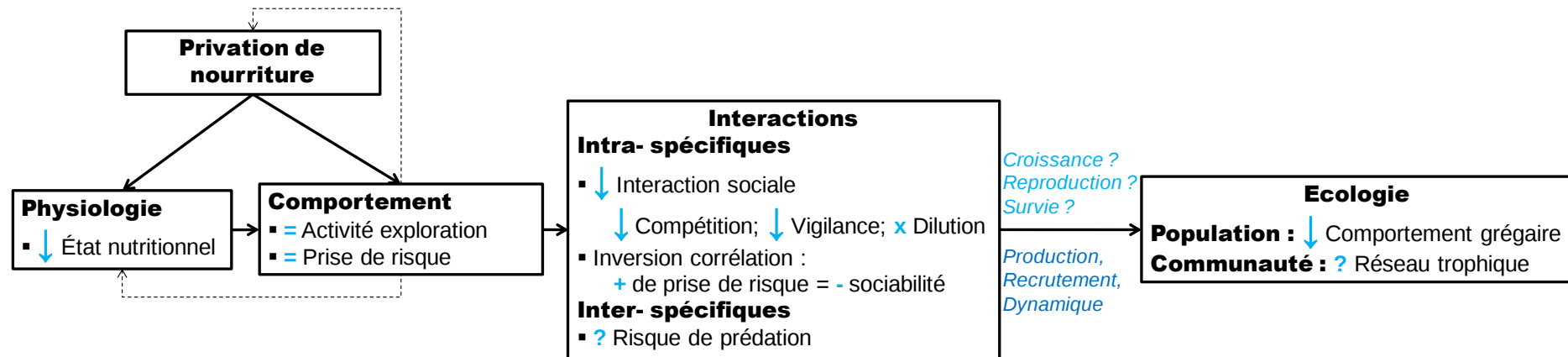


FIGURE II. Schéma conceptuel modélisant les effets directs et indirects de la privation de nourriture chez des juvéniles de bar européen (adapté de Peterson et al., 2017; Sih et al., 2010). = : non modifié; ? : possibles conséquences; ↓ : réduit ; x : perdu; + : plus ça augmente; - : plus ça diminue.

Les modifications du comportement induites par l'exposition à des hydrocarbures pétroliers dispersés chimiquement, mis en évidence dans les chapitres 2 et 3 de mon étude, peuvent également être reportées sur le schéma conceptuel comme suit :

Les individus exposés présentent une perturbation de l'activité d'exploration et du temps passé dans une zone à risques (chapitre 2; *box "Comportement"*, Fig. III). Ces comportements sont reconnus comme reflétant les capacités cognitives des poissons, leur permettant notamment de se créer et se remémorer une représentation spatiale de leur environnement. Aussi, ces résultats suggèrent un effet négatif de l'exposition aux hydrocarbures pétroliers sur les capacités cognitives du bar et son repérage dans l'espace. L'altération de ces capacités pourrait également avoir des conséquences sur l'interaction entre l'individu et son environnement et ses facultés à rétroagir sur les forçages environnementaux s'exerçant sur lui (Fig. III).

Les observations ont également révélé que même si la détection d'un stimulus n'est pas affectée, face à un stress aigue (*i.e.*, simulation d'une attaque de prédateur ou stimulus lumineux), les poissons ayant été exposés aux contaminants chimiques montraient une baisse d'activité moins marquée et un taux métabolique moins élevé par rapport aux contrôles (chapitre 3; *box "Interactions"*, Fig. III). L'altération de la réponse à un stimulus pouvant s'assimiler à l'attaque d'un prédateur dans le milieu naturel suggère que les individus exposés aux hydrocarbures pétroliers sont soumis à un risque accru de prédation (chapitre 3; *box "Interactions"*, Fig. III) et donc de possibles effets négatifs sur leur survie.

Par contre, les interactions intra-spécifiques ne semblent pas être affectées, avec une cohésion du groupe conservée, et augmentée suite à l'attaque d'un prédateur, de façon identique aux individus contrôles (*i.e.*, cohésion plus forte au sein du groupe), chez les individus exposés (chapitre 3; *box "Interactions"*, Fig. III).

L'évaluation des effets de l'exposition à des hydrocarbures pétroliers a également permis de mettre en évidence une altération de la biochimie interne de l'animal, avec la contamination des tissus par les HAP, nécessitant des processus de détoxification (chapitre 2 et 3; *box "Biochimie"*, Fig. III). Cette perturbation environnementale semble également réguler la physiologie animale et notamment le métabolisme énergétique en réduisant le taux métabolique de base des individus exposés (chapitre 3; *box "Physiologie"*, Fig. III).

L'ensemble de ces résultats suggèrent des conséquences directes sur la fitness des individus mais également des répercussions au niveau de la communauté, notamment dans les interactions trophiques (*box "Ecologie"*, Fig. III). En effet, si les individus sont moins à même de répondre à la pression de prédation en s'exposant d'avantage et en montrant une baisse moins marquée de leur activité face à une attaque, par rapport aux témoins, cela risque de créer un déséquilibre dans les relations prédateur-proie (chapitre 2 et 3; *boxes "Interactions"* et *"Ecologie"*, Fig. III). Nous pouvons illustrer cette idée par la figure IV: Si l'on simplifie les relations inter-spécifiques au sein d'un réseau trophique, chaque être vivant rattaché à cette chaîne trophique mange des organismes de niveau trophique inférieur dans le but d'acquérir de l'énergie (Fig. IV, A). Cependant, si suite à un événement de type déversement pétrolier les juvéniles de bar sont plus susceptibles à la prédation, leur population pourrait diminuer. Cela induirait alors une pression de prédation plus faible sur leurs proies qui, en retour, seraient plus à même de proliférer (Fig. IV, B). Ces proies de rang C pourraient également modifier leur pression de prédation sur leurs propres proies. Cependant, ce type d'interprétation intégratrice nécessiterait maintenant de développer des approches multi-espèces au sein des études d'impacts pour pouvoir réellement estimer les conséquences en cascades dans l'écosystème.

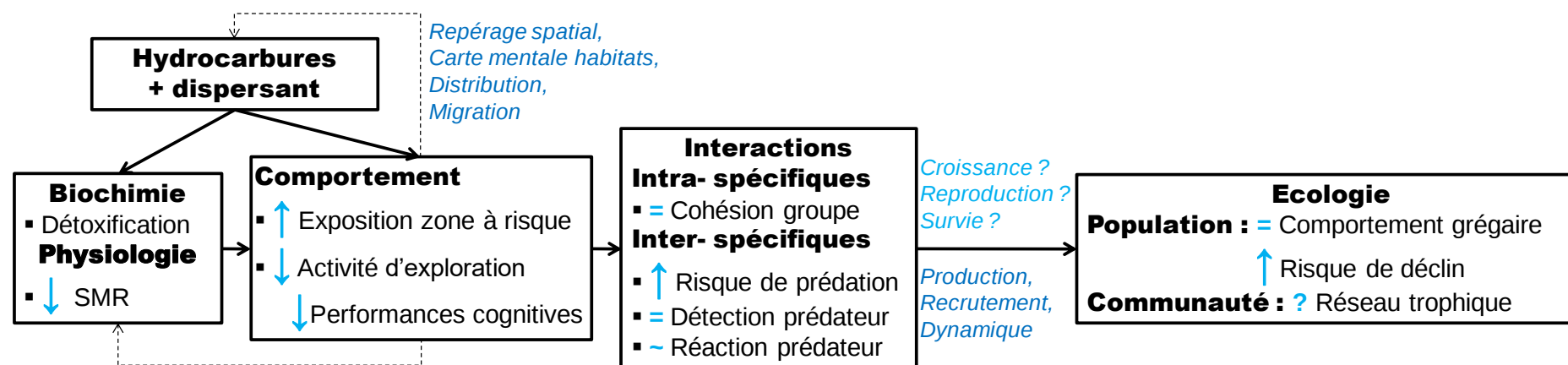


FIGURE III. Schéma conceptuel modélisant les effets directs et indirects de l'exposition à des hydrocarbures pétroliers dispersés chimiquement chez des juvéniles de bar européen (adapté de Peterson et al., 2017; Sih et al., 2010). ~ : altéré; ? : possible conséquences; ↓ : réduit; ↑ : augmenté; = : non modifié.

