REPRODUCTIVE AND CONDITION STATUS OF "COLD WATER" MARINE FISH: NEW INSIGHTS FROM A CHANGING ENVIRONMENT

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Universitat de Girona

Doctoral thesis

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Doctoral Program in the Environment

Supervised by

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Thesis submitted in fulfilment of the requirements to obtain the Degree of Doctor at the University of Girona

Universitat
de Girona

Dra. Marta Muñoz Frigola and Dr. Josep Lloret Romañach, from the University of Girona

DECLARE:

That the thesis entitled **Reproductive and condition status of "cold water" marine fish: new insights from a changing environment** presented by Alba Serrat Llinàs in the pursuance of a doctoral degree has been completed under our supervision and meets the conditions required to opt for an International Doctorate.

In witness thereof, we hereby sign this document.

Dra. Marta Muñoz Frigola

Dr. Josep Lloret Romañach

Marta

Lloret

Girona, 4 December 2018

Al mar, i aquells qui me l’han mostrat.

A la mare i al pare, a la iaia.

A Bahia Cinto.

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LIST OF PUBLICATIONS DERIVED FROM THIS THESIS

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Serrat, A., Lloret, J. & Muñoz, M. **Trade-offs between biological traits in a "cold-water" fish in the Mediterranean: the case of blue whiting (*Micromesistius poutassou*).** Manuscript under review.

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LIST OF ABREVIATIONS

This list compiles the abbreviations and acronyms used in this thesis

αATR	alpha atresia
AS	actively spawning
AT1	*Anisakis* spp. type I
AT2	*Anisakis* spp. type II
β	beta atresia
BAL	Balearic Sea
βATR	beta atresia
BF	batch fecundity
BLD	blood on capillaries
C	body cavity
CAO	cortical alveolar oocyte
CI	interval of confidence
cNO_i	total number of $stage_i$ oocytes corrected for shrinkage
CNR	circumnuclear ring
CNRO	circumnuclear ring previtellogenic oocyte
cOD_i	mean $stage_i$ oocyte diameter corrected for shrinkage
DEB	dynamic energy budget model
DV	development
Eα	early alpha atresia
EPVO1	early previtellogenic oocyte 1
EPVO2	early previtellogenic oocyte 2
EW	eviscerated weight
F	female
FS	fat stage
GLL	Gulf of Lion
GSI	gonadosomatic index
GW	gonad weight
GW_f	formalin-fixed gonad weight
HA	*Hysterotilacium aduncum*
HSI	hepatosomatic index
HYO	hydrated oocyte
$_i$	stage of oocyte development
I	intestines
IA	intensity of atresia

ICES	International Council for the Exploration of the Sea
IM	immature
IY	intensity of infection
$_j$	specimen
Kn	Le Cren's condition factor
L	oocyte long axis
L	liver weight
L3	third-stage larvae
L4	fourth-stage larvae
L_{50}	length at which 50% of the specimens are mature
$L\alpha$	late alpha atresia
LDW	lipid dry weight in the liver
LW	liver weight
LWW	lipid wet weight in the liver
M	male
MAB	most advanced batch
MAO	most advanced oocyte
MAT	gonad development phase
mdIY	median intensity of infection
MFSI	mesenteric fat somatic index
MFT	morphotype
MFW	mesenteric fat weight
MLS	minimum landing size
mnIY	mean intensity of infection
MNO	migratory nucleus oocyte
MON	month
Mu	muscle
n	number of samples
NaCl	sodium chloride
NI	non identified
NO_i	total number of stage$_i$ oocytes
NW	North-western
O	number of oocytes
OD	oocyte diameter
OD_i	stage$_i$ oocyte mean diameter
ODM	oocyte dry mass
ODv_i	volume-based stage$_i$ oocyte mean diameter

OPD$_i$	oocyte packing density of stage$_i$ oocyte
OSFD	oocyte size frequency distribution
OVW	ovary subsample weight
P	prevalence
PC	pyloric caeca
PG	primary growth
PR	parasite richness
PVO	previtellogenic oocyte
ρ	Spearman's coefficient of correlation, Rho
RBF	relative batch fecundity
ρo	density of the ovary
RS	regressing
RT	regenerating
S	oocyte short axis
SC	spawning capable
SD	standard deviation
SF	spawning fraction
Si	Stomach lumen
SS	spawning season
SSB	spawning stock biomass
SSI	spleenosomatic index
SST	sea surface temperature
SW	spleen weight
Sw	stomach wall
TEP	tetraphyllidean plerocercoid
TIS	tissue
TL	total length
TL-based cNO$_i$	relative number of stage$_i$ oocytes corrected for shrinkage standardized for total length
TW	total weight
VTO	vitellogenic oocyte
Vv$_i$	volume fraction of stage$_i$ oocyte diameter o of ovary element

SUMMARY

Cold water fish species in temperate and subtropical regions are exposed to stress caused by fishing and climate change. There is mounting concern regarding the status of many stocks of targeted or bycatch species and an assessment of their vulnerability to these extrinsic stressors is urgently required. Variations in species' responses to these stressors are determined not only by the nature of the stressor itself and the environmental context but also by species-specific attributes. However, for many marine species, data on their biological and ecological traits and on the factors controlling the variation of these traits is scarce or needs updating and, subsequently, this seriously limits our capacity to assess species sensitivity and to predict the species-specific impact risks and changes in productivity.

The survival of individuals is largely dependent on their health status (e.g. nutritional condition, absence of diseases, etc.) allowing the successful completion of their life cycle. On the other hand, the replenishment of populations depends on the success of the reproduction and recruitment. Faced with unfavourable environmental conditions, fish may respond with environmentally-mediated, self-regulatory processes of energy allocation – favouring health over reproduction, or vice versa – and these can have important effects on population dynamics.

In this context, the aim of this PhD Thesis was to generate critical knowledge on the main reproductive traits and health status of exploited fish species with an affinity for cold waters in the Gulf of Lions, Catalan Sea and Balearic Sea (NW Mediterranean) and Galician waters (Atlantic), inhabiting close to the warmer edge of their distribution area. Three case studies of "cold water species" were selected (*Argentina sphyraena*, *Micromesistius poutassou* and *Merluccius merluccius*) and, in each case, various biological traits – including the factors regulating early oogenesis, reproductive tactics, physical condition, parasitism and energy allocation trade-offs – were examined.

In addition to providing insights that fill gaps in our knowledge and update the available information on the reproductive tactics and health status of each of the selected species, special emphasis was placed on the following related aspects. In the first case-study involving *A. sphyraena*, differences were found in condition and reproductive capacity (egg quality and quantity) between two environmentally-different regions of the north-western Mediterranean Sea, suggesting that this species is better adapted to the colder and more productive Gulf of Lion waters than to the Balearic Sea warmer and less productive waters.

In the second case study, involving *M. poutassou* from the north-western Mediterranean, intensity-dependent relationships between parasitism by nematodes and fecundity, condition and spleen size are analysed. Parasitism appears to be higher in individuals with enhanced feeding ability (e.g. better-conditioned or larger specimens), and is related to lower batch fecundity and higher spleen size index, which is linked to immune activity. In the third case study, involving *M. merluccius,* oocyte development was quantified from the earliest oocyte stages and this revealed unexpected spawning-related dynamics, already at primary growth stages, which blurred the current definitions of fundamental terms related to egg production estimation methods.

This thesis contributes to the improvement of egg production estimation methods and to the understanding of fundamental biological mechanisms and their variability. But overall, the results are relevant not only from a theoretical point of view, but have implications for fisheries management and food safety; they support the need for a broader perspective when assessing stocks that takes the health status of fish populations into account.

Overall, the results of this thesis show the occurrence of distinct, condition-mediated trade-offs that suggest a certain degree of plasticity in the biological traits and support the idea that species-specific biological traits and plasticity partially influence a population’s sensitivity and response to external stressors such as climate change and fishing. Further research on the plasticity of these traits could tell whether this variability may, to some extent, buffer the impacts of short-term environmental pressures.

RESUM

A les regions temperades i subtropicals les espècies de peixos marins amb afinitat per a les aigües fredes i comercialment explotades poden estar patint estrès a causa de la pesca i el canvi climàtic. En aquest context, i donada la creixent preocupació per a l'estat del estocs ja sigui directament explotats o de captura accidental, esdevé fonamental avaluar la seva vulnerabilitat enfront les pressions esmentades. La resposta de les espècies a aquests factors d'estrès no només ve determinada per la pròpia naturalesa de la pressió i el context ambiental, sinó que també està condicionada pels atributs específics de cada espècie. No obstant, la manca de informació actualitzada sobre les característiques biològiques i ecològiques de moltes espècies marines, i sobre els factors que en controlen la seva variació, dificulta la tasca d'avaluar la sensibilitat de les espècies, predir el risc d'impactes i pronosticar canvis en la productivitat dels estocs.

L' estat de salut (estat nutricional, absència de malalties, etc.) dels individus determina l'èxit en el desenvolupament dels seus cicles vitals i les probabilitats de supervivència. Per altra banda, la renovació de les poblacions depèn de l'èxit de la reproducció i del posterior reclutament. En situacions ambientals desfavorables, els peixos poden respondre amb processos autoreguladors de la distribució de l'energia entre reproducció i salut, fet que pot repercutir a la dinàmica poblacional.

Aquesta tesi ha estat ideada per tal de generar coneixement crític sobre les principals característiques reproductives i l'estat de salut de les espècies explotades amb preferència per a aigües fredes, que viuen a l'hemisferi nord properes al límit sud de la seva àrea de distribució, en aquest cas al Golf de Lleó, la Mar Catalana i la Mar Balear. Es van seleccionar tres casos d'estudi de "espècies d'aigua freda" (*Argentina sphyraena*, *Micromesistius poutassou* i *Merluccius merluccius*) i es van examinar diferents trets biològics que comprenen des dels factors que regulen les etapes més inicials de la ovogènesi fins a diverses característiques reproductives, així com l'estat de condició, el parasitisme i els balanços en la distribució de l'energia.

A més de proporcionar coneixements dels que no es disposava i actualitzar la informació sobre les característiques reproductives i l'estat de salut de cadascuna de les espècies seleccionades, cada cas d'estudi s'ha focalitzat en un aspecte addicional relacionat. Així doncs, en el primer cas d'estudi, es van trobar diferencies en la capacitat reproductiva (qualitat i quantitat d'ous) i la condició de *A. sphyraena* de dues regions del nord-oest del Mediterrani, ambientalment diferents. Aquest fet suggereix que per a aquesta espècie son

més favorables les aigües del Golf de Lleó (més fredes i productives) que les de les Balears. En el segon cas d'estudi es van analitzar les relacions entre el parasitisme per nematodes i la reproducció i condició de *M. poutassou* al nord-oest del Mediterrani. Aparentment, els individus amb millors capacitats d'alimentació (ja sigui individus en millor condició o bé de talles grans) estan parasitats amb més freqüència i intensitat. Així mateix es va trobar una relació negativa amb la fecunditat i positiva amb l'activitat immunològica (estimada a partir de la mida relativa de la melsa). En el tercer cas d'estudi, es va quantificar el desenvolupament ovocitari de *M. merluccius* des dels estadis més inicials. D'aquesta manera es van detectar dinàmiques relacionades amb la posta ja en els estadis ovocitaris en creixement primari. Aquests resultats inesperats qüestionen les definicions actuals de conceptes fonamentals sobre l'estratègia reproductiva i que afecten directament els mètodes d'estimació de la producció d'ous.

A grans trets, els resultats d'aquesta tesi demostren l'existència de diversos equilibris dinàmics mediats per la condició del peix que suggereixen cert nivell de plasticitat en els trets biològics de les espècies i donen suport a la idea que les característiques biològiques especifiques de cada espècie i la seva plasticitat influeixen en la sensibilitat de les poblacions i determinen la seva resposta a pressions externes tals com el canvi climàtic i la pesca. Però per saber fins a quin punt aquesta plasticitat podria contribuir a esmorteir parcialment els impactes de les pressions ambientals a curt termini, cal més recerca .

En resum, aquesta tesi proporciona una base científica que pot ser rellevant no només per a científics sinó també per a la gestió de la pesca i per a la seguretat alimentaria, ja que dels resultats n'emergeix el suggeriment de dotar d'una perspectiva més amplia les avaluacions dels estocs, tot considerant l'estat de salut de les poblacions de peixos, al mateix temps que aporta contribucions per a la millora dels mètodes d'estimació de la producció d'ous i per a la comprensió de mecanismes biològics fonamentals i la seva variabilitat.

RESUMEN

En las regiones templadas i subtropicales las especies de aguas frías comercialmente explotadas están en riesgo de sufrir estrés a causa de la pesca i el cambio climático. En este contexto, y dada la creciente preocupación por el estado de muchos stocks pesqueros, es de urgente necesidad evaluar su vulnerabilidad a las ya mencionadas presiones extrínsecas. La variación de las respuestas de las especies a estos estresores no solo está determinada por la propia naturaleza del estresor y el contexto ambiental, sino que también viene condicionada por los atributos específicos de las especies. Sin embargo, para muchas especies escasea la información actualizada sobre los rasgos biológicos y ecológicos y sobre los factores que controlan su variabilidad, hecho que limita la evaluación de la sensibilidad de las especies, la predicción de su riesgo de impacto y el pronóstico de cambios de productividad.

Las probabilidades de supervivencia de los individuos están estrechamente relacionadas con su estado de salud (estado nutricional, ausencia de enfermedades, etc.) que condiciona el éxito en el desarrollo de sus ciclos vitales. Por otro lado, la reposición de las poblaciones depende del éxito de la reproducción y del reclutamiento. Ante situaciones ambientales desfavorables, los peces pueden responder con procesos de autorregulación de la asignación de la energía entre la reproducción y la salud, mediados por las condiciones ambientales y que pueden tener importantes consecuencias en la dinámica poblacional.

En este marco, la presente tesis doctoral tiene el objetivo de generar conocimiento crítico sobre las principales características reproductivas y el estado de salud de las especies explotadas con preferencia por aguas frías que viven cerca del límite sur de su área de distribución en el hemisferio norte, en este caso: el Golfo de León, el Mar Catalán y el Mar Balear Se seleccionaron tres especies de “agua fría” como casos de estudio (*Argentina sphyraena*, *Micromesistius poutassou* y *Merluccius merluccius*) y para cada uno de ellas se estudiaron diferentes rasgos biológicos, desde los factores que regulan la ovogénesis temprana hasta las distintas tácticas reproductivas, así como el estado de condición, el parasitismo y los balances en la distribución de la energía.

A parte de proporcionar nueva y actualizada información sobre las estrategias reproductivas y el estado de salud de estas especies, para cada caso de estudio se examinaron además distintos aspectos relacionados. De este modo en el primer caso de estudio se encontraron diferencias en la condición y la capacidad reproductiva (calidad y cantidad de huevos) de *A. sphyraena* de dos regiones ambientalmente diferentes del

noroeste del Mediterráneo. Los resultados sugieren que esta especie encuentra unas condiciones más optimas en el Golfo de León (aguas más frías y productivas) que en las Baleares (aguas más cálidas y oligotróficas). En el segundo caso de estudio se analizaron las relaciones entre parasitismo por nematodos y la reproducción y condición de *M. poutassou* en el noroeste del Mediterráneo. Aparentemente, los individuos con mayor habilidad para la obtención de alimento (individuos en mejor condición o de mayores tallas) presentaron mayor presencia de parásitos. Niveles altos de parasitismo se relacionaron con menor fecundidad y mayor actividad inmunológica. En el tercer caso de estudio, se cuantificó el desarrollo ovocitario desde los estadios más iniciales en *M. merluccius* y se hallaron inesperadas dinámicas relacionadas con la puesta desde los estadios de crecimiento primario de los ovocitos. Este hallazgo cuestiona las definiciones de conceptos fundamentales relacionados con los métodos para estimar la producción de huevos.

En general, los resultados de esta tesis muestran la existencia de distintos balances dinámicos mediados por el estado de condición, sugiriendo así cierto nivel de plasticidad de los rasgos biológicos que podría amortiguar hasta cierto punto los impactos de las presiones ambientales a corto plazo. Los resultados dan soporte a la idea de que los rasgos biológicos específicos de cada especie y su plasticidad influencia la sensibilidad de las poblaciones y sus respuestas a forzamientos externos tales como la pesca o el cambio climático.

Esta tesis proporciona una base científica que creemos que pude ser relevante no solo para científicos sino también para la gestión de pesca y la seguridad alimentaria. En esta línea, los resultados contribuyen a la mejora de los métodos de estimación de la producción de huevos y a una mejor comprensión de mecanismos biológicos fundamentales y de su variabilidad, y sugieren la necesidad de dotar de una perspectiva más amplia a las evaluaciones de los stocks pesqueros, considerando el estado de salud de las poblaciones de peces.

1.INTRODUCTION

This section provides an overview of the background of this thesis (topics, context and subjects).

1.1. THEORETICAL BACKGROUND

What are life history traits and why do they matter?

The population responses to external forcing is determined by the life history and biological traits and its plasticity (Bye, 1984; Quetglas et al., 2016). Life history traits are those major attributes of an organism's life cycle, e.g. production of offspring, growth, condition, life span, age- or size- at maturity and mortality (Stearns, 1992). In a specific environmental situation, these traits can have distinct phenotypic expression adapted to succeed on their life cycles, then we talk about tactics; whereas, the set of heritable life history traits of one species over the full range of environmental situations is known as the life history strategy (Wootton, 1998).

Individual survival chances of fish are highly reliant upon the health status, condition or fitness. The term "health", does not only refers to the absence of diseases but also to the normal functioning of the organism at a biochemical, physiological and behavioural level that allows the successful occurrence of life cycles (Depledge & Galloway, 2005), thus health variables are important for the future population success. Fish body condition, which is used as a proxy of fitness, is an estimate of nutritional state based on the relative amount of energy reserves (Jakob et al., 1996). Roughly, the oxidation of food liberates free energy that allows the synthesis of ATP (adenosine triphosphate) which powers all the metabolic processes. Once the basic metabolic requirements are met, the energy surplus is allocated to growth, reproduction and storage (Fig. 1). Energy is stored in the fish body principally in two kinds of compounds, i.e. proteins and lipids, that will be mobilized and used consecutively (first lipids and in a late stage during starvation, proteins) for future vital processes during periods of high expenditure or low nutritional intake. Condition can be estimated by different indicators ranging from simple morphometric measurements such as length-weight relationships, morphophysiological indicators such as relative liver weight and biochemical quantifications such as lipid content (Lloret et al., 2014). Fish with higher relative amount of energy reserves are considered to be in better condition, indeed an optimum fish condition is that amount of energy that allows not just the basic maintenance, but also the regular occurrence of reproduction and post-spawning physiological activity (Rijnsdorp, 1990).

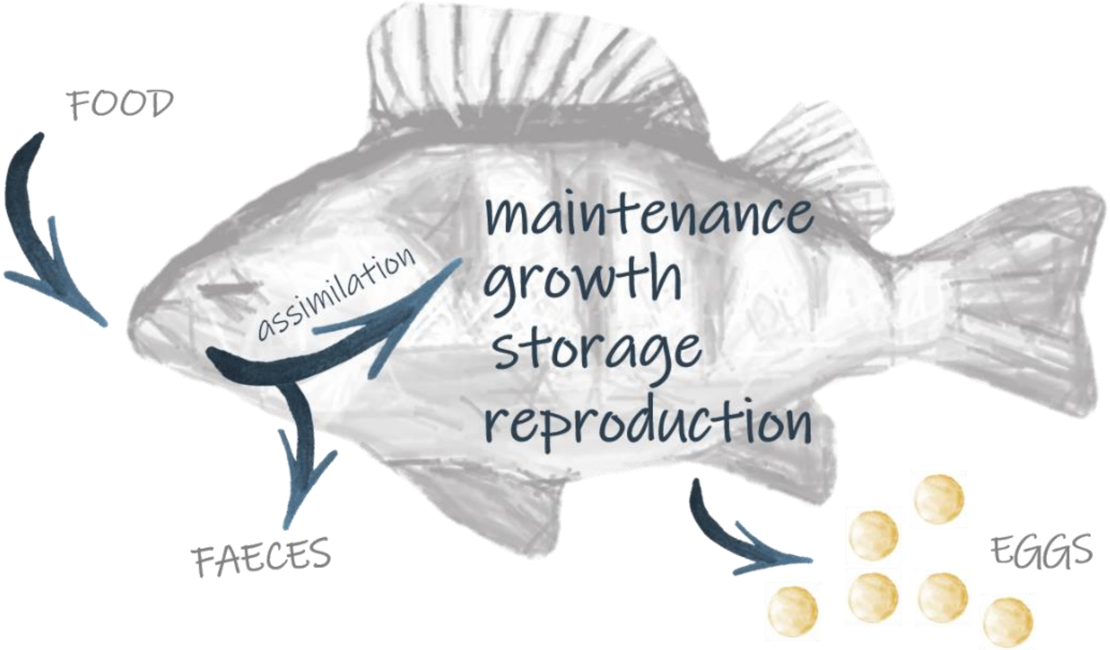


Fig 1. Scheme of energy allocation in fish. This figure is shown for illustrative purpose and arrows placement do not refer to anatomy but to energy fluxes. Adapted from McBride et al. (2013). Illustration: Alba Serrat Llinàs.

The temporal and spatial relationship between energy acquisition and energy allocation to reproduction determines the fish breeding strategy. Capital breeders are those that finance the reproductive output solely by previously acquired and stored energy; alternatively, income breeders fuel the reproduction by continuously acquired energy (Fig. 2) (Stephens et al., 2009). However, how reproduction costs are financed is suggested to be a continuum that goes from capital to income, including mixed strategies. Likely overlying this heritable trait, there is some degree of tactical flexibility that allows the maximization of life-time offspring production in response to environmental conditions (McBride et al., 2015). Actually, adaptive breeding strategy responses to seasonality have been suggested, i.e. strong seasonal environmental fluctuations promote capital behaviour whereas moderate and prolonged cycles are associated to income responses (Schultz & Conover, 1997). Deteriorated condition impairs normal gonad maturation and have been related to reduced reproductive potential in several fish species such as capelin (*Mallotus villosus*) and cod (*Gadus morhua*) in the North Atlantic (Lambert & Dutil, 2000; Orlova et al., 2010), and European hake (*Merluccius merluccius*) and bluemouth (*Helicolenus dactylopterus*) in the northwest Mediterranean (Lloret et al., 2008; Muñoz et al., 2010). Thus, the body condition has an influence on the reproductive processes (Rideout et al., 2005; Kennedy et al., 2007) and emerges as a key life history trait that is important for population success. In fact, there is increasing evidence that the maternal variables and the environmental conditions can truncate the direct relationship between spawning stock biomass (SSB) and reproductive potential which is a key parameter for stock assessment (Lloret et al., 2012).

