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Condition corporelle et conséquences sur la plasticité des traits d'histoire de vie chez les petits pélagiques de Méditerranée

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Abstract

Multiple changes have been described since 2008 in the Gulf of Lions ecosystem and particularly in small pelagic fish dynamic. In particular, the two main exploited species, i.e. anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) are now smaller and in poor condition while a third species, the sprat (*Sprattus sprattus*) strongly increased in terms of biomass and abundance. This PhD thesis investigated these changes through the analysis of small pelagic fish body condition (i.e individual energy stores) variations, its causes and consequences. Anchovy and sardine body condition was optimal in 2005 and 2006 and decreased after 2008 to remain steady at low level since 2010. Moreover, older sardine have particularly displayed poor body condition since 2008. A part of these changes is associated with changes in zooplankton concentration for both species, but also with both sea surface temperature and diatoms for sardine and Rhône outflow for anchovy. Thus, as bottom-up control was highlighted, the following chapter investigated potential changes in diet through isotope and stomach content analyses. Isotopic niche varied temporally and have been overlapping since 2010 for both anchovy and sardine with the sprat, resulting in a potential new trophic competition. Further, preys are also smaller and probably less energetic. Both studies may explain the poorer body condition by a reduced food supply. Although smaller energy stores are available, both species still allocate a large part of their energy to reproduction, as observed through e.g. gonado-somatic indices. This bias in energy allocation towards reproduction might impair survival and explain the disappearance of oldest mature sardine in the Gulf of Lions. Maternal effects were also underlined. Indeed, large individuals spawn more eggs while fatter individual spawn higher quality eggs. The decrease in size and condition has thus led to a lower production of eggs since 2010 for sardines while anchovy managed to maintain if not increase its egg production thanks to the expanding number of individuals and the decline of anchovy size and age at maturity. At a broader scale, we pointed out that fish body condition decreased in other Mediterranean areas such as the Adriatic Sea, the Catalan Sea or the Strait of Sicily. Nonetheless, body condition variations were not synchronous in the Mediterranean Sea, pointing out the importance of local factors in this quasi-enclosed basin. Furthermore, anchovy displays a better body condition in high energetic areas (fronts areas, local upwellings) when sardine prefers areas with high primary productivity. Those differences could be linked to the different reproductive cycles and trophic behaviours. This PhD thesis complements the previous work made on top-down processes and strengthens the bottom-up importance to determine forage fish body condition both in the Gulf of Lions and the Mediterranean Sea. Studying body condition allowed understanding the reasons of the sardine demographic truncation. Indeed, the older the individual the poorer the body condition, preventing them to achieve both reproduction and survival. This PhD thesis reaches new conclusions and improves knowledge on small pelagic fish dynamic in the Gulf of Lions. Although, further work is still necessary to estimate experimentally if a body condition threshold exists and to improve zooplanktonic monitoring to better understand the link between planktonic productivity and small pelagic fish dynamic. This will help to investigate deeply the ecological and economical consequences of small pelagic fish decreasing body condition in the Mediterranean.

Keywords: anchovy, sardine, sprat, body condition, environmental factors, trophic ecology, Mediterranean Sea, maternal effects, energetic trade-offs

Résumé

L'écosystème pélagique du Golfe du Lion a subi un changement très marqué de la dynamique de population des poissons petits pélagiques depuis 2008. L'anchois (*Engraulis encrasicolus*) et la sardine (*Sardina pilchardus*), exploités économiquement, sont devenus plus petits et plus maigres tandis que le sprat (*Sprattus sprattus*), non exploité car de faible valeur commerciale a fortement augmenté en abondance et en biomasse. Cette thèse analyse les changements observés en se basant sur la condition corporelle, i.e. les réserves énergétiques des individus, et les causes et conséquences de ses variations. La condition corporelle était optimale, en 2005 et 2006 pour l'anchois et la sardine, puis s'est dégradée depuis 2008 pour rester à de faibles niveaux depuis 2010. Les principaux paramètres expliquant les changements de condition corporelle sont la concentration zooplanctonique pour les deux espèces ainsi que la température de surface pour la sardine et le débit du Rhône pour l'anchois. Depuis 2008, les sardines les plus âgées semblent être les plus touchées par cette chute de condition. Le chapitre suivant a permis d'identifier des changements temporels des niches isotopiques qui sont aujourd'hui partagées par l'anchois et la sardine avec le sprat alors qu'elles ne l'étaient pas en 2004-2005. En plus de cette compétition potentielle, les proies aujourd'hui consommées sont de plus petite taille et probablement moins énergétiques. Ces deux résultats peuvent expliquer la plus faible condition actuellement observée par un apport nutritif actuellement réduit. Malgré les plus faibles réserves disponibles, les deux espèces investissent toujours autant dans la reproduction, voire même plus pour la sardine, comme suggéré par plusieurs proxys que sont l'indice gonado-somatique ou la durée de la période de reproduction. L'investissement énergétique semble donc se faire vers la reproduction au détriment de la survie, ce qui explique la disparition des sardines les plus âgées (celles en âge de se reproduire). Des effets maternels ont aussi été montrés, les plus grands individus pondant plus d'œufs et ceux en meilleure condition produisant des œufs de meilleure qualité. Le stock de sardine produit ainsi moins d'œufs depuis 2010 alors que l'anchois en produit plus, ce qui pourrait venir de l'augmentation du nombre d'individus et de l'abaissement de la taille et de l'âge à maturité. Dans une étude à large échelle, nous avons mis en évidence que la baisse de la condition est également perceptible dans d'autres zones de Méditerranée. Néanmoins, les variations de condition ne sont pas régies de façon synchrone en Méditerranée, pointant l'importance des facteurs locaux dans cette mer quasi-fermée. De plus, les anchois présentent une meilleure condition corporelle dans les zones de convergence des masses d'eaux (e.g. fronts, upwellings locaux) alors que les sardines préfèrent les zones de forte production primaire. Ces différences pourraient être liées aux différences dans le cycle de reproduction et dans le comportement alimentaire. Ces travaux de thèse complètent les précédents travaux faits sur les mécanismes top-down et soulignent ici le rôle que joue la ressource alimentaire (contrôle 'bottom-up') dans les variations de condition corporelle dans le Golfe du Lion et en Méditerranée. L'étude de la condition corporelle a aussi permis de comprendre les raisons de la troncation démographique observée chez la sardine. Malgré cela, il est toujours nécessaire d'estimer si un seuil létal de la condition corporelle existe chez nos espèces et d'améliorer le suivi zooplanctonique pour mieux comprendre le lien entre la productivité planctonique et la dynamique de population des petits pélagiques. Cette thèse permet une avancée notable dans la compréhension de la dynamique de population des petits pélagiques et permettra d'appréhender au mieux les répercussions écologiques et économiques de la baisse générale de leur condition dans toute la Méditerranée.

Mots clés : anchois, sardine, sprat, condition corporelle, paramètres environnementaux, écologie trophique, Méditerranée, effets maternels, compromis énergétique

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Chapter 1

Introduction

1.1 Généralités

Les océans et les mers sont exploités depuis plusieurs millénaires, que ce soit pour les ressources biologiques qu'ils contiennent (scène de pêche de poissons sur le littoral chez les Egyptiens dès 2000 ans avant JC) ou les avantages commerciaux qu'ils procurent (développement des routes maritimes plus rapides et sûres chez les phéniciens dès l'an 3000 avant JC). De nombreuses espèces ont ainsi commencé à être intensément exploités, comme par exemple la morue en Atlantique Nord-Ouest (Leavenworth, 2008) ou le hareng en Atlantique Nord-Est (Southward *et al.*, 1988). Mais c'est surtout durant le dernier siècle que l'exploitation des océans s'est diversifiée et a fortement augmenté (Jackson *et al.*, 2001), avec notamment la généralisation du poisson dans l'alimentation et l'arrivée du tourisme balnéaire, induisant un fort forçage anthropique. On peut aujourd'hui déjà voir les effets de la surexploitation des ressources avec l'effondrement de la morue ou du haddock (Hutchings and Myers, 1994), de la pollution avec les micro-plastiques ou les marées noires (Law *et al.*, 2014; Jambeck *et al.*, 2015) et du réchauffement climatique avec les migrations ou disparitions d'espèces induites par les changements d'habitats (Walther *et al.*, 2002; Dulvy *et al.*, 2008). A tel point que des expressions comme « le 6ème continent de plastique » ou encore la « gélification des océans » sont aujourd'hui employées couramment par les scientifiques et journalistes. Au sein de l'océan mondial, la mer Méditerranée est reconnue comme un hotspot de biodiversité, regroupant 10% d'espèces endémiques sur moins de 1% de la surface des océans (Quignard and Tomasini, 2000). Cependant, la Méditerranée est aussi très menacée car cette mer quasi-fermée est soumise à de forts changements climatiques (Lejeusne *et al.*, 2010), à une urbanisation croissante de son littoral regroupant déjà plus 150 millions de personnes (Coll *et al.*, 2012) et une forte pression de pêche (Colloca *et al.*, 2013). Ces changements impactent tous les compartiments biologiques (aussi bien végétaux que animaux) de même que les cycles biogéochimiques (construction de barrage sur les principaux fleuves, débit des fleuves moins réguliers, Adloff *et al.*, 2015). De plus, la situation géographique de la Méditerranée, entourée par pas moins de 23 pays différents rend difficile voire impossible une planification de l'espace ou une stratégie globale à large échelle spatiale.

Le compartiment des téléostéens, aussi bien pélagique que benthique, n'échappe pas à ces nouvelles contraintes. Ce sont les principales espèces ciblées par les pèches, avec divers engins de pêche comme la seine, la chalut ou encore la palangre. En Méditerranée comme ailleurs, le nombre et la puissance des navires, de même que leur efficacité, a fortement augmenté après la deuxième guerre mondiale et durant les années 1960, faisant passer les captures de 0,5 million de tonnes en 1960 à 1,4 million de tonnes au début des années 1990, dont 30% des captures representent les petits pélagiques. Cette tendance est la même à l'échelle planétaire avec une progression des captures de 30 millions de tonnes en 1960 jusqu'à 83 millions en 1990 (FAO, 2014). Quelque soit la zone de la Méditerranée, la plupart des stocks sont aujourd'hui surexploités (Tsikliras *et al.*, 2015), l'exemple le plus parlant étant celui du merlu (Colloca *et al.*, 2013). Avec le réchauffement des eaux Méditerranéennes, les aires de répartition des espèces natives subissent des contractions (Ben Rais Lasram *et al.*, 2010; Albouy *et al.*, 2013) ou des expansions (Sabatés *et al.*, 2006) et doivent de plus faire face à l'arrivée d'espèces invasives. En effet, le détroit de Gibraltar mais surtout le canal de Suez sont des portes d'entrées pour différentes espèces qui sont ainsi aujourd'hui considérées comme implantées en Méditerranée (13% d'espèces de poissons d'origine Lessepsienne sur les côtes Libanaise Harmelin-Vivien *et al.*, 2005, ou encore plus de 40 espèces invasives tout taxon confondus sur les côtes Ouest Méditerranéenne, Galil, 2009). D'autres espèces se distinguent par des changements de leur phénologie (modification de la temporalité des cycles biologiques, Edwards and Richardson 2004; Thackeray *et al.* 2010) ou des modifications de leur physiologie afin de s'adapter au changement climatique (Rijnsdorp *et al.*, 2009). Cela conduit les scientifiques à s'interroger sur le devenir de la plupart des espèces et des écosystèmes à court et moyen terme (Worm and Branch, 2012) aussi bien dans une perspective écologique (maintien de la biodiversité) que dans une optique socioéconomique (maintien des activités liées au monde de la mer, pêche et tourisme par exemple).

1.2 La variabilité démographique, caractéristique majeure des poissons petits pélagiques

Les poissons petits pélagiques sont largement étudiés en raison de leur distribution mondiale et de leur importance à la fois écologique et économique. Ces espèces font le lien entre les compartiments phyto-zooplantoniques et les niveaux trophiques plus élevés tels que les mammifères marins, les oiseaux ou encore le thon et le merlu (Cury *et al.*, 2000; Banaru *et al.*, 2013). Concernant l'aspect économique, ces poissons supportent 30% (FAO, 2011) des pêcheries mondiales, dans le but de fournir des denrées alimentaires pour les humains mais aussi des farines animales pour l'élevage (Metian, 2009).

Les populations de petits pélagiques sont connues pour montrer de très fortes fluctuations de biomasse, enregistrées dans certaines zones sur plusieurs centaines d'années (Valdés *et al.*, 2008), pouvant passer de plusieurs centaines de milliers de tonnes à pratiquement zéro dans les cas les plus extrêmes (Lluch-Belda *et al.*, 1989). Ces fortes variations entrainent des changements majeurs dans la structure de l'écosystème, avec des répercussions sur le niveau trophique supérieur dont ils représentent la nourriture et sur les niveaux trophiques inférieurs sur lesquels ils prédatent. De plus, de par leur importance économique, les très fortes fluctuations ont souvent des conséquences dramatiques pour les communautés de pêcheurs (e.g. sardine en Californie, Radovich, 1982), parfois à l'échelle de pays entiers (e.g. anchois du Pérou, Allison *et al.*, 2009).

Dans les zones les plus productives (forts upwellings, Benguela, Humboldt ou Californie par exemple), les populations de petits pélagiques varient principalement de façon asynchrone (par exemple, le stock de sardine augmente quand celui d'anchois décroît). Cependant, la spécificité des populations Méditerranéennes est qu'elles ne montrent pas clairement d'alternance de dominance inter-espèces, même si l'absence de suivi à long terme empêche de connaitre l'existence de potentiels cycles plus longs que 20 ans. La tendance générale est donc la même pour les anchois (*Engraulis encrasicolus*, L. 1758) et les sardines (*Sardina pilchardus*, W. 1792), même si des différences régionales apparaissent. En effet, des stocks sont surexploités dans certaines zones (anchois de la mer Adriatique, GFCM, 2015), considérés comme non surexploités dans d'autres (anchois de la mer Catalane, GFCM, 2015) ou encore parfois comme en déséquilibre écologique (sardine dans le Golfe du Lion, GFCM, 2015).

Les poissons petits pélagiques vivent dans des environnements spatialement et temporellement très hétérogènes au niveau des ressources qu'ils offrent et de leurs paramètres physico-chimiques. Ils ont donc adopté ce que l'on qualifie de 'stratégie fast' (Sih, 1987), basée sur un cycle de vie court et la production d'un grand nombre de jeunes, très tôt dans le cycle de vie avec une grande mortalité de ceux-ci. Les fortes fluctuations des poissons petits pélagiques sont ainsi expliquées par la grande variabilité de leur recrutement. Celui-ci est principalement déterminé par la survie des larves et des juvéniles qui est fonction de la disponibilité des ressources (hypothèse du Match-Mismatch, Cushing, 1990). Cette hypothèse a ensuite été reformulée par Bakun (1997) qui résume les processus physiques favorisant le recrutemment : i) un processus d'enrichissement supportant la production primaire, ii) un processus de concentration (zones de convergence, de front ou encore stratification des eaux et iii) la rétention des larves au sein de cet environnemnt favorable. En effet, des conditions défavorables sur 1 saison de ponte peuvent induire la perte de la quasi-totalité d'une cohorte, qui pour des espèces vivant entre 3 et 5 ans peut représenter jusqu'à 30 ou 40% des individus. L'inverse est aussi vrai si l'on se trouve dans une année de recrutement exceptionnel, avec une très forte augmentation du nombre d'individus et de la biomasse. Les deux phénomènes associés provoquent ainsi les grandes fluctuations interannuelles marquées par des périodes de fortes augmentations ou de fortes diminutions de la biomasse. C'est en ce point que la situation du Golfe du Lion est particulière, la biomasse étant stable à un niveau assez bas depuis 2008, tandis que le rapport biomasse sur abondance (poids moyen d'un individu) est en fort déclin. En effet, même si le recrutement fluctue, il est resté élevé (GFCM, 2014), c'est-à-dire que l'arrivée de nouveaux individus a été continue et qu'aucune année (excepté 2014 pour la sardine) n'a exhibé de faible taux de recrutement. Ceci est clairement visible sur le nombre d'individus présent dans le Golfe du Lion, qui a plutôt augmenté. Le problème de la chute du rapport biomasse sur abondance provient donc d'un problème au niveau des individus adultes.

De tels changements sont beaucoup plus rares que les importantes fluctuations des petits poissons pélagiques dues à la variabilité de leur recrutement. Ils peuvent résulter de la surpêche, qui de par sa sélectivité vers les individus les plus grands peut causer des troncations dans la structure en taille et en âge (Bianchi *et al.*, 2000; Berkeley *et al.*, 2004). De même, l'augmentation de la mortalité par la prédation naturelle (majoritairement due au thon rouge dans le Golfe du Lion) peut aussi être un autre type de contrôle top-down (contrôle par les niveaux trophiques supérieurs, c'est-à-dire les prédateurs, Hunt *et al.*, 2006) provoquant la disparition d'une partie bien précise des individus (Figure 1.1). Un effet bottom-up (Hunt *et al.*, 2006), c'est-à-dire d'un contrôle par la ressource (nourriture disponible) et les variables environnementales (Figure 1.1), peut lui aussi expliquer le fait que les adultes soient spécialement affectés, sachant qu'il existe des variations ontogéniques de l'alimentation chez les poissons petits pélagiques, avec des proies sensiblement différentes entre larves, juvéniles et adultes (Costalago *et al.*, 2012, 2014). De plus, les coûts énergétiques ne sont pas les mêmes, les adultes devant faire face à leur maintenance (survie), leur croissance mais aussi aux dépenses énergétiques très couteuses pour leur reproduction (Williams, 1966), chose que les juvéniles (non matures) ne font pas. Bien que rarement considérés, des effets supplémentaires (effets transversaux) tels que l'apparition de maladies, ont déjà été à l'origine d'un effondrement des populations de petits pélagiques (Whittington *et al.*, 2008) et devront être étudiés pour déterminer leur influence. Ainsi, malgré leur importance, l'état actuel des connaissances ne permet pas de connaître les tendances futures pour ces espèces qui subissent de plein fouet la pression du changement climatique et de la surexploitation, justifiant le gros travail encore nécessaire pour comprendre les fluctuations des petits pélagiques.

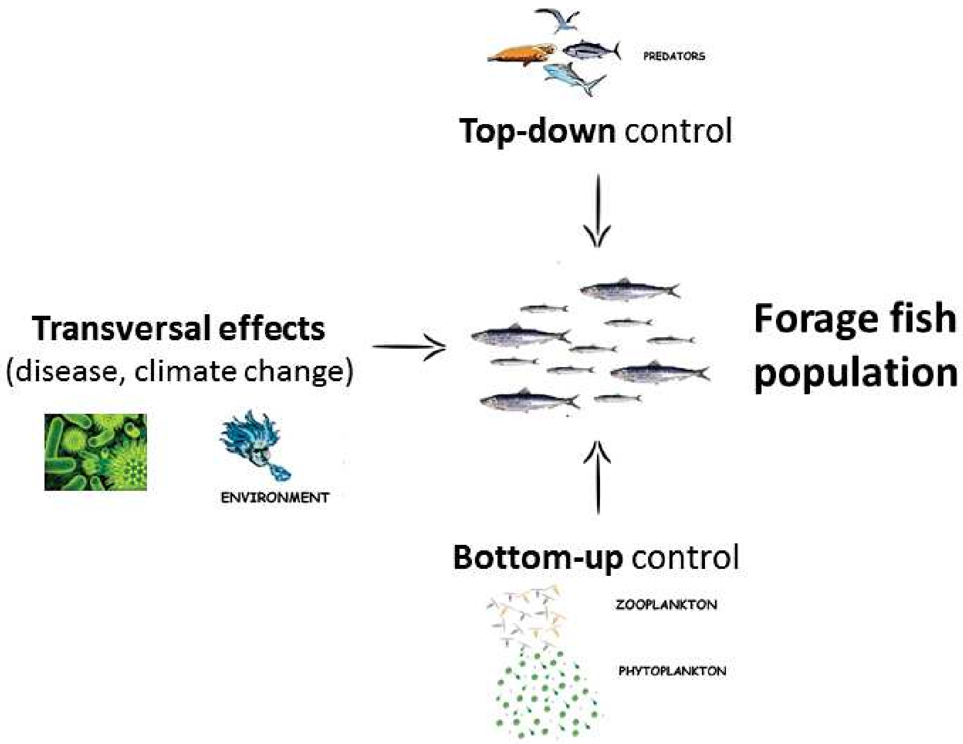

Figure 1.1 Schéma conceptuel des pressions pouvant s'exercer sur des populations de poissons petits pélagiques.

1.3 La condition corporelle : reflet des réserves énergétiques

Une des façons d'étudier la santé d'une population est de déterminer la condition corporelle des individus qui la compose. La condition corporelle est un estimateur des réserves énergétiques d'un individu (Jakob *et al.*, 1996), qui sont au centre du compromis entre les différents traits d'histoire de vie que sont la survie, la croissance et la reproduction principalement. La notion de compromis est très importante à garder en tête et vient du fait qu'aucun milieu n'offre de ressources ad-libitum pour les espèces qui l'occupent, chaque individu se retrouvant donc dans une situation d'énergie limitante (Stearns, 1989). En effet, le manque de ressources (quantitativement), leurs fluctuations au cours de l'année et la compétition inter ou intra espèces induisent une prise alimentaire non suffisante pour être toujours en situation de posséder des réserves énergétiques maximales. De ce fait découle la nécessité pour les individus de prioriser ses besoins afin de savoir quelle stratégie d'histoire de vie adopter, et donc dans quel trait d'histoire de vie investir, l'équilibre entre énergie assimilée et dépensée étant l'élément clé pour son adaptation au milieu naturel. Ainsi, l'allocation de l'énergie dépend des conditions climatiques, de la disponibilité alimentaire mais aussi des caractéristiques de l'individu, principalement son âge, sa taille et ses réserves énergétiques chez les poissons McBride *et al.* (2013). Tout en sachant que cela sera déterminant à posteriori pour la transmission de leur patrimoine génétique aux générations futures, i.e. pour la valeur sélective (fitness) de chaque individu et à terme la dynamique de population des différentes espèces (van Noordwijk and de Jong, 1986; Stearns, 1992).

L'étude de la condition corporelle n'est pas propre à la biologie marine, on trouve en effet un nombre important d'études utilisant ce type de mesure pour expliquer divers phénomènes, comme la baisse des performances reproductive chez les vaches (Pryce *et al.*, 2001), l'expansion des maladies chez les chats (Scarlett and Donoghue, 1998) ou encore les effets sur la migration des oiseaux (Brown and Sherry, 2006).

Cette énergie dont dispose les individus provient de l'oxydation de leur nourriture, qui va alimenter les processus vitaux de l'organisme (maintenance, synthèse de nouveaux tissus (anabolisme) par exemple) mais aussi la fabrication d'adénosine triphosphate (ATP, permettant le maintien du métabolisme). L'énergie restante sera stockée sous différentes formes de composants organiques (principalement lipides et protéines) pour pouvoir ensuite être mobilisée quand l'organisme en a le plus besoin (faibles ressources disponibles ou reproduction par exemple) lors d'une réaction appelée catabolisme. Chez tous les animaux, une part importante de l'énergie est stockée sous forme de lipides, forme permettant une mobilisation rapide de cette énergie dès que les réserves contenues dans le foie sont faibles. Parmi les lipides, les triacylglycerols (TAGs) sont la forme prédominante de stockage d'énergie (Lloret *et al.*, 2014) et sont connus pour varier durant la vie des poissons et durant le cycle annuel. Lors d'une phase de stress nutritionnel, c'est le glycogène et les lipides contenus dans le foie qui sont mobilisés en premier (phase 1 du jeûne), puis les lipides dans les muscles (phase 2 du jeûne appelée jeûne prolongé). Durant ces deux phases, les lipides de réserves seront oxydés et la concentration d'acides gras libre augmente dans le plasma limitant dans un premier temps la déplétion des protéines (Robin *et al.*, 1988; Cherel *et al.*, 1992). L'autre forme majeure de stockage est sous forme de protéine, dans la masse musculaire notamment. Contrairement aux lipides, cette énergie est difficilement mobilisable et demande plus d'énergie pour transformer les protéines en énergie directement disponible pour l'individu (1 gramme de protéine donnera moins d'énergie qu'un gramme de lipides, Reeds *et al.*, 1982). La mobilisation des protéines intervient donc en dernier lieu, lorsque la majorité des réserves lipidiques a été consommées, provoquant un amaigrissement significatif des individus (phase 3 du jeûne, considérée comme critique). Même si cette situation n'est pas irréversible, des changements métaboliques, de même que des changements comportementaux peuvent opérer et les individus doivent rapidement trouver des ressources afin de ne pas mourir d'épuisement (Goodman *et al.*, 1980; Le Maho *et al.*, 1988).

Chez les poissons comme pour la plupart des animaux, la différence dans la vitesse de mobilisation (mobilisation séquentielle) de ces deux types de substrats d'énergie (lipides et protéines) implique naturellement une utilisation majoritaire des lipides comme indicateurs des changements de condition. En effet, du fait de leurs variations rapides et importantes, des différences entre mois, entre populations ou entre zones sont facilement repérables (Rätz and Lloret, 2003). Même si les protéines sont parfois utilisées comme indicateur de la condition (McPherson *et al.*, 2011), leur dynamique est beaucoup plus stable et lisse les variations de condition, empêchant la détection de phénomènes à fine échelle.

1.4 Expliquer les variations de condition corporelle : le cas du Golfe du Lion

Cette thèse est inscrite au sein du projet EcoPelGol (Ecosystèmes Pélagique du Golfe du Lion) qui a pour objectif d'étudier la dynamique de population des petits pélagiques du Golfe du Lion, principalement représenté par l'anchois et la sardine. Au sein de ce projet, les processus top-down, bottom-up et les effets environnementaux ont été considérés en lien avec la distribution, la dynamique, la condition corporelle, les traits d'histoire de vie et les maladies des poissons petits pélagiques.

La condition corporelle peut être affectée aussi bien par des facteurs endogènes qu'exogènes. Plusieurs études ont déjà démontré des différences de conditions suivant l'âge ou le sexe des poissons (Lloret *et al.*, 2002; Ballón *et al.*, 2008) ou encore le lien avec les débarquements (Lloret *et al.*, 2004; Martín *et al.*, 2011) et les variables environnementales (Ballón *et al.*, 2008; Rosa *et al.*, 2010) régissant le milieu des espèces de petits pélagiques. Cette thèse s'intéresse principalement au Golfe du Lion (Figure 1.2), situé en mer Méditerranée Nord Occidentale, couvrant une superficie de 20400 km^2 depuis la frontière espagnole jusqu'à Marseille (Banaru *et al.*, 2014). Le plateau continental est particulièrement large (jusqu'à 60 kilomètres) et cisaillé de nombreux canyons qui additionnés aux forts vents permettent d'importantes remontées d'eau froide alimentant les nombreux upwellings locaux (Millot, 1990; Johns *et al.*, 1992). Les apports importants du Rhône et les upwellings locaux supportent une production primaire significative principalement dans la province néritique et en font l'une des zones les plus productives de Méditerranée (Palomera *et al.*, 2007). Ces caractéristiques font du Golfe du Lion une région très variable et hétérogène au niveau des paramètres environnementaux (forte variation de température, de ressources disponibles), qu'il sera important d'étudier en lien avec les fluctuations de condition corporelle observées.

Figure 1.2 Délimitation géographique du Golfe du Lion.

La condition corporelle de l'anchois (Figure 1.3), de la sardine (Figure 1.3) et du sprat a fortement varié dans le Golfe du Lion durant la période 1993-2014 (Figure 1.4). Pour les deux premières espèces citées, un pic de bonne condition corporelle a été atteint en 2006, pour ensuite décroître et atteindre des valeurs faibles (anchois) voire très faibles (sardine) aujourd'hui. La particularité de la situation dans le Golfe du Lion provient du prolongement dans le temps de cette période de mauvaise condition, qui dans le passé a déjà atteint de très faibles valeurs (par exemple 2003 pour l'anchois ou 1996 pour la sardine), mais sans jamais excéder deux années consécutives. Le sprat a lui aussi une condition qui fluctue mais qui reste stable autour de la valeur moyenne depuis 2007. Cette espèce n'est donc pas en bien meilleure condition que l'anchois ou la sardine. Cette situation inédite pose la question de ce qui peut aujourd'hui empêcher les poissons petits pélagiques de retrouver une condition corporelle semblable à celle qu'ils avaient au milieu des années 2000.

Figure 1.3 Photographie de l'anchois européen (*Engraulis encrasicolus*, A) et de la sardine européenne (*Sardina pilchardus*, B). D'après la thèse d'Aurélie Dessier (2015).

La condition corporelle n'est pas le seul paramètre biologique à avoir fortement varié ces dernières années. La biomasse est à de faibles niveaux pour l'anchois et la sardine alors qu'elle a fortement augmenté pour le sprat depuis 2008 (Figure 1.5). Le poids moyen est lui aussi faible pour les trois espèces et les débarquements se sont éffondrés entre 2008 et 2009 dans le Golfe du Lion et perdurent à de très faibles niveaux pour l'anchois et la sardine (Figure 1.5). Ceci est dû au manque de marché et aux bas prix pratiqués pour des poissons de petites tailles. Afin de conserver une activité rentable, les chalutiers pélagiques se sont maintenant tournés pour la plupart vers le chalutage profond. Tous les indicateurs écologiques et économiques soulignent donc une situation de déséquilibre prolongée au niveau du compartiment des poissons petits pélagiques du Golfe du Lion.

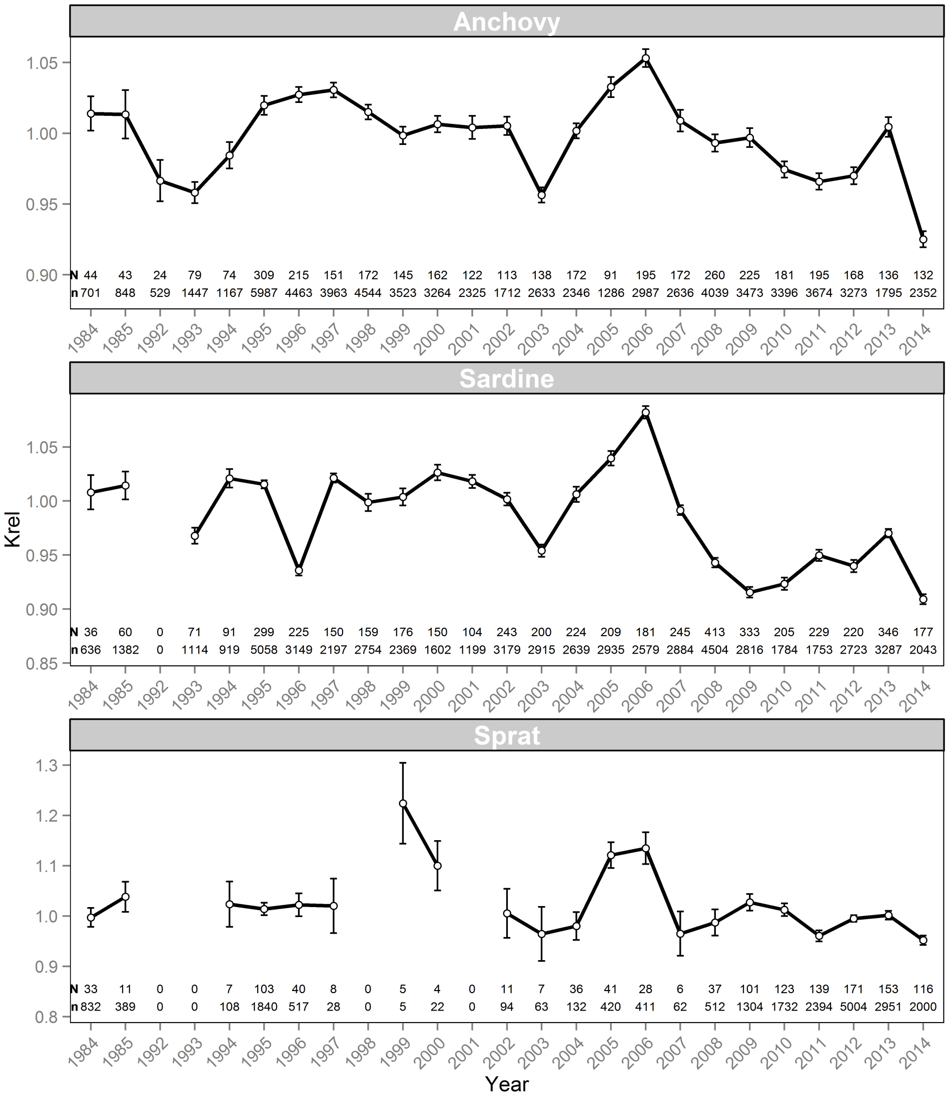

Figure 1.4 Variation de l'indice de condition relative (Krel) entre 1984 et 2014 dans le Golfe du Lion pour l'anchois, la sardine et le sprat. Adapté de Van Beveren et al. 2014.

La pêche est connue pour affecter la structure des populations exploitées, notamment en visant principalement les plus grands individus (Berkeley *et al.*, 2004; Planque *et al.*, 2010). Deux phénomènes peuvent se produire, le premier concerne la surexploitation sur toute la biomasse des reproducteurs qui peut ainsi abaisser le potentiel reproducteur. Mais on sait aussi que les individus les plus grands qui sont visés sont bien souvent les meilleurs reproducteurs (au travers des effets maternels comme une production plus importante d'œufs ou la production de larves de meilleure qualité par exemple), pouvant affecter le recrutement. Ces deux aspects pouvant conduire à l'effondrement des populations, les effets de la surexploitation par la pêcheries ont été étudiés. Dans le Golfe du Lion, l'activité de pêche (« pêche au bleu ») opérée principalement par les chalutiers pélagiques et les lamparos (senneur attirant le poisson

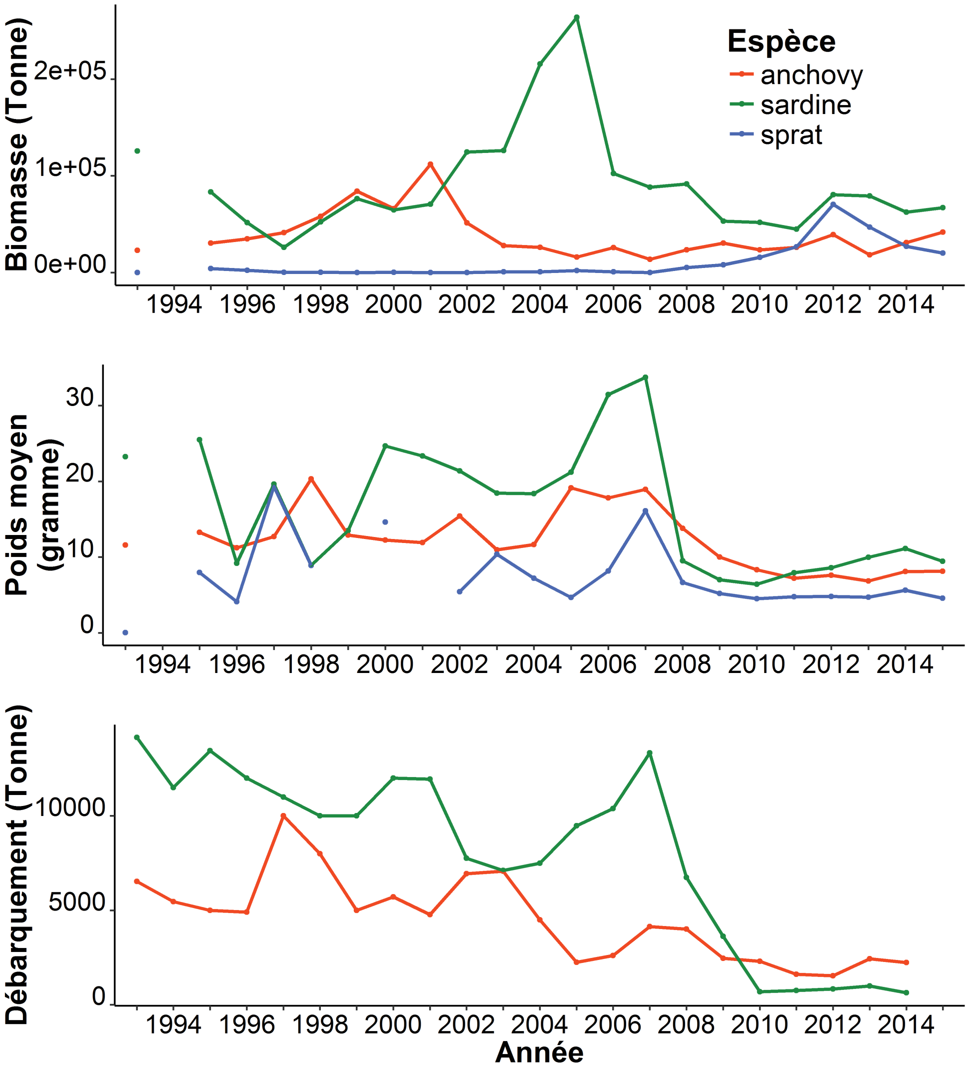

Figure 1.5 Evolution depuis 1993 à 2014 de la biomasse, le poids moyen et les débarquements de petits pélagiques dans le Golfe du Lion.

avec de la lumière), s'est effondrée avec la raréfaction des petits pélagiques de grande taille. Une étude rétrospective, réalisée par Van Beveren *et al.* (2016), a démontré que le seuil d'exploitation a toujours été compris entre 10 et 20% de la biomasse totale de petits pélagiques estimée dans le Golfe du Lion. De plus, les quantités débarquées actuellement sont les plus faibles depuis 150 ans, et ceux malgré le progrès technologique ayant amélioré l'activité de pêche depuis 1850. Basé sur la magnitude et le timing du taux d'exploitation en comparaison des changements biologiques observés, l'hypothèse d'une surexploitation des stocks de petits pélagiques dans le Golfe du Lion n'est pas considérées comme une cause majeure responsable de la situation observée actuellement. De même, le contrôle top-down exercé par les prédateurs principaux des petits pélagiques que sont les thons rouge (*Thunnus thynnus*) a aussi été quantifié (Van Beveren, in review). Leur pression de prédation ne représente que

1 à 2% de l'abondance totale d'anchois ou de sardine, et peut donc être considérée comme très faible. Cela ne veut pas dire que ce type de contrôle n'a aucun effet sur ce qu'il se passe actuellement dans le Golfe du Lion, plusieurs facteurs pouvant agir en synergie, mais aucun des deux contrôles top-down n'est la cause principale de la perte de biomasse et des individus les plus vieux.

Les deux espèces possédant une 'stratégie fast', il est attendu que les individus reproducteurs maximisent la reproduction au dépend de la survie. Le recrutement étant toujours élevé, même en période de faible condition, le compromis favorisant la reproduction pourrait bel et bien être présent. L'étude de l'évolution des traits d'histoire de vie se révèle être aussi une étape importante afin de comprendre la situation actuelle. Ainsi, la piste de la variabilité environnementale, au travers des processus bottom-up et son impact sur les traits d'histoire de vie est donc privilégiée afin de tenter de comprendre les changements observés depuis 2008 dans la communauté de poissons petits pélagiques du Golfe du Lion. En effet, par exemple, les stocks de sardine ont été qualifiés en ‘déséquilibre écologique' GFCM (2015), attestant de la mauvaise santé de cette espèce résultant des changements environnementaux.

Les changements environnementaux et les facteurs endogènes sont donc maintenant les pistes privilégiées pour expliquer les variations de condition. Peu d'études sur les changements environnementaux à long terme ont été menées dans le Golfe du Lion et tout particulièrement sur les compartiments planctoniques (Auger *et al.*, 2014). On sait que dans les régions voisines, des changements dans la quantité et la qualité du plancton ont été mis en lien avec le réchauffement des eaux notamment (mer Ligure, Molinero *et al.*, 2005, 2008, Mer Catalane et Mer des Baléares, de Puelles and Molinero, 2008; Calvo *et al.*, 2011). De tels changements ont très bien pu avoir lieu dans le Golfe du Lion, se répercutant sur les consommateurs du plancton que sont les poissons petits pélagiques.

1.5 Objectifs de la thèse

Les 5 parties de la thèse (Figure 1.6) traitent de façon variée et approfondie des fluctuations de la condition corporelle des petits pélagiques tout d'abord au sein du Golfe du Lion puis de la Méditerranée dans son ensemble. La première partie traitera des aspects méthodologiques pour évaluer au mieux la condition corporelle des petits pélagiques. En effet, les résultats des nombreuses études menées sur différentes espèces de poissons sont très hétérogènes, ne montrant pas toujours de relation significative entre les différents indices couramment utilisés et les réserves énergétiques des individus. Il était donc nécessaire d'étudier dans quelle proportion les différents types d'indices de condition (morphométrique, bioénergétique ou biochimique) reflètent la santé de nos espèces cibles. Ce travail méthodologique pourra ainsi servir dans la suite de ce travail de thèse et dans d'autres régions où sont distribués les anchois, sardines et autres sprats. **Il sera ainsi possible de connaître la façon optimale d'étudier la condition corporelle des poissons petits pélagiques.**

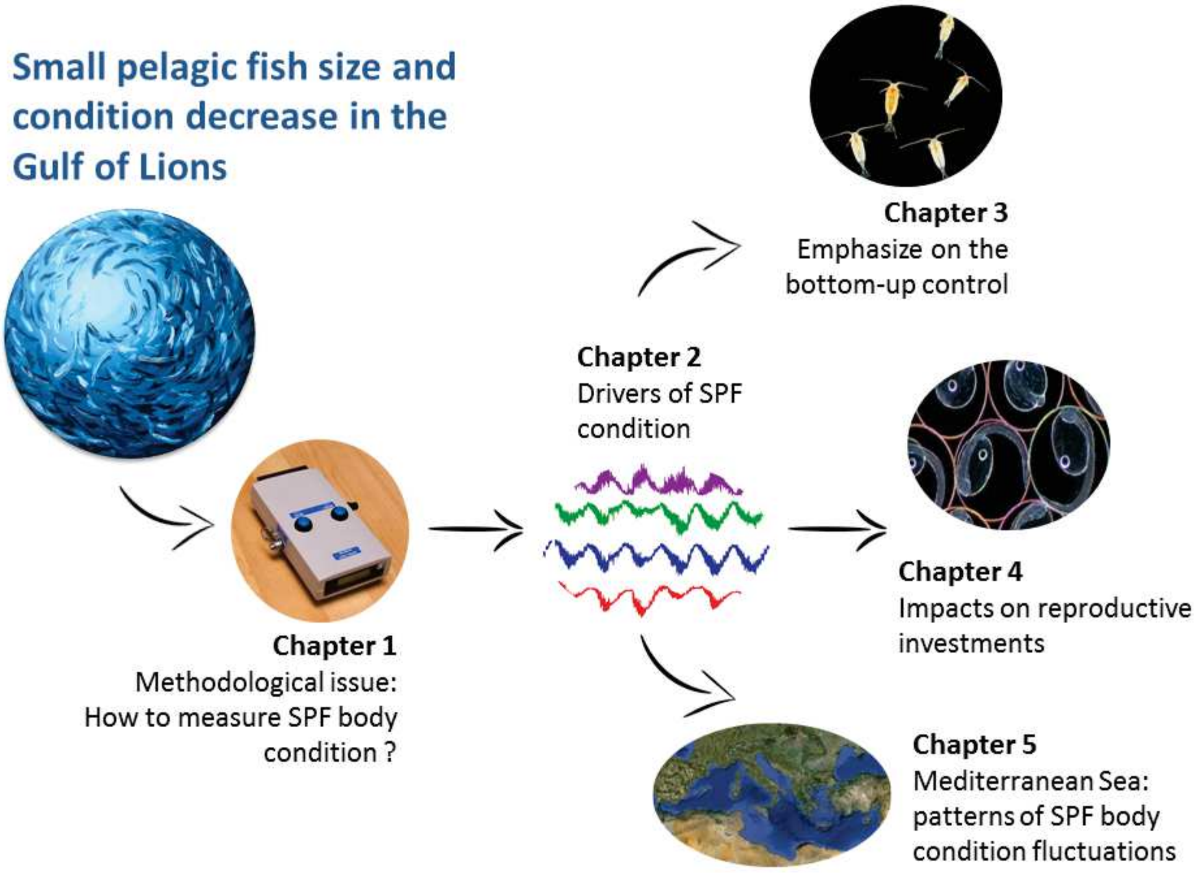

Figure 1.6 Structure et organisation de la thèse.

Le déterminisme de la condition corporelle des petits pétlagiques dans le Golfe du Lion a été étudié. Une série temporelle de données biologiques issues de la campagne océanographique PELMED et de données de pêche, ainsi que des données environnementales (débit du Rhône, concentration planctonique, température des eaux de surfaces) ont été utilisées afin de déterminer les facteurs endogènes et exogènes qui expliquent significativement les variations de condition corporelle des petits pélagiques. En regardant un large nombre de facteurs, ce chapitre vise à détecter des phénomènes et des tendances qui pourront être approfondies dans les chapitres suivants. A la suite de cette étude, l'hypothèse d'un contrôle bottom-up de la condition corporelle a été privilégiée et approfondie dans le chapitre suivant. De même, la disparition des individus les plus âgés soulignée dans une étude précédente nous a poussés à étudier le compromis entre trait d'histoire de vie des adultes.

L'hypothèse du contrôle bottom-up a été explorée à l'aide de deux méthodes complémentaires. Des changements d'alimentation auraient pu intervenir et expliquer les changements observés dans les populations de petits pélagiques. De même, des différences dans la capacité des différentes espèces à acquérir leur nourriture (et donc leur énergie), pourraient expliquer l'accroissement soudain du sprat si un avantage trophique (plus large spectre de proie par exemple) est mis en évidence. Sachant que les trois espèces étudiées se nourrissent dans le même compartiment zooplanctonique (e.g. mesozooplankton), l'augmentation du nombre de sprat pose aussi la question d'une compétition exacerbée entre les trois espèces. Cependant, les suivis planctoniques trop peu nombreux dans le Golfe du Lion ne permettant pas d'étudier si la ressource est limitante, uniquement des hypothèses seront émises sur ce point-là. Des contenus stomacaux ainsi que des rapports isotopiques (carbone et azote) reflétant les conditions contrastées (avant versus après les changements de condition, de croissance des différentes espèces de petits pélagiques) ont été analysés et aident à la compréhension des interactions trophiques interspécifiques et de l'effet bottom-up sur les populations de petits pélagiques.

La surmortalité des individus les plus âgés pose la question de l'allocation d'energie, notamment entre la survie et la reproduction. La faible condition corporelle détectée précédemment (principalement chez la sardine) a posé les bases d'un raisonnement autour du compromis énergétique lorsque les poissons possédent de faibles réserves. En effet, à moins d'être dans des conditions où les ressources sont ad libitum, tous les individus sont soumis à des compromis dans l'allocation de leurs réserves énergétiques entre leur survie, leur croissance et leur reproduction principalement. Le fait que la croissance soit plus faible depuis 2008 pour les deux espèces semble indiquer qu'il pourrait y avoir une réallocation de l'énergie vers d'autres fonctions. L'hypothèse d'une forte allocation vers la reproduction est aussi soutenue par des taux de recrutement particulièrement élevés. Le chapitre traitera de la résolution de ce compromis entre survie et reproduction chez l'anchois et la sardine dans des situations contrastées et en portant une attention particulière à leur stratégie de reproduction opposée (income vs capital breeder). De même, les effets maternels chez les deux espèces seront explorés afin de savoir si les capacités reproductives des deux populations sont affectées par la baisse de la condition corporelle observées ces dernières années.

Les changements observés dans le Golfe du Lion sont-ils uniques ou globaux à l'échelle Méditerranéenne ? Le but est de déterminer si les différentes populations de petits pélagiques sont soumises à un phénomène environnemental de grande échelle ou plutôt affectées par des facteurs locaux. Le dernier chapitre est ainsi une extension sur toute la Méditerranée et la mer Noire de l'étude des fluctuations de condition corporelle des populations de petits pélagiques ainsi que des facteurs environnementaux agissant dans les différentes régions. La zone d'étude s'étend du détroit de Gibraltar (mer d'Alboran) jusqu'à la Turquie (mer Noire et mer Léventine) et regroupe des données récoltées dans 9 GSA (zones géographiques) différentes sur les deux espèces les plus abondantes, l'anchois et la sardine. Ce chapitre tentera d'établir des comparaisons inter zones à la fois temporelle et spatiale pour savoir si des tendances communes dans les variations de la condition corporelle de petits pélagiques sont décelables à large échelle ou bien si les facteurs environnementaux locaux de chaque zone sont prédominants. De même, le côté spatial permettra de déterminer à l'échelle de la Méditerranée quelles sont les conditions propices pour expliquer les différences inter-zones de condition corporelle des petits pélagiques. Effectivement, le fort contraste environnemental entre les différentes zones étudiées permettra de mieux appréhender l'effet des différents facteurs environnementaux que lors d'études séparées à plus fine échelle.

Chapter 2

Matériels et Méthodes

2.1 La condition corporelle : méthodes et applications

La mesure de la condition corporelle peut se faire de différentes manières, chacune présentant des avantages et des désavantages. Sans vouloir faire une review exhaustive de tous les indices de condition utilisés, les méthodes largement utilisées que sont les indices morphométriques, bioénergétiques et biochimiques vont être détaillées.

Les indices morphométriques sont des mesures indirectes des réserves énergétiques par l'emploi de données de taille et de poids. Ils sont faciles d'utilisation et les données sont facilement obtenues en laboratoire ou lors des embarquements. Le principe de base repose sur le fait que pour deux poissons de même taille, le plus lourd sera considéré en meilleure condition. De même, les variations positive ou négative par rapport à la valeur de poids standard (pour une taille donnée) sont interprétées comme une indication des variations des réserves énergétiques de l'individu. L'indice morphométrique le plus couramment utilisée est celui de Fulton (K, Ricker, 1975), calculé en divisant le poids par la taille au cube (Poids/Taille^3). Cependant, il suppose une relation isométrique dans l'évolution du poids et de la taille, c'est à dire que le poids croît proportionnellement à la taille. Pourtant, l'isométrie est rare car pour des raisons fonctionnelles, mécaniques et physiologiques, la forme (et donc le poids) change avec la taille. Ce prérequis est pourtant quelquefois rencontré dans la nature, par exemple pour la morue (Lambert and Dutil, 1997b), mais pour la majorité des espèces qui présentent une croissance allométrique (croissance relative plus ou moins rapide du poids par rapport à la taille), l'utilisation de cet indice est erronée.

Pour les espèces à croissance allométriques, une relation puissance basée sur la relation taille-poids propre à chaque espèce est utilisée avec l'indice de condition relative (K_n, Le Cren, 1951, Poids = a*Taille^b). Cet indice permet de s'affranchir de l'effet de la taille sur la condition. Comme pour l'indice de Fulton, les valeurs de K_n varient autour de 1, et plus on se situe au-delà de 1 plus on est en bonne condition (et réciproquement en mauvaise condition). Les paramètres de la relation allométrique entre le poids et la taille peuvent varier entre différents stocks, entre les sexes ou les classes d'âge, empêchant les comparaisons des valeurs obtenues. Cependant, l'utilisation d'une équation globale permettant d'avoir un référentiel commun et de pouvoir comparer les différences de condition, a été appliqué avec succès (Pardoe *et al.*, 2008; Rideout and Morgan, 2010; Tarkan *et al.*, 2012).

D'autres indices morphométriques sont aussi employés, comme par exemple le poids relatif ou encore l'indice résiduel. Quel que soit l'indice morphométrique utilisé, il s'agit d'une mesure indirecte de la condition corporelle d'un individu, dont l'interprétation est soumise à controverse. En effet, alors que dans certaines études la corrélation entre les indices morphométriques et les réserves énergétiques apparaît clairement (Jakob *et al.*, 1996; Davidson and Marshall, 2010; McPherson *et al.*, 2011), dans certains cas une absence de corrélation a été mise en évidence (araignée, Jakob *et al.*, 1996, mammals Schulte-Hostedde *et al.*, 2005, bird Dombrowski *et al.*, 2003). Basé sur cette très forte hétérogénéité, à la fois entre les différents indices et les différentes espèces, il est aujourd'hui recommandé de calibrer les indice morphométriques avec des mesures directes de contenu lipidique (Green, 2001; Schulte-Hostedde *et al.*, 2005; Stevenson and Woods, 2006; Labocha *et al.*, 2013). Il est ainsi possible de savoir plus précisément ce que les variations de ces indices indirects reflètent au niveau de la physiologie des organismes étudiés.

Ces mesures directes, évaluant la quantité de réserve énergétiques stockée par l'individu, sont aussi utilisées pour mesurer la condition. L'analyses des lipides est la mesure directe la plus répandues dans la mesure de la condition chez les poissons (Adams, 1999; Shulman *et al.*, 2005; Davidson and Marshall, 2010). Si l'on compare aux mesures indirectes, ces indices sont chronophages et nécessite de connaître la physiologie de l'espèce étudiée compte tenu des différences de stockage d'énergie entre espèces. Par exemple, un individu appartenant aux clupéidés (et plus largement aux espèces pélagiques) stocke majoritairement l'énergie dans les muscles alors qu'une morue (Gadiformes, et plus largement les espèces démersales), stocke majoritairement leur énergie dans le foie. La mesure des réserves énergétiques ne se fera donc pas à partir du même tissu ou organe. Différentes méthodes chimiques existent pour isoler les lipides, basées sur les propriétés polaire et apolaire de différents solvants (chloroform/méthanol/eau) comme celles développées par Folch (Folch *et al.*, 1957) ou encore Bligh and Dyer (Bligh and Dyer, 1959). Ces mesures biochimiques ont été réalisée sur différents stades de développements des poissons (larve, Fraser, 1989, adultes, Lloret *et al.*, 2005;Røjbek *et al.*, 2014) et liée à des changements des différents traits d'histoire de vie (changement de croissance, dans la reproduction ou dans la survie) affectant à terme la dynamique de ces espèces. Elles sont considérées comme la référence en termes d'étude de la condition, et sont aujourd'hui couramment utilisées malgré leur coût et le temps nécessaire à leur réalisation. De nouvelles techniques plus rapides et moins chères sont apparues, comme l'utilisation d'un Fatmeter pour mesurer directement le contenu en eau d'un individu. Cette technique repose sur la corrélation négative entre le contenu en eau et le contenu lipidique, présente chez tous les poissons. On revient cependant à des estimations indirectes du contenu lipidiques, qui nécessite d'être calibrées et qui sont plus ou moins efficace suivant les espèces et les condition de manipulation (Vogt *et al.*, 2002; Davidson and Marshall, 2010; Goñi and Arrizabalaga, 2010; McPherson *et al.*, 2011).

Après avoir déterminé les avantages et désavantages liés à l'utilisation d'indices direct ou indirect, le type d'étude va aussi déterminer l'utilisation d'un type indice plutôt qu'un autre. Pour des analyses voulant évaluer les variations de condition sur une période temporelle longue ou à large échelle spatiale, nécessitant un nombre important d'échantillons, le coût et le temps nécessaire vont naturellement orienter les scientifiques vers le choix des indices morphométriques. De même, une analyse sur les données provenant de la pêche, contenant souvent uniquement des données taille-poids ne permettra que l'emploi d'indices morphométriques. Au contraire, pour des études ponctuelles nécessitant moins d'échantillons, le contenu lipidique sera privilégié et permettra de mettre en évidence des différences plus fortes que ne l'aurai permit l'emploi d'un indice morphométrique. Quoi qu'il en soit, pour l'utilisation d'un indice morphométrique, sa calibration est préalablement nécessaire afin de pouvoir interpréter les résultats obtenus en termes de processus physiologiques.

2.2 Measurement and analysis of small pelagic fish condition: a suitable method for rapid evaluation in the field

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Abstract

As condition is a key variable in population dynamics (especially for survival, growth and reproduction), the use of well-defined and accurate fish condition indices is capital. In particular, condition indices (morphometric, bioenergetic and biochemical) have never been compared and validated for the European anchovy *Engraulis encrasicolus*, the European pilchard *Sardina pilchardus* and the European sprat *Sprattus sprattus*. The accuracy of two indirect methods, the morphometric relative condition index K_n and the bioenergetics index determined with the Distell Fish fatmeter was investigated by comparing with a direct measure of relative lipid content carried out with a thin layer chromatography-flame ionization detector. Estimations from the fatmeter correlated quite well with the relative lipid contents of all species, regardless of the reproductive period ($R^2 = 0.69$ for anchovy, $R^2 = 0.75$ for sprat and $R^2 = 0.48$ for sardine). K_n correlated more poorly with relative lipid content ($R^2 = 0.22$ for anchovy and $R^2 = 0.41$ for sardine, ns for sprat), especially during the reproductive period, pointing out the difficulty for such an index to precisely reflect changes in fat allocation. During the reproductive period, changes in K_n could reflect other processes, such as changes in protein content. Therefore, these different types of commonly used indices do not reflect exactly the same type of energy stores. The high repeatability of the fatmeter was brought to light, so that only one measurement on each fish side may be necessary to evaluate the relative lipid content of a small pelagic fish. Finally, fatmeter measurements were not affected by freezing storage up to one month for anchovy ($R^2 = 0.66$) and sardine ($R^2 = 0.90$), making it possible to use frozen samples of both commercial and scientific survey. In contrast, the freezing storage for sprat should be avoided. Based on this study, the Fatmeter appears to be a suitable indirect method to assess condition and fat content of sardine and anchovy on a large number of individuals.

Keywords Fatmeter, Anchovy, Sardine, Sprat, Lipids, Mediterranean Sea

2.2.1 Introduction

Body condition is a key variable widely used in ecological studies particularly on fish, mammals and birds to define the nutritional or the physiological status of an individual (Bolger and Connolly, 1989; Stevenson and Woods, 2006). Commonly, body condition is defined as the quantity of nutrient reserves, which represents the quantity of metabolizable tissues exceeding those required for daily nutritional demands (Schulte-Hostedde *et al.*, 2001; Schamber *et al.*, 2009). Body condition indices thus inform on the quantity of energy extracted from the environment and can give for instance important insights on foraging behavior or prey distribution Lloret *et al.* (2014). They are also used as indicators of an individual's well-being which can affect its future performances (Stevenson and Woods, 2006; Wilson and Nussey, 2010). For example, individuals with larger nutritional reserves may have a greater survival rate, a larger reproductive success and a higher growth rate (Millar and Hickling, 1990), ultimately resulting in a link between body condition and fitness for several species Jakob *et al.* (1996). Measuring body condition is thus of the outmost importance for physiologists and ecologists to understand population dynamics (Schulte-Hostedde *et al.*, 2005) and monitor the status of fish stock (Lambert and Dutil, 1997a).

A large number of condition indices are available from literature. They can be sorted in three categories, namely, morphometric, bioenergetic and biochemical indices. While some studies demonstrated a correlation between bioenergetic (measure of the relative amount of fat in one fat depot, mainly in muscles) and biochemical indices (Vogt *et al.*, 2002; Nielsen *et al.*, 2005; Trudel *et al.*, 2005) or between biochemical and morphometric indices (Brown and Murphy, 2004; Pangle and Sutton, 2005), the use of three types of indices differs in terms of both methodology and meaning of the term condition. Morphometric indices are indirect and based on the assumption that for a given size, heavier individuals are in a better condition (Green, 2001). These indices assume that the bulk of available energy reserves is located in somatic and germ tissues, and then that the total weight is a suitable reflection of condition. They are extensively used because of their simplicity and have often been selected to monitor fish health (Lambert and Dutil, 1997a), investigate the effect of marine pollution (Bervoets and Blust, 2003) or manage fisheries (Cone, 1989). Direct estimations of fish condition can also be determined through lipid content, protein or ash quantification (Stevenson and Woods, 2006). As lipids are the first component of energy reserves to be mobilized (McCue, 2010), the majority of studies using direct estimations relies on them, but because of the complexity and the diversity of energy stores, proteins might also be crucial, especially when lipids concentrations are very low. Biochemical lipid quantification methods are time-consuming and expensive, leading to a preferential use of morphometric indices over biochemical ones (Cone, 1989; Schamber *et al.*, 2009). Finally, bioenergetics indices appear as a compromise between morphological and biochemical indices. They estimate lipid content through indirect measurements, e.g water content can be measured as it is strongly and inversely related to fat content (Craig *et al.*, 1978; Simat and Bogdanović, 2012), as is done by the fatmeter (Distell Fish Fatmeter; Kent, 1990). This electronic device provides some advantages because it is easy to use, portable, fast and non-destructive, allowing researchers to keep the individual alive or intact for further analyses. While several authors recommended validating indirect indices against direct measurements of condition, only few studies have investigated the accuracy of indirect indices for fish. When these validations were realized, significant relationships between biochemical and morphometric indices were sometimes missing (Nielsen *et al.*, 2005; McPherson *et al.*, 2011), pointing out that morphometric condition indices were not always indicative of energy reserves.

The aim of this study was firstly to compare three types of indices (morphometric, biochemical and bioenergetics) to understand the different information they provide. Comparative studies that involved three different types of condition indices are rare in literature and focused mostly on herring (Davidson and Marshall, 2010; McPherson *et al.*, 2011). The second aim was to determine a reliable method, easy and quick enough to monitor body condition of small pelagic fish at the population scale (i.e allowing rapid measurements of a large number of individuals).

In this study, for the first time, the use of an electronic instrument that makes non-invasive measurements of water content to determine the relative lipid content of fish (Distell Fish fatmeter MFM-992, Kent, 1990) was investigated for three widespread small pelagic fish species namely the European pilchard *Sardina pilchardus* (Walbaum 1792), the European anchovy *Engraulis encrasicolus* (L. 1758) and the sprat *Sprattus sprattus* (L. 1758). They are the most significant species of the Gulf of Lions in terms of biomass and economic value. Up until recently, the estimation of relative lipid content by a fatmeter was done on medium and large species such as Atlantic herring *Clupea harengus* (L. 1758), eel *Anguilla anguilla* (L. 1758) and carp *Cyprinus carpio* (L. 1758) (Davidson and Marshall, 2010; McPherson *et al.*, 2011; Klefoth *et al.*, 2013). However, the apparition of a new model aiming at smaller fish opened new perspectives on species which could not be monitored before.

In order to know if indirect condition indices reflect lipid storage, the fatmeter and a common morphometric index, the relative condition were compared with biochemical analyzes of lipid content realized with a thin layer chromatography, a direct method considered here as a benchmark. Furthermore, only one study has ever investigated the effects of storage duration on fatmeter measurements (herring, Vogt *et al.*, 2002). Here, various freezing durations were compared to determine precisely when differences were induced on fatmeter measurements. Moreover, the number of replications necessary to obtain reliable estimations of relative lipid content was explored for the first time using repeatability analyses.

2.2.2 Material and methods

Study area and fish sampling

In the Gulf of Lions (42°26′-43°12′N and 3 °09-5°27E), a total of 499 anchovies, 488 sardines and 187 sprats were sampled from January 2013 to February 2014. June and July samples came from a standardized acoustic survey (PELMED) onboard the RV "L'Europe", while the other samples were collected by commercial pelagic trawlers. For each sampling event, the same protocol was used, i.e. fish were randomly selected and measured directly onboard (scientific surveys) or one day after, in which case they were stored in ice (commercial fishing). For each fish, the total length (*TL*, to the nearest 1mm), total wet weight (*W*, to the nearest 1g), sex, maturity stage (based on macroscopic observation of the gonads) and fat content (with Distell Fish fatmeter) were recorded. The usual five maturity stages were also estimated (ICES, 2008): a first stage during which gonads are inactive, two intermediate stages during which they are active, a fourth stage corresponding to the spawning event and a fifth stage during which the gonads are inactive and recovering.

Morphometric index

As the three species exhibited an allometric growth pattern (Van Beveren *et al.*, 2014), the relative condition index K_n (Le Cren, 1951) was used as a proxy of individual fish condition (Green, 2001). Indeed, K_n prevents from the assumption of isometric growth and avoid a potential length effect. The index K_n was computed as:

$$K_n = \frac{W}{Wr}$$

where W is the mass of an individual and W_r is the theoretical mass of an individual of a given total length (*TL* in mm) predicted by a length-weight relationships ($W_r = \alpha TL^{\beta}$).

Length-weight relationships were calculated based on a 30-year dataset including more than 42,000 individuals and were characterized by α=0.00290.0001 and β=3.3020.007 for anchovy, α=0.00380.0001 and β=3.2410.011 for sardine, and α=0.00630.0005 and β=3.0220.003 for sprat.

Bioenergetic index (Distell fish fatmeter)

Based on the strong inverse relationship between water and fat content in fish (Craig *et al.*, 1978; Simat and Bogdanović, 2012), the microstrip sensor (microwave) of the fatmeter estimates the relative fat content (% lipids) of an individual from a permittivity calculation (Kent *et al.*, 1992). The MFM-992 fatmeter used in this study was equipped with a small sensor head (3 centimeters wide), making it more adapted to analyze small fish. For each species the calibration provided by the manufacturer was used (sardine2, anchovy2 and sprat2). Relative lipid content was measured twice on the same location on both sides of the fish (along the lateral line as recommended in user manual) and the average of these four measurements was used as the final value. Even if temperature does not affect fatmeter measurements (Klefoth *et al.*, 2013), all measurements were made following the same experimental conditions to avoid any potential bias.