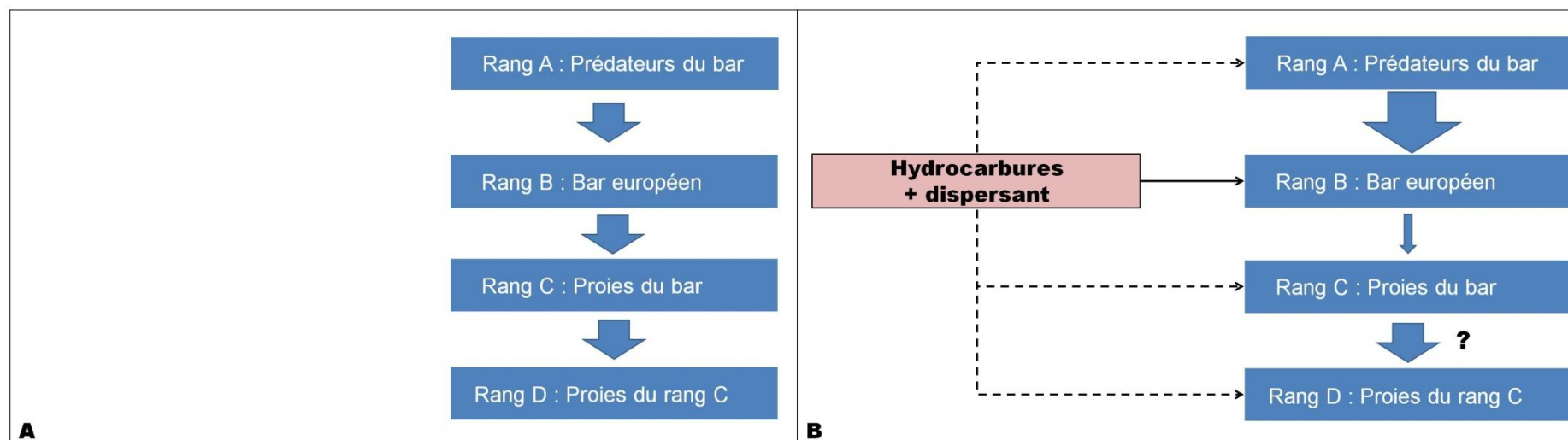


FIGURE IV. A: réseau trophique simplifié. B: possibles conséquences en cascade dans le réseau trophique si suite à une exposition aux hydrocarbures pétroliers dispersés chimiquement, les individus du rang B sont plus susceptibles à la prédation (adapté de Saaristo minna et al., 2018).

3) Evaluer la capacité de récupération post-stress des animaux pour avoir une estimation des impacts plus pertinente.

Afin d'évaluer les conséquences des effets induits par les perturbations environnementales, il semble primordial de pouvoir également estimer la durée pendant laquelle la fitness de l'individu et ses relations intra- et inter- populationnelles risquent d'être altérées. En d'autres termes, il est évidemment pertinent de déterminer dans quelles mesures les changements environnementaux vont avoir des impacts permanents ou bien ponctuels sur les organismes et d'évaluer la durée de ces effets. Pour cela il est essentiel de considérer l'étude d'une potentielle récupération post-exposition. En ce qui concerne notre première étude de cas, nous savons que les périodes de privation de nourriture dans le milieu côtier peuvent durer de quelques jours à plusieurs mois (Binner et al., 2008; Gingerich et al., 2010). Cependant, nous n'avons pas cherché à évaluer si les individus retrouvent leurs comportements initiaux après un retour aux conditions plus favorables. Les études disponibles dans la littérature suggèrent une récupération en mois de deux mois des performances affectées par le jeûne (Pastoureaud, 1991; Killen et al., 2013). Pastoureaud (1991) a notamment montré que deux semaines étaient nécessaires aux juvéniles de bar, à jeun depuis une semaine, pour retrouver leur appétit normal. Killen et al. (2013) ont mis en évidence une récupération de la vitesse moyenne de sprint en moins d'un mois. Cependant cette étude indique également une capacité de sprint altérée (*i.e.*, moins répétable, maximum réduit) sur la période de trente jours analysée. A notre connaissance il n'existe pas d'autre information quant à la durée nécessaire chez le bar pour récupérer ses comportements « normaux », suite à une privation de nourriture.

Concernant l'exposition aux hydrocarbures pétroliers dispersés chimiquement, nous avons tenté de mesurer le temps de récupération post-exposition des divers comportements analysés. La première étude suggère qu'après deux semaines de retour en condition normale, la durée passée dans la zone à risque du nouvel environnement était similaire à celle des poissons témoins (chapitre 2). La deuxième étude montre une tendance à la récupération sous 8 jours de la SMR et de la réaction aux stimuli lumineux. De plus nous avons cherché à établir une correspondance entre la cinétique de récupération des comportements initiaux et la décontamination des tissus. Cependant, les altérations comportementales significatives subsistaient encore en fin d'expérimentation alors que, les concentrations tissulaires en HAP étaient redevenues similaires au groupe témoins. Aussi, nous suggérons qu'il serait intéressant de poursuivre sur ce questionnement dans les travaux futurs. Dans l'ensemble, la présente

étude montre que les bars juvéniles exposés à un déversement d'hydrocarbures pétroliers présentent des troubles transitoires du comportement qui peuvent néanmoins avoir des conséquences majeures au niveau de la population. La pression de prédation étant la cause majeure de la mortalité chez les juvéniles, ces altérations transitoires sont susceptibles d'avoir des effets néfastes pour leur fitness.

4) Adapter des outils statistiques en composantes principales et multivariées pour objectiver les mesures et l'interprétation des analyses comportementales.

Une des difficultés rencontrées au cours de ces travaux de recherche a été de définir une technique objective d'identification des caractéristiques comportementales (*i.e.*, prise de risque, activité d'exploration, sociabilité) évaluées et ce, dans les différents tests mis en place. En effet, un challenge identifié dans la littérature comportementale est d'identifier les traits comportementaux ciblés par un test comportemental donné (Carter et al., 2013; Roche et al., 2016). Il existe en effet deux principaux problèmes :

- « One-to-many problem » : le même test est utilisé pour évaluer des traits comportementaux distincts. A titre d'exemple, le test du nouvel environnement, consistant à introduire un individu pour la première fois dans une arène inconnue, peut être utilisé aussi bien pour mesurer l'exploration (activité de nage) que l'audace (occupation des zones à risque) d'un individu.
- « Many-to-one problem » : plusieurs tests sont utilisés pour évaluer le même trait comportemental. Aussi, l'audace peut à la fois être évaluée dans un test du nouvel environnement ou bien un test du nouvel objet (approche vis-à-vis de l'objet inconnu).