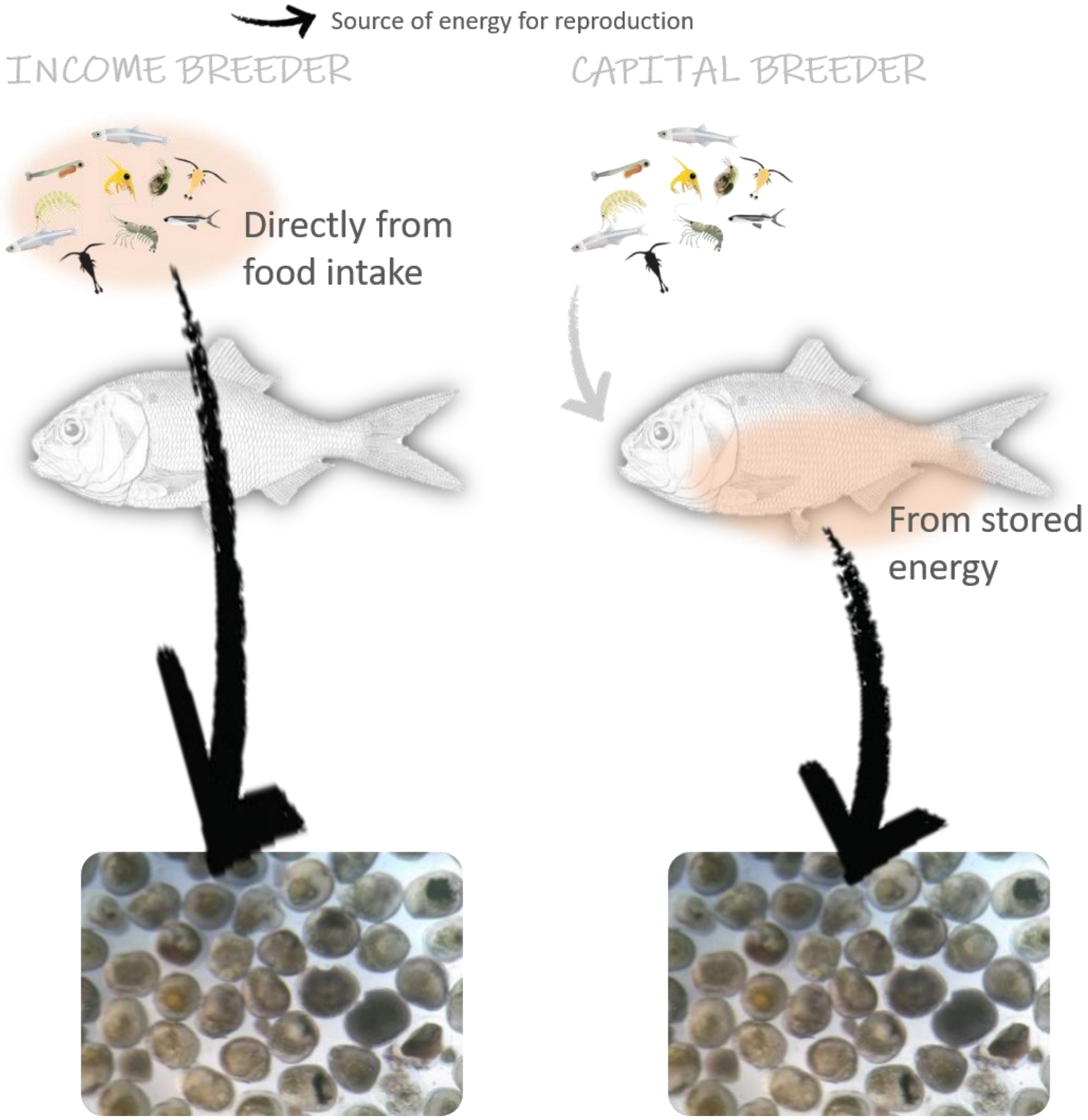


Fig 2. Breeding strategies of fish. The black arrow shows the energy allocation pathway in income breeders (A) and capital breeders (B), based on Stephens et al. (2009). Author: Alba Serrat Llinàs.

Population survival is determined by the relation between the replenishment of individuals through reproduction and the losses from mortality and emigration. Thus, the main outcome of any life history strategy is the reproductive potential, i.e. the ability of a fish stock to produce eggs and larvae that may eventually recruit into the adult population (Trippel, 1999). Despite in fisheries sciences the reproductive potential is classically assessed by the spawning stock biomass, its nature is more complex than that. For instance, it is constrained to a greater extent by egg production (Helfman et al., 2009), which includes egg quantity and quality. It is known that egg production is partially modulated by maternal effects (e.g. female size, body condition or physiology) (Alonso-Fernández & Saborido-Rey, 2011; Ohshimo et al., 2018) and ultimately influenced by experienced environmental conditions (Chambers, 1997; MacKenzie & Köster, 2004). However, we are still far from fully understanding egg production and the factors regulating its variability because it needs to be traced back to the underlying fundamental early processes during oogenesis (i.e. the new formation of eggs) (Fig. 3), which remains as a black box due to its complexity and the methodological challenges associated on studying this small cells (Kjesbu et al., 2011).

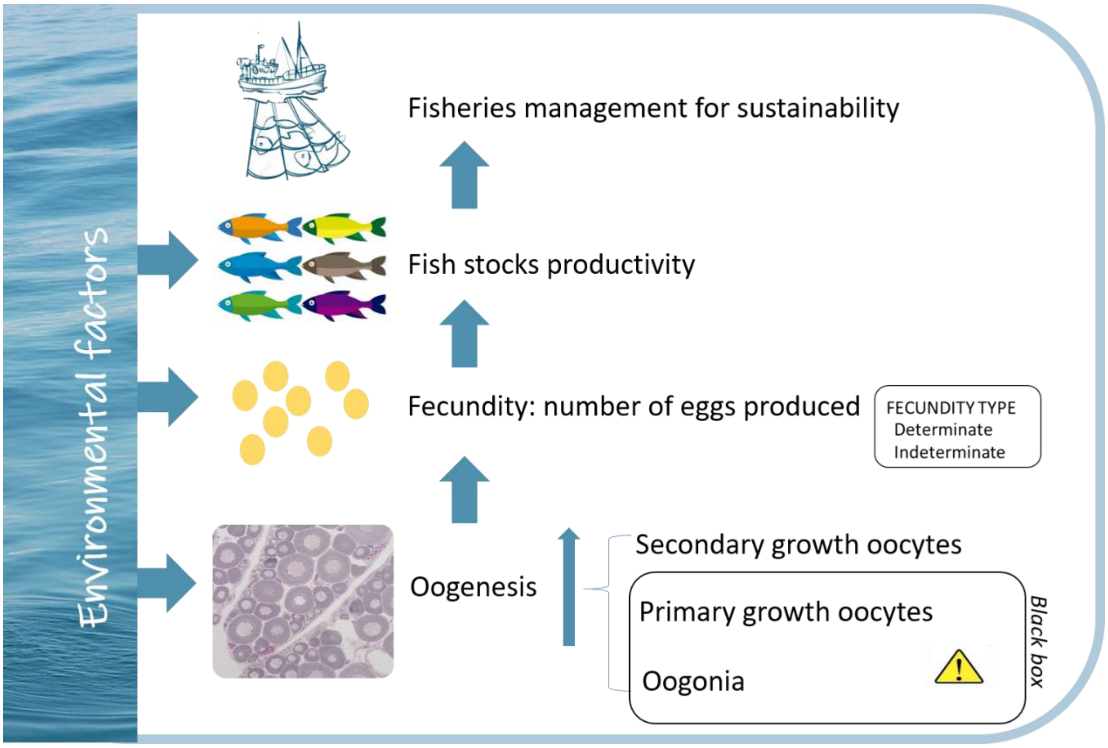


Fig 3. Tracing back fish productivity. Stock's reproductive potential is a key criterion for fisheries management and can be traced back to fundamental processes of early oogenesis which remain unclear. Author: Alba Serrat Llinàs.

Oogenesis is a universal but flexible process that underlies many reproductive traits. The course of oogenesis goes through three main steps: proliferation of oogonia in the lamellar germinal epithelium followed by development of successive primary and secondary oocyte stages, including ultimately oocyte maturation and ovulation (Grier et al., 2009). Along this steps the oocyte progress through successive oocyte stages (Fig. 4). Firstly, during the primary growth the oocytes are at a previtelogenic stage (PVO) and the folliculogenesis takes place. Secondary growth involves the formation of cortical alveoli (cortical alveoli oocyte stage, CAO) and the accumulation of yolk protein, i.e. vitellogenesis (vitellogenic oocyte stage, VTO). Finally, during oocyte maturation there is migration of the nucleus (MNO) and a steep increase on oocyte size due to the rapid intake of water, this is known as hydrated oocyte stage (HYO) (Grier et al., 2009) (Fig. 4).

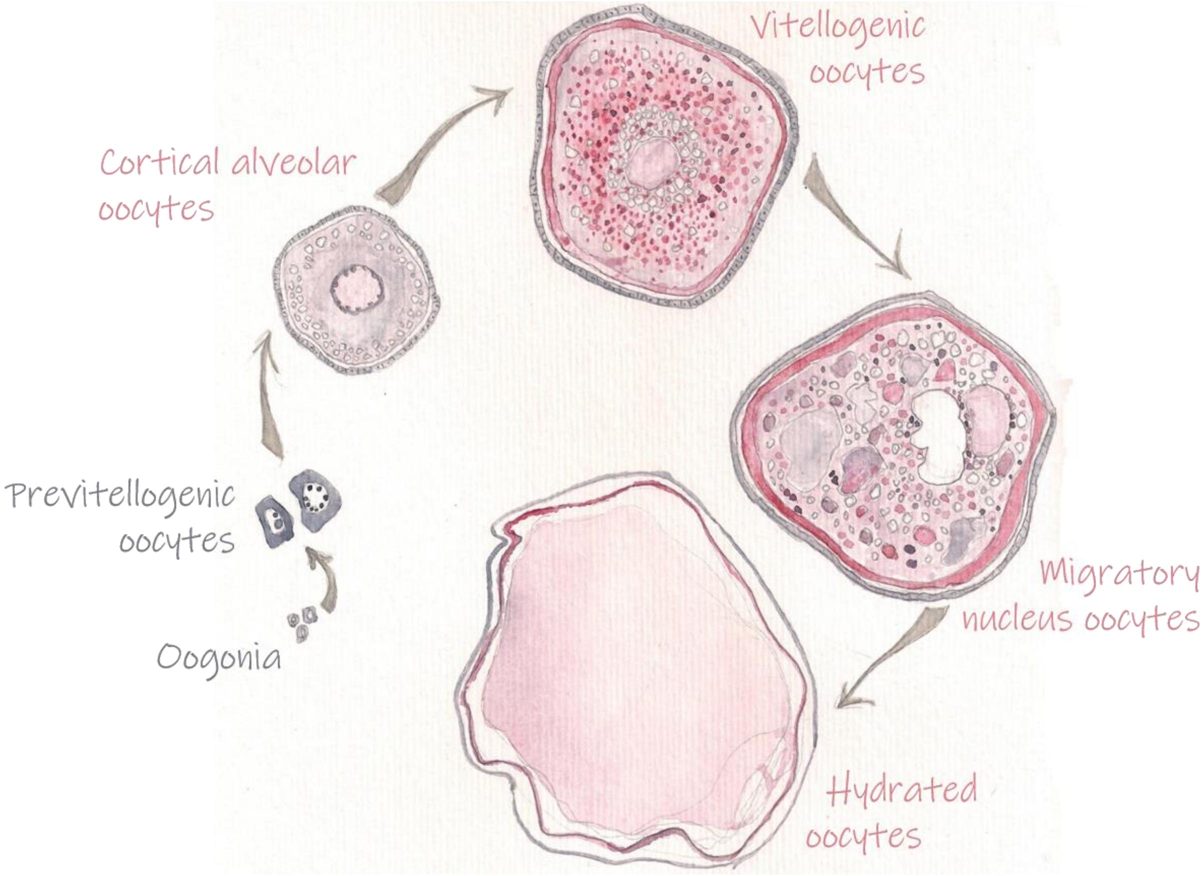


Fig 4. Scheme of oogenesis. Adapted from (Ganias & Lowerre-Barbieri, 2018). This figure is shown for illustrative purpose and oocytes are not scaled. Illustration: Alba Serrat Llinàs.

Traditionally the oocyte recruitment from primary to secondary growth has been described as synchronous, group synchronous or asynchronous (Wallace & Selman, 1981); however, these are misleading terms and it has been recently suggested to instead analyse the oocyte recruitment as unimodal/polymodal and continuous/discrete based on the shape of the oocyte size distribution frequency (Ganias & Lowerre-Barbieri, 2018). The temporal relation between oocyte recruitment from primary to secondary growth and the spawning season defines the fecundity pattern (or style), which goes from clear determinacy to indeterminacy (Hunter & Macewicz, 1985). In species showing a determinate fecundity, oocyte recruitment is completed before the onset of the spawning season; thus egg production, which is generally assessed by potential annual fecundity, can be estimated by the standing stock of pre-spawning secondary growth oocytes, since it is considered that it is in the beginning of this phase of development in which the oocytes that will be released during the spawning season are stored to be later matured in lots. Indeterminate species are, however, capable of recruiting oocytes to secondary growth all along the spawning season. Thus, direct knowledge of potential annual fecundity is unattainable because the total number of oocytes produced per season are not fixed prior, but should rather be approximated by multiplying the batch fecundity (number of eggs spawned in a single spawning event) for the number of batches released (Hunter et al., 1992). Therefore, the selection of the appropriate method for the estimation of egg production depends on the fecundity style in question (Fig. 3).

Generally, because food resources are limited, only one trait can be maximised at any time resulting in environmentally-mediated trade-offs of energy allocation (Stearns, 1989). One of the main trade-offs involves the traits related to reproduction and survival and it have relevant effects on populations dynamics (Gunderson, 1997). The decision of how to allocate energy between these traits is a function not just of food availability but also climatic conditions and individual features (McBride et al., 2015). These self-regulatory processes between reproduction and condition have an influence on host immunity (Rohlenová et al., 2011), e.g. higher investment in reproduction might decrease available energy to be invested in immune defence and subsequently facilitate parasite infection (Sheldon & Verhulst, 1996).

A higher parasite infection may have deleterious effects on fish health because it drains energy through distinct pathways (Walkey & Meakins, 1970) and subsequently impacts on fish reproduction (e.g. Heins & Baker (2003)); hence, the relationship between fish physiological status and parasitism is bidirectional. Therefore, ascertaining whether the physiological status of an organism is reflecting the effect of parasitism or the effect of several abiotic and biotic factors, is complicated due to the confounding effects (Rohlenová et al., 2011). In fact, energetic trade-offs as well as immune function and the dynamics of fish parasites, are influenced by water temperature (Rohde et al., 1995). Anomalies in the environment can result in diseases or sever infection levels because alterations in the balance between host resistance and parasite virulence and abundance (Iwanowicz, 2011). For instance, synergistic effects may result from the combination of higher temperatures and the parasitisation by certain species (e.g. Macnab & Barber (2012), Cereja et al. (2018)). Thus, the abundance and diversity of parasites in an organism is not only influenced by the host physiological status but also by the particular environment they inhabit, the climatic conditions and the anthropogenic activities such as species reintroduction (e.g. Horbowy et al. (2016)), the establishment of marine protected areas (e.g. Sasal et al. (2004)) or the fishing pressure (e.g. Wood et al. (2018)). Heteroxenus parasites (i.e. those that require more than two hosts to complete their live cycles), such as nematodes, due to the complexity of their life cycles are highly sensitive to external changes and can be regarded as comprehensive bioindicators of ecosystem's health and stability (Dzikowski et al., 2003).

It is known that several parasites may induce morphological and behavioural alterations (e.g. Barber et al. (2000)), result pathogenic or even turn into lethal, especially in severe infections (e.g. Poulin (2012); Woo & Buchmann (2012)). However, the actual virulence of parasites on their hosts health is mostly unknown and studies on its relation with the status of fish life history traits such as reproduction or condition are scarce and predominantly focused on freshwater species, e.g. Heins & Baker (2003) or Guidelli et al. (2011). Some studies have explored the relationship between parasitism and host condition in marine exploited fish species reporting divegent outcomes such as in hake, e.g. Ferrer-Maza et al. (2014) in the Mediterranean and Pascual et al. (2017) in te NE Atlantic; in cod, e.g. Horbowy et al (2016); in common sole, e.g. Durieux et al. (2007); or inred mullet, e.g. Ferrer-Maza et al. (2016). In fact, a recent meta-analysis of the condition-infection relationship in wild animals (Sánchez et al., 2018) showed a wide range of associations, from strongly positive to strongly negative, attributed to diverse underlying mechanisms. Regarding the relationships between parasitism and reproduction in marine fish, fewer studies are available but the reported responses of energy reserves in gonads, egg production and egg quality are also diverse (e.g. Sasal et al. (2001); Fogelman et al. (2009); Ferrer-Maza et al. (2014, 2015, 2016)). Thus despite the common assumption of negative consequences of parasite infection for host condition and reproduction, no generalization can be made and species-specific studies are needed for a better understanding of these relationships.

What are the relations between the environment and the life history traits?

Understanding the controls of biological processes is crucial to forecast productivity changes, especially in a context of climate change. Biological and ecological processes are directly and indirectly influenced by environmental factors. Temperature, winds, currents and precipitation are climatic factors affecting fish at all scales: from molecules, cells, individuals, populations, species, communities and up to ecosystems (Pörtner & Farrell, 2008; Rijnsdorp, 2009; Ottersen et al., 2010; Pörtner & Peck, 2010), hence these are determinants of marine fish populations dynamics. Temperature controls the metabolic processes rates because enzymatic reactions are strongly temperature-dependent. Fishes have evolved in order to optimize their physiological, ecological and reproductive performance in their thermal niche (Magnuson & DeStasio, 1997) (Fig. 5). Hence, sea warming is one of the main climate change-related driving forces causing changes in key life-history traits such as growth, condition and reproductive potential and in its phenology (e.g. Brander 1995; Rätz & Lloret 2003; Last et al. 2011; Petitgas et al. 2013). As an example, the timing of spawning behaviour, which is strongly dependent on sea water temperature, is expected to shift as a result of increased temperatures (Lett et al., 2010).

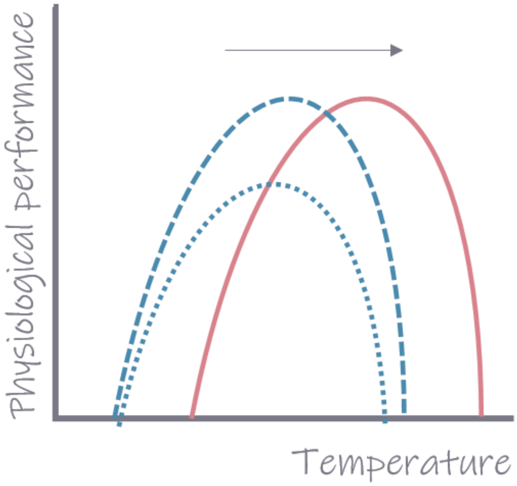


Fig 5. Temperature optimal range of physiological performance. Adapted from Willmer et al. (2009). This figure is shown for illustrative purpose and it is not scaled. Author: Alba Serrat Llinàs.

Extensive studies have reported effects of temperature on fish condition in different directions depending on the location of the population in relation to their distribution range. Positive relationships between temperature and body condition have been found in the coldest edge of the distribution area for several species such as Atlantic cod in north Atlantic waters (e.g. Rätz & Lloret (2003) or mackerel (*Scomber japonicus*) and jack mackerel (*Trachurus symmetricus*) in the northeast Pacific (Parrish & Mallicoate, 1995). On the contrary, negative relationships have been found close to the warmer edge of species distribution as for sole (*Solea solea*) in Portuguese waters (Vasconcelos et al., 2009) or sardine (*Sardina pilchardus*) in Iberian Atlantic waters (Rosa et al., 2010). Environmental changes also may have bottom-up effects through altered food availability as in the case of capelin (*Mallotus villosus*) condition in relation to the length of zooplankton reproduction season in the Barents Sea (Orlova et al., 2010). For instance, productivity blooms may be altered by modified sea currents and hydrodynamics such as up-welling periodicity and strength (Durrieu de Madron et al., 2011) or trophic-mismatches may result from temperature-induced phenological changes (Cushing, 1990). Additionally, synergetic effects may also occur, e.g. when temperature increase, metabolism tax rises and so do the energy demand and, as a consequence if food is limiting this may eventually lead to a reliance on stored energy (Otterlei et al., 1999).

Climate change affects fish demography because of changes in offspring production, growth and survival (e.g. O'Brien et al. (2000), Pörtner & Knust (2007)); distribution because of habitat shifts resulting in poleward movements (Walther et al., 2002); phenology due to advances in the timing of vital processes (Parmesan & Yohe, 2003); and ultimately affects the whole ecosystem and the human societies that exploit them (Fulton, 2011).

As a response to sea warming, species may shift their geographical distribution seeking for optimal environmental conditions (Cheung et al., 2009). Many studies have reported life-history traits in thermophilic species being enhanced with increased temperatures in transitional zones between temperate and subtropical waters such as the Mediterranean Sea and the Galician shelf, resulting in "meridionalization" of this areas due to expanded distribution and increased abundance of tropical species (Lloret et al., 2015), e.g. in the Mediterranean *Pomatomus saltatrix* (Sabatés et al., 2012), *Lampris guttatus* (Francour et al., 2010) or *Sphyraena viridensis* (Villegas-Hernández et al., 2014) have spread northwards. For instance, Villegas-Hernández et al. (2015b) suggested that increasing sea water temperatures contribute to increase the abundance of bluefish (*Pomatomus saltatrix*) in the northwest Mediterranean were this species benefits from local environmental conditions (e.g. better food availability) resulting in better conditioned specimens and the production of higher quality eggs.

What are cold-water species?

The latitudinal range of a species' distribution has been suggested as an indicator of species-specific thermal physiological preference (Neill, 1979, Thébaud & Blanchard, 2011). For the purpose of this thesis I considered cold-water species following the definitions in Lloret et al. (2015). This study established two groups of species: warm-water (those with an affinity to warmer waters than those of the north-western Mediterranean) and cold-water species (with an affinity for colder waters). Their approach was built on two criteria: the fish environment (tropical, subtropical, deep water and temperate; following Froese & Pauly (2015) and Hureau (2013)) and the mean latitude of their distribution. For temperate and deep-water fish, the mean latitude of their distribution area was located above the northern limit of the study area, and therefore based on the correlation between latitude and mean temperature in the north-western Mediterranean (Vargas-Yáñez et al., 2009), these species were categorized as cold-water species.

As already mentioned several studies have focused on warming effects on thermophilic species. Meanwhile, little attention has been paid to cold-water species that may be suffering physiological stress due to a reduction of their optimal habitat (Perry et al., 2005) and as a response shifting poleward or to deeper waters (Nye et al., 2009). In the northern hemisphere, pronounced changes are expected in the southern edges of reduction of their optimal habitat (Perry et al., 2005) and as a response shifting poleward or to deeper waters (Nye et al., 2009). In the northern hemisphere, pronounced changes are expected in the southern edges of species range where populations are inhabiting suboptimal conditions and are particularly sensitive to temperature (Pörtner & Peck, 2010). Stressed individuals may have a reduced food intake, lower body condition, lower growth rate, a less successful reproduction (or even failure) and suffer higher mortality (Schreck, 2000). In the Mediterranean Sea, while thermophilic species expand their areas of distribution, a contraction on the distribution area of cold-water species occurs (Quignard & Tomasini, 2000). In fact, a multidisciplinary study that collated fisheries data, gonads histological examinations, ichtyoplankton surveys, fishermen' ecological traditional knowledge and museum collections, suggested that sea-warming is partly responsible for the increase in the abundance of several warm-water species (e.g. *Dentex gibbousus*, *Lapris guttauts*, *Coryphaena hippurus*, *Thunnus alalunga, Seriola dumerili*, *Trachinotus ovatus*, *Sardinella aurita*) and the decrease in abundance of several cold-water fishes (e.g. *Alosa fallax*, *Argentina sphyraena*, *Molva macrophtalma*, *Sprattus sprattus*, *Molva molva*) in the northwestern Mediterranean sea (Lloret et al., 2015).