Repeatability of fatmeter measurements

Repeatability was assessed by testing the similarity between different fatmeter measurements (reliability of measurement). In addition, the number of measurements required to obtain an accurate estimate was determined (Lessells and Boag, 1987). Among all fish available and independently of the subsample for lipid analysis, a random sub-sample of 73 anchovies, 54 sardines and 50 sprats was taken, on which four measurements were made on both sides of each individual (n0= 8). Repeatability (*r*) was computed from the results of an ANOVA as:

$$r = \frac{s^2{}_A}{(s^2 + \mathrm{s}^2{}_A)}$$

where s^2 is the within-group variance component (MSW) and s^2_A is the among-group variance component based on the mean square among groups (MSA) and the mean square within groups (MSW) such as s^2_A = (MSA – MSW) / n0. Here, one group is equivalent to one individual, so all eight measurements. Repeatability values vary between 0 and 1, with a higher value indicating a better repeatability. To evaluate if the repeatability of the fatmeter measurements is size dependent, calculations were also performed per size class. The following classes were considered: small (TL < 105 mm), small intermediate ($105 \leq TL < 115$ mm), large intermediate ($115 \leq TL < 125$ mm) and large ($TL \geq 125$ mm).

Freezing impact on fatmeter measurements

Freezing can lead to water loss in fish tissues. For example, for Atlantic salmon, *Salmo salar* L. 1758, this effect has been documented and the water loss was quantified as ranging from 0.5 % to 5% of the initial individual's mass (Campañone *et al.*, 2001; Regost *et al.*, 2004). Therefore, freezing duration could influence fatmeter measurements. This effect was tested by comparing measurements on fresh individuals and on the same individuals after freezing at -21°C. Two freezing durations were considered: 5 and 31 days, using a sub-sample of 42 anchovies, 42 sardines and 42 sprats.

Chemical analyses of lipids

Immediately after morphometric and fatmeter measurements, a piece of muscle was removed from a sub-sample of 175 fish (60 anchovies, 60 sardines and 55 sprats). As lipids can be allocated both in the muscle and gonads during the reproductive period, gonads were sampled in addition to a piece of muscle for fish sampled in the reproductive period (i.e. 24 anchovies during summer and 30 sprats and 29 sardines during winter) to evaluate the link between fatmeter and total lipid content when a part of lipids is in gonads. The total weight of muscle and gonad of the fish was used to evaluate the relative part of each organ allowing to sum up their relative lipid content. Because in these three species of clupeids the weight of the liver and the stomach was too small in comparison to the other organs (<1%), they were not taken into account. Relative lipid content was thus estimated as follows: Lipid % = (Muscle Weight * Muscle lipid % + Gonad Weight * Gonad lipid % / Fish Weight). Outside the reproductive period, gonad weight was so small that the formula simplified in Lipid % = Muscle lipid %. Lipids were extracted from roughly 0.1g of each sampled tissue using a solvent mixture (chloroform-methanol 2:1, v/v) as described by Folch *et al.* (1957). Then, lipid extracts were spotted to SIII Chromarods (Iatron laboratories) using a 10 μl Hamilton air-tight glass syringe, and lipid classes were separated in a stepwise procedure using developing solvents of increasing polarity (Parrish, 1999). This method separates aliphatic hydrocarbons, sterol and wax esters, ketones, triacylglycerols, free fatty acids, free fatty alcohols, free sterols, diacylglycerols, acetone mobile polar lipids and phospholipids. Between each development, the Chromarods were partially scanned by flame ionization detection (FID) on an Iatroscan MK-VI

(Iatron Laboratories). Chromatograms were analysed using integration software (Peak Simple version 3.29, SRI). Lipid classes were quantified using standard calibration curves obtained for each lipid class. Only one replicate was made according to the high repeatability of the Iatroscan (Choy, pers comm) and all experiments were made by the same person. The proportion of Free Fatty Acids (FFA) was checked to make sure it was not too high ($<1\%$) and lipids had not been degraded due to bad conservation. For the purpose of this study, only total lipid content obtained by summation of individual lipid classes is presented.

Statistical analyses

Influences of sex and maturity stages on the relationship between condition indices were investigated per species with a covariance analysis (ANCOVA) including interactions. This was based on the full dataset and after having tested for the assumption of normality. As all species had a significant effect of maturity stages on the relationship between the relative condition index and Iatroscan values, additional analyses were performed by grouping maturity stages 1 and 5 as representative of resting period and maturity stages 2 to 4 as pre-spawning and spawning periods. This partition allowed us to take into account inactive versus active gonads.

We conducted linear regression analyses between biochemical estimations of relative lipid content and both fatmeter and K_n values. The coefficient of determination (r^2) was used to estimate the proportion of variability explained and the strength of the relationship between the different variables tested. Similarly, linear regression was also carried out per species on the fatmeter values before and after the freezing procedure to determine the relationship between relative lipid content as estimated on fresh fish and on fish having undergone freezing. Furthermore, an ANCOVA was performed to evaluate the potential difference between the regression slopes of the two different freezing durations. To confirm that for linear regression the underlying statistical assumptions were not violated, normality of residuals was assessed by plotting theoretical quantiles versus standardized residuals (Q–Q plots), and homogeneity of variance was evaluated by plotting residual versus fitted values. Analyses were performed with the statistical open source R software (R Development Core Team, 2013). Values are indicated as mean standard error (SE) and all statistical tests were performed at a significance level of 0.05.

2.2.3 Results

Relation between Kn and fatmeter values

Size and condition indices of the three species were summarized in Table 2.1. Interactions (e.g. between fatmeter and sex, fatmeter and maturity and sex and maturity) were not significant (ANCOVA, all $P > 0.05$), highlighting no effect of sex and maturity on the relationship between the two indirect indices. Considering all individuals, the relative condition index K_n was positively correlated with the fatmeter values for all three species (ANCOVA, all $P < 0.001$, respectively $F_{1,499} = 64.26$ for anchovy, $F_{1,488} = 374.34$ for sardine and $F_{1,187} = 43.04$ for sprat, Figure 2.1). However, R^2 values were low for sprat and anchovy (linear model, $R^2 = 0.27$ and 0.08, respectively), while it was higher for sardine (linear model, $R^2 = 0.44$). At the same time, maturity stage showed a significant effect on the relative condition index Kn for all 3 species (ANOVA, all $P < 0.001$, $F_{5,499} = 55.768$; $F_{5,488} = 22.425$ and $F_{5,187} = 14.786$, for anchovy, sardine and sprat, respectively). The higher the maturity stage, the higher the relative condition index for anchovy, while was the opposite for sardine and sprat. Conversely, sex had no significant effect (ANOVA, $P > 0.05$).

Table 2.1 Total length (LT), weight (W), relative condition index (K_n), fatmeter measurements and direct biochemical relative lipid content estimation (Iatroscan lipid content) recorded in the Gulf of Lions for anchovy, sardine and sprat.

Variables	MeanSD	Range	Sample Size
Anchovy			
LT (mm)	114.89 10.57	89-145	499
W (g)	9.12 2.64	4-19	499
K_n	0.97 0.09	0.81-1.20	499
Fatmeter measurments (%)	6.72 1.69	3.5-15.6	499
Iatroscan lipid content (%)	1.82 1.32	0.32-4.97	59
Sardine			
LT (mm)	125.51 12.58	95-160	488
W (g)	14.88 5.29	5-35	488
K_n	1.04 0.126	0.73-1.43	488
Fatmeter measurments (%)	9.98 3.41	4.9-23.1	488
Iatroscan lipid content (%)	2.41 1.65	0.15-6.66	55
Sprat			
LT (mm)	96.75 8.17	75-115	187
W (g)	6.66 1.92	2-12	187
K_n	1.09 0.16	0.72-1.48	187
Fatmeter measurments (%)	18.67 6.37	9.30-35.40	187
Iatroscan lipid content (%)	6.45 4.56	1.96-21.89	59

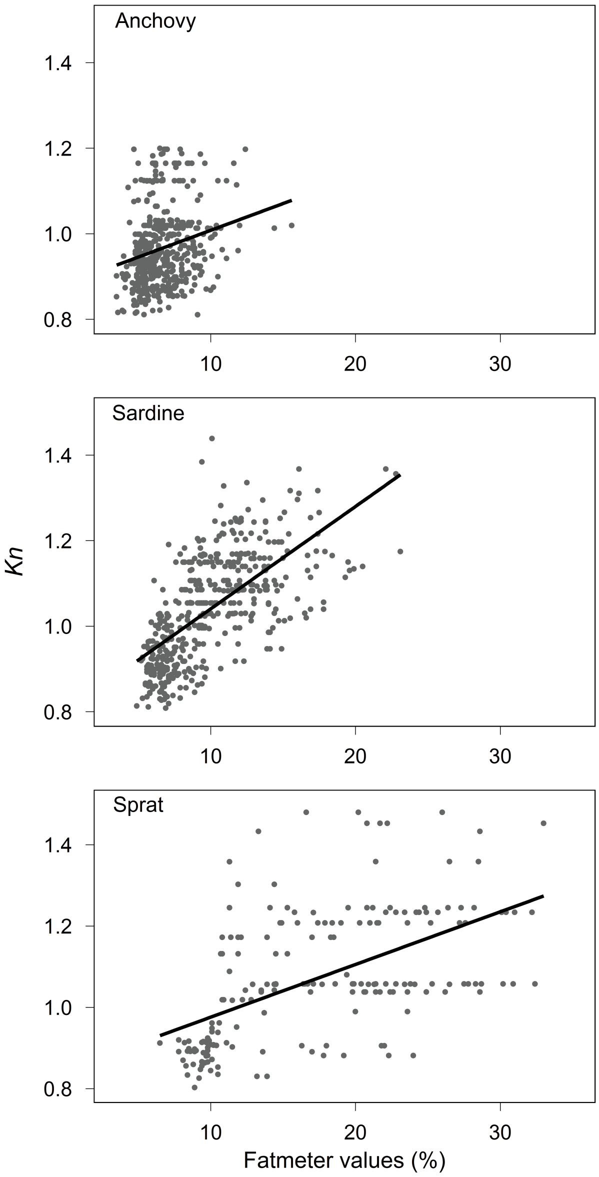


Figure 2.1 Relationship between the relative condition index (K_n) and fatmeter values for anchovy (y= 0.017x + 0.86), sardine (y= 0.025x + 0.79) and sprat (y= 0.010x+0.91). The lines indicate significant linear regressions.

Comparison with biochemical analyses

The sex did not show any significant effect on the relationships between biochemical index and fatmeter values or relative lipid content. Similarly, regardless of the species considered (ANCOVA, all $P > 0.05$). The relationship between biochemical index and fatmeter was not significantly affected by maturity stages for any of the three species (ANCOVA, all $P > 0.05$). Relative lipid contents evaluated by means of fatmeter and biochemical index were positively and highly correlated for all species (linear model, all $P < 0.001$, respectively n =60, R^2= 0.69 for anchovy; n =60, R^2= 0.48 for sardine and n =55, R^2= 0.75 for sprat) (Figure 2.2).

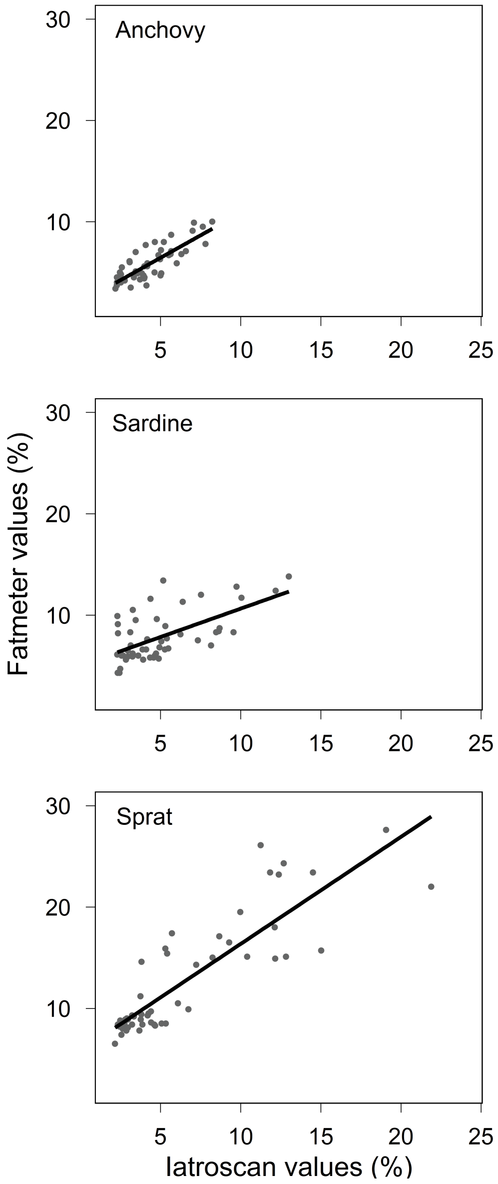


Figure 2.2 Relationship between direct biochemical measurements of relative lipid content (Iatroscan) and fatmeter measurements for anchovy (y= 0.85x + 2.17), sardine (y= 0.56x + 5.08) and sprat (y= 1.06x + 5.81). The lines indicate significant linear regressions.

On the contrary, the ANCOVA pointed out that maturity stages had a significant effect on the relationship between the biochemical index values and the relative condition index (respectively, $F_{5,48} = 13.17$, $P < 0.001$ for anchovy; $F_{5,47} = 4.94$, $P = 0.01$ for sardine and $F_{5,47} = 4.75$, $P = 0.03$ for sprat). Further analyses on the relationship between K_n and biochemical index were thus made by grouping data into two maturity categories: pre-spawning and spawning period versus resting period.

The relationships between K_n and biochemical index values during non-reproductive period were highly variable displaying significant positive correlation in sardine (linear model, n=25, $P = 0.002$, $R^2= 0.41$), weaker significant positive correlation in anchovy (linear model, n=29, $P = 0.02$, $R^2= 0.22$) but a lack of significant correlation for sprat (linear model, n=24, $P = 0.10$) (Figure 2.3).

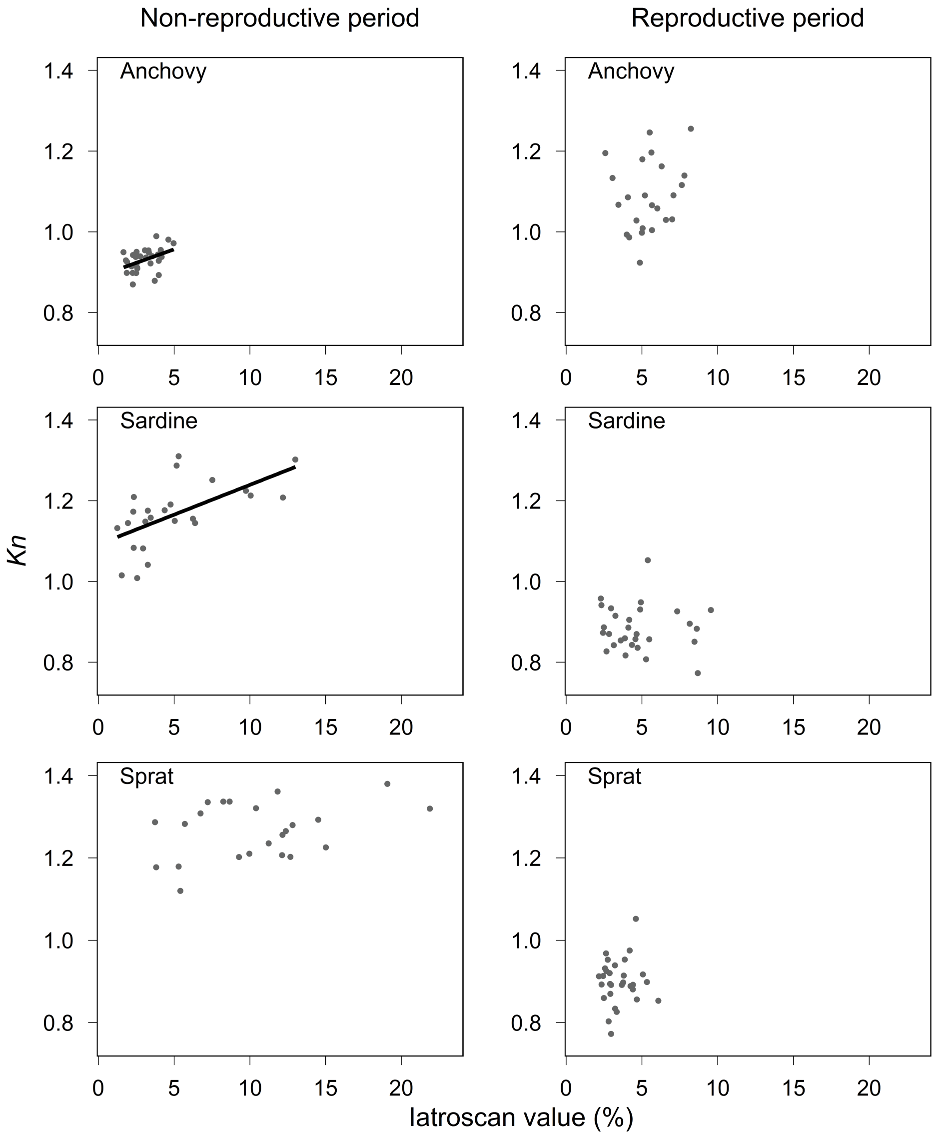


Figure 2.3 Relationship between biochemical measurements of relative lipid content (Iatroscan) and the relative condition index (K_n) depending on the reproductive status (resting reproductive period versus reproductive period) for anchovy (respectively y= 0.013x + 0.89 and non-significant correlation for reproductive period), sardine (respectively y= 0.015x + 1.09 and non-significant correlation for reproductive period), sprat (non-significant for the two periods). The lines indicate significant linear regressions.

During the reproductive period, the relationships between K_n and biochemical index values were all non-significant (linear model, all $P > 0.05$; Figure 2.3). The reproductive period for sardine and sprat induced lipid allocation from muscle to gonads (linear model, respectively, n=24, $P < 0.001$, $R^2 = 0.66$ for sardine and n=30, $P < 0.001$, $R^2 = 0.44$ for sprat) (Figure 2.4). The anchovy did not exhibit any significant pattern (linear model, P = 0.91) (Figure 2.4).

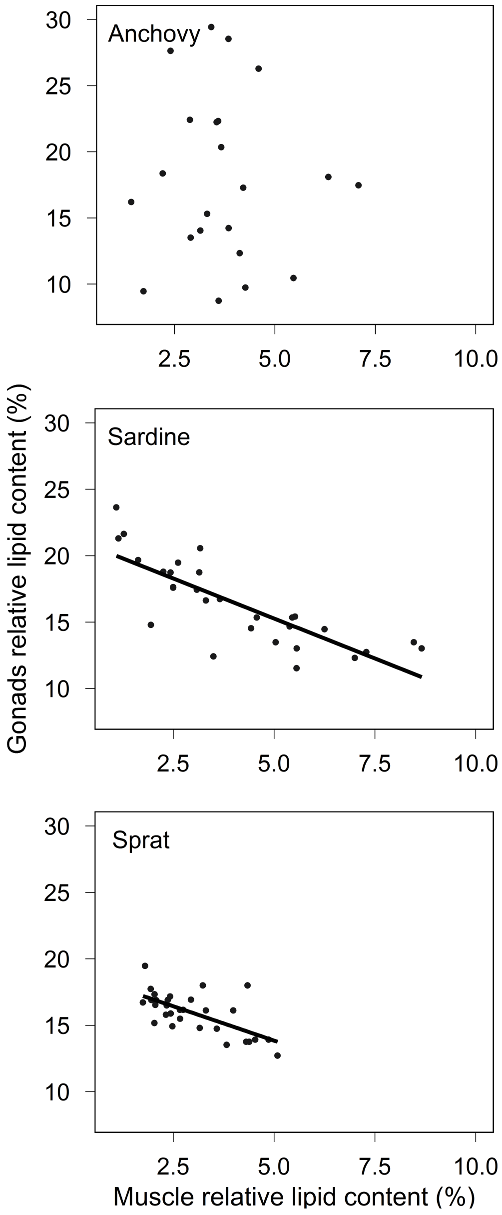


Figure 2.4 Relationship between biochemical measurements of muscle relative lipid content and gonad relative lipid content during the reproductive period for anchovy (y= -0.14x + 20.06), sardine (y= -1.70x + 22.14) and sprat (y= -1.03x + 19.02). The lines indicate significant linear regressions.

Repeatability of fatmeter measurement

The repeatability of the fatmeter was 0.91 for anchovy, 0.96 for sardine and 0.77 for sprat (Table 2.2, SI). Independently of the species, the repeatability of the fatmeter increased with fish size: r = 0.88 ($TL < 105$ mm, n= 17), 0.94 ($105 \leq TL < 115$ mm, n= 68), 0.95 ($115 \leq TL < 105$ mm, n= 41), 0.97 ($TL \geq 125$ mm, n= 23) (Table 2.3, SI).

Impact of freezing on fatmeter measurements

Fatmeter values from the two freezing durations were not significantly different for anchovy and sardine (ANOVA, respectively, $F_{3,38} = 0.249$, P = 0.62, n = 42 for anchovy and $F_{3,38}$ =0.367, P =0.55, n =42 for sardine). Fatmeter values obtained from fresh fish and frozen fish were positively correlated for both species (linear model, n = 42, P < 0.001, R^2 = 0.66 for anchovy; and n = 42, P < 0.001, R^2 = 0.90 for sardine, Figure 2.5). The slope of the regression was significantly different from 1 for anchovy (respectively 0.73±0.09), but not for sardine (0.95±0.06). On the contrary, a significant difference was detected for sprat between the regression slopes at 5 and 31 days of freezing (ANOVA, $F_{3,38}$ = 15.12, P < 0.001, n =42). Fatmeter values obtained from fresh and frozen sprats were positively correlated for both durations (linear model, n = 21, P < 0.001, R^2 = 0.53 and n = 21, P = 0.041, R^2 = 0.19 after 5 and 31-days of freezing) but the slopes differ between each other and from 1 (1.99±0.43 and 0.41±0.19 respectively for 5 and 31 days).

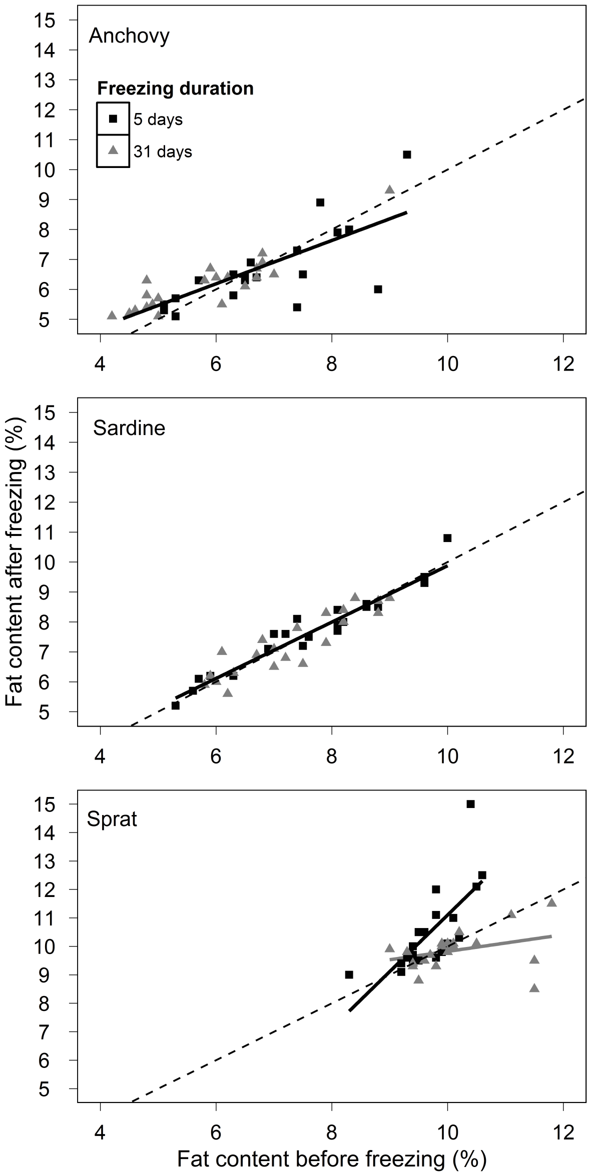


Figure 2.5 Positive linear relationship between relative lipid content measured before and after 5 or 31 days of freezing for anchovy (y= 0.74x + 1.73), sardine (y= 0.95x + 0.47) and sprat (respectively y= 1.99x – 8.78 and y= 0.42x + 5.6926).

2.2.4 Discussion

Comparison of condition indices

This study presents the first comparison of three different categories of condition indices (morphometric, bioenergetic and biochemical) applied to small pelagic fish (except for herring, Davidson and Marshall, 2010; McPherson *et al.*, 2011). Commonly, condition is used as a measure of the energetic status of animals. Individuals in better condition are thus supposed to have greater energy reserves than individual in poorer condition, although energy reserves could reflect a whole range of different energy storage types. For example, fat content is only one component of energy reserves, and measuring physiological condition can also include quantification of protein (Lambert and Dutil, 1997b; Sutton *et al.*, 2000). Proteins are indeed used as an energy source when fat reserves are exhausted (Love, 1970; McCue, 2010), which results in a greater decrease in mass than changes in fat content. Morphometric condition indices that are based on mass and length, such as the relative condition index (K_n) used here should thus vary with both fat and protein content. Therefore, these indices could reflect the shape (i.e the structure) of an individual rather than only its quantity of fat reserves (Stevenson and Woods, 2006). Bioenergetics and biochemical indices were used to estimate relative lipid content both indirectly and directly. Unsurprisingly, stronger correlations were thus found between the biochemical and the bioenergetics index than between these two and the K_n. These results also pointed out differences in correlation depending on the reproductive state of the fish.

During the non-reproductive period lipid storage is mainly located in muscle for clupeid species (Adams, 1999), while during the reproductive period, lipids are also allocated in tissues other than muscles, such as in particular in the developing gonads (Iles, 1984; McBride *et al.*, 2013). Taking into account the lipid content of muscle only during the non-reproductive period and both muscle and gonad during the reproductive period, the relative lipid content of the fish was precisely estimated. The high correlation between the biochemical index and the fatmeter measurements regardless of the species and period considered, has confirmed the ability of the fatmeter to reflect relative lipid content well. Previous studies have validated fatmeter calibrations to estimate muscle relative lipid content (Davidson and Marshall, 2010; McPherson *et al.*, 2011). Here, calibrations to estimate the relative lipid content of the entire fish were used and validated, providing evidences that these two types of measurements may be used to monitor the relative lipid content of small pelagic fish. Moreover, the results obtained by comparing fatmeter and direct lipid estimations with the small sensor head were similar to the results in other studies conducted on larger fish (Davidson and Marshall, 2010; McPherson *et al.*, 2011; Klefoth *et al.*, 2013), underlining the equally good accuracy of the two types of sensors. The correlations between the biochemical and the relative condition index were weaker or absent. As previously established by McPherson *et al.* (2011) for herrings, these results highlighted that the K_n does not measure exactly the same aspect of condition (especially during the reproductive period) as the bioenergetics and biochemical indices. Indeed, indices based on body weight, such as K_{n}, can obscure a seasonal pattern of fat reallocation between the different organs of the body (Fitzhugh *et al.*, 2010). This may explain the lack of a relationship between K_{n} and direct estimators of lipids for spawning individuals as has been previously observed for other species (Sutton *et al.*, 2000; Brown and Murphy, 2004; Óskarsson, 2008). Moreover, some authors have found a strong relationship between total muscle protein and body weight loss (Dygert, 1990; Brown and Murphy, 2004). This suggests that during the reproductive period, even if K_n did not correlate with relative lipid content, it can be a useful condition index as it measures protein loss which indicates that the fish consumes muscle. Thus, during the reproductive period changes in K_n might be less related to relative lipid content and reflect other processes, such as protein content changes, that define condition differently than just lipid content variations.

By looking at three different species of small pelagic fish, interspecific differences were exhibited in the strength of correlation between K_n and the biochemical index and the fatmeter. Sardine, which is generally larger than the two others species, showed always the best correlation. Similar R^2 between K_n and fatmeter measurements for herring (Vogt *et al.*, 2002), which has a bigger size and weight than sardine. This could be due to the large variations in size and weight of sardine, well reflected by large variations of K_n. Also, size and weight measurements to the nearest millimeter and the nearest gram induced coarser measurements for anchovy and sprat, due to their smaller size and weight. This issue prevented from catching all the variability between individuals, limiting the accuracy by which reserve variations can be estimated using morphometric measurements. This could explain the lower R^2 and the lack of relationship between K_n and the biochemical index for sprat. For this reason, small-sized species need more precise morphometric measurements to catch reserve variability.

The K_n and fatmeter values were also affected by the maturity stage, with an increasing condition with increasing maturity for anchovy and the inverse for sardine and sprat.

Differences in reproductive period (i.e late spring/early summer for anchovy and winter for sardine and sprat, Palomera *et al.*, 2007) may explain this contrasting patterns. In late spring/early summer, food is highly available and even if a part of the stored energy is allocated to gonad development, anchovy continues to feed and gain weight during reproduction, as already observed by Millán (1999). Similarly, lipids in the muscle are not depleted due to the abundance of food. On the contrary, planktonic food for sardine and sprat is at lower levels during winter, so that the energy allocation to the growing gonads induces a loss of weight and lipid depletion in muscles. As K_n is based on total weight, this difference in reproductive period could explain the opposite observed effect of maturity stage on this index.

The fatmeter appeared as a good indirect index to evaluate the lipid content of the three species of small pelagic fish studied. The results for K_n underlined that this index is a global indicator of condition, as it estimates both lipid and protein content rather than only lipids. The choice of an indirect index for further studies thus depends on the objectives of the study. As condition studies should define exactly what is really measured with indirect indices, further studies on anchovy, sardine and sprat are needed to determine if, as mentioned in Brown and Murphy (2004), K_n may also be directly correlated with protein content or other health indicators.

Repeatability of fatmeter measurements

Because of the advantages of the fatmeter, the repeatability of this instrument was investigated, which was shown to be excellent. The difference between measurements realized on the same fish was small, with the highest repeatability for sardine and anchovy. A smaller repeatability value for sprat might originate from their small and thin body. As indicated in the Distell user manual (Kent, 1990), permittivity calculations performed by the fatmeter are less exact for individuals with body thickness inferior to 1.5 centimeters, which was regularly the case for sprat in the Gulf of Lions. In general, the larger the fish, the greater was the repeatability between measurements, which is in agreement with Kent *et al.* (1992). Thus, only one measurement on each fish side is enough to evaluate the total lipid content of a small pelagic fish, corresponding to less than one minute of handling time. However, duplicated readings are encouraged to minimize errors, mainly for smaller and thinner species.

Freezing impact on fatmeter measurements

For anchovy and sardine, all experiments on freezing duration pointed out a significant and strong correlation between fatmeter measurements performed on fresh fish and frozen fish. The absence of a significant effect of freezing on fish water content and thus on fatmeter measurements was already documented for other species (e.g. Vogt *et al.*, 2002). Regost *et al.* (2004) showed that freezing over a timespan longer than one month does not change the water content of fish. This result allows dealing with up to one month frozen sardine and anchovy samples from research surveys or commercial catches. Except for 5-day frozen sprat wherefore an overestimation of lipid content was observed (i.e. a slope greater than 1), the regression slopes for the different freezing durations were always below 1. This could be due to defrosting. Mørkøre *et al.* (2002) indeed showed that a part of the water contained inside the fish body of the rainbow trout moved towards the body surface during defrosting. This surface water accumulation may lead to an overestimation of the water content, which could exceed the minor effect of water loss due to freezing. Additionally, the presence of ice crystals after freezing can lead to an overestimation of the water content by disturbing microwave dispersal (Kent, 1990). Finally, besides the lower accuracy and repeatability of sprat measurement the significant change in the slope for sprat after freezing could also be explained by individuals being relatively fatter than sardine and anchovy and thus containing less water. Therefore, small changes in their water content could have a large effect on fat content measurements. Consequently, the use of freezing storage for sprat samples needs to be considered with more caution.

2.2.5 Conclusion

This study provided the basis to decide which techniques are best to investigate the condition of small pelagic fish in the Gulf of Lions (and probably more generally in the Mediterranean Sea). The fatmeter appears as a good option to quickly and accurately track the lipid content of small pelagic fish. Despite contrasting results, the relative condition index (K_n) is not obsolete to track condition of small pelagic fish. This index indicates a correlation with the lipid content outside of the reproductive period but might also be sensitive to other parameters, such as fish protein content. The precision of morphometric measurements to the nearest gram and millimeter was found to be insufficient in the case of sprat, especially when compared to the other two species. Biochemical measurements of fat content are rarely practicable as routine analyses. For instance, lipid extraction is time consuming and samples need to be stored at low temperature (-20°C to -80°C), which is not always feasible on fishing boats. In contrast, the fatmeter has the advantages of being quick (1 minute per fish), portable, non-invasive, easy to use and giving the most reliable indirect index for small pelagic fish in our study. It can also be incorporated to field research and measurements can be taken on living fish (Cooke *et al.*, 2005). This study showed that the fatmeter can be used properly on small size fish, such as anchovy, sardine and sprat. Furthermore, fatmeter measurements are repeatable and can be realized on frozen samples.

2.2.6 Ackowledgements

The authors are grateful to the captain and the crew of RV "L'Europe", as well as all the scientists onboard for their assistance during PELMED surveys. PELMED surveys are cofinanced by Europe through the Data Collection Framework. We especially wish to thank all other fishing crews who contributed to the collection of samples at sea. We thank the two anonymous reviewers for their careful review of the manuscript and their excellent suggestions. This research was partly funded through the EcoPelGol project (Study of the Pelagic ecosystem in the Gulf of Lions), financed by France Filière Pêche (FFP). PB acknowledges doctoral fellowship support from the French Ministère de l'Education Nationale, de la Recherche et de la Technologie.

2.2.7 Supplementary data

Table 2.2 Analysis of variance for relative fat content in anchovy, sardine and sprat to estimate fatmeter repeatability (r) according to the different fish species.

Source of variation	df	Sum of square	Mean squares	F ratio
Anchovy				
Among anchovy	72	693.7	9.635	43.58*
Within anchovy	219	48.4	0.221	
Total	291	742.1		
Anchovy fatmeter repeatability = 0.91				
Sardine				
Among sardine	53	1594.1	30.077	107.8*
Within sardine	162	45.2	0.279	
Total	215	1639.3		
Sardine fatmeter repeatability = 0.96				
Sprat				
Among sprat	49	4323	88.22	14.66*
Within sprat	150	903	6.02	
Total	199	5226		
Sprat fatmeter repeatability = 0.77				

Table 2.3 Analysis of variance of relative fat content to estimate fatmeter repeatability according to the different class of size of pooled anchovy, sardine and sprat species in the Gulf of Lions.

Source of variation	df	Sum of square	Mean squares	F ratio
Small size class (size < 105mm)				
Among individuals	16	1477.9	92.37	31.96*
Within individuals	51	147.4	2.89	
Total	67	1625.3		
Large size class fatmeter repeatability = 0.88				
Small intermediate size class (105<size<115mm)				
Among individuals	67	12098	180.57	74.74*
Within individuals	204	493	2.42	
Total	271	12591		
Large size class fatmeter repeatability = 0.94				
Large intermediate size class (115<size<125mm)				
Among individuals	40	624.1	15.602	94.66*
Within individuals	123	20.3	0.165	
Total	163	644.4		
Large size class fatmeter repeatability = 0.95				
Large size class (size>125mm)				
Among individuals	22	1014.9	46.13	141.9*
Within individuals	69	22.4	0.33	
Total	91	1037.3		
Large size class fatmeter repeatability = 0.97				

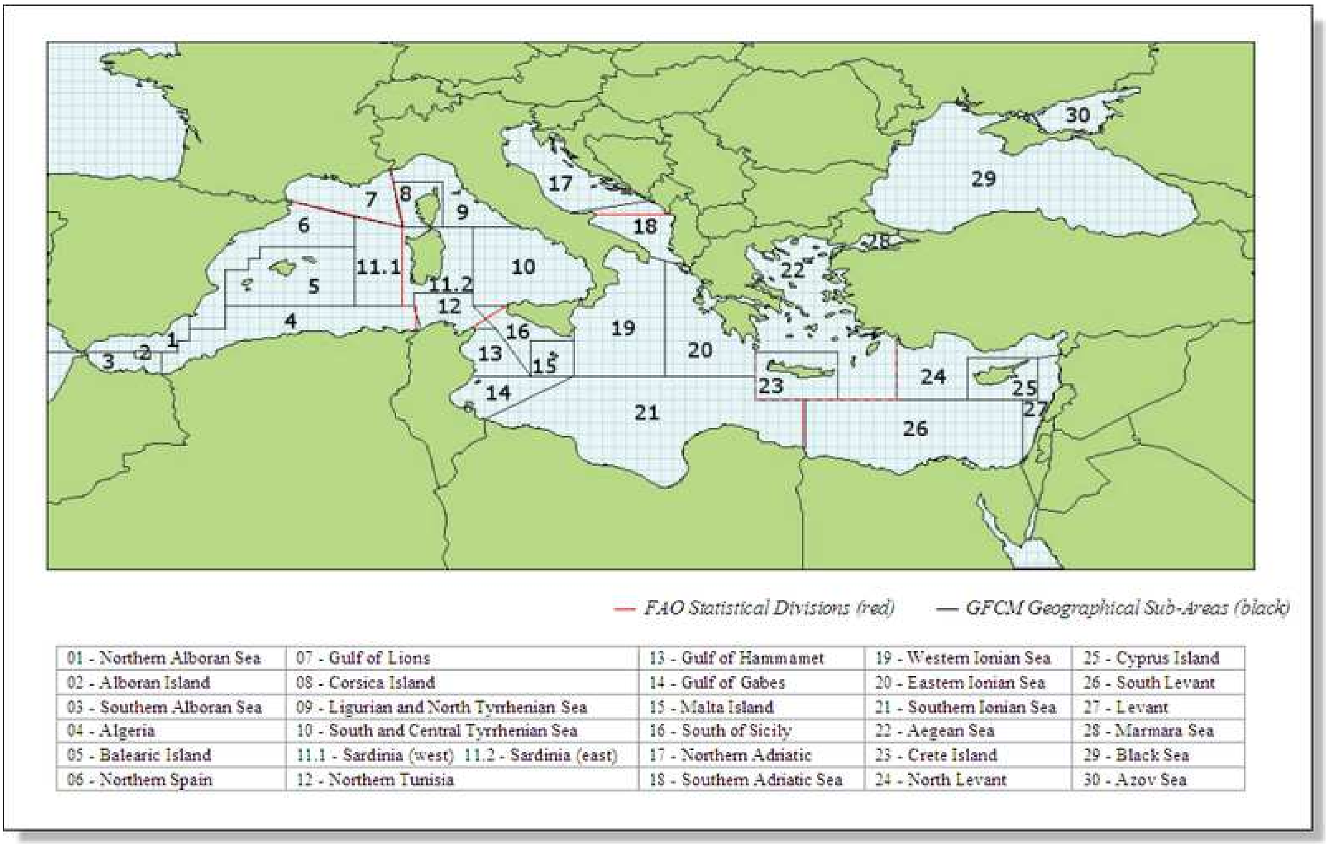
2.3 Echantillonnage des petits pélagiques en Méditerranée

Au cours de cette thèse, les études successives ont fait appel à des données de différentes origines. Un nombre important de données provient des campagnes scientifiques MEDIAS (MEDiterranean International Acoustic Survey), que ce soit pour les études spécifiques au Golfe du Lion ou bien l'étude à l'échelle de la Méditerranée. Mais un partenariat avec le monde de la pêche a aussi été créé pour avoir une résolution temporelle à plus fine échelle et ainsi avoir accès aux variations saisonnières des paramètres étudiés.

2.3.1 Echantillonnage scientifique

Présentation des campagnes MEDIAS

Les campagnes MEDIAS sont des campagnes scientifiques se déroulant durant environ un mois chaque année dans différentes zones cibles (Figure 2.6) avec comme but principal l'estimation des stocks de petits pélagiques par méthode acoustique (Doray et al. 2010).



01 - Northern Alboran Sea	07 - Gulf of Lions	13 - Gulf of Hammamet	19 - Western Ionian Sea	25 - Cyprus Island
02 - Alboran Island	08 - Corsica Island	14 - Gulf of Gabes	20 - Eastern Ionian Sea	26 - South Levant
03 - Southern Alboran Sea	09 - Ligurian and North Tyrrhenian Sea	15 - Malta Island	21 - Southern Ionian Sea	27 - Levant
04 - Algeria	10 - South and Central Tyrrhenian Sea	16 - South of Sicily	22 - Aegean Sea	28 - Marmara Sea
05 - Balearic Island	11.1 - Sardinia (west) 11.2 - Sardinia (east)	17 - Northern Adriatic	23 - Crete Island	29 - Black Sea
06 - Northern Spain	12 - Northern Tunisia	18 - Southern Adriatic Sea	24 - North Levant	30 - Azov Sea

Figure 2.6 Carte des sous-unités géographiques déterminées pour la Méditerranée et la mer Noire par la CGPM (Conseil général des pêches en Mediterranée).

Afin de pouvoir estimer les stocks de petits pélagiques, la biomasse et l'abondance des différentes espèces (mais principalement l'anchois, la sardine et le sprat) sont déduites des données acoustiques et des chalutages comme décrit dans le paragraphe suivant. L'intérêt d'avoir regroupé la plupart des pays du pourtour Méditerranéen autour du projet MEDIAS est d'avoir un protocole standardisé (même type de filet, radiale constante entre années, mois d'échantillonnage constant[1]) dans toutes les zones où se trouvent des poissons petits pélagiques en mer Méditerranée et en mer Noire. Autour de ce groupement, ce ne sont pas moins de 8 pays (Table 2.4) qui sont associés afin de fournir un état des lieux le plus précis possible de l'état de santé et de la viabilité des populations de poissons petits pélagiques à l'échelle Méditerranéenne.

Table 2.4 Résumé de toutes les données biologiques utilisées dans cette étude.

Country	Area	Species	Time Period	Data origin
Spain	North Alboran Sea GSA 01	Anchovy	2001-2013	MEDIAS
		Anchovy	1990-1992 & 2003-2013	Fisheries
		Sardine	2001-2013	MEDIAS
		Sardine	1990-1996 & 2003-2013	Fisheries
	Northern Spain GSA 06	Anchovy	1993-20113	MEDIAS
		Anchovy	2004-2013	Fisheries
		Sardine	1993-2013	MEDIAS
		Sardine	2004-2013	Fisheries
France	Gulf of Lions GSA 07	Anchovy	1993-2015	MEDIAS
		Anchovy	2005-2016	Fisheries
		Sardine	1993-2015	MEDIAS
		Sardine	1971-1983 & 2003-2016	Fisheries
Italy & Malta	Sicily Strait GSA15 & 16	Anchovy	1998-2013	MEDIAS
		Sardine	1998-2013	MEDIAS
Italy & Croatia	Northern Adriatic GSA 17	Anchovy	1998-2013	MEDIAS
		Anchovy	1975-2012	Fisheries
		Sardine	1998-2013	MEDIAS
		Sardine	1975-2012	Fisheries
Italy	Southern Adriatic GSA 18	Anchovy	1998-2013	MEDIAS
		Sardine	1998-2013	MEDIAS
Greece	Aegean Sea GSA 22	Anchovy	2003-2008	MEDIAS
		Sardine	2003-2008	MEDIAS
Turkey	North Levant GSA 24	Anchovy	2009-2011	Fisheries
Turkey	Black Sea SA 29	Anchovy	2011-2013	MEDIAS

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[1] excepté en Espagne où la campagne MEDIAS (juillet) a remplacé en 2009 la campagne EcoMed qui elle se déroulait en hiver

Le processus se décompose en plusieurs étapes. La première consiste à suivre des transects fixes pour balayer le plateau continental de la zone d'étude à l'aide d'un navire équipé d'un échosondeur (simple ou multiple faisceaux suivant les navires) permettant d'obtenir un échogramme pour différentes fréquences. La fréquence utilisée pour la détection des poissons est le 38kHz. Cet échogramme permet de connaître l'énergie attribuable aux poissons. Cependant, afin de savoir quels sont les poissons détectés dans chaque zone, des chalutages sont réalisés quand le nombre de détections est assez important sur au moins 2 miles nautiques. Ces chaluts vont servir de référence pour déterminer les assemblages d'espèces ainsi que la taille des individus. En effet, pour chaque chalut, la biomasse de chaque espèce est déterminée ainsi que le spectre de taille sur un sous échantillonnage aléatoire. Ceci pour avoir une idée la plus précise possible de la communauté de petits pélagiques présente à l'endroit du chalut à l'instant t. En résumé, la partie qui se fait à bord du bateau consiste en deux aspects, une part de détection acoustique et une part de chalutages qui seront ensuite associés sur la base chalut le plus proche (Figure 2.7).

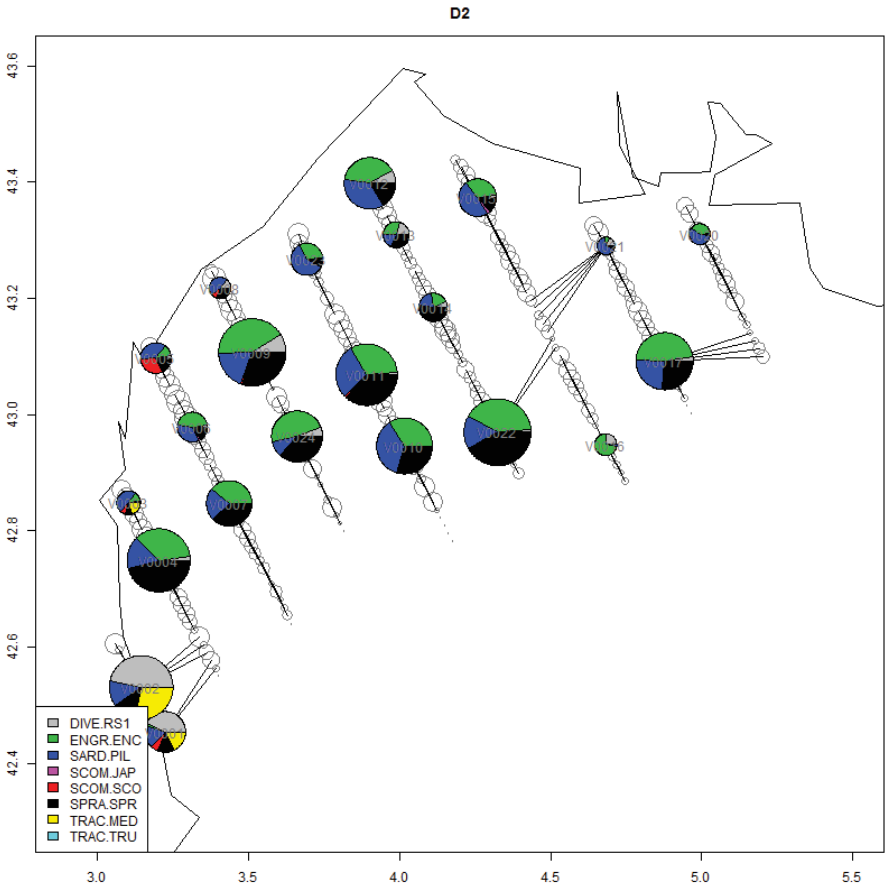

Figure 2.7 Carte EchoR associant les énergies acoustiques détectées aux chaluts les plus proches.

La phase suivante est la transformation de l'énergie acoustique détectée durant les radiales en une biomasse et une abondance de petits pélagiques tout en tenant compte de la distribution interspécifique et de la distribution en taille accessible grâce aux chalutages réalisés à proximité. Au terme du processus, la biomasse et l'abondance annuelle sont déterminées et permettent au niveau des instances (Conseil Général des Pêches pour la Méditerranée, CGPM) de statuer, de prendre des décisions et d'émettre des recommandations sur la santé et l'exploitation des différentes espèces de poissons petits pélagiques dans chacune des zones concernées.

Données biologiques récoltées sur MEDIAS

Les chalutages réalisés durant les campagnes PELMED permettent de réaliser en continu depuis 1993 des mesures biométriques individuelles, telles que la taille (au millimètre près), le poids (au gramme près) et des mesures physiologiques, telles que le sexe, l'état de maturation des gonades (échelle de 1 à 6, ICES, 2008). Depuis plus récemment (2013) le taux de gras est aussi mesuré de façon indirecte grâce à un fatmeter, dont les détails de fonctionnement ont été évoqués dans la partie précédente. Cette thèse m'a amener à participer à 4 campagnes PELMED entre 2013 et 2016, permettant de continuer ce qui était déjà en cours mais aussi de mettre en place des échantillonnages plus ponctuels comme détaillés juste après. Ces données provenant du Golfe du Lion ont permis principalement de calculer des indices de condition morphométriques (chapitre 2 et 5), mais aussi de disposer d'échantillons pour mesurer le taux de gras de façon biochimique ou pour l'étude de la reproduction de l'anchois dans le Golfe du Lion (chapitre 4). Des échantillonnages non réguliers entre 1994 et 2014 ont aussi permis de réaliser l'étude sur la variabilité de l'alimentation des petits pélagiques à l'aide des contenus stomacaux et des isotopes stables (chapitre 3).

2.3.2 Echantillonnage avec les professionnels de la pêcherie

Les campagnes scientifiques étant limitées à 1 mois dans l'année, des partenariats avec les pêcheurs ont été créés pour pouvoir étudier les variations saisonnières des paramètres biologiques afin d'avoir une vision plus représentatives de l'écosystème pélagique. Avec une contrepartie financière, financée principalement au travers les 'contrats bleus' (Fonds Européen pour la Pêche, FEP) puis le projet EcoPelGol, les professionnels ont ainsi fourni de nombreux échantillons. Pour un échantillonnage de qualité, les pêcheurs conservaient les individus destinés aux scientifiques sur un lit de glace, afin d'en éviter la dégradation très rapide. Dès le débarquement en criée, les poissons étaient rapatriés au laboratoire afin d'effectuer les mesures morphologiques, physiologiques et les prélèvements nécessaires aux analyses ultérieures (analyses de lipides par exemple) déjà précédemment décrits (chapitre 2, 4 et 5). Ce sont grâce à ces échantillons qu'ont notamment pu aboutir différents projets concernant la reproduction de la sardine (hiver, chapitre 4) ou encore la comparaison de plusieurs indices de condition corporelle durant les différentes saisons (section précédente). Tous le protocole pour les différents chapitres est résumé dans la figure 2.8, avec la description des principaux points abordés.

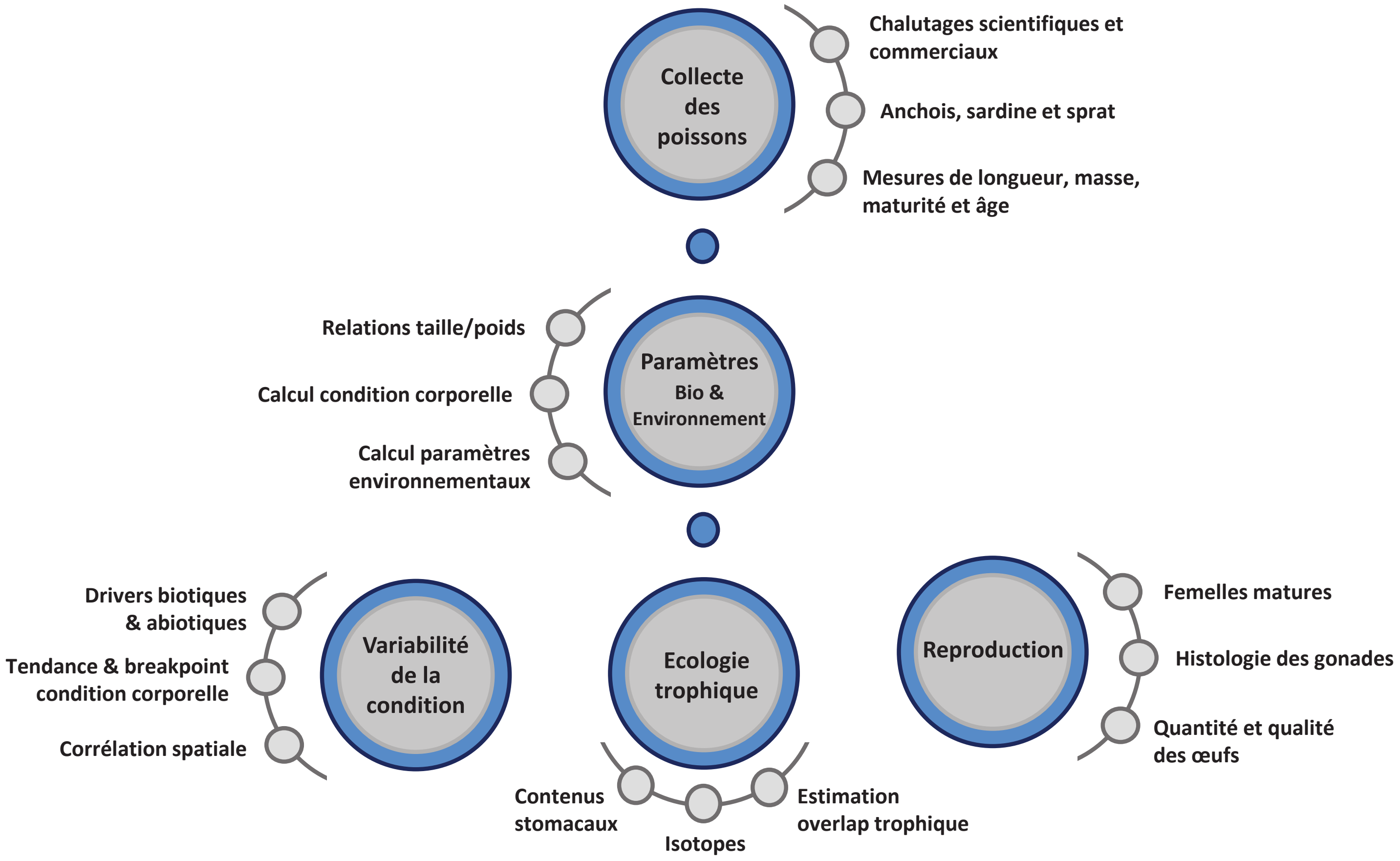

Figure 2.8 Résumé shématique des différents chapitres de la thèse.

Chapter 3

Influence of environmental variability and age on small pelagic fish body condition in the Gulf of Lions

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Preface

In the previous chapter we showed that the relative condition index adequately reflects fish condition (except for sprat), despite being mainly linked to protein instead of lipid concentration during reproductive period. Therefore, this index can now confidently be used to analyse the condition fluctuations of anchovy and sardine of the Gulf of Lions. Since 1993, scientific surveys and fisheries have been providing a huge quantity of biological data on both pelagic species in this area. Especially morphometric measurements are plentiful, i.e., fish length and body mass data. In a previous study, this dataset was already used to show a decrease in anchovy and sardine size and condition since 2007-2008. This raised several questions, such as which might be the drivers. But fish condition is affected by different types of drivers. On the one hand, there are endogenous drivers such as an individual's sex, maturity stage and age. On the other hand, environmental drivers such as temperature, food quantity and quality might also be important. Here, the large amount of morphometric data was used to compute the relative condition index for anchovy and sardine over the period 1993-2013, and to describe the annual condition cycle of each species. To determine whether for each species different periods of a certain state of condition can be delimitated, and to verify if endogenous variables (i.e., fish sex and age) have a significant effect on fish condition, a classification and regression tree analysis (CART) were also performed. On a decade of monthly data (2002-2011), general linear models (GLM) were used on deseasonalized time series of anchovy and sardine condition to predict their fluctuations in function of different environmental factors (i.e., Sea Surface Temperature, Rhône runoff, Western Mediterranean Oscillation index, Chlorophyll-a and the concentration of six groups of plankton). The plankton concentrations were obtained from validated models, due to the lack of sufficient in-situ plankton records in the Gulf of Lions. Thus, this chapter furnished some first answers for the reasons and causes of the currently reduced size, age and condition observed in the small pelagic fish populations in the Gulf of Lions.

Abstract

Endogenous and environmental variables are fundamental to explain fish condition variations. Based on more than 20 years of fish weight and length data, relative condition indices were computed for anchovy and sardine caught in the Gulf of Lions. Classification and regression trees (CART) were used to identify endogenous factors affecting fish condition and group years of similar condition together. Both species showed a similar annual cycle, condition being minimal in February and maximal in July. CART identified three groups of years of low, average and high condition, within which condition differed between age classes but not according to sex. In particular, during the period of low condition (mostly recent years), sardines older than 1-yr-old appeared more strongly affected than younger ones. Time series were analyzed using GLM to examine the effects of oceanographic abiotic (temperature, Western Mediterranean Oscillation (WeMO) and Rhône outflow) and biotic (chlorophyll-a and six plankton classes) factors on fish condition. The selected models explained 48% and 35% of the variance of anchovy and sardine condition, respectively. Sardine condition was negatively related to the temperature but positively to the WeMO, and mesozooplankton and diatoms concentration. A positive effect of mesozooplankton and Rhône runoff on anchovy condition was detected. The importance of increasing temperatures and reduced water mixing in the NW Mediterranean Sea, affecting planktonic productivity and thus fish condition by bottom-up control processes, was supported by these results. Changes in planktonic quality, quantity and phenology could lead to insufficient or inadequate food supply for both species.

Keywords Anchovy; Sardine; Relative condition factor; NW Mediterranean Sea; Endogenous effect; Environmental effect

3.1 Introduction

Body condition is widely used in ecological studies to determine the nutritional or physiological status of an individual (Bolger and Connolly, 1989; Stevenson and Woods, 2006) and is defined as the quantity of nutrient reserves, i.e. the quantity of accumulated energy reserves exceeding that required for daily nutritional demands (Schulte-Hostedde *et al.*, 2001; Schamber *et al.*, 2009). Body condition indices are thus used in order to evaluate the quantity of stored energy, giving an indication of an individual's well-being which can affect its future performances (Stevenson and Woods, 2006; Wilson and Nussey, 2010). For example, individuals with larger nutritional reserves may have a better chance to survive and a higher reproductive success or growth (Millar and Hickling, 1990), which may in turn affect the dynamics of the whole population (Jakob *et al.*, 1996; Adams, 1999). Many studies have used fish condition indices to monitor and investigate both pelagic and demersal fish population health and variability (Lambert and Dutil, 1997b; Lloret *et al.*, 2002; Shulman *et al.*, 2005; Ndjaula *et al.*, 2013). For example, anomalous conditions have been shown to strongly affect spawning and both egg quality and quantity of many fish species (Adams, 1999; Óskarsson *et al.*, 2002). Earlier sexual maturation, associated with smaller size as well as higher natural mortality, has also been shown to occur in low condition fish for *Clupea harengus* (Winters and Wheeler, 1994; Heino and Godø, 2002; Malzahn *et al.*, 2007) while the chances of becoming mature increase with good condition in *Hippoglossoides platessoides* (Morgan, 2004).

Fish condition may be linked to several factors, whether endogenous or exogenous (Shulman and Love, 1999; Lloret *et al.*, 2014). Reproductive state, sex or age are the main endogenous parameters known to affect condition. Drastic changes in population age/size structure have for instance resulted from differences between individuals of a single species. For example, a higher condition for females and older individuals led to an unbalanced sex-ratio or age distribution and increased population vulnerability to environmental stress (*Pagellus erythrinus*, Lloret *et al.*, 2002; *Merluccius gayi peruanus*Ballón *et al.*, 2008). Exogenous factors potentially impacting body condition are numerous as well, ranging from parasitism (Lambert and Dutil, 1997b; Barton *et al.*, 2002) to environmental conditions or food availability (Murphy *et al.*, 1990; Porath and Peters, 1997), highlighting the use of fish body condition as a measure of habitat quality and exogenous disturbance.

Anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) populations in the

Gulf of Lions supported important fisheries before exhibiting a major decline in the 2000s while the unexploited sprat (*Sprattus sprattus*) increased exponentially (GFCM, 2014). The biomass of these small pelagic and their role in the energy transfer from low (plankton communities) to high (e.g. marine mammals, tunas, seabirds) trophic levels underline the importance of these species (Cury *et al.*, 2000; Banaru *et al.*, 2013). In the Gulf of Lions, where anchovy and sardine biomass decreased, the number of sardines slightly increased and the number of anchovies remained steady due to the high abundance of small and young individuals (Van Beveren *et al.*, 2014). This underlines changes in the size and age-class distribution of both species. In parallel, fish condition started to decrease before the mean length and the mean age in both species, suggesting that condition and nutritional status might be an important factor explaining the currently observed population dynamics. Moreover, overexploitation was not retained as the prime force for this pelagic ecosystem shift, as bottom-up processes are the current major hypothesis for the change in small pelagic fish condition (Van Beveren *et al.*, 2014). Further investigations on the determinants of body condition are thus warranted to better understand the current situation of these stocks.

The aim of the present study was to analyze more precisely the variations in anchovy and sardine condition in the Gulf of Lions using a unique long-term dataset of morphometric (length and weight) and biological (age and sex) variables on more than 43,000 individuals from 1971 to 1978 and from 1993 to 2013. The first objective was to confirm the existence of different groups of years based on fish condition for both species using the largest dataset available in the Gulf of Lions. Secondly, according to the previously defined groups of years of fish condition, we investigated the potential effect of sex and age on condition of each species. Finally, we related the fluctuations in fish condition to environmental variables in order to determine potential exogenous drivers of the dynamics of small pelagic fish in the Gulf of Lions.

3.2 Material and Methods

3.2.1 Study area and data collection

This study took place in the Gulf of Lions located in the North Western part of the Mediterranean Sea (42°26′N, 3°00′E; 43°40′N, 5°28′E) and covering about 10,000 km². This area is composed by a wide continental margin (>60 km), bordered by numerous canyons (Millot, 1990). The Gulf of Lions is one of the most productive Mediterranean areas, dependent of Rhodanian inputs and strong mixing events induced by NW and

N winds and local upwellings (Palomera *et al.*, 2007). A nutrients gradient exists from East to West due to the Rhodanian input located at the East of the area (Darnaude *et al.*, 2004). Samples were collected from both scientific and commercial pelagic trawls. PELMED scientific surveys have been conducted each month of July on board the research vessel l'Europe to assess small pelagic fish biomass through acoustic methods from 1993 to 2013. Nine parallell fixed transects perpendicular to the coastline were carried out each year. Acoustic data allowed to detect fish occurrence and pelagic trawls were deployed to determine fish school composition when records of the presence of fish were long enough (> 2 nm). A random sample of fish in each trawl was collected and morphometric parameters, i.e. size (to the nearest millimetre) and body mass (to the nearest 0.1 gram), as well as age (otoliths reading), sex (by visual assessment) and maturity stage (by visual assessment according to ICES, 2008) were determined for each fish. Maturity stages were described from 1 to 6, with an increasing development of gonads from stages 2 to 4, spawning period during stage 5 and resting period during stages 6 and 1. To investigate the seasonality of fish condition, samples were collected on other periods of the year from commercial fisheries, and brought back to the lab for analyses. These samples were collected randomly in the Gulf of Lions from 1999 to 2013 (more regularly since 2005) during the different months of the year except months covered by the scientific survey (July). Samples consisted of one crate of fish randomly taken out of a pelagic trawl, before any sorting. Once in the lab, the same parameters as previously described for the scientific survey were collected. Data for sardine between 1971 and 1978 were also collected monthly by the commercial fleet and analyzed in the lab with the same accuracy. Thus, both periods (i.e 1971-1978 and 1993-2013) were judged comparable. In all fish sampling date, the length range was kept as large as possible to allow subsequent comparisons between periods and avoid bias in length-weight relationships computing.

3.2.2 Morphometric index of condition

As recommended by Froese in 2006 to compare condition within a given sample and as the two studied species exhibited an allometric growth pattern (Van Beveren *et al.*, 2014), we used for this study the relative condition index Kn (Le Cren, 1951; Bolger and Connolly, 1989; Blackwell *et al.*, 2000):

$$K_n = \frac{W}{Wr}$$

where Wr is the predicted weight of an individual of a given length TL ($W_r = \alpha TL^{\beta}$). Length-weight relationships were calculated separately for each month from each year (when the number of individuals was superior to 10) and the parameters αm and βm were then computed as geometric mean of all the α and β obtained. As no difference in weight-length relationship was found between sex and between juveniles versus adults (tested as interaction with fish length in separate linear models, e.g $\ln W \sim \ln LT$*Sex for sex effect), no distinction was made between males, females, juveniles and adults and a single length-weight relationship computed. Based on a 30-year dataset including more than 43,000 individuals (Table 3.1), the following values were obtained: αm=3.7.10-32.1.10-4 and βm=3.32.3.10-2, αm=9.1.10-39.2.10-4 and βm=3.14.2.10-2 for anchovy and sardine, respectively. Fitted β-values were in the usual range of 2.5 to 4 (Mendes *et al.*, 2004; Froese, 2006; Sinovčić *et al.*, 2008). By definition, the higher the index, the better the condition. As recommended by several authors (Schulte-Hostedde *et al.*, 2005; Stevenson and Woods, 2006; McPherson *et al.*, 2011), this morphometric index was comparable with direct assessment of total lipid content but outside the reproductive periods (Brosset *et al.*, 2015a). During the reproductive period, Brosset *et al.* (2015a) found that this index could reflect a more integrative measure of condition (such as lipids and proteins).

Table 3.1 Summary of dataset characteristics used in the study. For both species, number of individuals and size range inside parenthesis were showed for each month of the different groups of years identified.