Pour pallier à ces problèmes, j'ai décidé d'utiliser l'analyse en composante principale comme technique de réduction des données mesurées. Comme indiqué par Dingemanse et al. (2010) ou encore Carter et al. (2012), cette technique est pertinente pour traduire diverses mesures comportementales en un ou plusieurs indices comportementaux caractéristiques de l'espèce étudiée. Les composantes issues de ce type d'analyse sont constituées de variables mesurées qui corrélient entre elles et caractérisent un même comportement. La nomenclature et l'identification de la caractéristique comportementale associée à une composante se font de façon plus objective que sur une variable mesurée et prise individuellement. En effet, la composante est intégrant plusieurs mesures, il est possible de prendre en compte comment ces

variables corrèlent entre elles et le contexte dans lequel elles ont respectivement été mesurées afin de mieux déterminer la caractéristique comportementale associée à cette composante. Les mesures interprétées individuellement seraient redondantes et moins représentatives de la réponse comportementale globale de l'animal face au stimulus rencontré.

Ce type d'analyse, si il était d'avantage utilisé dans les études comportementales, pourrait également permettre de réduire un autre des problèmes majeurs identifiés dans la littérature du domaine, à savoir la "jingle-jangle fallacy" (Gosling, 2001; Toms et al., 2010; Carter et al., 2013; Roche et al., 2016). Ce terme fait référence aux irrégularités, répertoriées dans la littérature, quant à la qualification des traits analysés lors des tests comportementaux et qui induisent deux principales erreurs :

- L'erreur « jingle » : un même terme est utilisé pour qualifier des traits comportementaux distincts, mesurés avec différents tests. A titre d'exemple, le terme « audace » est utilisé pour qualifier à la fois la réponse à un nouvel objet, l'activité dans un nouvel environnement ou bien sous la pression d'un prédateur.
- L'erreur « jangle » : plusieurs termes sont attribués à un seul et même trait comportemental. Aussi, les termes "anxiété", "audace" et "exploration" sont utilisés pour qualifier la distance parcourue dans un test du nouvel environnement.

Les méthodes de réduction des données ne peuvent pas à elles seules résoudre ces problèmes et d'autres recommandations ont été émises dans la littérature comme par exemple le fait de limiter l'utilisation de nomenclatures spécifiques (*i.e.*, audace, exploration, sociabilité) aux mesures comportementales (Carter et al., 2013). Aussi, même si j'ai attribué certains termes spécifiques aux caractéristiques comportementales identifiées dans mes tests (*i.e.*, activité d'exploration, sociabilité), j'ai essayé de limiter leur utilisation en indiquant lorsque possible simplement le type de mesure réalisée (*i.e.*, temps passé dans la zone C ; chapitre 2). En outre, j'ai toujours essayé de définir clairement chaque trait comportemental en accord avec le contexte dans lequel il a été mesuré, comme suggéré par Réale et al. (2007).

Cette contextualisation ajoutée à l'utilisation d'une technique de réduction des données et un emploi limité de nomenclatures spécifiques aux traits comportementaux permettent de mieux borner l'interprétation de mes données.

La répétabilité de nos données n'ayant pas été testée, leur interprétation éthologique requiert quelques précautions.

Comme souligné dans le chapitre 1, mon travail de thèse ne remplit pas les exigences permettant de définir nos mesures comportementales comme des traits de personnalité. En effet, la personnalité animale fait référence aux différences de comportement inter-individuelles qui sont stables dans le temps (Sih et al., 2004a; Biro & Stamps, 2008; Carter et al., 2013; Roche et al., 2016). Dans le présent travail de thèse, les mesures n'ont été réalisées qu'une seule fois. Il est clairement indiqué que du fait de cette restriction, les réponses comportementales que nous avons étudiées ne peuvent pas être qualifiées de traits de personnalités (Chapitre 1). Cependant, l'utilisation de tests standards reconnus pour permettre la mesure de traits comportementaux spécifiques, nous permet de renforcer l'interprétation des réponses comportementales observées.

En outre, démontrer la stabilité temporelle d'une différence inter-individuelle requiert de répéter les mesures plusieurs fois dans le même contexte (Réale et al., 2007). Même si l'on évalue la répétabilité des mesures en les réalisant plusieurs fois dans le même contexte, il y a toujours de la variabilité entre les mesures. Cette variabilité temporelle entre les différentes mesures de la réponse comportementale peut résulter de l'accoutumance, la sensibilisation, l'acclimatation ou même la fatigue de l'animal (Greenberg & Mettke-Hofmann, 2001; Wahlsten, 2001). De plus, les phénomènes d'habituation et d'apprentissage peuvent rendre l'évaluation de la répétabilité très difficile. Les processus de familiarisation avec les conditions du test peuvent être exacerbés par de courts intervalles de temps entre les expériences (Dingemanse et al., 2002). Par exemple, Millot et al. (2009b) ont montré que le comportement de prise de risque dans un nouvel environnement changeait totalement entre le premier et le troisième test réalisés à 48h d'intervalle. En outre, tester la répétabilité d'une mesure comportementale comme la témérité face à la nouveauté (*e.g.*, test du nouvel objet) peut s'avérer délicat, si l'on considère que l'animal ne peut être confronté qu'une seule fois à la nouveauté. Bien que l'on puisse essayer de contourner le phénomène d'habituation en répétant le test en faisant, par exemple, varier la nouveauté (*e.g.*, utilisation d'un nouvel objet de forme et de couleur différente; Verbeek et al., 1994), l'animal pourrait s'habituer à la nouveauté elle-même. On peut se demander s'il est réellement possible de tester strictement la répétabilité d'un trait comportemental.

La généralisation de nos données à d'autres espèces reste à valider.

Deux aspects sont à prendre en considération lors de l'extrapolation de nos données à d'autres espèces et au milieu naturel. D'une part, il est important de considérer que les réponses observées dans ces travaux de recherche pourraient être spécifiques au bar. Il ne serait pas surprenant que d'autres espèces, avec des écologies différentes (*e.g.*, poissons pélagiques, abyssaux), réagissent différemment aux mêmes perturbations. Par exemple, il a été montré que deux espèces exposées de façon similaire à des hydrocarbures pétroliers présents dans le sédiment durant leur développement embryonnaire, présentaient des différences dans l'altération de leur activité locomotrice. En effet suite à l'exposition, le medaka japonais (*Oryzias latipes*) était plus enclin à augmenter son activité locomotrice (Le Bihanic et al., 2014) alors que le poisson zèbre (*Danio rerio*) avait tendance à la diminuer (Vignet et al., 2014b). D'autre part, pour l'extrapolation de nos données au milieu naturel, il faut tenir compte du fait que nous avons basé nos expérimentations sur des lots de poissons issus des écloseries Aquastream (France) et Les poissons du soleil (France) et présentent donc un certain niveau de domestication. Cependant, à chaque génération les éleveurs réintroduisent des animaux sauvages. Aussi, il est important de noter que des études cherchant à évaluer l'influence de la domestication sur le comportement du bar européen ont suggéré que celle-ci n'affecte pas le comportement natatoire (*i.e.*, répartition spatiale, vitesse de nage) (chap.7; Millot, 2008). Il a également été observé que les souches sauvages et domestiquées présentaient des comportements similaires de prise de risque, d'exploration, d'activité et de réaction de fuite face aux stimulus de prédation (Millot et al., 2009a, 2009b).