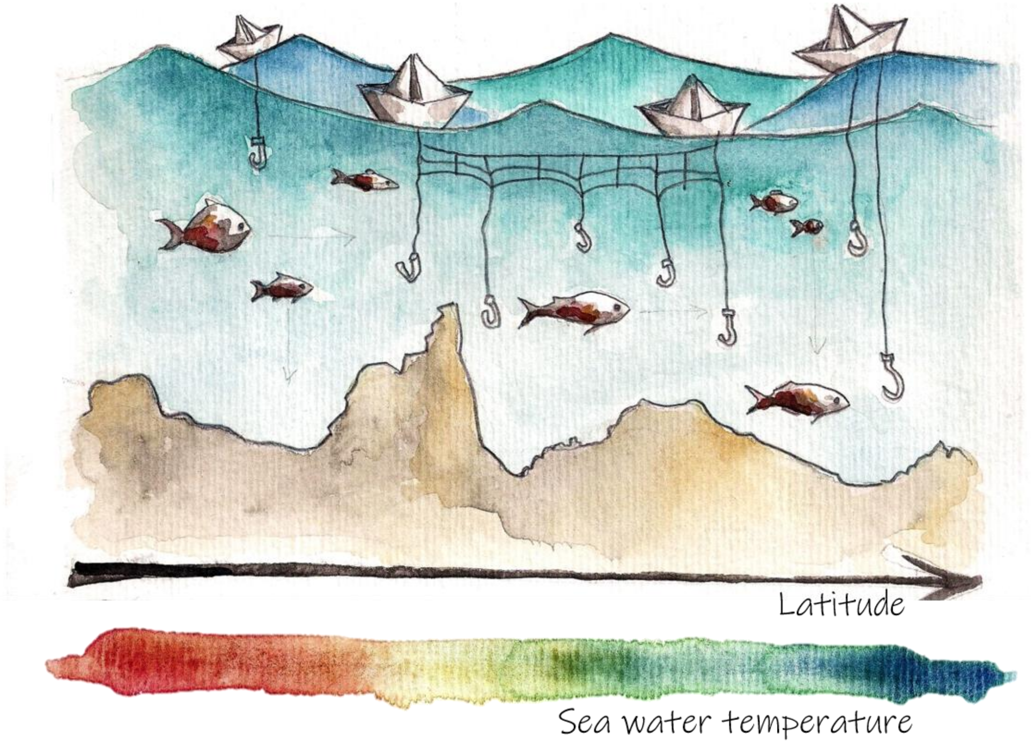


Fig 6. Pressures over exploited "cold-water" species. This figure is shown for illustrative purpose, representing fish under fishing pressure and shifting poleward seeking for colder water. Illustration: Alba Serrat Llinàs.

Moreover, exploited fish species with an affinity for cold waters are currently in an especially vulnerable situation in relatively temperate seas and oceans where they are threatened not only by increasing sea temperatures but mainly by overfishing (Fig. 6). Overfishing and several human-related impacts have probably contributed in the decline, range retraction and disappearance of certain cold-water species (Brander, 2010). But in such species, the relative importance of temperature and fishing as drivers of populations fluctuation is controversial (Worm & Myers, 2004), and furthermore, it is difficult to separate their respective effects which can act synergistically (Eero et al., 2011, Hjermann et al., 2004), e.g. increasing environmental fluctuations in reproductive success reduce resilience to fishing in cod (Kuparinen et al., 2014; Wang et al., 2014). Moreover, overexploitation, which imply either fishing mortality rates exceeding the maximum sustainable yield (Worm et al., 2006) or unbalanced harvesting (Jorgenson, 2007), i.e. non-random fishing (Heino & Godø, 2002), has been related to increased fish populations' sensitivity to environmental variability in certain species such as cod (Ottersen et al., 2006) and it can also induce evolutionary changes in life history traits, e.g. timing of spawning (Wright & Trippel, 2009), egg production (Botsford et al., 2011), growth rate and length at maturity (Kuparinen & Merilä, 2007).

2. APPROACH AND AIMS

This section outlines the approach, the main objectives and the structure of this PhD thesis.

2.1. APPROACH

The stress situation that exploited marine fish species with affinity for cold waters may be suffering due to the synergistic effects between the ongoing climate change and exploitation, is expected to be especially critical in the transition zone between subtropical and temperate regions. Bearing in mind the latter statement, this thesis is primarily motivated by the lack of knowledge regarding health and reproduction of exploited cold-water fish species, two key biological traits that play a key role in the survival of individuals and the replenishment of populations, and that are affected by both, fishing and environmental conditions. Altogether, understanding the species population status and their vulnerability to external forcing needs of accurate data on the expression of their biological and life history traits. Indeed, studies on their reproductive tactics (e.g. fecundity style, egg quantity and quality, timing of reproduction, spawning season duration, breeding strategy, oocyte development pattern, size at maturity) are needed to evaluate the reproductive potential of fish (Koslow et al., 1995). This information, complemented with data on their health status (body condition and parasitism), may serve as a basis to future assessments of population resilience, adaptation and vulnerability to extrinsic pressures, which is crucial for the effective management and conservation of exploited cold water species (Jakobsen et al. 2009; Lloret et al. 2014). The overall purpose was then to provide critical missing knowledge on key biological and life history traits of cold water species inhabiting these transition waters.

Three case studies were selected looking for exploited “cold-water” marine fish species inhabiting the transition between subtropical and temperate waters (north of the Iberian Peninsula) (Fig. 7) where the impact of climate change on the marine ecosystems is likely to be critical because of the observed sea warming and altered hydrodynamics in the last decades (see Methodology: Study Area). As stated above, cold-water species were defined following Lloret et al. (2015). In addition, reported evidences of large fluctuations on their abundance (see Methodology: Study species) and the feasibility of obtaining sufficient samples were criteria also taken into account on the selection of the case studies. The three selected species were *Argentina sphyraena*, *Micromesistius poutassou* and *Merluccius merluccius*. Bibliography was consulted to validate that the definition of the selected species as cold water species is in accordance with existing studies (i.e. Southward & Mattacola (1980); Jolivet et al. (2012); de Pontual et al. (2013); Lloret et al. (2015).

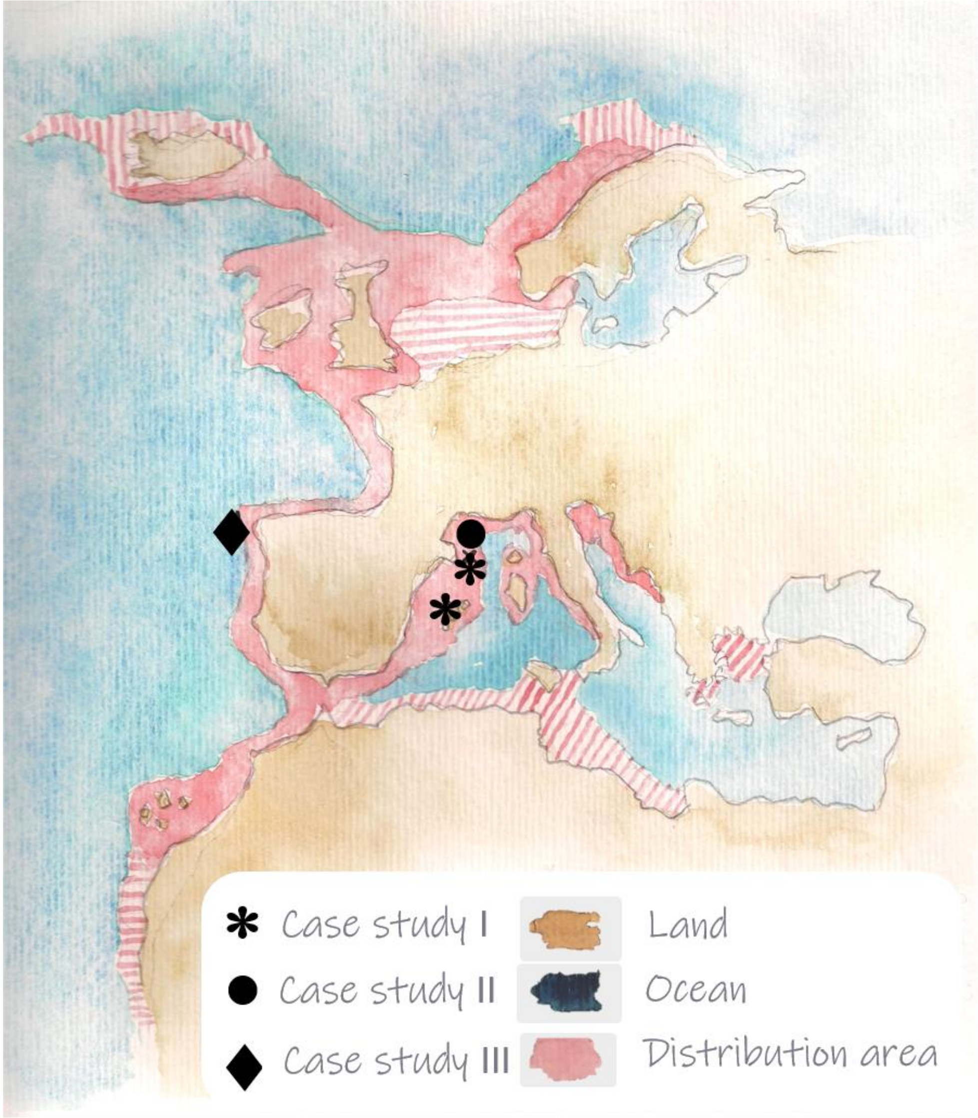


Fig 7. Sampling area of the case studies. The species distribution area is shown in an illustrative way (details can be found in the Methodology). In plain pink is the distribution area where the distribution of the three species are overlaps, in vertical pink lines the areas were only *Argentina sphyraena* is present, in diagonal pink lines the area where only *Micromesistius poutassou* is presents and in horizontal red pink lines the area where only *Merluccius merluccius* is present). The sampling location of each case study is also pointed (detailed sampling area is provided in the corresponding section of each case study). Illustration: Alba Serrat Llinàs.

These are species of different commercial importance (listed below in increasing order of marketable value), which translates into the degree of available knowledge on their biology, ecology and demography. Accordingly, the set of specific objectives addressed was different for each case study. For the first two species, samples were collected from the Mediterranean, and reproduction tactics and condition status were described following standard procedures and subsequently their trade-offs were analysed.

Besides this, in the first case study the reproduction and condition descriptions of *A. sphyraena* in the Gulf of Lion were additionally compared to a small sample from the Balearic sea, an environmentally-differing region. Because of the reported regression of this species in relation to sea warming in the NW Mediterranean (Lloret et al., 2015) it was expected to find better condition and reproductive potential in the colder and more productive Gulf of Lion waters than in the warmer and less productive waters of the Balearic Sea. In this first case study parasites were not considered because on a preliminary examination of 40 randomly chosen specimens only two fish showed a metazoan parasite each. In the second case study the assessment of the condition status of *M. poutassou* was complemented with the evaluation of parasitic infestation. Taking into account that parasitism effects on fish host are generally intensity-dependent (Sindreman, 1987) and that nematodes are reported to be one of the most widespread parasites of marine teleost and one of the most common and abundant parasites of blue whiting in several regions (e.g. Kusz & Treder (1980), Fernandez et al. (2005), Cruz et al. (2007)), this study focused on nematodes. Moreover, they are relatively easy to monitor and could be included in long term fish health assessments. Owing to the potential risk of human diseases associated with the consumption of fish infected by certain nematodes (i.e. Anisakids) extensive research has been done on the prevalence of this parasites in exploited species, but there are not many studies dealing with the relationships between parasitism, condition and reproduction (see Introduction: Theoretical background). Meanwhile, the last case study does not follow the same scheme but seeks a different aim which is specifically focused on the reproduction. This was prompted by the experienced difficulties on firmly state the fecundity style of the two first case study species and by the up-to-date generalized controversy on this issue (Ganias & Lowerre-Barbieri, 2018). I selected an extensively studied species from which already processed samples were available and used them to apply advanced methodologies to analyse in detail the early stages of egg production that may account for its variability and that may help not only to understand the underling fecundity style but also to discuss the process of oocyte recruitment that occurs in many other fish species. Descriptions of the case study species and study areas are provided in the Methodology chapter.

2.2 AIMS

The specific aims of each case study are as follows:

CASE STUDY I: Lesser silver smelt (*Argentina sphyraena*, Linnaeus 1758) in the NW Mediterranean. Specific objectives:

i) To provide new data on their reproductive tactics and condition status

ii) To investigate the self-regulatory processes between reproduction and condition

iii) To explore the regional variability of life history traits between two areas environmentally-differing

CASE STUDY II: Blue Whiting (*Micromesistius poutassou*, Risso 1827) in the NW Mediterranean. Specific objectives:

i) To provide new data on their reproductive tactics, condition status and parasitism

ii) To investigate the self-regulatory processes of the energy allocation patterns between reproduction and health

CASE STUDY III: European hake (*Merluccius merluccius*, Linnaeus 1758) in the North Atlantic. Specific objectives:

i) To improve the understanding of oocyte recruitment variability and fecundity style regulation underlying egg production

2.3. STRUCTURE OF THE THESIS

This thesis consists of seven chapters, following the traditional structure of Introduction, Aims and Approach, Methodology, Results, General Discussion, Conclusions and Bibliography. The Introduction describes the framework in which this research lays and defines the main topics and context. The Aims and Approach states the main goals and the outline of the thesis. The Methodology chapter provides information on the study species, study areas and methodological procedures. The Results chapter is divided in three main sections, corresponding each to one case study. For each case study a specific background and specific methodology are provided. Outputs derived from the specific objectives of each case study are showed and discussed in detail in every corresponding section of the Results chapter. The General Discussion chapter at the end of this dissertation integrates the outcomes from the three case studies which are finally interpreted from a broader perspective that may apply for other exploited cold water species inhabiting the southern area of their distribution range. A Conclusion chapter and the Bibliographic reference list end this volume. Supplementary information can be found in the Annex I.

3. METHODOLOGY

This chapter outlines the materials and methods used for the development of this thesis. The basic information on the study species and areas is provided and main methodological procedures are described in a generalized way. Some of the parameters and procedures here described apply for the three case studies, whilst others are only used in one of the sections. Particular details on the specific methodology as well as the data analysis are provided for each case study in the corresponding section from the Results chapter.

3.1. STUDY SPECIES

Argentina sphyraena

The lesser silver smelt (*Argentina sphyraena*, Linnaeus 1758) (Fig. 8) is a bathydemersal species, distributed along the Eastern Atlantic coast, from Norway to Western Sahara, and in the Western Mediterranean Sea. It occurs in the continental shelf and upper slope at depths of 50-500m (Halliday, 1969b), where it is caught as by-catch in mixed trawl fisheries.

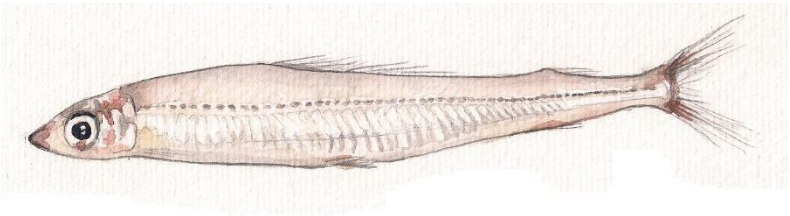


Fig 8. The lesser silver smelt, *Argentina sphyraena*. This figure is shown for illustrative purpose it is not scaled. Illustration: Alba Serrat Llinàs

This is a by-catch species and there is no stock assessment or capture production statistics available. Information on the biology of *A. sphyraena* is scarce and mostly based on Atlantic samples: a macroscopic description of population and reproductive parameters of North Atlantic populations (Halliday 1969 a, b, c), observations of meristic and basic morphometric traits (Schmidt, 1906; Cohen, 1958, 1961; Lee, 1963) and one study of age and growth in the Adriatic Sea (Ferri et al., 2017). Its preys are benthic species and the main feeding season is from May to November, while in May-June planktonic species were important preys (Halliday, 1969b). No spawning longitudinal migrations but bathymetric ontogenic migrations have been reported. Length at maturity is 14-16 cm for males and 16.5-18 cm for females (Halliday, 1969b). Spawning in the Atlantic takes place from March to June and the upward trend in water temperature after the winter minimum seems to act as a cue (Halliday, 1969b). A similar spawning season was reported for *Argentina silus* in the Atlantic (Bergstad, 1993). Halliday (1969a) suggested a batch spawning pattern for *A. sphyraena* but the reproductive strategy has not yet been defined and there is no estimation of its fecundity; likewise, no histological studies have been carried out to describe its oocyte recruitment pattern. Regarding energy storage, Halliday (1969a) observed that mesenteric fat was the main energy store for the species. Its population in the Western Mediterranean Sea showed a decreasing trend in abundance during recent decades, inversely correlated to the rise in sea water temperature (Lloret et al., 2015).

Micromesistius poutassou

The blue whiting, *Micromesistius poutassou* (Risso, 1827) (Fig. 9), is a cold-water mesopelagic gadoid species distributed along the continental slope of the North East Atlantic Ocean from the Iberian Peninsula and northwest of the Mediterranean Sea in the south to the Barents Sea in the north.

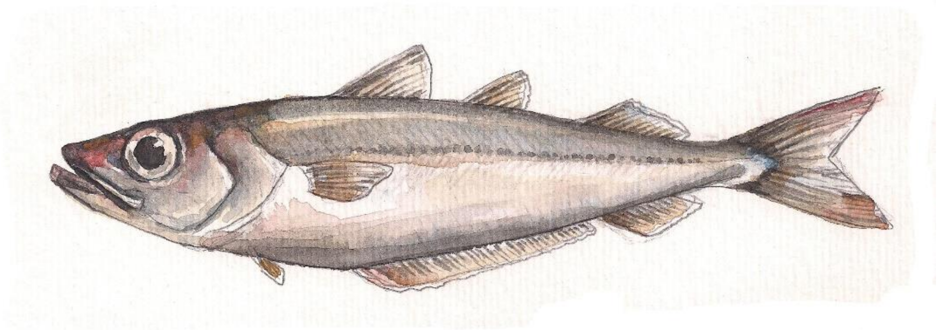


Fig 9. The blue whiting, *Micromesistius poutassou*. This figure is shown for illustrative purpose it is not scaled. Illustration: Alba Serrat Llinàs.

This is a widely consumed species. In 2016 its global capture production was almost 1300000 t (FAO 2019), from which 2000 t were from the Mediterranean where it is caught mostly by trawlers and where it is one of the most important fish in terms of landings (GFCM, 2016). Despite its commercial importance, information regarding its biology is nor abundant, nor modern and it is mostly referred to the Atlantic stock. In the Atlantic, blue whiting takes dial vertical migrations from 100-200 m during the night to deeper waters during the day (300-500 m) and seasonal migrations between the feeding grounds (late spring-summer) and the spawning area (early spring) (Bailey, 1982). Blue whiting spawning distribution in the North Atlantic is regulated by the oceanographic conditions, i.e. the subpolar gyre (Hatún et al., 2009). Spawning takes place in winter-spring between the 9º-10º isotherms (Mazhirina, 1978; Hátún et al., 2009). In the Portuguese coast spawning activity peaks in February coinciding with the highest values of condition index (Fulton's K) in winter (Gonçalves et al., 2017). Juveniles are distributed in shallower waters on the continental shelf and maturation takes place at length 18-20 cm (Bailey, 1982) and spawning typically starts at 19-24 cm length (ICES 2007). Females achieve greater lengths than males (Trenkel et al., 2015). Eggs are spawned in batches (Mazhirina, 1978) and deposited in cold water below the thermocline at 400-600 m, and larval condition has been linked to turbulence and wind mixing events (Kloppmann et al., 2001, 2002). The blue whiting main preys are zooplanktonic crustaceans (copepods, euphasids and decapods larvae) and fish larvae (Cabral & Murta, 2002) and its main predators are large fish such as hake (*Merluccius merluccius*) and cetaceans (Heino & Godø, 2002), thus it has an important role in the pelagic ecosystem. The population structure of blue whiting is poorly understood but there are evidences that support the existence of two independent populations in the North Atlantic (Pointin & Payne, 2014).

Regarding the Mediterranean stock, little is known: it reproduces in winter (December-March) (Palomera et al., 1983), recruits (<12cm total length) reach the sea floor in May-June at 150-350 m depth and no clear indications of major migrations exist however Spanish and Italian ports landings show some degree of seasonality increasing in autumn and winter (Bas & Calderon-Aguilera, 1989). Its population dynamics, stock size and landings, have experienced large fluctuations during the last decades sometimes but not always linked to large environmental changes (temperature, salinity, phytoplankton and zooplankton) (Payne et al., 2012) and in the Atlantic Ocean its populations are now slowly recovering from a sharp decrease (ICES 2015b). In the Mediterranean the landings of blue whiting have decreased during the last two decades from almost 30000 tons down to 2000 tons in 2016 (General Fisheries Commission for the Mediterranean, 2016).

Merluccius merluccius

The European hake (*Merluccius merluccius*, Linnaeus 1758) (Fig. 10) is a gadoid species widely distributed along the north east Atlantic from the Southern cost of the Black Sea to the Northern Africa and the Mediterranean Sea (Froese & Pauly, 2015). In the southern and western Europe, it is widely consumed and is one of the most important target species of commercial fisheries mostly caught by demersal trawlers, long-lines and bottom-set gillnets, i.e. in 2016 its global capture production was almost 150000 t (FAO, 2019).

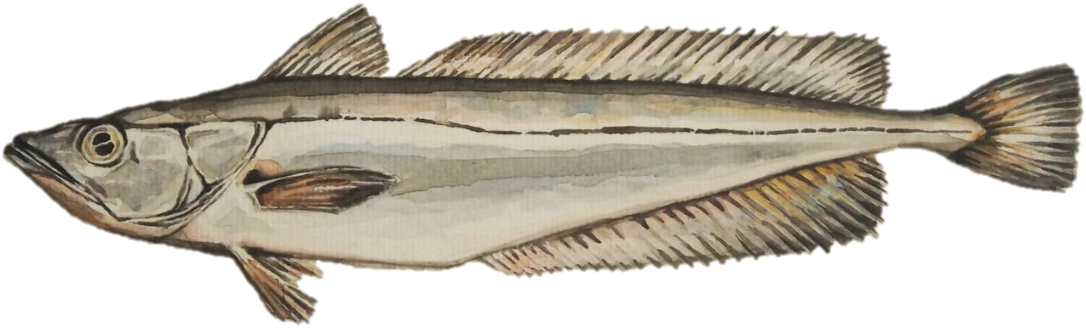


Fig 10. The European hake, *Merluccius merluccius*. This figure is shown for illustrative purpose it is not scaled. Illustration: Alba Serrat Llinàs.