Species	Period	Group of years	January	February	March	April	May	June	July	August	September	October	November	December
Sardine	1971 -1978		434 (118-201)	468 (134-208)	230 (137-203)	347 (142-210)	381 (129-213)	272 (135-212)	321 (118-215)	206 (150-207)	546 (133-216)	524 (126-210)	369 (131-210)	347 (138-206)
	1993 -2013	Good condition			192 (127-181)		118 (142-182)	51 (163-200)	997 (80-205)	20 (150-205)				
		Average condition		143 (140-200)					2909 (70-205)	65 (110-180)				
		Low condition	722 (90-187)	1055 (79-200)	872 (75-190)	1583 (95-193)	555 (109-194)	1149 (74-190)	4675 (70-210)	715 (76-183)	521 (96-189)	692 (91-181)	1702 (81-193)	868 (93-194)
Anchovy	1993 -2013	Good condition			82 (105-175)	101 (105-135)	91 (125-165)	112 (120-170	973 (70-185)					60 (143-182)
		Average condition		60 (135-180)		11 (155-180)	24 (100-175)		2785 (55-185)			220 (70-170)	5 (120-125)	
		Low condition	779 (67-168)	799 (68-159)	1227 (79-173)	1101 (70-171)	853 (90-158)	1659 (86-167)	5407 (40-180)	1323 (93-155)	1220 (78-166)	663 (72-148)	1469 (71-162)	536 (96-160)

3.2.3 Environmental parameters in the Gulf of Lions

Small pelagic populations are subject to considerable fluctuations caused by environmental variability (Bakun, 1997), mainly due to their relatively short life span (3-5 years). Satellite-derived sea surface temperature (°C SST) and surface chlorophyll a (Chl-a mg.m-3) were extracted from MODIS-aqua data (http://oceancolor.gsfc.nasa.gov). The Rhône outflow was computed with the daily inputs of the Grand Rhône, measured at the Beaucaire station, and provided by the Compagnie Nationale du Rhône (CNR). The Western Mediterranean Oscillation index (WeMO; Martin-Vide and Lopez-Bustins, 2006) was computed as the daily differences in standardized surface atmospheric pressure values between San Fernando (Spain) and Padua (Italy) (López-Bustins, pers. comm). This index integrates fluctuations of temperature, rainfall and wind mixing. Positive values reflect low temperature and high river runoff and wind mixing while negative values reflect the opposite. As environmental variability also affects primary and secondary production, the planktonic community was included in our analyses. Daily plankton concentrations estimates were extracted from the coupled physical-biogeochemical model SYMPHONIE-Eco3m-S. The physical model SYMPHONIE (Marsaleix *et al.*, 2009, 2011, 2012) is based on a 3-D primitive equation, free surface model, with hydrostatic and Boussinesq approximations. The biogeochemical model Eco3m-S (Baklouti *et al.*, 2006b,a; Auger *et al.*, 2011) is a multi-nutrient and multi-plankton functional type model that simulates the dynamics of several biogeochemical elements (carbon, nitrogen, phosphorus, silica and chlorophyll) and plankton groups (picophytoplankton (0.7-2μm); nanophytoplankton (2-20μm, high taxonomic heterogeneity); microphytoplankton (20-200μm, dominated by diatoms); nanozooplankton (5-20μm, small ciliates and flagellates bacteriophage); microzooplankton (20-200μm, ciliates and large flagellates) and mesozooplankton (>200μm, mainly copepods species)). The coupled model was previously used to study the dynamics of plankton communities impacted by freshwater discharge and their role in carbon export on the Gulf of Lions (Auger *et al.*, 2011), as well as the interannual biogeochemical variability linked to atmospheric and hydrodynamic forcing in the Gulf of Lions from climatological (Herrmann *et al.*, 2014) and statistical (Auger *et al.*, 2014) points of view. In this study, we used a horizontal curvilinear mesh, giving a resolution smaller than 1 km on the Catalan and western Gulf of Lions shelf and ~2 km near the eastern coast of the Gulf of Lions. Initialization and boundary conditions were provided by the NEMOMED8 model (Herrmann *et al.*, 2010). The coupled model was forced by the atmospheric ARPERA model outputs (Herrmann and Somot, 2008). MODIS chlorophyll-a monitoring started in 2002, and the biogeochemical simulation that provided 6-classes plankton concentrations was produced over the period 2001-2011. Therefore, we extracted morphometric data for the Gulf of Lions from January 2002 to December 2011 with the advantage that this decade covered all the different types of years (i.e good, average or low) of both anchovy and sardine conditions in the Gulf of Lions. All these variables were averaged per month over the entire Gulf of Lions from January 2002 to December 2011.

3.2.4 Data analyses

Two components of the large time-series dataset were studied in this paper. On the one hand, we described the seasonal cycle of anchovy and sardine condition. On the other hand, linking fish condition and environmental drivers allowed understanding fish condition fluctuations in the Gulf of Lions. Therefore, the time series was decomposed into:

$$\text{Series} = \text{Trend} + \text{Seasonality} + \text{Residuals}$$

First, we studied the seasonal signal. A seasonal cycle of fish condition was computed for anchovy over the whole studied period (1993-2013). Because of a big temporal gap of 14 years in the dataset (from 1979 to 1992), sardine data were divided into two periods (1971-1978 and 1993-2013). Seasonal cycles were then estimated by period and compared using a Generalized Linear Model (GLM) including a period effect and the interaction between month and period.

The dataset showed irregularities in the sampling scheme (e.g. different monthly sample sizes, missing data for some months, Table 1). To avoid any influence of the sampling month on the endogenous and exogenous factors possibly affecting fish condition, we deseasonalized and scaled the fish condition data. Deseasonalized values were obtained by removing median values estimated with the seasonal cycle of condition for each species. All values were then scaled by dividing each value by the standard deviation (SD) of the corresponding month. To investigate the potential effect of endogenous factors (sex and age), we then used the Classification And Regression Tree (CART) approach of Breiman *et al.* (1984). Decision trees were built by recursively partitioning our dataset into increasingly homogeneous subgroups based on fish condition values. Each split is defined by a simple rule based on a single explanatory variable, and each final group is characterized by mean values of fish condition. Two separate CARTs were applied for the two fish species. Year was also added as an explanatory categorical variable in order to define precisely groups of years according to fish condition.

In addition, we evaluated the link between environmental variables and fish condition over a decade using data available from January 2002 to December 2011. As we were interested in the origin of the trend in fish condition, we deseasonalized environmental data to reduce risks of spurious correlation which could be due to a seasonal cycle. Following the same procedure applied for fish condition, environmental variables were (i) deseasonalized by removing median values of the raw data and (ii) scaled by dividing by the SD. The standardized environmental time series were implemented as explanatory variables in a GLM approach.

Because the present paper concentrated on the factors influencing fish condition, we did not consider interactions between the explanatory variables. Model residuals were visually assessed for normality and an untransformed response with a normal error structure was found to be appropriate. As the sample size (n) was small compared to the number of predictor variables (K) ($\frac{n}{K} < 40$), the Akaike's Information Criterion (AIC) was corrected for small sample sizes and the best model was selected according to the AICc (Hurvich and Tsai, 1989). GLMs were used to detect potential effects of environmental parameters on fish condition as follows:

$$Condition = \mu + \alpha SST + \beta Chla + \gamma Rhonerunoff + \delta WeMO + \theta Zoomeso + \lambda Zoomicro + \nu Zoonano + \xi Syn + \sigma Diatom + \rho Nano + \varepsilon$$

where μ is the intercept and ε the error relative to a normal distribution.
Variance inflation factors (VIF) were calculated between all the environmental parameters to detect high-dimensional collinearity. When collinearity was identified between covariates, the covariates with the highest VIF were sequentially removed from the model until the highest VIF value was less than 5 Zuur *et al.* (2007). The same model was run for both anchovy and sardine.

All statistical analyses were performed with R version 3.0.2 (R Development Core Team, 2013). Values are indicated as mean standard error (SE) and all statistical tests were performed at a significance level of 0.05. As autocorrelation has been recognized as inflating the probability of a type I error in hypotheses tests, causing biases in variable selection, and violating the assumption of independence of error terms in regression models, we also checked for temporal autocorrelation in both anchovy and sardine condition time series; but this was not significant.

3.3 Results

3.3.1 Annual cycle of fish condition

The period and the interaction between period and month were significant for sardine (GLM, d.f. =1, P <0.001 and GLM, d.f.=11, P <0.001, respectively), indicating changes in the phenology of the condition and the need to run separate analyses for each period. The condition values of each month (excluding April) were significantly higher for the 1971-1978 years (Figure 3.1). Anchovy showed a minimal condition in winter, i.e. during January (0.89±0.002) and February (0.89±0.003), and a peak in the beginning of the summer, i.e. in June (1.05±0.002) and July (1.05±0.001). Sardine exhibited a similar pattern to that of anchovy for the minimum (0.85±0.006) and the maximum (1.08±0.002) of condition during the 1993-2013 years, while the maximum value of condition occurred later (e.g. 1.15±0.005 in September and 1.16±0.005 in October) during the earlier years (1971-1978).

3.3.2 Fish condition partition and endogenous factor

Regression trees (CART analyses) were used to partition fish condition data in relation to year, age and sex. They revealed year and age as explanatory variables for both species while sex did not show any significant effect (Figure 3.2). Year had the greatest effect on anchovy condition with the highest values for years from 1995 to 2007 (except 2003) (Figure 3.2). In this group, a secondary partition separated the highest values (for years 2005 and 2006) from the others. Three groups of years were thus identified for anchovy (Figure 3.2). Within these groups of years, condition was then partitioned according to age. While no effect of age was demonstrated in the low condition years, anchovy condition tended to slightly decrease with age during the years of average and high condition (Figure 3.2). Year was also the most important variable explaining sardine condition. Indeed, three groups of years were identified: years of low condition composed by 1993, 1996, 2003 and 2008-2013, years of high condition (2005-2006, as for anchovy) and years of intermediate condition values (1994, 1995, 1997-2002, 2004 and 2007) (Figure 3.2).

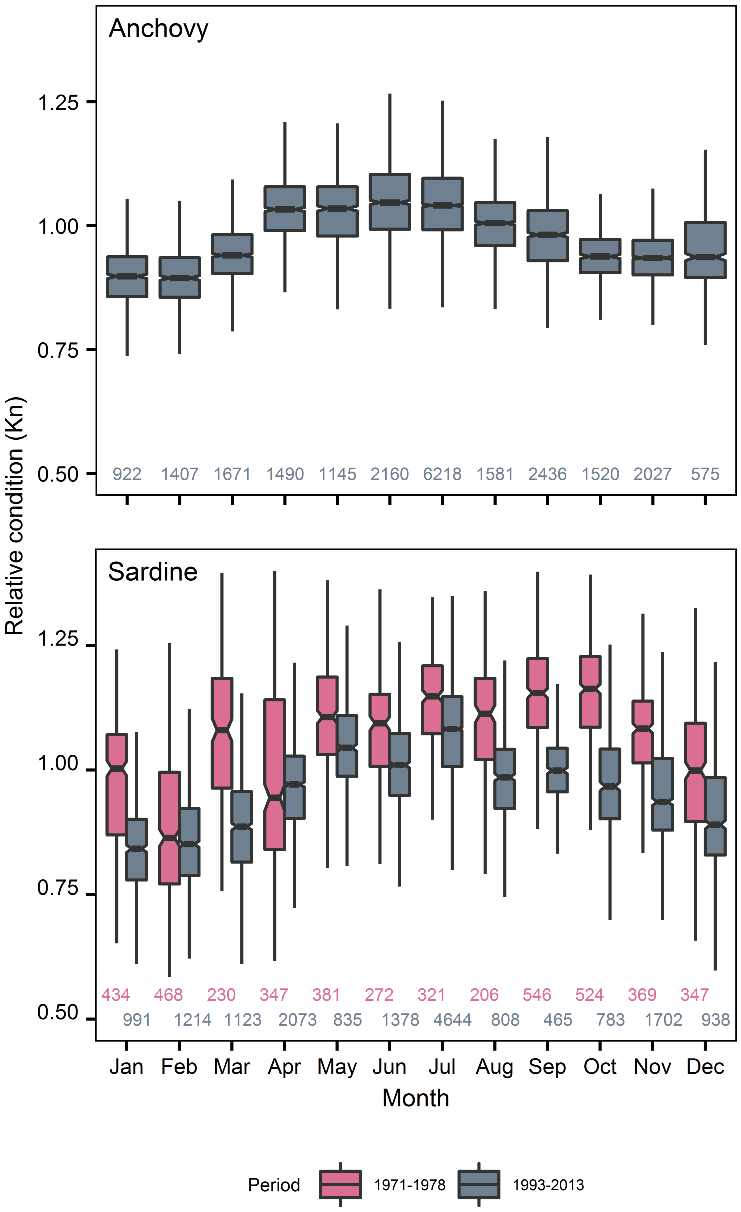

Figure 3.1 Seasonal cycle of the relative condition for anchovy and sardine. For sardine, the condition was significantly higher during 1971-1978 years for all month (except April). The number of individuals sampled during each month is included at the bottom of the boxplot.

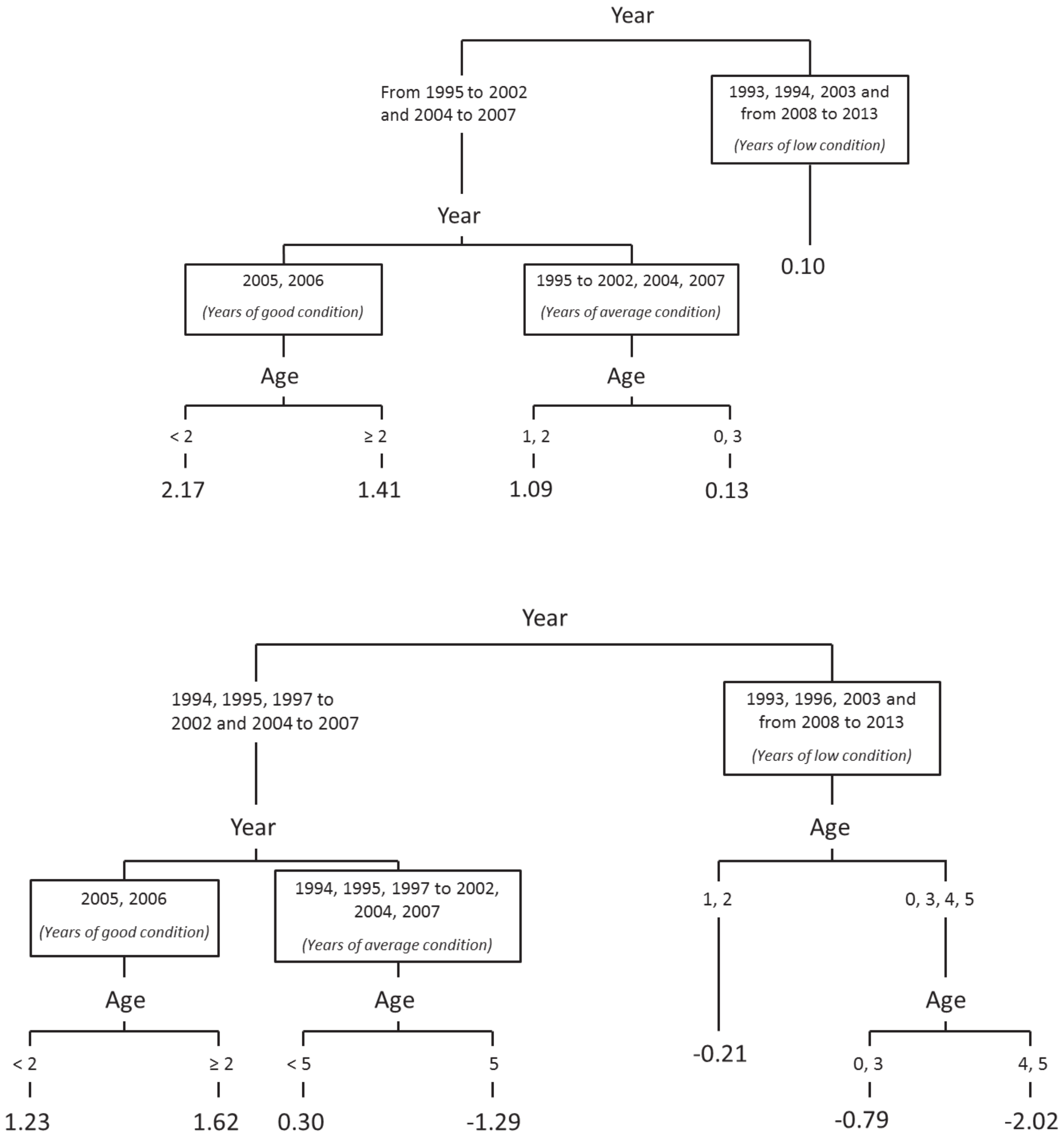

Figure 3.2 Results of the classification and regression tree assessing the importance of year, age and sex to anchovy (up) or sardine (down) condition values. The tree is split off on the values of one covariate at a time such that the overall variance in the dependent variable is minimized at each split. Terminal nodes indicate the value of fish standardized condition assigned to the node. The different groups of years identified according to the fish condition were highlighted in boxes.

Age then determined the following nodes. In the years of good condition, sardine condition increased from age 0 to 1 and was higher and steady in individuals older than 2 (Figure 3.3). Even if age did not affect condition in the intermediate years (except for individuals older than 5 which had a low condition, although this was based on 11 individuals only), a similar pattern of sardine condition with age was found during years of average condition (Figure 3.3). On the contrary, in the years of low condition, which comprised the last 6 years, condition seemed to decrease sharply with increasing age after 1 year old. Sardine of age 3, 4 or 5 exhibited the worse condition observed in the dataset (Figure 3.3).

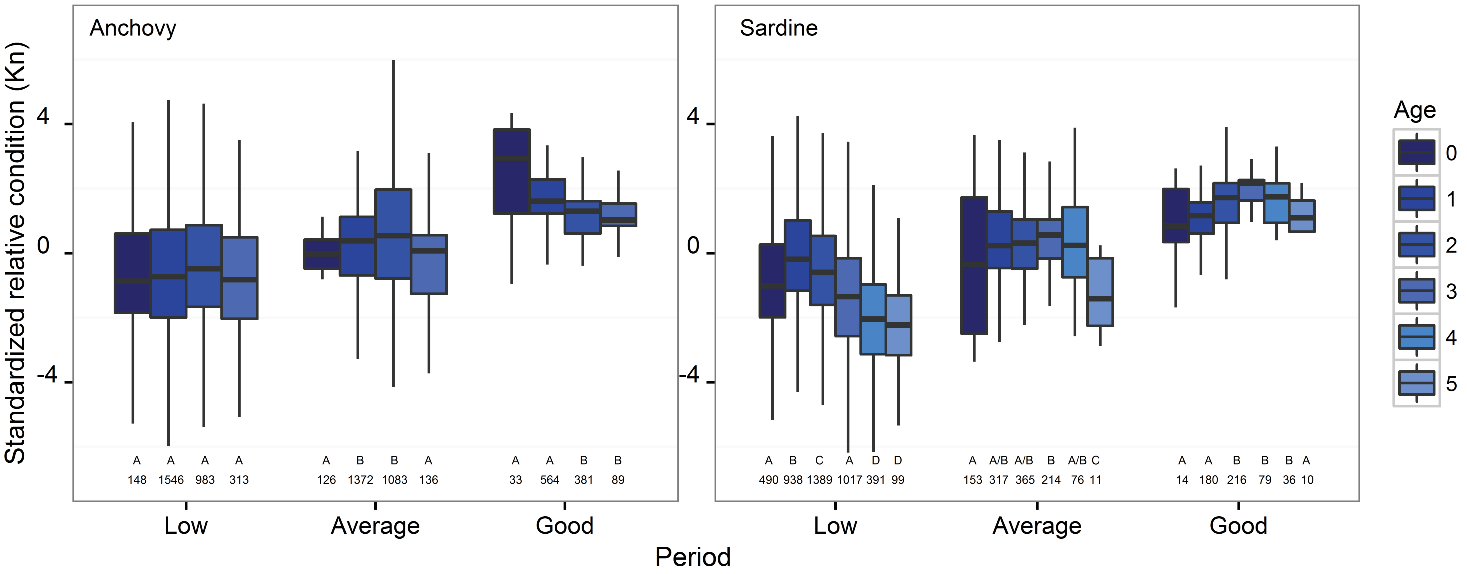

Figure 3.3 Boxplot of the standardized relative condition in function of age for anchovy and sardine. The number of individuals of each age class is included below each boxplot and letters indicate box age-classes that are statistically different for each group of years.

3.3.3 Fish condition and environmental parameters

Variance inflation factors were calculated to determine the variables with high collinearity. As the picophytoplankton showed a value of 8.42, this variable was removed from the model for each species. Other variables showed a maximum VIF of 4.58 and did not introduce a major bias in our analyses. The AICc selected models including mesozooplankton and Rhône runoff for anchovy, and mesozooplankton, temperature, WeMO and diatoms concentration for sardine. Anchovy condition was significantly and positively influenced by the concentration of mesozooplankton, and the Rhône

Runoff (Table 3.2). For sardine, the GLM revealed a significant positive relationship of condition with the mesozooplankton concentration, the WeMO and the diatom concentration while there was a negative effect of temperature (Table 3.2). Final models selected with AICc explained 48% and 35% of the variance for anchovy and sardine (r^2_{adj}), respectively.

Table 3.2 Results of the AICc selected GLMs performed on anchovy and sardine. The relative condition index was taken as a function of Sea surface temperature (SST), Chlorophyll a concentration (Chl a), Rhone runoff, Western Mediterranean Oscillation (WeMO), Mesozooplankton concentration (Mesozoo), Nanozooplankton concentration (Nanozoo), Microzooplankton concentrations (Microzoo), Nanophytoplankton concentration (Nanophyto) and Microphytoplankton concentration (Diat). All data were standardized and GLMs were performed with a Gaussian distribution and identity link.

	Anchovy				Sardine			
Parameter	Estimate	s.e	*t*	*p*-value	Estimate	s.e	*t*	*p*-value
Intercept	0.168	0.146	1.179	0.235	-1.215	0.388	-3.3448	0.005
Mesozoo	0.273	0.056	4.871	<0.001	0.646	0.142	3.369	<0.001
SST	-	-	-	-	-0.749	0.261	-3.004	0.005
Rhone runoff	0.306	0.109	2.903	0.006	-	-	-	-
WeMO	-	-	-	-	0.601	0.226	2.921	0.007
Chl a	-	-	-	-	-	-	-	-
Nanophyto	-	-	-	-	-	-	-	-
Diat	-	-	-	-	0.396	0.112	3.620	<0.001
Nanozoo	-	-	-	-	-	-	-	-
Microzoo	-	-	-	-	-	-	-	-

3.4 Discussion

3.4.1 Annual cycle of anchovy and sardine condition

Monthly variations of fish condition showed similar patterns for anchovy and sardine. Condition was maximal in summer and minimal in winter, as has been described in other Mediterranean areas (Basilone *et al.*, 2006; Zlatanos and Laskaridis, 2007; Simat and Bogdanović, 2012) and in the Gulf of Lions by lipid analysis (Pethybridge *et al.*, 2014). However, while sardine exhibited a strong difference in condition between winter and summer, seasonal variations in condition were lower for anchovy. Such differences between species might be explained by different spawning strategies and the seasonality of prey abundance. Summer vs. winter represent periods of high vs. low energy intake for both species due to increased food (Lefevre *et al.*, 1997) determined by prey availability and quality in spring/summer. However, expenses differ greatly between the 2 species. Indeed, anchovies are income breeders (i.e they acquire energy concurrently to spawning period) while sardines are capital breeders (i.e they store energy before spawning period, see Ganias *et al.*, 2007b; McBride *et al.*, 2013; Pethybridge *et al.*, 2014). At the beginning of spring, fish condition of both species increase because of higher prey availability, but a part of that energy storage is then directly allocated to the gonads during summer for anchovies.

On the contrary, energy storage of sardine, and thus its condition, continues to increase during summer because no spawning event happens during that season. Similarly, the progressive decline in food quantity at the end of summer (Lefevre *et al.*, 1997) induced a reduction in condition for both species. The seasonal difference in body condition is thus stronger for sardines, which alternate a season of high intake and low expenses (summer) with a season of low intake and high expenses (winter), than anchovies, which alternate high intake and expenses with low intake and expenses. As a result, the decrease in condition is more drastic in sardines and one can wonder if a delayed increase in food abundance might affect sardines to the point of impairing their survival. We hypothesized that after February, in both the 70′s and recent decades, sardines were able to recover their fat reserves as they stopped the lipid allocation to gonads(Lefevre *et al.*, 1997). However, we highlighted differences for sardine between periods (we also investigated another methodology using two different LW relationships, one per period, and direct comparison between their parameters gave similar conclusions than the comparison of body condition based on a single LW relationship for both periods). First, the condition of this species was always higher in the 70′s (excluding April). Secondly, while their maximal condition was in autumn during the first period, it occurred at the beginning of summer during the recent years. As the breeding season is likely to be the same over time (Lee, 1961; Palomera *et al.*, 2007), this suggests a potential change in feeding condition. In particular, a change in the phenology of the primary and secondary production, usually marked by a second bloom of phytoplankton in September in the Gulf of Lions (Lefevre *et al.*, 1997), might explain the change in small pelagic fish condition.

3.4.2 Fish condition and endogenous characteristics

For the two species, different clusters of condition values were clearly separated in accordance with the general pattern of condition variability previously found (Van Beveren *et al.*, 2014), highlighting the low anchovy and sardine condition since 2008.

Another study in the same area pointed out differences in condition between sexes for some demersal species like Mullus barbatus or Pagellus erythrinus (Lloret *et al.*, 2002). However, no variation between sexes was found in our study or according to previous study on lipid content (Pethybridge *et al.*, 2014). Thus, for anchovy and sardine of the Gulf of Lions, a high or low fish condition cannot be due to one of the two sexes.

Significant variations between age classes depended upon years. Sardine condition always increased similarly from age-0 to age-1 and either increased afterwards (years of high condition), remained steady (years of average condition) or decreased dramatically (years of low condition). These results might provide some explanation about the current changes in size and age structure of sardine, with recent dominance of small and young individuals in the Gulf of Lions (Van Beveren *et al.*, 2014). Sardine condition decreases after July, possibly because of a lack of food followed by the start of the breeding season later on, which mobilizes a high quantity of lipid reserves. The lack of sufficient fat reserves for old sardines could lead to a higher mortality at the end of the winter. Although anchovy also exhibited a pattern of condition changing with age and according to groups of years, the trend was not as pronounced. This could be both because of a smaller number of age classes and a lower parameter variability than sardine due to measurements to the nearest millimeter (Brosset *et al.*, 2015a). A shift in quantity and/or availability of zooplanktonic prey species between years could have affected the condition of older and larger adults.

3.4.3 Fish condition and environmental parameters

The relation between fish condition and environmental data was investigated from 2002 to 2011, a period where environmental data were available and encompassed years of both high and low condition for anchovy and sardine. High sardine condition was linked with high WeMO values, corresponding with low temperature and strong Rhône runoff and wind mixing, i.e. a regime of enhanced primary and secondary productivity. As the statistical model also confirmed this negative effect of temperature on sardine condition, the positive effect of WeMO is likely to be mostly related to planktonic productivity. Variations in sea temperature may affect the fish directly (by physiological stress) or indirectly (through changes on the ecosystem production and prey availability, Brett, 1979; Lloret *et al.*, 2014). Indeed, temperature is a proxy of planktonic productivity. Warmer temperatures can reduce upwelling and water mixing and may therefore reduce and move earlier the late summer energy peak (Vidussi *et al.*, 2011; Calvo *et al.*, 2011), preventing sardine and anchovy to find sufficient food resources before entering the prolonged winter season. Moreover, the development of cold water copepods during the winter could also be affected by warmer water (Halsband-Lenk *et al.*, 2002; Hinder *et al.*, 2014) leading to low copepod abundance as demonstrated in the Balearic Sea (de Puelles *et al.*, 2004). A similar case has already been observed along the Portuguese coast for sardine (Rosa *et al.*, 2010), where temperature explained a sharp decline in their condition as it changed prey availability and phenology during the year. Similarly, anchovy condition was positively affected by the Rhône river outflow. The Rhône is the main source of runoff in the Gulf of Lions (and the Western Mediterranean) and enhances planktonic production by strongly increasing nutrients inputs (Lefevre *et al.*, 1997). Its positive effect can thus be explained by favoring feeding conditions for both species, increasing energy reserves and improving fish condition.

Our results on the significant effect of mesozooplankton support the close relationship between food supply and fish condition. As expected, the mesozooplankton estimated from a coupled model outputs showed a significant positive relationship with fish condition. This could be explained by the fact that small pelagic fish derive the bulk of their dietary carbon mainly from copepods and euphausiids (Plounevez and Champalbert, 2000; Garrido *et al.*, 2008; Nikolioudakis *et al.*, 2014; Costalago and Palomera, 2014). Sardine condition was also affected by a variation in diatom concentration. The consumption of phytoplankton and especially diatoms was already underlined in the Gulf of Lions (Plounevez and Champalbert, 2000). Furthermore, fish condition is a function of food quality and/or food quantity. Food quantity is important, as demonstrated by the decreased food supply during different invasions of jellyfish, which were associated with a sharp decline in condition of different species of small pelagic fish (Shulman *et al.*, 2005; Daskalov and Mamedov, 2007; Sabatés *et al.*, 2010). In our study, local (temperature, river runoff, wind mixing and surface chlorophyll a) and regional (Western Mediterranean Oscillation) environmental conditions could have such an impact on the trophic web and thus on fish condition. Additionally, prey quality in term of species composition can also have an important effect on fish condition. For example, differences in lipid content of fish according to copepod diversity were found in other sardine populations (Garrido *et al.*, 2008) and also in populations of capelin (Orlova *et al.*, 2010) in the Atlantic Ocean. In the case of the Gulf of Lions, there is currently insufficient knowledge on potential variations in the planktonic communities to be able to establish a clear link with the observed changes in fish condition.

However, several authors pointed out changes in the composition and phenology of zooplankton associated with environmental fluctuations in Mediterranean areas surrounding the Gulf of Lions, especially the Ligurian and the Balearic Sea (see Molinero *et al.*, 2005, 2008; de Puelles and Molinero, 2008; Calvo *et al.*, 2011; Auger *et al.*, 2014). The overall trend in the North Western Mediterranean Sea is characterized by an increasing temperature, decreasing wind stress and water mixing (Calvo *et al.*, 2011). Such changes induce a longer stratification period, which leads to a reduced nutrient supply to the upper layers and results in changes in the phytoplanktonic and zooplanktonic compositions as demonstrated by Auger *et al.* (2014) in the Gulf of Lions. In such cases, small-sized phytoplankton should dominate, decreasing diatom concentration, affecting grazing efficiency of copepods and ultimately copepod size and egg production (Halsband-Lenk *et al.*, 2002; Ianora *et al.*, 2003). As anchovy mainly feeds on large copepods and sardine on large copepods and diatoms, fish condition should benefit more from diatom-based food chains (Lloret *et al.*, 2014) than from the more heterotrophic food chains occurring in summer and early fall (small-sized phytoplankton composed out of e.g. flagellates and ciliates). In this way, the prevalence of small copepods may be insufficient to satisfy the entire energetic demand of the fish. The differences in the annual cycle of the condition of sardine between 1971-1978 and 1993-2013 also support possible changes in environmental conditions. A change in the phenology of the primary and secondary production, usually marked by a second bloom of phytoplankton in September in the Gulf of Lions (Lefevre *et al.*, 1997), might explain the observed changes in sardine condition. Bosc (2004) evidenced changes in the timing and magnitude of the blooms in the north-western Mediterranean using satellite images. In particular they showed a quasi-absence of an autumnal bloom over the Seawifs images period (1998-2001) while it was clearly marked over the CZCS images period (1978-1986). They ascribed these differences to environmental changes. Warmer water during the period 1993-2013 could reduce or delay the second bloom of phyto and zooplankton, and prevent sardine to remain in good condition until September or October. This strong dependency of condition upon mesozooplankton may be the explanation of the observed shift in the phenology of the sardine condition between the two periods (1971-1978 vs 1993-2011). However, the lack of historical data on chlorophyll a and plankton composition for the period 1971-1978 prevented us from comparing with current seasonal signals of plankton production. Further studies are needed in the Gulf of Lions to investigate the evolution of the planktonic community and to evaluate the link between the different mesozooplankton components and fish condition.

3.5 Conclusion

This long term study allowed us to assess the main drivers of the condition of the two main small pelagic fish in the Gulf of Lions. Our results are in agreement with previous studies which highlighted the importance of bottom-up processes for anchovy and sardine condition (Shulman *et al.*, 2005; Rosa *et al.*, 2010; Nikolioudakis *et al.*, 2012). Sardine clearly exhibited differences in condition between groups of years, with decreasing and very low values for individuals of two years old or more during the low condition years of 1993, 1996, 2003 and from 2008 to 2013. Differences were less evident for anchovy which showed only small dissimilarities between age classes for the different groups of years. Thus, the low condition of older individuals, mainly for sardine remains one of the main hypotheses for the current disappearance of old individuals in the Gulf of Lions. Environmental factors, such as temperature and Rhône runoff, were essential to explain small pelagic fish condition, probably through their indirect impact on primary production and thus on food availability. Furthermore, mesozooplankton was the major parameter driving significant fluctuations in condition for both species. Also, there was a positive effect of diatom concentration on sardine. Therefore, mesozooplankton appears to be an essential aspect for understanding small pelagic fish condition and further studies are needed to investigate the impact of this plankton compartment on small pelagic fish condition more precisely. The recent increase in sprat abundance and biomass (Van Beveren *et al.*, 2014) may lead to an increase in trophic competition, which would also need to be investigated to completely understand the current and prolonged poor condition of anchovy and sardine.

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Chapter 4

Linking small pelagic dietary shifts and ecosystem changes in the Gulf of Lions

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Preface

Chapter 3 showed that anchovy and sardine condition were significantly and mainly driven by different planktonic groups, such as mesozooplankton and diatoms. The former group is chiefly composed out of copepods (e.g. Paracalanidae, *Microsetella* spp. and Oncaeidae) and cladocerans, which are also the major part of the small pelagic fish diet. Thus, this indicates that small pelagic fish condition is as expected linked to their food sources. Specifically, condition might change if food quantity and/or quality alter. However, in the Gulf of Lions data on plankton (total biomass or composition) is extremely limited, so that the effect on fish condition is hard to investigate directly and it is unclear whether resources became limiting and/or changed greatly in composition around the year that fish condition and size decreased (2007). Nonetheless, some indications were found in adjacent Mediterranean areas that plankton changes did indeed occur around 2007. But although regional plankton data is lacking, the hypothesis that a change in fish condition occurred because of a change in plankton quality can be verified by analyzing changes in small pelagic diet. That is, as these species are filter-feeders, they are indicators of the NW Mediterranean zooplankton community. Therefore, this chapter was aimed at describing and quantifying the trophic changes of sardine, anchovy and sprat over the two last decades, using two complementary methods. We analyzed stomach contents of anchovy sampled in 1994, 2011 and 2012 and sardine caught in 2007, 2011 and 2012. Also, stable isotopes analyses were performed between 2004 and 2014 for anchovy, sardine and sprat. Both methods combined enabled us to study potential resource competition and the evolution of prey composition. These results were then linked to the previously identified periods of condition (e.g., low for anchovy and sardine since 2007/2008). Additionally, the trophic characteristics of sprat might help explain their population increase.

Abstract

Since 2008, a severe decrease in size and body condition together with a demographic truncation was observed in sardine (secondarily in anchovy) population of the Gulf of Lions (NW Mediterranean Sea). In parallel, sprat biomass, which had been negligible before, increased tenfold. All these changes have strongly affected the regional fisheries. Using trophic and isotopic data from contrasted periods of low versus high growth and condition, we investigated potential changes in diet and interspecific feeding interactions through time. Evidence of resource partitioning was found between sprat and both anchovy and sardine in 2004 and 2005. Since 2010, the isotopic niches of the three species tend to overlap, suggesting higher risk of competition for food resources. The wider trophic niche of sprat indicates higher prey diversity or occurrence of prey species that could feed on different organic matter sources. Anchovy and sardine diet varied through time, with a high proportion of large copepods or cladocerans in periods of high growth and condition (1994 and 2007 respectively) versus a dominance of small copepods in the present (2011-2012). Furthermore, an important reduction in prey diversity was also identified in the diet of both anchovy and sardine during the recent period. All the results support the hypothesis that changes in small pelagic fish growth, size and body condition and ultimately biomass could be due to a bottom-up control characterized by changes in food availability and increasing potential trophic competition.

Keywords Trophic ecology - Anchovy - Sardine – Sprat – Dietary overlap – NW Mediterranean

4.1 Introduction

Small pelagic fish species are characterized worldwide by important temporal fluctuations of their abundance and biomass, generally ruled by environmental changes in marine ecosystems (Schwartzlose *et al.*, 1999; Alheit and Niquen, 2004). Bottom-up control, driven by changing ocean conditions was therefore often argued as the main hypothesis to explain these regime shifts (Cury and Shannon, 2004), but changes in plankton quantity and quality is difficult to confirm due to a lack of sufficient observations of the planktonic community in both time and space.

In the Gulf of Lions, sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and sprat (*Sprattus sprattus*) the three main small pelagic species, act as a critical link between planktonic production and top predators (Banaru *et al.*, 2013). Additionally, anchovy and sardine support pelagic trawling and purse seine fisheries (between 30 and 50% of the total landings in this area, (Banaru *et al.*, 2013), making them both ecologically and economically essential (Palomera *et al.*, 2007). During the last decade, a decrease in size and body condition was observed for anchovy and sardine while at the same time, sprat biomass which had been negligible before, increased tenfold (Van Beveren *et al.*, 2014). These changes made sardine and anchovy commercially less interesting, so that landings dropped dramatically. Surprisingly, the recruitment has remained high and these modifications primarily affected the adults, with a disappearance of older age classes, especially for sardine (Brosset *et al.*, 2015b).

Such changes in the small pelagic fish community are far less common than changes in recruitment and might result from selective pressure from fishing or natural predation or modifications in prey availability and/or quality. Yet, fishing pressure does not appear to be the main driver of these changes, as these populations were/are not overfished (GFCM, 2015) and exploitation rates have remained low over the last 2 decades (i.e. at around 10 to 20% in average without exceeding 40%, see Van Beveren *et al.*, 2016). Predation pressure from Atlantic Bluefin tunas, the main top predator of those small pelagic fish in this area, has been recently evaluated to be < 2% (Van Beveren, 2015), indicating that a top-down control is unlikely. While predation due to natural predator or fishing remained at low levels for these species, body condition in sardine and anchovy has been shown to be strongly affected by mesozooplankton abundance (Brosset *et al.*, 2015b), advocating for a bottom-up control as the most probable source of small pelagic fish changes in the NW Mediterranean. Unfortunately, plankton records are scarce in the Gulf of Lions, and no time series is currently available to test for such a bottom-up control.

Stomach content analyses and stable isotope analyses (SIA) are two of the main approaches for investigating feeding habits and trophic interactions (Darnaude *et al.*, 2004; Post *et al.*, 2007). Stomach content analyses document recently consumed food items and permit a quantitative and qualitative snapshot of the diet (Hyslop, 1980). SIA are complementary to stomach content analyses and allow obtaining an integrated measure of the assimilated food over the previous months depending on the variability of prey and their stable isotope ratios, the fractioning and the isotopic turnover. Values of δ^{15}N may be related to the trophic level of an individual, while the δ^{13}C ratio indicates the primary production sources, that is, the different feeding environments (coastal/oceanic, pelagic/benthic) used by consumers (Vander Zanden and Rasmussen, 1999, 2001). Combining stomach content analyses and SIA has become an effective tool to investigate changes in trophic structure. Hence, their joint use contributes to the further understanding of how an ecosystem may be affected by changes in interspecific interactions (Caut *et al.*, 2006).

Several studies have provided important information on feeding habits and diets of these 3 species in the Gulf of Lions (Plounevez and Champalbert, 2000; Costalago *et al.*, 2012, 2014; Pethybridge *et al.*, 2014; Le Bourg *et al.*, 2015). However, they were usually limited to one species or a given period, so that the investigation of potential temporal changes along the last 15 to 20 years is still missing. The aim of this study was to investigate the hypothesis that recent changes observed in anchovy, sardine and sprat populations from the Gulf of Lions might result from diet changes. In this study, we propose to investigate changes in the feeding habits between periods of contrasted growth and condition (before and after 2008) defined by Van Beveren *et al.* (2014). In particular, isotopic interspecific overlap, trophic niche width and prey type were examined through time. Indeed, knowing that sardines, sprats and anchovies strongly co-occur in terms of spatial distribution in the Gulf of Lions (Saraux *et al.*, 2014), trophic overlap between species might result in food competition if resources become limited (Hardin, 1960). Moreover, the niche width, i.e. an index of prey diversity estimated through the range of δ^{15}N and δ^{13}C values or Shannon's diversity may be used to determine how generalist a population might be in terms of diet and feeding areas (Newsome *et al.*, 2007). Finally, prey species composition might have a strong effect on the energy intake of predators (Beaugrand *et al.*, 2003; Blanchard *et al.*, 2012).

4.2 Material and methods

4.2.1 Study area

The Gulf of Lions (42°26′-43°40′N and 3°00′-5°27′E; Figure 4.1) is located in the North Western Mediterranean Sea and is characterized by a large continental shelf (Millot, 1990). Shallow waters between 0 and 200 meters associated with Rhône river discharge and coastal upwelling due to Northern winds support high productivity, making it one of the most productive areas of the Mediterranean Sea (Minas and Minas, 1989; Lefevre *et al.*, 1997). A decreasing trend in the concentration of nutrients exists from East to West and from coastal to deeper waters (Banaru *et al.*, 2013). The particulate organic matter and phyto- and zooplankton communities at the base of the food webs show inter annual, seasonal and spatial variations in terms of composition and stable isotope ratios inside this area (Darnaude *et al.*, 2004; Banaru *et al.*, 2014; Espinasse *et al.*, 2014b). These variations may be related to the terrestrial and anthropogenic river inputs, to currents and wind forcing influencing the hydrography of the area, as well as to changes in the phyto- and zooplankton communities in terms of species composition and size classes (Rau *et al.*, 1990; Harmelin-Vivien *et al.*, 2008; Banaru *et al.*, 2014).

4.2.2 Sample collection

Fish and zooplankton samples were collected during MERLUMED and Pelagic Mediterranean (PELMED) scientific surveys during the summer under a similar protocol. Fish were sampled with a pelagic trawl with a small-mesh cod-end (mesh length 5 mm, ISO 1107) and towed at an approximate speed of 4 knots over 30 min periods. All specimens were selected randomly from hauls and their standard length was determined to the nearest mm, as well as their mass (to the nearest g) and sex. Numerous stations were sampled each year in the Gulf of Lions (i.e inshore/offshore, West and East) to cover the complete area (Figure 4.1) and to avoid bias of interannual differences due to spatial heterogeneity. In 2004, 2005 and 2014, zooplankton was sampled using a vertical WP2 net (200 μm mesh size) at each trawl station (4 stations in 2004 and 2005 and 10 in 2014, respectively). Zooplankton samples were stored in frozen sea water to be used for SIA as bulk. Using a combination of previously published data (Costalago *et al.*, 2012; Le Bourg *et al.*, 2015) and new samples that were analyzed for this study, stable isotope values were available for June and July of 2004, 2005, 2008, and 2010-2014 (except for sprat in 2008 which was not sampled). Stomach content data were available in 2011-2012 for both anchovy and sardine (previously published data; n = 118 & 104 for sardine and anchovy respectively; Le Bourg *et al.*, 2015) as well as prior to population changes: in 2007 for sardine (n=156; Costalago *et al.*, 2012) and in 1994 for anchovy (n=50, unpublished).

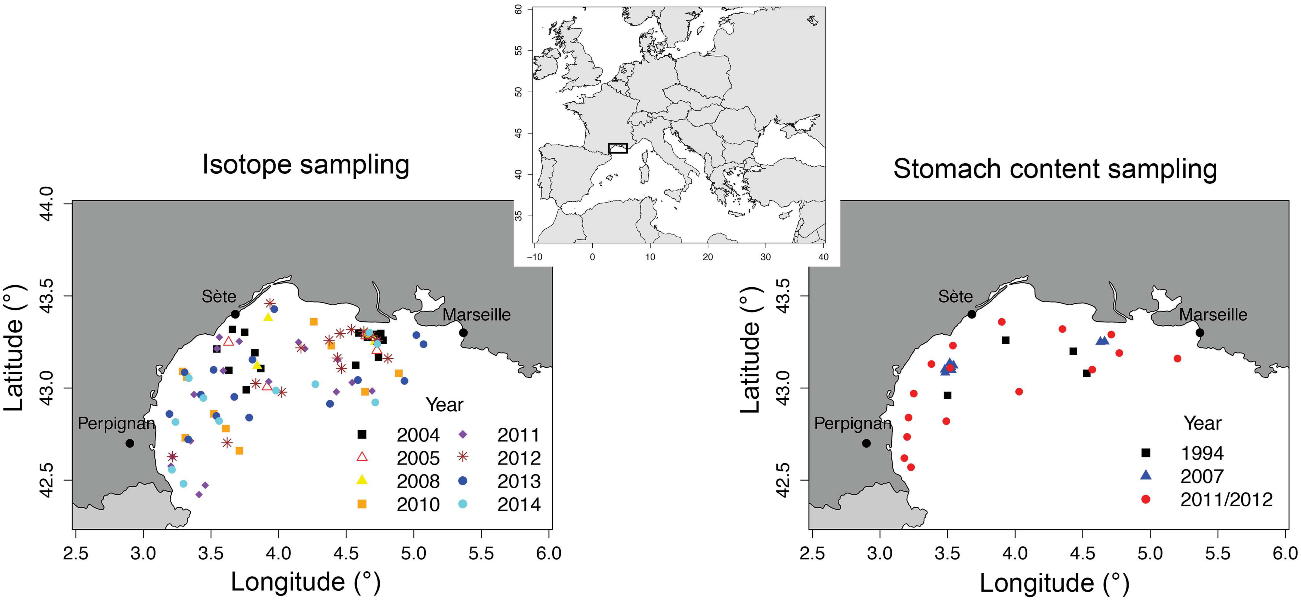

Figure 4.1 Sampling sites in the Gulf of Lions (Northwestern Mediterranean Sea). The left panel indicates isotope sampling locations and the right one stomach content sampling locations.

4.2.3 Stable isotope analyses

Fish isotope analyses were conducted on a piece of ~1 cm^3 of white muscle that had been kept frozen at -80°C (Sweeting *et al.*, 2005). Both fish white muscle and zooplankton samples were freeze-dried during 48h and grounded into a fine powder before being encapsulated in a tin cup and sent for SIA analysis to the LIENSs laboratory (La Rochelle, France). An acidification step was necessary for zooplankton samples to remove any ^{13}C-enriched carbonates (DeNiro and Epstein, 1978). A subsample was acidified with 1% of HCl, rinsed with distilled water and dried to determine the δ^{13}C ratio while an untreated subsample was used for δ^{15}N analysis. Three zooplankton replicates were performed from each sampled site for both δ^{13}C and δ^{15}N. A continuous-flow isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific) coupled to an elemental analyzer (Flash EA1112, Thermo Scientific) was used to perform stable isotope measurements. Results were expressed in parts per thousand (‰) relative to

Vienna PeeDee Belemnite and atmospheric N_2 for δ^{13}C and δ^{15}N, respectively, using the equation:

$$X = (\frac{Rsample}{Rstandard} - 1) * 10^3$$

where X is ^{13}C or ^{15}N and R is the isotope ratio ^{13}C/^{12}C or ^{15}N/^{14}N, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicated a precision of 0.2‰ for both δ^{13}C and δ^{15}N values.

Variation in lipid content among fish species can introduce a bias in carbon stable isotope analyses (Post *et al.*, 2007). Therefore, the potential effect of lipids on δ^{13}C values of fish samples was corrected by applying the procedure of Post *et al.* (2007) when the C/N ratio was >3.5 (which was mainly the case for sprat). Thus, the time and uncertainty due to lipid extraction were reduced. For all years, fish length ranges were kept similar (from 10 to 15 cm, Table 1.) to avoid any bias due to ontogenetic changes.

4.2.4 Stomach content analyses

A similar protocol was applied on all analyses of stomach contents regardless of the year (see details in Costalago and Palomera, 2014; Le Bourg *et al.*, 2015). Briefly, fish were frozen at -20°C immediately after being caught to stop digestive processes. Then, the gut was removed and stored into alcohol (95%) at the laboratory. Each stomach was carefully opened and all prey species were placed in a Petri dish and identified and quantified under a stereo microscope to the lowest possible taxonomic level, depending on the digestion state. Unidentified preys were excluded from the statistical analyses. A single difference in protocol occurred, as fish were analysed individually in all years except for sardines in 2007, for which fish of a given station were pooled together, i.e. between 16 and 20 stomachs were pooled together. In that case the associated sardine length corresponded to the mean length of all individuals from a given sample. Prey dry weight was derived from literature (see Le Bourg *et al.*, 2015).

Prey importance was assessed using the percentage of occurrence (%O, proportion of stomachs where the prey species was found), percentage in number (%N, ratio between the number of one prey species and the total number of prey), percentage in dry weight (%DW, ratio between the weight of one prey species and the total weight of prey). These percentages were then combined to calculate the Index of Relative Importance (IRI, Pinkas, 1971), necessary to compute the %IRI, which allows an integrated comparison between dietary items of the same species (Hyslop, 1980; Cortés, 1997):

$$\%IRI = \frac{IRI}{\sum_{a=1}^{n} IRI} * 100$$

where *IRI* = %O x (%N + %DW) and n is the number of prey species. Because of potential disparities in species or groups of species names between 1994 and 2011-2012, we carefully linked all prey names to current species name. Due to a lack of correspondence between periods, few species were not retained. However these species/groups of species, all had very low %*IRI* (< 0.2%), so that their absence could not significantly affect the computation of %*IRI* and trophic niche width (see below). The trophic niche width was measured for each period, using the exponential of Shannon's entropy, N1 = exp(H), where H is the Shannon-Wienner diversity index: $-\sum_{i=1}^{q} pi * ln * (pi)$ and *pi* is the proportion of *IRI* (Hill, 1973; Medina *et al.*, 2015).

4.2.5 Data analyses

Determinants of isotopic values

To investigate the potential effect of species, year, fish length, latitude, longitude and coastal/offshore gradient on δ^{13}C and δ^{15}N values, we used the Classification And Regression Tree (CART) approach of Breiman *et al.* (1984). Decision trees were built by recursively partitioning our dataset into increasingly homogeneous subgroups of isotope values. Each split is defined by a simple rule based on a single explanatory variable, and each final group is characterized by its mean isotope values. Two separate CARTs were applied for fish δ^{13}C and δ^{15}N values. As less stations and years were sampled for zooplankton, we only tested annual differences in δ^{13}C and δ^{15}N to look for interannual variability (2004, 2005 and 2014) using one-way analyses of variance (ANOVA).

Isotopic niches

Standard ellipse areas corrected for small sample size (SEA$_C$, Jackson *et al.*, 2011), which define the isotopic niche space of 40% of typical individuals within the group based on bivariate normal distributions, were calculated as a measure of the mean core population isotopic niche in order to analyze resource partitioning among species over time. However, because both the shape and size of the area filled by the points influence this estimate, we first scaled isotope values as recommended by Cucherousset and Villéger (2015) to obtain an informative assessment of isotopic overlap. Then, niche overlaps between the different species were determined using SEA$_C$. Niche overlap varied between 0 (no overlap) and 1 (one species is included in the other species stable isotope space). Additionally to niche overlap, the size of standard ellipses was also compared between species and between years for each species. To do that, bayesian standard ellipse areas (SEA$_B$) were calculated, which allowed for robust statistical comparisons. In addition, the probability of Bayesian ellipses, which were larger or smaller relative to the compared group was computed (e.g p, the proportion of ellipses in 2011 that were lower than 2012, see Jackson *et al.* (2011) for more details). We considered two SEA$_B$ to be significantly different when more than 95% of the posterior estimates of one group were smaller than those of another group (Turner *et al.*, 2010). Estimation via Bayesian inference allowed to make robust comparisons among data sets comprising different sample sizes (Syväranta *et al.*, 2013).

Temporal variation in stomach contents

To describe the temporal variations in stomach contents of anchovy and sardine, we applied a principal component analysis (PCA), which is a multivariate analysis of individuals' stomach contents in function of prey using a correlation matrix. For each predator species, only prey constituting > 2% of %IRI in at least one of our data subsets were considered for the analysis, except for non-identified copepods. Empty stomachs were excluded from this analysis. To deal with different sampling strategies for sardine stomach contents between 2007 and 2011/2012, the 8 stations of 2007 (representing each between 16 and 20 individuals) were weighted by the number of corresponding individuals in the PCA analysis. The PCAs were performed on the dry weight (%DW) of the different prey ingested for each individual. Similar results were obtained when PCAs were performed on the proportion of frequency (%N), showing the robustness of these results.

All statistical analyses were performed with R version 3.0.2. Values are indicated as mean standard error (SE) and all statistical tests were performed at a significance level of 0.05. All data were tested for normality and heteroscedasticity using Kolmogorov-Smirnov and Levene's tests.

4.3 Results

4.3.1 Factors influencing isotope values

Fish size, nitrogen and carbon stable isotope ratios were determined for 330 anchovies, 327 sardines and 236 sprats (Table 4.1 & Figure 4.2). Using a CART analysis, 85% of the variability in the δ^{13}C values were significantly explained by the variables year and species, whereas spatial locations of capture inside the Gulf of Lions (latitude and longitude), fish length and the coastal vs offshore gradient were not retained (Figure 4.3). The year variable had the greatest effect, and δ^{13}C values were mainly split accordingly (Figure 4.3). First, the maxima for 2004 and 2005 were separated from all other years. In this small group, a secondary partition separated high δ^{13}C sprat values from anchovy and sardine values. From all remaining years (2008 to 2014), the years 2010 and 2011 showed the most negative values, especially for sprat (Figure 4.3). Years 2008, 2012, 2013 and 2014 had intermediate δ^{13}C values, with no differences between the three species studied. Between 2004 and 2014, the δ^{13}C isotope values of all three species decreased progressively, but less for sardine and anchovy (-2‰) than for sprat (-3‰) (Figure 4.2).

Table 4.1 Mean standard length (in mm ± standard deviation, sd) and number of samples analysed (n) for stable isotopes of *Engraulis encrasicolus*, *Sardina pilchardus* and *Sprattus sprattus*.

Year	Engraulis encrasicolus Standard length (mm)		Sardina pilchardus Standard length (mm)		Sprattus sprattus Standard length (mm)	
	Mean ± sd	n	Mean ± sd	n	Mean ± sd	n
2004	113 ± 16	65	131 ± 22	83	106 ± 6	14
2005	121 ± 15	29	129 ± 18	25	114 ± 8	8
2008	115 ± 4	15	136 ± 4	17	NA	NA
2010	110 ± 4	32	100 ± 5	33	87 ± 8	32
2011	103 ± 19	61	117 ± 14	51	96 ± 8	57
2012	97 ± 5	36	111 ± 13	38	95 ± 8	50
2013	108 ± 17	43	104 ± 18	38	103 ± 8	39
2014	111 ± 14	49	121 ± 15	42	94 ± 9	36

According to the CART, 39% of the variability for the δ^{15}N signal was accounted for by year and species variables (Figure 4.3). In 2005 and 2011 to 2013, δ^{15}N values were lower for anchovy and sprat in comparison to sardine (Figure 4.3). In contrast, during all others years (2004, 2008, 2010 and 2014), sardine and sprat had more similar δ^{15}N values than anchovy. Only in anchovy in certain years did other variables affect isotope values. Indeed, anchovy values in 2004, 2008, 2010 and 2014 also depended on the sampling location, in particular the longitude, with lower values in the East. Moreover, in the Western part during those years, anchovy δ^{15}N values increased with size (Figure 4.3). However, even if year was significant in the CART analysis (Figure 4.3), during our study period only a slight difference in trophic levels was observed for any species (δ^{15}N range), with no clear temporal trend (Figure 4.2).

Similarly to fish, the δ^{13}C values of the bulk of zooplankton were higher in 2004 and 2005 in comparison to 2014 (ANOVA, $p< 0.001$) while δ^{15}N values remained stable over time (ANOVA, $p> 0.05$; See supplementary material 4.6).

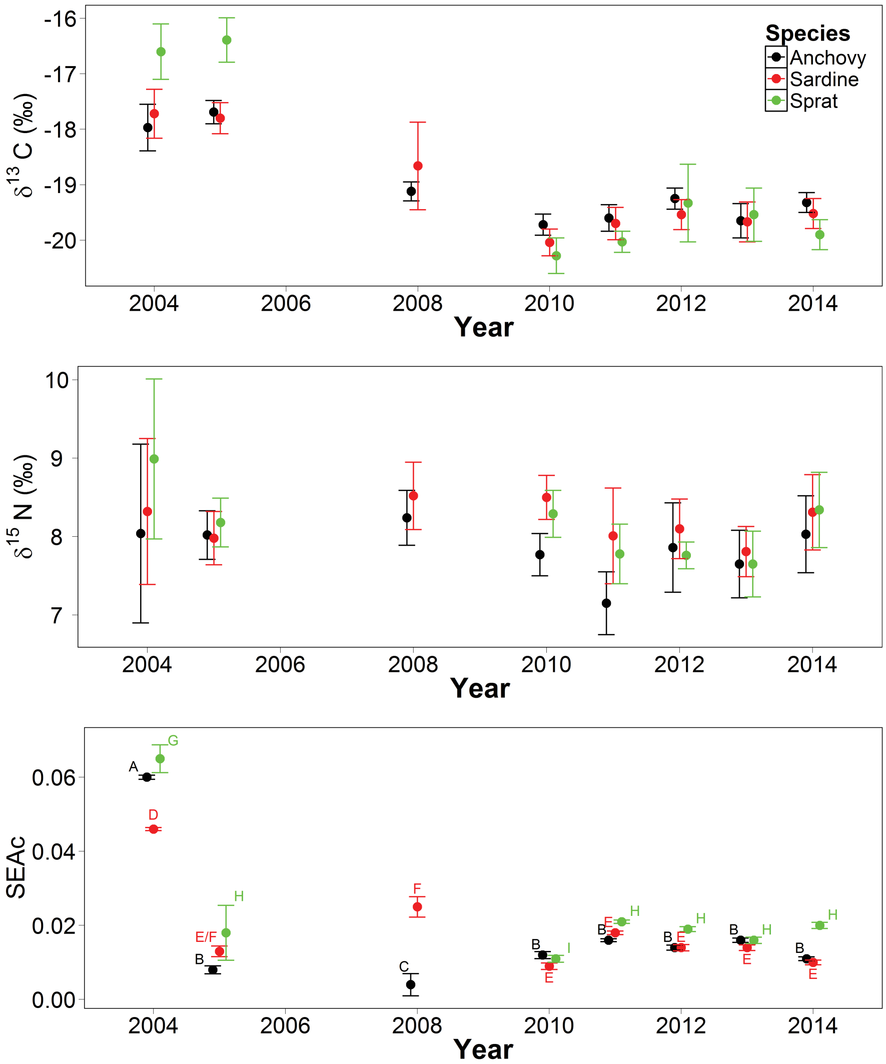

Figure 4.2 Summary of a) δ^{13}C, b) δ^{15}N values (mean, s.d., in ‰) and c) trophic niche width of the 3 species over time in the Gulf of Lions. SEA_C (SEA_C: standard ellipse area) are indicated in ‰². The Bayesian area estimate of the standard ellipse metrics (SEA_B) that present differences between years for each species are indicated by superscripts.

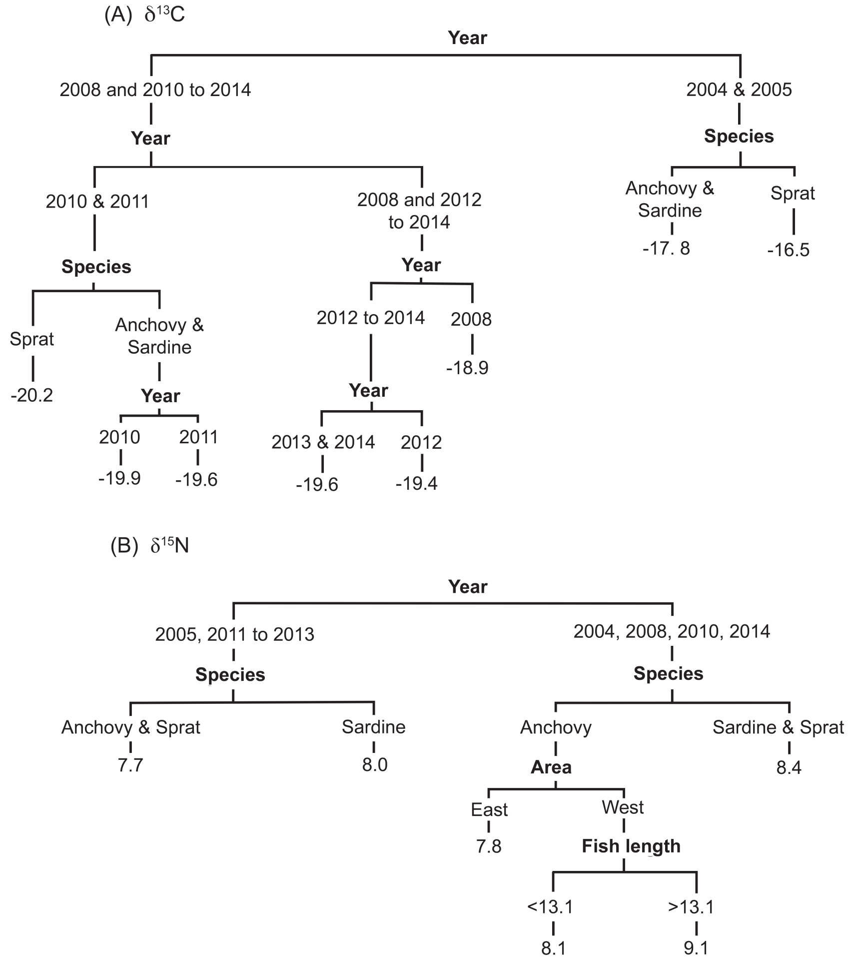

Figure 4.3 Classification and regression tree assessing the importance of year, species, fish length, latitude, longitude and coastal/offshore gradient on (A) δ^{13}C values and (B) δ^{15}N values. Trees are split off on the values of one covariate at a time such that the overall variance in the dependent variable is minimized at each split. Terminal nodes indicate the value of assigned δ^{13}C or δ^{15}N to the node.

4.3.2 Temporal changes in isotopic niche width

The niche width of the three species varied notably between years (Figure 4.2). The anchovy isotopic niche width decreased between 2004 and 2008 (SEA_B: $P < 0.001$) and then increased to remain steady at middle values (SEA_B: $P > 0.05$, Figure 4.2). The sardine isotopic niche width also decreased between 2004 and 2005 (SEA_B: $P < 0.001$), but increased between 2005 and 2008 (SEA_B: $P < 0.01$) to then decrease again in 2010 (SEA_B: $P < 0.01$) and finally remain steady until 2014 (SEA_B: $P > 0.05$, Figure 4.2). The isotopic niche width of sprat decreased between 2004 and 2010 (SEA_B: $P < 0.01$) and subsequently increased and stabilized from 2011 to 2014 (SEA_B: $P > 0.05$, Figure 4.2). In 2010 and 2013, the SEA_B width was similar between all three species (SEA_B: P

>0.05, Figure 4.2). On the contrary, during years 2004, 2005, 2011, 2012 and 2014, sprat always had a larger SEA_B than sardine and anchovy (SEA_B: $P < 0.05$, Figure 4.2). Thus, sprat always had a larger or equivalent isotopic niche width when compared to anchovy or sardine. In 2008, when only two species were available, sardine SEA_B was significantly larger than the anchovy one (SEA_B: $P < 0.001$).

4.3.3 Temporal changes in isotopic niche overlap

Figure 4 displays the bayesian ellipse areas (SEA_C) that represent the isotopic niche of the three small pelagic species in a scaled isotopic niche space. Two patterns were observed: firstly, sprat was segregated from sardine and anchovy during the two earliest years (2004 and 2005, Figure 4.4); second, the overlap of sprat isotopic niche on sardine's was present until 2014 (Table 4.2). Sprat also overlapped strongly with anchovy in 2012 and 2013, but no clear temporal trend was evidenced (Table 4.2). In contrast, the trophic niche overlap of sardine and anchovy was high in 2004 and 2005 (Table 4.2). In 2008, the total anchovy niche even fell within the one from sardine. However, during the subsequent years (2010-2014), overlap was much lower except for 2013 (at least halved compared to before 2010, Table 4.2). For each species, the isotopic location of the SEA_C differed among years (Figure 4.4).

Table 4.2 Standard ellipse overlaps for each combination of species. The values represent the percentage of overlap between the standard ellipse areas in the scaled stable isotope space. Each number in the cell refers to the percentage of overlap of the area of the species indicated first (e.g. 68% is the percentage of ellipses of sardine that are overlapped with the ellipses of the anchovy while 52% is the percentage of the ellipses of anchovy that are overlapped with sardine).