La prise en compte du comportement stéréotypé observé chez le bar exposé à un environnement nouveau permet de mieux analyser la dynamique exploratoire.

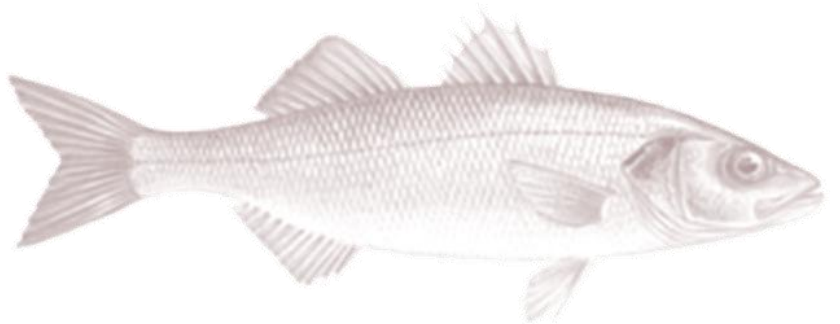
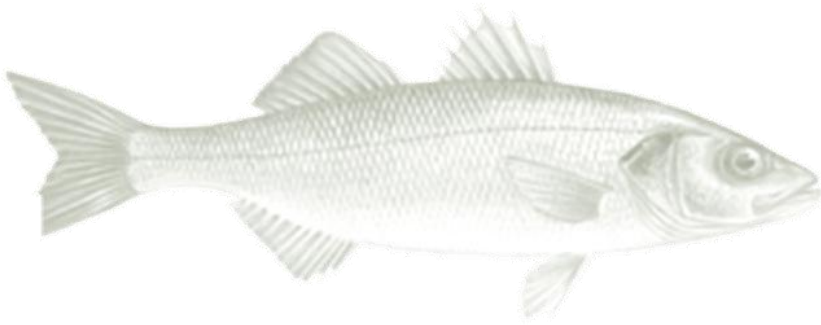
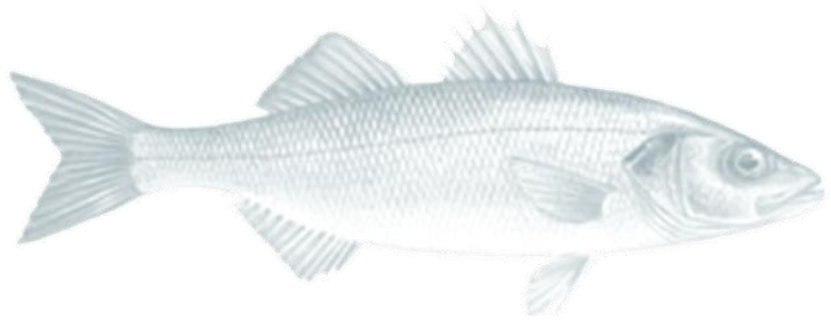
L'analyse des réponses comportementales nous a permis d'évaluer les effets des perturbations environnementales sur l'individu, comme détaillé précédemment, mais cela nous a également permis d'identifier des comportements stéréotypés chez le bar Européen.

Au cours des tests du nouvel environnement (open field tests ; chapitre 1 et 2), les juvéniles de bar ont systématiquement exprimé une première période d'agitation (<1min). Suite à cela, les individus ont montré une période de prostration, avec une diminution notable de l'activité natatoire voir une complète immobilité. Ce comportement disparaît sur une période d'environ

20 minutes après le début de l'exposition à l'environnement nouveau (chapitre 2). Les individus reprennent peu à peu une activité exploratoire en favorisant la nage le long des parois de l'arène expérimentale (thigmotaxie). Même si plusieurs études rapportent une augmentation graduelle de l'activité d'exploration en réponse à une exposition à un nouvel environnement chez divers taxons (*i.e.*, mammifères, oiseaux, téléostéens; Archer & Birke, 1983; Lister, 1990; Renner, 1990, rats; Griffin & Guez, 2014; Jacquin et al., 2017, Trinidadian guppies), à notre connaissance seulement trois autres études ont documenté cette évolution temporelle dans le comportement exploratoire d'un nouvel environnement chez le bar (Milot et al., 2009a; Benhaïm et al., 2013; Ferrari, 2014). Leurs résultats indiquent une reprise de l'activité exploratoire en moins de 40 minutes. Nos observations indiquent qu'il ne faut pas considérer l'évaluation de comportements autres que ceux rattachés à l'exploration chez le bar Européen, dans les 20 premières minutes post-introduction dans un nouvel environnement. Sur ce principe, nous avons laissé les individus s'acclimater à l'arène expérimentale durant 1h avant d'évaluer l'activité et la cohésion du groupe en réponse à la simulation d'une attaque de prédateur (chapitre 3).



PERSPECTIVES



Ce travail de thèse a abouti sur l'obtention de résultats qui ont été reportés dans le schéma conceptuel sur lequel s'appuyait l'étude. Les changements de comportements individuels y sont étudiés, en tenant compte de leur valeur intégrative de l'état interne de l'animal et de leurs rôles dans les dynamiques écologiques (interactions intra- et inter- populationnelles). Cependant, les différentes observations soulèvent certains questionnements qu'il serait intéressant de pouvoir approfondir dans de futures études. Premièrement, l'effet de narcose suggéré par les altérations comportementales résultant de l'exposition à des hydrocarbures pétroliers, pourrait être étudié plus finement. Deuxièmement, les changements dans les conditions environnementales ne surviennent pas forcément de façon isolée et il serait enrichissant de développer une approche multi-stress pour évaluer de possibles effets synergiques. Troisièmement, il serait pertinent d'affiner notre approche en identifiant la personnalité de l'individu au sein du groupe et les possibles conséquences de ses changements sur la dynamique du groupe.

Etudier plus finement l'effet narcose

Les altérations comportementales observées suite à l'exposition aux hydrocarbures pétroliers suggèrent un état de narcose chez les juvéniles de bar (chapitre 2). Il serait intéressant de pouvoir évaluer plus finement ce phénomène et ses conséquences sur l'individu. Le mécanisme d'action des composés narcotiques, tels que les hydrocarbures aromatiques polycyclique (HAP), consiste en leur introduction dans la bicouche lipidique des membranes biologiques (van Brummelen et al., 1998). Leur interférence avec la fluidité membranaire des cellules neuronales perturbe particulièrement les fonctions du système nerveux central et les voies de signalisation cellulaire (van Wezel & Opperhuizen, 1995; van Brummelen et al., 1998; Barron et al., 2004; Incardona et al., 2004; Hsieh et al., 2006; Gonçalves et al., 2008). Le comportement animal est particulièrement corrélé avec les niveaux des neurotransmetteurs cérébraux, les capacités sensorielles et les fonctions enzymatiques cérébrales (Alanärä et al., 1998; Elofsson et al., 2000; Hofmann & Fernald, 2000; Höglund et al., 2001; Scott & Sloman, 2004).