Due to its marketable value it has been broadly studied. The averaged life-span is of 10 years (Cohen et al., 1990). In the Galician coast, length at maturation is between 35 and 50 cm (Domínguez-Petit et al., 2008). A protracted spawning season has been documented including assumingly two main peaks per year in the Galician coast, the Bay of Biscay and the Mediterranean (Murua & Motos, 2006; Domínguez-Petit, 2007; Recasens et al., 2008; Korta et al., 2010b). This is the longest spawning season documented for *Merluccius* genus. Generally, the species is considered a batch spawner (Murua & Saborido-Rey, 2003) with an asynchronous oocyte development and continuous indeterminate fecundity (Korta et al., 2010a) and an income breeding strategy (Domínguez-Petit et al., 2010). For the southern stock and the Mediterranean, a high degree of variability in several reproductive traits has been reported (e.g. Korta et al. (2010b), Ferrer-Maza et al. (2014), Soykan et al. (2015), Kahraman et al. (2017), Khoufi et al. (2014)) but despite its economic and ecological value more fundamental reproductive aspects are still barely touched upon (Murua et al., 1998; Domínguez-Petit, 2007). Large fluctuations of recruitment occur in relation to extreme oceanographic and environmental conditions (Sánchez & Gil, 2000). European hake has an important ecological role on its ecosystem and adults are considered top predators feeding mainly on fish (e.g. blue whiting, horse mackerel, silver pout, etc.), crustaceans and molluscs (Velasco & Olaso, 2000). Dial vertical migrations have been related to feeding behaviour, close to the surface during the night and inhabiting close to the bottom during daytime (Cohen et al., 1990; De Pontual et al., 2012). Seasonal reproductive migrations from south to north have been reported for the northern stock (Persohn et al., 2009), whereas studies on the southern stock only show bathymetric seasonal and ontogenic migrations (Fariña et al., 1997). Within its Atlantic distribution two stocks are assumed: the northern stock (North-east Atlantic down to Bay of Biscay: ICES Divisions IIIa, Subareas II, IV, VI and VII and Divisions VIIIa, b, d) and the southern stock (from Galician shelf to Gulf of Cadiz: ICES Division sVIIIc and IXa). The boundary between both stocks is located in the Cap Breton Canyon (Bay of

Biscay), however there is a lack of genetic or geographic evidences of a biological separation between northern and southern populations (Roldan et al., 1999; Mattiucci et al., 2004; Castillo et al., 2005; Piñeiro et al., 2007), thus the Galician shelf can be considered a transition zone. Recovery management plans were applied in both stocks because they were critically overfished (ICES, 2004). After this, the northern is currently recovered whilst the southern stock exploitation stills unsustainable and shows reduced reproductive capacity (ICES, 2008).

3.2. STUDY AREA

The North-western Mediterranean Sea

The Mediterranean Sea is a semi-enclosed sea, generally described as oligotrophic (Raymont, 1980) and a surface water temperature that ranges from 13ºC in winter to 27ºC in summer, being homothermic below 200m depth (12.5-14.5 ºC, 38.0-39.5% salinity and 4.5-5 ml/l oxygen concentration, by Hopkins 1985). Temperature and salinity increases from north to south and from west to east whereas productivity shows the opposite trend. The north western Mediterranean Sea includes the Catalan Sea and the Gulf of Lion and it is a highly productive area (Estrada, 1996) considered an Essential Fish Habitat due to its meteorological, hydrographical and oceanographic peculiarities. The hydrodynamics of these area are strongly influences by the Northern Current that runs along the continental slope from the north east to the south west (Millot, 1990). This area has a narrow continental shelf (6 km wide) that becomes wider in the Gulf of Lion (75km); strong northern and north-eastern winds during winter create termohaline convection, mechanic mixing of the water column and upwelling of nutrient-rich deep water (Rodriguez, 1982). There are important hydrographic inputs from the main rivers supplying sediments, nutrients and organic matter (mainly from the Rhone and Ebre rivers) that stimulate the primary production (Lochet & Leveau, 1990).

The fisheries landings in this area show important inter-annual fluctuations that may be influenced by environmental factors (Bas & Calderon-Aguilera, 1989; Lloret et al., 2000). Based on projections of climate change this is considered to be a climate change “hot-spot” (Durrieu de Madron et al., 2011) because its effects are likely to become apparent earlier and to be more pronounced in the Mediterranean Sea than in other oceans (Bethoux et al., 1999; Coll et al., 2010) and to interact with other anthropogenic pressures (Lejeusne et al., 2010). In fact, in recent decades in the NW Mediterranean Sea a rapid sea warming has been registered with Surface temperatures increasing at an average rate of 0.04 +- 0.01 ºC year-1 (Diaz-Almela et al., 2007). In this context, the north-western Mediterranean Sea has been defined as a *cul-de-sac* for those temperate species that are expected to shift their range northwards as they seek optimal environmental conditions but, eventually, encounter land boundaries (Lasram et al. 2010). The PhD Thesis has considered three different areas of the NW Mediterranean: Gulf of Lion, Catalan Sea and Balearic Sea.

The Galician coast

The Galician coast (north-western corner of the Iberian Peninsula) is located in the north east Atlantic. In this area two water masses can be found: an upper layer (< 1000 m depth) of Northeast Atlantic Central Waters with temperatures between 10.5 and 12ºC and salinity 35.45-35.75 and the lower layer (>1000 m depth) characterised by the Mediterranean Sea run-off water (OSPAR Commission, 2000). Generally, this is a highly productive area due to its latitude and environmental and oceanographic conditions which results in large phytoplankton blooms in spring and summer (e.g. Casas et al. (1999)) that support the high fishery yields of this region. The hydrodynamics in this region are influenced by the Eastern North Atlantic Upwelling System (Fraga, 1981) and during spring and summer northern winds favour the renewal of waters and the advection of colder deep water rich in nutrients (Rios et al., 1992). Important freshwater inputs from continental run-off are also a main supplier of nutrients and organic matter (Villegas-Ríos et al., 2011).

3.3. SAMPLING

Specimens were caught monthly by commercial bottom trawlers and also by trawl surveys on the study areas (Gulf of Lion and Galician shelf). Commercial catches were landed at the main ports from the regions, i.e. Roses, Port de la Selva, Llançà, Palamós and Blanes in the NW Mediterranean. Samples were obtained shortly after landed, stored in cooling containers, immediately transported to the laboratory and processed. Samples obtained during the trawl surveys (MEDITS GSA007 and GSA005) were immediately processed on board. Samples for the third case study were collected, processed and provided by the Instituto de Investigaciones Marinas (IIM-CSIC) on the frame of the project DREAMER, Recruitment Dynamic of European Hake (CTM2015-66676-C2-1-R), in the Galician shelf (Atlantic Ocean). The number of samples, as well as the main variables registered, can be consulted in Table 1.

Table 1. General sampling scheme. Summary of the sampling areas, dates, effort and of the variables analysed on each case study. TL: Total length, EW: eviscerated weight, Kn: condition factor, HSI: hepatosomatic index, MFSI: mesenteric-fat somatic index, FS: fat stage, SSI: spleen-somatic index, MAT: gonad development phase, L50: length at maturity, OSFD: oocyte size frequency distribution, BF: batch fecundity, RBF: relative batch fecundity, ODW: oocyte dry weight, OPD_i: $stage_i$ oocyte packing density, NO_i: number of $stage_i$ oocytes, I_A: intensity of atresia, P_A: prevalence of atresia.

		Case study			
General data		**1. *Argentina sphyraena* (Mediterranean)**		**2. *Micromesistius poutassou* (Mediterranean)**	**3. *Merluccius merluccius* (Atlantic)**
Sampling location		Gulf of Lion	Balearic Sea	Gulf of Lion	Galician shelf
Date		June 2015 -July 2016	June -July 2016	June 2015 -July 2016	December 2011 - November 2012
Sample size (n)		713	77	635	162
Females (n)		354	52	350	162
Variables analised		**n**	**n**	**n**	**n**
Condition	TL, EW	713	77	635	162
	Kn	713		635	
	HSI			635	
	MFSI	713	0		
	FS	713	77		
	GSI	673	0	635	162
	Lipid content	10		30	
Health	SSI			532	
	parasitism			174	
Reproduction	MAT	713	77	635	162
	L50			635	
	Whole-mounts				
	OSFD	37	6	30	
	BF, RBF	37	6	30	
	ODW	37	20	37	
	Histology	20	0	100	
	OD	350 oocytes	0	1652 oocytes	16000 oocytes
	OPD, No_i			30	162
	atresia (I_A, P_A)	20	0	55	162

3.4. CONDITION AND HEALTH INDICATORS

The energetic fitness (condition) was approximated using morphometric and organosomatic indicators.

Morphometric indicators

The morphometric estimation was based on the Le Cren's relative condition factor (Kn) which measures the deviation of an individual from the averaged weight at length in the respective sample (Lloret et al., 2014) as follows: Kn = EW / EWe, where EW is the eviscerated weight of one fish and EWe is the estimated weight of that fish derived from the weight-length relationship obtained from all the individuals sampled. The benchmark of Kn is 1, i.e. fish above or below 1 are respectively in better or worse condition respectively than the average population.

Organosomatic indicators

Organosomatic indices were calculated for each individual as a function of EW. As a measure of stored energy, and depending on the main deposit tissue of each species, hepatosomatic index (HSI) or mesenteric-fat somatic index were calculated respectively from the liver weight (LW) and the mesenteric fat weight (MFW) as HSI = 100 x LW / EW and MFSI = 100 xMFW / EW Energy allocated to reproduction was estimated through the gonadosomatic index (GSI) from the gonad weight (GW) as GSI= 100 x GW / EW.

The spleen, as a secondary lymphatic organ, plays a role in immune response against parasite infection (Lefebvre et al., 2004). It is suggested that a large spleen may reflect the ability to respond to parasite infection or may indicate high immunological activity against already established infection (Skarstein et al., 2001; Ottova et al., 2005). Thus the spleen-somatic index (SSI), i.e. relative size of the spleen, was calculated as SSI = 100 x SW / EW where SW is the spleen weight and EW is the eviscerated weight.

Lipid content verification

In order to identify which are the main lipid deposits the lipid distribution in the fish body tissues was examined. Lipid content in tissues was extracted and quantified following the Soxhlet method for lipid extraction. This procedure uses a Soxhlet extraction unit and hexane as solvent. Briefly, the solvent is heated in the flask of the unit and the vapours rise through a thimble containing the sample and are liquefied in a water-cooled condenser. This methodology was described by (Shahidi, 2001) and reviewed in Lloret et al. (2014)

Parasitism

The whole fish viscera and the left side muscle were examined under the stereomiscroscope. Parasites were removed and based on observations of their morphology and internal structure on the optical microscope were identified to the lowest possible taxonomical level following keys and descriptions from specific bibliography (see Results, Case Study II, Methodological approach).

3.5. REPRODUCTION

Gonads development phases

Sex and gonads development phases (MAT) were macroscopically determined using standardized terminology (Brown-Peterson et al. 2011), i.e. immature (IM), developing (DV), spawning capable (SC), actively spawning (AS), regressing (RS) and regenerating (RT). Posteriorly, MAT was validated on histological sections.

Gonads fixation

Gonads were fixed in 4% buffered formaldehyde.

Histological processing of gonads

A cross slide of the central area of the left lobe of each fixed ovary was dehydrated, cleared with histoclear II and embedded in paraffin, histologically sectioned at 4 µm (the protocol was obtained from Vila (2010) and can be consulted in the annex II) and stained with haematoxylin and eosin (Kiernan, 1999).

Oocyte staging

Based on cytoplasmic processes, oocytes were classified into stages of oocyte development following existing literature (Lowerre-Barbieri et al., 2011a), i.e. previtellogenic (PVO), cortical alveoli (CA), vitellogenic (VTO), migratory nucleous (MNO) and hydrated oocytes (HYO). On this standard basis the oocyte classification scheme was adapted to meet the aims of each case study (see Methodological approach subsections of Results chapter).

Oocyte diameter

Oocyte short (S) and long (L) axes were measured on micrographs taken from histological slides and, subsequently, individual arithmetic oocyte diameter was calculated as OD = (L+S)/2.

Oocyte packing density and number of stage$_i$ oocytes

$$OPD_{ij} = log\left[Vv_{ij} \times \left(\frac{1}{\rho o}\right) \times \left\{\left(1 + k_{ij}\right)^3 /(8 \times k_{ij})\right\}\right] + 12.28 - 3 \times log(cODv_{ij})$$

The Oocyte Packing Density (OPD) (Kurita & Kjesbu, 2009), the number of oocytes per gram of ovary, was estimated for every stage ($_i$) of oocytes present in the ovary applying the refined formula of Korta *et al.* (2010):

OPD_{ij}: Stage$_i$-specific oocyte packing density by female (j)

Vv_{ij}: Volume fraction of stage$_i$ oocytes by female (j)

ρ_o: Specific gravity of the ovary

k_{ij}: Mean shape factor of stage$_i$ oocytes by female (j)

$cODvi_i$: Mean stage$_i$ volume-based oocyte diameter by female (j), corrected for shrinkage

According to Delesse's principle, area fraction and volume fraction are proportional. Thus the amount of stage$_i$ oocytes, i.e. the volume fraction of stage$_i$ oocytes (Vv_i), was estimated from the area fraction computed on grid-overlaid photomicrographs of histological sections taken every two fields (Fig. 11). The area fraction was estimated as the ratio between grid points hitting the stage$_i$ oocytes and the total points hitting the sectioned tissue (excluding hits on empty spaces and outside of the ovarian wall). In each case study, the required number of counting fields per sample was defined in former pilot study aiming for a deviation from Vv normalized mean no higher than ± 0.05 (see Annex I, e.g. Fig S1), this resulted in a compromise between accuracy of Vv estimations and time consumption. The specific gravity of the ovary was obtained from Kurita and Kjesbu, (2009), i.e. being set at 1.061 and 1.072 for ovaries showing PVO/MNO and CA/EVTO/VTO as the most advanced oocyte stage, respectively. The oocyte shape factor (k) was given from the ratio between oocyte L and S axis, i.e. k = L/S, and mean stage$_i$-specific oocyte shape factor thereafter calculated for each female (K_{ij}). Only those oocytes sectioned through the nucleus were considered. Stage$_i$-specific mean volume-based oocyte diameter (OD_{vi}) was then estimated as $OD_{vi} = [\Sigma^{n_i}_{j=1}(OD_{ij})^3/n_i)]^{1/3}$ (Kurita & Kjesbu, 2009). The number of oocytes to be measured to ensure a deviation from K normalized mean below ± 0.05 was based on tests previously performed (see Annex I, e.g. Fig S17). To deal with oocyte shrinkage derived from histological processing, oocyte diameter was turned into the formalin-fixed dimensions ($cODv_i$) by applying a correction factor. Finally, the number of oocytes by stage (i) in each ovary (j) was calculated from OPD_{ij} and formalin-fixed gonad weight (GW_{fj}) as follwos: $NO_{ij} = OPD_{ij} \times GWf_j$.

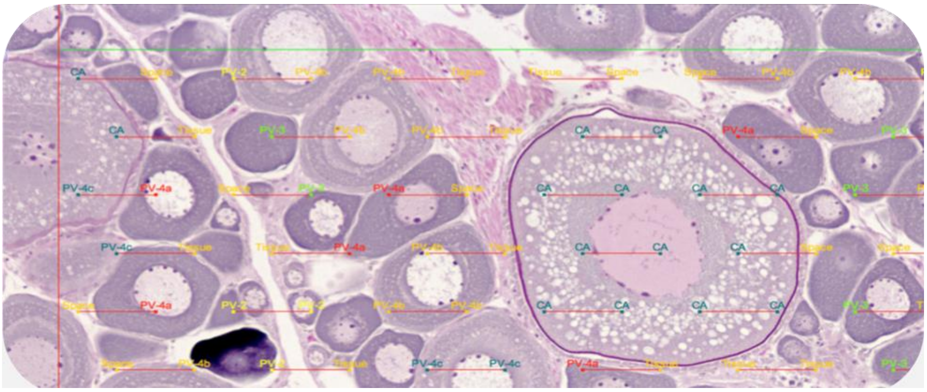


Fig 11. Estimation of volume fraction on grid-overlaid histological section. Micrograph from *Merluccius merluccius* ovary.

Relative intensity of atresia

Relative intensity (I_A) of alpha-atresia (αATR) (Fig. 12) was calculated as the number of αATR oocytes divided by the total number of vitellogenic oocytes (healthy and atretic) counted on three non-consecutive random fields of each specimen's histological slides.

Egg quality

The mean dry mass of HYO (ODM) from AS ovaries was used as a proxy of egg quality and it was estimated for each female, after drying two replicates of HYO (n=50) at 110 ºC for 24 h.

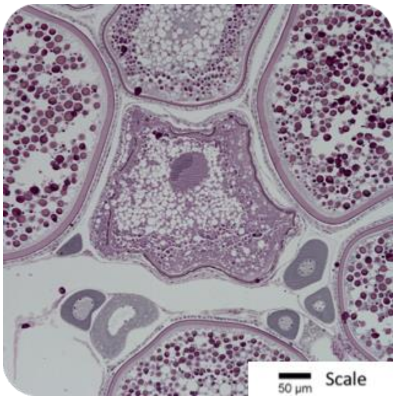


Fig 12. Micrograph of alpha-atresia. Taken at 20x on histological slides of *Merluccius merluccius* ovaries.

Oocyte size frequency distribution and batch fecundity

Oocyte size frequency distribution (OSFD) from whole-mounts was obtained using the gravimetric method: a subsample from the central area of each ovary was removed, weighed (OVW) and washed to separate oocytes from connective tissue. Subsequently oocytes were sorted according to their size using a sieves system with different mesh sizes (generally, 125 µm – 800 µm), and, using a computer-aided image analysis, oocytes were then counted and measured. The reproductive capacity was assessed according to the batch fecundity (BF), i.e. number of eggs spawned per batch, which was estimated from the number of oocytes (O) in the most advanced batch (MAB) calculated from the whole-mounts as BF = GW x (O_{MAB} / OVW).

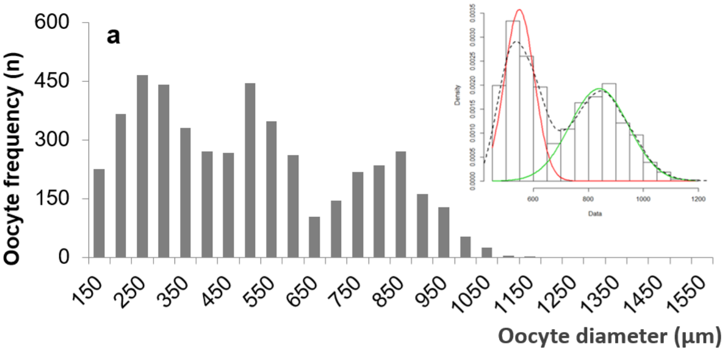


Fig 13. Example of oocyte size frequency distribution. From whole-mounts of ovaries of a spawning capable female of *Micromesistius poutassou*.

Identifying and isolating the oocytes belonging to the MAB was easy when it consisted of HYO (i.e. AS ovaries) because generally a gap appeared on the OSFD between the MAB and the rest of the oocytes, but on SC ovaries OSFD showed an overlapped multimodal distribution that could be described with a two or three-component mixture model. In these cases, the number of oocytes belonging to the next batch (those with a 95% probability of belonging to the last component) was estimated using an algorithm of the *mixtools* package (Benaglia et al 2009) for R software (www.r-projecte.org) (e.g. Fig 13), that has been successfully applied in our previous works (Ferrer-Maza et al., 2014). In those AS females showing post-ovulatory follicles, BF was estimated from the preceding batch

Image analysis

Micrograph resolution, microscope magnifications and camera model varied according to the specific features of each case study, thus these information is given on each corresponding Methodological approach subsection in the Results chapter. Image analysis of histological sections was undertaken using the software ImageJ (Schneider et al., 2012), and the ObjectJ plugin (https://sils.fnwi.uva.nl/ bcb/objectj). Oocyte size and shape were measured using a slightly modified version of the Elliptical oocytes project (https://sils.fnwi.uva.nl/bcb/objectj/ examples/oocytes/Oocytes), while grid counting was performed using the Weibel Grid Cell project (https://sils.fnwi.uva.nl/bcb/objectj/examples//Weibel/MD/weibel.html). Image analyses of whole-mounts were performed using a computer-aided image analysis system (ZEISS ZEN Imaging software, www.zeiss.com).

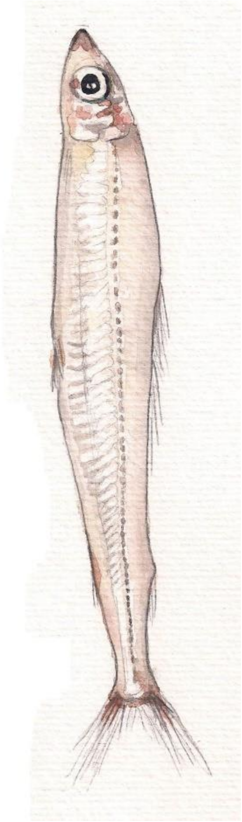
Data analysis

Most of the data analyses were performed using R version 3.2.3 (http://www.rproject.org/) and P<0.05 was considered a significant result. Statistical tests varied between case study and consequently are detailed in the respective subsection of the Results chapter. Due to the right-skewed distribution of parasites the statistical analyses of the related parameters were executed using the software Quantitative Parasitology 3.0 (Reiczigel & Rózsa, 2010) which was developed to manage the left biased frequency distribution of parasites.

4. RESULTS

This chapter is structured in three sections, each one containing one of the three case studies that constitute the main body of the thesis. Supplementary information is attached as an Annex at the end of the document.

4. 1. CASE STUDY I



The **lesser silver smelt** (*Argentina sphyraena*) from the NW Mediterranean Sea[1]

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[1] Serrat, A., Muñoz, M. & Lloret, J. (2018). **Condition and reproductive strategy of the *Argentina sphyraena*, a cold-water species in the Mediterranean Sea.** Published in Environmental Biology of Fish, 101: 1082-1096. DOI: 10.1007/s10641-018-0763-x

Condition and reproductive strategy of the *Argentina sphyraena*, a cold-water species in the Mediterranean Sea

4.1.1. OVERVIEW

We evaluate the condition and reproductive status of the lesser silver smelt, *Argentina sphyraena* in the north-western Mediterranean Sea. We describe for the first time several life history traits of this species, i.e. condition, reproductive cycle, fecundity, breeding strategy, oocyte recruitment and egg quality, and evaluate the links between these traits. We also contrast condition and reproduction parameters in two study areas with different environmental conditions: the Gulf of Lion and the Balearic Sea. Our results indicate that *A. sphyraena* is a capital breeder with group-synchronous oocyte development and determinate fecundity. Energy requirements for reproduction are mostly met by lipids stored in the mesentery which is the main fat deposit and a good indicator of condition; this means that condition can be estimated visually and easily using a fat scaling technique. Our results show that, in terms of condition and reproduction, this species performs better in the colder and more productive Gulf of Lion than in the warmer and less productive Balearic Sea, as evidenced by the amount of stored energy and reproductive strategy. These findings suggest that suboptimal environmental conditions may have a negative impact on the condition and reproduction strategy of cold-water species, which will ultimately affect their abundance and distribution.

4.1.2. SPECIFIC BACKGROUND

Plasticity in reproductive traits (e.g. fecundity, spawning seasonality) and in the trade-offs between condition, reproduction and growth could be a response to environmental condition as it has been reported for anchovies and sardines (Brosset et al., 2016), Atlantic cod (Dutil & Lambert, 2000) and herring (Engelhard & Heino, 2006). This plasticity is crucial in a context of climatic change, especially in a semi-enclosed and highly disturbed sea such as the Mediterranean, which is likely to be one of the regions most impacted by current sea warming (Lejeusne et al., 2010), in conjunction with other anthropic pressures. Cold-water fish species inhabiting temperate seas may be particularly impacted by sea warming as well as fishing. One such species inhabiting the Mediterranean is the lesser silver smelt (*Argentina sphyraena*, Linnaeus 1758) which is showing a decreasing trend in abundance during recent decades, inversely correlated to the rise in sea water temperature (Lloret et al., 2015). Information on the biology of *A. sphyraena* is scarce and there are very few studies based on Mediterranean populations (Lee, 1963; Ferri et al., 2017). The reproductive strategy of *A. sphyraena* has not yet been defined and there is no estimation of its fecundity; likewise, no histological studies have been carried out to describe its oocyte recruitment pattern. Regarding energy storage, Halliday (1969a) observed that mesenteric fat was the main energy store for the species. Visual assessments of mesenteric fat have been shown to be an efficient and accurate predictor of body lipid content in anchovy and sardine (van der Lingen and Hutchings 2005) and, although this technique has not been widely used in other fish species, we decided to use and verify the technique in this study. Overall, prior to this study, no comprehensive and up-to-date information existed on key life history traits of *A. sphyraena*, such as condition and reproduction.