Year	Sardine| Anchovy	Anchovy| Sardine	Anchovy| Sprat	Sprat| Anchovy	Sardine| Sprat	Sprat| Sardine
2004	68	52	0	0	0	0
2005	58	89	0	0	0	0
2008	100	15	NA	NA	NA	NA
2010	0	0	0	0	50	41
2011	12	14	8	6	41	35
2012	14	13	69	52	43	30
2013	69	63	65	65	54	49
2014	32	27	9	5	70	32

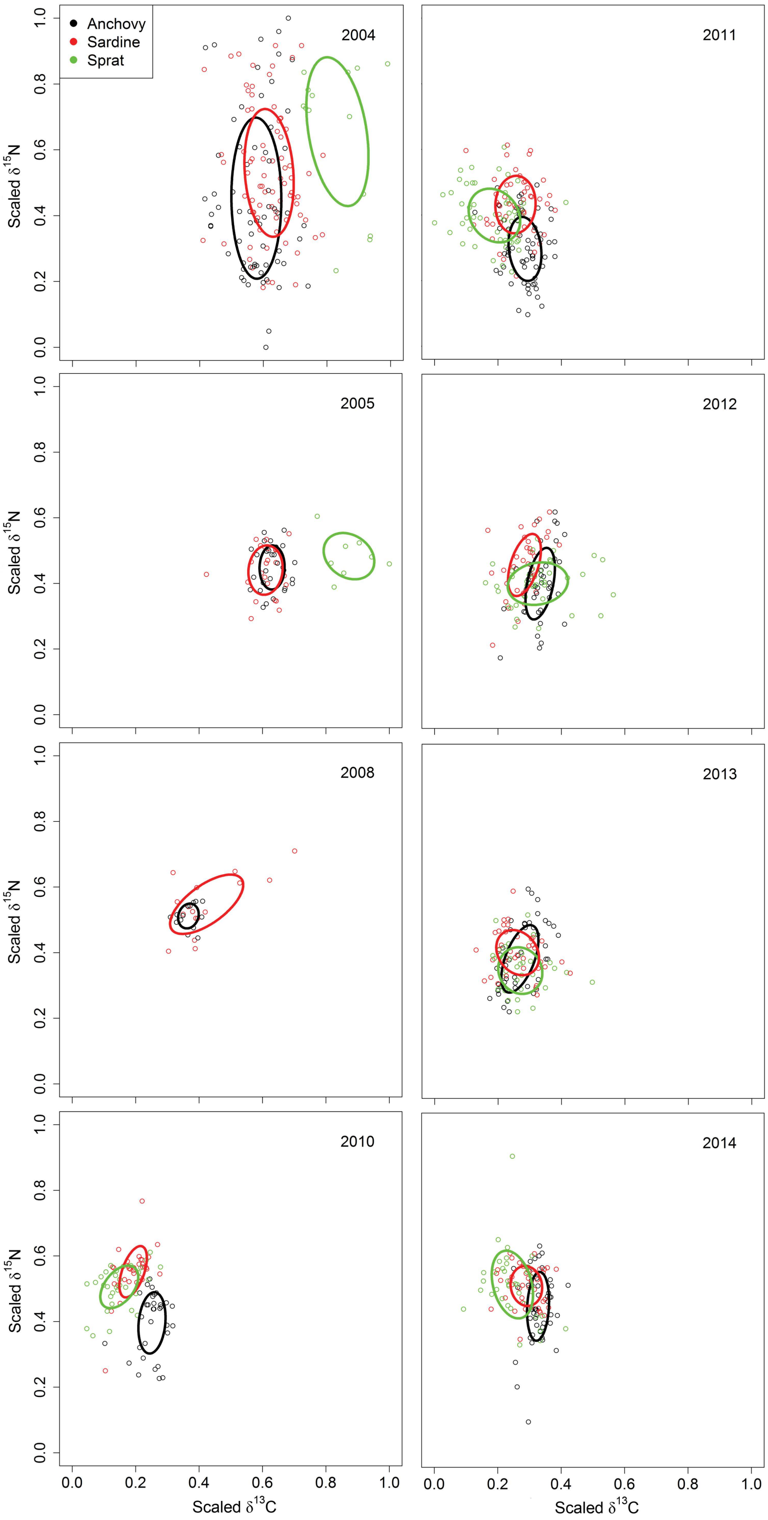

Figure 4.4 Species scaled isotopic niche space between 2004 and 2014, represented by solid bold lines based on the area of the standard ellipses corrected to small samples sizes (SEA_c). Stable carbon and nitrogen isotope values are from the muscle of adult anchovy, sardine and sprat.

4.3.4 Size, area and temporal variations in diet composition

The diet of sardine and anchovy was mainly zooplanktivorous, with some traces of phytoplankton consumption (diatoms) in 2011-2012 for both species (see Tables 4.3 and 4.4). The anchovy diet in 1994 was characterized by the dominance of *Acartia clausi* (18.23 %IRI), *Microsetella* spp (13.52 %IRI), Clauso/Paracalanidae (13.22 %IRI), *Oncaea* spp. (12.67 %IRI) and Euphausiacaea larvae (7.31 %IRI) (Table 4.3). Copepods also dominated the anchovy diet in 2011-2012, but anchovy fed almost only on two species: *Microsetella* spp (34.78 %IRI) and *Oncaea* spp. (27.52 %IRI). In 2007, sardine diet was composed of copepods (60.31 %IRI, mainly *Euterpina acutifrons*, *Microsetella* spp. and *Temora stylifera*) and cladocerans (31.48 %IRI). In contrast, cladocerans were almost absent in 2011-2012, while *Microsetella* spp. (29.34 %IRI), *Oncaea* spp. (24.55 %IRI) and *Corycaeus* spp. (21.64 %IRI) dominated the sardine diet (Table 4.4). For both sardine and anchovy, we also found a strong increase in the proportion of Corycaeidae during years 2011 and 2012.

The trophic niche width, measured with the exponential of Shannon's entropy was highest in the earliest period studied for both species (10.84 in 1994 versus 5.57 in 2011-2012 for anchovy (Table 4.3), and 8.54 in 2007 versus 6.46 in 2011-2012 for sardine, see Table 4.4).

Table 4.3 Summary of stomach content analysis for *E. encrasicolus*. %N: Percent number; %W: percent estimated dry weight; %O: percent frequency of occurrence; %IRI: percent Index of Relative Importance; *Exp*(H): trophic niche width determinate with stomach content.

		1994				2011 and 2012			
Prey type		%N	%W	%O	%IRI	%N	%W	%O	%IRI
Crustacea									
Copepods	*Acartia clausi*	6.60	30.36	57.90	18.23	2.64	7.98	18.27	2.29
	Clauso-Paracalanidae	13.54	19.49	52.63	13.22	6.36	3.68	15.38	1.83
	Centropages typicus	9.76	4.82	50	6.21	3.60	3.64	21.15	1.81
	Corycaeus spp.	1.72	2.05	55.26	1.78	4.45	23.81	43.27	14.46
	Clytemnestra	0.02	<0.01	2.63	<0.01	0.08	<0.01	1.92	<0.01
	Candacia spp.	4.00	8.15	44.74	4.63	0.90	1.10	10.58	0.25
	Oithona spp.	1.48	0.07	42.11	0.55	0.12	<0.01	5.77	<0.01
	Oncaea spp.	15.30	0.39	94.74	12.67	20.15	15.43	65.38	27.52
	Microsetella spp.	16.08	0.22	97.37	13.52	26.38	13.34	74.04	34.78
	Euterpina acutifrons	1.25	0.36	94.74	1.30	2.01	0.13	36.54	0.89
	Temora stylifera	1.22	0.41	42.11	0.59	0.02	<0.01	0.96	<0.01
	Unidentified Copepods	23.26	0.78	71.05	14.55	15.36	18.69	32.69	13.16
Cladoceran		2.79	5.36	73.68	3.37	0.33	<0.01	9.62	0.04
Ostracod		0.29	0.03	44.73	0.08	1.29	0.29	22.12	0.41
Tunicata									
	Appendicularia	-	-	-	-	0.12	<0.01	0.96	<0.01
Protists									
	Diatoms	-	-	-	-	9.21	9.60	3.85	0.86
Chaetognatha									
	Chaetognatha	0.05	0.01	2.63	<0.01	-	-	-	-
Larvae									
Crustacae	Euphausiacae	0.21	26.96	31.57	7.31	2.34	3.31	7.69	0.45
	Decapoda	0.35	0.19	34.21	0.16	1.89	1.47	11.54	0.46
Mollusca	Gasteropodae	<0.01	0.03	5.26	<0.01	1.98	0.04	10.58	0.25
	Bivalve	0.02	0.30	42.11	0.11	1.68	0.29	23.08	0.54
Eggs									
	Anchovy egg's	0.15	0.09	47.37	0.10	1.1	0.49	16.35	0.31
	Other fish egg's	0.24	0.42	50	0.28	0.14	<0.01	2.88	<0.01
		Exp(H) = 10.84				*Exp*(H) = 5.57			

Table 4.4 Summary of stomach content analysis for *S. pilchardus*. %N: Percent number; %W: percent estimated dry weight; %O: percent frequency of occurrence; %IRI: percent Index of Relative Importance; *Exp*(H): trophic niche width determinate with stomach content.

		1994				2011 and 2012			
Prey type		%N	%W	%O	%IRI	%N	%W	%O	%IRI
Crustacea									
Copepods	*Acartia clausi*	-	-	-	-	3.58	8.14	0.12	1.74
	Clauso-Paracalanidae	3.26	6.78	62.50	4.36	7.07	5.05	0.41	5.95
	Centropages typicus	-	-	-	-	1.51	1.55	0.13	0.49
	Corycaeus spp.	4.09	4.11	75.00	4.28	8.61	30.10	0.46	21.64
	Clytemnestra	-	-	-	-	0.43	0.16	0.03	0.02
	Candacia spp.	0.16	18.27	12.50	1.60	0.13	1.04	0.03	0.04
	Oithona spp.	-	-	-	-	0.16	0.04	0.04	<0.01
	Oncaea spp.	11.58	0.61	100	8.48	17.61	12.27	0.68	24.55
	Microsetella spp.	6.31	11.11	87.50	10.60	24.65	10.09	0.70	29.34
	Euterpina acutifrons	3.82	19.27	62.50	10.03	9.12	4.43	0.36	5.87
	Temora stylifera	2.01	30.95	50.00	11.46	-	-	-	-
	Unidentified Copepods	10.73	2.92	100.00	9.50	9.43	6.06	0.25	4.77
Cladoceran		43.64	1.63	100	31.48	1.08	0.70	0.11	0.24
Ostracod		-	-	-	-	1.09	1.55	0.12	0.39
Tunicata									
	Appendicularia	0.08	0.06	12.50	0.02	-	-	-	-
Protists									
	Diatoms	8.39	0.79	75.00	4.79	5.85	9.00	0.11	2.03
Chaetognatha									
	Chaetognatha	-	-	-	-	<0.01	<0.01	<0.01	<0.01
Larvae									
Crustacae	Euphausiacae	-	-	-	-	-	-	-	-
	Decapoda	0.54	2.63	37.5	0.83	0.09	0.76	0.05	0.05
Mollusca	Gasteropodae	-	-	-	-	0.12	<0.01	0.02	<0.01
	Bivalve	1.29	0.19	50.00	0.51	1.09	1.04	0.09	0.24
Eggs									
	Anchovy egg's	-	-	-	-	5.40	6.87	0.11	1.68
	Other fish egg's	-	-	-	-	2.80	0.83	0.21	0.91
		Exp(H) = 8.54				*Exp*(H) = 6.46			

In the PCA performed on the dry weight (%W) of the different prey of anchovy, the first (F1) and second (F2) component represented respectively 35% and 25% of the total variance (Figure 4.5). F1 represented a gradient whereby *Oncaea* spp was opposed mainly to *Acartia clausi* and Clauso/Paracalanidae species. F2 represented a gradient opposing *Microsetella* spp to *Acartia clausi* and Clauso/Paracalanidae species in anchovy diet. The majority of anchovy from 1994 had negative values on both axes while the majority of anchovy from 2011-2012 had positive values. Thus, we deduced an increasing proportion in dry weight of *Microsetella* spp and *Oncaea* spp and a decreasing proportion of *Acartia clausi* and Clauso/Paracalanidae species in the anchovy diet from 1994 to 2011-2012 (Figure 4.5). In the PCA carried out on the sardine data, F1 and F2 represented 44% and 18% of the total variance respectively (Figure 4.5). *Microsetella* spp was opposed to *Oncaea* spp, *Corycaeus* spp and cladocerans on F1 axis, while the second axis showed a clear separation between the cladocerans and other prey species. The two time periods were opposed on the second axis reflecting a decreasing gradient of cladocerans and an increasing proportion of *Oncaea* spp, *Corycaeus* spp and *Microsetella* spp in the sardine diet from 2007 to 2011-2012. Fish length (4 length classes of 1.5 cm from 10 to 16 cm) as well as sampling area (3 areas; West, Central and East) were plotted on the PCA, but both factors displayed no trend.

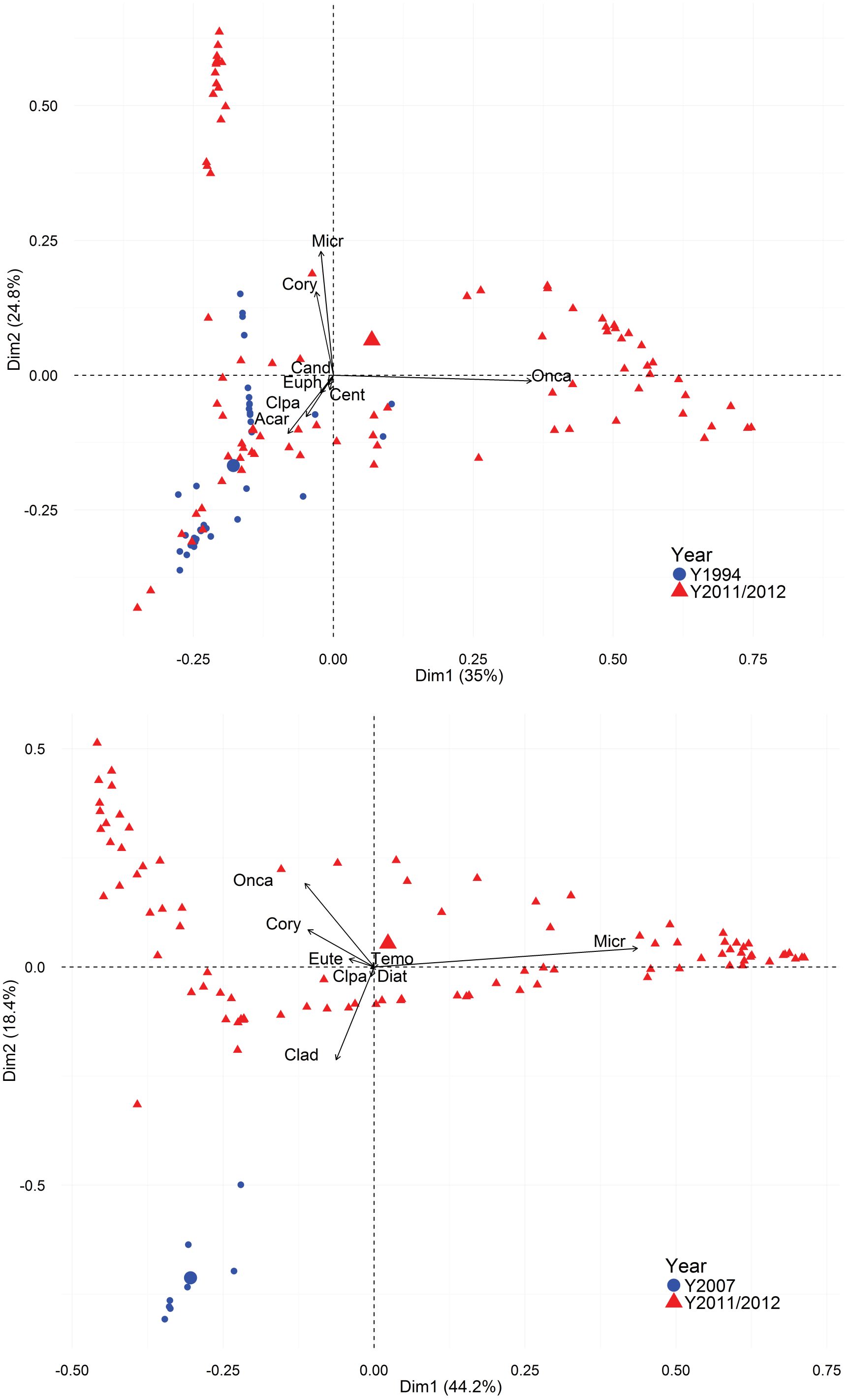

Figure 4.5 Principal component analysis of the dry weight percentage of the main anchovy (up) and sardine (down) prey in the Gulf of Lions. Cent: *Centropages typicus*; Cand: *Candacia* spp; Clad: cladoceran; Temo: *Temora stylifera*; Cory: *Corycaeus* spp; Micr: *Microsetella* spp; Eute: *Euterpina acutifrons*; Diat: Diatoms; Clpa: Clauso-Paracalanidae; Onca: *Oncaea* spp. Barycenters of the years (Y) are added as supplementary variables.

4.4 Discussion

Data availability does not allow us to describe the trophic ecology along the last 20 years, but we can contrast the present situation to data collected before the drastic changes in demography occurred. The comparison of small pelagic trophic data: (i) showed wider isotopic niche in sprat compared to sardine and anchovy; (ii) tended to confirm the hypothesis of changes in the anchovy and sardine diets and (iii) suggested a recent increase in the trophic overlap between the different small pelagic fish species of the Gulf of Lions.

Using SIA and isotope-derived metrics to study interspecific trophic differences, we showed that sprat had a larger isotopic niche width (in 2004, 2005, 2011, 2012 and 2014) than sardine and anchovy or at least equivalent (in 2010 and 2013), mainly due to the broad range of δ^{13}C. Assuming that wider sprat isotopic niche reflects higher variability in individual diets (Matthews and Mazumder, 2004), sprat intraspecific trophic competition may be lower than those of other species. Additionally, stomach content analyses tended to indicate a slightly more diversified sprat diet (Le Bourg *et al.*, 2015). These results are particularly interesting from a population dynamics viewpoint. Indeed, sprat biomass has considerably increased since 2008, while the ratio biomass/abundance of both sardine and anchovy has considerably declined (GFCM, 2015). Previous studies have shown that those changes were mainly driven by reduced growth, age truncation and condition and bottom-up processes were the most likely drivers (Van Beveren *et al.*, 2014). Being a generalist feeder with low intraspecific food competition might thus have conferred important advantages to sprat over sardine and anchovy, especially to adapt to environmental changes. This might also explain why sprat body condition was more stable, while sardine and anchovy condition exhibited stronger ups and downs, possibly in reaction to prey variability (Van Beveren *et al.*, 2014).

This study also depicts a considerable reduction in both sardine and anchovy isotopic niche through time. The carbon isotopic composition of fish muscle is mainly related to the ingested preys (Hobson, 1999). As previously stated, strong changes in δ^{13}C values of small pelagic fish and zooplankton were observed along years. Differences between years in fish stable isotope values may be due to inter-annual differences in the planktonic community and/or the organic matter, the latter being at the basis of the food web. The Gulf of Lions is further known as a highly variable area in water circulation, productivity and stable isotope ratios of plankton community (André *et al.*, 2005; Espinasse *et al.*, 2014b), which could add variability in fish isotopic signature. Yet, similarly to previous findings obtained with a different methodology (e.g fatty acid profiles, Pethybridge *et al.*, 2014), we showed that isotopic values did not differ between sampling areas except for δ^{15}N in anchovy during some years, underlining the weak influence of the sampling area on the isotopic values at the scale of the present study. This allows us to reasonably assume that differences were not due to sampling bias, even if the sampling locations varied from year-to-year. A reduction of the anchovy isotopic niche was observed between 2004 and 2013, although the largest shrinkage occurred between 2004 and 2005. Nevertheless, this considerable change in isotopic niche area between the two years has to be considered with caution, due to the lower sampling size in 2005 in comparison to 2004 which reduces the precision of the estimated mean of niche width (see Syväranta *et al.*, 2013). The largest reduction in sardine isotopic niche was observed between 2008 and 2013, concomitantly with the decrease in sardine size, condition and biomass.

Moreover, stomach contents also confirmed a significant reduction in the trophic niche width of both anchovy and sardine (25% and almost 50%, respectively). Specifically, both species displayed a much narrower diet during recent years (2011-2012) compared to previous time periods (1994 or 2007). This might have resulted from a decrease in the availability of certain prey species, downplaying their role in the diet of the small pelagics, as indicated by temporal differences in composition and size range of consumed prey. The sampling methodology has been kept as consistent as possible during the study, especially in terms of season, fishing gear and daylight time. Hence, changes could only be related to variables such as year, area and fish size. However, no size class or sampling area effects were detected during analyses, so that observed changes are likely to be due to a year effect. For adult anchovy and sardine, copepods were, as in most other studies in the Mediterranean (see Tudela and Palomera, 1997; Borme *et al.*, 2009; Nikolioudakis *et al.*, 2012), by far the most important prey item, regardless of the year. However, there was a prey size reduction between both periods. In 1994, large copepods (size >1 mm, *Acartia* spp., *Candacia* spp., *Clauso*/*Paracalanus* spp. and *Centropages typicus*), attained almost 50% of the relative importance index (%IRI) and 65% in relative dry weight (%W). This contrasted with 2011-2012 years when they represented barely more than 5% of the relative importance index and 15% in relative dry weight (Le Bourg *et al.*, 2015). Large copepods in the anchovy diet were replaced in 2011-2012 by small copepods species (size <1 mm, such as Coryceidae, *Microsetella* spp. and *Oncaea* spp.), which increased threefold. The proportion of small and large copepods in 1994 was in agreement with another study from 1995 and 1996, performed in the same area and during the same period Plounevez and Champalbert (2000). Marked changes between 1994 and 2011/2012 in the anchovy diet also concerned other prey groups, such as cladocerans that became practically absent in recent years, larvae (mainly Euphausiacea) that also decreased while diatoms appeared. The decrease of large Euphausiacea larvae, recognized as important anchovy prey in other areas such as the Humboldt current (Ayón *et al.*, 2011), might have accentuated the deficiency in large prey species.

Similarly, small copepods dominated sardine diet in recent years, while cladocerans were the dominant prey in 2007. Assuming that larger copepods have a higher energy content (Dumont *et al.*, 1975; Vijverberg and Frank, 1976; Zarubin *et al.*, 2014), current nutritional conditions of anchovy and sardine might have thus strongly decreased compared to pre-2008, potentially explaining the strong demographic changes observed in both species after 2008. Furthermore, fish were sampled in July, when adults need to accumulate their fat supply to survive the next winter (Wiegand, 1996; Sánchez-Gómez, 2013). Therefore, the lack of large copepods in their stomachs could reflect difficulties to build a sufficient fat to survive an energetically demanding winter, especially so for sardines which reproduce at that moment. This might thus explain the apparent adult overmortality detected for sardine (less pronounced for anchovy, Van Beveren *et al.*, 2014). However, in order to better understand the impact of the changes in sardine and anchovy diet, a detailed investigation of zooplankton variations in terms of quantity/quality and fish energetics would be required.

Nonetheless, considering the usual importance of large copepods in the diet of anchovy and sardine, these changes probably reflect a potential decrease in their availability, which might result either from an increase in competition between fish species or a decrease in abundance of large copepods. First, the increase in sprat abundance and biomass might have drawn more intensive interspecific competition. Indeed, all three species have been shown to inhabit the same areas and to spatially co-occur in the Gulf of Lions, except for some deeper grounds where only anchovy occurs (Saraux *et al.*, 2014).

The hypothesis of an increased interspecific competition during the last decade is supported by the changes in the degree of overlap and segregation in the isotopic niches of the fish species. Indeed, there was clear isotopic niche segregation between sprat and the two other species in 2004 and 2005, indicating that sprat could have exploited different species groups from anchovy and sardine during these still prosperous years. On the contrary, sprat diet has overlapped significantly with anchovy and/or sardine diet since 2010. Conversely, the overlap between anchovy and sardine was strong in 2004, 2005 and 2008, but was nearly inexistent in 2010, 2011, 2012 and 2014. These stable isotope analyses are in accordance with stomach content analyses, which also provided evidence for potential strong competition between sprat, sardine and anchovy (Le Bourg *et al.*, 2015). So, this study underlined the importance of new food sharing in the context of the recent increase of the sprat population. The special case of 2013 might be caused by very limited food resources (quantitatively or/and qualitatively), forcing the three species to highly exploit prey species with similar stable isotope ratios (possibly similar prey species).

Second, beyond prey size spectrum changes, we also showed the decreasing richness of small pelagic fish diet with time. This result was in agreement with observed and modeled changes in the composition and the phenology of zooplankton in Mediterranean areas surrounding the Gulf of Lions (Balearic Sea: Auger *et al.*, 2014; de Puelles and Molinero, 2008; Ligurian Sea: Molinero *et al.*, 2005, 2008). Recent low prey diversity and small prey sizes suggest that recent environmental changes affected the planktonic production. This study, despite some missing values in some years, showed a decreasing trend in δ^{13}C values for zooplankton. This is also in agreement with other studies, which underlined similar isotopic values in 2010 and in 2014, and most importantly strictly lower values in 2010 than in 2004 and 2005 (Banaru *et al.*, 2013; Espinasse *et al.*, 2014a; Strady *et al.*, 2015). Although isotopic variations may result from a multitude of drivers, strong differences documented in this study could support important changes in the planktonic community and/or in the organic matter sources. Auger *et al.* (2014) showed that climatic and environmental variations may induce changes in size structure of the plankton community, while Rau *et al.* (1990) showed that small phytoplankton (pico and nano phytoplankton) had lower δ^{13}C values than micro phytoplankton. Interestingly, possible reduction in δ^{13}C values for zooplankton between 2004/2005 and 2010 correspond to the period of the drastic changes in small pelagic fish populations (Van Beveren *et al.*, 2014). As already observed in the Benguela ecosystem, where shifts between anchovy and sardine regimes were caused by changes in the availability of mesozooplankton prey mediated through changes in environmental conditions (Shannon *et al.*, 2004), growth and condition of sardine and secondarily anchovy in the Gulf of Lions are also likely to be mediated by a bottom-up control.

4.5 Conclusion

This study supports the hypothesis that changes in small pelagic fish growth, size and body condition could be due to a bottom-up control characterized by changes in food availability or/and increasing potential trophic competition, two other factors that might influence the feeding success and the energy allocation. Our results illustrate the utility of comparing feeding habits between periods using a combination of short (stomach content) and longer term (SIA) indicators. This not only helps to understand the current ecosystem fluctuations in the Gulf of Lions, but also provides an insight in the trophic dynamics of the pelagic ecosystem, possibly allowing a more efficient monitoring of marine food-web evolution.

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4.6 Supplementary material

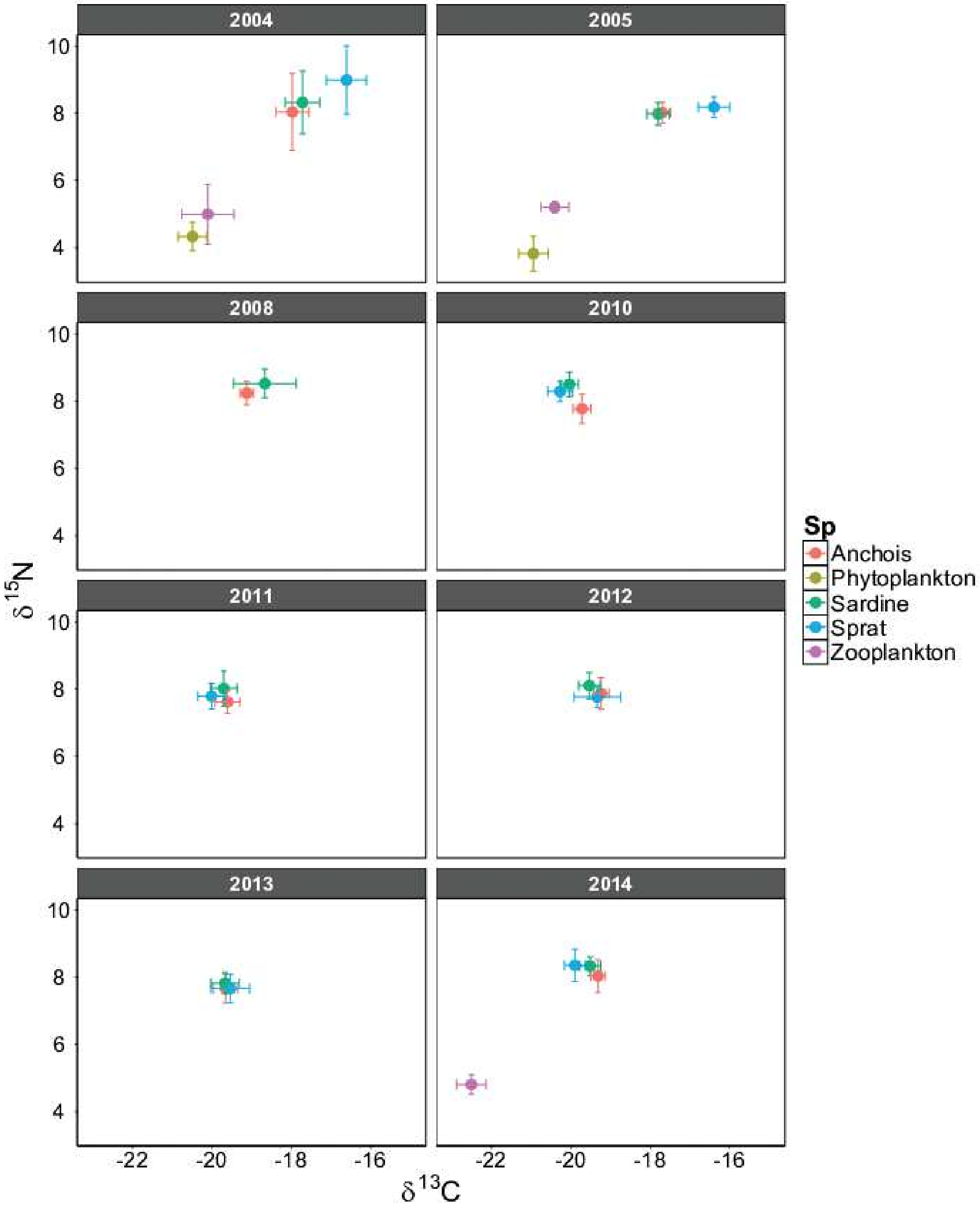

Figure 4.6 A dual isotope plot representing the different small pelagic fish species sampled each year and the basal trophic levels (phytoplankton and zooplankton). All the different groups represented by the mean δ^{13}C and δ^{15}N values (SD).

Chapter 5

Body reserves mediate trade-offs between life history traits: new insights from small pelagic fish reproduction

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Preface

Previous chapters investigated the reasons for a decreasing small pelagic fish condition in the Gulf of Lions, highlighted the main role of food resources and indicated the potential negative impact of an increasing sprat population on the anchovy and sardine population. In the following chapter, we will examine the consequences of a decrease in size and condition of anchovy and sardine on their reproductive capacities. Indeed, as anchovy and sardine are smaller and store less energy, essential life history traits such as growth, survival and reproduction could also be affected. For example, some studies already demonstrated that fish can skip reproductive events when their condition starts hampering survival and reproduction. However, little knowledge is available on this for our species of interest.

The idea that survival and reproduction of small pelagic in the Gulf of Lions might have been strongly affected is reinforced by several other studies linking reproductive output on size, maturity and condition. Generally, such studies focused on females because it was assumed that reproductive success was mostly driven by maternal investments in egg production. Females determine the size of the energetic reserves available to the embryo and future larvae. For example, the egg quantity and quality was shown to increase with female size and age. However, the effect of maternal condition on both egg quantity and quality remained largely unknown for small pelagic fish. This chapter aimed at verifying this effect, using two different data sources.

During a first experimental part, females of both sardine and anchovy during their respective reproductive periods (i.e., summer 2015 for anchovy and winter 2014/2015 for sardine) will be investigated. The effects of size and maternal condition on both batch fecundity and egg quality (i.e., eggs dry mass) will be studied. Additionally, these analyses will allow us to make the link between maternal condition and the formation of atresia (degenerating oocytes which will not be spawned). We included a second part in this chapter to show the approximately decadal (2003 and 2015) variation of both the reproductive period and reproductive effort of anchovy and sardine. The reproductive effort will be calculated based on length at maturity and the gonadosomatic index. This will allow us to quantify the variability in this variable, and to assess small pelagic fish recruitment could be affected. This study also has the advantage that an overall recommendation could be given concerning the potential use of fish maternal condition as a proxy of future reproductive success? If maternal condition would be a good proxy of reproductive effort, it could be incorporated in future fish stock evaluations.

Abstract

Limited resources in the environment prevent individuals to simultaneously optimize all life history traits, resulting in trade-offs. In particular, the cost of reproduction is well known to negatively affect energy investment in growth and maintenance. Here, we investigated these trade-offs during contrasted periods of high versus low fish body condition (before/after 2008) in the Gulf of Lions. Female reproductive allocation and performance in anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) were examined based on morphometric historical data from the 70s and from 2003 to 2015. Additionally, potential maternal effects on egg quantity and quality were examined in 2014/2015. After 2008, the gonadosomatic index increased for sardine and remained steady for anchovy, while a strong decline in mean length at first maturity indicated earlier maturation for both species. Regarding maternal effects, for both species egg quantity was positively linked to fish size but not to fish lipid reserves, while the opposite was true for egg quality. Atresia prevalence and intensity were rather low regardless of fish condition and size. Finally, estimations of annual total numbers of eggs spawned indicated a sharp decrease for sardine since 2008 but a slight increase for anchovy during the last 5 years. This study revealed a biased allocation towards reproduction in small pelagic fish when confronted with a really low body condition. This highlights that fish can maintain high reproductive investment potentially at the cost of other traits which might explain the present disappearance of old and large individuals in the Gulf of Lions.

Keywords trade-off; life history; maternal effect; NW Mediterranean; anchovy; sardine

5.1 Introduction

Reproduction and maintenance require important energy investments in all animal species (Jensen, 1996; Cox *et al.*, 2010). In their environment, individuals find finite resources, limiting their energy gain and bringing out trade-offs, preventing simultaneous maximization of all life-history traits (Stearns, 1989, 1992). In particular, the main trade-off involves the cost of reproduction (Stearns, 1989), represented by negative correlations between the current reproductive effort and both maintenance and future reproduction (Williams, 1966). In a situation of food shortage and low individual energy reserves, this might lead to extreme choices, such as either temporarily stopping reproduction (skipping of breeding event) or maintaining reproduction at the cost of survival (Jørgensen *et al.*, 2006). In many populations, the trade-off between the investments in the different life history traits may be impacted by strong changes in size or condition, and individuals could give a preference to one or another (e.g. birds, Bêty *et al.* (2003), mammals, Shero *et al.* (2015) and fish, Adams, 1999).

For instance, in small pelagic fish, female batch fecundity (the number of eggs per batch) rises with increasing body size (Peck *et al.*, 2013). This suggests that female size and growth rate could have a marked effect on reproductive output (similar conclusions were demonstrated for turtles Rowe (1994) or daphnia, Lampert, 1993). This is known as the maternal effect, i.e. when the phenotype of an organism is influenced not only by its own genes and the environment, but also by the characteristics of its mother, which might increase or decrease the chance of survival. Also, studies on diverse species have demonstrated that young females may not produce eggs of the same quality (Lampert, 1993; Ganias, 2009) or as many eggs as older females per unit of maternal biomass (i.e., relative fecundity, Martin, 1995). Besides a decrease in size and age, a drop in body condition might also influence reproduction. Condition is commonly considered as the quantity of nutrient reserves and a proxy of fitness (Lloret *et al.*, 2014), determining the survival and reproductive capacity of individuals and populations. Indeed, energy is usually the main limiting factor preventing individuals from maximizing life-history traits (Hirshfield and Tinkle, 1975; Reznick, 1985; Brown *et al.*, 2004). Some studies have shown that a better body condition of the females may allow the allocation of energy surplus to reproduction (increasing reproductive outputs in quantity and quality), thereby influencing population growth (Naulleau and Bonnet, 1996; Blums *et al.*, 2002; Hamel *et al.*, 2010). In contrast, species reducing their reproductive potential (e.g. birds, Chastel *et al.* (1995) or fish Lambert and Dutil, 2000) or skipping the spawning period (e.g. turtle, Rivalan *et al.* (2005) or fish, Rideout and

Tomkiewicz, 2011) are the most common negative trade-off effects observed. Those contrasted findings usually fit the life-history predictions that species with slower life-history paces should allocate more into survival and future reproduction, while short-lived species should dedicate most of their energy towards reproduction (Stearns, 1989). While the trade-off between somatic and reproductive functions has been long considered (Williams, 1966; Hirshfield and Tinkle, 1975; Stearns, 1989; Skibiel *et al.*, 2013), especially in the context of the slow-fast life-history gradient, less information is available on how capital vs. income breeding (use of energy stored before reproduction or acquired during reproduction) might affect the resolution of this conflict.

Small pelagic fish are relatively short-lived species, known to commonly face strong variations in abundance and biomass (Cole and Mcglade, 1998). In particular, anchovy and sardine are important forage fish, which form a key component of the pelagic ecosystems (Cury *et al.*, 2000; Banaru *et al.*, 2013), and constitute the prey of numerous predators (e.g tuna, marine mammals and seabirds). Small pelagic fish also support the most important fisheries in the world and employ a significant number of people (FAO, 2014). The ratio of biomass to abundance (i.e. the mean population weight) of the anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) populations in the Gulf of Lions has dramatically decreased since 2008, while simultaneously a pronounced increase in both abundance and biomass of another small pelagic fish species, namely sprat (*Sprattus sprattus*) has occurred (Van Beveren *et al.*, 2014). The decline went along with a marked decrease in size, age and condition as well as reducing food resources for both species (Van Beveren *et al.*, 2014). This raised several questions regarding i) the resolution of the reproduction vs. maintenance trade-off in a situation of low energetic reserves and ii) the effects on the anchovy and sardine population dynamics, specifically through their reproductive capacities. Anchovy and sardine share the Gulf of Lions as an important spawning area, but their spawning characteristics and habitats are clearly differentiated (Palomera *et al.*, 2007). On one hand, anchovy reproduces during late spring and summer when water temperatures reach values between 17°C and 23°C and is an income breeder (Somarakis *et al.*, 2004; Palomera *et al.*, 2007). On the other hand, sardine is a capital breeder reproducing in cold water (temperatures ranging between 12 and 14 °C) from December to March (Ganias *et al.*, 2007a; Pethybridge *et al.*, 2014). Despite their opposite reproductive strategies, the two species are batch spawners (i.e. they release eggs in batches over a protracted spawning season, Millán, 1999; Ganias *et al.*, 2004). Despite for evident socioeconomic reasons, these two species thus offer a unique opportunity to investigate the cost of reproduction in a situation of food shortage in two species sharing very similar characteristics but for their capital vs. income breeding strategies.

The main purpose of this study was to investigate the trade-off between the different life-history traits, to assess whether small pelagic fish maintain a high reproductive effort, which could potentially affect their maintenance, or not. The answer to this question could allow us to better understand the recent fluctuations in the small pelagic fish populations of the Gulf of Lions. To do so, we used length at first maturity (L_{50}), the gonadosomatic index (GSI) and reproductive period durations as indices of reproductive investment. We investigated their temporal changes between 2003 and 2015, a period in which drastic changes in body condition were observed. In a second step, biometry and gonad analyses were performed over one spawning season, to investigate the effect of adult size and condition on the reproductive capacity of both species, i.e the maternal effects. Sampling effort and analyses were limited to females as their reproductive capacity is the main driver of the population dynamics, caused by the high cost of egg production relatively to the energy needed for producing sperm (Coleman and Jones, 2011; McBride *et al.*, 2013). All these parameters are frequently considered for fisheries research and management, but are rarely reported simultaneously over a long-term period for multiple species. Linking fish condition to reproductive capacity could greatly aid the understanding of the population dynamics of sardine and anchovy. In turn, such understanding could benefit management of these economically valuable species.

5.2 Material and Methods

5.2.1 Fish sampling

A total of 8,887 female anchovies were randomly collected from 2003 to 2015. Female sardines (N= 10,541) were caught between 1971 and 1978 (N= 2,192) and 2004 to 2016 (N= 8,259). Samples were obtained from scientific surveys (PELMED & MEDITS, from 2003 onwards) and commercial trawlers operating in the Gulf of Lions (North Western Mediterranean Sea, during all years). Total body length (LT, to the nearest mm), body mass (M, to the nearest 0.1 g), eviscerated body mass (ME, to the nearest 0.1 g), sex and gonad mass (MG, to the nearest 0.1 g) were recorded for each individual. Maturity stages were determined by visual examination of the gonads, using a six-stage key in which stage 1 indicates immature individuals, stages 2 to 4 illustrate 3 steps of increasing development of gonads, stage 5 shows the spawning period and stage 6 features the post-spawning period (ICES, 2008). Fish at stage 2 and above were considered to be adults, forming the putative spawning population. Fish in stages 3-5 were assumed to be showing reproductive activity. An additional analysis was performed on 108 sardines sampled during the 2014-2015 winter and 126 anchovies collected during the 2015 summer. Individuals were either dissected onboard (PELMED) or placed in plastic bags filled with ice to be transported to the laboratory (trawlers), where they were immediately dissected. One gonad was fixed in 4% buffered formaldehyde for histological processing and oocyte quantity and quality estimation, as recommended by Rakka and Ganias (2015). A piece of muscle was also removed and frozen at -80°C for further lipid content determination.

5.2.2 Historical changes in reproductive patterns

First, we investigated the duration and timing of reproduction along the studied years, using the percentage of mature individuals (stage 5) spawning every month. A lack of data in some months for some of the years prevented accurate yearly representations. Data were thus pooled into two equal time periods (2008-2011 and 2012-2015) to increase sample sizes and representativeness (n $\geq$ 50 in any given month). These breeding cycles were then compared to previously published data from 1965 (Aldebert and Tournier, 1971). Additionally, data on the mean breeding stage of sardines collected in 1959 (Lee, 1961) were also used for comparative purposes. In order to confirm these results, a second approach to assess breeding phenology is presented by using gonadosomatic index (*GSI*, see below) seasonality in the 70s and between 2002 and 2015 for sardine and between 2003 and 2015 for anchovy.

Two measures were used to describe small pelagic fish reproductive investment. The gonadosomatic index was calculated using the following formula:

$$GSI = \frac{MG}{ME} * 100$$

where MG is the gonad weight and ME the fish eviscerated weight, respectively.

Length at first maturity (L_{50}), i.e. body length at which 50% of the individuals were mature, was estimated per spawning season between 2003 and 2015 for anchovy and from 1971 to 1976 and 2003 to 2016 for sardine. To do so, annual maturity ogives were created per species, plotting the proportion of mature individuals relative to fish length. Generalized Linear Models (GLM) with a binomial error distribution and a logit link were used to approximate the ogive, with the proportion of mature fish (m) as the dependent variable and the length classes (L_C, 0.5 cm) as the independent variable. The models had the general form:

$$\text{Logit}(E[\text{m}]) = a + b\text{L}_C$$

where a and b are the intercept and slope of the ogive, respectively. L_{50} for each year was derived from the estimated parameters:

$$L_{50} = \frac{-a}{b}$$

5.2.3 Fish muscle lipid content

Fish condition was estimated by muscle lipid content for anchovy and sardine collected during their spawning season, respectively in the 2015 summer and the 2014-2015 winter. Liver was not investigated here as its weight was too small in comparison to the weight of other organs (<1%) and these two species are renowned to store lipids in the muscle. Roughly 0.1 g of muscle was sampled in order to extract lipids using a solvent mixture (chloroform–methanol 2:1, v/v), as described by Folch *et al.* (1957) and analysed by flame ionization detection (FID) on an Iatroscan (Brosset *et al.*, 2015a). For the purpose of this study, only total lipid content obtained by summation of individual lipid classes is presented.

5.2.4 Reproductive capacity

Histological determination and follicular atresia

After fixation, one gonad was cut transversely along its midsection and embedded in paraffin before being sliced into 5-10 μm sections and stained with both haematoxylin-eosin and Mallory's trichrome stains. The latter staining method highlights the zona radiata and its continuity and facilitates the detection of degenerating oocytes which will not be spawned, i.e. atretic oocytes (Muñoz *et al.*, 2010).

Histological analyses allowed us to select individuals used in fecundity and egg quality analyses. First, we used the terminology employed by Brown-Peterson *et al.* (2011) to describe the developmental stage of the oocytes; immature (fish that have not reached sexual maturity), regenerating (mature but reproductively inactive individuals), developing (fish with gametes that are beginning to develop), spawning capable (fish with advanced gametes that are ready for spawning), actively spawning (oocytes in migratory nucleus or hydration stage) or regressing (massive atresia which indicates the end of the reproductive cycle). Only fish in the actively spawning stage were

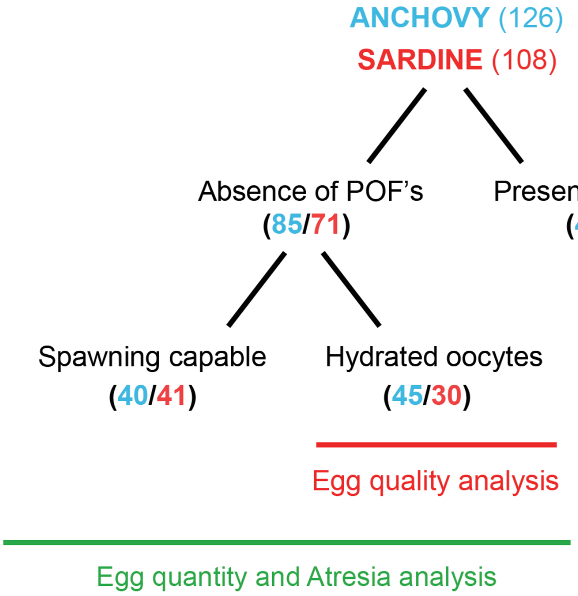

Figure 5.1 Sample size for fecundity analyses for anchovy (blue) and sardine (red).

retained for later analyses on egg quality, whereas both spawning capable and actively spawning stages were kept for estimations of batch fecundity. To avoid underestimations of batch fecundity histological analyses were used to check for the presence of postovulatory follicles (POFs), which reveal if spawning had already started. Egg quality analyses were performed with hydrated oocytes. For the experimental part of this study, 108 female sardines were analyzed, including 41 individuals classified as spawning capable and 30 individuals with hydrated eggs. Over the 126 female anchovy, 45 individuals hold gonads with hydrated oocytes and 40 were retained as spawning capable individuals (Fig 5.1).

In a second step, histological analyses were also performed to study atresia. Atresia were quantified with two different measures: the prevalence of atresia (PA), calculated as the proportion of females with α-atretic oocytes, and the relative intensity of atresia (IA), determined for females exhibiting atretic oocytes as the number of α-atretic oocytes divided by the total number of vitellogenic oocytes. Three different gonad areas were analyzed for both indices and the mean of the three areas was used as the relative intensity of atresia.

Fecundity and egg quality

To assess fecundity, subsamples of the central part of the ovary were weighted before being washed to separate the oocytes from the connective tissue (Lowerre-Barbieri and Barbieri, 1993). Anchovy and sardine are batch spawners, so their fecundity was estimated in terms of Batch Fecundity (*BF*), defined as the number of eggs spawned per batch (Murua *et al.*, 2003). Three sieves with a mesh size ranging from 600 μm to 250 μm were used to sort the oocytes by size. Oocyte size distribution followed a bimodal distribution and eggs belonging to the next batch were counted based on their size as defined in (Ganias *et al.*, 2004; Ferrer-Maza *et al.*, 2016) for anchovy. Counting of oocytes was performed with an image analysis system (Image-Pro Plus 5.1; http://www.mediacy.com). The *BF* was estimated with the following formula:

$$BF = MG * \frac{O}{SW}$$

where *MG* is the gonad weight after fixation, *O* is the number of oocytes counted in the subsample of the ovary and *SW* is the subsample weight. In addition, the relative batch fecundity (*RBF*) was computed as the *BF* divided by the eviscerated weight of the fish (g).

Oocyte quality, another proxy of reproductive success (Brooks *et al.*, 1997), was assessed by estimating the mean oocyte dry mass for each fish, obtained by drying two replicates of 100 hydrated oocytes during 24h at 110°C.

Estimation of the number of eggs spawned by the populations

An estimation of the total population egg production for each year and species was calculated by combining (I) the number of fish per length class in the population, (II) maturity ogives, (III) length-dependent batch fecundity, and (IV) the number of spawning events (or batches) per length class during the spawning season in the same formula:

$$\text{Egg number} = \sum_{li}(n_{li} * M_{li} * BF_{li} * \frac{Spawning\,duration}{Batch\,period_{li}})$$

With n_{li} the number of fish in length class li, M_{li} the percentage of mature individuals in length class li, BF_{li} the batch fecundity for length class li and $\frac{Spawning\,duration}{Batch\,period_{li}}$ the number of spawning events for length class li.

n_{li} was obtained from PELMED acoustic surveys, which took place during summer (i.e, the reproductive period of anchovy). However, as sardines reproduce between December and March, size structure information needed to be corrected for this species.

Therefore, the theoretical sizes of sardine in winter were estimated from their sizes of the preceding summer recorded during PELMED, using a growth correction formula. Given that small pelagic fish grow mainly during summer, seasonal growth variability was accounted for using Somers' model based on 2003-2014 otolith data (Somers, 1988; García-Berthou *et al.*, 2012). Adult mortality was assumed to be size independent between summer and the following winter, as mortality was assumed to occur mostly in the first weeks of life. M_{li} was obtained from annual maturity ogives (see historical changes in reproductive patterns), while BF_{li} was inferred from fecundity analyses (see fecundity and egg quality).

The number of spawning events was obtained by combining the spawning duration (obtained from annual reproduction cycles depending on the studied period; see historical changes in reproductive patterns) and the between-batches period. The latter was size-dependent and obtained for sardine from (Ganias *et al.*, 2003): individuals smaller than 13cm spawn every 17 days, those ranging between 13 and 16cm spawn every 12.25 days and those greater than 16cm spawn every 7.81 days. Regarding anchovy, the spawning frequency changes with age, and was derived from Dynamic Energy Budget (DEB) modeling (García and Palomera, 1996; Pethybridge *et al.*, 2013). This number of batches from DEB modeling is close to direct observations (García and Palomera, 1996). Age was transformed into size using mean length at age (Van Beveren *et al.*, 2014). Due to the size decrease during the last decade, separate values were determined before and after 2008. Therefore, age 1 anchovy spawning every 5.26 days correspond to a size range between 11.5 and 13cm before 2008, and between 10 and 11.5cm after 2008. Age 2 individuals spawning every 4.35 days fall into a size range of 13 to 15cm before 2008, and 11.5 to 14.5cm after 2008. Age 3 anchovy spawning every 4.17 days have a size superior to 15.5cm before 2008 and 14.5cm after 2008 (Van Beveren *et al.*, 2014). Egg quality was not included in this simulation because of the difficulties to link morphometric to biochemical condition measurements during the reproductive period (Brosset *et al.*, 2015b).

5.2.5 Data analyses

We conducted linear regression analyses between total muscle lipid content and both egg quantity and quality. The coefficient of determination (R^2) was used to estimate the proportion of variability explained and the strength of the relationship between the different variables tested. Similarly, linear regressions were also carried out per species between the intensity of atresia and the muscle lipid content and fish length.

Generalized linear models with a binomial error distribution were conducted to investigate the relationships between fish length and condition and atresia prevalence. All residuals were tested for normality and homogeneity of variance, and transformed if necessary. *GSI* annual cycles (base period 1973-1978 plus 2002-2015 for sardine and 2004-2015 for anchovy) were used to generate *GSI* anomalies calculated as the difference between the observed *GSI* of each fish for a given month, and the mean *GSI* of that month over the entire dataset. Then, in order to investigate interannual variation in GSI, the analyses were restricted to the spawning months where at least 25% of the population was spawning (as defined in the results on breeding phenology). For both species, *GSI* interannual variability and differences between time periods were tested using one-way analyses of variance (ANOVA). For the calculation of the number of eggs spawned annually per species, 1000 simulations were performed in order to incorporate the uncertainties derived from fecundity and maturity at length as well as spawning duration. Parameters for which an estimation of the associated uncertainty was available (i.e. M_{li}, BF_{li}) were randomly drawn from a uniform distribution whose range was defined by the 95% confidence interval calculated from the size-maturity and size-BF glm results. Spawning duration was also randomly drawn according to the range inferred from results of the reproduction annual cycles.

All statistical analyses were performed with R version 3.0.2 (R Development Core Team, 2013). Values are indicated as means standard errors (SE) and all statistical tests were performed at a significance level of 0.05.

5.3 Results

Historical perspective of reproductive patterns

Based on the percentage of actively spawning individuals, anchovy spawning season lasted longer (5 months) and started earlier (between April to August, Fig 5.2a.) during the two recent time periods (2008 to 2011 and 2012 to 2015) than in 1965 or 2005-2006 (3 or 4 months between May-June and August, Fig 5.2a.). The gonadosomatic index also indicated that anchovy reproductive period was delimited between April and August (Fig 5.2c.). In contrast, sardine spawning season remained relatively stable over the different time periods, lasting 5 months (November to March, Fig 5.2b.). Small variations were registered in 2008-2011, when the season started slightly earlier, as about 25% of the population spawned already in October. Also, a small part of the population (~10-15%) was still spawning in April during the 2 recent periods (2008-2011 and 2012-2015). The sardine spawning season also lasted from October to March of the 70s and 2002-2015, according to annual *GSI* cycle (Fig 5.2d.).

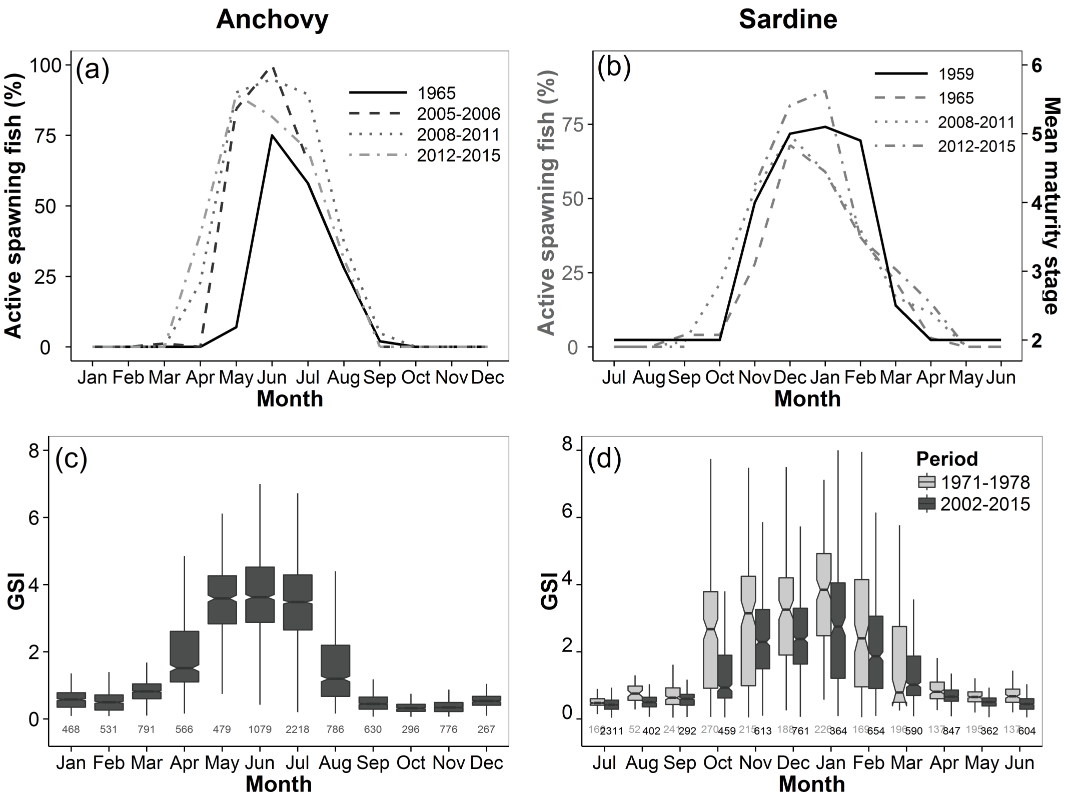

Figure 5.2 Breeding cycles in different years or periods for anchovy (left side) and sardine (right side). The top panels represents the proportion of mature females in stage 5 (i.e. individuals actively spawning), except for 1959 data on sardines (adapted from Lee, 1961) which shows the mean maturity stage in all mature females. Data from 1965 (sardines and anchovies) are derived from Aldebert and Tournier (1971). The bottom panels display the *GSI* index.

Length at maturity (L_{50}) estimated for the spawning seasons 2002 to 2015 using yearly GLMs showed strong interannual variations (Fig 5.3). Results for sardine indicated a major downward shift in L_{50} during the late 2000s, from 12.1cm during 2002-2008 to 9.6cm since 2009. For anchovy, L_{50} first increased from 2003 to 2007, then strongly declined in 2008 and at last stabilized around 9.3cm.

Sardine *GSI* anomalies significantly fluctuated between years ($p < 0.001$, Fig 5.4). In the 70s, *GSI* anomalies fluctuated around small positive values without any temporal trend. In contrast, during the more recent period a clear significant rise (linear model, $p < 0.001$) in *GSI* anomalies values appeared, from strong negative values in 2008/2009 (mean= -0.81/-0.99) to the highest values in the series in 2015 (mean= 1.73). Anchovy *GSI* also exhibited significant variations between years ($p < 0.001$, Fig 5.4), with an alternation of negative and positive anomalies, and a slight increase between the two periods (2004-2005 vs. 2008-2015, $p < 0.01$).

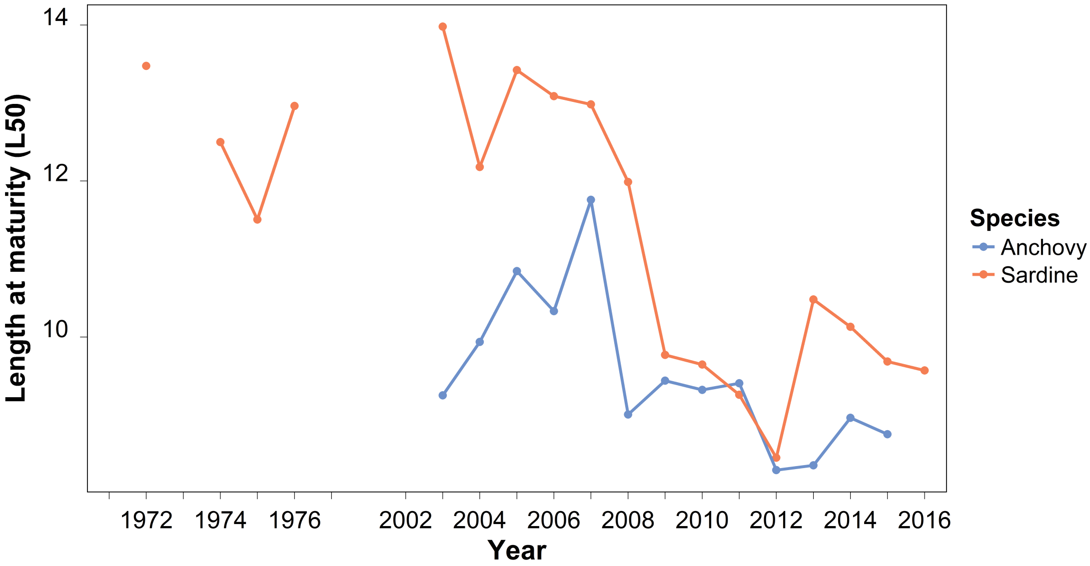

Figure 5.3 Interannual variability in length at first maturity (L_{50}) between 2003 and 2015 for anchovy and sardine.

Effects of size and condition on batch fecundity and atresia

Female sardines egg numbers ranged from 537 to 4,486 eggs per batch while anchovy ovaries had between 1,492 and 9,406 eggs per batch. Both species exhibited a significant and positive relationship between gonad fresh weight and batch fecundity (egg number; n=71, $R^2 = 0.34$, $p < 0.001$, n=85, $R^2 = 0.61$, $p < 0.001$, for sardine and anchovy, respectively). Batch fecundity was positively related to the total length of

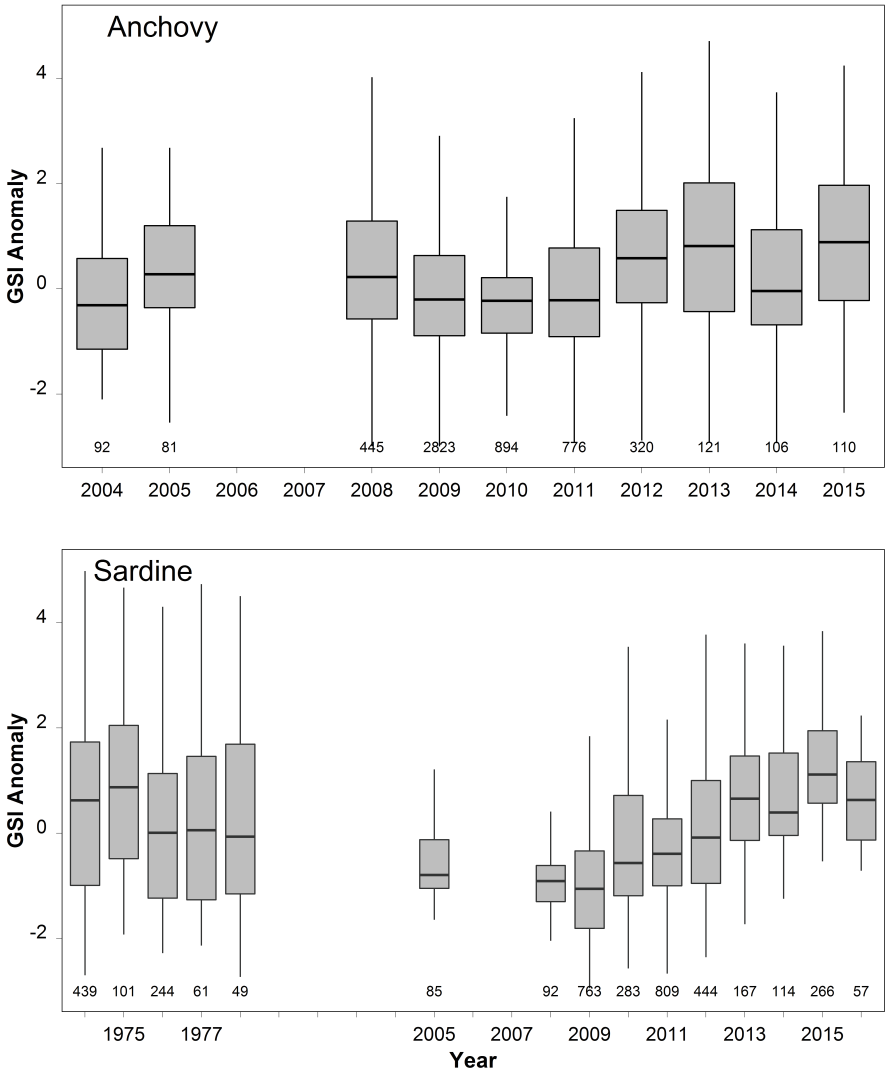

Figure 5.4 Interannual variability in *GSI* anomaly values for anchovy (2003-2015) and sardine (1974-2015).

fish (n=71, R^2=0.38, p <0.001, n=85, R^2=0.53, p <0.001, for sardine and anchovy, respectively, Fig 5.5) as was the relative batch fecundity, although not significantly for anchovy (n=71, R^2=0.10, P = 0.03; n=85, R^2=0.05, P = 0.07 for sardine and anchovy, respectively). In the contrary, neither batch fecundity (n=71, p =0.24 and n=85, p =0.07 for sardine and anchovy, respectively) nor relative batch fecundity (n=71, p =0.12 and n=85, p =0.67 for sardine and anchovy, respectively) was related to muscle lipid content. Sardine ovaries with atretic oocytes had a low prevalence (Pa = 29.3 and 6.7, Table 5.1) and intensity (RIa = 13.5 and 3.4, Table 5.1), for both spawning capable and actively spawning stages. Similar observations were made for anchovy, with atresia prevalence ranging from 17.8% for spawning capable individuals to 22.5% for actively spawning individuals (Table 5.1). Intensity of atresia was slightly higher than for sardine, with values from 13.1% for spawning capable individuals to 16.9%

for actively spawning individuals (Table 5.1). No significant relationship between prevalence or intensity of atresia and both fish condition and length was found for either species (Table 5.1).

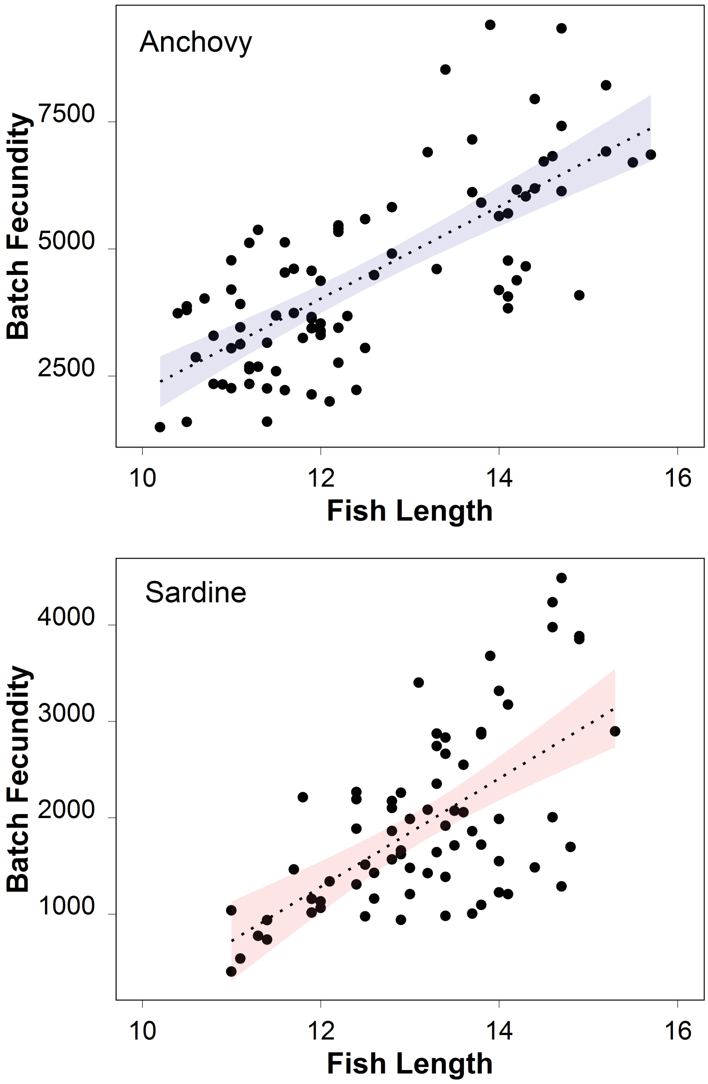

Figure 5.5 Relationship between batch fecundity (*BF*) and fish length for anchovy and sardine. Lines indicate significant linear regressions, while shaded zones correspond to the 95% confidence interval.