Nous pourrions envisager de conserver une approche comportementale en choisissant des tests permettant d'évaluer les capacités de détections des stimuli (positifs ou négatifs) et d'estimer si l'intégration de ces signaux par le système nerveux central conduit à une réponse comportementale appropriée ou non suite à l'exposition aux hydrocarbures pétroliers. Dans

cette optique, des tests en canal à double flux pourraient être réalisés. Ces tests permettraient d'évaluer le positionnement des individus entre deux courants contenant des odeurs différentes (*e.g.*, prédateurs, congénères, nourriture) et d'établir ainsi leur capacité de détection et d'analyse de ces stimuli.

D'autre part, nous pourrions songer à combiner ces approches comportementales avec des techniques d'histologie ou de neurobiologie. Les systèmes sensoriels des animaux exposés pourraient être observés afin d'évaluer si les voies de signalisation cellulaire sont affectées. En effet, nous pourrions regarder si les tissus et notamment les membranes d'organes sensoriels comme les bulbes olfactifs ou les nerfs optiques sont altérés suite à une exposition aux hydrocarbures pétroliers. Ces observations pourraient faire l'objet d'un suivi post-exposition afin d'affiner notre analyse de la récupération suite au stress.

Aborder une approche multi-stress pour évaluer de possibles effets synergiques

Les facteurs de stress environnementaux sont susceptibles d'influer sur les organismes par des processus uniques, cumulatifs ou synergiques (Adams, 2005). Les forçages environnementaux sont multiples et de diverses natures, naturelle ou anthropique. Ces forçages peuvent agir simultanément sur l'écosystème. Aussi, il est possible que plusieurs facteurs environnementaux interagissent les uns avec les autres et causent ensemble des effets synergiques. Bien que le besoin d'évaluer les effets interactifs des multiples facteurs de stress soit prouvé, il persiste un manque d'information au sujet de la possible synergie de leurs conséquences sur les organismes marins. Les études multi-stress en sont à leurs prémices, mais il semble indispensable de les développer dans un futur proche pour pouvoir mieux comprendre les conséquences des multiples changements environnementaux sur les organismes et l'écosystème dans son ensemble.

Mon travail de thèse a consisté à évaluer séparément, les effets de la privation de nourriture et d'une exposition à des hydrocarbures pétroliers, sur les poissons et à interpréter les changements comportementaux observés en les replaçant dans un contexte écologique intégrateur. Nous pourrions étudier si l'association de ces deux facteurs a un effet supérieur à la somme de leurs effets pris séparément. Il serait intéressant de savoir si, en réponse à la privation de nourriture, des poissons dans un état de narcose, suite à une exposition aux

hydrocarbures pétroliers, présentent une réponse comportementale similaire à celle que nous avons observée. On peut en effet, se demander si la narcose affecte la plasticité comportementale de l'individu et si cela a un effet sur le changement de compromis que nous avons observé entre une baisse de la sociabilité et l'augmentation probable du risque de prédation. Aussi, même si l'exposition aux hydrocarbures pétroliers ne semble pas affecter la cohésion du groupe, on peut se demander si des individus à jeun ne changeraient pas de stratégie sociale par rapport à ce que nous avons observé. Enfin, nous pouvons nous demander si l'augmentation du temps d'exposition dans la zone à risque, observée chez les poissons exposés aux hydrocarbures pétroliers, serait accentuée par le jeûne.

Identifier la personnalité de l'individu au sein du groupe

Si la méthode de tracking vidéo utilisée dans cette étude est parfaitement fonctionnelle pour le suivi d'un individu isolé, certaines limites techniques ont été rencontrées durant cette thèse. Des progrès techniques restent encore à faire pour permettre d'étudier les comportements individuels au sein d'un groupe de plusieurs individus sans marquage externe. Ce suivi est pour l'instant trop imprécis pour être totalement exploité. Par exemple, lorsque des individus sont trop proches ils peuvent être confondus et il n'est donc pas possible d'avoir le suivi individuel de chaque membre du groupe sur toute la période de l'expérimentation. Pourtant, il serait intéressant de pouvoir aller plus loin dans ces analyses en identifiant possiblement des individus moteurs au sein du groupe ou bien de pouvoir identifier les précurseurs d'un signal anti-prédateur transmis au reste du groupe.

Il est dans les objectifs actuels des études comportementales de considérer justement ces approches individuelles dans le contexte social d'un groupe. Il a été suggéré que le positionnement spatial des individus à l'intérieur du groupe est particulièrement influencé par leurs différences de personnalité mais aussi leur taux métabolique et leur capacité de nage (Killen et al., 2012; Marras et al., 2012; Metcalfe et al., 2016). Par exemple, la personnalité audacieuse des individus est souvent associée à un large registre aérobie et à un positionnement à l'avant dans le groupe. Ce positionnement leur permet d'avoir un meilleur accès à la nourriture et donc une croissance supérieure, et ceci malgré un coût métabolique supérieur, qui est compensé par un registre aérobie plus important que les autres (Krause et al., 1992; Krause, 1993a, 1993b).

Par ailleurs, le positionnement des individus au sein du groupe joue un rôle primordial dans la diffusion des informations entre les membres (Marras et al., 2012). Lorsqu'une information est perçue, sa transmission peut prendre la forme d'une chaîne de réaction dans le groupe (Oliveira et al., 2017). Par exemple, lorsqu'une menace de prédateur est perçue, elle est souvent détectée initialement par des individus situés en périphérie du groupe. Le(s) poisson(s) détectant le prédateur va(ont) montrer un comportement défensif en réagissant à cette menace (*i.e.*, modification de l'orientation et/ou vitesse de nage par rapport à la position du prédateur). Le comportement anti-prédateur est perçu par les membres voisins qui à leur tour réagissent et diffusent encore plus largement l'information d'une menace imminente dans le groupe, (Oliveira et al., 2017). Cependant, si des perturbations environnementales modifient la personnalité des individus et/ou leur positionnement au sein du groupe, on peut supposer que la diffusion des informations et la réponse comportementale du groupe face à des facteurs de stress comme la prédation, pourraient être altérées.

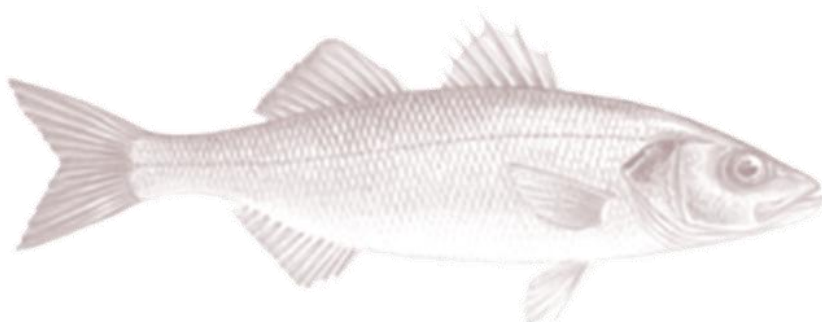
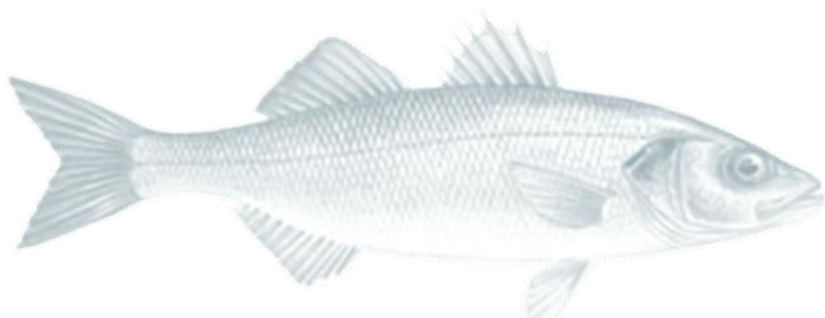
Le positionnement des individus dans le groupe est susceptible d'être contexte-dépendant et de varier si les conditions environnementales changent. Des questions subsistent donc quant aux possibles impacts des changements environnementaux sur la personnalité des membres du groupe, et donc leur influence sur les actions collectives. Il a par exemple été montré qu'une pénurie de nourriture peut modifier la position des individus au sein du groupe. Les poissons positionnés à l'avant du groupe se placent à l'arrière lorsqu'ils sont rassasiés, afin de réduire le risque de prédation et le coût énergétique de la nage associés à la position frontale dans le groupe (Krause et al., 1992). Cependant, ce changement d'organisation entre les membres du groupe a possiblement des conséquences sur les actions collectives et la diffusion d'information.