Hence, in this work we investigate the two biological variables of condition and reproduction in *A. sphyraena*. More specifically we aim (i) to describe for the first time the reproductive strategy, spawning characteristics and condition of this species in the north-western (NW) Mediterranean Sea; (ii) to analyse the interrelationships between these life history traits; (iii) to explore simple and accurate condition indicators such as the mesenteric fat scale; and (iv) to explore regional variability of condition and reproduction, comparing two areas in the NW Mediterranean which have different environmental conditions.

4.1.3. METHODOLOGICAL APPROACH

Fish sampling

Samples were collected monthly in the Gulf of Lion (GLL) region (NW Mediterranean Sea, Fig. 14) from June 2015 to July 2016 from commercial bottom trawlers landing at the four main Catalan fishing harbours (Llançà, Roses, Palamós and Blanes; Fig. 14) and also during the MEDITS GSA007 trawl survey, at depths ranging from 50 to 520 m. In order to account for regional variability, additional samples were collected from the Balearic Sea (BAL) region (north-eastern Spain) by the MEDITS GSA005 trawl survey (Fig. 14) in June-July 2016. The two sampling areas differ in terms of environment and fishing: the GLL has a lower sea water temperature as shown by the monthly mean sea surface temperatures (SST) (André et al., 2005); greater primary productivity (Estrada, 1996; Siokou-Frangou et al., 2010) as shown in the distribution of phytoplankton biomass as satellite derived Chl *a* reported by D'Ortenzio and Ribera d'Alcalà (2009) and revised by Siokou-Frangou et al. (2010); and higher trawl fishing pressure than the BAL, with trawling intensity on the Balearic shelf and slope being much lower than along the peninsular bottoms (Ordinas & Massutí, 2009).

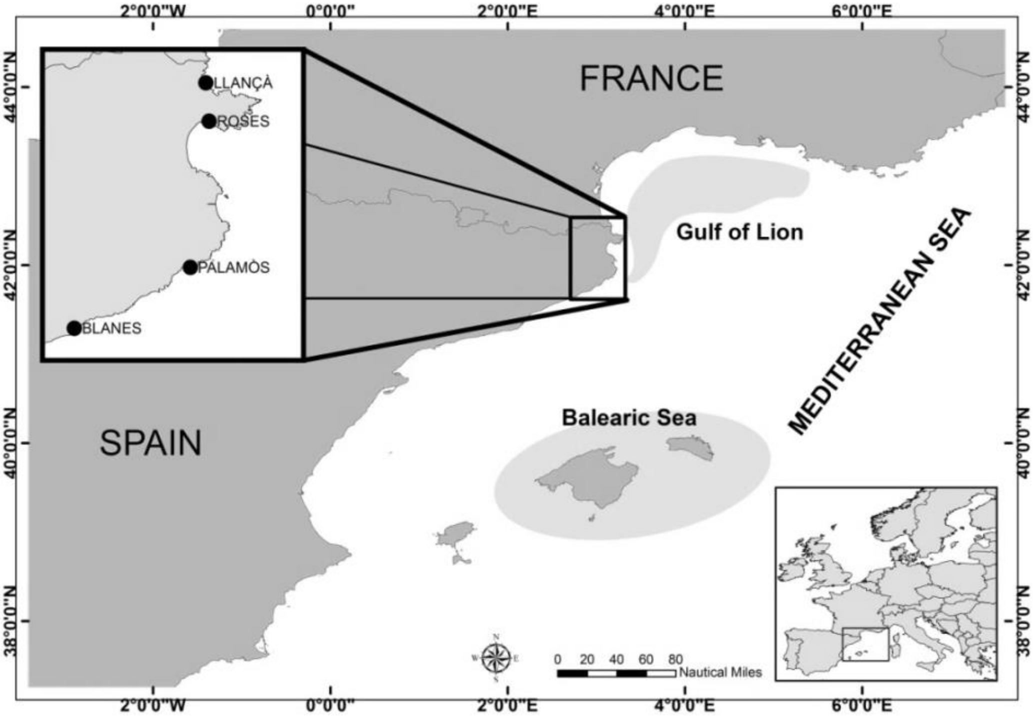


Fig 14. Sampling area of case study I. Map of the north-western Mediterranean, showing the two sampling areas within the Gulf of Lion and the Balearic Sea, and the ports where the samples from commercial catches were collected. Author: Víctor Àguila Gimeno.

Once in the laboratory, total length (TL, ± 0.1 cm) and total weight (TW, ± 0.01g) were recorded. Thereafter, samples were dissected and eviscerated in order to record eviscerated body weight (EW, ± 0.01g), liver weight (LW, ± 0.1 mg), gonad weight (GW, ± 0.1 mg) and mesenteric fat weight (MFW, ± 0.1 mg). Sex and gonad development phases (MAT) were macroscopically determined using the scale defined by Brown-Peterson et al. (2011). Subsequently, the gonads were fixed in 4% buffered formaldehyde for histological assessment of fecundity and egg quality estimation. Additionally, the whole left muscle of each fish was removed and frozen at -20ºC for further lipid content quantification in the laboratory (see following section).

Condition estimation

Halliday (1969a) identified mesenteric fat as the main lipid store in *A. sphyraena,* and therefore we used it as the main condition indicator for this species. Nevertheless, prior to our analyses, we verified lipid compartmentalization by analysing the lipid ratio content in the mesentery and muscle of ten regenerating (RT) females, since lipid depots are filled up at this maturity phase (see Fig. 15). Quantification of total lipid was performed only in these tissues since, in *A. sphyraena*, the liver and gonads are too small to have a relevant role in lipid storage. Lipid content was determined following the Soxhlet method (Shahidi, 2001). Given that in these ten females the absolute fat content in the muscle of these individuals averaged 0.44 g compared to 3.36 g in the mesenteric fat (this is, mesenteric fat accounted for more than 80% of the total lipids of the specimen), we confirmed Halliday's findings (1969a) that mesenteric fat is the main lipid deposit in *A. sphyraena* and that muscle constitute a minor deposit; therefore, we proceeded to analyse mesenteric fat as the main indicator of condition for this species.

Table 2. Mesenteric fat stages. Five–points scale for visual fat staging of *Argentina sphyraena*. Scale adapted from Halliday (1969a).

Fat stage	Criteria
0	Virtually no fat; spleen clearly visible
1	Spleen obscured; fat deposit narrow, extending less than half the distance from the stomach to the origin of the pelvic fins
2	Fat deposit narrow, although more than half the width of the body cavity; extending posteriorly more than half the distance from the stomach to the origin of the pelvic fins
3	Deposit thick and wide, virtually filling ventral part of body cavity from the stomach to the origin of the pelvic fins.
4	Fat virtually filling the whole body cavity posterior to stomach. Tip of fat deposit almost reaching the anus.

The relative mesenteric fat content of the specimens was visually assessed by assigning a fat stage (FS) on a 5-point scale (Table 2), adapted from a 7-point scale used by Halliday (1969a). Subsequently, the mesenteric fat was carefully removed from each specimen and weighed, and the mesenteric fat somatic index (MFSI) was calculated. The accuracy of the visual fat staging technique was then verified (see data analyses section).

Reproductive potential estimation

Progress in the reproductive cycle was estimated by calculating the Gonadosomatic index (GSI) as described in the Methodology chapter.

The ovaries of this species have the tendency to decompose when the fish is manipulated, especially at spawning capable phase. For this reason, the number of specimens that allowed histological sectioning (8-10 µm) was limited (n = 20). Oocyte development stages were described following Lowerre-Barbieri et al. (2011); oocyte diameter (OD) was measured on histological sections for 50 oocytes randomly chosen per oocyte stage (only oocytes sectioned through the nucleus were considered) (see image analyses procedures on the Methodology chapter). Due to the higher degree of shrinkage during histological processing, hydrated (HYO) OD was measured on whole mounts. Intensity of alpha-atresia (I_A) was estimated from three non-consecutive random fields of each specimen.

In order to determine the spawning pattern (total spawner *vs*. batch spawner) and the type of fecundity (determinate *vs*. indeterminate), eleven females in the spawning capable (SC) phase were selected, each of similar size (TL of about 165 mm) and from samples taken approximately every two weeks throughout the spawning season (December – May). As described in the Methodology, a subsample of each ovary was used to count and measure oocytes on whole-mounts and oocyte size frequency distributions (OSFD) were obtained.

Since our preliminary results showed the *A. sphyraena* is a batch spawner with determinate fecundity, batch fecundity (BF) and relative batch fecundity (RBF) were estimated. Estimations of BF were carried out using the gravimetric method combined with image analysis (Murua et al., 2003) following the same procedure as described previously. As the presence of postovulatory follicles (POF) could not be checked histologically in all the samples due to the fragility of the ovaries, hydrated actively spawning ovaries could bias the results. To avoid underestimation of BF, just those SC females which did not show signs of hydration (HYO can be distinguished macroscopically) were selected to assess reproductive capacity. These criteria were met by 43 females (37 from the GLL and 6 from the BAL).

The OSFD showed an overlapped multimodal distribution that could be described with a two-component mixture model. In order to accurately calculate the number of oocytes belonging to the next batch (those with a 95% probability of belonging to the second component), we applied an algorithm of the *mixtools* package (Benaglia et al., 2009) for *R* software (www.r-projecte.org)

Egg quality was estimated from the mean dry mass (ODM) of the hydrated oocytes of actively spawning females (those showing HYO) (n_{GLL} = 36, n_{BAL} = 18) as described in the Methodology chapter.

Data analyses

The first step was to describe and analyse the data on reproduction and condition, and any relationships between them, based on the whole set of samples obtained in the GLL Following this, some of these parameters were compared between the two study areas.

First of all, in order to look for differences between sexes regarding the parameters TL, GSI and MFSI, a non-parametric Mann-Whitney U test was applied, as normality of data had already been tested and rejected using the Saphiro-Wilk test. A Spearman's Rank Correlation test was used to investigate the possible relation between reproduction parameters (GSI, BF, RBF and ODM), condition (MFSI) and size (TL). Any of these variables that showed significant correlation ($\rho > 0.5$) were subsequently fitted to regression models. In addition, a Kruskall-Wallis Test was performed to test differences in the distribution of MFSI during the different ovarian development phases.

Removing and weighing mesenteric fat is very time consuming and, for this reason, visual assessments were made of each specimen using a 5-point scale of fat stages so that we could test the reliability of the scale. This was tested as follows: ANOVA was used to find whether, on average, MFSI was statistically different between categories of the visual scale.

Differences in TL between study areas were tested using a Mann-Whitney Wilcoxon W-test. The response of BF, ODM and FS was respectively modelled by multiple linear regression analysis with TL (explanatory continuous variable), MAT (explanatory categorical factor), MON (explanatory categorical factor) and Z (explanatory categorical factor) and their first order interactions as covariates. The backward elimination procedure was applied so that only those explanatory variables that contributed significantly to the model ($p < 0.05$) were retained.

To compare BF between sampling areas, firstly the regression model approach was used to eliminate the influence of size and month by estimating the adjusted means of BF for the variation of the covariates. Subsequently, the adjusted means were contrasted between sampling areas using a pairwise Tukey HSD test. A Welch's t-test was applied to analyse sampling-area differences in egg quality, estimated by hydrated ODM. In the regressions of BF and ODM only those females at spawning capable maturity phase were considered. In order to study sampling-area variations in condition, FS mean values were plotted throughout ovarian development phases for both areas. A pairwise Tukey HSD was used to contrast FS least square means (adjusted for MAT, TL and month –MON- based on the regression model output) between sampling areas. All statistical analyses were performed using *R* software, and the level of statistical significance adopted was p-value < 0.05.

4.1.4. OUTCOMES

Condition and reproduction

Of the 713 specimens examined in the Gulf of Lion (GLL), 335 were females and 378 males. Mann-Whitney Wilcoxon W-test showed significant differences between sexes in GSI ($W = 72390.5$, $p < 0.001$ and MFSI ($W = 64427.5$, $p < 0.001$). The GSI ranged from 0.05 to 20.25 (mean ± SD = 3.33 ± 3.86) in females and from 0.01 to 9.87 (mean ± SD = 2.74 ± 2.78) in males, and showed a negative correlation (Spearman's $\rho = -0.64$, $n = 673$, $p < 0.001$) with MFSI. Values of MFSI ranged from 0 to 12.23 (mean ± SD = 2.19 ± 2.32) in females and from 0 to 7.12 (mean ± SD = 1.40 ± 1.68) in males, and showed a significant positive correlation with TL ($\rho = 0.33$, $n = 673$, $p < 0.001$).

Regarding the annual reproductive and condition cycle, the monthly frequency-distribution of ovarian development phases and GSI showed a seasonal pattern that is the inverse of the MFSI pattern (Fig. 15). Spawning capable gonads appeared from December to May concurring with the highest values of GSI and the lowest values of MFSI, indicating that mesenteric fat depots were transferred to the gonads. Moreover, a Kruskal-Wallis Test revealed significant differences in the distribution of MFSI during the different gonad development phases ($\chi^2 = 386.48$, $df = 5$, $p < 2.2e{-16}$). Due to the difficulty of staging males macroscopically, GSI was used as the main indicator of testis development. The same seasonal pattern was observed for both sexes.

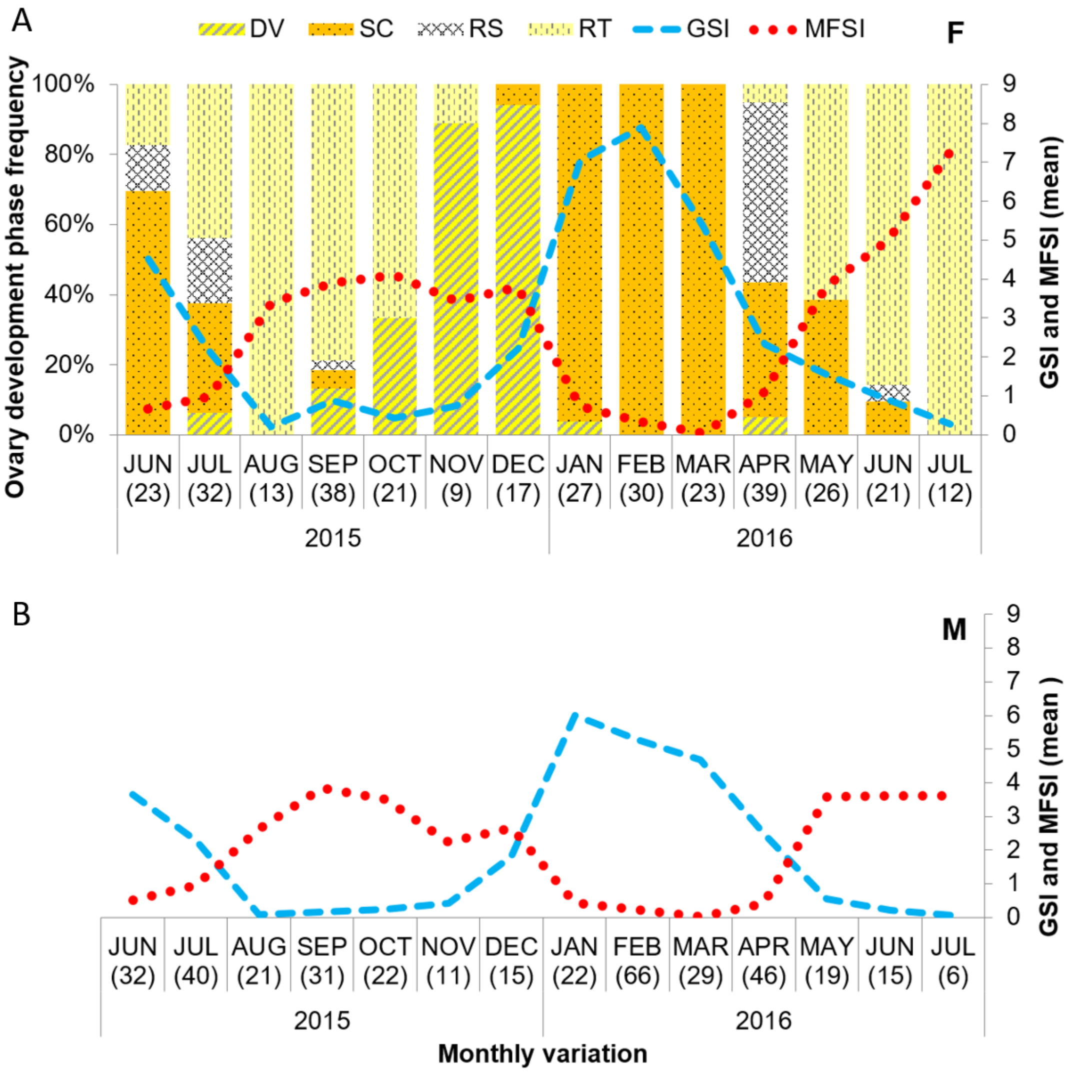


Fig 15. Reproductive cycle of *Argentina sphyraena* in the Gulf of Lion. Monthly frequency (percent abundance) of gonadal development phases and mean variation in the gonadosomatic index (GSI) and the mesenteric fat somatic index (MFSI), for females (F) and males (M). Gonadal development phases: developing (DV), spawning capable (SC), regressing (RS) and regenerating (RT). Number of samples per month in brackets.

With regard to the visual scale for mesenteric fat estimation the mean MFSI was significantly different between fat stages (FS) (ANOVA, $F_{1,667} = 1966$, $p < 2.2e-16$) increasing from stage 0 to 4 (Fig. 16). Furthermore, a positive relationship between MFSI and TL was found ($MFSI = -7.79 + 0.07 \cdot TL$, $R^2 = 0.32$, $n = 713$, $p < 0.0005$).

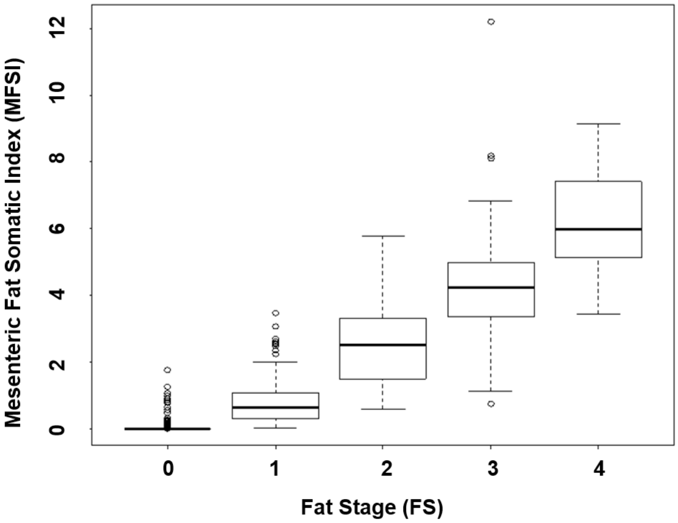


Fig 16. Validation of mesenteric fat visual scale. Box-plots of mesenteric fat somatic index (MFSI) through the different stages of mesenteric fat visual scale for sampled specimens of *Argentina sphyraena.*

The oocyte mean diameter increased gradually in line with the stage of development (Table 3). Oocytes at different development stages were simultaneously present during different phases of ovarian development, indicating (together with diameter-frequency distributions, see below) that secondary growth oocyte are not developed synchronously. Some SC ovaries that allowed for histological sectioning, showed postovulatory follicles but no hydrated oocytes, indicating multiple spawns. In all ovarian phases and throughout the entire spawning season, levels of atresia were very low (I_A = 4.19 ± 4.78%).

Table 3. Oocyte diameter of *Argentina sphyraena.* Oocyte stage mean diameter and standard deviation (±SD). Oocyte diameters measured on histological slides in exception of HY which was measured on whole mounts. PGO: primary growth oocytes, CAO: cortical alveolar oocytes, VTO1: vitellogenic 1 oocytes, VTO2: vitellogenic 2 oocytes, VTO3: vitellogenic 3 oocytes, MNO: migratory nucleus oocytes, HYO: hydrated oocytes.

	Diameter (µm)	
Oocyte stage	**Mean**	**SD**
PG	91.2	24.6
CAO	238.7	55.8
VTO1	372.2	46.5
VTO2	472.6	51.0
VTO3	654.1	69.6
MNO	760.2	57.1
HYO	1305.1	65.0

The OSFD obtained from whole mounts of SC ovaries, showed a polymodal distribution (Fig. 17) following Ganias & Lowerre-Barbieri (2018). In the samples for which the whole set of sieves was used (125-700 µm), three modes could be observed. The first component (125-300 µm) corresponded to the pool of cortical alveoli oocytes (CAO) and showed higher frequencies in early spawning capable ovaries than in ovaries from later in the spawning season. The second (300-675 µm) mode corresponded to vitellogenic oocytes (VTO1, VTO2, VTO3); and the third (675-1000 µm) contained the most advanced oocytes (migratory nucleus, MN). All actively spawning ovaries showed a final separate component (1000-1500µm) corresponding to the batch of hydrated oocytes (HYO) ready to be released during ovulation. The relative abundance of CAO and VTO1 pooled together (<450um) decreased as the spawning season progressed, with the highest values in January close to the spawning peak, falling to very low values in May (Fig. 18) when spawning is almost over. This suggests that the standing stock of early secondary growth oocytes is not replaced during the spawning season.

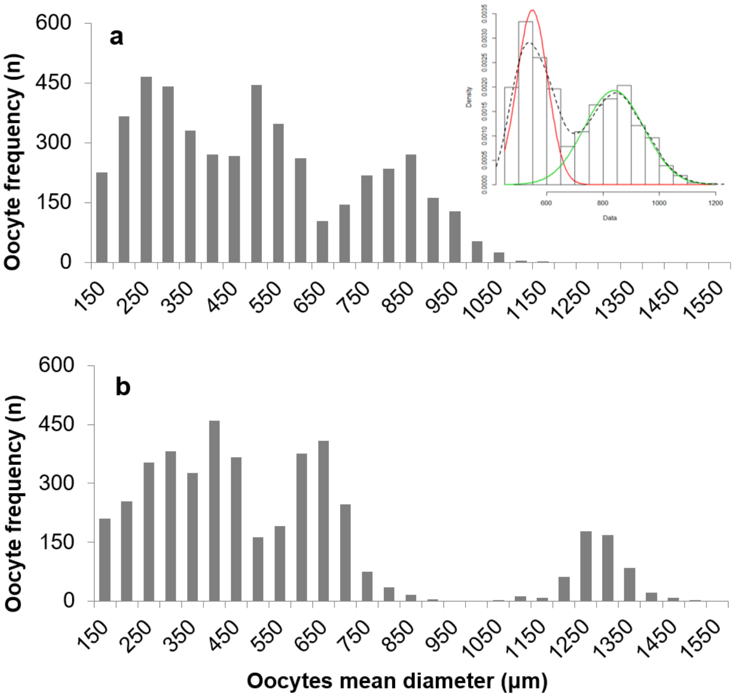


Fig 17. Oocyte diameter frequency distribution (for oocytes >150 µm) shown by most of the female *Argentina sphyraena* in spawning capable phase. Example a corresponds to one female with most advanced oocytes in migratory nucleus stage (TL = 176 mm), example b corresponds to one female with hydrated oocytes (TL = 159 mm). Since SC females showed a three-component overlapping mixture distribution, oocytes with 95% probability of belonging to the last component (small chart) were considered as being from the next batch.