Effects of size and condition on egg quality

Mean egg dry mass was established for hydrated females only, and estimated at 0.039 (±0.006) and 0.052 (±0.016) mg per egg for sardine and anchovy, respectively. For both species, this index of egg quality was positively correlated with fish muscle lipid content, but more strongly for anchovy (n=45, R^2 =0.71, p <0.001, Fig 5.6) than for sardine (n=30, R^2 =0.59, p <0.001, Fig 5.6). No relationship was found between egg quality and fish size for either species (n=30, p =0.21 and n=45, p =0.10 for sardine and anchovy, respectively).

Table 5.1 Prevalence of atresia (Pa, %) and mean relative intensity of atresia (RIa, %) for both stages of ovarian development studied in anchovy and sardine. RIa was estimated on fish presenting atresia only to avoid incorporating a large number of 0. n represents the total number of fish on which atresia was investigated. P-values of the relationships between fish Pa or RIa and fish condition and length are given in the table. NA indicates that a statistical analysis was not performed due to a too low sample size.

Species	Maturity stage	n	P_a	Condition vs P_a	Length vs P_a	Mean R_{Ia}	Condition vs R_{Ia}	Length vs R_{Ia}
Anchovy	Spawning capable	40	22.5%	0.26 (ns)	0.28 (ns)	13.1%	0.56 (ns)	0.81 (ns)
	Actively spawning	45	17.8%	0.71 (ns)	0.54 (ns)	16.9%	0.67 (ns)	0.90 (ns)
Sardine	Spawning capable	41	29.3%	0.81 (ns)	0.60 (ns)	13.5%	0.77 (ns)	0.68 (ns)
	Actively spawning	30	6.7%	0.30 (ns)	0.95 (ns)	3.4%	NA	NA

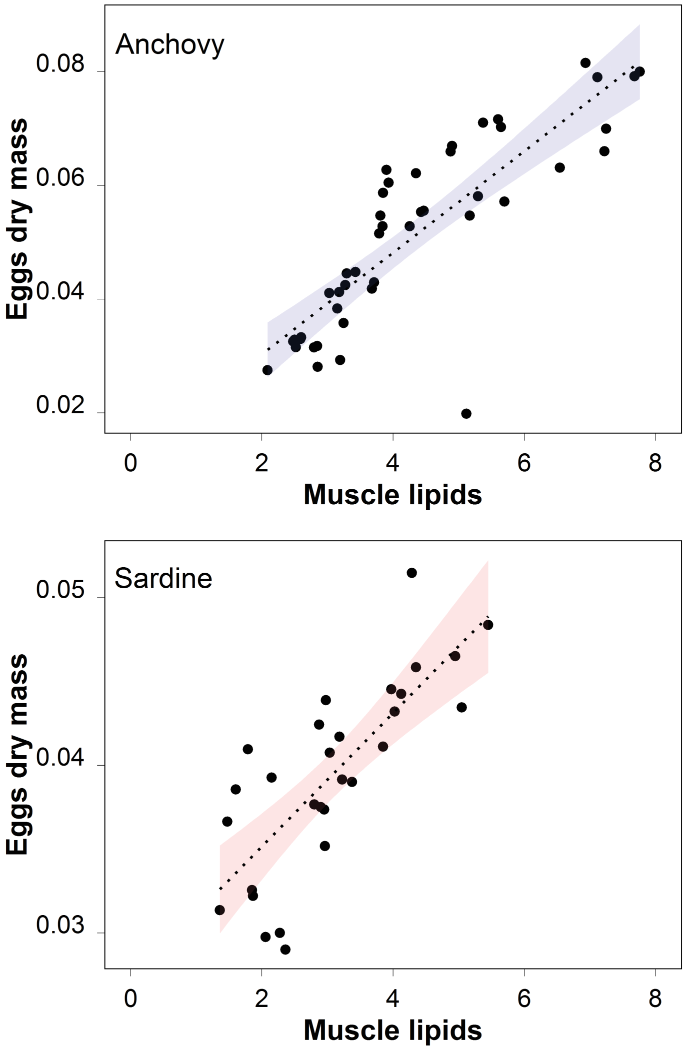

Figure 5.6 Relationship between egg quality and fish condition measured as the total lipid content in muscle for anchovy and sardine. Lines indicate significant linear regressions, while shaded zones correspond to the 95% confidence interval.

Number of eggs spawned by small pelagic fish

To calculate the annual total number of eggs produced per population, sardine spawning season was set to 5 or 6 months, based on previous results. Simulations for anchovy were realized for a spawning season duration ranging from 3 to 5 months. From 2004 to 2006, the number of eggs increased and was high (between $7.02.10^{14}$ to $1.47.10^{15}$ eggs for 2004-2006) but declined by one third until 2008 to then remain steady at low level (between $5.58.10^{14}$ to $5.28.10^{14}$ eggs for 2008-2016, Fig 5.7). Anchovy egg number production showed large interannual fluctuations with a low eggs number between 2004 and 2008 (between $4.63.10^{14}$ and $6.18.10^{14}$ eggs, Fig 5.7) while the number of eggs produced between 2009 and 2015 was higher ($8.81.10^{14}$ to $1.45.10^{15}$ eggs, Fig 5.7).

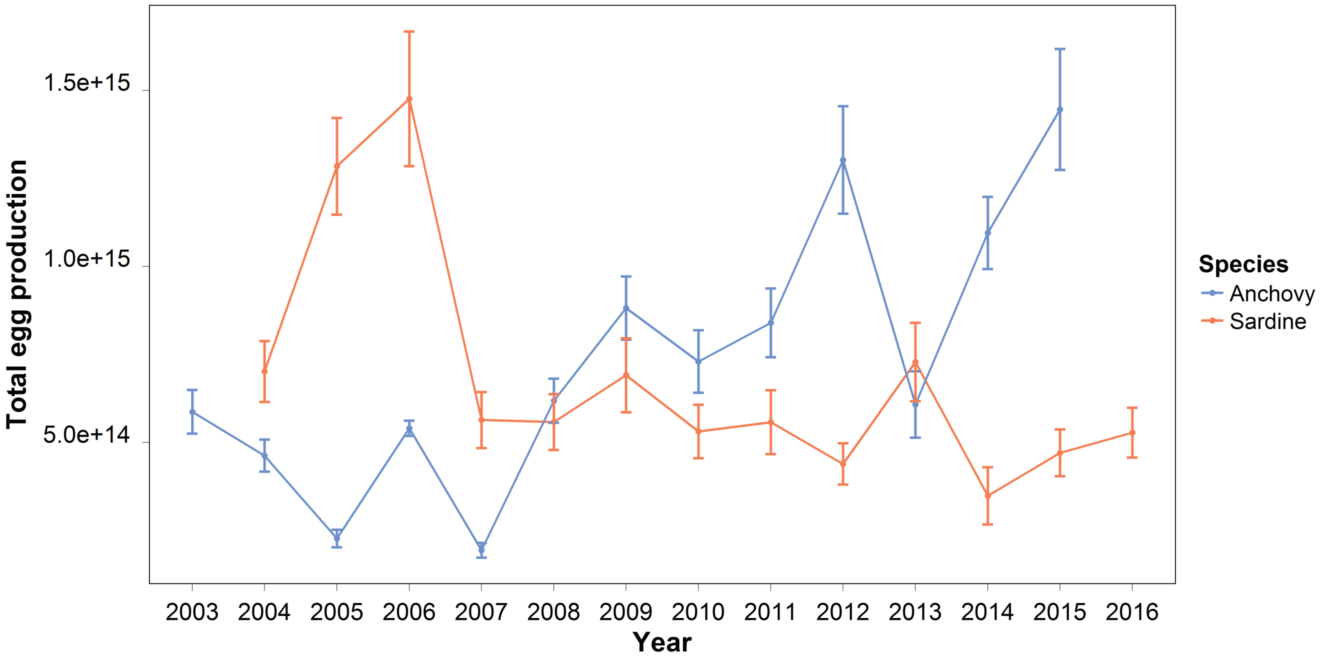

Figure 5.7 Total egg number spawned in the Gulf of Lions between 2003 and 2016 for anchovy and sardine. Error bars represent the standard error associated to each year.

5.4 Discussion

Reproduction is costly, leading to a trade-off between reproductive investment, survival and growth (Roff and Fairbairn, 2007). The part of ingested energy remaining after allocation to metabolic process, is allocated to both somatic growth and reproductive investments, which are hence in mutual competition. Recently, an increase of trophic overlap between small pelagic fish species was observed in the Gulf of Lions at the same time as a decrease in fish body condition (Van Beveren *et al.*, 2014), supporting the hypothesis that food resources could be currently more limiting than previously (Brosset *et al.*, 2015b, 2016). In such context of food shortage, one might wonder how the trade-off between reproduction and growth or maintenance has been dealt with in both species, especially in light of their fast life-history pace as well as their opposite breeding strategies. To investigate reproductive allocation, we used a combination of three measurements: 1) the length of the breeding season, 2) the age or size at which they first reproduce and 3) the weight of the gonad relatively to the individual total weight.

First, the spawning period seems to have been slightly extended compared to previous studies from the 60s (Lee, 1961; Aldebert and Tournier, 1971). In particular, anchovy starts reproducing a bit earlier, so starting in end April rather than May. These changes could be an adaptation to increasing sea temperatures, which induce physiological and environmental changes. Anchovy spawning was shown to be induced by temperatures higher than 17°C (Palomera *et al.*, 2007), so that advanced warming water (Vargas-Yáñez *et al.*, 2010) could promote earlier gonad maturation. Moreover, an increase in temperature earlier in the season might move phytoplankton and zooplankton development forward. An earlier start of reproduction of the small pelagic fish might thus be an adaptive strategy to match with their food resources ('match/mismatch hypothesis', Cushing, 1990). As reproductive performance is known to increase with age (constraint (Curio, 1983), restraint (Williams, 1966) and selection (Smith, 1981) hypotheses), especially in terms of breeding duration (Rödel *et al.*, 2004; Roff and Fairbairn, 2007), spawning period was expected to be shorter for younger female than older ones (Somarakis *et al.*, 2006; Pecquerie *et al.*, 2009). Surprisingly, none of our studied species displayed a shorter spawning period despite a rejuvenation of the population, suggesting high reproductive investment for both species.

Further, our results suggest a decrease in length at first maturity of both species. Sardine length at maturity was high during the 70′s and early 2000′s, but has since 2009 decreased strongly. This indicates that sardine matured at a consequently smaller size during each spawning of the recent years. An abrupt change in size at first maturity from 2007 to 2008 has also been observed in anchovy, which matured at extremely small size since 2012. While a decrease in L_{50} happens usually progressively (Silva *et al.*, 2006), here its decline was very fast around 2008 in both species, confirming the high plasticity of their reproductive characteristics as already observed for other short-lived species, such as Daphnia (Stibor and Lüning, 1994), fish (Bøhn *et al.*, 2004) or toad (Morey and Reznick, 2000). Under unfavourable environmental condition (reduced growth), organisms should adjust size at maturity to maximize fitness (Stearns and Koella, 1986). For instance, growth reduction often leads to earlier reproduction at a smaller size (Alm, 1959) in short-lived species. Similarly, under reduced adult survival, selection should favour genotypes capable of reproducing earlier, at a smaller size and with a higher reproductive effort (Monaghan, 2008). Accordingly, sardine reproductive effort (as measured through the *GSI*) showed a strong increase during the last seven years, anchovy *GSI* increasing as well though less strongly. Such increase in reproductive effort might be a response to the decreasing proportion of large females, which usually produce more eggs. By devoting more energy per individual to reproduction, both populations try to maintain their total reproductive output. However, this strategy could be deleterious to later adult survival. Moreover, the gradual increase over the last years in sardine *GSI* (and also but slower so for anchovy) may reflect the progressive increase in the number of individuals able to invest highly in reproduction, supporting the idea that there was a selection favouring this phenotype.

Our results thus indicate that when confronted to low energy resources, small pelagic fish seem to increase reproductive allocation. This might be an important source of demographic changes. By maintaining a prolonged reproductive period and high reproductive effort (advancing age at maturity and maintaining if not increasing *GSI*) when total energy decreases, small pelagic fish seem to favour their reproductive output over somatic growth. These results support life-history theory, as short-lived species are expected to favour reproduction over survival (Stearns, 1989), as previously shown in a large number of fish species (e.g. in vendace, Bøhn *et al.* (2004) or herring, Rajasilta *et al.*, 2015). Contrary to longer-lived species able to safeguard their own survival by ceasing to breed at any time (e.g. amphibian, Muths *et al.* (2010) and reptile, Shine and Brown, 2008), such a strategy could greatly affect other small pelagic life-history traits and explain the recent reduction of growth rates and sardine adult survival highlighted by Van Beveren *et al.* (2014). This might even be further amplified by the fact that the survival cost of reproduction is known to be higher in individuals maturing at smaller size and earlier age (Roff, 2002).

Despite similarities, our results also highlighted differences between the two species. Indeed, the increase in anchovy *GSI* values was much lower than sardine's one. This has to be put in relation with the steeper decline in growth and condition in sardines compared to anchovies, as well as the adult overmortality which only occurred in sardines (Van Beveren *et al.*, 2014). Such results could come from their reproductive strategies, anchovy being an income breeder and sardine is a capital breeder (Somarakis *et al.*, 2004; Ganias *et al.*, 2007b). If we assume that growth is mainly realized during spring and summer (Ganias, 2014), when planktonic resources are more important, anchovy strategy allows them to take advantages of high resource availability to invest in both somatic growth and reproduction. In contrast, sardine has to store energy and incur costs due to accumulating fat store and maintaining storage compound to then spend this capital during winter (Jönsson, 1997). Following breeding at the end of winter, sardine reserves should thus be exhausted making them more vulnerable to lower food availability (whether due to a general decrease in prey quantity or quality). Together with really low energy stores, high reproductive investment at a period at which food is scarce could deeply lower the survival and prevent the majority of sardine from surviving past their first reproductive season, explaining the observed disappearance of large sardine since 2008 in the Gulf of Lions (Van Beveren *et al.*, 2014). Our results thus suggest potential differences in the effect of reproductive investment on other life-history traits depending on breeding strategies (income/capital), which would merit further investigation.

Results also suggest significant preovulatory maternal condition effects on the egg quantity and quality of sardine and anchovy. Reproductive features are similar to numerous species of birds (Pellerin *et al.*, 2016), daphnia (Lampert, 1993), snakes (Baron *et al.*, 2013) or turtles (Rowe, 1994). As the number of eggs increases linearly with fish size for both species and the relative batch fecundity also increases with body mass for sardine, reproductive capacity can therefore be assumed to be higher in large individuals. This emphasizes the hypothesis that the reproductive potential of a species is highly dependent of large individuals (i.e dependent on the age-structure of the population, e.g. see Cooper *et al.*, 2013) as previously reported for many taxa (e.g. De Roos *et al.*, 2003). However, contrasting with several other species, e.g. birds (Chastel *et al.*, 1995) or reptile (Naulleau and Bonnet, 1996), anchovy and sardine females in better condition did not produce more eggs relative to their weight than females in a poorer condition. On the contrary, egg quality was dependent on female lipid content. As a trade-off between the number and size of offspring might emerge in situations of low resource levels (Stearns, 1992), our results might indicate that small pelagic fish give priority to the size of their eggs and thus their viability. Thus, maternal condition may be relevant for the survival of egg and larvae. Indeed, Riveiro *et al.* (2000) demonstrated the link between larval survival in hatching condition and the egg quality underlining its importance in larvae survival rate and in the short-term fish recruitment. Consequently, we may reasonably think that last years' egg quality of sardine and anchovy was affected by the decrease of adult body condition (Van Beveren *et al.*, 2014). While a positive size effect had been previously detected on egg quality in cod (Trippel, 1998) but also in turtles (Rowe, 1994) or birds (Kvalnes *et al.*, 2012), none of our species displayed such relationship. For both species, maternal condition rather than size seems to define egg quality. Moreover, maternal condition rules oocytes resorption mechanism, e.g. in insects (Ohgushi, 1996) or fish (Kurita *et al.*, 2003), depending on fat quantity. However, the occurrence and intensity of atresia were not related to fish condition in any of the two species, underlining that fish condition seemed to have no effect on the phenomenon of oocytes resorption. This could also be due to the fact that in indeterminate fecundity species, the atresia is really important in the regression phase, and this factor is much more relevant than the condition or any other feature of the mother to determine atresia intensity.

In light of the current small pelagic fish situation in the Gulf of Lions, characterised by small sardine and anchovy in poor condition (Van Beveren *et al.*, 2014; Ferrer-Maza *et al.*, 2016), our results indicate that the individual reproductive potential could be strongly affected both in terms of quantity and quality. Indeed, despite that no studies focusing on the Mediterranean Sea have measured egg quality as oocyte dry mass, preventing any comparison, the current batch fecundity of sardines and anchovies in the Gulf of Lions appeared quite low in comparison to other stocks. Also, sardine batch fecundity estimations from the Gulf of Lions were lower than those estimated from the Eastern Mediterranean Sea (Ganias *et al.*, 2004), which ranged from 2,000 to 12,000 eggs per batch. Anchovy batch and relative fecundity were slightly lower but in the same range as other estimations made in the Mediterranean Sea (between 4,500 and 8,200 eggs, (Pethybridge *et al.*, 2013) or between 981 and 21,750 eggs, (Ferrer-Maza *et al.*, 2016) or for other species of the genus Engraulis (*Engraulis japonicus*, mean relative batch fecundity is 572 oocytes.g^{-1} of fish, Yoneda *et al.*, 2013).

However, earlier maturation could potentially lead to a higher number of breeders and compensate at the population level the decrease in individual reproductive capacity. This was previously described in other short-lived species such as Daphnia (Stibor, 1992) or insect (Blanckenhorn, 1999). Indeed, the back calculated yearly population egg number production values indicated that anchovy egg production has been slightly higher since 2009 and was not affected by low resource levels and smaller fish dominance. On the contrary, the change in L_{50} was not sufficient to counteract the more pronounced disappearance of large and old individuals in sardines. Indeed, the model highlighted an estimated fourfold reduction of the sardine egg number in the Gulf of Lions between years when large sardines dominated (i.e. 2005-2006) and when small sardines dominated (i.e. since 2008). As sardine and anchovy condition has decreased since 2007 and influences egg quality, we suggest that along egg number reduction, egg quality also decreased. This enhances the idea of a stronger degradation of sardine reproductive capacities paralleling the decline in the lipid reserves of the stock compared to anchovy. Nevertheless, no data were currently available to obtain an accurate estimation of recruitment for both species, leaving as a challenge for future studies to test whether egg number and quality explain a significant part of recruitment variability of small pelagic fish.

Long-lived species are able to prioritize their energy allocation between life history traits and usually favour their own condition over their propagules' condition (Stearns, 1989). For example, fish (McBride *et al.*, 2013) as well as birds (Linden and Møller, 1989) or reptile (Bull and Shine, 1979) are able to skip or delay breeding under poor environmental condition to attempt to maximize fitness by allocating resources optimally among growth, maintenance, and future reproduction. By opposition short-lived anchovy and sardine clearly guided the trade-offs between reproduction and survival towards a maintenance (if not an increase) of their reproductive investment even during a poor condition period in the Gulf of Lions. According to the costs associated with reproduction, females favouring reproduction led to a reduced growth and a reduction in survival and might explain the current lack of large old small pelagic fish in the Gulf of Lions. Even if reproduction was prioritized, egg quantity and quality decreased between 2006 and 2015 for sardine. While the effect of decreasing sardine egg production on its recruitment could not be investigated in this study, these findings brought to light strong evidence on the need to consider fish reproductive and condition characteristics altogether. Both are essential to understand forage fish fluctuations and evaluate the long-term sustainability of the forage fish stocks.

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Chapter 6

Spatio-temporal patterns and environmental controls of small pelagic fish body condition from contrasted Mediterranean areas

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Preface

Previous chapters of this thesis focused specifically on the Gulf of Lions. However, small pelagic fish are widespread throughout the Mediterranean Sea. In both the Northern and Southern parts (e.g. the Algerian coast and the Adriatic and Aegean Sea) different populations were identified. A large scale study is necessary to know if similar and/or synchronous fluctuations in the anchovy and sardine population/condition occurred in those areas, when compared to the Gulf of Lions. This chapter is an initiative of the GFCM (General Fisheries Commission for the Mediterranean) to join efforts of researchers from different countries and the entire area around the Mediterranean to the study of small pelagic fish (here anchovy and sardine). Indeed, the fluctuations of these fish have both important economic (e.g. a fisheries crisis) and ecological consequences.

An inter-area comparison is possible because both sardine and anchovy are regularly sampled in several of the Mediterranean regions. A large amount of data is also important to study temporal and spatial drivers of small pelagic fish condition at a Mediterranean scale. In this last chapter, morphometric data (e.g. length and weight) were provided through collaboration with other countries surrounding the Mediterranean Sea. The relative condition index, already validated and used in chapter 1 and 2, was computed for several areas. This factor was then linked to environmental factors, information derived from satellite data. By gathering data and researchers from all this area, this study is the first to focus on small pelagic fish body condition at the Mediterranean scale and aims to define whether the recent overall decrease is truly a common issue at a basin scale.

Abstract

Small pelagic fish are among the most ecologically and economically important marine fish species and are characterized by large fluctuations all over the world. In the Mediterranean Sea, low catches and biomass of anchovies and sardines have been described in some areas during the last decade, resulting in important fisheries crises. Therefore, we studied anchovy and sardine body condition variability, a key index of population health and its response to environmental and anthropogenic changes. Wide temporal and spatial patterns were investigated by analyzing separately data from scientific surveys and fisheries in eight Mediterranean areas between 1975 and 2015. Results showed that anchovy and sardine body condition as well as maximum size in some areas sharply decreased in most Mediterranean areas along years (except in the Northern Alboran Sea). Despite this general pattern, well-marked environmental differences between sub-regions were highlighted by several analyses and variations in body condition were not found to be homogeneous over all the Mediterranean Sea. Further, other analyses revealed that except for the Adriatic where major changes towards a lower body condition were concomitant with a decrease in river runoffs and chl-a concentration, no concomitant environmental regime shift was detected in other areas. Together, these analyses highlighted the current poor body condition of almost all small pelagic fish populations in the Mediterranean. Yet, global environmental indices could not explain the observed changes and the general decrease in condition might more likely come from regional environmental and/or anthropogenic (fishing) effects. A prolonged state of poor fish body condition, together with an observed reduced size and early age-at-maturity may have strong ecological, economic and social consequences all around the Mediterranean Sea.

Keywords Anchovy; Sardine; Fish health; Marine monitoring, Time series

6.1 Introduction

Ocean systems are currently going towards changing environmental conditions especially due to the impact of anthropogenic climate change (Hoegh-Guldberg and Bruno, 2010). In particular, the Mediterranean Sea has been identified as one of the most responsive regions to climate change (Giorgi, 2006), as well as to direct human impacts such as pollution (Halpern *et al.*, 2008). Specifically, recent studies using climate models already highlighted the warming of this sea (both at the surface and in deep waters) and the increasing frequency of extreme events (Bethoux *et al.*, 1990; Theocharis, 2008; Vargas-Yáñez *et al.*, 2008, 2010). Large scale perturbations, such as climate change, are known to affect all biological levels, directly (e.g. by affecting physiological thresholds) and/or indirectly (e.g. by modifying food resources; Jørgensen, 1992), resulting in spatio-temporal changes in the abundance of exploited fish in the Mediterranean Sea (see e.g. Lloret *et al.*, 2015) and elsewhere (see e.g. Drinkwater *et al.*, 2010).

Pronounced small pelagic fish population fluctuations are observed all over the world, especially in the upwelling areas (Baumgartner *et al.*, 1992; Chavez *et al.*, 2003). In the Mediterranean, those variations have been linked to recruitment (Martín *et al.*, 2011), spawning (Agostini and Bakun, 2002; Basilone *et al.*, 2013) or larval survival (Garcia *et al.*, 1998). Such major population changes can have important impacts on the dynamics of marine ecosystems, as these forage fish represent an important biomass at mid-trophic levels, making them a key-component of the ecosystem (Cury *et al.*, 2000; Coll and Libralato, 2012). Thus, the dynamics of this functional group are important both to maintain the integrity of the marine ecosystem, and for socioeconomic stability in the region. The main reason for the renowned population fluctuations of small pelagic is their short life span, as well as their susceptibility to environmental variability (Cushing and Dickson, 1976). Importantly, these characteristics make them excellent indicators of climate-driven environmental changes in marine systems (Drinkwater *et al.*, 2010; Peck *et al.*, 2013). The two most abundant small pelagic fish species in the Mediterranean Sea, the European sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*), have been exploited for a long time (Lleonart and Maynou, 2003) and are still intensely exploited, accounting for 35 to 50% of the reported catch in this area (GFCM, 2015). In recent years, a general decreasing trend has been observed in the landings of the small pelagic fish stocks in different parts of the Mediterranean Sea (GFCM, 2014; Vasilakopoulos *et al.*, 2014), leading to an important fisheries crisis. In addition to this, alarming biological signals also appeared, such as a decrease in growth and body condition of small pelagics in the Gulf of Lions (Van Beveren *et al.*, 2014), and a higher risk of recruitment failure with increasing temperature in the Northern Spain area (Maynou *et al.*, 2014).

Body condition is an important individual physiological trait of marine organisms, influencing other life history traits, such as growth, reproduction (e.g. egg size and number, age at first maturity) and mortality (Lloret *et al.*, 2014). Specifically, body condition indices are proxies of the quantity of stored energy, evaluating individual's health status and fitness(Schulte-Hostedde *et al.*, 2001; Wilson and Nussey, 2010). For example, survival, growth and reproductive success are theoretically higher for individuals in better condition (Millar and Hickling, 1990), resulting in an important link between average population body condition and future population success (Jakob *et al.*, 1996; Adams, 1999). Recently, more evidence emerged showing that variations in body condition can affect ecological processes at scales ranging from individuals to ecosystems (Rätz and Lloret, 2003; Lloret *et al.*, 2014; Van Beveren *et al.*, 2014). Consequently, fish body condition can in part determine ecosystem functioning and fisheries yield, so that this factor can constitute a valuable tool in stock assessment and management (Lloret *et al.*, 2012). Furthermore, condition indices can be computed easily from collected length and weight data, available in numerous areas and at large temporal scale. It has already been demonstrated that environmental factors, such as temperature, food availability (Brosset *et al.*, 2015b) or parasitism (Ferrer-Maza *et al.*, 2016) influence fish condition through direct and/or indirect effects. For example, a decrease in body condition due to a lack of food or an increase of metabolic costs under higher temperature has already been advocated in several studies (see e.g. Pörtner and Knust, 2007; Brosset *et al.*, 2015b). Changes in water stratification and currents are known to strongly influence plankton productivity and could thus indirectly act on the body condition of planktivorous small pelagic fish (Costalago *et al.*, 2014; Le Bourg *et al.*, 2015; Brosset *et al.*, 2016). Furthermore, these effects are easily visible because of the fast response of fish condition to environmental changes (Peck *et al.*, 2013).

However, to our knowledge, a large scale study focusing on small pelagic fish body condition has never been realised in the Mediterranean Sea, so that at present it is still unclear if the recent decreases are truly a common issue. In this study, data of anchovy and sardine body condition from eight Mediterranean areas were compiled for the first time to compare the temporal trend in body condition of the different studied stocks and to assess whether a general decline took place. We also focused on the environmental (including climatic) factors that could potentially affect fish condition, taking into account spatial factors. A dataset of morphometric (i.e. length and weight) variables of more than 250,000 individuals analysed from 1975 to 2016 enabled us to compute the individuals' morphometric condition index, which assumes that for a given length a heavier fish is in better condition.

6.2 Material and Methods

6.2.1 Study areas

Mediterranean sub-areas (GSA) were selected following General Fisheries Commission for the Mediterranean (GFCM) delimitations adopted for stock assessment from the western-most Alboran Sea to the Black Sea in the East (Figure 6.1). The Mediterranean Sea is known to be oligotrophic, even if an important variability in productivity is visible with a West to East gradient and enhanced primary productivity areas due to strong river discharge as in the NW Mediterranean, with the Rhone in the Gulf of Lions and the Ebro in the Catalan Sea, or the Po river in the Northern Adriatic Sea.

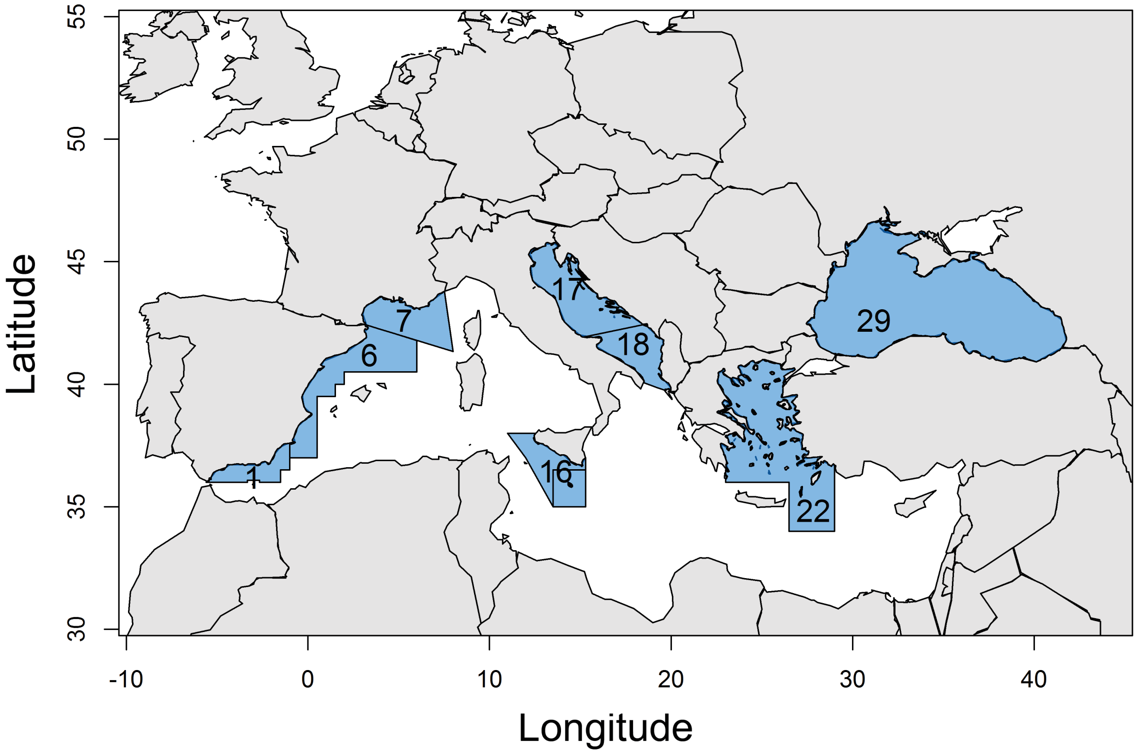

Figure 6.1 Main Geographical Subareas of the Mediterranean and Black Seas used in this study according to their number defined by the General Fisheries Commission for the Mediterranean (1: Northern Alboran Sea; 6: Northern Spain area; 7: Gulf of Lions; 16: Strait of Sicily (gathered GSA 15 and 16); 17: Northern Adriatic Sea; 18: Southern Adriatic Sea; 22: Aegean Sea; 29: Black Sea).

In the North Western part of the Mediterranean Sea the Gulf of Lions is the most productive area and covers about 20400 km^2 (Banaru *et al.*, 2013). This area has a wide continental margin (>60 km) and depends on Rhodanian inputs, strong mixing events induced by NW and N winds. A gradient of nutrients takes place from East to West due to the Rhodanian inputs located to the East of the area (Darnaude *et al.*, 2004). Also, local upwellings support the high productivity (Millot, 1990). The Northern Spain area, grouping the Catalan Sea and the Gulf of Valencia, is oceanographically connected with the Gulf of Lions through the Northern current flowing to the south-west which enhances the production in the north Catalan Sea. Models have indeed highlighted larval connectivity between the northern spawning grounds of anchovy in the Gulf of Lions and the Catalan Sea due to the hydrodynamic characteristics in the area mediated by the North current and the filament transport in mesoscale eddies (Ospina-Alvarez *et al.*, 2012). The north Catalan Sea has a narrow shelf that widens in front of the Ebro river delta (>60km wide) where it is strongly influenced by water discharge and wind mixing allowing phytoplankton to find necessary nutrients, especially during summer period when the water column is strongly stratified (Palomera *et al.*, 2007). In the south of Spain, the Northern Alboran Sea is the southern- and western-most area in our study, and it is a transitional zone between Mediterranean and Atlantic waters. Two gyres, occurring in the central basin of the Alboran Sea, as well as several upwellings in the north coast associated with vertical and lateral nutrient inputs, make this area productive (Macías *et al.*, 2008).

The Adriatic Sea was divided in two areas: the Northern Adriatic Sea and the Southern Adriatic Sea. The Northern Adriatic is characterized by shallow depths and high river discharge, particularly due to the Po River outflow, which has a major impact on the phytoplankton biomass due to the high nutrients loads (Artioli *et al.*, 2008). Furthermore, local upwelling events and eddies, that contribute to spread the Po river discharge offshore, enhance the primary production (Russo and Artegiani, 1996). A decreasing trend in nutrient concentration and in production is observed from North to South (Zavatarelli *et al.*, 1998): the Southern Adriatic is in fact deeper, mostly influenced by eastern Mediterranean waters and exhibits an oligotrophic character with lower phytoplankton abundance and biomass (Zavatarelli *et al.*, 1998). The Strait of Sicily is the area where the Western and the Eastern Mediterranean basins connect, playing a crucial role in determining the water-mass exchanges. Main water masses present in the Strait of Sicily might be divided in a surface layer composed of Atlantic water flowing eastward, and intermediate and deep layers mainly composed of Levantine intermediate water, and transitional eastern Mediterranean deep water flowing in the opposite direction. Their encounter creates local upwellings which enrich the upper water layers, enhancing primary production and creating favorable environmental conditions for small pelagic fish (Basilone *et al.*, 2013; Bonanno *et al.*, 2014).

The North Aegean Sea is among the most productive areas of the Eastern Mediterranean Sea. Black Sea waters, river runoffs and the large continental shelf contribute to increase primary productivity of this area, explaining the high small pelagic catches in that area and its importance as a spawning ground for both anchovy and sardine. In the Eastern part of the Mediterranean, the North Levant Sea is characterized by the highest temperatures in the Mediterranean, together with high salinity values and extreme oligotrophy. The Black Sea is almost completely isolated, being connected to the Mediterranean only through the Bosphorus Strait. It is therefore mostly influenced by freshwater inputs from rivers (Özsoy and Ünlüata, 1997) and it is characterized by low water circulation and anoxic waters below the oxycline at about150 meters depth. Only anchovy is found in the Black Sea, where this species is known to migrate between the North where they reproduce and feed to the South where they overwinter.

6.2.2 Fish sampling

Two types of sampling were done. One used commercial vessels (pelagic trawlers and/or purse seiners) all over the year and the other, scientific surveys at sea, carried out once per year and dedicated to small pelagic fish abundance estimation (called Mediterranean International Acoustic Survey (MEDIAS) since 2008). During the acoustic surveys fish were caught with pelagic trawls. Summarized information on fish sample sizes, temporal coverage and month of collection are provided in Table 6.1. During scientific surveys, parallel fixed transects perpendicular to the coastline were followed each year to collect data in the same area. Echosounders enabled to detect fish traces and a pelagic trawl was deployed to determine fish composition when these traces were long enough. The same protocol was adopted for both types of samples. A random sample of anchovies or sardines from the catch was directly measured on board or brought to the lab on land for subsequent analyses. Fish measurements included fish total length (TL, from the nearest mm to the nearest 0.5 cm for scientific survey or from the nearest mm to nearest cm for commercial samples depending on areas) and total weight (W, to the nearest g for both types of samples).

Table 6.1 Summary of all biological data used in this study by area.

Area	Species	Time period	Data origin	Sampling month	Nb of individuals
GSA1 Northern Alboran Sea	Anchovy	2001-2009	ECOMED	November-December	666
		2009-2013	MEDIAS	June-July	182
	Anchovy	1990-1992 & 2003-2013	Fisheries	All months	12,126
	Sardine	2001-2009	ECOMED	November-December	1,600
		2009-2013	MEDIAS	June-July	742
	Sardine	1990-1992 & 2003-2013	Fisheries	All months	21,539
GSA6 Northern Spain	Anchovy	1993-2009	ECOMED	November-December	6,726
		2009-2013	MEDIAS	June-July	4,649
	Anchovy	2004-2013	Fisheries	All months	7,049
	Sardine	1993-2009	ECOMED	November-December	6,316
		2009-2013	MEDIAS	June-July	8,923
	Sardine	2004-2013	Fisheries	All months	13,157
GSA7 Gulf of Lions	Anchovy	1993-2015	MEDIAS	June-July	8,525
	Sardine	1993-2015	MEDIAS	June-July	9,464
GSA 15&16 Strait of Sicily	Anchovy	1998-2013	MEDIAS	June-August	1,331
	Sardine	1998-2013	MEDIAS	June-August	1,572
GSA17 Northern Adriatic Sea	Anchovy	1998-2013	MEDIAS	July-October	14,137
	Anchovy	1975-2012	Fisheries	All months	50,826
	Sardine	1998-2013	MEDIAS	July-October	7,550
	Sardine	1975-2012	Fisheries	All months	27,164
GSA18 Southern Adriatic Sea	Anchovy	1998-2013	MEDIAS	June-September	1,849
	Sardine	1998-2013	MEDIAS	June-September	1,046
GSA22 Aegean Sea	Anchovy	2003-2008	MEDIAS	June-July	8,574
	Sardine	2003-2008	MEDIAS	June-July	2,848
GSA29 Black Sea	Anchovy	2011-2013	MEDIAS	November-March	3,678

6.2.3 Condition and maximum size estimations

Morphometric index of condition

Condition factors are mainly based on length–mass relationships (Bolger and Connolly, 1989). While Fulton's condition factor assumes isometric growth or growth without changes in body proportions, resulting in condition factors that are often length- and species-dependent, the relative condition factor according to Le Cren (1951) compares actual weight to a standard weight predicted by weight-length regression, based on the population from which the fish was sampled (Basilone *et al.*, 2006; and references therein). As the two studied species exhibited an allometric growth pattern (Brosset *et al.*, 2015a), we used for this study the more suitable relative condition index (K_n, Le Cren, 1951) rather than Fulton's index:

$$K_n = \frac{W}{W_r}$$

where W is the observed weight of an individual of a given length and W_r is the predicted weight of an individual of a given length TL calculated with ($W_r = \alpha TL^{\beta}$). By definition, the values of the relative condition factor are distributed around 1 and the higher the value the better the condition.

To investigate temporal fluctuations, length-weight relationships were computed separately for each area and species. Yet, during spatial analysis, all individuals belonging to anchovy or sardine were pooled together to compute the parameters of a single non-linear regression for each species. The advantage of a single regression curve for all individuals of one species is the possibility to compare the values between the different stocks (i.e. standardization of the indicators for inter-ecosystem comparisons). As recommended by several authors (Schulte-Hostedde *et al.*, 2005; Stevenson and Woods, 2006; McPherson *et al.*, 2011), this morphometric index is related to total lipid content, and its use is validated for individuals outside the reproductive period; during the reproductive period, the variation of condition seemed to reflect both lipid and protein variations which represent also a global measure of condition (Brosset *et al.*, 2015a). Maximum size of both species was estimated each year by computing the 95% quantile of fish size distribution rather than the maximal size observed, making this values less sensitive to extreme individuals.

6.2.4 Environmental data

Daily sea surface temperature (SST, in °C), chlorophyll a (Chl-a, in mg.m-3) and Eddy kinetic energy (Eke, measuring turbulence in $cm^2.s^{-2}$) were obtained from satellite data. These three environmental variables were chosen due to their large coverage in space and time and because they are relevant to explain differences in productivity, water mixing and temperature patterns affecting fish body condition. SST comes from the version 5 of the AVHRR Oceans Pathfinder SST data set obtained from the NOAA website (http://podaac.jpl.nasa.gov). Surface chlorophyll a was similarly extracted using MODIS-aqua data from the OceanColor web site (http://oceancolor.gsfc.nasa.gov). Kinetic energy was extracted from Aviso database. All variables were extracted on the basis of the different area coordinates given by the GFCM. All these variables were averaged monthly over each area.

6.2.5 Data analyses

Fish body condition and maximum size time series analysis

All fish body condition and maximum size time series were analysed together to identify the main characteristics of the time series, as well as to compare them. Correspondence in terms of trend, periodicity or inflection points among the different areas could indicate that small pelagic fish condition or size exhibits common pattern of temporal variation at the Mediterranean scale. As Spanish scientific survey shifted from winter to summer in 2009, we accounted for this change by calculating the mean annual body condition cycle of each species from fisheries data and then adding the obtained winter/summer difference to winter values (i.e. before 2009). This allowed having a continuous time series from 1993 to 2013 with comparable values before and after 2009.

Trends, periodicity and annual cycle of body condition

Twelve MEDIAS (Northern Alboran Sea, Northern Spain area, Gulf of Lions, Northern Adriatic Sea, Southern Adriatic Sea and Strait of Sicily) and two fishery time series (Adriatic) were long enough to investigate temporal changes for both species. In order to fill some existing gaps in the time series, interpolations had to be applied using cubic splines or linear interpolations. The different condition time series for both species and each location were analyzed to detect potential long term trends with Eigen vector filtering (EVF, Colebrook, 1978). This method was used to estimate the general trend of each time series, and to estimate the percentage of variance explained by each one of these trends. Briefly, for each time series, an autocovariance matrix was constructed by shifting the series between 1 and 4 years (to retain fluctuations beyond the seasonal signal). The series' trend is computed by a principal component analysis performed on this matrix (use of its first axis).

Periodicity analyses on the other hand were performed only on fisheries data from the Adriatic Sea which displayed the only long-enough time series for both species (from 1975 to 2012). Fishery time series exist for the Northern Alboran Sea (2003-2013), the Northern Spain area (2004-2013) and the Gulf of Lions (2005-2016) but were not long enough to be used in this type of analyses. To do so, wavelets were used: this analysis not only decomposes the variance of a time series over frequencies, but it does it also over time domains and tolerates non-stationary data. To detect low and high frequencies, β surrogate test were used since it is deemed to be well-suited to ecological time series (Rouyer *et al.*, 2008). Further details on the methodology can be found in Van Beveren *et al.* (2016). Final wavelet power spectrum was presented as a time-frequency plot, colors indicating intensities of match between wavelets and time series (from blue=low variance to grey=high variance). Additionally to periodicity analyses, temporal changes in the timing of the peak of condition in the Adriatic Sea were investigated. The timing of the peak of body condition was computed as the mean number (1 for January, 2 for February, etc) of the three months with highest condition during each year where all data were available.

Covariability of small pelagic fish body condition in the Mediterranean

In order to know if nearby fish body condition time-series covary, the spatial decorrelation scales of fish body condition time series from fisheries data was estimated. We used a combination of paired time series correlation analyses in function to the separation distance between sites. A nonlinear was fitted to compute the distance where correlations are reduced to 50% of the estimated correlation at a distance of 0 km ($d_{0.5}$, see Batchelder et al., 2012 for further details). We account for year-to-year temporal autocorrelation by using estimated number of effective degrees of freedom (typically ∼10% smaller than the number of years in the time series).

Linking environmental changes with fish condition

To detect temporal changes in fish condition that might have resulted from environmental changes, and to compare periods of major changes between time series, we used a change point analysis (inflection point). Fish condition time series were analyzed using the strucchange package (Zeileis *et al.*, 2013) to assess the year(s) (and their 95% confidence intervals) of statistically significant changes in the level of subsets of fish condition time series. The Bayesian Information Criterion (BIC) was consulted to assess a penalty for the number of segments used to describe the data and thus have an objective criterion to determine the most parsimonious number of breakpoints. We performed this test for each species and area in order to check if there was a synchrony between the fluctuations of small pelagic fish condition in different Mediterranean Sea areas. The same method was used to detect environmental breakpoints and check their synchrony with fish biological parameters breakpoints.

A principal component analyses (PCA) was implemented to describe the multivariate relationships among the annual values of both fish biological parameters (fish body condition and maximum size) and environmental factors (SST, Chl-a and Eke). A correlation-based matrix on Euclidean distance among all variables was used. The

PCA allowed reducing into a few dimensions the variability of the population and environment parameters over areas. Since sardine is not present in the Black Sea, this area was not considered in sardine principal component analysis.

All statistical analyses were performed with R version 3.0.2 (R Development Core Team, 2013). Values are indicated as mean standard error (SE) and all statistical tests were performed at a significance level of 0.05.

6.3 Results

Annual fish condition and maximum size trends

Anchovy and sardine mainly displayed a decreasing trend in their body condition in most studied areas (Figure 6.2). Anchovy body condition showed a pronounced decrease in the Gulf of Lions (63% of deviance explained), in both Adriatic Sea areas (72% and 78% of deviance explained in the North and South part, respectively) and in the Strait of Sicily (60% of deviance explained). While the decreasing trend has been linear along years since 1998 in the Southern Adriatic Sea, anchovy condition has started to drop only since 2004 in the Strait of Sicily and in the Northern Adriatic and since 2007 in the Gulf of Lions. Anchovy body condition was increasing before being stable in the Northern Alboran Sea and stable over the time series in the Northern Spain area (43% and 46% of deviance explained). All areas except the Northern Alboran Sea displayed an overall decreasing trend for sardine body condition (Figure 6.2). Similarly to anchovy, the decrease was mostly linear in the Southern Adriatic, and began later in the 2000s in other areas (in 2004, 2007, 2007 and 2009 in respectively the Northern Spain area, the Gulf of Lions, the Northern Adriatic and the Strait of Sicily, Figure 6.2). The deviance explained by this trend was relatively high in all areas (from 57% to 85%), except for the Strait of Sicily where it only explained 49%. The trend was different in the Northern Alboran Sea, where sardine body condition was stable before it greatly increased during the last two years (Figure 6.2).

As for fish condition, the Northern Alboran Sea did not exhibit pronounced trend in fish maximum size and the same applies to the Northern Spain area (between 40 and 50% of deviance explained, Figure 6.3). The Gulf of Lions (especially after 2008), and Northern Adriatic (linearly) on the other hand showed a strong negative trend for both species and a high percentage of deviance explained (Figure 6.3). The decrease was also observed but less pronounced in the Southern Adriatic Sea (Figure 6.3). Finally, a decrease in sardine size only was found in the Strait of Sicily, though not really strong (49% of deviance explained, Figure 6.3).

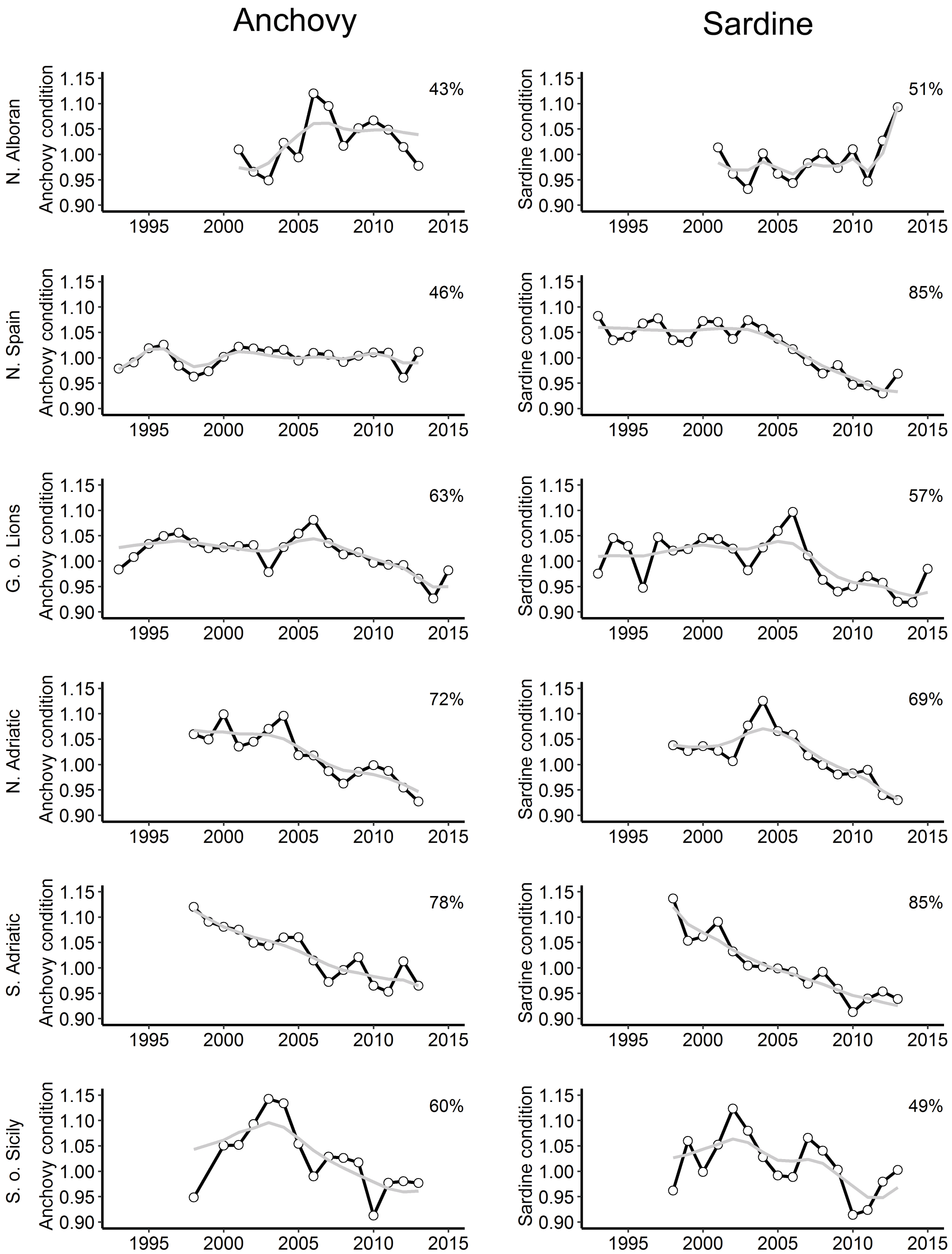

Figure 6.2 Time series of the annual anchovy and sardine condition of the six different areas studied from MEDIAS data. The black line with markers represents the time series and the grey line the trend from Eigen Vector Filtering. The percentage of deviance explained by the main trend is indicated in the upper right corner.

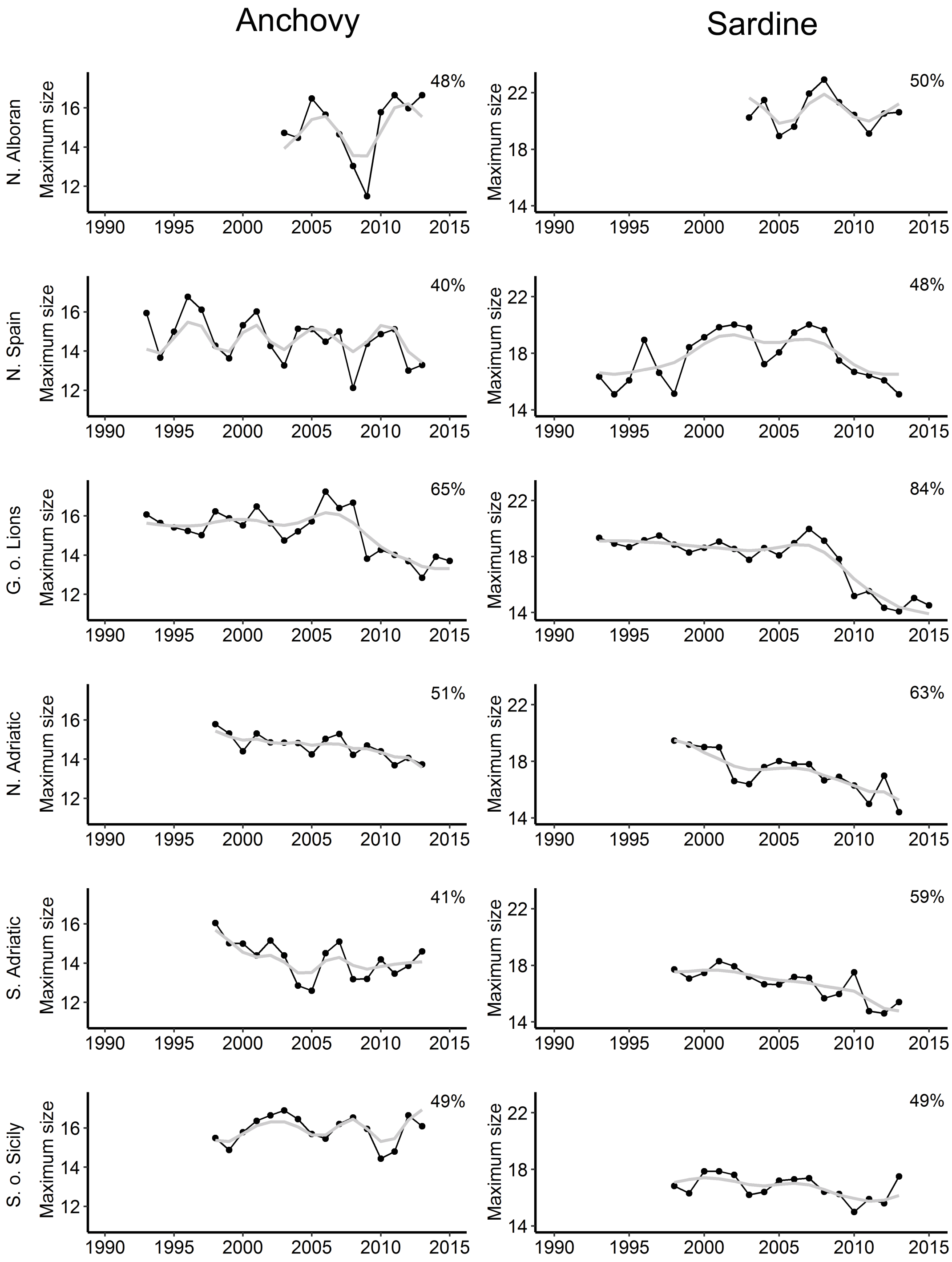

Figure 6.3 Time series of the annual anchovy and sardine maximum size of the six different areas studied from MEDIAS data. The black line with markers represents the time series and the grey line the trend from Eigen Vector Filtering. The percentage of deviance explained by the main trend is indicated in the upper right corner.

Fish condition and environmental breakpoint analysis

Fish body condition time series showed between zero and two statistically significant breakpoints, which mostly occurred in the middle of the 2000's (Figure 6.4). The last breakpoints in sardine condition (consistently towards lower condition values) occurred simultaneously between 2006 and 2008 in all areas (except in the Northern Alboran Sea and the Strait of Sicily where no breakpoint occurred, Figure 6.4). Regarding anchovy, the last breakpoints were much more widely spread in time between 2005 and 2009 (Figure 6.4). Breakpoints in maximum fish size appeared in the areas where fish body condition have also shifted and often occurred simultaneously or just after those ones (Figure 6.4). Regarding the environment, no breakpoints were detected in either the Eke time series, nor in the climatic indices (except for AMO in 1995) nor in the chl-a concentration of certain areas (Figure 6.4). Depending on the area, SST had one or two breakpoints. The first ones co-occurred around 1996 while the second one only occurred in 2006 in Sicily and in 2010 in both Adriatic areas. The three river discharges also showed breakpoints, the first one being similar around 2002 but the second one only occurred in the Adriatic in 2008 (Figure 6.4). In the Northern Adriatic, the anchovy decreasing body condition led to a breakpoint in 2004, which coincided with the decline in Po river runoff and chl-a for which breakpoints have been also detected in the same period, in 2002 and 2003, respectively (Figure 6.4). The breakpoint in sardine condition reflecting a drop occurred in 2006 (Figure 6.4). In the Southern Adriatic, the both anchovy decreasing breakpoint co-occurred with a decrease in chl-a. Sardine's first decreasing breakpoint co-occurred with a decrease in chl-a while the second sardine condition decreasing breakpoint co-occurred with an increase of temperature (Figure 6.4). Apart from the Adriatic, only two breakpoints of fish body condition and size time series co-occur with any environmental breakpoint (Figure 6.4). An increase in sardine size occurred with increasing SST in the North Spain area while a decreasing anchovy body condition has been concomitant with decreasing chl-a quantity and increasing SST in the strait of Sicily (Figure 6.4).

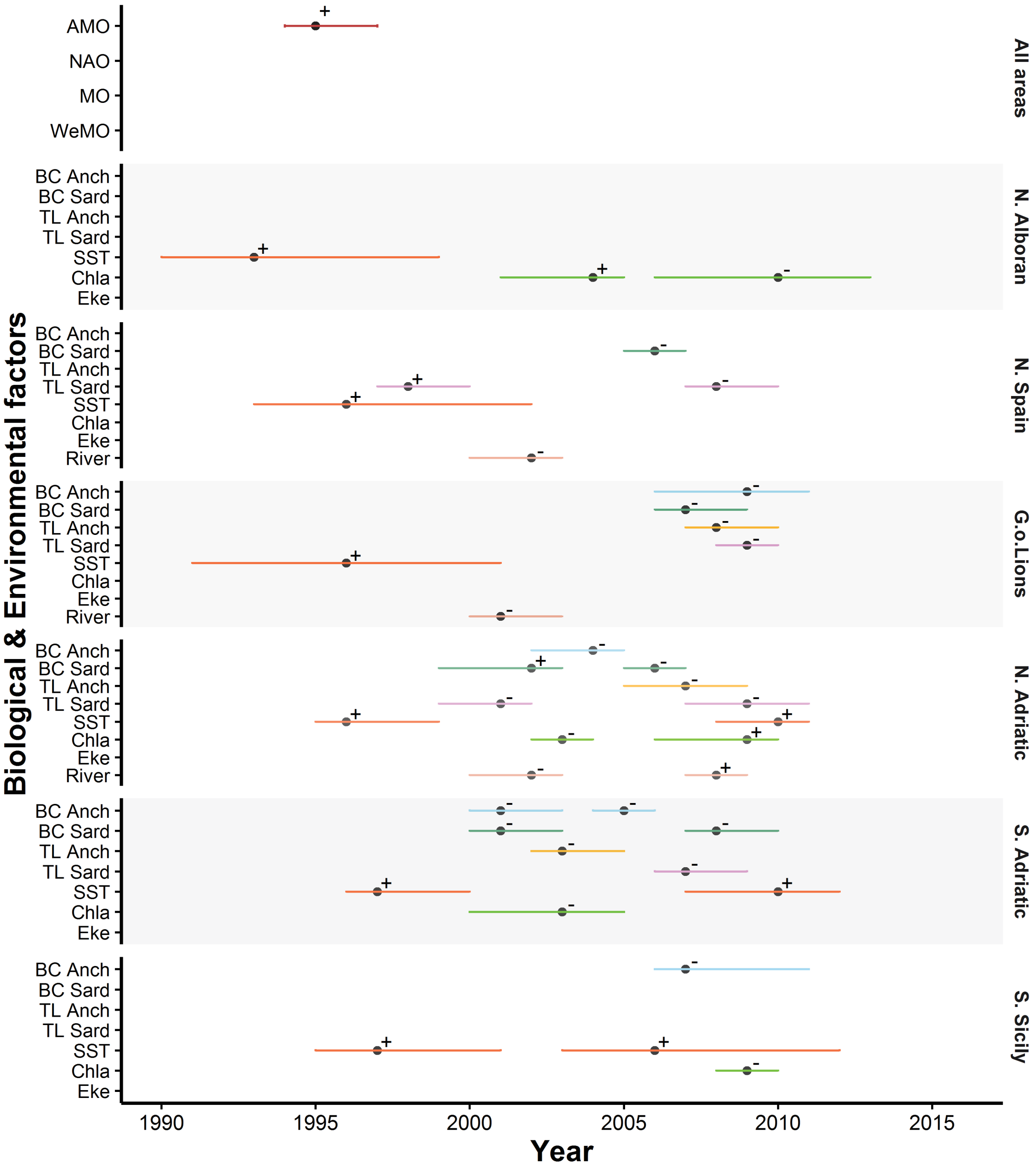

Figure 6.4 Breakpoint analyses of fish biological parameters from MEDIAS data and environmental factors time series per Mediterranean Sea region (BC and TL mean body condition and total length, respectively). Horizontal lines indicate the confidence interval around the detected breakpoint and the sign (plus or minus) indicated the direction of the breakpoint toward an increase or a decrease.

Focus on both fish condition temporal periodicity and phenology analyses in the Adriatic
Time series of monthly body condition from 1975 to 2012 derived from fisheries data in the Adriatic Sea displayed long term decreases. A first drop occurred in the mid-80s for both species. It is followed by a slight decrease (1995) and increase (2005) for anchovy and a final decrease between 2008 and 2012 only for sardine (from 42% to 48% of deviance explained, Figure 6.5A). Periodicities at lower frequency were also observed for anchovy with periods of 3 years between 1996 and 2012 or 6-7 years between 1975 and 1995 (Figure 6.5A). Periods of 6-7 years were detected all along the temporal windows in sardine body condition (Figure 6.5A). The annual cycles during the entire period is well pronounced, as revealed by wavelet analyses (Figure 6.5A), but the peak in condition moved earlier in the season for both species along the series, with a stronger change for anchovy (from October/November in the 1970′s to June/August in the 2000′s) than for sardine (from September/October in the 1970′s to July at the end of the 2000′s, Figure 6.5B).

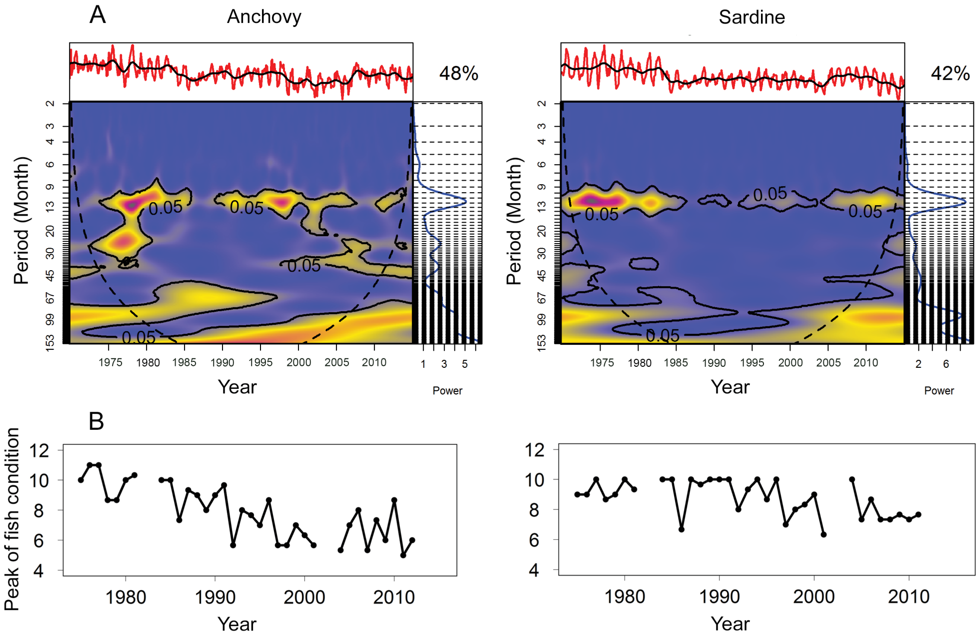

Figure 6.5 Wavelet and peak of condition analyses for the Adriatic condition time series from fisheries data. In the part A, the main panels are the wavelet power spectrum. Power values range from blue (low) to grey (high). The black striped line forming a cone delimits the region not influenced by edge effects. Continuous black lines show 5% significant areas. The top panel represents the standardized data time series (in red) as well as the EVF analysis of this series (in black). The right panel is the global spectrum. The part B is the yearly evolution of the moment of the maximum fish condition. Peak was averaged on the three months with higher values of condition.