Par ailleurs, les relations entre traits de personnalités définissant les interactions intra-populationnelles, sont à la base de la structure des populations et sont donc particulièrement pertinentes à suivre dans l'étude d'impact des changements environnementaux. L'analyse des différences de personnalités au sein d'un groupe, pourrait également nous permettre de pousser plus loin l'étude des corrélations entre traits comportementaux comme abordé dans le chapitre 1. Suite à l'identification de différence de personnalités au sein d'un groupe et de possibles corrélations entre traits comportementaux, il serait enrichissant d'évaluer comment les perturbations environnementales peuvent moduler ces liens. Au cours des 10-15 dernières

années, les études sur la personnalité animale se sont intensifiées et un concept associé est né, le concept de syndrome comportemental (Gosling, 2001; Sih et al., 2004a; Dingemanse & Réale, 2005; Réale et al., 2007; Gosling, 2008; Réale Denis et al., 2010). Ce concept est défini comme un ensemble de comportements corrélés soit dans un contexte comportemental donné (*e.g.*, corrélation entre les comportements d'alimentation dans différents habitats), soit dans différents contextes (*e.g.*, corrélation entre les comportements alimentaires, anti-prédateurs, agressifs; Sih et al., 2004b). Pour un même syndrome, les individus présentent différents types de comportements (*e.g.*, plus agressifs vs moins agressif). Un syndrome comportemental reflète donc des différences entre individus constantes dans de multiples situations (Sih et al., 2003, 2004a; Bell, 2007; Castanheira et al., 2013). Cependant, les changements environnementaux pourraient modifier les relations entre les comportements définissant un syndrome. Il a été suggéré que des syndromes comportementaux peuvent être régis soit par des mécanismes communs, soit indépendants (Sih et al., 2004a). Si les comportements corrélant entre eux sont sous l'influence de mécanismes communs (*e.g.*, effet d'un gène pléiotropique) leur corrélation sera plus difficilement découplée que pour deux traits comportementaux gouvernés par des mécanismes indépendants. Comme identifié dans le chapitre 1, les comportements de prise de risque et de sociabilité semblent corrélés et leur relation serait dépendante des conditions environnementales. Aussi, il serait intéressant de chercher à confirmer l'existence de ce syndrome comportemental chez le bar Européen.



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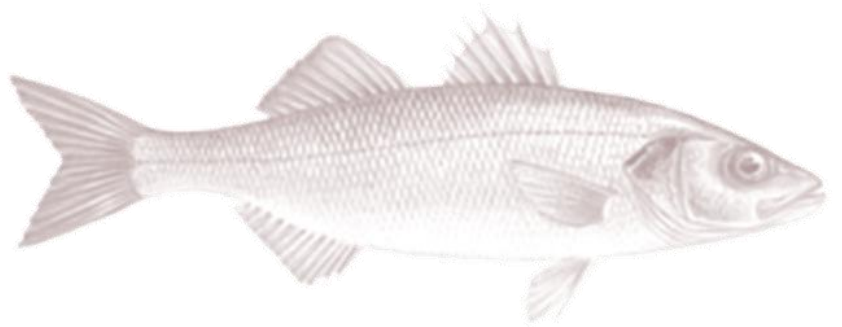
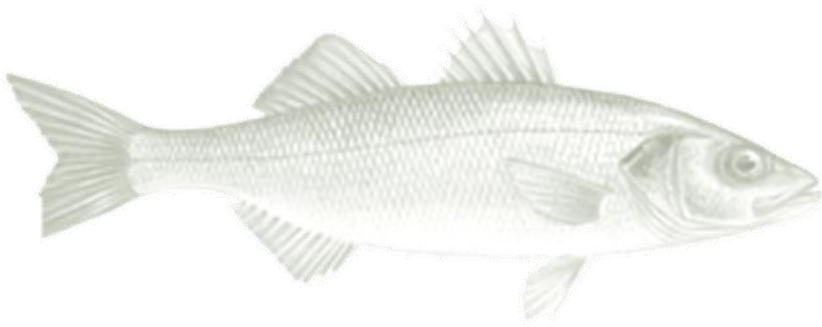
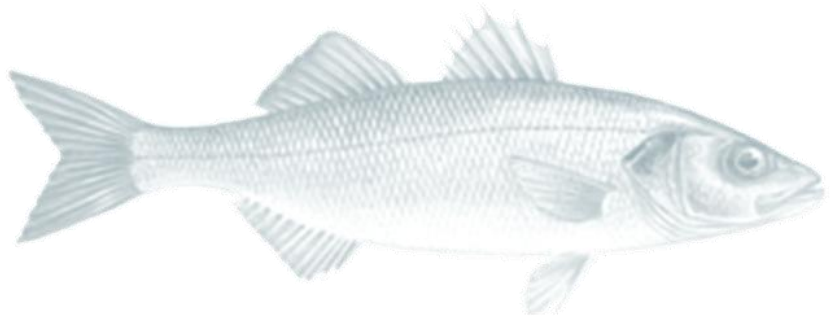
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ANNEXES



Annexe A - Publications et actes de congrès générés par ce travail

Publications

- **Aimon, C.**, Le Bayon, N., Le Floch, S., Claireaux, G., 2019. Food deprivation reduces social interest in the European sea bass *Dicentrarchus labrax*. J. Exp. Biol. jeb.190553. <https://doi.org/10.1242/jeb.190553>
- **Aimon, C.**, Lebigre, C., Albert, L., Le Bayon, N., Le Floch, S., Claireaux, G., 2019. Effects of dispersant treated oil upon exploratory tendency in juvenile European sea bass (*Dicentrarchus labrax*). (in progress)
- **Aimon, C.**, Lebigre, C., Le Floch, S., Lacroix, C., Le Bayon, N., Claireaux, G., 2019. Effects of dispersant treated oil upon group cohesion and anti-predator response in juvenile European sea bass (*Dicentrarchus labrax*). (in progress)
- Ollivier, H., **Aimon, C.**, Zambonino, J.L., Claireaux, G., Effect of chronic exposure to mild hypercapnia on the metabolic rate and behaviour in the European sea bass (*Dicentrarchus labrax*). (in progress)