With regard to BF; it ranged from 533 to 2987 eggs (mean ± SD = 1253 ± 567) and had a significant positive correlation to TL, fitting the following regression: $log(BF) = 5.33 + 0.01 \cdot TL$ (R^2=0.41, p-value < 0.0005),and a significant positive correlation to EW, fitting the following equation: $log\ (BF) = 6.34 + 0.034 \cdot EW$ (R^2 = 0.48, p-value < 0.0005). RBF ranged from 29.79 to 122.20 (mean ± SD = 63.05 ± 23.37) eggs per gram of EW. Estimations of ODM as an indicator of egg quality, ranged from 0.1195 to 0.3870 (mean ± SD = 1.95 E^{-4} ± 0.49 E^{-4}). Neither RBF nor ODM showed significant correlation to any of the other parameters we analysed.

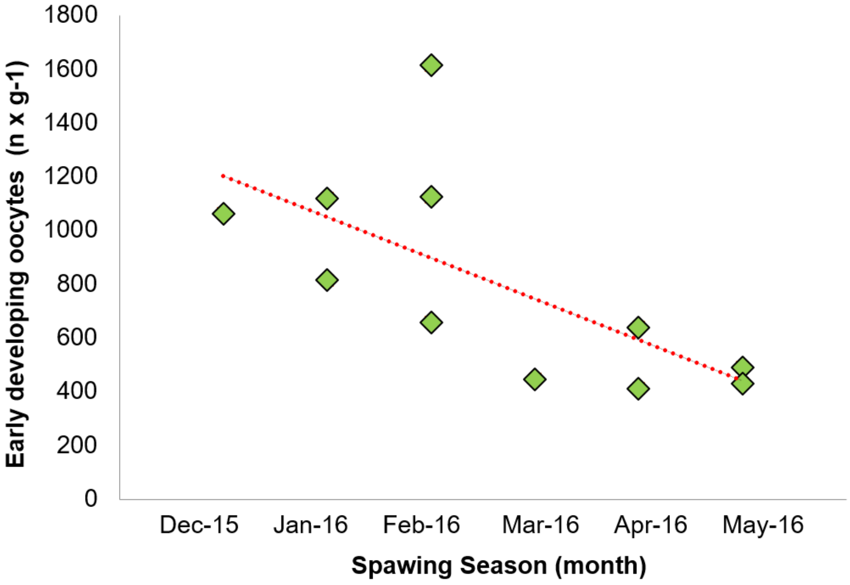


Fig 18. Tendency of early secondary growth oocytes in *Argentina sphyraena*. Relative abundance of early secondary growth oocytes (number of cortical alveolar and vitellogenic1 oocytes per gram of ovary) through the spawning season for spawning capable females sampled in Gulf of Lion (R2 = 0.4399, n = 11).

Regional variability

A total of 77 specimens (52 females, 25 males) were sampled in the Balearic Sea (BAL). As shown in Fig. 19, timing in gonadal development was different between sampling areas. While the spawning season in the GLL was already over by June, 30% of the sampled individuals in the BAL were still in SC phase. For this reason, in order to analyse differences in condition (FS) and reproductive potential (BF and ODM) between areas we compared samples from BAL (June-July 2016) to a subset from GLL including only those individuals sampled during the spawning season (December-May 2016, n = 354, from which 166 were females and 188 males). When necessary, data was adjusted to account for the influence of months (see below).

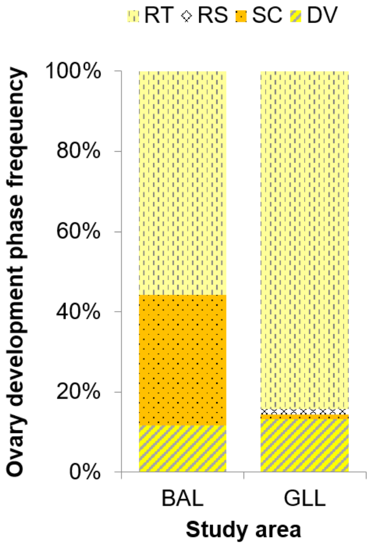


Fig 19. Snapshot of regional reproductive status of *Argentina sphyraena*. Frequency (percent abundance) of gonadal development phases and mean variation in the mean mesenteric fat visual stage (FS) for sampled specimens in Gulf of Lion (GLL, n = 79) and Balearic Sea (BAL, n = 77), in June-July 2016. Gonadal development phases: developing (DV), spawning capable (SC), regressing (RS) and regenerating (RT).

Although BAL specimens were in average of longer size (mean ± SD = 174.66 ± 22.74 mm, n = 77) than those from the GLL (mean ± SD = 159.27 ± 23.47 mm, n = 354), a Mann-Whitney Wilcoxon W-test showed that, overall, there were no significant differences in size between the specimens from the two study areas and actually the size range in GLL was wider and reached higher and lower values (97 – 216 mm) than in BAL (123-202 mm). In BAL, the BF ranged from 719 to 3104 (mean ± SD = 2561 ± 1050, n = 6) and the ODM ranged from 9 E^{-5} to2.75 E^{-4} (mean ± SD = 1.82 ± 0.50 E^{-4}, n = 18).

The multiple linear regression incorporating TL, Z and their interaction as explanatory variables, was found to be the best fit model, accounting significantly for 67% of the deviance in BF (n_{GLL} = 37; n_{BAL} = 6). Regarding FS, the predictors retained were MAT, MON, TL and Z, and the model accounted for 47% of the deviance (n_{GLL} = 354; n_{BAL} = 77) (Table 4). Any of the predictor variables contributed significantly to the deviance of ODM (n_{GLL} = 36; n_{BAL} = 18).

Table 4. Multiple linear regression of batch fecundity and fat stage of *Argentina sphyreana.* Analysis of deviance for the multiple linear regression fitted to batch fecundity (BF) and fat stage (FS) using as explanatory variables the total length (TL), month (MON), zone (ZONE), gonad development phase (MAT), and their first order interactions (just the significant explanatory variables are shown). DF: degrees of freedom, MS: mean square.

Response variable	**Explanatory variable**	**DF**	**MS**	**F**	**P**	**Adjusted R^2**
BF	TL	1	12243687	58.57	<0.000	
	ZONE	1	4649918	22.24	<0.001	
	TL:ZONE	1	1616896	7.73	<0.05	
	Residuals	39	209040			
	Model	3			<0.001	0.67
FS	TL	1	76.24	109,605	<0.001	
	ZONE	1	26.43	38	<0.001	
	MAT	1	15.95	22,937	<0.001	
	MONTH	2	41,073	59,049	<0.001	
	Residuals	228	0.696			
	Model	8			<0.001	0.47

Mean FS estimated from the visual scale followed similar variation patterns along the ovarian development phases in both areas, showing higher values in GLL (n = 354) than in BAL (n = 77) (Fig. 20). In fact, a *post hoc* Tukey test showed that adjusted mean FS (estimated least square –ls-mean- values adjusted for MAT, TL and MON based on the regression output) FS was significantly higher (p<0.05) in GLL (ls mean ± SE = 1.65 ± 0.10, n = 354) than in BAL (ls mean ± SE = 1.31 ± 0.16, n = 77).

A post hoc Tukey test showed that the BF adjusted mean was significantly higher (p<0.05) in BAL (ls mean ± SE = 1984.86 ± 222.58, n = 6) than in GLL (ls mean ± SE = 1321 ± 75.49, n = 37). Egg quality estimated from ODM showed higher values in GLL (mean ± SD = 1.95 E^{-4} ± 4.87E^{-5}, n = 36) than in BAL (mean ± SD = 1.81E^{-4} ± 4.95E^{-5}, n = 18), even though the Mann-Whitney Wilcoxon W-test did not show a significant difference between sampling areas probably due to the small number of samples from BAL. Note that ls mean is just used with the purpose of comparing the adjusted values between sampling areas, but reference values for further studies should be based on the arithmetic mean (see above).

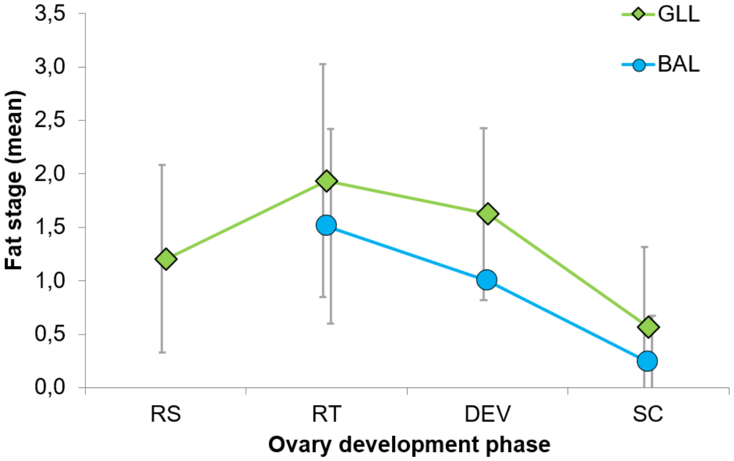


Fig 20. Regional variability of condition of *Argentina sphyraena*. Variation of mesenteric fat stage (FS) with the gonad development phase for specimens sampled in Gulf of Lion (GLL, n = 354) and Balearic Islands (BAL, n = 77). Mean mesenteric fat stage (on a five-point scale from 0 to 4) plotted against gonad development phases: regenerating (RT), developing (DV), spawning capable (SC) and regressing (RS).

4.1.5. CASE STUDY DISCUSSION

This study provides important new data on reproduction and condition of *A. sphyraena*. Regarding condition, we confirmed that mesenteric fat is the major lipid deposit in *A. sphyraena*, as had previously been suggested by Halliday (1969a). MFSI correlates negatively with GSI and exhibits opposing seasonal variability, indicating that energy demand for reproduction is met mainly by the mesenteric fat stores, in line with a capital breeding tactic. This suggests the importance of mesenteric fat for reproductive purposes of species that display these stores, such as *A. sphyraena*. During the spawning season mesenteric fat stores are depleted and this could leave the fish in a vulnerable situation to predation, diseases and unpredictable environmental stressors, lowering the chances of fish survival (Lloret et al., 2014). At the end of spring, when spawning is nearly finished, mesenteric fat slowly begins to be replaced until it reaches maximum values in September when there is a demand of energy reserves to develop the gonads. This fits with the feeding season described in Atlantic waters from May to November (Halliday, 1969c), when resources are acquired and accumulated. MFSI increased significantly with increased size of specimens of *A. sphyraena*, indicating an enhanced acquisition of energy resources in larger specimens. This effect of size on the energy accumulation rate may be connected with migration to deeper water, where they may find new food resources. Halliday (1969b) previously reported a change in growth pattern related to this migration.

This study confirmed that visual assessment of relative mesenteric fat amount is an accurate predictor of stored energy for this species. This fat staging technique is efficient, quick and easy to apply, it does not require expensive equipment extra than what can be found in standard fish biology laboratories and it can be done in the field. In spite of all these advantages, which have previously been described by van der Lingen and Hutchings (2005), the technique has not been widely applied to date. In surveys, where time and equipment are in short supply and a large number of samples are available (Lloret et al., 2014), the incorporation of a validated visual fat staging technique (to be used in place of the time-consuming removal and weighing of mesenteric fat) would provide sufficiently accurate and interesting information on condition, at almost no extra cost, for fish species accumulating fat stores in their mesenteries, such as the European anchovy *Engraulis encrasicolus* (Ferrer-Maza et al., 2016) and the Far Eastern sardine *Sardinops sagax* (van der Lingen & Hutchings, 2005)..

Concerning reproduction biology, the results presented here such as the OSFD and the decreasing trend in the relative number of early secondary growth oocytes, indicates that there is no replacement of the standing stock of these oocytes during the spawning season. Moreover, the observations of low values of atresia, sparsely distributed throughout the reproductive season, and the presence of regressing specimens showing ovaries with a high number of POFs but no relevant atresia levels reinforces the idea that the potential annual fecundity in *A. sphyraena* is already fixed before the onset of spawning. Altogether, this provides evidence for the first time that, following the criteria established by Murua & Saborido-Rey (2003), *A. sphyraena* in the NW Mediterranean Sea is an iteroparous batch spawner with group synchronous development of oocytes and determinate fecundity. In addition, this species shows a capital breeding strategy, since energy requirements for reproduction are met mainly at the expense of lipid stored in the mesentery prior to the spawning season.

The macroscopic monthly frequency of gonad development phases and the histological analysis coupled with the seasonal variation of the GSI index, revealed that the spawning period of *A. sphyraena* collected in the Gulf of Lion (GLL) takes place in the winter-spring period, from December to May, with a peak of spawning activity in February-March. In general, it is known that several inheritable components and exogenous cues interact in the control of spawning timing and duration, among which, photoperiod commonly trigger oocyte maturation (Migaud et al., 2010), and water temperature (Kjesbu, 1994) and energy resources (Thorpe, 1994) act as major regulators. Specifically, temperature affects the speed of gonadal development, thus it is expected that populations from warmer waters would reach the spawning capable phase earlier than those from colder northern waters (reviewed in Lowerre-Barbieri *et al.*, 2011). This is supported by comparison of our results with observations on populations of *A. sphyraena* inhabiting the colder waters and higher latitudes of the eastern Atlantic, where spawning season was registered from March to June (Halliday, 1969c), a spawning season that is both later and shorter than the one we observed in the GLL. However, although in the Balearic Sea (BAL) the timing of reproduction would be expected to be slightly more advanced since the water is warmer, 30% of the sampled specimens in June are still spawning, whereas in GLL the spawning was, by then, completed. This suggests that factors other than temperature, e.g. condition, and their interaction, may be playing a major role in regulating the spawning season and may counteract the effect of temperature. In fact, for other cold-water species such as cod, it has been observed that poor conditioned adults delay spawning time by up to two weeks (reviewed in Kjesbu, 2009). Thus, changes in temperature or in condition may also drive changes in spawning timing which may compromise the release of offspring in optimal environmental conditions for larval survival and ultimately affect reproductive success and have demographical consequences (Yamahira, 2004)

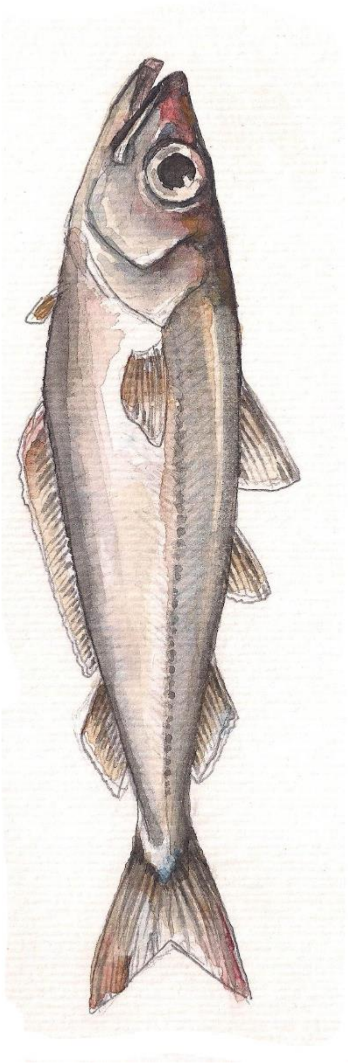
The results of this study point to differences in the condition status and reproductive strategies among populations of *A. sphyraena* inhabiting areas with different environmental conditions. Indeed, *A. sphyraena* specimens sampled in the BAL were in poorer condition in all phases of gonad development than those from the GLL, probably as a result of (i) more limited food availability in the BAL, since productivity is lower there than in the GLL which has important river runoff and where wind mixing enhances primary productivity (Estrada, 1996), or (ii) warmer sea water in the BAL (Bianchi & Morri, 2000). Metabolic rates and enzymatic processes in ectotherms species increase with ambient temperature and therefore higher rates of lipid accumulation and depletion are expected in warmer waters (Cossins & Bowler, 1987). Thus, in an oligotrophic environment where condition is constrained by low food intake, higher temperature might cause a critical loss of energy reserves (Sogard & Olla, 1996).

Regarding reproductive traits, in BAL the number of eggs per batch was significantly higher than in GLL while in the latter area hydrated oocytes were of better quality (ODM) despite not statistically significant. Thus, under the more favourable environmental conditions of the GLL (with higher productivity and lower temperature), the cold-water species *A. sphyraena* is in better condition (i.e. it has higher energy stores) and spawns smaller number of eggs but these are of slightly better quality ODM than under the less favourable conditions (with lower productivity and higher temperature) of BAL. In BAL, the decreased egg quality is compensated by greater batch fecundity. Similar changes in reproductive strategy have already been described in the NW Mediterranean Sea, in relation to the availability of energy resources for anchovies and sardines (Brosset et al., 2016) and in relation to thermal regime for the bastard grunt, *Pomadasys incisus* (Villegas-Hernández et al., 2015a) a thermophilic species showing different reproductive strategy among populations from contrasting thermal regimes i.e. spawning better quality eggs at the expense of quantity at the warmer study area. This supports the idea that “cold-water” fish threatened by environmental constraints, such as sea warming, are in worse condition and might have lower reproductive potential than those inhabiting waters without these constraints (Marshall & Frank, 1999; Lambert et al., 2003). Consequently, their abundance could be affected which partly relates to the documented contraction of the southern limit of the distribution area and the consequent shift northwards or in depth (Perry et al., 2005) as a response to climate change, in cold-water species inhabiting relatively temperate seas in the northern hemisphere. Altogether, these results could be accounting for the abundance reduction of *A. sphyraena* and other cold-water fish species (e.g. *Alosa fallax* or *Molva macrophthalma*) found by Lloret et al. (2015) in the GLL.

Even though the life history traits of the populations of *A. sphyraena* in the GLL and the BAL analysed in this work show that this cold-water species displays a certain amount of plasticity which may help it to cope with environmental variability, our observations showed that the persistence of populations in suboptimal conditions results in reduced biological performance. Moreover, fishing, together with climate forcing, might be another major pressure causing demographic changes and shaping life history traits, but it might be difficult to distinguish fishing impacts from environmental impacts (Perry et al., 2010).

Therefore, increased efforts should be made to regularly monitor the condition and reproduction of “cold-water” fish, using standardized indicators, in order to follow the evolution of the health status of their populations, to distinguish the effects of multiple threats and to apply specific criteria to better manage these particular species.

4. 2. CASE STUDY II



The **Blue whiting** (*Micromesistius poutassou*) from the NW Mediterranean Sea[2]

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[2] Serrat, A., Lloret, J. & Muñoz, M. **Trade-offs between biological traits in a "cold-water" fish in the Mediterranean: the case of blue whiting (*Micromesistius poutassou*).** Manuscript under review.

Trade-offs between biological traits in a "cold-water" fish in the Mediterranean: the case of blue whiting (*Micromesistius poutassou*)

4.2.1. OVERVIEW

A combination of traditional and emerging methodologies was used to assess the trade-offs of several biological and life history traits (parasitism, reproduction and condition) of *Micromesistius poutassou* in the Mediterranean Sea, a commercially exploited "cold-water" species. The use of histological and gravimetric methods revealed mixed evidences of indeterminate and determinate fecundity type, e.g. lack of a hiatus between the previtellogenic and vitellogenic oocytes and a decrease in the number of developing oocytes along the spawning season. Moreover, there seems to be condition-mediated compensations between the egg quality and quantity. Considering the fact that larger fish spawn more eggs and that the minimum landing size is lower than the size at maturity, we provide recommendations to be used in future stock assessments. Local environmental conditions of the Gulf of Lion may account for geographical differences of infection in blue whiting. Relationships between parasitism and reproduction and condition occur mainly on high intensities of infection: a lower batch fecundity (affecting reproductive potential), a higher hepatosomatic index and a higher spleen somatic index. The complex trade-offs between parasitism, reproduction and condition need to be considered to understand the future of cold-water species such as blue whiting in the context of global change.

4.2.2. SPECIFIC BACKGROUND

Life history traits are interrelated by dynamic trade-offs of energy allocation mediated by several extrinsic and intrinsic factors resulting in balanced fluctuations (Stearns, 1989). However, unusual environmental conditions and anthropogenic pressures may disrupt these self-regulatory processes (e.g. Rueda *et al.*, 2015). One of the main trade-offs involves the cost paid in future reproduction and in health or maintenance (Gunderson, 1997). To deal with fluctuations in the energy invested in reproduction, fish can adopt different reproductive tactics, i.e. phenotypical expression of its reproductive traits in a particular environmental situation (Kjesbu, 2009). The latter may translate into shifts in fish productivity which result in variations in stock abundance and determine its sensitivity to fishing mortality, thus the reproductive tactics are closely connected to the population dynamics and have implications for a sustainable stock exploitation (Pavlov et al., 2009). Health variables such as parasitism and energy reserves of fish are important for the future population success. However, there is no a single fish health measurement rather it can be assessed by a variety of criteria depending on the study and ideally a set of indicators is to be used to get a more realistic picture. Condition is a proxy of fitness and under the assumption that fish in better condition have larger energy reserves, it can be estimated by morphometric, morphophysiological and biochemical indicators (Lloret et al., 2014).

Additionally, because of their potential effect on the fish live cycle, parameters describing the infection by macroparasites could also be included in health assessments as they are relatively easy to monitor (Lloret et al., 2012). Parasites are those organisms which benefit at the expenses of their host draining the host energy directly, e.g. use of host-derived resources, or indirectly, e.g. immune response, and ultimately altering the host homeostasis (Bush, 2000). Understanding the factors that influence the bidirectional relationship between parasitism and host life-history traits is challenging not just because of its complex nature but also because of the difficulty on quantifying the effects of parasitism (Sindermann, 1987) and on separating the impacts of the multiple stressors that are acting simultaneously (e.g. Ukwa *et al.*, 2018). It is believed that the coevolution of the host-parasite interaction over time may have resulted in a dynamic equilibrium between parasite virulence and the host resistance (Barret, 1986). However, under the influence of climate change, this equilibrium may be broken, e.g. the temperature is one of the strongest abiotic factors modifying host-parasite relationships as it may have direct effects over hosts and parasites altering their distribution ranges, immunocompetence, virulence and transmission rates, but also indirect effects through the modification of the environment, impacting host life history traits and altering the occurrence of parasite infection (Marcogliese, 2008). Because macroparasites generally have complex life cycles, they can also provide information about their hosts, habitats and the structure of the ecosystem (Marcogliese, 2004), and they can as well be used as biological tags for stocks differentiation (Mackenzie & Hemmingsen, 2015) or as indicators of heavy metal pollution (Abdel-Ghaffar et al., 2014). Additional motivations for their monitoring are related to public health because the ingestion of raw fish infected with anisakid L3 larvae may cause anisakiasis in humans (e.g. Arizono *et al.*, 2012).

Despite the commercial importance of blue whiting, *Micromesistius poutassou* (Risso, 1827), little information is available regarding its biology (collected in the Methodology section, e.g. Bailey (1982); Bas & Calderon-Aguilera (1989)), especially scarce in the Mediterranean. Based on existing studies, the nematodes are of the most common and abundant parasites of blue whiting in several regions, e.g. Kusz & Treder (1980), Fernandez et al. (2005), Cruz et al. (2007). Extensive bibliography is available regarding the prevalence and intensity of Anisakidae nematodes in blue whiting (Molina-Fernández et al., 2018), but the few studies existing on the infection by other nematodes are mostly from the Atlantic and not updated (MacKenzie, 1979). In terms of the relationship between parasitism and condition and reproduction that could give us some insights on the effects of parasites on their hosts biological performance, little information is available for marine exploited fish (see Introduction section).