Environmental parameters determining pelagic fish body condition in the Mediterranean

In the PCA performed on anchovy body condition, maximum size and environmental factors, the first (F1) and second (F2) components accounted for respectively 40% and 22% of the total variance (Figure 6.6). Positive values of F1 represented areas with high mean annual SST while positive values of F2 indicated areas with high mean annual chl-a. Anchovy mean annual body condition and maximum size were positively related to mean annual Eke rather than to mean annual chl-a and temperature in the Mediterranean Sea. In the PCA performed on sardine, F1 and F2 represented 37% and 28% of the total variance, respectively (Figure 6.6). F1 represented the contrast between mean annual temperature and mean annual chl-a, while F2 is mostly characterized by the mean annual Eke. Contrary to anchovy, sardine annual body condition and maximum size were strongly related to annual mean chl-a, opposed to annual mean temperature and almost independent of Eke. For both species, even if interannual variability within areas was sometimes important (e.g. Northern Alboran Sea), points of a same area were closer to each other than to points from other areas, indicating a spatial variability much higher than the temporal one (Figure 6.6). This regional coherency was especially visible for sardine where areas were ordered according to the latitudinal gradient (from South to North) on the first component and where the northern the area, the higher the sardine body condition (except for the Northern Alboran Sea, Figure 6.6). Particularly, the Strait of Sicily and the Northern Alboran Sea were totally isolated, mainly due to their strong Eke values. A last point of interest relies on the discrimination of the different areas, such as the Gulf of Lions against the Alboran Sea for sardine or Sicily against Northern Adriatic for anchovy (Figure 6.6), which indicates that regions inhabited by anchovy and sardine display different environmental conditions.

Covariability of small pelagic fish body condition in the Mediterranean

Covariability of both anchovy and sardine body condition declined exponentially with increasing distance between harbors, as expected (Figure 6.7). The 50% decorrelation distance of the pairwise correlation between GSAs was estimated as 948 km (845-1075 km, 95% CI) for anchovy and 843 km (732-1031 km, 95% CI) for sardine. However, some harbors separated by small distance could also display a low Pearson correlation coefficient (e.g. between Split and Chioggia for anchovy, respectively on the eastern and western coast of the Northern Adriatic Sea, Table 6.2), indicating occasional strong local variability in fish body condition, mainly for anchovy (Table 6.2).

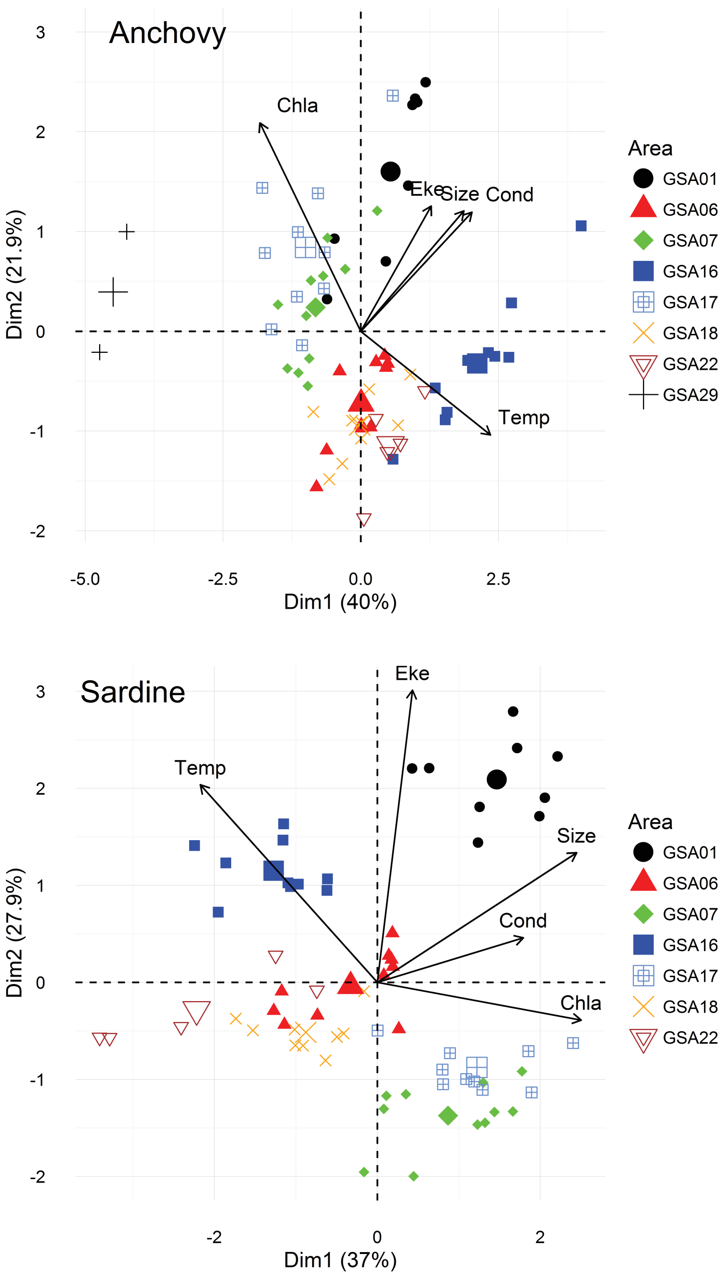

Figure 6.6 Principal component analysis for (a) anchovy and (b) sardine from MEDIAS data showing the relative importance of two fish biological parameters and three environmental factors of 8 different Mediterranean areas, with the variance explained by each axis between brackets. Larger symbol reflects the barycenter of each area. No sardine data were available for the Black Sea, so this area was excluded from this analysis.

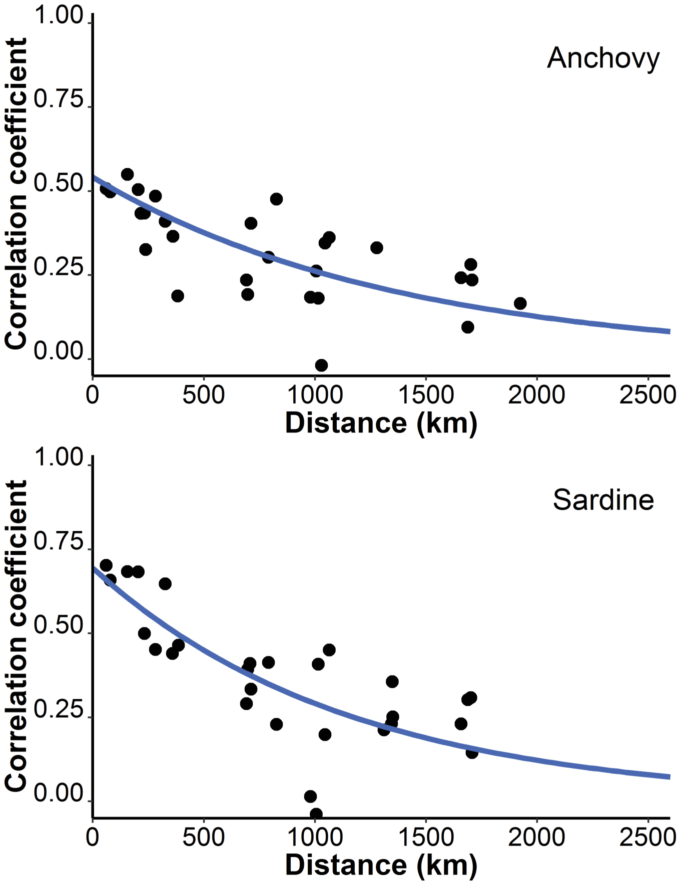

Figure 6.7 Exponential decline over distance of pairwise correlations between anchovy and sardine body condition time series from 8 different harbors (5 in Adriatic Sea: Split, Ancona, San Benedetto, Chioggia and Porto Garibaldi; 2 in Northern Alboran Sea: Màlaga and Torrevieja, 1 in Northern Spain area: Tarragona and 1 in the Gulf of Lions: Sète).

Table 6.2 Pairwise correlation (Pearson coefficient) between fish body condition time series from 9 different harbors. Orange data come from anchovy and blue data from sardine. Harbors are classified from West to East (Málaga; Torrevieja, Tarragona; Sète; Chioggia, Porto Garibaldi, Ancona, San Benedetto, Split). Statistical significances of correlation are indicated by bold values.

	Málaga	Torrevieja	Tarragona	Sète	Chioggia	Garibaldi	Ancona	Benedetto	Split
Málaga		NA	**0.24**	0.18	0.10	**0.24**	**0.28**	**0.24**	0.17
Torrevieja	**0.44**		NA	NA	NA	NA	NA	NA	NA
Tarragona	**0.29**	**0.46**		**0.41**	**0.26**	0.18	**0.35**	**0.36**	**0.33**
Sète	**0.41**	**0.41**	**0.65**		**0.38**	0.21	**0.3**	**0.48**	-0.02
Chioggia	0.30	0.23	-0.04	0.33		**0.51**	**0.51**	**0.49**	0.19
Garibaldi	0.23	0.21	0.02	**0.39**	**0.70**		**0.55**	**0.44**	**0.37**
Ancona	0.31	**0.36**	0.20	**0.41**	**0.68**	**0.68**		**0.50**	**0.33**
Benedetto	0.15	0.25	**0.45**	0.23	**0.45**	**0.50**	**0.66**		**0.43**
Split	NA	NA	NA	NA	NA	NA	NA	NA	

6.4 Discussion

Anchovy and sardine exhibited a decreasing trend in body condition in most of the studied areas, i.e. four or five over six for anchovy and sardine, respectively. None of the studied species in the different Mediterranean areas displayed a long term increase in body condition. The general decrease in small pelagic fish body condition is in agreement with other observations, such as a decreasing biomass of several stocks and weakened biological state for both species at the Mediterranean scale (Tsikliras *et al.*, 2015; Vasilakopoulos *et al.*, 2014; Vilibić *et al.*, 2016). This is particularly clear in some areas, such as the NW Mediterranean, where small sized less fecund individuals (McBride *et al.*, 2013) in a low health status (i.e. lower lipid content and higher intensity of certain parasites, Ferrer-Maza *et al.*, 2016) are dominant in the population.

Additionally, sardine body condition dropped synchronously in 2006 and 2007 in four areas, indicating potential broad-scale causes affecting nearly simultaneously the majority of studied stocks. The range of breakpoint years was more spread between areas for anchovy than sardine, as body condition dropped between 2004 and 2009. Further, maximum size, a relevant parameter to monitor population changes due to its impact on survival, growth and reproduction (e.g. larger fish are more fecund, McBride *et al.*, 2013) also decreased concomitantly with fish body condition for both species in most areas. A general and rapid change affecting the entire Mediterranean Sea could thus be involved to explain the synchrony observed. Small pelagic fish body condition is known to be primarily affected by food availability, especially the zooplanktonic compartment which constitutes the bulk of small pelagic fish preys (Basilone *et al.*, 2006; Brosset *et al.*, 2015b). Lower quality and/or quantity in food resources may have negatively affected fish growth, lowering overall mean and maximum fish size. Changes in the zooplanktonic community have been observed in different parts of the Mediterranean. For example, a shift towards smaller zooplankton biomass in 2003 in the Adriatic (Mozetič *et al.*, 2012) or a change towards smaller plankton species in the Northern Spain area since 2000 related to increasing temperature and stratification (Calvo *et al.*, 2011) were described. Few data are available in other areas, even if similar changes are suspected to have occurred in for example the Gulf of Lions (Auger *et al.*, 2014). Although chl-a is a proxy of primary production available thanks to satellite data, longer precise plankton time series (with at least main functional groups) are required to link food availability to fish body condition and size fluctuations.

Even if a general decrease in body condition was visible for both species, this pattern could not be explained with global environmental factors, such as climatic indices which did not display synchronous breakpoints with fish body condition. Furthermore, the importance of regional factors was highlighted by both the results of the PCAs and the analyses of spatial covariability. For the latter, a 50% decorrelation distance of about 900 kms (i.e. 950 and 850 kms for anchovy and sardine, respectively) was detected. In other words, small pelagic fish body condition may not vary in synchrony at the Mediterranean scale, but rather at the scale of sub-regions. Because of the small number of time series and the resulting uncertainties of the underlying fish condition autocorrelation functions, we can only make a preliminary and rough comparison with correlograms for other species. Nevertheless, when we compare those correlograms to the spatial autocorrelation of zooplankton (biomass and abundance or even phenology), which represents the main prey of small pelagic fish and greatly influence their body condition (Brosset *et al.*, 2015b), similar values up to 950 kilometers were found (Batchelder *et al.*, 2012; Mackas *et al.*, 2012). For example, Mackas *et al.* (2012) computed a 50% decorrelation distance around 1000 km in the Mediterranean and NE Atlantic zooplankton phenology time series, while Batchelder *et al.* (2012) found decorrelation to occur between 643 and 673 km depending on the length of zooplankton abundance time series used, so some values in the vicinity of those found for anchovy and sardine in this study. As mentioned above, this strong regionalization of the environment in the Mediterranean Sea was also confirmed by the results of the PCAs. For both species, areas were mostly marked by spatial (inter-area) variability and were aggregated into three main groups: the eastern areas (the Strait of Sicily and the Aegean Sea), the northern areas (the Gulf of Lions and the Northern Adriatic) and the southern areas (the Southern Adriatic and the Northern Spain area). The Alboran Sea mainly stands apart, probably due to the strong influence of the Atlantic waters which are characterized by low deviations on the annual average values of biological parameters (Renault *et al.*, 2012), and are richer in zooplankton biomass (Youssara and Gaudy, 2001), creating a relatively stable environment. Areas were thus distinct in terms of demographic and environmental characteristics, a result in coherence with the scale of about 900kms displayed by covariability analyses. Although the Mediterranean Sea is strongly affected by large atmospheric transfers (Vargas-Yáñez *et al.*, 2008; Martín *et al.*, 2011), forceful influences are also attributable to more local factors, such as river runoff, which induce contrasted conditions at small temporal and spatial scales. As a consequence, more local drivers had to be investigated in order to better understand these fluctuations. For example, anchovy optimal habitat is usually seen to be related to high frontal activities and river inputs (Agostini and Bakun, 2002;

Giannoulaki *et al.*, 2012; Carpi *et al.*, 2015), resulting in strong water mass mixing. River runoffs are known to potentially affect fish condition (Lloret *et al.*, 2004), but because of a lack of appropriate measures to evaluate correctly river discharges in the different areas (i.e. when no major river is present), this parameter was not taken into account in the PCA but it will necessitate to be correctly incorporated in further spatial work. Sardine is known to prefer colder waters than anchovy (Palomera *et al.*, 2007), explaining the opposition between its body condition and high temperature. Chlorophyll a plays an important role on sardine body condition which may be explained by the fact that sardine also feeds on diatoms additionally to zooplankton when anchovy do not (Costalago and Palomera, 2014; Nikolioudakis *et al.*, 2014) even if phytoplankton consumption remains minor. Yet, despite the important covariation of environmental parameters with body condition and size of sardine and anchovy to explain spatial differences, synchronies in the breakpoints of fish body condition and other environmental factors considered were only rare in most of the areas. This might come from the importance of even more local factors (e.g. small rivers runoff, local upwellings) or the lack of data on small pelagic fish main preys (e.g. zooplankton species). Another explanation could be the delay and/or the complexity of the interactions between environmental variations and fish body condition responses.

The higher level of detection of breakpoints and environmental relationships in the Adriatic (more specifically river runoff, chlorophyll a and SST for that area) might be related to its geographic situation. The Adriatic Sea (especially in the northern part) is a quasi-closed basin in comparison to more open areas (e.g. the Gulf of Lions or the Catalan Sea), which might reinforce the effects of local environmental factors. Further, the length of the fisheries monthly time series in the Adriatic (37 years) also enabled us to study long-term patterns in terms of periodicity. Small pelagic fish body condition is known to display a clear annual pattern together with a strong low frequency signal in the major upwelling regions of the world (Lluch-Belda *et al.*, 1989; Chavez *et al.*, 2003; Lindegren *et al.*, 2013). In addition, it's seasonality also changed in both species. The condition's peak moved earlier over the years, modifying fish energy storage during the year as already underlined by Zorica *et al.* (2013). This may results from environmental changes in the Adriatic Sea between 1975 and 2012 (as underlined for the period 1997 to 2015 in the breakpoint analysis). Phytoplankton production mostly depends on nutrients, light and temperature. As warmed waters promoted earlier phytoplankton development and stronger water stratification led to a reduced second peak of primary production (Edwards and Richardson, 2004), primary production and zooplankton bloomed earlier. This may explain why the peak in fish energy storage shifted toward earlier months for both species. The timing of the peak in anchovy body condition, even if shifted earlier (from October/November to June) still occurs during the reproductive period (late spring and summer). Thus, anchovy can allocate large reserves to reproduction, a phase highly demanding on energy (Williams, 1966) and still survive to the following winter. On the contrary, sardine has to store a maximum of energy before winter reproduction when few resources are available. However, body condition peaked just before reproduction until the mid 90's (September/November) and now peaks in July, longer before reproduction takes place, preventing sardine to reach the reproductive period with maximal fat storage. This difference can make the sardine population more sensitive to current environmental changes in the Adriatic Sea, as has already been observed in the Gulf of Lions, where sardine has less energy to both overwinter and reproduce. Longer time series on a monthly basis will be required to provide conclusions for other areas, and see if the Adriatic Sea situation is unique or if the body condition's annual peak has moved earlier in all areas.

Apart from environmental factors, fishing can also affect fish condition and size, which is important to stress as most of the stocks are overexploited in the Mediterranean (GFCM, 2015). Fishing can impact fish condition through overfishing on their main preys, as demonstrated for demersal species (Hiddink *et al.*, 2005). As small pelagic fish depend on planktonic preys, which are not exploited, such a process remains however very unlikely. A more likely process relies on the catchability that is often size and condition-dependent. Indeed, fisheries-induced selection is known to magnify shifts toward young, small and more quickly maturing individuals in targeted populations (Audzijonyte *et al.*, 2013), but difficulties remain in distinguishing phenotypical responses from true evolutionary changes. Phenotypic plasticity deals with the adaptive response of an organism faced with fishing pressure and/or changing environment. As fishing can reduced fish density, resulting in a decrease in food and space competition, faster growth and earlier maturation can occur. Alternatively, faster growth and earlier maturation can be a consequence of changing genetic composition of population for certain trait values, fishing also determines evolutionary dynamic. The decreasing trend in body size may be also due to an evolutionary response to intensive size selective fishing in overexploited Mediterranean areas. This appears unlikely for the Gulf of Lions because of the asynchrony between fishing pressure and the decline in size and condition and the historical low harvest rates (Van Beveren *et al.*, 2016). However, major changes observed in other areas might be explained by the combined effect of exploitation and environmental changes, especially in areas where anchovy and sardine were clearly overexploited, such as the Adriatic Sea (FAO, 2014). Unfortunately, no proper long-term information on fishing effort directed to each stock was available, preventing us to quantify ecological feedbacks of such life-history changes. Indeed, effort monitoring programs differ between countries and Mediterranean fisheries are often opportunistic (multispecific in some areas), making it impossible to quantify precisely fishing effort on all these stocks.

Based on scientific surveys and fisheries data linked to environmental data, this study evaluates for the first time the temporal and spatial variability in body condition of the two main small pelagic fish at the Mediterranean scale. The results highlighted a general decrease in fish body condition and maximum size, probably related to changes in planktonic quantity and/or quality. The forecasted trend for the near future is an increase in mean Mediterranean SST, sea surface salinity and water stratification (Vargas-Yáñez *et al.*, 2010; Adloff *et al.*, 2015), favoring lower size classes of plankton (Herrmann *et al.*, 2013). In such conditions, small pelagic fish condition may remain poor or even continue to decline as smaller plankton has lower energetic values (Vijverberg and Frank, 1976; Zarubin *et al.*, 2014). Further, due to a lack of reliable data, this analysis did not take into account the exploitation pressure, that we know is high in several stocks and that might well explain part of the decline in some areas. This decreasing trend in the condition of small pelagic fish may have long-lasting negative effects on the Mediterranean fisheries through an effect on life-history traits such as growth, reproduction or natural mortality hampering stock productivity and hence future fisheries yield, as well as on other ecosystem components such as predators preying on small pelagic fish (e.g. Wanless *et al.*, 2005). Considering fish population body condition through scientific surveys and fisheries landings samplings should now be gathered with environmental regular monitoring (including planktonic sampling) and considered in order to implement and provide effective and efficient management plans in the Mediterranean Sea.

The forecasted trend for the near future is that of an increasing mean Mediterranean Sea surface temperature, sea surface salinity and water stratification (Vargas-Yáñez *et al.*, 2010; Adloff *et al.*, 2015), favoring lower size classes of plankton (Herrmann *et al.*, 2013). In such conditions, small pelagic fish condition may stay really poor or even continue to decline. Smaller plankton will induce a strengthening of reduced fish body condition values due to its lower energetic values (Vijverberg and Frank, 1976;

Zarubin *et al.*, 2014). Anchovy body condition was mainly linked to Eke, which may also decrease due to stronger and longer water stratification period during summer. As well as sardine, this means that anchovy body condition is expected to stay low or decrease during future decades. This could finally have an impact on other life history traits: lower growth, lower reproductive potential or higher natural mortality, which would hamper the productivity of stocks and hence future fisheries yield. Moreover, if condition of small pelagic fish is decreasing, it may affect negatively the condition status of other ecosystem components such as fish predating on these small pelagic fish or even birds (Wanless *et al.*, 2005). Finally, if the condition of these pelagic fish which provide high quantities of omega 3 to human consumers decrease, this means less omega 3 available in the future for the consumers. Thus, either markets could import small pelagic fish from other place, disturbing local fisheries economies or the potential health benefits of eating small pelagic fish could be reduced (Lloret *et al.*, 2016).

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Chapter 7

Synthèse, discussion, limites et perspectives

L'objectif de ce doctorat était d'examiner la situation actuelle de la communauté des poissons petits pélagiques du Golfe du Lion au travers une approche d'écophysiologie se basant sur l'étude de la variabilité de la condition corporelle. Pour cette thèse, il fallait donc tout d'abord développer une méthodologie de suivi de la condition, ce qui n'avait pas encore été fait chez les poissons petits pélagiques. Concernant la variabilité de la condition corporelle, ce ne sont pas uniquement les **causes** (mécanismes régulant la condition corporelle) de ces changements qui ont été étudiées, mais aussi ses **conséquences** (décisions d'allocation d'énergie entre les traits d'histoire de vie) afin d'avoir une vision la plus globale possible. La totalité du processus devait être appréhendée principalement grâce à l'étude des trois espèces principales (l'anchois, la sardine et le sprat) afin de comprendre pourquoi de tels changements (perte des individus les plus âgés; baisse drastique de la condition corporelle et de la taille moyenne) ont pu avoir lieu en seulement quelques années pour l'anchois et la sardine.

7.1 Rappel des principaux résultats

Le fatmeter est un outil efficace pour suivre les réserves lipidiques des poissons petits pélagiques et présente l'avantage de ne pas être dépendant de la période de reproduction. Cet instrument permet des mesures répétables et un gain de temps par rapport aux méthodes biochimiques. Il a aussi été utilisé lors des nouveaux suivis débutés pendant la thèse (e.g. suivi pour l'épidémiologie des sardines ou encore sur les sardines en captivité). Malheureusement, si cela a servi pour des études ponctuelles et la mise en place d'un suivi sur les campagnes Pelmed pour les 3 espèces, l'absence de données historiques nous a conduit à nous intéresser aux indices morphometriques. Concernant les indices morphométriques, qui sont les seuls à pouvoir être utilisés sur les données historiques, on a montré que pour nos espèces d'intérêts, ils sont corrélés au taux de lipides hors période de reproduction. La moindre corrélation durant la période de reproduction pouvant s'expliquer par le fait que durant cette période les lipides sont pratiquement épuisés et que les variations de masse sont dépendantes des protéines qui sont consommées.

Dans le Golfe du Lion, les anchois et les sardines sont en mauvaise condition depuis 2008. Les sardines les plus âgées (au-delà de 1 an) présentent un déficit de réserves énergétiques depuis 2008 quand l'on compare à ce qui était observé auparavant, cet effet sur les individus les plus âgés étant non visible chez l'anchois. Ceci pourrait expliquer la disparition des individus les plus âgés dans la population, le déficit en énergie pouvant ne pas suffire à soutenir l'allocation d'énergie pour les différents traits d'histoire de vie. En effet, nous avons montré que l'hypothèse d'un investissement important d'énergie par les petits pélagiques dans leur reproduction et la production de leurs gamètes malgré une faible condition corporelle est très probable. L'énergie dévouée aux autres traits d'histoire de vie s'en voit ainsi très réduite, notamment pour la sardine qui se reproduit en hiver. Malgré ce maintien d'un fort investissement, les performances reproductives au niveau individuel sont affectées par la réduction de la taille moyenne des individus (production d'œufs moins importante) ainsi que par la réduction de leur condition corporelle (œufs de moins bonne qualité). Cependant, au niveau de la population, le nombre d'œufs total produit dans le golfe du Lion a augmenté pour les anchois dû à l'augmentation du nombre d'individus mais a fortement chuté pour la sardine. Mais le fait que le recrutement ne semble pas varier souligne l'importance primordiale des conditions environnementales présentes au moment de la ponte par rapport au nombre d'œufs produit.

Dans le Golfe du Lion, la température semble agir négativement sur la condition de la sardine tandis que le débit du Rhône est un facteur positif significatif chez l'anchois. Mais c'est la quantité de plancton (ressource alimentaire) qui a été mise en avant comme driver principal expliquant les fluctuations de condition corporelle chez les deux espèces. Lors de l'étude spécifique sur l'évolution temporelle de l'alimentation des petits pélagiques, des changements dans le régime alimentaire et une nouvelle compétition interspécifique potentielle à partir de 2010 ont été mis en évidence. Le sprat, dont l'abondance a fortement augmenté depuis 2008 et dont la niche isotopique recouvre celle de l'anchois et de la sardine, pourrait être un compétiteur pour les ressources zooplanctoniques empêchant les deux dernières espèces citées de maximiser leur consommation des ressources. Cette ressource zooplanctonique semble en plus avoir évolué avec aujourd'hui la dominance d'espèces deux fois plus petites, donc moins énergétiques qu'avant les changements observés dans les populations de petits pélagiques. Ces deux changements ont pu agir en synergie et créer un déséquilibre dans l'apport nutritif chez les petits pélagiques, profitant au sprat dont le régime alimentaire plus généraliste lui aurait permis de devenir très abondant voir majoritaire dans le Golfe du Lion.

La situation en Méditerranée est marquée par une quasi généralisation de la baisse de la condition corporelle et de la taille maximum des individus. L'effondrement de la condition s'est fait dans les années 2000 pour les deux espèces, mais concorde rarement avec un shift environnemental la même année. De plus, on note une absence de pattern à l'échelle du bassin Méditerranéen dans les séries temporelles de condition corporelle, renforçant l'hypothèse de l'importance majeure de facteurs locaux, qui peuvent être environnementaux ou anthropique (e.g. surpêche ou pollutions locales). La condition corporelle des anchois est dépendante de la turbulence des zones (énergie cinétique) quand celle de la sardine dépend plus directement de la quantité de chlorophylle a, permettant une première explication des différences observées entre les deux espèces.

7.2 Discussion

Que ce soit en Méditerranée ou ailleurs, on observe des phénomènes de changement climatique et des changements écologiques associés en cours. On peut citer par exemple l'augmentation des températures, de la stratification verticale des eaux ou encore la réduction de la formation des eaux profondes. Ces changements agissent à la fois directement par un forçage physique (e.g. la température agissant sur la physiologie du poisson), indirectement au travers du réseau trophique par le phénomène de cascade trophique ou encore à travers une combinaison de processus (e.g. 'la triade de Bakun', Bakun, 1997).

L'effet indirect majeur de ces changements se traduit par une perturbation de la production primaire, avec des décalages dans les cycles annuels de développement, des substitutions d'espèces dominantes et des pics de productivité moins élevés (Edwards and Richardson, 2004; Hoegh-Guldberg and Bruno, 2010). Les changements dans la structure de la communauté planctonique se répercutent ensuite sur la communauté des poissons petits pélagiques qui dépendent directement du plancton de par leur régime alimentaire planctonophage, comme mit en évidence dans de nombreux cas (Ayón *et al.*, 2011; Lluch-Belda *et al.*, 1992). En Méditerranée, les populations d'anchois et de sardine montrent globalement une baisse de leur abondance et de leur biomasse (Tsikliras *et al.*, 2015). Cependant, quelques particularités sont à noter. Tout d'abord, en Méditerranée, les anchois et les sardines ne varient pas en asynchronie (Palomera *et al.*, 2007). De plus, alors que les populations de petits pélagiques fluctuent habituellement en fonction de leur recrutement, celui-ci ne s'est pas effondré ni pour l'anchois ni pour la sardine lors des changements de dominance entre espèces dans le Golfe du Lion. Le nombre de recrues est toujours important, mais les individus âgés au-delà de 1 an ont pratiquement disparu de la population. L'hypothèse d'une migration de ces individus vers la mer Catalane en suivant le plateau continental et le courant ligure a été écartée par Van Beveren (2015) en comparant les tailles des individus des deux zones. Les recherches ont donc majoritairement tourné autour de l'hypothèse d'une surmortalité des adultes induite par un contrôle bottom-up puisque les contrôles top-down, qu'ils soient anthropique (pêche) ou naturel se sont révélés être très faibles et sans changements notables sur les dernières années (excepté le thon rouge mais dont l'impact est n'est pas la cause principale des changements observés, Van Beveren, 2015).

Importance de la taille et de l'âge des individus composant la population

Effectivement, au moins chez la sardine, les individus âgés de plus d'un an se sont révélés en très mauvaise condition corporelle depuis 2008 par rapport aux valeurs précédemment observées. Ajouté à cela, la vitesse de croissance est réduite (Van Beveren *et al.*, 2014) aboutissant à une population constituée d'individus plus jeunes et plus petits. On peut faire l'hypothèse que les individus âgés de plus d'un an sont aujourd'hui soumis à un taux de mortalité plus élevé qu'auparavant, en raison de leurs plus faibles réserves énergétiques, provoquant une troncation démographique. Or, il a été montré et il est couramment discuté que la sélection d'individus plus petits et/ou plus jeunes à l'intérieur d'une population peut conduire à un affaiblissement de la capacité de résilience de celle-ci face au changement environnemental (Planque *et al.*, 2010). Ceci peut rendre l'effondrement des populations plus probable, plus rapide et plus durable dans le temps. En effet, de manière générale, chez les téléostéens, ce sont les plus grands individus qui possèdent les meilleures capacités migratrices, les meilleurs taux d'échappement à la prédation et qui produisent plus d'œufs et de meilleure qualité (même si les différences entre individus sont réduites chez les petits pélagiques par rapport à des espèces plus longévives, Beamish *et al.*, 2006). Concernant les capacités reproductrices, cela a été montré spécifiquement pour l'anchois et la sardine au cours de cette thèse. Il est aujourd'hui trop tôt pour conclure si les populations d'anchois et de sardine du Golfe du Lion sont vraiment fragilisées par la troncation démographique aujourd'hui observée (pour rappel, une sardine peut vivre jusqu'à 7 ans et un anchois jusqu'à 5 ans dans le Golfe du Lion). On peut tout de même noter que le poids moyen des individus des deux espèces stagne à de faibles niveaux depuis 2008, soulignant la part importante des petits individus. Le fait qu'aucune réaugmentation forte de la biomasse ne soit visible peut être un signe que l'absence des individus les plus grands est préjudiciable et n'arrive pas à être compensé par l'augmentation du nombre de petits individus.

Quels paramètres environnementaux sont importants pour les populations de poissons petits pélagiques ?

La ressource alimentaire (production primaire et secondaire) ainsi que la température et le débit du Rhône ont été soulignés comme significatifs pour expliquer les variations de condition corporelle de nos deux espèces d'intérêts dans le Golfe du Lion. Excepté en mer Noire pour le sprat (Shulman *et al.*, 2005), aucune autre étude n'a étudié les déterminants environnementaux de la condition corporelle des petits pélagiques. Pourtant, les conditions environnementales de l'habitat des poissons petits pélagiques sont déterminantes pour la survie des jeunes stades affectant le succès du recrutement (Bonanno *et al.*, 2013) et pour les stades adultes (Basilone *et al.*, 2006).

Plusieurs études ont ainsi recherché les environnements favorables pour les petits pélagiques en Méditerranée, ce qui peut fortement influencer leur condition et être mis en parallèle de nos recherches. Concernant les zones très oligotrophes, en mer Tyrrhénienne, les anchois et les sardines préfèrent les zones très productives et sous influence des apports terrestres (Bonanno *et al.*, 2016). Les anchois montrent en plus une tendance à se retrouver proche de la côte dans des zones semi-fermées (Golfe) favorisant la présence de fortes quantités de nourriture et la rétention des œufs et des jeunes stades (Bonanno *et al.*, 2016). Dans le détroit de Sicile et dans le nord de la mer Egée, la disponibilité des ressources et la profondeur révèlent l'importance des zones peu profondes où la productivité est localement favorisée (Bonanno *et al.*, 2014). Ceci est confirmé par les fortes concentrations d'anchois et de sardine au niveau des gyres anticycloniques caractéristiques du nord de la mer Egée (Giannoulaki *et al.*, 2005). Pour les zones plus productives comme la mer Catalane, l'abondance et les débarquements de petits pélagiques sont liés à la concentration de chlorophylle a, la bathymétrie et la température (Bellido *et al.*, 2008), mais aussi au débit des rivières (Lloret *et al.*, 2004). Ceci est confirmé par Salat (1996) qui note en plus l'importance des zones de front comme paramètre essentiel de l'habitat optimal des anchois.

Ces quelques exemples montrent la tendance générale des poissons petits pélagiques à favoriser les zones avec d'importants apports terrestres et une productivité associée importante, quel que soit le niveau d'oligotrophie dans les différentes zones. Le plancton ne pouvant pas se déplacer activement contre le courant, il est limité aux zones enrichies en nutriments, donc proche de l'embouchure des rivières, dans les zones frontales ou lors d'événements d'upwellings locaux. Même si le Golfe du Lion fait partie des zones les plus productives de Méditerranée, contrastant avec d'autres zones, l'habitat optimal de toutes les populations de petits pélagiques semble homogène en Méditerranée (Bellido *et al.*, 2008; Bonanno *et al.*, 2014). Cela pourrait permettre de déterminer des zones essentielles pour ces espèces, qui seraient utilisées dans la gestion de la pêcherie et pour minimiser son impact sur les différents stocks.

Concernant la partie de la thèse qui a évalué les drivers de la condition corporelle dans toute la Méditerranée, quelques différences entre anchois et sardine ont été entrevues. La condition corporelle des anchois était majoritairement affectée par les zones de fronts quand la condition corporelle de la sardine l'était plutôt par la quantité globale de chlorophylle a. Ceci est confirmé par les études précédemment citées, qui faisait état d'une importance primordiale de la productivité primaire avec une tendance plus spécifique pour l'anchois qui préfère les zones de fort mélange des eaux. Cette différence pourrait s'expliquer par le fait que l'anchois se reproduit en été quand les eaux sont à leur maximum de stratification. Dans les zones de front (e.g. en mer Catalane, Salat, 1996) ou dans les zones semi-fermées avec de forts apports terrestres (e.g. mer Tyrrhénienne, Bonanno *et al.*, 2016), la ressource disponible pour les jeunes stades mais aussi leur rétention dans une zone favorable est maximisée. La sardine quant à elle profiterait des zones très productives pour accumuler un maximum de réserves énergétiques sans avoir à se soucier des conditions environnementales pour sa descendance. Il a tout de même été observé que les sardines peuvent aussi favoriser des zones de convergence comme les gyres (e.g. en mer Egée et dans le détroit de Sicile, Giannoulaki *et al.*, 2005). Cela pourrait provenir de l'avantage que procure ces zones qui concentrent le plancton pour des espèces filtreuses, qui tirent un maximum de bénéfices énergétiques dans ces zones où la nourriture est accumulée comme discuté dans le paragraphe suivant. Une autre hypothèse pour le choix des zones de front par l'anchois pourrait aussi venir de la composition du plancton dans ces zones. Si celui-ci est différent et notamment composé d'espèces plus grandes, cela avantagerait leur mode de nutrition, principalement basé sur la chasse active avec des proies plus facilement détectables. Cependant, l'hypothèse d'une différence de composition planctonique dans ces zones reste encore à éxaminer.

Compétition entre petits pélagiques dans le Golfe du Lion

Le chapitre portant sur l'alimentation a mis en évidence une compétition trophique potentielle entre les 3 espèces étudiées pour tenter de mieux comprendre la quasi absence des individus les plus âgés. Même si l'existence réelle de cette compétition ne pourra être vérifiée que si des données sur la ressource sont acquises, la notion de compétition mérite d'être discutée. La compétition est un mécanisme densité-dépendant, qui influence la dynamique de population de nombreux téléostéens et notamment celle des anchois et des sardines (Schwartzlose *et al.*, 1999; Barange *et al.*, 2005; van der Lingen *et al.*, 2006). Deux types de compétition sont présents, la **compétition interspécifique** et la **compétition intraspécifique**, qui agissent tous deux au niveau de la disponibilité de la **ressource alimentaire** et de l'**espace**.

Chez les petits pélagiques du Golfe du Lion, les proies zooplanctoniques sont très similaires entre les trois espèces et celles-ci partagent globalement les mêmes aires de répartition (Saraux *et al.*, 2014). L'altération des paramètres biologiques chez l'anchois et la sardine (croissance, condition corporelle, Van Beveren *et al.*, 2014) pourrait découler de ce chevauchement comme cela a déjà été mis en évidence dans d'autres écosystèmes (e.g. en mer Baltique entre le sprat et le hareng, Casini *et al.*, 2011). Malgré de la variabilité interannuelle et si l'on excepte la très forte valeur de 2012, le nombre d'individus (toutes espèces confondues) reste stable dans le Golfe du Lion durant les 10 dernières années (Van Beveren *et al.*, 2014). De même, les aires de répartitions, même si il y'a de la variabilité interannuelle, ne se réduisent ou ne s'étendent pas pour aucune des espèces de façon générale (Saraux *et al.*, 2014). La densité d'individus par kilomètre carré ne changeant pas, on peut donc raisonnablement penser que la **compétition pour l'espace** n'a pas ou que très peu joué sur la situation actuellement observée dans le Golfe du Lion.

L'aspect trophique pourrait quant à lui avoir plus d'importance. Vu que le nombre total d'individus n'a que peu varié, si l'intensité de la compétition pour chaque couple d'espèce est identique, le partage des ressources entre les trois espèces devrait être stable. Or, la compétition entre différentes paires d'espèces n'est que rarement voire jamais exactement identique dans la nature. Dans le Golfe du Lion, durant les années où le sprat devient important (voir dominant) en abondance, celui-ci présente des chevauchements de niches isotopiques plus importants avec l'anchois et la sardine que le chevauchement entre anchois et sardine. L'augmentation du nombre de sprats a ainsi pu provoquer une augmentation des interactions trophiques interspécifiques et donc potentiellement de l'intensité de la **compétition interspécifique**. Pour aller plus loin et discuter de façon plus exhaustive l'effet des interactions interspécifiques sur les petits pélagiques, des analyses supplémentaires ont été effectuées. Sur chaque chalut des campagnes scientifiques PELMED depuis 1993, les valeurs de condition corporelle ont été calculées pour chaque espèce. Par espèce, les chaluts où celle-ci était seule ont été regroupés. La même chose a été faite lorsque l'espèce était associé à 1 et 2 autres espèces, afin de calculer une condition moyenne de l'espèce suivant ces trois types d'interactions. Ces analyses ont été faites par période (bonne, moyenne ou mauvaise, définies par Van Beveren *et al.*, 2014; Brosset *et al.*, 2015b). De même, par période, la condition de l'espèce suivant sa proportion en poids dans le chalut a été calculée. La figure 7.1 résume pour chaque espèce et par période sa condition corporelle en fonction du nombre d'espèces présentes dans le coup de chalut (et qui nous donne une estimation des espèces présentes dans la zone). Excepté pour l'anchois durant les périodes dites moyenne et mauvaise, la condition ne semble pas décroitre avec le nombre d'espèces présentes dans la zone. La figure 7.2 confirme l'effet négatif de la présence du sprat sur la condition de l'anchois lors de la période de mauvaise condition quand la présence de la sardine n'a pas d'effet. La présence du sprat ne semble par contre pas affecter la condition de la sardine quelle que soit la période. Ceci est assez surprenant car c'est la population de sardine qui est la plus affectée au niveau de sa condition corporelle par la situation actuelle. La forte abondance de sprat pourrait n'être donc qu'un cofacteur et non pas la raison principale de la mauvaise santé de la population de sardine. Ainsi on peut supposer que ce n'est pas l'arrivée du sprat qui a provoqué l'effondrement de la population de la sardine mais plutôt la niche écologique laissée vacante par la sardine qui a été exploitée par le sprat.

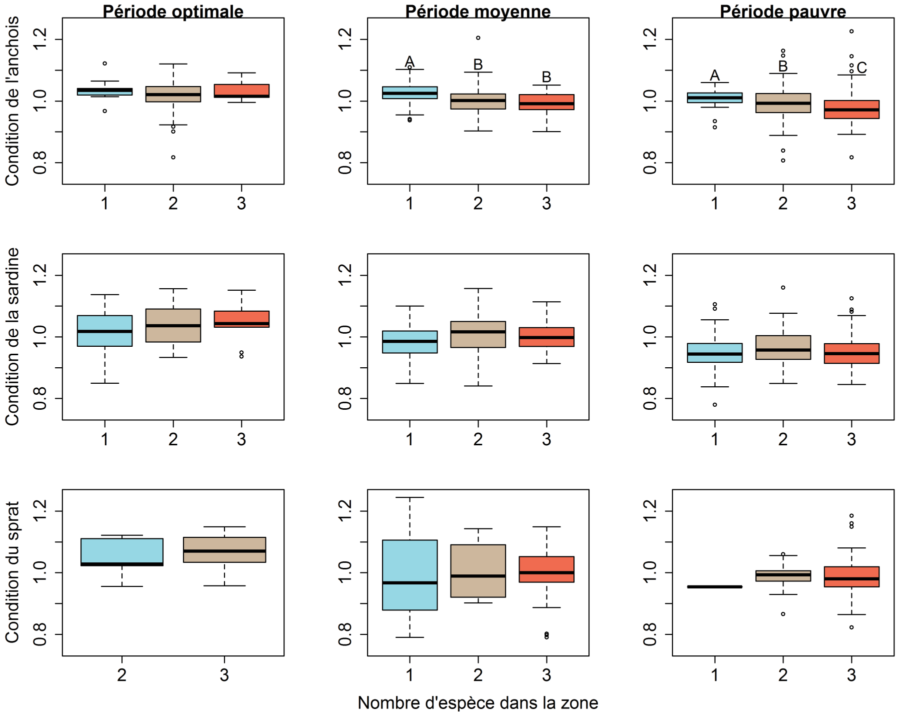

Figure 7.1 Condition corporelle de l'anchois, de la sardine et du sprat en fonction du nombre d'espèces dans le chalut (1: chalut monospécifique, 2: chalut avec deux espèces et 3: chalut avec les trois espèces) pour chaque période. Les lettres indiquent quand les différences entre les différentes conditions sont significatives. Rappel : bonne (2005-2007); moyenne (1993-2004); mauvaise (2008-2016).

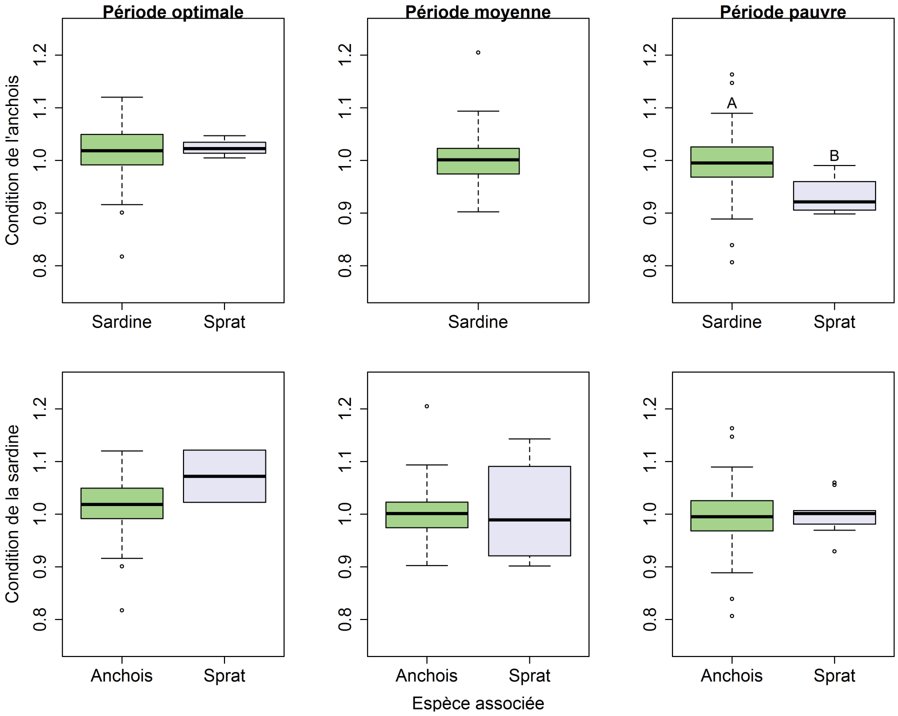

Figure 7.2 Condition corporelle de chaque espèce en fonction de l'espèce associée pour chaque période. Les lettres indiquent quand les différences entre couple d'espèces sont significatives. Rappel : bonne (2005-2007); moyenne (1993-2004); mauvaise (2008-2016).

Concernant la **compétition intraspécifique**, elle a pu augmenté sachant que le nombre d'anchois et de sardine est stable alors que la ressource zooplanctonique semble avoir diminuée. Pour le sprat, elle a fortement augmenté avec la hausse de son abondance. Ceci peut expliquer pourquoi le sprat n'est pas en condition optimale avec des valeurs de condition corporelle très peu supérieures à 1 comme mis en évidence par Van Beveren *et al.* (2014). Cependant, cette hypothèse reste à confirmer (données manquantes pour l'instant) mais les même constatations ont été faites en mer Baltique, où le sprat possède une croissance et une condition corporelle suboptimale quand son abondance augmente (Mollmann *et al.*, 2005). Dans les deux cas, la compétition intraspécifique semble être un élément majeur de la dynamique de population du sprat au travers son effet sur sa croissance et sa condition. Les analyses par chalut (figure 7.3) mettent aussi en évidence une relation négative de la condition corporelle avec sa propre proportion (en poids) dans le chalut chez le sprat lors de la période dite mauvaise. Ce n'est pas visible durant les autres périodes car la proportion de sprat ne dépasse que deux fois 25% dans un chalut avant 2008 et est très souvent proche

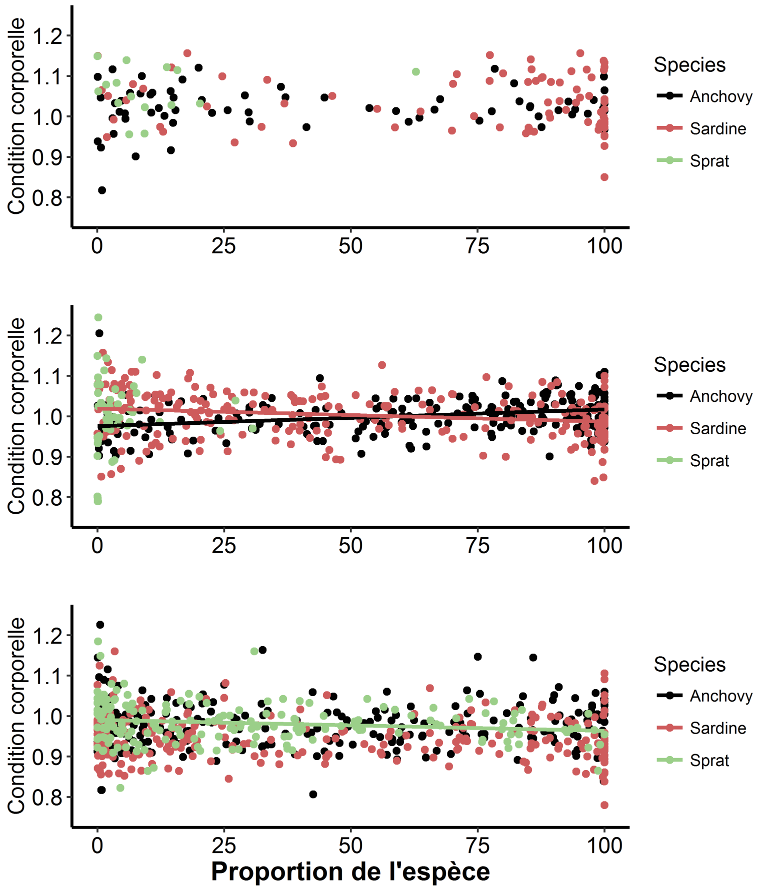

Figure 7.3 Condition corporelle de chaque espèce en fonction de sa proportion (en poids) dans la zone pour chaque période (Haut: bonne (2005-2007); milieu: moyenne (1993-2004); bas: mauvaise (2008-2016)). Les lignes indiquent quand la relation linéaire est significative.

de 0%. Ceci ne nous permet donc pas de savoir si la même relation était présente à ce moment-là. L'anchois montre lui plutôt une augmentation ou une stagnation de sa condition corporelle en fonction de sa proportion dans la zone. La sardine a elle plutôt tendance à voir sa condition légèrement baisser quand sa proportion dans la zone augmente, mais chez ces deux dernières espèces, les variations sont plus faibles ou moins évidentes que pour le sprat.

Ces analyses préliminaires ont le mérite de souligner que le sprat pourrait être sensible à la compétition intraspécifique alors que la sardine semblerait y être que peu sensible et l'anchois pas du tout. Ces résultats sont cependant à nuancer car lors des chalutages les espèces peuvent montrer des taux d'échappement différents qui peuvent biaiser les résultats de présence/absence ou les proportions des différentes espèces par rapport à la réalité dans la zone. De plus, ces analyses sont plutôt basées sur l'aspect qualitatif (basées notamment sur le nombre d'espèces, figures 7.1 & 7.2) et pour vraiment étudier le phénomène de densité-dépendance il faudrait avoir accès aux effectifs de chaque espèce, ce qui n'est pas possible pour l'instant. Les interactions inter et intra spécifiques sont cependant un aspect qui reste à étudier et qui pourrait permettre à l'aide d'analyses statistiques supplémentaires de mieux décrire et connaître les relations au sein d'une même espèce et entre les différentes espèces de petits pélagiques.

Pourquoi la sardine semble-t-elle plus fragilisée que l'anchois ?

Tout au long des différentes études, la sardine a semblé être beaucoup plus affectée que l'anchois par la situation actuelle. Brièvement, les variations de condition corporelle se sont faites dans une gamme de valeur plus importante, de même que les variations de biomasse et les classes d'âge d'ordinaire présentes ont été réduites à des individus de 0 à 2 ans (Van Beveren *et al.*, 2014). L'investissement reproducteur a augmenté quand il est stable pour l'anchois et enfin pour les dernières années, la condition corporelle de la sardine est dépendante de l'âge, ce qui n'est pas forcément évident pour l'anchois. Les particularités de chaque espèce peuvent ainsi être discutées et mises en lien avec les changements précédemment décrits.

Sur les dernières années, cette thèse et des travaux déjà réalisés (Le Bourg *et al.*, 2015) ont montré une forte ressemblance du régime alimentaire entre les deux espèces, puisque majoritairement composé de copépodes (excepté en 2007 pour la sardine). L'absence de différences majeures permet de supposer l'implication d'autres facteurs au delà de la quantité et la qualité de la ressource. Il est intéressant de noter que des différences existent au niveau du comportement d'alimentation et de la dépense énergétique des deux espèces (van der Lingen *et al.*, 2006). Même si l'espèce de sardine utilisée pour la comparaison avec l'anchois est la sardine sud-américaine (*Sardina sagax*), celle-ci est très proche de *Sardina pilchardus*. Si les deux espèces peuvent alterner entre les modes d'alimentation, l'anchois maximise son gain énergétique avec un mode d'alimentation dit de chasse active ("particulate feeding") alors que la sardine le maximise avec un mode d'alimentation filtreur ("filter feeding") (van der Lingen *et al.*, 2006). Le choix entre les deux modes d'alimentation est dépendant de la concentration et/ou de la taille des proies. Quand les proies sont très abondantes, le mode filtreur est très rentable alors que lorsque la concentration en proies est faible, le mode de chasse actif sera plus efficace. Si l'on met cela en lien avec l'évolution de la productivité planctonique dans le Golfe du Lion, qui baisse sur les dernières décennies (Calvo *et al.*, 2011; Herrmann *et al.*, 2014), le mode de chasse active de l'anchois pourrait être le plus adapté et défavoriser les sardines qui dépensent plus d'énergie pour se nourrir avec ce mode de nutrition. Cette augmentation de la dépense énergétique pourrait être en partie contrebalancée par le fait que le plancton est aujourd'hui plus petit et que dans ce cas le mode d'alimentation filtreur semble plus rentable (van der Lingen *et al.*, 2006). De plus, les sardines sont aussi connues pour adapter leur vitesse de nage proportionnellement à la concentration en proies. Les sardines pourraient ainsi nager moins vite pour dépenser moins d'énergie lorsqu'elles filtrent et ne serait pas si désavantagées par rapport à l'anchois. Au niveau de l'assimilation des proies, peu de différences sont notables entre les deux espèces pour le carbone qui permet de produire l'énergie (van der Lingen *et al.*, 2006). Des expérimentations supplémentaires utilisant cette fois *Sardina pilchardus* comme modèle d'étude sont aujourd'hui nécessaires pour déterminer plus précisement si des différences significatives d'efficacité trophique existent réellement entre les deux espèces.

L'alimentation est aussi le facteur clé expliquant l'alternance entre anchois et sardine dans les régions de grands upwellings permanent. En présence majoritaire de copépodes de grande taille et d'euphausiacés, les anchois sont favorisés dans l'écosystème du Humboldt (Ayón *et al.*, 2011) et du Benguela (van der Lingen *et al.*, 2006) et dominent en abondance et en biomasse. Dans le cas contraire, c'est-à-dire avec majoritairement des copépodes de petite taille, c'est la sardine qui domine en abondance et en biomasse. Une étude récente a mis en évidence en mer Catalane l'existence de deux réseaux alimentaires parallèles pour les deux espèces au moins durant l'été

(Cardona *et al.*, 2015), où les anchois se nourrissent principalement sur une chaine alimentaire basée sur les dinoflagellés et les cyanobactéries alors que les sardines se nourrissent plutôt sur une chaine à diatomées. Les diatomées constituant en général le gros phytoplancton, qui est aujourd'hui moins abondant dans le Golfe du Lion, cela tend à confirmer que le manque de gros plancton peut bien être une des causes majeures de la plus grande fragilité de la sardine par rapport à l'anchois.

La reproduction, qui est un trait d'histoire de vie très couteux en énergie (Williams, 1966), et donc l'allocation d'énergie qui en résulte est aussi essentielle pour appréhender les différences entre les deux espèces. Comme déjà énoncé, la sardine se reproduit en hiver, et alloue principalement l'énergie qu'elle a accumulée pendant le printemps et l'été pour le développement des gonades. L'anchois lui se reproduit en été et l'énergie nécessaire au développement des gonades provient principalement de la nourriture qui est consommée instantanément et non de ses réserves. Avec le décalage d'un mois plus tôt des pics de productivité primaire et secondaire (Herrmann *et al.*, 2014) ainsi qu'une quantité moindre de plancton (Herrmann *et al.*, 2014), les sardines pourraient avoir plus de difficulté à maintenir une quantité suffisante d'énergie pour l'hiver. L'anchois lui pourrait être moins affecté puisque sa reproduction se fait quand la ressource alimentaire est toujours suffisamment abondante. Puisque l'on a vu que les sardines investissaient encore plus dans leur reproduction aujourd'hui, la disparition spécifique à la sardine des individus âgés de plus d'un an (i.e. celles qui sont en âge de se reproduire) pourrait simplement découler de leur incapacité à allouer suffisamment d'énergie à la fois pour la reproduction et la survie.

Les différences dans le comportement de nutrition, la physiologie (e.g. l'efficacité d'assimilation) et la période de reproduction nous permettent de mieux comprendre pourquoi de forts changements sont visibles chez la sardine. Dans le même temps, les caractéristiques de l'anchois lui permettent de limiter l'effet des changements environnementaux. De nouvelles avancées pourront être obtenues en étudiant précisément la physiologie de ces deux espèces ou au moins de la sardine en mésocosme, perspective qui sera abordée plus loin dans cette partie.

Quand les différents facteurs agissent ensemble : stress cumulé potentiel sur les poissons petits pélagiques

Même si les différents facteurs (prédation, pêcheries) pris à part les uns des autres peuvent paraître peu impactants sur les populations d'anchois et de sardines, ils peuvent agir ensemble. C'est-à-dire que l'effet des différents facteurs s'additionne ou même agit en synergie pour au final devenir significatif. Alors que les recherches en écologie ont largement documenté l'effet individuel de nombreux facteurs de stress sur les populations, beaucoup moins de travaux ont considéré l'effet cumulatif de facteurs multiples. Pourtant, les milieux naturels sont presque toujours soumis simultanément à des stress multiples (Halpern *et al.*, 2008). Dans le milieu marin, cela a déjà été montré avec l'effet cumulé des pêcheries, de la pollution et de l'augmentation de l'apport de nutriment (Hsieh *et al.*, 2006; Brander, 2007; Crain *et al.*, 2008; Halpern *et al.*, 2008), qui ont déstabilisé l'écosystème par leur effet combiné. Les zones côtières et de plateau continental sont les plus touchées, et il est fortement recommandé de prendre en compte un maximum de facteurs simultanément dans la gestion de ces zones (Crain *et al.*, 2008; Halpern *et al.*, 2008).

Cependant, considérer l'effet cumulé des différents stress reste un challenge car de nombreux cas ont suscité des débats sur l'importance de chaque facteurs, notamment entre les pêcheries et le forçage environnemental (Finney *et al.*, 2002; Beaugrand *et al.*, 2003). Par exemple, il a été montré que les pêcheries peuvent avoir un effet significatif même quand le déclin de la population n'est pas marqué (Hsieh *et al.*, 2006). En général, l'effet de multiple facteurs est considéré comme une addition de leur deux effets individuels (effet additif, Sanderson *et al.*, 2002). Mais d'autres expériences ont montré que différents stress peuvent agir en synergie, c'est-à-dire que l'effet d'un facteur peut être amplifié (effet synergique) ou diminuer (effet antagoniste) par un autre facteur, compliquant l'évaluation commune de l'effet de multiple facteurs (voir la Figure 7.4). On sait aussi que les facteurs varient en intensité suivant les espèces et les zones (en fonction de facteurs additionnels non pris en compte, Rouyer *et al.*, 2014), le calcul des effets cumulés reste largement spéculatif et rend l'établissement de relations générales très difficiles (Crain *et al.*, 2008; Darling and Côté, 2008). De plus, l'effet de la combinaison des différents facteurs peut lui aussi varier spatialement, temporellement et en fonction de seuils (Coles and Jokiel, 1978). Le but final est d'obtenir un indice qui combine les différents facteurs et qui serait comparable avec d'autres mesures, afin d'éviter les échecs dans la gestion des stocks dus à l'incertitude sur les différents facteurs et notamment leur interaction (e.g. pêcheries et environnement, Ludwig *et al.*, 1993).

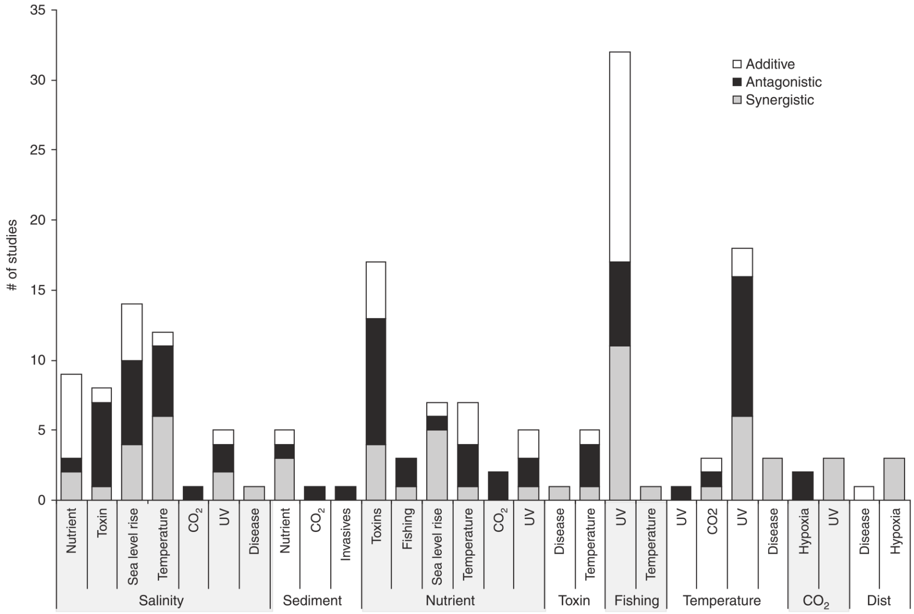

Figure 7.4 Distribution en fréquence des différents types d'intéractions (additive, synergique et antagoniste) par paire de stress (d'après Crain et al., 2008).

Afin d'obtenir de meilleurs résultats dans la gestion et la conservation des écosystèmes, une meilleure compréhension et une meilleure prise en compte de l'effet cumulatif des différents stress sont nécessaires. C'est-à-dire que dans le cas du Golfe du Lion, la pêche, la prédation, éventuellement la pollution et les agents pathogènes doivent toujours être considérés comme pouvant être des effets cumulatifs. Ainsi un effet d'un ou plusieurs parasites pourrait se cumuler avec la mauvaise condition des poissons par exemple. Des poissons en bonne santé peuvent résister à ces parasites, mais lorsqu'ils sont en mauvaise condition ce parasite pourrait arriver à affecter de nombreux individus, agissant ainsi en synergie avec leur mauvaise condition. Un autre exemple est celui des cocktails de polluants, lorsque différent polluants comme les PCB, les microplastiques et les métaux lourds peuvent agir en synergie et directement affecter les mécanismes physiologiques des poissons. Ces facteurs ne peuvent donc pas être totalement écartés pour expliquer la situation des populations de petits pélagiques. Des études supplémentaires sont aujourd'hui nécessaires pour déterminer la nature des interactions. Si l'on a plutôt des interactions de type additif, réduire l'effet d'un des facteurs se répercutera forcément sur la population visée. Si ce sont plutôt des facteurs agissant en synergie, les résultats attendus seront réduits ou augmentés en fonction de l'interaction entre les facteurs. Connaître ces interactions est nécessaire car la gestion des océans ne peut plus être faite en considérant les facteurs séparément. Considérer les effets cumulés est une opportunité réelle d'améliorer les résultats obtenus dans la conservation des écosystèmes marins en réduisant une source majeure d'incertitude (Sala *et al.*, 2000) et pourrait être aujourd'hui appliqué à l'écosystème pélagique du Golfe du Lion.

Au delà de l'écologie des poissons petits pélagiques :

1. Conséquences écologiques, socio-économiques et régulations potentielles de la pêche en Méditerranée

La notion de cascade trophique regroupe les effets des variations en biomasse et en nombre d'un niveau trophique sur les échelons trophiques inférieurs et supérieurs. Les variations des anchois, des sardines et des sprats ont donc des répercussions à la fois sur le compartiment zooplanctonique et les prédateurs de par leur place d'espèce clé de voute de l'écosystème pélagique (Pikitch *et al.*, 2014). Dans d'autres écosystèmes, les variations de l'abondance ou de la biomasse des poissons petits pélagiques ont provoqué l'effondrement de populations d'oiseaux marins (Cury *et al.*, 2011), de mammifères marins (Pikitch *et al.*, 2014) ou une intensification de la production secondaire par la baisse de la pression de prédation. Des phénomènes similaires pourraient être observés au niveau du Golfe du Lion. Comme expliqué précédemment, les thons et les mammifères marins n'exploitent qu'une infime proportion des populations de petits pélagiques (Van Beveren, 2015; Queiros, 2016), donc une baisse de la biomasse de celles-ci ne devrait pas fortement affecter ces prédateurs-là. Le retour du thon rouge a d'ailleurs été observé alors que l'on avait une baisse de la biomasse des anchois et des sardines. L'absence d'étude dans le Golfe du Lion sur les oiseaux marins ne permet pas de tirer de conclusions concernant ce compartiment trophique, cependant si l'on se base sur les observations faites dans d'autres écosystèmes (Cury *et al.*, 2011), leur abondance pourrait stagner ou même légèrement décroitre avec la légère baisse de la biomasse totale de petit pélagique mais aussi à cause de la baisse de leur valeur nutritive (due à la baisse de la condition corporelle des poissons). Enfin, concernant l'effet sur les niveaux trophiques inférieurs, la situation actuelle découlant très certainement d'un contrôle bottom-up et non d'un effondrement du recrutement des petits pélagiques, la pression de prédation exercée par les petits pélagiques n'a pas du diminuer sachant que leur biomasse totale n'a que peu varié. Aucune surabondance de production secondaire (zooplancton) n'a en effet été notée dans le Golfe du Lion et corrobore le fait que la diminution des petits pélagiques vient d'un problème au niveau de leur ressource alimentaire qui s'est propagé dans le réseau trophique.