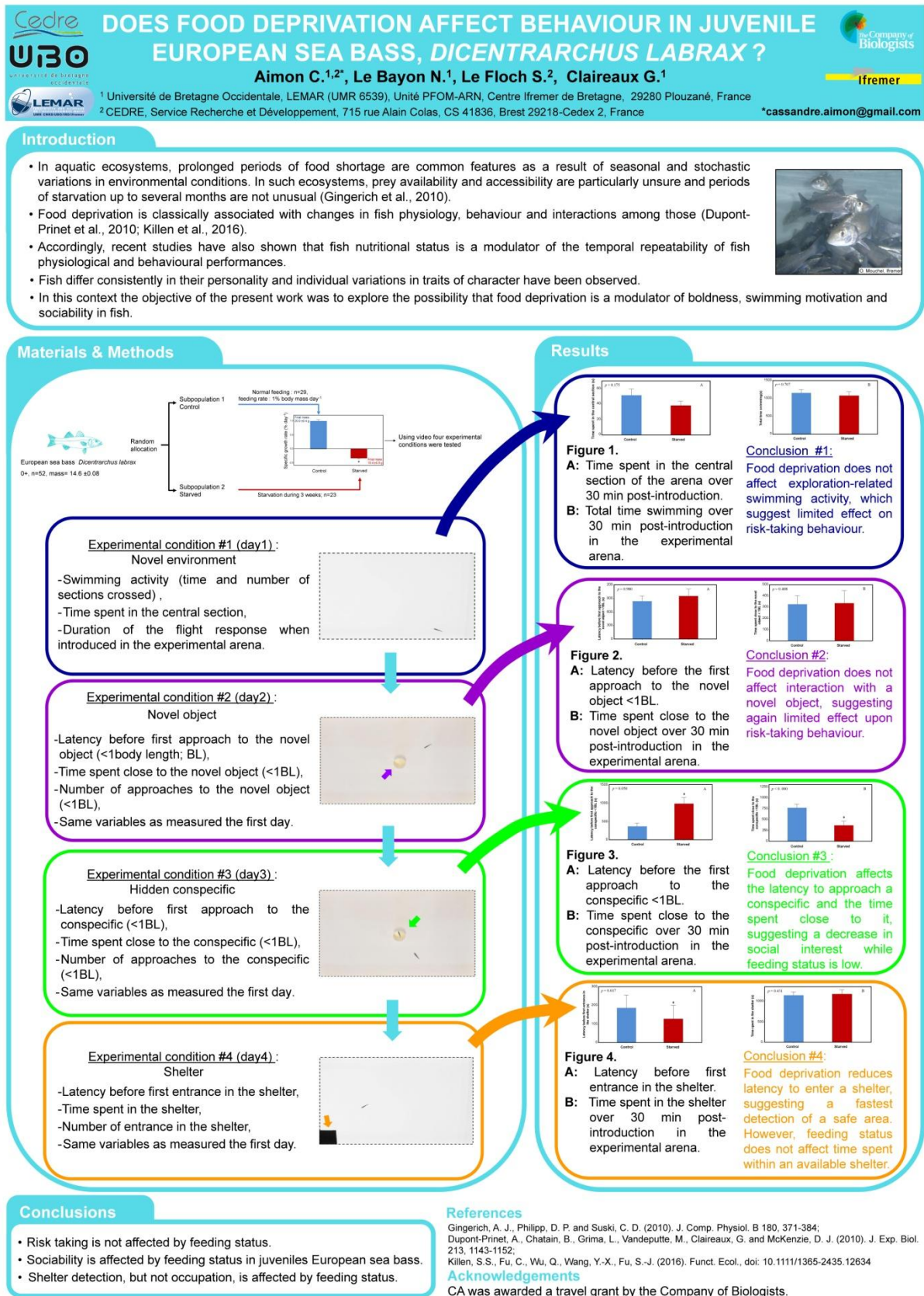
Communications orales

- **Aimon, C.**, Lebigre, C., Albert, L., Le Bayon, N., Le Floch, S., Claireaux, G., 2017. Effect of dispersant-treated oil on the behaviour of the European sea bass, *Dicentrarchus labrax*. Society for experimental Biologists annual meeting, 2017, Göteborg, Suède.
- **Aimon, C.**, Ollivier, H., Claireaux, G., 2018. Behavioural and physiological response to ocean acidification makes juvenile European sea bass more relaxed. Society for experimental Biologists annual meeting, Florence, Italie.