Populations of exploited cold-water species such as the blue whiting in the Mediterranean are in a poor situation because of the isolated and synergistic effects of both, overfishing and climate change (e.g. Lloret *et al.*, 2015). In this sense and as we already pointed out in the introductory chapter, the NW of the Mediterranean Sea, which has colder waters than the rest, may become a *"Cul de Sac"* because the predicted range shifts are physically constrained (Lejeusne et al., 2010) and therefore these species would be unable to escape from suboptimal environmental conditions and suffer physiological stress (Lloret et al., 2015). In fact, during the last two decades the Mediterranean landings of blue whiting species have decreased from almost 30000 tons down to 2000 tons in 2016 (GFCM, 2016). Actually, variation in blue whiting population parameters has shown links to fluctuation of environmental and hydrodynamic factors (Trenkel et al., 2015; Martin et al., 2016) and overfishing (Bas & Calderon-Aguilera, 1989; Rueda et al., 2015). Thus, it is necessary to assess the biological traits and the trade-off among them in cold-waters species inhabiting the warmer edge of their distribution such as the blue whiting in the Mediterranean.

In this study we focused on *M. poutassou* from the NW Mediterranean as a case study of an exploited cold-water species in a warming sea. Our purpose was (i) to evaluate its reproductive tactics and health status (including condition and parasitism by nematodes[3]) and (ii) to investigate the trade-offs between these life history traits. Some of the parameters here analysed have never before been described for the blue whiting. We described the fluctuation of the main energy deposits, the reproductive cycle, the breeding

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[3] Despite other parasites (e.g. monogeneans or crustaceans) could be more relevant in terms of energetic drain and direct effects on the host, here we focused on nematodes because of (i) in general parasite effects seems to be intensity-dependent and (ii) previous studies have reported the nematodes as one of the most abundant parasites of blue whiting. It has also been taken into account that they are relatively easy to monitor and thus could be a new parameter to include in fish health assessments. Moreover, their potential risk for human health makes of them an interesting subject of study. Extensive details are given in the Approach and aims section.

strategy, the egg production and quality, the fecundity type and the infection by nematodes. Subsequently the interrelationship between the aforementioned variables was studied to assess the trade-offs among them and the relative mass of the spleen was included as an indicator of the immune activity (Press & Evensen, 1999). Theoretically, a higher spleen index corresponds to a higher immune activity (Smith & Hunt, 2004). Aiming for a precise and comprehensive assessment, a combination of traditional and emerging methodologies was used, e.g. egg production was estimated through gravimetric methods and also through histological methods applying the advanced oocyte packing density (OPD) theory (Kurita & Kjesbu, 2009). Altogether, the results of this study aim to help to understand and predict the effects of climate change and to develop integrated management strategies for the sustainable exploitation of the stock.

4.2.3. METHODOLOGICAL APPROACH

Fish sampling

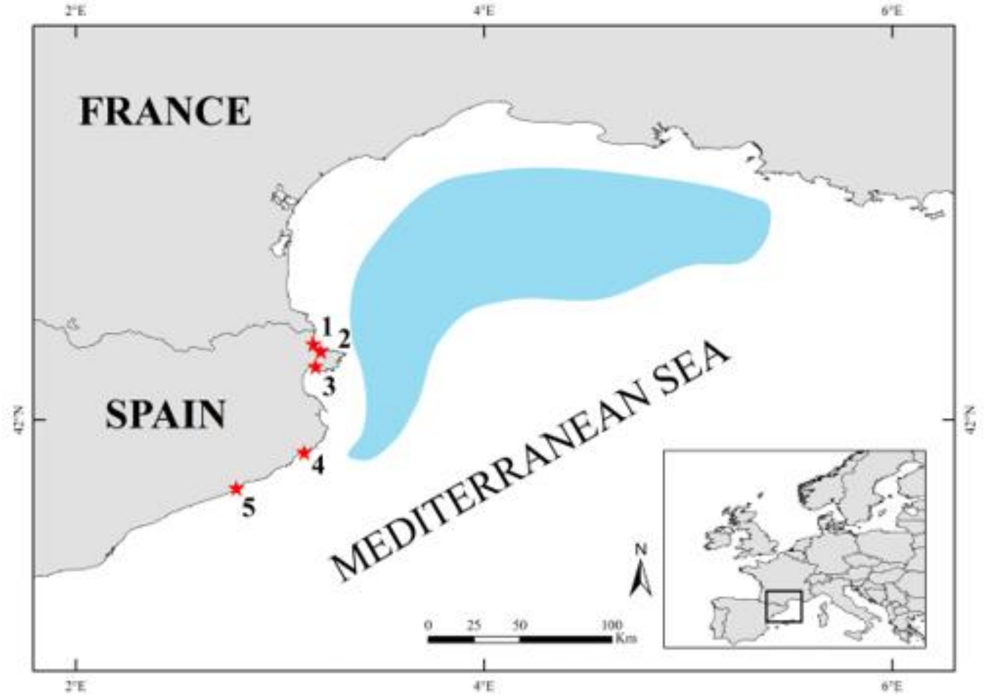


Fig 21. Sampling area of Case study II. Map showing the Gulf of Lion where the presently examined females of blue whiting (*Micromesistius poutassou*) were captured (blue area) (May 2015 - July 2016), and the landing ports (red stars): (1) Llançà, (2) Port de la Selva, (3) Roses, (4) Palamós, (5) Blanes. Author: Víctor Àguila Gimeno.

Blue whiting specimens were collected monthly aiming to 40 specimens per month, from commercial bottom trawlers fishing in the Gulf of Lion (NW Mediterranean Sea) at 50-520 m depths, from June 2015 to July 2016. Individuals were obtained shortly after being landed at the five main Catalan fishing harbours of the region (Fig. 21). Total length (TL, ± 0.1 cm), total body weight (TW, ± 0.01 g), eviscerated body weight (EW, ± 0.01g), liver weight (LW, ± 0.1 mg), gonad weight (GW, ± 0.1 mg) and spleen weight (SW, ± 0.1 mg) were recorded. Sex and gonads development phases (MAT) were macroscopically determined using standardized terminology (Brown-Peterson et al. 2011). The gonads were removed and the left lobe was fixed in 4% buffered formaldehyde for histological assessment of fecundity and egg quality estimation. Additionally, the liver of each fish was frozen at -20ºC for further lipid content quantification in the laboratory (see following section).

Condition

The energetic fitness (condition) was approximated using morphometric (i.e. Le Cren's relative condition factor, Kn) and organosomatic indicators (i.e., hepatosomatic index, HSI). Given that the liver is the major lipid storage in many gadoids species (e.g., Lambert and Dutil, 1997), the HSI was calculated for each individual. Prior to that, we verified lipid ratio content in the liver of 30 developing (DV) females, since in this MAT lipid depots have already been filled up, following the Soxhlet method. Our results confirmed the main role of the liver in energy, i.e. about the 70% of the liver wet weight and the 90% of the liver dry weight corresponded to lipids (Fig. 22). The Kn was positively related to HSI ($R^2 = 0.62$, $p < 2.2e-16$) (see Annex I: Fig. S1), thus to avoid redundancy only HSI was used as the condition indicator for the analysis of this study.

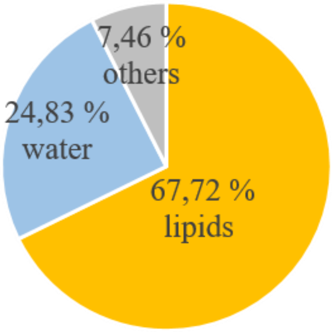


Fig 22. Liver proximate composition in blue whiting (*Micromesistius poutassou*) from Gulf of Lion. Liver lipid, water and others content is shown as percentage of liver wet weight, analysed in developing females (n = 30) applying the Soxhlet's method.

Spleen-somatic index (SSI), which describes the relative size of the spleen, was calculated. Among other numerous functions, the spleen of vertebrates has a role on immune response (Press & Evensen, 1999) and variations in its mass in relation to parasite richness and load have been reported for different taxa (Seppänen et al., 2009; Marteinson et al., 2017).

Reproduction

The energy allocated to reproduction was estimated by calculating the gonadosomatic index (GSI), this also provided overviews on the progress in the fish reproductive cycle.

Apart from haematoxylin and eosin, histological sections (4 µm) were additionally stained with Mallory to enable the detection of the cortical alveoli (Muñoz et al., 2010), which in this species are reported to be small and located in a narrow layer stuck to the wall (Mazhirina, 1978). Sections were screened for validation of the assigned MAT (Fig. 23) based on the most advanced oocyte stage and the presence of post ovulatory follicles (POF), alpha-atresia (αATR) and beta-atresia (βATR) and the relative amount of connective tissue (TIS) and vascularization (BLD) (see below).

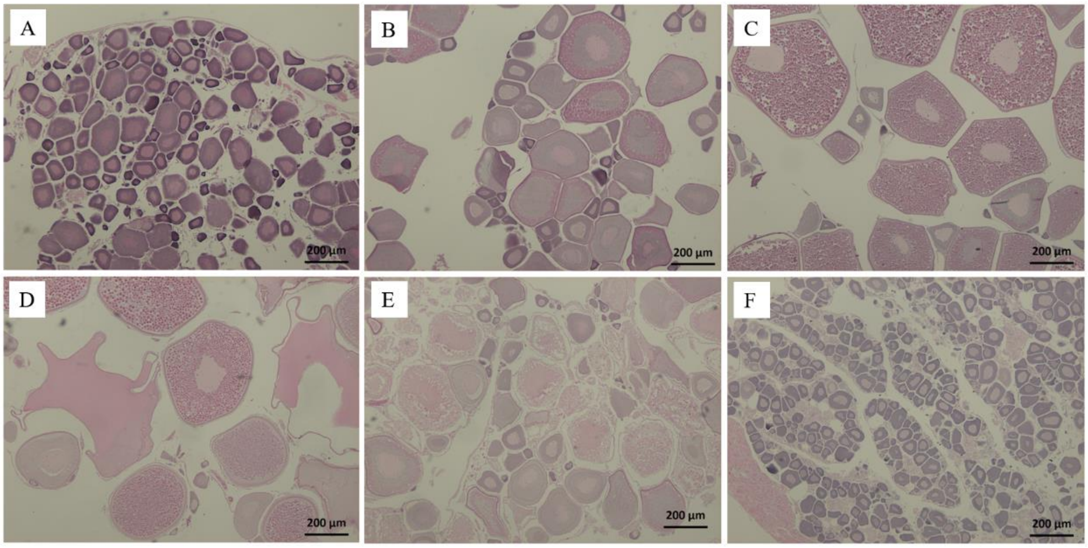


Fig 23. Photomicrographs of histological slides of ovaries of blue whiting (*Micromesistius poutassou*) from the Gulf of Lion. Immature (A), Developing (B), Spawning capable (C), Actively spawning (C), Regressing (D) and Regenerating (E) ovaries embedded in paraffin and stained with haematoxylin-eosin. Scale bar is shown.

Based on cytoplasmic processes the stages ($_i$) of oocyte development (Table 5, Fig. 26) were adapted from existing literature (Mazhirina, 1978; Kjesbu, 1987; Lowerre-Barbieri et al., 2011a). The probability that an individual was mature was predicted as a function of body length fitting binary maturity observations (immature, mature) and length (1 cm length classes) to binary logistic models, based on which the L_{50} was estimated (the body length at which 50% of the individuals were mature).

Table 5. Histological descriptions of ovaries of *Micromesistius poutassou*. Terminology of histological features seen under the light microscope, split into oocytes in different development stages and other, relevant ovary structures detected.

Oocyte development stage	**Histological features**
Previtelogenic 1 (EPVO1)	Oocytes are small with a prominent nucleus. The cytoplasm is strong and uniformely stained.
Previtelogenic 2 (EPVO2)	Oocytes increase in size. Around the peripheral zone of the nucleus there are 5-6 large nucleoli. Cytoplasm is evenly stained.
Presence of circumnuclear ring (CNRO)	Localized RNA zones in the cytoplasm, turn into a deep staining RNA ring. The RNA ring migratesto the periphery and gets narrower. Numerous small nucleoli. The follicular evelop become visible.
Cortical alveoli (CAO)	The first vaccuoles, not always visible, appear between the residuals of the RNA ring. Small vacuoles in a thin layer stuck to the chorion. The number of small nucleoli rises. Defined perinuclear zone. Chorion becomes visible.
Vitelogenic 1 (VTO1)	Small yolk granules distributed in a narrow ring in the periphery of the cell. Well defined perinuclear zone.
Vitelogenic 2 (VTO2)	Yolk granules increase in number and size. The perinuclear zone stills visible.
Vitelogenic 3 (VTO3)	The whole cytoplasma is filled with large yolk granules . The layer of granulosa cells is clearly visible and the chorion is intensely stained.
Migratory nucleus (MNO)	Yolk granules increase in size and the nucleus migrate to the periphery. Thick zona radiata.
Hydrated (HYO)	Oocytes become completely hydrated and homogeneously stained. Irregular shape due to shrinkage. Thin chorion.
Post-ovulatory follicle (POF)	Follicular envelop after ovulation
Alpha-atresia (αATR)	Breaks appear in the chorion, cytoplasm disorganized
Beta-atresia (βATR)	No corion or yolk granules left, high degree of vacuolization

A subset (n = 30) of spawning capable (SC) and actively spawning (AS) females was randomly selected for detailed description of reproductive tactics (oocyte development pattern and fecundity type) through the analysis of histological sections and whole-mounts. For each female, seven micrographs (counting fields) of ovary histological sections were taken at 4x magnifications using a digital camera (OLYMPUS U-CMAD-2AxioCam Icc1) mounted on a light microscope (OLYMPUS BX40) with a resolution of 0.855 px/µm. The number of counting fields required to reliably estimate the volume fraction (Vv) was assessed on a pilot study on two females (one in SC and one in AS phase) aiming for a deviation from normalized grand mean of Vv_i (estimated by grid counting as a function of increasing number of counting fields, from 1 to 15) lower than 0.05 (see Annex I: Fig. S2). These micrographs were used to estimate $stage_i$ oocyte diameter (OD_i) and Vv. A total of 10 oocytes (see Annex I: Fig. S2) for pilot study on the number of oocytes to be measures) were measured to estimate the mean volume based oocyte diameter (ODv_i) which was then turned into the formalin-fixed dimensions ($cODv_i$) by applying a correction factor developed by Saber *et al.* (2015). A grid (418 number of points and 70 µm probe line length) was overlaid on the micrographs and the Vv of $stage_i$ oocytes, POF, ATR, TIS and BLD was estimated. The $stage_i$ OPD (OPD_i) (Kurita & Kjesbu, 2009) was calculated and used to estimate the total number of $stage_i$ oocytes (cNO_i), which was standardized for fish TL as TL-based $cNO_i = cNO_i/TL^3$. It was not feasible to get reliable histological measurements of HYO size due to their irregular shape resulting from high shrinkage. The aforementioned calculations followed Korta *et al.* (2010), and can be consulted in the Methodology section. Finally, this subset of 30 females was expanded with 25 extra females randomly selected from the spawning season period to evaluate the evolution of the relative intensity of αATR (I_A) throughout the spawning season.

Following Domínguez-Petit *et al.* (2015) the gravimetric method was applied on whole-mounts of ovaries subsamples from the same subset to count and measure oocytes and obtain oocyte size frequency distribution (OSFD) (see Methodology chapter) that could help to assess the type of fecundity (determinate vs. indeterminate). Based on the data obtained from the gravimetric method, the reproductive capacity was assessed from the batch fecundity (BF) estimated on whole-mounts. Because of the multimodal overlapped OSFD the algorithm from the *mixtools* package (Benaglia et al., 2009) for *R* software (www.r-projecte.org) was applied in order to accurately calculate the number of oocytes belonging to the most advanced batch (MAB) (those with a 95% probability of belonging to the second component. In those AS females showing POF, the BF was estimated from the preceding batch. Additionally, BF was also estimated from the stereological data as cNO_{MAB} which is the sum of the oocytes belonging to the MAB (in this case which oocyte stages composed the MAB was decided based on the OSFD).

The mean dry mass of HYO (ODM) from AS ovaries was used as a proxy of egg quality and it was estimated for each female as described in the Methodology chapter.

Parasitism

Nematode parasitism was evaluated in a subset selected aiming for 30 randomly chosen blue whiting individuals per month when it was possible. This resulted in a subset of 174 fish, which was considered a representative subsample. The entire viscera were removed and subsequently the body cavity and the internal organs -i.e. the stomach, the intestine, the pyloric caecum, the liver, the gonads right lobe and the gallbladder-, were examined under a stereomicroscope. The left side muscle was filleted and crushed between two methacrylate plaques to be observed onto a transillumination platform. All nematode parasites were collected, washed with a saline solution (0.8% NaCl), observed alive and, when necessary, cleared in lactophenol for a better observation of internal structures. Finally, the nematodes were fixed and preserved in 70% ethanol.

Based on their morphology and internal structures, nematodes were classified into morphotypes (MFT_i) and subsequently identified to the lowest possible taxonomic level (Naidenova & Nikolaeva, 1968; Petter & Maillard, 1988; Berland, 1989; Køie, 1993; Petter & Cabaret, 1995; Moravec, 2007; Santos et al., 2009). Since nematode parasite species identification is mostly based on adult features, larvae could not always be identified to the species level.

Individual intensity of infection (IY) was calculated as the number of specimens of nematodes (IY_{nem}) or of a particular MFT (IY_{MFT}) found in a single host, and the mean intensity (mnIY) was calculated as the average in the whole sample of infected hosts. The median intensity (mdIY) and its 95% confidence interval (CI) were also determined. The prevalence (P) of nematodes (P_{nem}) and of a particular MFT (P_{MFT}) was estimated as the proportion of fish infected with it (Bush et al., 1997). Parasite richness (PR) was considered to be the number of MFT present in each host.

Data analyses

After rejecting normality and homoscedasticity by Saphiro-Wilk and Levene's test respectively, the differences of mean values of TL, Kn, HSI, GSI and SSI was checked between sex and between mature and immature using the Mann–Whitney U test and between MAT applying the Krsukal-Wallis test followed by the post-hoc Dunn test with Bonferroni adjustment. The possible relationships between reproduction and condition parameters, - i.e. TL, HSI, GSI, SSI, lipid percentage of liver dry weight (LDW), lipid percentage on liver wet weight (LWW), percentage of water in liver and IY_{nem}-, was assessed using the Spearman's Rank Correlation coefficient and, when significant correlations were found, the relationship was fitted to linear regression models. When differences aroused in the previous comparisons of the somatic indices between sex or MAT, the relationship between de somatic indices for the levels of these factors was explored on a generalized linear model (GLM) where the equality of slopes was tested. Subsequently, if the equality of slopes was rejected, the correlation was assessed separately for sex and/or for those gonad development phases that were significant in the model.

Typically, most hosts show small or null parasite infection while few hosts show high infection levels, in front of these aggregated (right-skewed) distribution of parasites many statistical methods become obsolete (Rózsa et al 2000). For this reason, the software *Quantitative Parasitology 3.0* (Reiczigel and Rózsa, 2005), which was developed to manage the left biased frequency distribution of parasites, was used to report the prevalence and the mean and median intensity with their 95% CI of parasites, as well as to compare parasite infection across categories of the factors sex, MAT and site of infection (SITE). The mdIY was compared applying the Mood's median and the equality of intensity distribution by and the stochastic equality test. For factors including several levels, i.e. MAT and site of infection, P was compared applying the Fisher's exact test, while in two-level factors, i.e. sex, P comparisons were performed using the Unconditional exact test. A Mann-Whitney U test was used to analyse differences of PR.

Based on the results from the first explorations, GLMs (gamma family by log-link function) were used to determine the effects of the covariables TL, MAT and sex on the variation of the reproduction and condition parameters (GSI, BF, ODM, HSI, SSI) when necessary. The most parsimonious model was selected based on the smallest AIC in a backwards stepwise procedure using the *stepAIC()* function from the *MASS* package. The *k* parameter was set to 3.8415 to impose a threshold of 5% significance level for the inclusion of the model variables. The Table S1 shows which of the explanatory variables had significant effect on the SSI, GSI, HSI; while no effects on BD and ODM was found. Thus, SSI, GSI and HSI were adjusted for the indicated explanatory variables and the possible differences between infected/uninfected hosts or between hosts showing high or low infection levels (over/below mdIY) were tested by adjusted means pairwise contrast using the *emmeans()* and *contrast()* functions of the *emmeans* package. While for BD and ODM the differences were analysed by Mann-Whitney U test. The Spearman's Rank Correlation coefficient was used to assess the possible relationship between the variable response and the intensity of infection.

Residuals were checked for model assumptions of independence, normality, linearity and homoscedasticity (residuals vs. fitted plot and Normal Q-Q plot). All statistical analyses (except for those already mentioned) were performed using R version 3.2.3 (https://www.r-project.org/) and the level of statistical significance adopted was $p < 0.05$.

4.2.4. OUTCOMES

Of the 635 blue whiting specimens examined 351 were females, 266 were males and on 19 it was not possible to determine the sex. The overall L_{50} was estimated at 190 mm and no immature individuals were found with TL > 206 mm (Fig. 24).

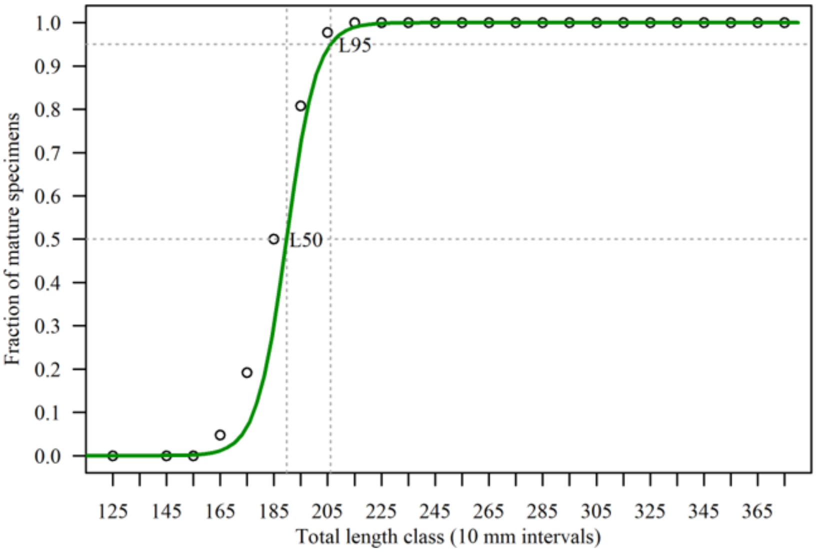


Fig 24. Frequency of mature specimens of blue whiting (*Micromesistius poutassou*) from the Gulf of Lion as a function of body size class. Logistic curve is shown in the plot. The size at 50% maturity (L50) was estimated at 190 mm and the size at 95% maturity (L95) at 206 mm.

Table 6. Condition and reproductive variables for *Micromesisitus poutassou*. Values and comparison tests between sex and maturity stage. Mean, standard deviation (SD) minimum (min) and maximum (max) values of total length (TL), condition factor (Kn), hepatosomatic index (HSI), spleen-somatic index (SSI), gonadosomatic index (GSI), batch fecundity (BF), relative BF (RBF), number of oocytes in the most advanced batch (cNO_{MAB}) and oocyte dry mass (ODM). Number of analysed samples (n). The statistics of significant comparison tests (w for Mann–Whitney U test and K for Kruskal-Wallis) and p-values (p) are reported.