Dès que les stocks de petits pélagiques ont baissé dans les différentes zones de Méditerranée, les pêcheurs (de part une surexploitation des stocks) ont été accusés comme les principaux acteurs de cet effondrement, suivi par une crise de la pêcherie. A titre d'exemple, l'activité socio-économique générée par la pêche du poisson bleu se résume aujourd'hui à une dizaine de chalutiers pour tout le Golfe du Lion quand on en dénombrait plus de 60 avant 2007 (GFCM, 2015). Cette crise a été accentuée par le fait que le sprat qui est maintenant en quantité importante dans le Golfe du Lion depuis 2008 est un poisson de petite taille et sans valeur économique, donc difficilement valorisable. De plus, la plupart des espèces commercialisables du Golfe du lion étant déjà pleinement exploitées, les possibilités de report de l'effort de pêche étaient réduites voire inexistantes. Beaucoup de bateaux se sont quand même tournés vers la pêche au merlu, déjà surexploité dans le Golfe du Lion (GFCM, 2015), mettant encore plus en péril ce stock et l'activité économique en découlant en augmentant encore plus la pression anthropique. Cependant, comme montré dans la littérature et dans cette thèse, la Méditerranée fait face à une grande variété de l'état des stocks de poissons petits pélagiques. En effet, certains sont surexploités (e.g. l'anchois en mer d'Alboran et en mer Catalane ou encore la sardine en Adriatique, Colloca *et al.*, 2013) quand d'autres sont classés en déséquilibre écologique (sardine dans le Golfe du Lion, GFCM, 2015) ou encore comme pêché durablement (anchois en mer Egée, Colloca *et al.*, 2013). Historiquement, la Méditerranée est une zone où peu de mesures ont été mises en place (Smith and Garcia, 2014), où les quotas n'existent pas hormis pour le thon rouge (Fromentin *et al.*, 2014) et où les tailles limites de capture sont limitées à quelques espèces (dont les anchois (9 cm) et les sardines (11 cm), Mora *et al.*, 2009). Concernant les petits pélagiques, la taille minimale de capture pour l'anchois de 9cm et de 11cm pour la sardine doivent être respectées, ainsi qu'un nombre maximum de jours en mer pour les bateaux sous licence.

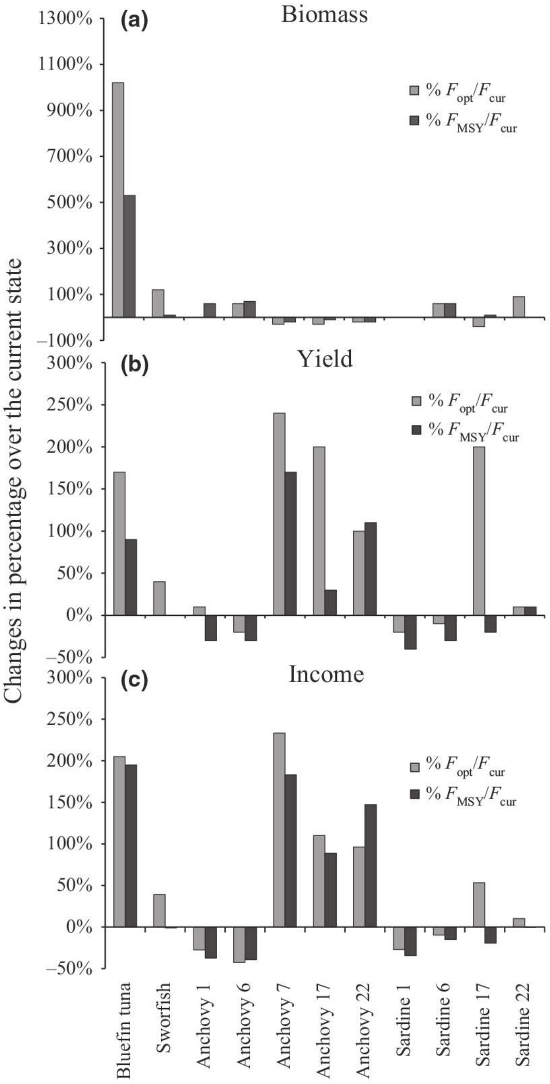

Figure 7.5 Changements estimés (%) pour une mortalité par pêche à taille optimale (F_{Lopt}) ou au rendement maximum durable (F_{MSY}) par rapport à la situation actuelle pour la biomasse des stocks, le rendement de la pêcherie et le revenu des pêcheurs (d'après Colloca *et al.*, 2013). La situation actuelle a été calculée avec les valeurs obtenues avec le régime de pêche aujourd'hui en cours dans les différentes zones (F_{cur}).

Plusieurs travaux se sont penchés sur les effets que pourraient avoir certaines mesures sur les pêcheries de petits pélagiques, notamment la mise en place de mortalité par pêche à taille optimale (F_{Lopt}) ou de stratégie visant à atteindre une mortalité par pêche au rendement maximum durable (F_{MSY}, Colloca *et al.*, 2013). Les deux types de scénarios montrent des résultats variables, étant dépendant de la zone ou de l'espèce ciblée. Les paramètres étudiés (biomasse, rendement et revenus) sont montrés dans la figure 7.5 et montre bien l'hétérogénéité de la situation des stocks de petits pélagiques. Concernant l'aspect économique, la gestion sous F_{MSY} et F_{Lopt} serait bénéfique uniquement pour l'anchois dans le Golfe du Lion, en Adriatique et en mer Egée, la sardine en mer Egée et inefficace dans les autres zones. Du côté écologique, la biomasse n'augmente pas forcément (figure 7.5), et ces mesures semblent apparemment inefficaces pour le rétablissement des stocks. Même si très peu d'études ont été aujourd'hui menées sur cette problématique en Méditerranée, ces travaux ont le mérite de faire apparaître la complexité de la gestion des stocks dans cette zone, partagée entre une efficacité faible et hétérogène des différentes mesures et la difficulté d'une gestion multi-pays pour la plupart des stocks. La gestion des stocks de petits pélagiques est aussi rendue difficile par leurs fortes variations interannuelles de biomasse et d'abondance. Cela induit un décalage entre la détection de phénomènes, de tendances versus la situation en cours. D'autres travaux doivent donc être menés en utilisant notamment d'autres modèles qui pourraient être plus adaptés aux poissons petits pélagiques afin d'envisager d'autres mesures pour réduire au maximum l'effet de la pêche. Le tout en conservant cette activité économique essentielle au développement et à la prospérité de nombreuses régions du pourtour Méditerranéen.

2. Vers une approche écosystémique des pêcheries de poissons petits pélagiques ?

L'approche écosystémique des pêches repose sur la considération de paramètres biologiques, écologiques et des relations interspécifiques dans la gestion des ressources exploitées (Pikitch, 2004; Jennings, 2005) afin d'atteindre une gestion durable. Concrètement, cela se fait en incluant les informations environnementales (e.g habitat), biologiques (e.g. taille à maturité) et les interactions interspécifiques (e.g. relation proies-prédateurs) comme paramètres dans l'évaluation du stock, dans les modèles prédictifs et au moment de la prise de décision. Concernant les écosystèmes pélagiques, cette approche est déjà utilisée par exemple en mer Baltique pour le hareng en tenant compte de la température et de quantité de zooplancton pour les prévisions de biomasse des 5 années suivantes (ICES, 2015). La mortalité découlant de la prédation de la morue est aussi incorporée dans l'évaluation de stock du hareng et du sprat (ICES, 2015). La condition corporelle est un paramètre encore rarement utilisé, même si son ajout dans certaines évaluations par l'ajustement de la valeur de la mortalité naturelle (morue en mer Baltique) a montré son importance. Par exemple, les résultats concernant la biomasse du stock, le recrutement et la mortalité par pêche sont jusqu'à 40% différents de ceux issus du modèle ne tenant pas compte de la condition corporelle des morues (Casini *et al.*, 2016).

Pour le Golfe du Lion, les évaluations des stocks de petits pélagiques sont faites pour l'anchois en prenant en compte sa biomasse estimée par acoustique. On cherche des tendances sur le long terme et des points de références (biomasse limite et biomasse de précaution) qui vont permettre de définir si l'on tend plutôt vers un stock réduit ou un stock en bonne santé (GFCM, 2015). Pour la sardine, c'est un modèle de production de surplus à deux stades qui est basé sur les biomasses estimées par les campagnes scientifiques, le calcul du surplus (recrutement + croissance - mortalité naturelle) et les captures et qui fournira un indice de biomasse pour les adultes et les recrues ainsi qu'un taux d'exploitation permettant d'estimer la santé des différents stocks. Basé sur cette thèse et la littérature, une situation avec une pauvre condition corporelle et une croissance ralentie a été signalée dans la plupart des GSA de Méditerranée. On s'attend donc à une augmentation de la mortalité naturelle des poissons petits pélagiques. Plus spécifiquement pour le Golfe du Lion, la pression de prédation des prédateurs majeurs est maintenant correctement estimée (Van Beveren, 2015) et l'accès aux séries temporelles de phytoplancton est en cours de développement et permettra d'avoir une idée du type de zooplancton présent. Alors que la mortalité naturelle est difficile à estimer et souvent fixée comme taille dépendante chez les petits pélagiques, les principaux paramètres jouant sur la mortalité naturelle adulte (excepté les concentrations planctoniques en cours d'analyses) sont aujourd'hui disponibles pour le Golfe du Lion et pourraient commencer à être intégré dans les évaluations de stocks afin d'être comparées aux autres modèles. La condition corporelle n'agit pas que sur la mortalité naturelle, mais aussi sur d'autres paramètres. On a montré au cours de cette thèse qu'elle est liée à la qualité des œufs, mais elle peut aussi agir sur les chances de devenir mature (Morgan, 2004) ou la biomasse des reproducteurs (Dutil and Lambert, 2000). Ces effets peuvent aussi affecter les résultats de l'évaluation des stocks et l'estimation de points de référence. Comme la plupart des zones présentent des tendances très marquées pour des paramètres influençant la mortalité naturelle, il est fortement recommandé dans le futur de tenir compte de ces paramètres dans l'évaluation des stocks (Casini *et al.*, 2016). Cependant, par rapport à la Baltique par exemple (où l'influence des eaux Atlantique et la température expliquent la majeure partie de la variance), la situation dans le bassin Méditerranéen est très complexe du fait de la forte biodiversité, de la grande complexité du réseau trophique ainsi que du forçage environnemental multi-facteurs. Beaucoup d'efforts sont donc encore nécessaires pour obtenir ces paramètres dans toutes les zones de Méditerranée, mais la plus-value apportée par une approche plus globale comme l'approche écosystémique des pêches permettrait d'améliorer les résultats obtenus ainsi que les prédictions, tout en sachant que les données pour calculer la condition corporelle sont disponibles pour toutes les zones.

7.3 Limites et perspectives

Au-delà de ce travail de thèse, en plus des pistes de départ qui n'ont pas pu être explorées par manque de temps, de nouvelles idées ont émergé et devront être creusées afin d'approfondir les travaux déjà effectués au sein du projet EcoPelGol.

Le principal débat et la principale limite tourne autour des indices de condition. Dans cette thèse, le proxy de la condition est l'indice de Le Cren, qui est un indice morphometrique. Or on sait que ces indices sont des proxies des réserves énergétiques et non pas une mesure directe. On n'observe donc pas directement les variations des réserves énergétiques (i.e. la condition corporelle) de l'individu, mais les variations de sa masse en tenant compte de sa croissance (car l'on divise la masse de l'individu par sa masse théorique dépendant de sa longueur). Même si l'on a vu que l'indice de Le Cren est corrélé aux mesures du taux de lipides total, des différences peuvent résulter du fait que l'on travaille sur des mesures indirectes. Tout d'abord, il peut y avoir un décalage dans le temps entre les variations du taux de lipides et du poids, ce dernier variant plus fortement quand ce sont les protéines qui commencent à être consommées (phase 3 du jeûne) et variant donc quand les lipides sont déjà faibles, donnant un signal retardé. De plus, la partie méthodologie a mis en évidence une grande variabilité de l'indice de condition pour un même taux de lipide, et donc que les variations de l'indice de condition ne reflètent pas de manière parfaite les variations du taux de lipides. Il faut donc garder en tête que les variations observées de l'indice de condition ne sont pas le reflet exact en quantité et dans le temps des variations de quantité de lipides, et peuvent donc fournir une vision incomplète de la réalité. Cependant, contrairement à de nombreuses autres études, les indices ont tout de même été validés face à des mesures directes de lipides et sont fiables pour nos espèces étudiées. Malgré les quelques limites inhérentes à l'utilisation de tout indice indirect qu'il est nécessaire de considérer lors de l'interprétation, l'utilisation faite dans cette thèse de ces indices est robuste. Cette partie de la thèse a aussi permis de développer l'utilisation du Fatmeter pour mesurer le taux de lipides chez l'anchois, la sardine et le sprat. Ainsi, depuis le début de la thèse, un suivi de la condition des petits pélagiques a été initié et a permis de démarrer une série temporelle qui sera poursuivie dans les années à venir au sein de l'Ifremer de Sète.

Le chapitre 3 a mis en évidence des changements dans l'alimentation des poissons petits pélagiques, que ce soit avec l'utilisation des isotopes stables ou des contenus stomacaux. Cependant, une limite de ce travail est l'absence de suivi temporel et spatial in situ du plancton dans le Golfe du Lion empêchant de tester la notion de compétition trophique, car elle reste impossible à démontrer sans des indices de quantité et de qualité du compartiment planctonique. Autrement dit, est ce que la ressource commune aux trois espèces principales de petits pélagiques du Golfe du Lion est limitante ou non. En premier lieu, ce manque de données sur la quantité et la qualité du plancton dans le Golfe du Lion pourrait en partie être comblé par l'adaptation de l'algorithme PHYSAT (analyse de la couleur de l'eau des images satellites) pour le Golfe du Lion. Cet algorithme est utilisé à large échelle (par exemple sur tout le bassin Nord Atlantique, Alvain *et al.*, 2005) mais nécessite une calibration spécifique lorsque l'on s'intéresse à des zones plus restreintes et surtout aussi hétérogènes que le Golfe du Lion. Même si l'utilisation des algorithmes PHYSAT ne donne accès uniquement qu'aux principaux groupes phytoplanctoniques (résultats préliminaires pour le Golfe du Lion, Figure 7.6) et dépend de la couverture spatio-temporelle des satellites SeaWiFS et MODIS, cette approche permettrait une réelle avancée dans la connaissance des fluctuations planctoniques dans le Golfe du Lion.

Figure 7.6 Séries temporelles préliminaires obtenues avec PHYSAT pour les principaux groupes de phytoplancton dans le Golfe du Lion entre 2002 et 2015.

En effet, les deux types principaux de chaine trophique, reposant soit sur des diatomées (gros phytoplancton) ou des synechococcus et dinoflagellés (petit phytoplancton) sont connus pour favoriser différents types de zooplancton. Le couplage de la donnée de dominance et de la quantité phytoplanctonique permettrait donc de tracer le type et la quantité de plancton disponibles dans le Golfe du Lion et de permettre de mieux appréhender la notion de compétition entre les différentes espèces de poissons petits pélagiques. Il pourrait être ainsi plus facile de déterminer si la ressource s'est amoindrie et est devenue limitante et donc si la compétition interspécifique est réelle ou non. De plus, un croisement de ces données avec des séries temporelles obtenues par le laboratoire de Villefranche sur Mer permettra d'avoir une vision plus complète des modifications temporelles du zooplancton dans le Golfe du Lion. Même si les points d'échantillonnage du laboratoire océanographique de Villefranche sur Mer ne sont pas situés sur le plateau continental du Golfe du Lion, les séries temporelles ont démarré depuis les années 1960. En plus de la grande quantité de données sur l'aspect temporel, l'identification spécifique du plancton permettrait d'affiner le lien entre qualité du plancton (sa composition spécifique) et condition des poissons petits pélagiques. L'effet de la qualité du plancton sur la condition des poissons étant peu étudié, établir des comparaisons entre ces deux aspects permettrait de savoir s'il s'agit d'un paramètre clé pour expliquer les fluctuations de condition chez les poissons planctonophages. Si oui, ce paramètre pourrait être considéré dans les modèles halieutiques pour les régions où ces données sont disponibles ou correctement modélisées.

Une autre perspective découlant du chapitre portant sur l'alimentation des poissons petits pélagiques est celle concernant l'aspect énergétique des proies chez l'anchois et la sardine. Pour rappel, les contenus stomacaux ont montré que les proies sont toujours majoritairement des copépodes quelle que soit la période temporelle mais les espèces consommées aujourd'hui sont deux fois plus petites[1]. De précédentes analyses en énergétique des copépodes ont mis en évidence un apport énergétique positivement corrélé à la taille des espèces (Vijverberg and Frank, 1976; Zarubin *et al.*, 2014). Sachant que la taille des proies a baissé en comparant les différentes périodes temporelles, l'apport énergétique serait aujourd'hui moindre en comparaison de ce qui était ingéré avant. Il pourrait donc en découler une baisse des réserves énergétiques disponibles chez chaque individu, avec potentiellement de fortes conséquences sur sa stratégie d'allocation de l'énergie comme nous avons pu le souligner dans le chapitre 4 concernant les aspects de reproduction. C'est justement sur cette notion de déficit

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[1] même si pour la sardine les copépodes étaient moins consommés que les cladocères en 2007, les espèces de copépodes consommées aujourd'hui sont beaucoup plus petites

de réserves énergétiques, que l'on a utilisé pour faire l'hypothèse d'une surmortalité potentielle des adultes qu'il faudrait aussi travailler et qui est une limite à notre travail. En effet, aucun lien empirique entre condition corporelle et mortalité n'a été trouvé dans la littérature ou n'a pu être calculé expérimentalement durant cette thèse car il aurait fallu avoir des sardines en mésocosme (et sous différentes conditions contrôlées). Cela aurait permis d'étudier des seuils de condition 'létaux' pour chaque espèce (valeur de condition en dessous de laquelle il présente un risque de mortalité plus élevée que la moyenne). Afin de vérifier ces hypothèses, des expériences en mésocosmes ont débuté récemment sur la sardine.

Une fois la période d'acclimatation réussie, différents lots de sardine ont été soumis à des régimes alimentaires différents. Dans un premier temps, des granulés ont été distribués afin de tester l'évolution de la condition corporelle des individus. Les résultats préliminaires obtenus ont déjà mis en évidence que à taille égale dans des conditions de photopériode, de température et de salinité identiques, les sardines en bassin et nourries aux granulés ont un poids bien supérieur à celui des sardines en milieu naturel (Figure 7.7). Ce premier résultat est encourageant et permet de penser que les conditions nutritionnelles ne seraient effectivement pas optimales actuellement dans le Golfe du Lion, ne permettant pas une bonne croissance et l'établissement d'une grande quantité de réserves énergétiques chez l'anchois et la sardine.

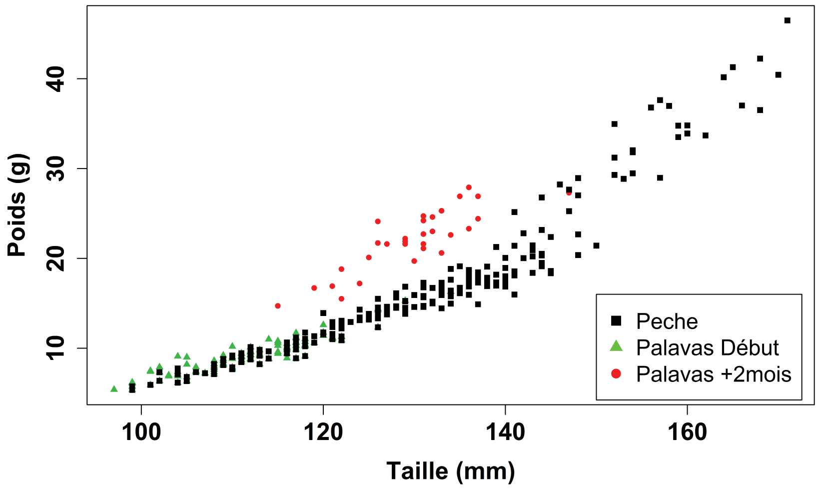


Figure 7.7 Biplot représentant l'évolution du poids en fonction de la taille pour les sardines en milieu naturel versus celles en captivité au début de l'expérimentation et après deux mois de nourrisage aux granulés.

Dans un futur proche, il serait intéressant de lancer des cultures de copépodes afin de tester l'effet de différents cocktails de copépodes sur la condition corporelle des sardines, tout en étant beaucoup plus proche de l'alimentation réelle des sardines. Ce travail permettrait de savoir si le changement de régime alimentaire aperçu au chapitre 3 (changement de dominance d'espèces de proies du zooplancton dans les contenus stomacaux) peut être responsable de l'effondrement de la condition corporelle. Au-delà du travail sur l'effet du régime alimentaire, des mesures physiologiques axées sur la bioénergétique musculaire, allant du métabolisme in vivo (utilisation de couloir de nage et de sonde à oxygène) à la bioénergétique mitochondriale (mesure de la consommation d'oxygène et de la production d'ATP par plusieurs techniques) permettraient d'estimer la balance énergétique à différents niveaux d'intégration sur ces sardines. Cela servirait pour aborder les questions écologiques avec un aspect beaucoup plus global et intégratif. De plus, un suivi régulier de la condition des individus permettrait de détecter l'existence de valeurs critiques (seuil létal) et de confirmer si oui ou non nos individus actuellement en faible condition corporelle sont dans des gammes de valeurs où ils seraient soumis à une surmortalité. L'existence d'un seuil de condition corporelle pour le déclenchement de la reproduction a été mis en évidence chez certains clupéidés (hareng, Engelhard and Heino, 2006). Une autre hypothèse à tester serait de regarder l'effet de la nourriture et de la condition sur un potentiel report de la reproduction à partir d'un certain seuil de réserves lipidiques. Pour y répondre, un projet de recherche a été soumis et cet aspect sera une partie d'une thèse commençant en Octobre 2016.

Cela permettrait ainsi de répondre plus précisément à la question de la disparition des individus les plus âgés, marquée principalement chez la sardine. Le fait d'avoir des sardines en mésocosme ouvre aussi de nouvelles perspectives concernant l'étude de plusieurs effets combinés (étude multistress) concernant la température, la densité d'individus mais aussi différents composés toxiques dont les concentrations seraient connues (PCB, métaux lourds, microplastique). Leurs conséquences sur la condition mais aussi plus largement sur la croissance et potentiellement la reproduction des individus pourront ainsi être mieux appréhendées. Concernant l'influence des contaminants, celle-ci n'a pas pu être étudiée pendant cette thèse. Sachant que le Golfe du Lion est la zone la plus polluée de Méditerranée (Harmelin-Vivien *et al.*, 2012), cet aspect pourrait être essentiel. Les effets des polluants sur les poissons peuvent être directs quand ils affectent leur physiologie (Heath, 1995) ou indirect si ils affectent le plancton qui est leur ressource alimentaire en entrainant des changements d'abondance ou même d'espèce Uriarte and Villate (2005). Peu d'études sur la bioaccumulation (augmentation de la concentration de polluants de l'environnement vers le premier niveau trophique) des polluants par le plancton ont été réalisées. Lors d'une étude portant spécifiquement sur les PCB, il a était montré que leur bioaccumulation est faible (Tiano *et al.*, 2014) et se répercuterait que faiblement sur le niveau trophique supérieur que sont les petits pélagiques. Si l'on s'intéresse à l'effet direct, les concentrations des principaux polluants (PCB, PBDE, Hg) dans la chair des petits pélagiques du Golfe du Lion sont à peine plus élevées que dans d'autres zones de Méditerranée moins polluées (Golfe de Naples, Naso *et al.*, 2005; Mer Catalane, Bocio *et al.*, 2007). Les concentrations en microplastiques ne sont pas non plus beaucoup plus élevées dans le Golfe du Lion que ailleurs en Méditerranée (Faure *et al.*, 2015). Il serait donc intéressant de regarder les cocktails de polluants, pouvant avoir un impact significatif. Même si rien n'a été analysé durant cette thèse, des échantillons de muscle et de gonades ont été prélevés sur une centaine de poissons de chaque espèce et sont destinés à être analysés dans un futur proche pour mieux comprendre l'effet des polluants sur l'écosystème pélagique du Golfe du Lion.

Lors de l'étude à l'échelle Méditerranéenne, il est dommage de noter l'absence de données provenant de pays bordant la rive Sud de la Méditerranée limitant l'étude à la rive Nord. Plusieurs études, portant sur les espèces invasives (Zenetos *et al.*, 2005) ou encore les changements d'habitats (Albouy *et al.*, 2012; Hattab *et al.*, 2014) ont montré des différences entre les deux rives, que ce soit dans la rapidité des processus ou les espèces concernées. L'absence de données sur la rive Sud limite donc les conclusions que l'on peut faire, surtout sur de potentielles différences avec ce qui est visible au Nord. Même si des similitudes semblent apparaître, avec au Sud des sardines plus petites et plus maigres sur les côtes Algériennes (Ben Smail, comm. Pers), des données sur plusieurs zones auraient permis de voir si le même phénomène avec une baisse de la condition et de la taille des individus agit comme au Nord et si oui si il existe des différences spatiales et temporelles. Cela représente un challenge pour le futur, qui en impliquant le maximum de pays du pourtour Méditerranéen permettrait de réaliser une synthèse intéressante de l'état écologique des différents stocks de petits pélagiques présents en mer Méditerranée.

Enfin, un aspect rarement considéré et étudié dans la dynamique de population en milieu marin est l'impact du parasitisme et des organismes pathogènes. Concernant les petits pélagiques, une étude a déjà mis en évidence le fait qu'un pathogène ait contribué à l'effondrement d'une population de sardine sur les côtes Australiennes (herpès-virus, Whittington *et al.*, 2008) en aggravant leur état de santé. D'autres études ont mis en évidence des mécanismes induisant des pertes de poids ou des croissances ralenties par les pathogènes (Ferrer-Maza *et al.*, 2014, 2016). Une étude préliminaire menée dans le Golfe du Lion, avec comme but la description générale et la quantification d'agents infectieux grâce à un large spectre de détection de bactérie et d'organismes pathogènes a été menée. Malgré la difficulté due à l'absence de symptômes d'alerte, justifiant la recherche générale d'organismes, cette étude a souligné la présence de coccidies (protiste parasitaire) dans les foies des sardines du Golfe du Lion. Ces coccidies sont présentes chez environ 1/3 des sardines avec une intensité d'infection très variable (allant du foie de sardine complètement rempli de coccidies à celui n'en contenant que quelques-uns). Le but ultime de ce travail sera de déterminer l'effet exact des coccidies sur les sardines, afin de savoir si celles-ci représentent un facteur de stress ou non. Une future hypothèse de travail concerne la place qu'elles occupent dans le foie, réduisant la quantité et/ou la taille des hépatocytes nécessaires à la synthèse et au stockage de glycogène. Des comparaisons avec d'autres écosystèmes (le Golfe de Gascogne et la mer Adriatique) vont être lancées et serviront de point de comparaison. Si la présence de coccidies se révèle être un cas unique pour le Golfe du Lion, il sera urgent de prendre ce paramètre en considération. En effet, le peu de connaissance sur le cycle de vie et les mécanismes d'infections par les coccidies chez les poissons soulève beaucoup de questions. Quels sont les symptômes d'une infection par ces parasites ? La sardine est-elle un porteur sain ? Est-elle l'hôte définitif ? Autant de questionnements qui vont entrainer dans un futur proche la collaboration de plusieurs domaines (parasitologie, écologie, physiologie) afin d'avancer sur la compréhension du rôle des coccidies dans la situation actuelle du Golfe du Lion.

Ce travail de thèse ayant montré l'intérêt d'étudier la condition corporelle chez les petits pélagiques, notamment pour l'estimation de la santé des populations et son lien avec la fonction de reproduction, il serait intéressant d'utiliser cette mesure de façon plus régulière et généralisée. Détecter des tendances dans la santé des populations, identifier des périodes particulières ou encore estimer si la condition sera suffisante pour le rétablissement d'un stock pourrait ainsi aider à mieux comprendre comment les stocks de poissons réagissent au changement environnemental et anthropogénique. Alors que la biomasse, l'abondance, la taille ou encore la croissance sont des critères clés déjà utilisés pour les évaluations de stocks, combiner la condition corporelle à ces paramètres pourrait améliorer les évaluations de stock et les processus de veilles et de suivis écologiques (Lloret *et al.*, 2012) et ainsi optimiser la gestion des stocks de toutes espèces exploitées. De façon générale, même si de nouvelles pistes méritent aujourd'hui d'être explorées afin d'améliorer notre connaissance de l'écosystème pélagique, ce travail a permis de faire avancer la compréhension de la dynamique de population des principaux petits pélagiques dans le golfe du Lion et en Méditerranée.

7.4 Conclusion

Ce travail de thèse a permis d'identifier deux niveaux de variation dans la communauté des poissons petits pélagiques du Golfe du Lion depuis 1993, mettant en évidence des variations inter et intraspécifique. Une modification de l'environnement et plus précisément de la productivité primaire et secondaire est responsable de l'actuelle forte abondance et biomasse du sprat et du déclin de l'anchois et de la sardine. La thèse a aussi fourni des précisions quant aux changements plus marqués chez la sardine comme décrit par Van Beveren *et al.* (2014), celle-ci allouant des réserves énergétiques vers la reproduction en hiver, quand la ressource est faible, pouvant entrainer une surmortalité. Au contraire, pour l'anchois les ressources disponibles durant la reproduction semblent être suffisantes pour allouer suffisamment d'énergie aux différents traits d'histoire de vie. De plus, la sardine semble plus sensible au réchauffement des eaux de surface que l'anchois. Les deux espèces ne sont donc pas égales face aux changements environnementaux. Des différences ont aussi été vu au niveau intraspécifique, les sardines montrant une différence de condition en fonction de l'âge contrairement à l'anchois. Enfin, la situation en Méditerranée est majoritairement tournée vers un affaiblissement de la condition corporelle pour les deux espèces, même si aucune synchronie n'a pu être établie à l'échelle du bassin entier. Même si la majorité des conclusions renforce l'hypothèse du changement de régime environnemental et donc d'un contrôle "bottom-up" de la dynamique de population des petits pélagiques, il ne faut pas oublier que les différents facteurs ne sont pas exclusifs et que la plupart agissent en synergie. En effet, l'importance des contrôles top-down peut sembler minime, mais l'effet cumulé de la pêche, de la prédation naturelle mais aussi de facteurs non considérés dans cette thèse comme les polluants pourrait avoir joué un rôle non négligable renforçant la situation créée par le changement très probable de la communauté planctonique.

Affiner les relations entre la condition corporelle des petits pélagiques et l'environnement mises en évidence durant cette thèse est encore aujourd'hui nécessaire si l'on veut à terme pouvoir inclure ce paramètre afin d'améliorer les modèles de gestion de stocks comme suggéré et démontré par Casini *et al.* (2016) avec la morue. Pour cela, on sait qu'il est primordial d'avoir un monitoring précis et fiable que ce soit pour les variables biologiques ou environnementales. L'utilisation du Fatmeter en routine pour le Golfe du Lion est ainsi un premier pas vers un suivi plus régulier et précis de la condition corporelle des petits pélagiques. De même, le développement de la partie hydrologie sur la campagne PELMED réalisée chaque année permettra d'avoir une vision globale des processus en cours au sein du Golfe du Lion à défaut d'avoir un monitoring à haute fréquence qui demanderait la mise en place d'une bouée instrumentalisée.

Cette thèse fait suite à une situation inédite constatée à la fois de la part des professionnels de la pêcherie chalutière pélagique et des scientifiques. Des explications aux préoccupations des professionnels ont donc été apportées au cours de ce travail (et plus largement au sein du projet EcoPelGol) concernant l'avenir de l'écosystème pélagique et de l'activité économique en découlant. Les changements observés étant principalement bottom-up, la difficulté réside à savoir si les conditions actuelles vont s'inscrire dans la durée ou si ce n'est qu'une période passagère. Il serait maintenant intéressant de mêler projections climatiques et adaptations physiologiques de nos espèces d'intérêt afin de pouvoir se projeter dans divers scénarios. Néanmoins, les travaux menés au cours de ces trois années soulignent l'importance de projets globaux tels que le projet EcoPelGol, qui au travers de la prise en compte d'un maximum de paramètres, a permis de grandement améliorer la compréhension de l'écosystème pélagique du Golfe du Lion.

References

Adams SM. (1999) Ecological role of lipids in the health and success of fish populations. *In* Lipids in Freshwater Ecosystems, edited by Arts MT, Wainman BC. Springer New York. pp. 132–160

Adloff F, Somot S, Sevault F, Jordà G, Aznar R, Déqué M, Herrmann M et al. (2015) Mediterranean Sea response to climate change in an ensemble of twenty first century scenarios. Climate Dynamics 45:2775–2802. doi: 10.1007/s00382-015-2507-3

Agostini VN, Bakun A. (2002) 'Ocean triads' in the Mediterranean Sea: physical mechanisms potentially structuring reproductive habitat suitability (with example application to European anchovy, *Engraulis encrasicolus*). Fisheries Oceanography 11:129–142. doi: 10.1046/j.1365-2419.2002.00201.x

Albouy C, Guilhaumon F, Araújo MB, Mouillot D, Leprieur F. (2012) Combining projected changes in species richness and composition reveals climate change impacts on coastal Mediterranean fish assemblages. Global Change Biology 18:2995–3003. doi: 10.1111/j.1365-2486.2012.02772.x

Albouy C, Guilhaumon F, Leprieur F, Lasram FBR, Somot S, Aznar R, Velez L et al. (2013) Projected climate change and the changing biogeography of coastal Mediterranean fishes. Journal of Biogeography 40:534–547. doi: 10.1111/jbi.12013

Aldebert Y, Tournier H. (1971) La reproduction de la sardine et de l'anchois dans le Golfe du Lion. Revue des Travaux de l'Institut des Pêches Maritimes 35:57–75

Alheit J, Niquen M. (2004) Regime shifts in the Humboldt Current ecosystem. Progress in Oceanography 60:201–222. doi: 10.1016/j.pocean.2004.02.006

Allison EH, Perry AL, Badjeck MC, Neil Adger W, Brown K, Conway D, Halls AS et al. (2009) Vulnerability of national economies to the impacts of climate change on fisheries. Fish and Fisheries 10:173–196. doi: 10.1111/j.1467-2979.2008.00310.x

Alm G. (1959) Connection between maturity, size, and age in fishes. Report from the Institute of Freshwater Research, Drottingholm 40:5–145

Alvain S, Moulin C, Dandonneau Y, Bréon FM. (2005) Remote sensing of phytoplankton groups in case 1 waters from global SeaWiFS imagery. Deep Sea Research Part I: Oceanographic Research Papers 52:1989–2004. doi: 10.1016/j.dsr.2005.06.015

André G, Garreau P, Garnier V, Fraunié P. (2005) Modelled variability of the sea surface circulation in the North-western Mediterranean Sea and in the Gulf of Lions. Ocean Dynamics 55:294–308. doi: 10.1007/s10236-005-0013-6

Artioli Y, Friedrich J, Gilbert AJ, McQuatters-Gollop A, Mee LD, Vermaat JE, Wulff F et al. (2008) Nutrient budgets for European seas: A measure of the effectiveness of nutrient reduction policies. Marine Pollution Bulletin 56:1609–1617. doi: 10.1016/j.marpolbul.2008.05.027

Audzijonyte A, Kuparinen A, Gorton R, Fulton EA. (2013) Ecological consequences of body size decline in harvested fish species: positive feedback loops in trophic interactions amplify human impact. Biology Letters 9:20121103. doi: 10.1098/rsbl.2012.1103

Auger PA, Diaz F, Ulses C, Estournel C, Neveux J, Joux F, Pujo-Pay M et al. (2011) Functioning of the planktonic ecosystem on the Gulf of Lions shelf (NW Mediterranean) during spring and its impact on the carbon deposition: a field data and 3-D modelling combined approach. Biogeosciences 8:3231–3261. doi: 10.5194/bg-8-3231-2011

Auger PA, Ulses C, Estournel C, Stemmann L, Somot S, Diaz F. (2014) Interannual control of plankton communities by deep winter mixing and prey/predator interactions in the NW Mediterranean: Results from a 30-year 3D modeling study. Progress in Oceanography 124:12–27. doi: 10.1016/j.pocean.2014.04.004

Ayón P, Swartzman G, Espinoza P, Bertrand A, Ayon P, Swartzman G, Espinoza P et al. (2011) Long-term changes in zooplankton size distribution in the Peruvian Humboldt Current System: conditions favouring sardine or anchovy. Marine Ecology Progress Series 422:211–222. doi: 10.3354/meps08918

Baklouti M, Diaz F, Pinazo C, Faure V, Quéguiner B. (2006a) Investigation of mechanistic formulations depicting phytoplankton dynamics for models of marine pelagic ecosystems and description of a new model. Progress in Oceanography 71:1–33. doi: 10.1016/j.pocean.2006.05.002

Baklouti M, Faure V, Pawlowski L, Sciandra A. (2006b) Investigation and sensitivity analysis of a mechanistic phytoplankton model implemented in a new modular numerical tool (Eco3M) dedicated to biogeochemical modelling. Progress in Oceanography 71:34–58. doi: 10.1016/j.pocean.2006.05.003

Bakun A. (1997) Patterns in the ocean: ocean processes and marine population dynamics. California Sea Grant, La Jolla, CA, and Centro de Investigaciones Biologicas del Noroeste, La Paz, BCS, Mexico 17:1945–1946. doi: 10.1016/S0278-4343(97)00037-X

Ballón M, Wosnitza-Mendo C, Guevara-Carrasco R, Bertrand A. (2008) The impact of overfishing and El Niño on the condition factor and reproductive success of Peruvian hake, *Merluccius gayi peruanus*. Progress in Oceanography 79:300–307. doi: 10.1016/j.pocean.2008.10.016

Banaru D, Carlotti F, Barani A, Grégori G, Neffati N, Harmelin-Vivien M, BÄnaru D et al. (2014) Seasonal variation of stable isotope ratios of size-fractionated zooplankton in the Bay of Marseille (NW Mediterranean Sea). Journal of Plankton Research 36:145–156. doi: 10.1093/plankt/fbt083

Banaru D, Mellon-Duval C, Roos D, Bigot JLL, Souplet A, Jadaud A, Beaubrun P et al. (2013) Trophic structure in the Gulf of Lions marine ecosystem (North-Western Mediterranean Sea) and fishing impacts. Journal of Marine Systems 111-112:45–68. doi: 10.1016/j.jmarsys.2012.09.010

Barange M, Coetzee JC, Twatwa NM. (2005) Strategies of space occupation by anchovy and sardine in the southern Benguela: The role of stock size and intra-species competition. ICES Journal of Marine Science 62:645–654. doi: 10.1016/j.icesjms.2004.12.019

Baron JP, Le Galliard JF, Ferrere R, Tully T. (2013) Intermittent breeding and the dynamics of resource allocation to reproduction, growth and survival. Functional Ecology 27:173–183. doi: 10.1111/1365-2435.12023

Barton BA, Morgan JD, Vljayan M. (2002) Physiological and condition-related indicators of environmental stress in fish. 656 pp.

Basilone G, Bonanno A, Patti B, Mazzola S, Barra M, Cuttitta A, Mcbride R. (2013) Spawning site selection by European anchovy (*Engraulis encrasicolus*) in relation to oceanographic conditions in the Strait of Sicily. Fisheries Oceanography 22:309–323. doi: 10.1111/fog.12024

Basilone G, Guisande C, Patti B, Mazzola S, Cuttitta A, Bonanno A, Vergara AR et al. (2006) Effect of habitat conditions on reproduction of the European anchovy

(*Engraulis encrasicolus*) in the Strait of Sicily. Fisheries Oceanography 15:271–280. doi: 10.1111/j.1365-2419.2005.00391.x

Batchelder HP, Mackas DL, O'Brien TD. (2012) Spatial-temporal scales of synchrony in marine zooplankton biomass and abundance patterns: a world-wide comparison. Progress in Oceanography 97-100:15–30. doi: 10.1016/j.pocean.2011.11.010

Baumgartner T, Soutar A, Riedel W. (1992) Natural time scales of variability in coastal pelagic fish populations of the California Current over the past 1500 years: Response to global climate change and biological interaction. California Sea Grant Rep. for 1995:31–37

Beamish R, McFarlane G, Benson A. (2006) Longevity overfishing. Progress in Oceanography 68:289–302. doi: 10.1016/j.pocean.2006.02.005

Beaugrand G, Brander KM, Alistair Lindley J, Souissi S, Reid PC. (2003) Plankton effect on cod recruitment in the North Sea. Nature 426:661–664. doi: 10.1038/nature02164

Bellido JM, Brown AM, Valavanis VD, Giráldez A, Pierce GJ, Iglesias M, Palialexis A. (2008) Identifying essential fish habitat for small pelagic species in Spanish Mediterranean waters. Hydrobiologia 612:171–184. doi: 10.1007/s10750-008-9481-2

Ben Rais Lasram F, Guilhaumon F, Albouy C, Somot S, Thuiller W, Mouillot D. (2010) The Mediterranean Sea as a 'cul-de-sac' for endemic fishes facing climate change. Global Change Biology 16:3233–3245. doi: 10.1111/j.1365-2486.2010.02224.x

Berkeley SA, Hixon Ma, Larson RJ, Love MS. (2004) Fisheries Sustainability via protection of age structure and spatial distribution of fish populations. American Fisheries Society 29:23–32. doi: 10.1577/1548-8446(2004)29[23:FSVPOA]2.0.CO;2

Bervoets L, Blust R. (2003) Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: Relationship with fish condition factor. Environmental Pollution 126:9–19. doi: 10.1016/S0269-7491(03)00173-8

Bethoux JPP, Gentili B, Raunet J, Tailliez D. (1990) Warming trend in the Western Mediterranean deep water. Nature 347:660–662. doi: 10.1038/347660a0

Bêty J, Gauthier G, Giroux JF. (2003) Body condition, migration, and timing of reproduction in snow geese: a test of the condition dependent model of optimal clutch size. The American Naturalist 162:110–121. doi: 10.1086/375680

Bianchi G, Gislason H, Graham K, Hill L, Jin X, Koranteng K, S. Manickchand-Heileman IP et al. (2000) Impact of fishing on size composition and diversity of demersal fish communities. ICES Journal of Marine Science 57:558–571

Blackwell BG, Brown ML, Willis DW. (2000) Relative weight (Wr) status and current use in fisheries assessment and management. Reviews in Fisheries Science 8:1–44. doi: 10.1080/10641260091129161

Blanchard JL, Jennings S, Holmes R, Harle J, Merino G, Allen JI, Holt J et al. (2012) Potential consequences of climate change for primary production and fish production in large marine ecosystems. Philosophical transactions of the Royal Society of London. Series B, Biological Sciences 367:2979–89. doi: 10.1098/rstb.2012.0231

Blanckenhorn WU. (1999) Different growth responses to temperature and resource limitation in three fly species with similar life histories. Evolutionary Ecology 13:395–409. doi: 10.1023/A:1006741222586

Bligh EG, Dyer WJ. (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911–917. doi: 10.1139/o59-099

Blums P, Clark RG, Mednis A. (2002) Patterns of reproductive effort and success in birds: path analyses of long-term data from European ducks. Journal of Animal Ecology 71:280–295. doi: 10.1046/j.1365-2656.2002.00598.x

Bocio A, Domingo JL, Falco G, Llobet JM. (2007) Concentrations of PCDD/PCDFs and PCBs in fish and seafood from the Catalan (Spain) market: Estimated human intake. Environment International 33:170–175. doi: 10.1016/j.envint.2006.09.005

Bøhn T, Sandlund OT, Amundsen PA, Primicerio R. (2004) Rapidly changing life history during invasion. Oikos 106:138–150. doi: 10.1111/j.0030-1299.2004.13022.x

Bolger T, Connolly PL. (1989) The selection of suitable indices for the measurement and analysis of fish condition. Journal of Fish Biology 34:171–182. doi: 10.1111/j.1095-8649.1989.tb03300.x

Bonanno A, Barra M, Basilone G, Genovese S, Rumolo P, Goncharov S, Popov S et al. (2016) Environmental processes driving anchovy and sardine distribution in a highly variable environment: the role of the coastal structure and riverine input. Fisheries Oceanography 25:471–490. doi: 10.1111/fog.12166

Bonanno A, Giannoulaki M, Barra M, Basilone G, Machias A, Genovese S, Goncharov S et al. (2014) Habitat selection response of small pelagic fish in different environments. Two examples from the oligotrophic Mediterranean Sea. PLoS ONE 9:e101498. doi: 10.1371/journal.pone.0101498

Bonanno A, Zgozi S, Cuttitta A, El Turki A, Di Nieri A, Ghmati H, Genovese S. (2013) Influence of environmental variability on anchovy early life stages (*Engraulis encrasicolus*) in two different areas of the Central Mediterranean Sea. Hydrobiologia 701:273–287

Borme D, Tirelli V, Brandt SB, Fonda Umani S, Arneri E. (2009) Diet of *Engraulis encrasicolus* in the northern Adriatic Sea (Mediterranean): ontogenetic changes and feeding selectivity. Marine Ecology Progress Series 392:193–209. doi: 10.3354/meps08214

Bosc E. (2004) Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from 4 years of SeaWiFS observations. Global Biogeochemical Cycles 18:1–17. doi: 10.1029/2003GB002034

Brander KM. (2007) Global fish production and climate change. Proceedings of the National Academy of Sciences of the United States of America 104:19709–19714. doi: 10.1073/pnas.0702059104

Breiman L, Friedman JH, Olshen R, Stone CJ. (1984) Classification and regression trees. Chapman and Hall, London

Brett JR. (1979) Environmental factors and growth. Fish physi Edition. No. 8 in Fish physiology, (eds W.S. Hoar D.J. Randall), Academic Press, New York. pp. 599–675

Brooks S, Tyler CR, Sumpter JP. (1997) Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7:387–416. doi: 10.1023/A:1018400130692

Brosset P, Fromentin JM, Ménard F, Pernet F, Bourdeix JH, Bigot JL, Van Beveren E et al. (2015a) Measurement and analysis of small pelagic fish condition: a suitable method for rapid evaluation in the field. Journal of Experimental Marine Biology and Ecology 462:90–97. doi: 10.1016/j.jembe.2014.10.016

Brosset P, Le Bourg B, Costalago D, Banaru D, Van Beveren E, Bourdeix J, Fromentin J et al. (2016) Linking small pelagic dietary shifts and ecosystem changes in the Gulf of Lions. Marine Ecology Progress Series 554:157–171. doi: 10.3354/meps11796

Brosset P, Ménard F, Fromentin JM, Bonhommeau S, Ulses C, Bourdeix JH, Bigot JL et al. (2015b) Influence of environmental variability and age on the body condition of small pelagic fish in the Gulf of Lions. Marine Ecology Progress Series 529:219–231. doi: 10.3354/meps11275

Brown DR, Sherry TW. (2006) Food supply controls the body condition of a migrant bird wintering in the tropics. Oecologia 149:22–32. doi: 10.1007/s00442-006-0418-z

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. (2004) Toward a metabolic theory of ecology. Ecology 85:1771–1789

Brown ML, Murphy BR. (2004) Seasonal dynamics of direct and indirect condition indices in relation to energy allocation in largemouth bass *Micropterus salmoides* (Lacèpede). Ecology of Freshwater Fish 13:23–36. doi: 10.1111/j.0906-6691.2004.00031.x

Brown-Peterson NJ, Wyanski DM, Saborido-Rey F, Macewicz BJ, Lowerre-Barbieri SK. (2011) A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries 3:52–70. doi: 10.1080/19425120.2011.555724

Bull JJ, Shine R. (1979) Iteroparous animals that skip opportunities for reproduction. The American Naturalist 114:296–303. doi: 10.2307/2678832

Calvo E, Simo R, Coma R, Ribes M, Pascual J, Sabatés A, Gili JM et al. (2011) Effects of climate change on Mediterranean marine ecosystems: The case of the Catalan Sea. Climate Research 50:1–29. doi: 10.3354/cr01040

Campañone LA, Salvadori VO, Mascheroni RH. (2001) Weight loss during freezing and storage of unpackaged foods. Journal of Food Engineering 47:69–79. doi: 10.1016/S0260-8774(00)00101-1

Cardona L, Martinez-Inigo L, Mateo R, Gonzalez-Soles J. (2015) The role of sardine as prey for pelagic predators in the western Mediterranean Sea assessed using stable isotopes and fatty acids. Marine Ecology Progress Series 531:1–14. doi: 10.3354/meps11353

Carpi P, Martinelli M, Belardinelli A, Russo A, Arneri E, Coluccelli A, Santojanni A. (2015) Coupling an oceanographic model to a Fishery Observing System through mixed models: the importance of fronts for anchovy in the Adriatic Sea. Fisheries Oceanography 24:521–532. doi: 10.1111/fog.12126

Casini M, Eero M, Carlshamre S, Lövgren J. (2016) Using alternative biological information in stock assessment: condition-corrected natural mortality of Eastern Baltic cod. ICES Journal of Marine Science 73:fsw117. doi: 10.1093/icesjms/fsw117

Casini M, Kornilovs G, Cardinale M, Mollmann C, Grygiel W, Jonsson P, Raid T et al. (2011) Spatial and temporal density dependence regulates the condition of central Baltic Sea clupeids: Compelling evidence using an extensive international acoustic survey. Population Ecology 53:511–523. doi: 10.1007/s10144-011-0269-2

Caut S, Roemer GW, Donlan CJ, Courchamp F. (2006) Coupling stable isotopes with bioenergetics to estimate interspecific interactions. Ecological Applications 16:1893–1900. doi: 10.1890/1051-0761(2006)016[1893:CSIWBT]2.0.CO;2

Chastel O, Weimerskirch H, Jouventin P. (1995) Body condition and seabird reproductive performance: A study of three petrel species. Ecology 76:2240–2246. doi: 10.2307/1941698

Chavez FP, Ryan J, Lluch-Cota SE, C MÑ. (2003) From anchovies to sardines and back: multidecadal change in the Pacific Ocean. Science 299:217–221. doi: 10.1126/science.1075880

Cherel Y, Robin JP, Heitz A, Calgari C, le Maho Y. (1992) Relationships between lipid availability and protein utilization during prolonged fasting. Journal of Comparative Physiology B 162:305–313. doi: 10.1007/BF00260757

Cole J, Mcglade J. (1998) Clupeoid population variability, the environment and satellite imagery in coastal upwelling systems. Reviews in Fish Biology and Fisheries 8:445–471. doi: 10.1023/A:1008861224731

Colebrook JM. (1978) Continuous plankton records-zooplankton and environment, northeast Atlantic and North-Sea, 1948-1975. Oceanologica Acta 1:9–23

Coleman SW, Jones AG. (2011) Patterns of multiple paternity and maternity in fishes. Biological Journal of the Linnean Society 103:735–760. doi: 10.1111/j.1095-8312.2011.01673.x

Coles SL, Jokiel PL. (1978) Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. Marine Biology 49:187–195. doi: 10.1007/BF00391130

Coll M, Libralato S. (2012) Contributions of food web modelling to the ecosystem approach to marine resource management in the Mediterranean Sea. Fish and Fisheries 13:60–88. doi: 10.1111/j.1467-2979.2011.00420.x

Coll M, Piroddi C, Albouy C, Ben Rais Lasram F, Cheung WWL, Christensen V, Karpouzi VS et al. (2012) The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. Global Ecology and Biogeography 21:465–480. doi: 10.1111/j.1466-8238.2011.00697.x

Colloca F, Cardinale M, Maynou F, Giannoulaki M, Scarcella G, Jenko K, Bellido JM et al. (2013) Rebuilding Mediterranean fisheries: a new paradigm for ecological sustainability. Fish and Fisheries 14:89–109

Cone RS. (1989) The need to reconsider the use of condition indices in fishery science. Transactions of the American Fisheries Society 118:510–514. doi: 10.1577/1548-8659(1989)118<0511:TNTRTU>2.3.CO;2

Cooke SJ, Crossin GT, Patterson DA, English KK, Hinch SG, Young JL, Alexander RF et al. (2005) Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon: Development and validation of a technique. Journal of Fish Biology 67:1342–1358. doi: 10.1111/j.1095-8649.2005.00830.x

Cooper WT, Barbieri LR, Murphy MD, Lowerre-Barbieri SK. (2013) Assessing stock reproductive potential in species with indeterminate fecundity: Effects of age truncation and size-dependent reproductive timing. Fisheries Research 138:31–41. doi: 10.1016/j.fishres.2012.05.016

Cortés E. (1997) A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. Canadian Journal of Fisheries and Aquatic Sciences 54:726–738. doi: 10.1139/cjfas-54-3-726

Costalago D, Navarro J, Álvarez-Calleja I, Palomera I. (2012) Ontogenetic and seasonal changes in the feeding habits and trophic levels of two small pelagic fish species. Marine Ecology Progress Series 460:169–181. doi: 10.3354/meps09751

Costalago D, Palomera I. (2014) Feeding of European pilchard (*Sardina pilchardus*) in the northwestern Mediterranean: from late larvae to adults. Scientia Marina 78:41–54. doi: 10.3989/scimar.03898.06D

Costalago D, Palomera I, Tirelli V. (2014) Seasonal comparison of the diets of juvenile European anchovy *Engraulis encrasicolus* and sardine *Sardina pilchardus* in the Gulf of Lions. Journal of Sea Research 89:64–72. doi: 10.1016/j.seares.2014.02.008

Cox RM, Parker EU, Cheney DM, Liebl AL, Martin LB, Calsbeek R. (2010) Experimental evidence for physiological costs underlying the trade off between reproduction and survival. Functional Ecology 24:1262–1269

Craig JF, Kenley MJ, Talling JF. (1978) Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxidation and proximate analysis. Freshwater Biology 8:585–590. doi: 10.1111/j.1365-2427.1978.tb01480.x

Crain CM, Kroeker K, Halpern BS. (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters 11:1304–1315. doi: 10.1111/j.1461-0248.2008.01253.x

Cucherousset J, Villéger S. (2015) Quantifying the multiple facets of isotopic diversity: new metrics for stable isotope ecology. Ecological Indicators 56:152–160. doi: 10.1016/j.ecolind.2015.03.032

Curio E. (1983) Why do young birds reproduce less well? Ibis 125:400–404. doi: 10.1111/j.1474-919X.1983.tb03130.x

Cury P, Bakun A, Crawford R, Jarre A, Quiñones R, Shannon L, Verheye H. (2000) Small pelagics in upwelling systems: patterns of interaction and structural changes in wasp-waist ecosystems. ICES Journal of Marine Science 57:603–618. doi: 10.1006/jmsc.2000.0712

Cury P, Shannon L. (2004) Regime shifts in upwelling ecosystems: Observed changes and possible mechanisms in the northern and southern Benguela. Progress in Oceanography 60:223–243. doi: 10.1016/j.pocean.2004.02.007

Cury PM, Boyd IL, Bonhommeau S, Anker-Nilssen T, Crawford RJM, Furness RW, Mills JA et al. (2011) Global seabird response to forage fish depletion: One-Third for the birds. Science 334:1703–1706. doi: 10.1126/science.1212928

Cushing DH. (1990) Plankton production and year class strength in fish populations: an update of the match mismatch hypothesis. Advances in Marine Biology 26:249–293

Cushing DH, Dickson RR. (1976) The biological response in the sea to climatic changes. *In* Advances in Marine Biology. Volume 1. pp. 1–122

Darling ES, Côté IM. (2008) Quantifying the evidence for ecological synergies. Ecology Letters 11:1278–1286. doi: 10.1111/j.1461-0248.2008.01243.x

Darnaude AM, Salen-Picard C, Polunin NVC, Harmelin-Vivien ML. (2004) Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138:325–332. doi: 10.1007/s00442-003-1457-3

Daskalov GM, Mamedov EV. (2007) Integrated fisheries assessment and possible causes for the collapse of anchovy kilka in the Caspian Sea. ICES Journal of Marine Science 64:503–511. doi: 10.1093/icesjms/fsl047

Davidson D, Marshall CT. (2010) Are morphometric indices accurate indicators of stored energy in herring *Clupea harengus*? Journal of Fish Biology 76:913–929. doi: 10.1111/j.1095-8649.2010.02546.x

de Puelles MLF, Molinero JC. (2008) Decadal changes in hydrographic and ecological time-series in the Balearic Sea (western Mediterranean), identifying links between climate and zooplankton. ICES Journal of Marine Science 65:311–317. doi: 10.1093/icesjms/fsn017

de Puelles MLF, Valencia J, Vicente L. (2004) Zooplankton variability and climatic anomalies from 1994 to 2001 in the Balearic Sea (Western Mediterranean). ICES Journal of Marine Science 61:492–500. doi: 10.1016/j.icesjms.2004.03.026

De Roos AM, Persson L, McCauley E. (2003) The influence of size-dependent life-history traits on the structure and dynamics of populations and communities. Ecology Letters 6:473–487. doi: 10.1046/j.1461-0248.2003.00458.x

DeNiro MJ, Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506. doi: 10.1016/0016-7037(78)90199-0

Dombrowski P, Bourgeois JC, Couture R, Linard C. (2003) Estimation of carcass fat and protein in Northern pintails (*Anas Acuta*) during spring migration. Journal of Wildlife Diseases 39:620–626. doi: 10.7589/0090-3558-39.3.620

Drinkwater KF, Beaugrand G, Kaeriyama M, Kim S, Ottersen G, Perry RI, Pörtner HO et al. (2010) On the processes linking climate to ecosystem changes. Journal of Marine Systems 79:374–388. doi: 10.1016/j.jmarsys.2008.12.014

Dulvy NKN, Rogers SISSI, Jennings S, Stelzenmüller V, Dye SSR, Skjoldal HRH, Stelzenmuller V et al. (2008) Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. Journal of Applied Ecology 45:1029–1039

Dumont HJ, Van de Velde I, Dumont S. (1975) The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 19:75–97. doi: 10.1007/BF00377592

Dutil JD, Lambert Y. (2000) Natural mortality from poor condition in Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 57:826–836. doi: 10.1139/f00-023

Dygert PH. (1990) Seasonal changes in energy content and proximate composition associated with somatic growth and reproduction in a representative age-class of female English sole. Transactions of the American Fisheries Society 119:791–801. doi: 10.1577/1548-8659(1990)119<0791:SCIECA>2.3.CO;2

Edwards M, Richardson A. (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 430:881–884. doi: 10.1038/nature02808

Engelhard G, Heino M. (2006) Climate change and condition of herring (*Clupea harengus*) explain long-term trends in extent of skippend reproduction. Oecologia 149:593–603. doi: 10.1017/s00442-006-0483-3

Espinasse B, Carlotti F, Zhou M, Devenon JL. (2014a) Defining zooplankton habitats in the Gulf of Lion (NW Mediterranean Sea) using size structure and environmental conditions. Marine Ecology Progress Series 506:31–46. doi: 10.3354/meps10803

Espinasse B, Harmelin-Vivien M, Tiano M, Guilloux L, Carlotti F. (2014b) Patterns of variations in C and N stable isotope ratios in size-fractionated zooplankton in the Gulf of Lion, NW Mediterranean Sea. Journal of Plankton Research 36:1204–1215. doi: 10.1093/plankt/fbu043

FAO. (2011) Review of the state of world marine fishery resources. FAO Fisheries and Aquaculture Department, Rome, Italy. 334 pp.

FAO. (2014) The state of world Fisheries and Aquaculture (SOFIA) 2014. 200 pp.

Faure F, Saini C, Potter G, Galgani F, de Alencastro LF, Hagmann P. (2015) An evaluation of surface micro- and mesoplastic pollution in pelagic ecosystems of the Western

Mediterranean Sea. Environmental Science and Pollution Research 22:12190–12197. doi: 10.1007/s11356-015-4453-3

Ferrer-Maza D, Lloret J, Muñoz M, Faliex E, Vila S, Sasal P. (2014) Parasitism, condition and reproduction of the European hake (*Merluccius merluccius*) in the northwestern Mediterranean Sea. ICES Journal of Marine Science 71:1088–1099. doi: 10.1093/icesjms/fst217

Ferrer-Maza D, Lloret J, Muñoz M, Vila S, Faliex B, Sasal P. (2016) Links between parasitism, energy reserves and fecundity of European anchovy, *Engraulis encrasicolus*, in the northwestern Mediterranean Sea. Conservation Physiology 4:1–13

Finney BP, Gregory-Eaves I, Douglas MSV, Smol JP. (2002) Fisheries productivity in the northeastern Pacific Ocean over the past 2,200 years. Nature 416:729–733. doi: 10.1038/416729a

Fitzhugh GR, Wuenschel MJ, McBride RS. (2010) Evaluation of bioelectrical impedance analysis (BIA) to measure condition and energy allocated to reproduction in marine fishes. Journal of Physics: Conference Series 224:12137. doi: 10.1088/1742-6596/224/1/012137

Folch J, Lees M, Stanley GHS. (1957) A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497–509

Fraser AJ. (1989) Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Canadian Journal of Fisheries and Aquatic Sciences 46:1868–1873. doi: 10.1139/f89-235

Froese R. (2006) Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology 22:241–253. doi: 10.1111/j.1439-0426.2006.00805.x

Fromentin JM, Bonhommeau S, Arrizabalaga H, Kell L. (2014) The spectre of uncertainty in management of exploited fish stocks: the illustrative case of Atlantic bluefin tuna. Marine Policy 47:8–14

Galil BS. (2009) Taking stock: inventory of alien species in the Mediterranean Sea. Biological Invasions 11:359–372

Ganias K. (2009) Linking sardine spawning dynamics to environmental variability. Estuarine, Coastal and Shelf Science 84:402–408. doi: 10.1016/j.ecss.2009.07.004

Ganias K. (2014) Biology and ecology of sardines and anchovies. CRC Press. 394 pp.

Ganias K, Nunes C, Stratoudakis Y. (2007a) Degeneration of postovulatory follicles in the Iberian sardine *Sardina pilchardus*: Structural changes and factors affecting resorption. Fishery Bulletin 105:131–139

Ganias K, Somarakis S, Koutsikopoulos C, Machias A. (2007b) Factors affecting the spawning period of sardine in two highly oligotrophic Seas. Marine Biology 151:1559–1569. doi: 10.1007/s00227-006-0601-0

Ganias K, Somarakis S, Machias A, Theodorou A. (2004) Pattern of oocyte development and batch fecundity in the Mediterranean sardine. Fisheries Research 67:13–23. doi: 10.1016/j.fishres.2003.08.008

Ganias K, Somarakis S, Machias A, Theodorou AJ. (2003) Evaluation of spawning frequency in a Mediterranean sardine population (*Sardina pilchardus sardina*). Marine Biology 142:1169–1179. doi: 10.1007/s00227-003-1028-5

Garcia A, Cortes D, Ramirez T. (1998) Daily larval growth and RNA and DNA content of the NW Mediterranean anchovy *Engraulis encrasicolus* and their relations to the environment. Marine Ecology Progress Series 166:237–245

García A, Palomera I. (1996) Anchovy early life history and its relation to its surrounding environment in the Western Mediterranean basin. Scientia Marina 60:155–166

García-Berthou E, Carmona-Catot G, Merciai R, Ogle DH. (2012) A technical note on seasonal growth models. Reviews in Fish Biology and Fisheries 22:635–640

Garrido S, Ben-Hamadou R, Oliveira PB, Cunha ME, Chicharo MA, Van Der Lingen CD. (2008) Diet and feeding intensity of sardine *Sardina pilchardus*: Correlation with satellite-derived chlorophyll data. Marine Ecology Progress Series 354:245–256. doi: 10.3354/meps07201

GFCM. (2014) Report of the working group on stock assessment of small pelagic species. 258 pp.

GFCM. (2015) Report of the working group on stock asessment of small pelagic species (WGSASP). 82 pp.