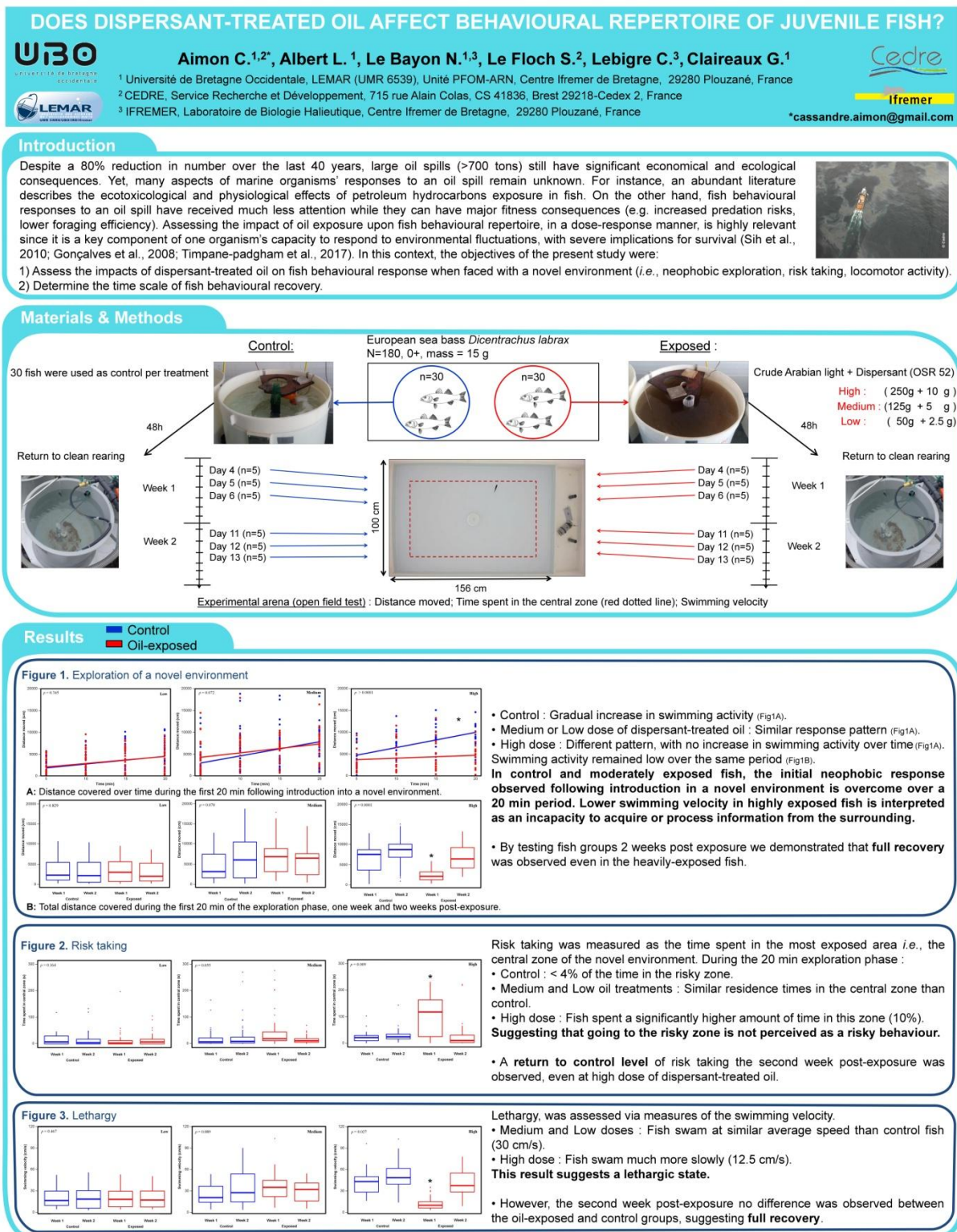
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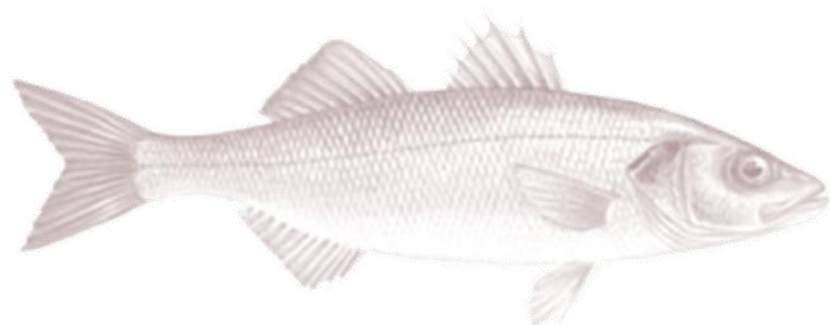
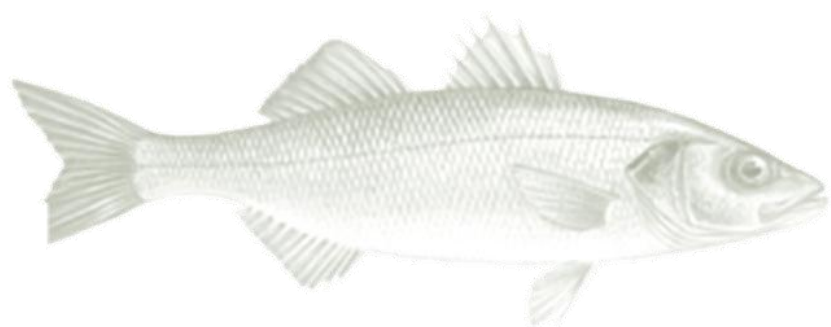
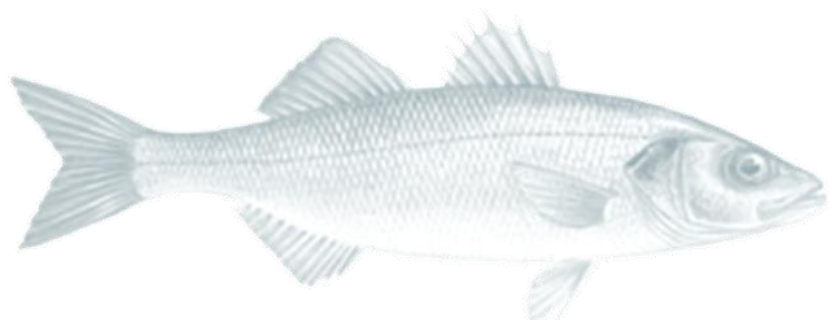
- **Aimon, C.**, Le Bayon, N., Le Floch, S., Claireaux, G., 2016. Does food deprivation affect behaviour in juvenile European sea bass, *Dicentrarchus labrax*? Society for experimental Biologists annual meeting, Brighton, Royaume-Uni.
- **Aimon, C.**, Albert, L., Le Bayon, N., Le Floch, S., Lebigre, C., Claireaux, G., 2018. Does dispersant-treated oil affect behavioural repertoire of juvenile fish? Society for experimental Biologists annual meeting, Florence, Italie et Journée de l'EDSML, février 2018.

Annexe B - Poster congrès annuel de la Society of Experimental Biology de 2016, Brighton, Royaume-Uni



Annexe C - Poster congrès annuel de la Society of Experimental Biology de 2018, Florence, Italie





Titre : Effet de l'environnement sur les stratégies comportementales du bar Européen *Dicentrarchus labrax*.
Cas d'une pénurie de nourriture et d'une marée noire

Mots clés : Plasticité comportementale, sociabilité, exploration, prise de risque, privation de nourriture, marée noire

Résumé : Les écosystèmes marins sont soumis à une large palette de forçages naturels ou d'origine anthropique. Face à ces forçages, les organismes marins font notamment appel à la plasticité phénotypique afin de préserver leur valeur sélective. Dans le cadre de cette thèse, je m'intéresse plus particulièrement à la plasticité comportementale des juvéniles de bar Européens en réponse à deux stress environnementaux, la privation de nourriture et l'exposition à des hydrocarbures pétroliers. L'objectif principal de ce travail est d'analyser les conséquences de ces perturbations à travers une approche intégrative permettant d'évaluer les effets directs au niveau individuel, mais aussi les répercussions indirectes possibles au niveau populationnel et des communautés.

Les tests comportementaux : mis en place ont permis d'évaluer trois traits comportementaux, la sociabilité, la prise de risque et l'exploration. D'un point de vue analytique, une analyse en composante principale a été appliquée afin d'objectiver l'identification des comportements et leur interprétation. Les résultats expérimentaux montrent que le jeûne réduit la sociabilité et que l'exposition aux hydrocarbures pétroliers peut conduire à des altérations de la réponse anti-prédateur. Ces résultats suggèrent des effets néfastes sur la fitness des individus avec des répercussions possibles sur les dynamiques écologiques via une altération des relations intra- (grégarité) et inter-spécifiques (prédateurs/proie). Ces travaux de recherche illustrent combien les régulations comportementales permettent de relier les effets des perturbations environnementales à de multiples niveaux d'organisation, de l'individu à l'écosystème.

Title : Effect of the environment on the behavioural strategies of the European sea bass *Dicentrarchus labrax*.
Cases of food shortage and oil spill

Keywords : Behavioural plasticity, sociability, exploration, risk-taking, food-deprivation, oil spill

Abstract : Marine ecosystems are under a wide range of natural or anthropogenic forcings. In response to these forcings, marine organisms rely notably on their phenotypic plasticity to preserve their fitness. In this thesis, I am particularly interested in the behavioural plasticity of juvenile European sea bass in response to two environmental stressors, food deprivation and exposure to petroleum hydrocarbons. The main objective of this work is to evaluate the consequences of these disturbances through an integrative approach that assesses direct effects at the individual level, but also possible indirect impacts at the population and community levels.

The behavioural tests implemented allowed the evaluation of three behavioural traits : sociability, risk-taking and exploration. From an analytical point of view, a principal component analysis was applied in order to objectify the identification of behaviours and their interpretation. Experimental results show that fasting reduces sociability and that exposure to petroleum hydrocarbons can lead to alterations in the anti-predator response. These results suggest adverse effects on the fitness of individuals with possible repercussions on ecological dynamics through altered intra- (gregarious) and inter-specific (predator/prey) relationships. This research illustrates how behavioural regulations can link the effects of environmental disturbances to multiple levels of organization, from the individual to the ecosystem.