Giannoulaki M, Iglesias M, Tugores MP, Bonanno A, Patti B, De Felice A, Leonori I et al. (2012) Characterizing the potential habitat of European anchovy *Engraulis encrasicolus* in the Mediterranean Sea, at different life stages. Fisheries Oceanography 22:69–89. doi: 10.1111/fog.12005

Giannoulaki M, Machias A, Somarakis S, Tsimenides N. (2005) The spatial distribution of anchovy and sardine in the northern Aegean Sea in relation to hydrographic regimes. Belgian Journal of Zoology 135:151–156

Giorgi F. (2006) Climate change hot-spots. Geophysical Research Letters 33:L08707. doi: 10.1029/2006GL025734

Goñi N, Arrizabalaga H. (2010) Seasonal and interannual variability of fat content of juvenile albacore (*Thunnus alalunga*) and bluefin (*Thunnus thynnus*) tunas during their feeding migration to the Bay of Biscay. Progress in Oceanography 86:115–123. doi: 10.1016/j.pocean.2010.04.016

Goodman MN, Larsen PR, Kaplan MM, Aoki TT, Young VR, Ruderman NB. (1980) Starvation in the rat. Effect of age and obesity on protein sparing and fuel metabolism. The American Journal of Physiology 239:277–286

Green AJ. (2001) Mass/length residuals: measures of body condition or generators of spurious results. Ecology 82:1473–1483. doi: 10.1890/0012-9658(2001)082[1473:MLRMOB]2.0.CO;2

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF et al. (2008) A global map of human impact on marine ecosystems. Science 319:948–952. doi: 10.1126/science.1149345

Halsband-Lenk C, Hirche HJ, Carlotti F. (2002) Temperature impact on reproduction and development of congener copepod populations. Journal of Experimental Marine Biology and Ecology 271:121–153. doi: 10.1016/S0022-0981(02)00025-4

Hamel S, Gaillard JM, Yoccoz NG, Loison A, Bonenfant C, Descamps S. (2010) Fitness costs of reproduction depend on life speed: empirical evidence from mammalian populations. Ecology Letters 13:915–935. doi: 10.1111/j.1461-0248.2010.01478.x

Hardin G. (1960) The competitive exclusion principle. Science 131:1292–1297

Harmelin-Vivien M, Bitar G, Harmelin, Jean-Georges Monestiez P. (2005) The littoral fish community of the Lebanese rocky coast (eastern Mediterranean Sea) with emphasis on Red Sea immigrants. Biological Invasions 7:625–637

Harmelin-Vivien M, Bodiguel X, Charmasson S, Loizeau V, Mellon-Duval C, Tronczyński J, Cossa D. (2012) Differential biomagnification of PCB, PBDE, Hg and Radiocesium in the food web of the European hake from the NW Mediterranean. Marine Pollution Bulletin 64:974–983

Harmelin-Vivien M, Loizeau V, Mellon C, Beker B, Arlhac D, Bodiguel X, Ferraton F et al. (2008) Comparison of C and N stable isotope ratios between surface particulate organic matter and microphytoplankton in the Gulf of Lions (NW Mediterranean). Continental Shelf Research 28:1911–1919. doi: 10.1016/j.csr.2008.03.002

Hattab T, Albouy C, Lasram FBR, Somot S, Le Loc'h F, Leprieur F. (2014) Towards a better understanding of potential impacts of climate change on marine species distribution: A multiscale modelling approach. Global Ecology and Biogeography 23:1417–1429. doi: 10.1111/geb.12217

Heath AG. (1995) Water Pollution and Fish Physiology. *In* Water Pollution and Fish Physiology. pp. 67–78

Heino M, Godø OR. (2002) Fisheries-induced selection pressures in the context of sustainable fisheries. Bulletin of Marine Science 70:639–656

Herrmann M, Diaz F, Estournel C, Marsaleix P, Ulses C. (2013) Impact of atmospheric and oceanic interannual variability on the Northwestern Mediterranean Sea pelagic planktonic ecosystem and associated carbon cycle. Journal of Geophysical Research: Oceans 118:5792–5813. doi: 10.1002/jgrc.20405

Herrmann M, Diaz F, Estournel C, Marsaleix P, Ulses C, Adloff F, Diaz F. (2014) Impact of climate change on the northwestern Mediterranean Sea pelagic planktonic ecosystem and associated carbon cycle. Journal of Geophysical Research: Oceans 119:5815–5836. doi: 10.1002/jgrc.20405

Herrmann M, Sevault F, Beuvier J, Somot S. (2010) What induced the exceptional 2005 convection event in the northwestern Mediterranean basin? Answers from a modeling study. Journal of Geophysical Research: Oceans 115:C12051. doi: 10.1029/2010JC006162

Herrmann MJ, Somot S. (2008) Relevance of ERA40 dynamical downscaling for modeling deep convection in the Mediterranean Sea. Geophysical Research Letters 35:L04607. doi: 10.1029/2007GL032442

Hiddink JG, Jennings S, Kaiser MJ. (2005) Do haddock select habitats to maximize condition? Journal of Fish Biology 67:111–124

Hill MO. (1973) Diversity and evenness: a unifying notation and its consequences. Ecology 54:427–432. doi: 10.2307/1934352

Hinder SL, Gravenor MB, Edwards M, Ostle C, Bodger OG, Lee PLM, Walne AW et al. (2014) Multi-decadal range changes vs. thermal adaptation for north east Atlantic oceanic copepods in the face of climate change. Global Change Biology 20:140–146. doi: 10.1111/gcb.12387

Hirshfield MF, Tinkle DW. (1975) Natural selection and the evolution of reproductive effort. Proceedings of the National Academy of Sciences 72:2227–2231

Hobson KA. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326. doi: 10.1007/s004420050865

Hoegh-Guldberg O, Bruno JF. (2010) The impact of climate change on the world's marine ecosystems. Science 328:1523–1528. doi: 10.1126/science.1189930

Hsieh Ch, Reiss CS, Hunter JR, Beddington JR, May RM, Sugihara G. (2006) Fishing elevates variability in the abundance of exploited species. Nature 443:859–862. doi: 10.1038/nature05232

Hunt GL, McKinnell S, Hunt Jr. GL, McKinnell S, Hunt GL, McKinnell S. (2006) Interplay between top-down, bottom-up, and wasp-waist control in marine ecosystems. Progress in Oceanography 68:115–124. doi: 10.1016/j.pocean.2006.02.008

Hurvich CM, Tsai CL. (1989) Regression and time series model selection in small samples. Biometrika 76:297–307. doi: 10.1093/biomet/76.2.297

Hutchings JA, Myers RA. (1994) What can be learned from the collapse of a renewable resource? Atlantic cod, *Gadus morhua*, of Newfoundland and Labrador. Canadian Journal of Fisheries and Aquatic Sciences 51:2126–2146

Hyslop EJ. (1980) Stomach contents analysis: a review of methods and their application. Journal of Fish Biology 17:411–429. doi: 10.1111/j.1095-8649.1980.tb02775.x

Ianora A, Poulet SA, Miralto A. (2003) The effects of diatoms on copepod reproduction: a review. Phycologia 42:351–363. doi: 10.2216/i0031-8884-42-4-351.1

ICES. (2008) Report of the Workshop on Small Pelagics (*Sardina pilchardus*, *Engraulis encrasicolus*) maturity stages (WKSPMAT). ICES CM 2008/ACOM, 10-14 November 2008, Mazara del Vallo, Italy. p. 82

ICES. (2015) ICES WGBFAS REPORT 2015 Report of the Baltic Fisheries Assessment Working Group (WGBFAS). 14–21 pp.

Iles TD. (1984) Allocation of resources to gonad and soma in Atlantic herring *Clupea harengus* L. Fish Reproduction: strategies and tactics:331–347

Jackson AL, Inger R, Parnell AC, Bearhop S. (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80:595–602. doi: 10.1111/j.1365-2656.2011.01806.x

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH et al. (2001) Historical overfishing and the recent collapse of coastal ecosystems. science 293:629–637

Jakob EM, Marshall SD, Uetz GW. (1996) Estimating Fitness: A Comparison of Body Condition Indices. Oikos 77:61–67. doi: 10.2307/3545585

Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, Narayan R et al. (2015) Plastic waste inputs from land into the ocean. Science 347:768–771

Jennings S. (2005) Indicators to support an ecosystem approach to fisheries. Fish and Fisheries 6:212–232. doi: 10.1111/j.1467-2979.2005.00189.x

Jensen AL. (1996) Beverton and Holt life history invariants result from optimal trade-off of reproduction and survival. Canadian Journal of Fisheries and Aquatic Sciences 53:820–822

Johns B, Marsaleix P, Estournel C, Véhil R. (1992) On the wind-driven coastal upwelling in the Gulf of Lions. Journal of Marine Systems 3:309–320. doi: 10.1016/0924-7963(92)90008-V

Jönsson KI. (1997) Capital and income breeding as alternative tactics of resource use in reproduction. Oikos 78:57–66. doi: 10.2307/3545800

Jørgensen C, Ernande B, Fiksen Ø, Dieckmann U. (2006) The logic of skipped spawning in fish. Canadian Journal of Fisheries and Aquatic Sciences 63:200–211. doi: 10.1139/f05-210

Jørgensen T. (1992) Long-term changes in growth of North-east Arctic cod (*Gadus morhua*) and some environmental influences. ICES Journal of Marine Science 49:263–278. doi: 10.1093/icesjms/49.3.263

Kent M. (1990) Hand-held instrument for fat/water determination in whole fish. Food Control 1:47–53. doi: 10.1016/0956-7135(90)90121-R

Kent M, Lees A, Christie RH, Robert AL, Christie H. (1992) Seasonal variation in the calibration of a microwave fat: water content meter for fish flesh. International Journal of Food Science Technology 27:137–143. doi: 10.1111/j.1365-2621.1992.tb01189.x

Klefoth T, Skov C, Aarestrup K, Arlinghaus R. (2013) Reliability of non-lethal assessment methods of body composition and energetic status exemplified by applications to eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*). Fisheries Research 146:18–26. doi: 10.1016/j.fishres.2013.03.010

Kurita Y, Meier S, Kjesbu OS. (2003) Oocyte growth and fecundity regulation by atresia of Atlantic herring (*Clupea harengus*) in relation to body condition throughout the maturation cycle. Journal of Sea Research 49:203–219. doi: 10.1016/S1385-1101(03)00004-2

Kvalnes T, Ringsby TH, Jensen H, Sæther BE. (2012) Correlates of egg size variation in a population of house sparrow *Passer domesticus*. Oecologia 171:391–402. doi: 10.1007/s00442-012-2437-2

Labocha MK, Schutz H, Hayes JP. (2013) Which body condition index is best? Oikos 123:111–119. doi: 10.1111/j.1600-0706.2013.00755.x

Lambert Y, Dutil JD. (1997a) Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? Canadian Journal of Fisheries and Aquatic Sciences 54:104–112. doi: 10.1139/f96-149

Lambert Y, Dutil JD. (1997b) Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. Canadian Journal of Fisheries and Aquatic Sciences 54:2388–2400. doi: 10.1139/f97-145

Lambert Y, Dutil JD. (2000) Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. Canadian Journal of Fisheries and Aquatic Sciences 57:815–825

Lampert W. (1993) Phenotypic plasticity of the size at first reproduction in Daphnia: the importance of maternal size. Ecology 74:1455–1466. doi: 10.2307/1940074

Law KL, Morét-Ferguson SE, Goodwin DS, Zettler ER, DeForce E, Kukulka T, Proskurowski G. (2014) Distribution of surface plastic debris in the eastern Pacific Ocean from an 11-year data set. Environmental Science Technology 48:4732–4738

Le Bourg B, Banaru D, Saraux C, Nowaczyk A, Le Luherne E, Jadaud A, Bigot JL et al. (2015) Trophic niche overlap of sprat and commercial small pelagic teleosts in the Gulf of Lions (NW Mediterranean Sea). Journal of Sea Research 103:138–146. doi: 10.1016/j.seares.2015.06.011

Le Cren ED. (1951) The length-weight relationship and seasonal cycle in gonad weight and Ccondition in the Perch (*Perca fluviatilis*). Journal of Animal Ecology 20:201–219. doi: 10.2307/1540

Le Maho Y, Robin JP, Cherel Y. (1988) Starvation as a treatment of obesity: the need to conserve body protein. Physiology 3:21–24

Leavenworth WB. (2008) The Changing Landscape of Maritime Resources in Seventeenth-Century New England. International Journal of Maritime History 20:33–62. doi: 10.1177/084387140802000103

Lee JY. (1961) La sardine du Golfe du Lion *Sardina pilchardus sardina*. Revue des Travaux de l'Institut des Pêches Maritimes 25

Lefevre D, Minas HJ, Minas M, Robinson C, Le B. Williams PJ, Woodward EMS. (1997) Review of gross community production, primary production, net community production and dark community respiration in the Gulf of Lions. Deep Sea Research Part II: Topical Studies in Oceanography 44:801–832. doi: 10.1016/S0967-0645(96)00091-4

Lejeusne C, Chevaldonné P, Pergent-Martini C, Boudouresque CF, Pérez T. (2010) Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. Trends in Ecology Evolution 25:250–260. doi: 10.1016/j.tree.2009.10.009

Lessells CM, Boag PT. (1987) Unrepeatable repeatabilities: a common mistake. The Auk 104:116–121. doi: 10.2307/4087240

Lindegren M, Checkley DM, Rouyer T, MacCall AD, Stenseth NC. (2013) Climate, fishing, and fluctuations of sardine and anchovy in the California Current. Proceedings of the National Academy of Sciences 110:13672–13677. doi: 10.1073/pnas.1305733110

Linden M, Møller AP. (1989) Cost of reproduction and covariation of life history traits in birds. Trends in Ecology Evolution 4:367–371

Lleonart J, Maynou F. (2003) Fish stock assessments in the Mediterranean: state of the art. Scientia Marina 67:37–49

Lloret J, de Sola LG, Souplet A, Galzin R. (2002) Effects of large-scale habitat variability on condition of demersal exploited fish in the north-western Mediterranean. ICES Journal of Marine Science 59:1215–1227. doi: 10.1006/jmsc.2002.1294

Lloret J, Faliex E, Shulman GE, Raga JA, Sasal P, Muñoz M, Casadevall M et al. (2012) Fish health and fisheries, implications for stock assessment and management: the Mediterranean example. Reviews in Fisheries Science 20:165–180. doi: 10.1080/10641262.2012.695817

Lloret J, Galzin R, de Sola L, Souplet A, Demestre M. (2005) Habitat related differences in lipid reserves of some exploited fish species in the north-western Mediterranean continental shelf. Journal of Fish Biology 67:51–65. doi: 10.1111/j.0022-1112.2005.00708.x

Lloret J, Palomera I, Salat J, Sole I. (2004) Impact of freshwater input and wind on landings of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) in shelf waters surrounding the Ebre (Ebro) River delta (north-western Mediterranean). Fisheries Oceanography 13:102–110. doi: 10.1046/j.1365-2419.2003.00279.x

Lloret J, Rätz HJ, Lleonart J, Demestre M. (2016) Challenging the links between seafood and human health in the context of global change. Journal of the Marine Biological Association of the United Kingdom 96:29–42

Lloret J, Sabatés A, Muñoz M, Demestre M, Solé I, Font T, Casadevall M et al. (2015) How a multidisciplinary approach involving ethnoecology, biology and fisheries can help explain the spatio-temporal changes in marine fish abundance resulting from climate change. Global Ecology and Biogeography 24:448–461

Lloret J, Shulman G, Love RM. (2014) Condition and Health Indicators of Exploited Marine Fishes. John Wiley Sons, Oxford, U.K. 247 pp.

Lluch-Belda D, Crawford RJM, Kawasaki T, MacCall AD, Parrish RH, Schwartzlose RA, Smith PE. (1989) World-wide fluctuations of sardine and anchovy stocks: the regime problem. South African Journal of Marine Science 8:195–205

Lluch-Belda D, Schwartzlose Ra, Serra R, Parrish R, Kawasaki T, Hedgecock D, Crawford RJM. (1992) Sardine and anchovy regime fluctuations of abundance in four regions of the world oceans: A workshop report. Fisheries Oceanography 1:339–347. doi: 10.1111/j.1365-2419.1992.tb00006.x

Love RM. (1970) The chemical biology of fishes. With a key to the chemical literature.:547

Lowerre-Barbieri SK, Barbieri LR. (1993) A new method of oocyte separation and preservation for fish reproduction studies. Fishery Bulletin-National Oceanic and Atmospherix Administration 91:165

Ludwig D, Hilborn R, Walters C. (1993) Uncertainty, resource exploitation, and conservation: lessons from history. Science 260:17–36. doi: 10.1126/science.260.5104.17

Macías D, Bruno M, Echevarría F, Vázquez A, García CM. (2008) Meteorologically-induced mesoscale variability of the North-western Alboran Sea (southern Spain) and related biological patterns. Estuarine, Coastal and Shelf Science 78:250–266. doi: 10.1016/j.ecss.2007.12.008

Mackas DL, Greve W, Edwards M, Chiba S, Tadokoro K, Eloire D, Mazzocchi MG et al. (2012) Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. Progress in Oceanography 97:31–62

Malzahn A, Aberle N, Clemmesen C, Boersma M. (2007) Nutrient limitation of primary producers affects planktivorous fish condition. Limnology and Oceanography 52:2062–2071

Marsaleix P, Auclair F, Duhaut T, Estournel C, Nguyen C, Ulses C. (2012) Alternatives to the Robert-Asselin filter. Ocean Modelling 41:53–66. doi: 10.1016/j.ocemod.2011.11.002

Marsaleix P, Auclair F, Estournel C. (2009) Low-order pressure gradient schemes in sigma coordinate models: The seamount test revisited. Ocean Modelling 30:169–177. doi: 10.1016/j.ocemod.2009.06.011

Marsaleix P, Auclair F, Estournel C, Nguyen C, Ulses C. (2011) An accurate implementation of the compressibility terms in the equation of state in a low order pressure gradient scheme for sigma coordinate ocean models. Ocean Modelling 40:1–13. doi: 10.1016/j.ocemod.2011.07.004

Martin K. (1995) Patterns and mechanisms for age-dependent reproduction and survival in birds. American Zoologist 35:340–348. doi: 10.1093/icb/35.4.340

Martín P, Sabatés A, Lloret J, Martin-Vide J. (2011) Climate modulation of fish populations: the role of the Western Mediterranean Oscillation (WeMO) in sardine (*Sardina* *pilchardus*) and anchovy (*Engraulis encrasicolus*) production in the north-western Mediterranean. Climatic Change 110:925–939. doi: 10.1007/s10584-011-0091-z

Martin-Vide J, Lopez-Bustins JA. (2006) The Western Mediterranean Oscillation and rainfall in the Iberian Peninsula. International Journal of Climatology 26:1455–1475. doi: 10.1002/joc.1388

Matthews B, Mazumder A. (2004) A critical evaluation of intrapopulation variation of δ13C and isotopic evidence of individual specialization. Oecologia 140:361–371. doi: 10.1007/s00442-004-1579-2

Maynou F, Sabatés A, Salat J. (2014) Clues from the recent past to assess recruitment of Mediterranean small pelagic fishes under sea warming scenarios. Climatic Change 126:175–188. doi: 10.1007/s10584-014-1194-0

McBride RS, Somarakis S, Fitzhugh GR, Albert A, Yaragina NA, Wuenschel MJ, Alonso-Fernández A et al. (2013) Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish and Fisheries 16:46–59. doi: 10.1111/faf.12043

McCue MD. (2010) Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comparative Biochemistry and Physiology Part A: Molecular Integrative Physiology 156:1–18. doi: 10.1016/j.cbpa.2010.01.002

McPherson LR, Slotte A, Kvamme C, Meier S, Marshall CT. (2011) Inconsistencies in measurement of fish condition: a comparison of four indices of fat reserves for Atlantic herring (*Clupea harengus*). ICES Journal of Marine Science 68:52–60. doi: 10.1093/icesjms/fsq148

Medina A, Goñi N, Arrizabalaga H, Varela JL. (2015) Feeding patterns of age-0 bluefin tuna in the western Mediterranean inferred from stomach-content and isotope analyses. Marine Ecology Progress Series 527:193–204

Mendes B, Fonseca P, Campos A. (2004) Weight-length relationships for 46 fish species of the Portuguese west coast. Journal of Applied Ichthyology 20:355–361. doi: 10.1111/j.1439-0426.2004.00559.x

Metian AGJTM. (2009) Fishing for feed or fishing for food: increasing global competition for small pelagic forage fish. AMBIO: A Journal of the Human Environment 38:294–302. doi: 10.1579/08-A-574.1

Millán M. (1999) Reproductive characteristics and condition status of anchovy *Engraulis encrasicolus* L. from the Bay of Cadiz (SW Spain). Fisheries Research 41:73–86. doi: 10.1016/S0165-7836(99)00010-7

Millar JS, Hickling GJ. (1990) Fasting endurance and the evolution of mammalian body size. Functional Ecology 4:5–12. doi: 10.2307/2389646

Millot C. (1990) The Gulf of Lions' hydrodynamics. Continental Shelf Research 10:885–894. doi: 10.1016/0278-4343(90)90065-T

Minas M, Minas HJ. (1989) Primary production in the Gulf of Lions with considerations to the Rhone River inputs. Water Pollution Research Reports 32(5) 13:112–125

Molinero JC, Ibanez F, Nival P, Buecher E, Souissi S. (2005) North Atlantic climate and northwestern Mediterranean plankton variability. Limnology and Oceanography 50:1213–1220

Molinero JC, Ibanez F, Souissi S, Buecher E, Dallot S, Nival P. (2008) Climate control on the long-term anomalous changes of zooplankton communities in the Northwestern Mediterranean. Global Change Biology 14:11–26. doi: 10.1111/j.1365-2486.2007.01469.x

Mollmann C, Kornilovs G, Fetter M, Koster F. (2005) Climate, zooplankton, and pelagic fish growth in the central Baltic Sea. ICES Journal of Marine Science 62:1270–1280. doi: 10.1016/j.icesjms.2005.04.021

Monaghan P. (2008) Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society of London B: Biological Sciences 363:1635–1645

Mora C, Myers RA, Coll M, Libralato S, Pitcher TJ, Sumaila RU, Zeller D et al. (2009) Management effectiveness of the world's marine fisheries. PLoS Biology 7:e1000131. doi: 10.1371/journal.pbio.1000131

Morey S, Reznick D. (2000) A comparative analysis of plasticity in larval development in three species of spadefoot toads. Ecology 81:1736–1749. doi: 10.1890/0012-9658(2000)081[1736:ACAOPI]2.0.CO;2

Morgan MJ. (2004) The relationship between fish condition and the probability of being mature in American plaice (*Hippoglossoides platessoides*). ICES Journal of Marine Science 61:64–70. doi: 10.1016/j.icesjms.2003.09.001

Mørkøre T, Hansen A, Unander E, Einen O. (2002) Composition, liquid leakage, and mechanical properties of farmed rainbow trout: variation Between fillet sections and the impact of ice and frozen storage. Journal of Food Science 67:1933–1938. doi: 10.1111/j.1365-2621.2002.tb08749.x

Mozetič P, Francé J, Kogovšek T, Talaber I, Malej A. (2012) Plankton trends and community changes in a coastal sea (northern Adriatic): Bottom-up vs. top-down control in relation to environmental drivers. Estuarine, Coastal and Shelf Science 115:138–148. doi: 10.1016/j.ecss.2012.02.009

Muñoz M, Dimitriadis C, Casadevall M, Vila S, Delgado E, Lloret J, Saborido-Rey F et al. (2010) Female reproductive biology of the bluemouth *Helicolenus dactylopterus dactylopterus*: spawning and fecundity. Journal of Fish Biology 77:2423–2442. doi: 10.1111/j.1095-8649.2010.02835.x

Murphy BR, Brown ML, Springer TA. (1990) Evaluation of the Relative Weight (Wr) Index, with New Applications to Walleye. North American Journal of Fisheries Management 10:85–97. doi: 10.1577/1548-8675(1990)010<0085:EOTRWW>2.3.CO;2

Murua H, Kraus G, Saborido-Rey F, Witthames PR, Thorsen A, Junquera S. (2003) Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy

Muths E, Scherer RD, Lambert BA. (2010) Unbiased survival estimates and evidence for skipped breeding opportunities in females. Methods in Ecology and Evolution 1:123–130. doi: 10.1111/j.2041-210X.2010.00019.x

Naso B, Perrone D, Ferrante MC, Bilancione M, Lucisano A. (2005) Persistent organic pollutants in edible marine species from the Gulf of Naples, Southern Italy. Science of the Total Environment 343:83–95. doi: 10.1016/j.scitotenv.2004.10.007

Naulleau G, Bonnet X. (1996) Body condition threshold for breeding in a viviparous snake. Oecologia 107:301–306. doi: 10.1007/BF00328446

Ndjaula HON, Gerow KG, van der Lingen CD, Moloney CL, Jarre A. (2013) Establishing a baseline for evaluating changes in body condition and population dynamics of sardine (*Sardinops sagax*) in the southern Benguela ecosystem. Fisheries Research 147:253–263. doi: 10.1016/j.fishres.2013.06.006

Newsome SD, del Rio C, Bearhop S, Phillips DL. (2007) A niche for isotopic ecology. Frontiers in Ecology and the Environment 5:429–436. doi: 10.1890/060150.1

Nielsen D, Hyldig G, Nielsen J, Nielsen HH. (2005) Lipid content in herring (*Clupea harengus*, L.): influence of biological factors and comparison of different methods of analyses: solvent extraction, Fatmeter, NIR and NMR. LWT - Food Science and Technology 38:537–548. doi: 10.1016/j.lwt.2004.07.010

Nikolioudakis N, Isari S, Pitta P, Somarakis S. (2012) Diet of sardine *Sardina pilchardus*: an 'end-to-end' field study. Marine Ecology Progress Series 453:173–188. doi: 10.3354/meps09656

Nikolioudakis N, Isari S, Somarakis S. (2014) Trophodynamics of anchovy in a non-upwelling system: direct comparison with sardine. Marine Ecology Progress Series 500:215–229. doi: 10.3354/meps10604

Ohgushi T. (1996) A reproductive tradeoff in an herbivorous lady beetle: egg resorption and female survival. Oecologia 106:345–351. doi: 10.1007/BF00334562

Orlova E, Rudneva G, Renaud P, Eiane K, Savinov V, Yurko A. (2010) Climate impacts on feeding and condition of capelin *Mallotus villosus* in the Barents Sea: Evidence and mechanisms from a data set spanning 30 years. Aquatic Biology 10:105–118. doi: 10.3354/ab00265

Óskarsson GJ. (2008) Variation in body condition, fat content and growth rate of Icelandic summer-spawning herring *Clupea harengus* L. Journal of Fish Biology 72:2655–2676. doi: 10.1111/j.1095-8649.2008.01886.x

Óskarsson GJ, Kjesbu OS, Slotte A. (2002) Predictions of realised fecundity and spawning time in Norwegian spring-spawning herring (*Clupea harengus*). Journal of Sea Research 48:59–79. doi: 10.1016/S1385-1101(02)00135-1

Ospina-Alvarez A, Parada C, Palomera I. (2012) Vertical migration effects on the dispersion and recruitment of European anchovy larvae: From spawning to nursery areas. Ecological Modelling 231:65–79. doi: 10.1016/j.ecolmodel.2012.02.001

Özsoy E, Ünlüata Ü. (1997) Oceanography of the Black Sea: A review of some recent results. Earth-Science Reviews 42:231–272. doi: 10.1016/S0012-8252(97)81859-4

Palomera I, Olivar MP, Salat J, Sabatés A, Coll M, García A, Morales-Nin B. (2007) Small pelagic fish in the NW Mediterranean Sea: An ecological review. Progress in Oceanography 74:377–396. doi: 10.1016/j.pocean.2007.04.012

Pangle KL, Sutton TM. (2005) Temporal changes in the relationship between condition indices and proximate composition of juvenile *Coregonus artedi*. Journal of Fish Biology 66:1060–1072. doi: 10.1111/j.0022-1112.2005.00660.x

Pardoe H, Thórdarson G, Marteinsdóttir G. (2008) Spatial and temporal trends in condition of Atlantic cod *Gadus morhua* on the Icelandic shelf. Marine Ecology Progress Series 362:261–277

Parrish CC. (1999) Determination of Total Lipid, Lipid Classes, and Fatty Acids in Aquatic Samples. *In* Lipids in Freshwater Ecosystems, edited by Arts MT, Wainman BC. Springer New York. pp. 4–20

Peck MA, Reglero P, Takahashi M, Catalán IA. (2013) Life cycle ecophysiology of small pelagic fish and climate-driven changes in populations. Progress in Oceanography 116:220–245. doi: 10.1016/j.pocean.2013.05.012

Pecquerie L, Petitgas P, Kooijman SALM. (2009) Modeling fish growth and reproduction in the context of the Dynamic Energy Budget theory to predict environmental impact on anchovy spawning duration. Journal of Sea Research 62:93–105. doi: 10.1016/j.seares.2009.06.002

Pellerin S, Paquette SR, Pelletier F, Garant D, Bélisle M. (2016) The trade-off between clutch size and egg mass in tree swallows *Tachycineta bicolor* is modulated by female body mass. Journal of Avian Biology 47:500–507. doi: 10.1111/jav.00725

Pethybridge H, Bodin N, Arsenault-Pernet EJ, Bourdeix JH, Brisset B, Bigot JL, Roos D et al. (2014) Temporal and inter-specific variations in forage fish feeding conditions in the NW Mediterranean: lipid content and fatty acid compositional changes. Marine Ecology Progress Series 512:39–54. doi: 10.3354/meps10864

Pethybridge H, Roos D, Loizeau V, Pecquerie L, Bacher C. (2013) Responses of European anchovy vital rates and population growth to environmental fluctuations: An individual-based modeling approach. Ecological Modelling 250:370–383. doi: 10.1016/j.ecolmodel.2012.11.017

Pikitch EK. (2004) Ecology: Ecosystem-Based Fishery Management. Science 305:346–347. doi: 10.1126/science.1098222

Pikitch EK, Rountos KJ, Essington TE, Santora C, Pauly D, Watson R, Sumaila UR et al. (2014) The global contribution of forage fish to marine fisheries and ecosystems. Fish and Fisheries 15:43–64. doi: 10.1111/faf.12004

Pinkas L. (1971) Food habits study. Fishery Bulletin 152:5–10

Planque B, Fromentin JM, Cury P, Drinkwater KF, Jennings S, Perry RI, Kifani S. (2010) How does fishing alter marine populations and ecosystems sensitivity to climate? Journal of Marine Systems 79:403–417. doi: 10.1016/j.jmarsys.2008.12.018

Plounevez S, Champalbert G. (2000) Diet, feeding behaviour and trophic activity of the anchovy (*Engraulis encrasicolus*, L.) in the Gulf of Lions (Mediterranean Sea). Oceanologica Acta 23:175–192. doi: 10.1016/S0399-1784(00)00120-1

Porath MT, Peters EJ. (1997) Use of Walleye relative weights (Wr) to assess prey availability. North American Journal of Fisheries Management 17:628–637. doi: 10.1577/1548-8675(1997)017<0628:UOWRWW>2.3.CO;2

Pörtner HO, Knust R. (2007) Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance. Science 315:95–97. doi: 10.1126/science.1135471

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG. (2007) Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189. doi: 10.1007/s00442-006-0630-x

Pryce JE, Coffey MP, Simm G. (2001) The relationship between body condition score and reproductive performance. Journal of Dairy Science 84:1508–1515

Queiros Q. (2016) Pression de predation des mammiferes marins (dauphins) sur les poissons petits pelagiques dans le Golfe du Lion. Master thesis. University of Marseille. 46 pp.

Quignard JP, Tomasini JA. (2000) Mediterranean fish biodiversity. Biologia Marina Mediterranea 7:1–66

Radovich J. (1982) The collapse of the California sardine fishery. What have we learned? CalCOFI Report. 56–77 pp.

Rajasilta M, Eklund J, Hänninen J, Vuorinen I, Laine P. (2015) Female Baltic herring *Clupea harengus* allocate resources from growth to reproduction in poor feeding conditions. Journal of Fish Biology 86:575–591. doi: 10.1111/jfb.12577

Rakka M, Ganias K. (2015) Assessing species and stage specific effects of preservation on fish oocytes over different temporal scales. Mediterranean Marine Science:533–537

Rätz HJ, Lloret J. (2003) Variation in fish condition between Atlantic cod *Gadus morhua* stocks, the effect on their productivity and management implications. Fisheries Research 60:369–380. doi: 10.1016/S0165-7836(02)00132-7

Rau G, Teyssie JL, Rassoulzadegan F, Fowler S. (1990) $^{13}C/^{12}C$ and $^{15}N/^{14}N$ variations among size-fractionated marine particles: implications for their origin and trophic relationships. Marine Ecology Progress Series 59:33–38. doi: 10.3354/meps059033

Reeds PJ, Wahle KWJ, Haggarty P. (1982) Energy costs of protein and fatty acid synthesis. Proceedings of the Nutrition Society 41:155–159. doi: 10.1079/PNS19820025

Regost C, Jakobsen JV, Rørå AMB. (2004) Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. Food Research International 37:259–271. doi: 10.1016/j.foodres.2003.12.003

Renault L, Oguz T, Pascual A, Vizoso G, Tintoré J. (2012) Surface circulation in the Alborán Sea (Western Mediterranean) inferred from remotely sensed data. Journal of Geophysical Research: Oceans 117:2156–2202

Reznick D. (1985) Costs of reproduction: an evaluation of the empirical evidence. Oikos:257–267

Ricker WE. (1975) Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191:1–382

Rideout RM, Morgan MJ. (2010) Relationships between maternal body size, condition and potential fecundity of four north-west Atlantic demersal fishes. Journal of Fish Biology 76:1379–1395. doi: 10.1111/j.1095-8649.2010.02570.x

Rideout RM, Tomkiewicz J. (2011) Skipped spawning in fishes: more common than you might think. Marine and Coastal Fisheries 3:176–189. doi: 10.1080/19425120.2011.556943

Rijnsdorp AD, Peck MA, Engelhard GH, Möllmann C, Pinnegar JK. (2009) Resolving the effect of climate change on fish populations. ICES Journal of Marine Science 66:1570–1583. doi: 10.1093/icesjms/fsp056

Rivalan P, Prévot-Julliard AC, Choquet R, Pradel R, Jacquemin B, Girondot M. (2005) Trade-off between current reproductive effort and delay to next reproduction in the leatherback sea turtle. Oecologia 145:564–574. doi: 10.1007/s00442-005-0159-4

Riveiro I, Guisande C, Lloves M, Maneiro I, Cabanas JM. (2000) Importance of parental effects on larval survival in *Sardina pilchardus*. Marine Ecology Progress Series 205:249–258

Robin JP, Frain M, Sardet C, Groscolas R, Maho YL. (1988) Protein and lipid utilization during long-term fasting in emperor penguins. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 254:61–68

Rödel H, Bora A, Kaiser J, Kaetzke P, Khaschei M, Von Holst D. (2004) Density-dependent reproduction in the European rabbit: a consequence of individual response and age-dependent reproductive performance. Oikos 104:529–539. doi: 10.1111/j.0030-1299.2004.12691.x

Roff DA. (2002) Life history evolution, Volume 7. Sinauer Associates Sunderland. 256 pp.

Roff DA, Fairbairn DJ. (2007) The evolution of trade-offs: where are we? Journal of evolutionary biology 20:433–447

Røjbek MC, Tomkiewicz J, Jacobsen C, Støttrup JG. (2014) Forage fish quality: seasonal lipid dynamics of herring (*Clupea harengus* L.) and sprat (*Sprattus sprattus* L.) in the Baltic Sea. ICES Journal of Marine Science 71:56–71. doi: 10.1093/icesjms/fst106

Rosa R, Gonzalez L, Broitman BR, Garrido S, Santos AMP, Nunes ML. (2010) Bioenergetics of small pelagic fishes in upwelling systems: relationship between fish condition, coastal ecosystem dynamics and fisheries. Marine Ecology Progress Series 410:205–218. doi: 10.3354/meps08635

Rouyer T, Fromentin J, Stenseth NC, Cazelles B. (2008) Analysing multiple time series and extending significance testing in wavelet analysis. Marine Ecology Progress Series 359:11–23. doi: 10.3354/meps07330

Rouyer T, Fromentin JM, Hidalgo M, Stenseth NC. (2014) Combined effects of exploitation and temperature on fish stocks in the Northeast Atlantic. ICES Journal of Marine Science 71:1554–1562. doi: 10.1093/icesjms/fsu042

Rowe JW. (1994) Reproductive variation and the egg size-clutch size trade-off within and among populations of painted turtles (*Chrysemys picta bellii*). Oecologia 99:35–44. doi: 10.1007/BF00317081

Russo A, Artegiani A. (1996) Adriatic sea hydrography. Scientia Marina 60:33–43

Sabatés A, Martín P, Lloret J, Raya V. (2006) Sea warming and fish distribution: the case of the small pelagic fish, *Sardinella aurita*, in the western Mediterranean. Global Change Biology 12:2209–2219. doi: 10.1111/j.1365-2486.2006.01246.x

Sabatés A, Pagès F, Atienza D, Fuentes V, Purcell JE, Gili JM. (2010) Planktonic cnidarian distribution and feeding of *Pelagia noctiluca* in the NW Mediterranean Sea. Hydrobiologia 645:153–165. doi: 10.1007/s10750-010-0221-z

Sala OE, Chapin III FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E et al. (2000) Global Biodiversity Scenarios for the Year 2100. Science 287:1770 – 1774. doi: 10.1126/science.287.5459.1770

Salat J. (1996) Review of hydrographic environmental factors that may influence anchovy habitats in northwestern Mediterranean. Scientia Marina 60:21–32

Sánchez-Gómez S. (2013) Advances in the knowledge of condition and energy budgets along the life cycle of two small pelagic fishes from the Western Mediterranean. Master thesis.

Sanderson EW, Jaiteh M, Levy Ma, Redford KH, Wannebo AV, Woolmer G. (2002) The human footprint and the last of the wild. BioScience 52:891. doi: 10.1641/0006-3568(2002)052[0891:THFATL]2.0.CO;2

Saraux C, Fromentin JM, Bigot JL, Bourdeix JH, Morfin M, Roos D, Van Beveren E et al. (2014) Spatial structure and distribution of small pelagic fish in the Northwestern Mediterranean Sea. PLoS ONE 9:e111211. doi: 10.1371/journal.pone.0111211

Scarlett JM, Donoghue S. (1998) Associations between body condition and disease in cats. Journal of the American Veterinary Medical Association 212:1725–1731

Schamber JL, Esler D, Flint PL. (2009) Evaluating the validity of using unverified indices of body condition. Journal of Avian Biology 40:49–56. doi: 10.1111/j.1600-048X.2008.04462.x

Schulte-Hostedde aI, Millar JS, Hickling GJ. (2001) Evaluating body condition in small mammals. Canadian Journal of Zoology 79:1021–1029. doi: 10.1139/z01-073

Schulte-Hostedde AI, Zinner B, Millar JS, Hickling GJ. (2005) Restitution of mass size residuals: validation body condition indices. Ecology 86:155–163. doi: 10.1890/04-0232

Schwartzlose RA, Alheit J, Bakun A, Baumgartner TR, Cloete R, Crawford RJM, Fletcher WJ et al. (1999) Worldwide large-scale fluctuations of sardine and anchovy populations. South African Journal of Marine Science 21:289–347. doi: 10.2989/025776199784125962

Shannon LJ, Field JG, Moloney CL. (2004) Simulating anchovy-sardine regime shifts in the southern Benguela ecosystem. Ecological Modelling 172:269–281. doi: 10.1016/j.ecolmodel.2003.09.011

Shero MR, Krotz RT, Costa DP, Avery JP, Burns JM. (2015) How do overwinter changes in body condition and hormone profiles influence Weddell seal reproductive success? Functional Ecology 29:1278–1291. doi: 10.1111/1365-2435.12434

Shine R, Brown GP. (2008) Adapting to the unpredictable: reproductive biology of vertebrates in the Australian wet-dry tropics. Philosophical Transactions of the Royal Society of London B: Biological Sciences 363:363–373. doi: 10.1098/rstb.2007.2144

Shulman GE, Love RM. (1999) The biochemical ecology of marine fishes, a. j. sout Edition, Volume 36 of *Advances in Marine Ecology*. Academic Press, London

Shulman GE, Nikolsky VN, Yuneva TV, Shchepkin GSMY, Shchepkina AM, Ivleva EV, Dobrovolov OAYS et al. (2005) Fat content in Black Sea sprat as an indicator of fish food supply and ecosystem condition. Marine Ecology Progress Series 293:201–212. doi: 10.3354/meps293201

Sih A. (1987) Predators and prey lifestyles: an evolutionary and ecological overview. Predation: direct and indirect impacts on aquatic communities 31:203–224

Silva A, Santos MB, Caneco B, Pestana G, Porteiro C, Carrera P, Stratoudakis Y. (2006) Temporal and geographic variability of sardine maturity at length in the northeastern Atlantic and the western Mediterranean. ICES Journal of Marine Science 63:663–676. doi: 10.1016/j.icesjms.2006.01.005

Simat V, Bogdanović T. (2012) Seasonal changes in proximate composition of anchovy (*Engraulis encrasicolus*, L.) from the central Adriatic. Acta Adriatica 53:125–131

Sinovčić G, Keč VČ, Zorica B. (2008) Population structure, size at maturity and condition of sardine, *Sardina pilchardus* (Walb., 1792), in the nursery ground of the eastern Adriatic Sea (Krka River Estuary, Croatia). Estuarine, Coastal and Shelf Science 76:739–744. doi: 10.1016/j.ecss.2007.07.037

Skibiel AL, Speakman JR, Hood WR. (2013) Testing the predictions of energy allocation decisions in the evolution of life-history trade-offs. Functional Ecology 27:1382–1391

Smith ADDM, Garcia SM. (2014) Fishery management: contrasts in the Mediterranean and the Atlantic. Current Biology 24:810–812. doi: 10.1016/j.cub.2014.07.031

Smith JNM. (1981) Does high fecundity reduce survival in Song Sparrows? Evolution:1142–1148

Somarakis S, Ganias K, Siapatis A, Koutsikopoulos C, Machias A, Papaconstantinou C. (2006) Spawning habitat and daily egg production of sardine (*Sardina pilchardus*) in the eastern Mediterranean. Fisheries Oceanography 15:281–292. doi: 10.1111/j.1365-2419.2005.00387.x

Somarakis S, Palomera I, Garcia A, Quintanilla L, Koutsikopoulos C, Uriarte A, Motos L. (2004) Daily egg production of anchovy in European waters. ICES Journal of Marine Science 61:944–958. doi: 10.1016/j.icesjms.2004.07.018

Somers I. (1988) On a seasonally oscillating growth function. Fishbyte 6:8–11

Southward AJ, Boalch GT, Maddock L. (1988) Fluctuations in the herring and pilchard fisheries of Devon and Cornwall linked to change in climate since the 16th century. Journal of the Marine Biological Association of the United Kingdom 68:423–445. doi: 10.1017/S0025315400043320

Stearns SC. (1989) Trade-offs in life-history evolution. Functional ecology 3:259–268

Stearns SC. (1992) The evolution of life histories, Volume 249. Oxford University Press Oxford. 262 pp.

Stearns SC, Koella JC. (1986) The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution:893–913

Stevenson RD, Woods WA. (2006) Condition indices for conservation: new uses for evolving tools. Integrative and Comparative Biology 46:1169–1190. doi: 10.1093/icb/icl052

Stibor H. (1992) Predator induced life-history shifts in a freshwater cladoceran. Oecologia 92:162–165. doi: 10.1007/BF00317358

Stibor H, Lüning J. (1994) Predator-induced phenotypic variation in the pattern of growth and reproduction in *Daphnia hyalina* (Crustacea: Cladocera). Functional Ecology 8:97–101. doi: 10.2307/2390117

Strady E, Harmelin-Vivien M, Chiffoleau JF, Veron A, Tronczynski J, Radakovitch O. (2015) ^{210}Po and ^{210}Pb trophic transfer within the phytoplankton-zooplankton-anchovy sardine food web: a case study from the Gulf of Lion (NW Mediterranean Sea). Journal of Environmental Radioactivityadioactivity 143:141–151

Sutton SG, Bult TP, Haedrich RL. (2000) Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic Salmon Parr. Transactions of the American Fisheries Society 129:527–538. doi: 10.1577/1548-8659(2000)129<0527:RAFWBW>2.0.CO;2

Sweeting CJ, Jennings S, Polunin NVC. (2005) Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. Functional Ecology 19:777–784. doi: 10.1111/j.1365-2435.2005.01019.x

Syväranta J, Lensu A, Marjomäki TJ, Oksanen S, Jones RI. (2013) An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. PLoS ONE 8:e56094. doi: 10.1371/journal.pone.0056094

Tarkan AS, Gaygusuz Ö, Gürsoy Gaygusuz Ç, Saç G, Copp GH. (2012) Circumstantial evidence of gibel carp, *Carassius gibelio*, reproductive competition exerted on native fish species in a mesotrophic reservoir. Fisheries Management and Ecology 19:167–177. doi: 10.1111/j.1365-2400.2011.00839.x

Thackeray SJ, Sparks TH, Frederiksen M, Burthe S, Bacon PJ, Bell JR, Botham MS et al. (2010) Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. Global Change Biology 16:3304–3313. doi: 10.1111/j.1365-2486.2010.02165.x

Theocharis A. (2008) Do we expect significant changes in the thermohaline circulation in the Mediterranean in relation to the observed surface layers warming. Climate warming and related changes in Mediterranean marine biota:25–30

Tiano M, Tronczynski J, Harmelin-Vivien M, Tixier C, Carlotti F. (2014) PCB concentrations in plankton size classes, a temporal study in Marseille Bay, Western Mediterranean Sea. Marine Pollution Bulletin 89:331–339. doi: 10.1016/j.marpolbul.2014.09.040

Trippel EA. (1998) Egg size and viability and seasonal offspring production of young Atlantic cod. Transactions of the American Fisheries Society 127:339–359

Trudel M, Tucker S, Morris JFT, Higgs DA, Welch DW. (2005) Indicators of energetic status in juvenile coho salmon and chinook salmon. North American Journal of Fisheries Management 25:374–390. doi: 10.1577/M04-018.1

Tsikliras AC, Dinouli A, Tsiros VZ, Tsalkou E. (2015) The Mediterranean and Black Sea fisheries at risk from overexploitation. PLoS ONE 10:e0121188. doi: 10.1371/journal.pone.0121188

Tudela S, Palomera I. (1997) Trophic ecology of the European anchovy *Engraulis encrasicolus* in the Catalan Sea (northwest Mediterranean). Marine Ecology Progress Series 160:121–134

Turner TF, Collyer ML, Krabbenhoft TJ. (2010) A general hypothesis-testing framework for stable isotope ratios in ecological studies. Ecology 91:2227–2233. doi: 10.1890/09-1454.1

Uriarte I, Villate F. (2005) Differences in the abundance and distribution of copepods in two estuaries of the Basque coast (Bay of Biscay) in relation to pollution. Journal of Plankton Research 27:863–874

Valdés J, Ortlieb L, Gutierrez D, Marinovic L, Vargas G, Sifeddine A. (2008) 250 years of sardine and anchovy scale deposition record in Mejillones Bay, northern Chile. Progress in Oceanography 79:198–207. doi: 10.1016/j.pocean.2008.10.002

Van Beveren E. (2015) Population changes in small pelagic fish of the Gulf of Lions: a bottom-up control? Phd thesis, Université de Montpellier

Van Beveren E, Bonhommeau S, Fromentin JM, Bigot JL, Bourdeix JH, Brosset P, Roos D et al. (2014) Rapid changes in growth, condition, size and age of small pelagic fish in the Mediterranean. Marine Biology 161:1–14. doi: 10.1007/s00227-014-2463-1

Van Beveren E, Fromentin JM, Rouyer T, Bonhommeau S, Brosset P, Saraux C. (2016) The fisheries history of small pelagics in the Northern Mediterranean. ICES Journal of Marine Science 73:1474–1484. doi: 10.1093/icesjms/fsw023

van der Lingen CD, Hutchings L, Field JG. (2006) Comparative trophodynamics of anchovy *Engraulis encrasicolus* and sardine *Sardinops sagax* in the southern Benguela: are species alternations between small pelagic fish trophodynamically mediated? African Journal of Marine Science 28:465–477. doi: 10.2989/18142320609504199

van Noordwijk AJ, de Jong G. (1986) Acquisition and Allocation of Resources: Their Influence on Variation in Life History Tactics. The American Naturalist 128:137–142

Vander Zanden MJ, Rasmussen JB. (1999) Primary consumer δ13c and δ15n and the trophic position of aquatic consumers. Ecology 80:1395–1404. doi: 10.1890/0012-9658(1999)080[1395:PCCANA]2.0.CO;2

Vander Zanden MJ, Rasmussen JB. (2001) Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. Limnology and Oceanography 46:2061–2066

Vargas-Yáñez M, Jesús García M, Salat J, García-Martínez MC, Pascual J, Moya F. (2008) Warming trends and decadal variability in the Western Mediterranean shelf. Global and Planetary Change 63:177–184. doi: 10.1016/j.gloplacha.2007.09.001

Vargas-Yáñez M, Moya F, García-Martínez MC, Tel E, Zunino P, Plaza F, Salat J et al. (2010) Climate change in the Western Mediterranean Sea 1900-2008. Journal of Marine Systems 82:171–176. doi: 10.1016/j.jmarsys.2010.04.013

Vasilakopoulos P, Maravelias CD, Tserpes G. (2014) The alarming decline of Mediterranean fish stocks. Current Biology 24:1643–1648. doi: 10.1016/j.cub.2014.05.070

Vidussi F, Mostajir B, Fouilland E, Le Floc'h E, Nouguier J, Roques C, Got P et al. (2011) Effects of experimental warming and increased ultraviolet B radiation on the Mediterranean plankton food web. Limnology and Oceanography 56:206

Vijverberg J, Frank TH. (1976) The chemical composition and energy contents of copepods and cladocerans in relation to their size. Freshwater Biology 6:333–345

Vilibić I, Keč VČ, Zorica B, Šepić J, Matijević S, Džoić T. (2016) Hydrographic conditions driving sardine and anchovy populations in a land-locked sea. Mediterranean Marine Science 17:1–12

Vogt A, Gormley T, Downey G, Somers J. (2002) A Comparison of Selected Rapid Methods for Fat Measurement in Fresh Herring (*Clupea harengus*). Journal of Food Composition and Analysis 15:205–215. doi: 10.1006/jfca.2002.1049

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin JM et al. (2002) Ecological responses to recent climate change. Nature 416:389–395

Wanless S, Harris MP, Redman P, Speakman JR. (2005) Low energy values of fish as a probable cause of a major seabird breeding failure in the North Sea. Marine Ecology Progress Series 294:8

Whittington RJ, Crockford M, Jordan D, Jones B. (2008) Herpesvirus that caused epizootic mortality in 1995 and 1998 in pilchard, *Sardinops sagax neopilchardus* (Steindachner), in Australia is now endemic. Journal of Fish Diseases 31:97–105

Wiegand MD. (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. Reviews in Fish Biology and Fisheries 6:259–286. doi: 10.1007/BF00122583

Williams GC. (1966) Natural selection, the costs of reproduction, and a refinement of Lack's principle. American naturalist 100:687–690

Wilson AJ, Nussey DH. (2010) What is individual quality? An evolutionary perspective. Trends in Ecology Evolution 25:207–214. doi: 10.1016/j.tree.2009.10.002

Winters GH, Wheeler JP. (1994) Length-specific weight as a measure of growth success of adult Atlantic herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Sciences 51:1169–1179. doi: 10.1139/f94-116

Worm B, Branch TA. (2012) The future of fish. Trends in Ecology and Evolution 27:594–599. doi: 10.1016/j.tree.2012.07.005

Yoneda M, Kitano H, Selvaraj S, Matsuyama M, Shimizu A. (2013) Dynamics of gonadosomatic index of fish with indeterminate fecundity between subsequent egg batches: application to Japanese anchovy *Engraulis japonicus* under captive conditions. Marine Biology 160:2733–2741. doi: 10.1007/s00227-013-2266-9

Youssara F, Gaudy R. (2001) Variations of zooplankton in the frontal area of the Alboran Sea (Mediterranean Sea) in winter 1997. Oceanologica Acta 24:361–376. doi: 10.1016/S0399-1784(01)01154-9

Zarubin M, Farstey V, Wold A, Falk-Petersen S, Genin A. (2014) Intraspecific differences in lipid content of calanoid copepods across fine-scale depth ranges within the photic layer. PLoS ONE 9:e92935. doi: 10.1371/journal.pone.0092935

Zavatarelli M, Raicich F, Bregant D, Russo A, Artegiani A. (1998) Climatological biogeochemical characteristics of the Adriatic Sea. Journal of Marine Systems 18:227–263. doi: 10.1016/S0924-7963(98)00014-1

Zeileis A, Leisch F, Hornik K, Kleiber C, Hansen B. (2013) strucchange: Testing, Monitoring, and Dating Structural Changes

Zenetos A, Çinar ME, Pancucci-Papadopoulou MA, Harmelin JG, Furnari G, Andaloro F, Zibrowius H. (2005) Annotated list of marine alien species in the Mediterranean with records of the worst invasive species. Mediterranean Marine Science 6:63–118

Zlatanos S, Laskaridis K. (2007) Seasonal variation in the fatty acid composition of three Mediterranean fish: sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and picarel (*Spicara smaris*). Food Chemistry 103:725–728. doi: 10.1016/j.foodchem.2006.09.013

Zorica B, Vilibić I, Keč VČ, Šepić J. (2013) Environmental conditions conducive to anchovy (*Engraulis encrasicolus*) spawning in the Adriatic Sea. Fisheries Oceanography 22:32–40. doi: 10.1111/fog.12002

Zuur AF, Ieno EN, Smith GM. (2007) Analysing ecological data, Volume 1. Springer New York. doi: 10.1007/978-0-387-45972-1

Appendices

Publications

Accepted (ant chronological order)

- Brosset P., Lloret J., Muñoz M., Fauvel C., Van Beveren E., Marques V., Fromentin J.-M., Ménard F., Saraux C. (In press). Body reserves mediate trade-offs between life history traits: new insights from small pelagic fish reproduction. Royal Society Open Science.
- Van Beveren, E., Keck, N., Fromentin, J.-M., Stéphanie, L., Boulet, H., Labrut, S., Baud, M., Bigarré, L., Brosset, P., and Saraux, C. (In press). Can pathogens alter the population dynamics of sardine in NW Mediterranean? Mar Biol.
- Brosset P., Le Bourg B., Costalago D., Bănaru D., Van Beveren E., Bourdeix J-H., Fromentin J-M., Ménard F., Saraux C. (2016). Linking small pelagic dietary shifts and ecosystem changes in the Gulf of Lions. Mar Ecol Prog Series 554:157-171
- Van Beveren, E., Fromentin, J.-M., Rouyer, T., Bonhommeau, S., Brosset, P., and Saraux, C. (2016). The fisheries history of small pelagics in the Northern Mediterranean. ICES Journal of Marine Science. 73 (6): 1474-1484
- Brosset P., Ménard F., Fromentin J.M., Bonhommeau S., Ulses C., Bourdeix J.H., Bigot J.L., Van Beveren E., Roos D., Saraux C. (2015). Influence of environmental variability and age on the body condition of small pelagic fish in the Gulf of Lions. Mar Ecol Prog Ser 529: 219-231
- Brosset P., Fromentin J-M., Ménard F., Pernet F., Bourdeix J-H., Bigot J-L., Van Beveren E., Pérez Roda M.A., Choy S., Saraux C. (2015). Measurement and analysis of small pelagic fish condition: A suitable method for rapid evaluation in the field. J Exp Mar Biol Ecol 462:90–97
- Van Beveren E., Bonhommeau S., Fromentin J.-M., Bigot J.-L., Bourdeix J.-H., Brosset P., Roos D., Saraux C. (2014). Rapid changes in growth, condition, size and age of small pelagic fish in the Mediterranean. Mar Biol. 161(8): 1809-1822

Submitted

- Brosset Pablo, Fromentin Jean-Marc, Van Beveren Elisabeth, Lloret Josep, Marques Virginie, Basilone Gualtiero, Bonanno Angelo, Carpi Piera, Donato Fortunata, Čikeš Keč Vanja, De Felice Andrea, Ferreri Rosalia, Giráldez Ana, Gücü Ali, Iglesias Magdalena, Leonori Iole, Palomera Isabel, Somarakis Stylianos, Ticina Vjeko, Torres Pedro, Ventero Ana, Zorica Barbara, Ménard Frédéric, Saraux Claire. Spatio-temporal patterns and environmental controls of small pelagic fish body condition from contrasted Mediterranean areas. Progress in Oceanography.
- Van Beveren Elisabeth, Fromentin Jean-Marc, Bonhommeau Sylvain, Nieblas Anne-Elise, Metral Luisa, Brisset Blandine, Jusup Marko, Bauer Robert, Brosset Pablo, Saraux Claire. Prey predator interactions in the face of management regulations: changes in Mediterranean small pelagics are not due to increased tuna predation. Canadian Journal of Fisheries and Aquatic Sciences.

Conference presentations

Ecology & Behaviour (Poster Session, May 2014, Montpellier)

What are the main determinants of the small pelagic fish condition in the Gulf of Lions?

Pablo Brosset, Frédéric Ménard, Jean-Marc Fromentin and Claire Saraux

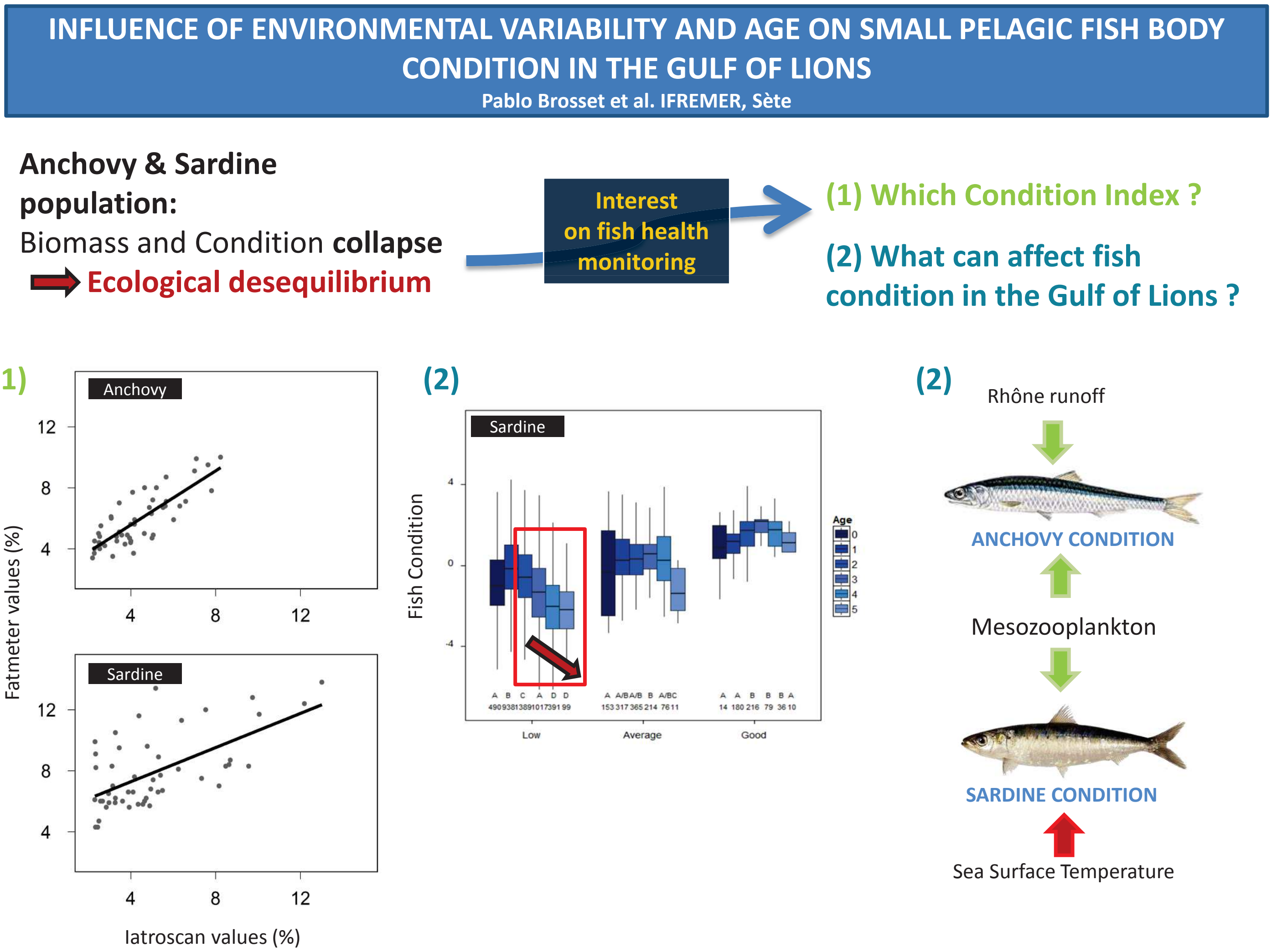
Abstract

Along with important changes in stock biomass and population structure, recent research highlighted long term fluctuations of small pelagic fish body condition in the Gulf of Lions indicating different periods during which anchovy and sardine were in an average (1992-2004), good (2005-2007) or poor (2008-2013) condition. Body condition is defined as the quantity of nutrient reserves and widely used in ecological studies to determine the nutritional or physiological state of an individual and give information about its future performances. In order to better understand the fluctuations observed in these two species, a detailed analysis was conducted to determine endogenous or environmental drivers of body condition. Research surveys and fishermen sampling allowed to establish a dataset including morphometric (i.e length and weight; used to calculate the Le Cren condition index) and physiological (e.g sex, age) characteristics of more than 30000 individuals (anchovy, sardine, sprat) from 1971 to 2013. Males and females were in similar condition regardless of the period (1992-2004, 2005-2007, 2008-2013). On the other hand, we found a significant interaction between age and period. Age-0 sardines were in much better condition during the last period than before, supporting recent observations of high recruitment levels. Further, condition decreased sharply with increasing age in the last period, offering potential causes to the observed disappearance of larger and older sardines. For all species, body condition exhibited both a clear seasonal pattern, peaking at the beginning of summer and interannual fluctuations. While both seasonal and interannual fluctuations were positively correlated respectively with the Western Mediterranean Oscillation (integrative indicator of river runoff, temperature and wind mixing) and the Rhone runoff in anchovies, they were mainly explained by temperature and chlorophyll a in sardines. These results show how body condition can offer insights into potential mechanisms driving population dynamics and its link with the environment.

Conservation Physiology of Marine Fishes (Poster Session, May 2015, Montpellier)

Influence of environmental variability and age on small pelagic fish body condition in the Gulf of Lions

Pablo Brosset, Frédéric Ménard, Jean-Marc Fromentin, Elisabeth Van Beveren and Claire Saraux



INFLUENCE OF ENVIRONMENTAL VARIABILITY AND AGE ON SMALL PELAGIC FISH BODY CONDITION IN THE GULF OF LIONS

Pablo Brosset et al. IFREMER, Sète

Anchovy & Sardine population:
Biomass and Condition **collapse**
➡ **Ecological desequilibrium**

Interest on fish health monitoring

(1) Which Condition Index ?

(2) What can affect fish condition in the Gulf of Lions ?

Figure .1 Abstract graphique (Pecha Kucha)

Colloque de l'Association Française d'Halieutique (Oral Session, July 2015, Montpellier)

Influence des variables environnementales et de l'âge sur la condition corporelle des petits pélagiques du Golfe du Lion

Pablo Brosset, Frédéric Ménard, Jean-Marc Fromentin, Sylvain Bonhommeau, Caroline Ulses, Jean-Hervé Bourdeix, Jean-Louis Bigot, Elisabeth Van Beveren, David Roos, Claire Saraux

Résumé

Les stocks d'anchois (Engraulis encrasicolus) et de sardine (Sardina pilchardus) du Golfe du Lion sont affectés depuis 2008 par une baisse drastique de la biomasse et des changements dans leurs distributions en taille et en âge. Une des hypothèses principales repose sur un problème de condition corporelle des poissons, qui influence directement l'investissement entre les traits d'histoire de vie. L'indice de condition relative a été calculé à partir de données (poids/taille) collectées de 1993 à 2013 sur plus de 43 000 poissons. Le cycle annuel de la condition des deux espèces est similaire, avec une condition minimale en février et maximale en juin. Des arbres de décisions (CART) ont permis d'identifier trois groupes d'années, de bonne, moyenne et mauvaise condition et de montrer que le sexe n'a pas d'influence sur celle-ci. De plus, la baisse de condition observée depuis 2008 chez les anchois et les sardines est plus marquée chez les sardines les plus âgées, un phénomène peut-être en lien avec le déséquilibre démographique observé. Les séries temporelles ont ensuite été analysées à l'aide d'un modèle linéaire généralisé (GLM) afin de rechercher les effets potentiels sur la condition des variables abiotiques (température, Oscillation Ouest Méditerranéenne (WeMO) et débit du Rhône) et biotiques (Chlorophylle a et six classes de plancton). Le GLM met en évidence une forte corrélation positive de la condition des deux espèces avec le compartiment mesozooplanctonique. Pour les sardines, les diatomées, la WeMO ont une influence positive quand la température a une influence négative. Pour l'anchois, un effet positif du débit du Rhône est souligné. Ces résultats supportent le fait que l'actuelle augmentation des températures et la réduction des phénomènes d'upwelling généralisée en Méditerranée Nord-Ouest, affectant la productivité primaire et secondaire pourrait se répercuter sur la condition des petits pélagiques.

ICES Annual Science Conference 2016 (Poster Session, September 2016, Riga)

Body reserves mediate trade-offs between life history traits: new insights in small pelagic fish reproduction

Brosset Pablo, Jean-Marc Fromentin, Claire Saraux

Abstract

Limited resources in the environment prevent individuals to simultaneously optimize all life history traits, resulting in trade-offs. In particular, the cost of reproduction is well known to negatively affect energy investment in growth and maintenance. Here, we investigated these trade-offs during contrasted periods of high versus low fish body condition (before/after 2008) in the Gulf of Lions. Female reproductive allocation and performance in anchovy (Engraulis encrasicolus) and sardine (Sardina pilchardus) were examined based on morphometric historical data from the 70s and from 2003 to 2015. Additionally, potential maternal effects on egg quantity and quality were examined in 2014/2015. After 2008, the gonadosomatic index increased for sardine and remained steady for anchovy, while a strong decline in mean length at first maturity indicated earlier maturation for both species. Regarding maternal effects, for both species egg quantity was positively linked to fish size but not to fish lipid reserves, while the opposite was true for egg quality. Atresia prevalence and intensity were rather low regardless of fish condition and size. Finally, estimations of annual total numbers of eggs spawned indicated a sharp decrease for sardine since 2008 but a slight increase for anchovy during the last 5 years. This study revealed a biased allocation towards reproduction in small pelagic fish when confronted with a really low body condition. This highlights that fish can maintain high reproductive investment potentially at the cost of other traits which might explain the present disappearance of old and large individuals in the Gulf of Lions.

Reports and working groups

EcoPelGol project Final report (March 2016, Sète)

Saraux C., Van Beveren E., Brosset P., Fromentin J-M.

Understanding of small pelagic fish fluctuations in the Gulf of Lions. Test of bottom-up and top-down processes, effect of environmental conditions on small pelagic fish abundance, condition, distribution and life-history traits, epidemiology.

ICES working group on fish condition (WKFICON, November 2016, Girona)

The WKFICON workshop is aimed at opening up new directions in marine fish condition research with a potential impact on the assessment and management of exploited resources and marine ecosystems. It will bring together marine scientists with different expertise in the field of marine fish condition to discuss on recent advances in that field, the gaps in knowledge, the future research needs and the use of fish condition as indicator of population and marine ecosystem conditions. It will also foster collaboration among scientists working on exploited fish condition from an ecological and fisheries management perspective.