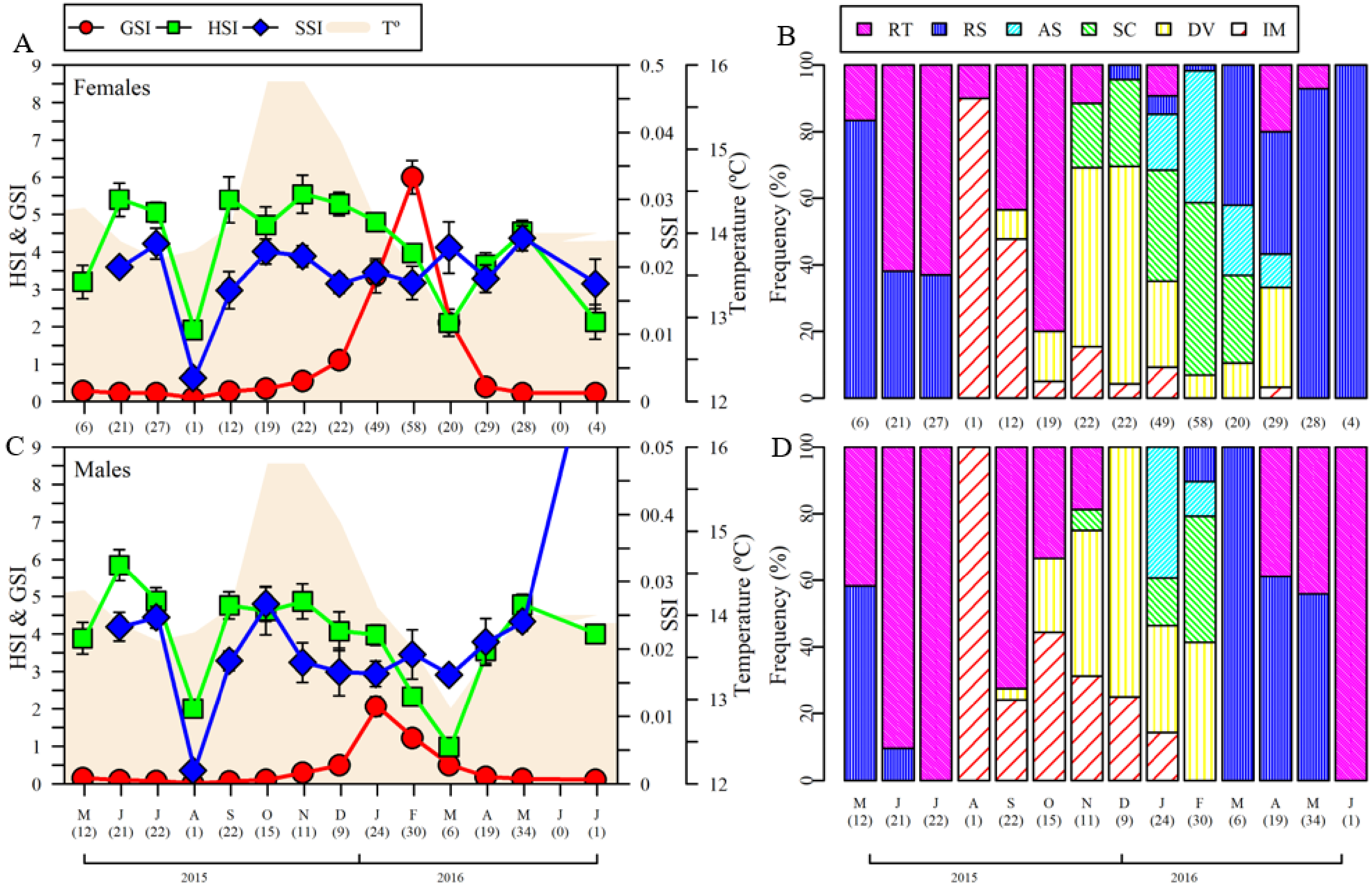
Variable	n	mean	SD	min	max	Statistic	p
TL	635	247.46	45.86	134	380		
Immature	83	175.06	14.28			W=454	< 2.2e-16
Mature	552	258.49	38.08				
Females	350	262.18	42.76			W=40480	< 0.05
Male	266	253.92	30.19				
DV	103	251.16	42.96			K=118.27	< 2.2e-16
SC	80	239.38	38.50				
AS	53	228.42	25.51				
RS	125	284.38	34.62				
RT	170	262.55	26.20				
Kn	635	1.02	0.09	0.74	1.27		
HSI	635	4.06	1.93	0.52	11.29		
Immature	83	2.37	1.11			W=8761	< 2.2e-16
Mature	552	4.32	1.89				
Female	350	4.22	1.94			W=71804	< 2.2e-16
Male	266	3.94	1.91				
DV	103	4.63	2.15				
SC	80	4.24	1.73				
AS	53	3.91	1.76				
RS	125	3.93	1.98				
RT	170	4.70	1.71				
SSI	532	2.03E-02	1.17E-02	5.84E-04	9.44E-02		
DV	93	1.9E-02	1.1E-02			K=26.498	2.51E-05
SC	69	1.8E-02	1.4E-02				
AS	47	1.8E-02	1.3E-02				
RS	104	2.3E-02	1.2E-02				
RT	163	2.2E-02	1.0E-02				
GSI	635	1.2	2.3	0.00	16.72		
Immature	82	0.13	0.14			W=8233	< 2.2e-16
Mature	550	1.36	2.42				
Female	350	1.82	2.86			W=56649	< 0.001
Male	266	0.5	0.72				
DV	103	0.69	0.69			K=19.454	6.40E-04
SC	80	3.44	2.20				
AS	53	6.29	3.66				
RS	124	0.29	0.34				
RT	170	0.17	0.14				
BF	30	13570	18248	492	78379		
cNO_{MAB}	30	35814	45178	748	186061		
RBF	30	3002.04	2933.09	47.18	14509.53		
ODM	37	1.73E-05	7.62E-06	2.00E-06	2.83E-05		

The Table 6 shows the range, the mean and the SD values of the analysed parameters and the significant differences between sexes and between immature and mature specimens. In average, mature females were larger than males. The HSI was higher in mature individuals than in immature; differences of mean HSI aroused between MATs being higher in regenerating (RT) and DV specimens.

The GSI differed between immature and mature fish and between sexes being higher in mature females; as expected, there were differences between MATs, being highest in SC and AS specimens. As seen in the GLM (Table S2), the relationship between GSI and HSI showed non-equal slopes between MATs and sexes (see Annex I: Fig. S3). In AS females the GSI showed a strong positive correlation with the HSI ($\rho = 0.6$, $p < 0.0001$) while for DV, SC, regressing (RS) and RT individuals these indices correlated negatively ($\rho = -0.12$, $p = 0.007$). A negative correlation was found between GSI and LWW ($\rho = -0.57$, $p < 0.05$) indicating that these fat depots accumulated in the liver are invested in gonadal development. The SSI showed differences between MATs achieving greater values in RS and RT females (Table 6) and it was negatively correlated with GSI ($\rho = -0.22$, $p = 0.015$). This correlation was stronger in SC fish ($\rho = -0.4$, $p = 0.0019$).

The fluctuation of the GSI and the monthly frequency of MAT (see Fig. 23 for ovarian development phase micrographs) in mature specimens showed that the gonads began to develop in October, coinciding with the maximum on sea water temperature (Fig. 25A,B). The spawning took place from January to April (Fig. 25C,D) peaking in January for males and in February for females and followed by a sharp decrease (coinciding with the maximum on sea water temperature) to minimum values of reproductive activity from April on it. The HSI decreased during the gonadal development, increased at the end of the spawning season and fluctuated modestly around 4.5 during the rest of the year. The SSI decreased during the period of gonad development and raised after the peak of spawning activity. It should be noted that in August almost all the samples obtained were immature and that the sharp decrease of both condition indices (HSI and SSI) recorded for mature individuals during this month came from a single female and a single male (Fig. 25c). Immature individuals appeared only from August to April (see Annex I: Fig. S4).

Fig 25. Reproductive cycle for females (A, B) and males (C, D) of *Micromesistius poutassou* from Gulf of Lion. (A, B) Monthly frequency of gonad development phases. (C, D) Variation on the gonadosomatic (GSI), hepatosomatic (HIS) and spleen-somatic (SSI) indices and sea-water temperature (Tº) at -80 m. Gonad development phases: immature (IM), developing (DV), spawning capable (SC), actively spawning (AS), regressing (RS) and regenerating (RT). Sea water temperature (at -80m) was obtained from l'Estartit meteorological station (http://meteolestartit.cat/).


A
GSI
HSI
SSI
Tº
Females
HSI & GSI
SSI
Temperature (ºC)
0 1 2 3 4 5 6 7 8 9
0 0.01 0.02 0.03 0.04 0.5
12 13 14 15 16
(6) (21) (27) (1) (12) (19) (22) (22) (49) (58) (20) (29) (28) (0) (4)
B
RT
RS
AS
SC
DV
IM
Frequency (%)
0 20 40 60 80 100
(6) (21) (27) (1) (12) (19) (22) (22) (49) (58) (20) (29) (28) (4)
C
Males
HSI & GSI
SSI
Temperature (ºC)
0 1 2 3 4 5 6 7 8 9
0 0.01 0.02 0.03 00.4 0.05
12 13 14 15 16
M J J A S O N D J F M A M J J
(12) (21) (22) (1) (22) (15) (11) (9) (24) (30) (6) (19) (34) (0) (1)
2015
2016
D
Frequency (%)
0 20 40 60 80 100
M J J A S O N D J F M A M J
(12) (21) (22) (1) (22) (15) (11) (9) (24) (30) (6) (19) (34) (1)
2015
2016

The cODv$_i$ increased gradually in line with the stage of oocyte development ($_i$) (Table 7, Fig. 26). Oocytes at different development stages were simultaneously present during different MATs (Fig. 23) and the OSFD showed a polymodal pattern (see below).

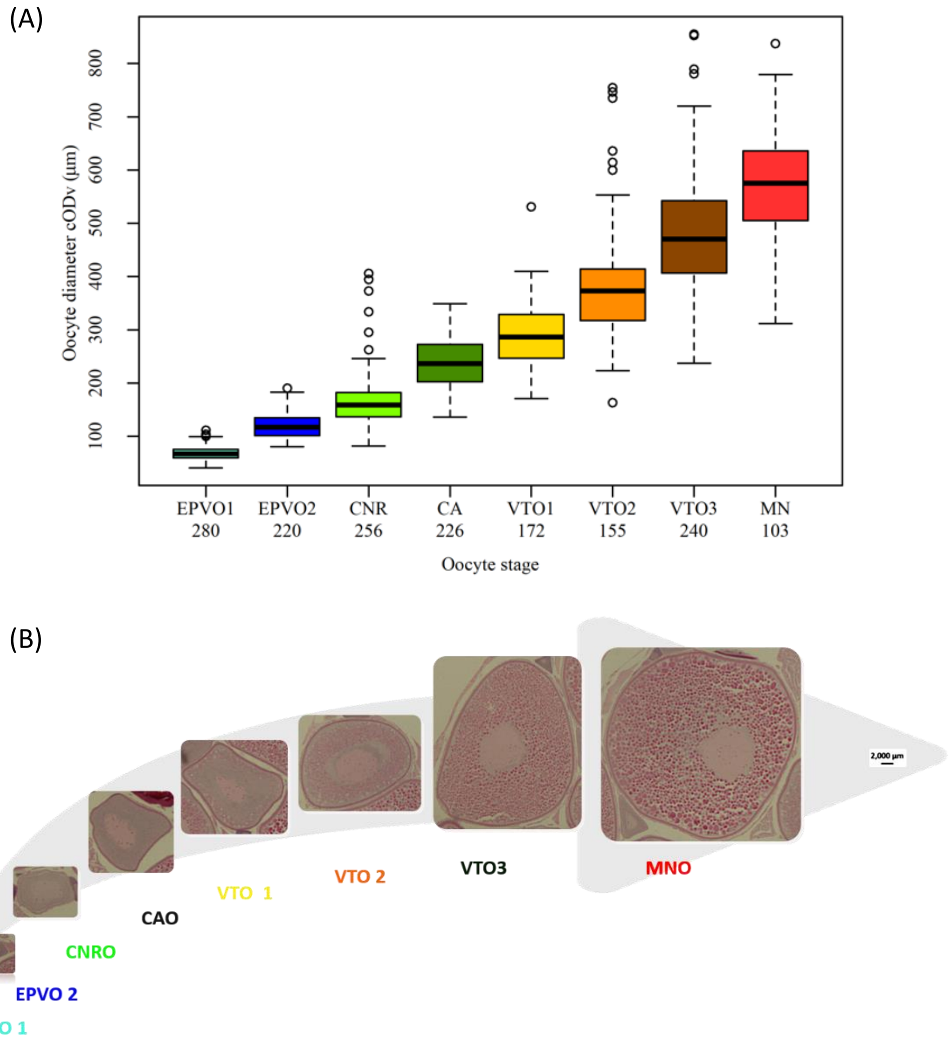


Fig 26. Stage-specific oocyte diameter in blue whiting (*Micromesistius poutassou*) from the Gulf of Lion. (A) Volume-based oocyte diameter (ODv$_i$) measured from histological slides and corrected for shrinkage in paraffin. Number of measured oocytes are shown in x-axis second line. Oocyte stages: previtellogenic oocytes 1 (EPVO1), previtellogenic oocytes 2 (EVPO2), previtellogenic oocytes with circumnuclear ring (CNRO), cortical alveoli oocytes (CAO), vitellogenic 1 oocytes (VTO1), vitellogenic 2 oocytes (VTO2), vitellogenic 3 oocytes (VTO3), migratory nucleus oocytes (MNO). (B) Oocyte stages micrographs.

Table 7. Stage$_i$ oocyte diameter of *Micromesistius poutassou* . Volume based oocyte diameter (ODv$_i$). ODv$_i$ is non-corrected and cODv$_i$ is corrected for shrinkage, applying correction factors given for paraffin-embedded material (Saber et al., 2015).

Oocyte development stage	ODvi (µm) (Mean ± SD)	cODvi (µm) (Mean ± SD)
Previtelogenic 1 (EPVO1)	34.00 ± 6.04	68.94 ± 7.384
Previtelogenic 2 (EPVO2)	81.04 ± 13.68	130.00 ± 17.75
Presence of circumnuclear ring (CNRO)	107.20 ± 15.96	163.96 ± 20.72
Cortical alveoli (CAO)	164.13 ± 21.51	237.85 ± 27.92
Vitelogenic 1 (VTO1)	213.57 ± 38.99	302.06 ± 50.61
Vitelogenic 2 (VTO2)	276.73 ± 58.32	384.06 ± 75.70
Vitelogenic 3 (VTO3)	347.72 ± 59.11	476.15 ± 76.72
Migratory nucleus (MNO)	415.06 ± 68.52	563.56 ± 88.94

As shown in Fig. 27A, the TL-based cNO$_i$ of early secondary growth oocytes (CAO + VTO1) decreased after the peak of spawning activity was reached suggesting that the standing stock of oocytes is not replaced after this point. In the same line, the TL-based cNO$_i$ of advanced vitellogenic oocytes (VTO2-MNO) decreased during the spawning season (Fig. 27B) suggesting that the total fecundity decreases with each spawning event.

No significant differences of BF, ODM, $Vv_{\alpha ATR}$ and $Vv_{\beta ATR}$ aroused between SC and AS females, thus for the following analysis they were considered as a single group of ovaries with maturing oocytes. The mean I_A increased across the spawning season reaching values of 100% on RS females at the end of the spawning season (Fig. 27D). The OSFD obtained from the whole-mounts followed a multimodal distribution with no hiatus separating the yolked oocyte stock from the reservoir of unyolked oocytes, indicating a continuous oocyte development (Fig. 27C). In AS ovaries a separate mode with large (>700 um) HYO appeared.

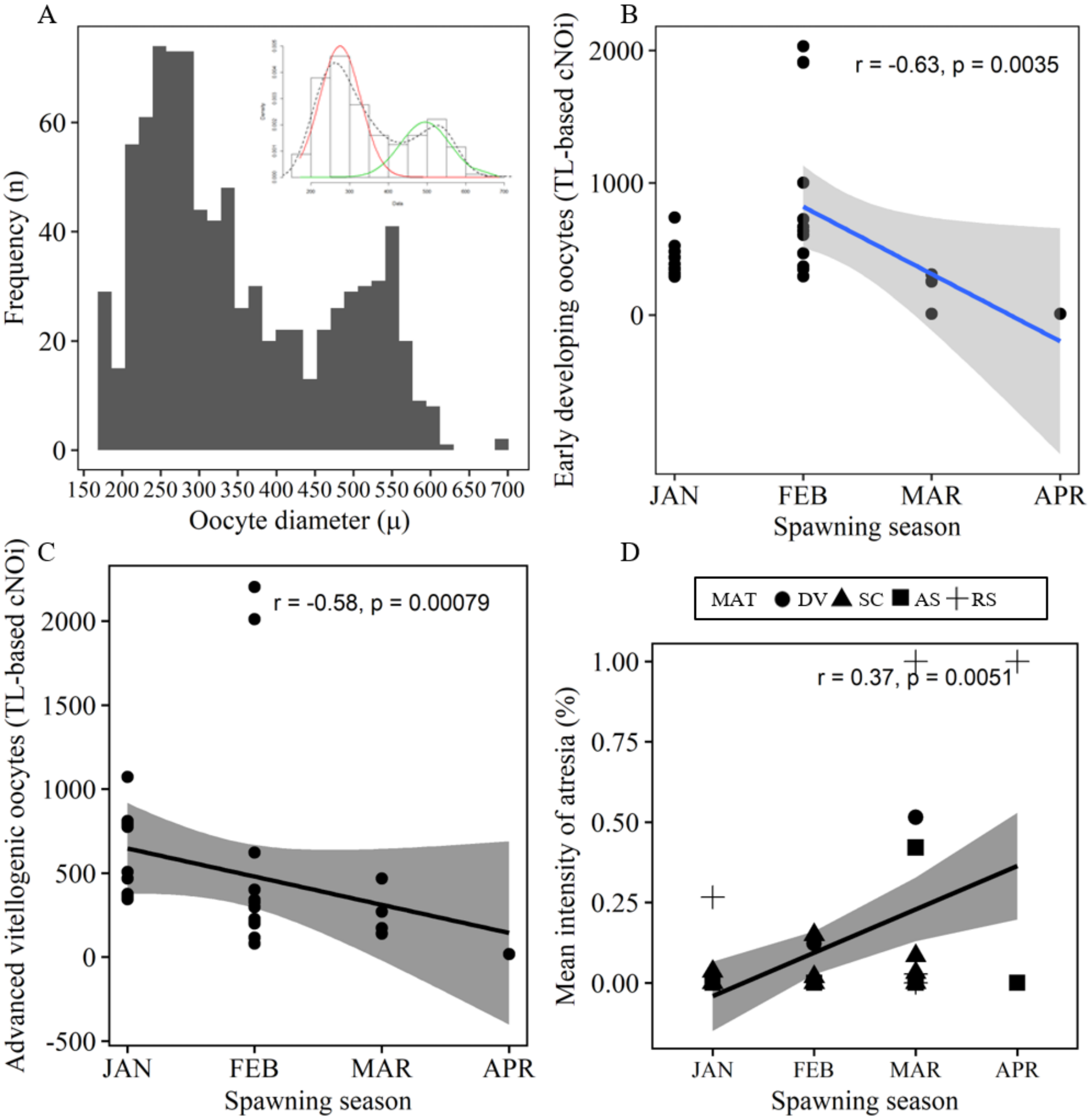


Fig 27. Criteria to assess the fecundity type (from Dominguez-Petit et al., 2015) applied *to Micromesistius poutassou* from Gulf of Lion. Monthly variation, throughout the spawning season, of (A) the abundance of early developing oocytes (cortical alveoli + vitellogenic 1), (B) the number of advanced vitellogenic oocytes (vitellogenic 2-3 + migratory nucleous), (C) incidence of atresia. (D) Oocyte size-frequency distribution and overlapped mixture distribution (small chart). Gonad development phases: developing (DV), spawning capable (SC), actively spawning (AS), regressing (RS).

The mean ± SD values of BF, RBF, cNO_{MAB} and ODM can be consulted in the Table 6. The $_{wm}BF$ correlated positively to TL (ρ = 0.31, p = 0.04). The cNO_{MAB} showed a strong positive correlation to BF (ρ = 0.62, $p < 2.2E-16$; Fig. 28) and to TL (ρ = 0.53, p = 0.002). The ODM showed a negative correlation ($p < 0.05$) with HSI (ρ = -0.49) and with GSI (ρ = -0.46).

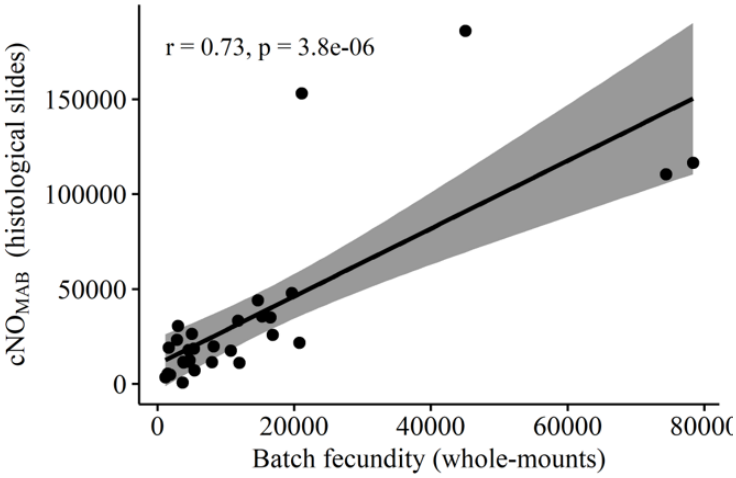


Fig 28. Relationship between number of oocytes in the most advanced batch (cNO_{MAB}) and batch fecundity (BF) in blue whiting (*Micromesistius poutassou*) from Gulf of Lion. The cNO_{MAB} was estimated based on histological slides. The BF was estimated based on whole-mounts. Both parameters were estimated over 30 SC and AS females sampled during the spawning season in 2016 (January -April). The outliers belong to the largest females, both in AS phase and sampled at the beginning of the spawning season (January). The Spearman Correlation Coefficient (r) and the p-values (p) are also shown. The shaded area reflects the 95% confidence band.

Of the 635 specimens sampled, 174 were examined for parasitism by nematodes. The 70.5% of the examined hosts were infected with at least one nematode, with an individual IY_{nem} ranging from 1 to 251 nematodes. The $mnIY_{nem}$ was estimated at 6.48 (95%CI = 4.11-15.2) and the $mdIY_{nem}$ at 2, 5 (95%CI = 2-3). In total, 790 nematodes belonging to 15 MFTs were registered (Table 8). The number of MFTs doesn't reflect the real number of species which may be higher because specimens belonging to different species may have been identified only to genus level or it may as well be lower because different larval stage of the same species may have been identified as distinct MFTs. Of the 15 MFTs, 8 were identified to genus or species level while 7 were left as non-identified (NI) MFTs (see Annex I: Fig. S5-S14).

Table 8. Nematodes (and cestode plerocercoid) found in *Micromesistius poutassou* from Gulf of Lion. MFT, morphotype. n, sample size. P, prevalence. CL 95% CI, Clopper-Pearson 95% confidence limits. YI, intensity of infection. BCa 95% CI, 2000 Bootstrap BCa 95% confidence limits. Min, minimum value. Max, maximum value. 95%CI, 95% confidence interval. Parasite stage: second-stage larvae (L2), third-stage larvae (L3), forth-stage larvae (L4), adult (A), and, plerocercoid larvae (P). Site of infection: body cavity (C), liver (L), stomach lumen (Si), embedded in the stomach wall (Sw), pyloric caecum (PC), muscle (Mu), and, intestines (I). Host sex: female (F) and male (M). Ovarian development phases (Host MAT): immature (IM), developing (DV), spawning capable (SC), actively spawning (AS), regressing (RS), and, regenerating (RT).

Morphotype	Species	Parasite stage	Site of infection	Host sex	Host MAT	Infected (n=174)	P (%)	CL 95% CI	Intensity				
									Min-Max	Mean	BCa 95%CI	Median	95% CI
Nematodes	**All**	**All**	**C, L, Si, Sw,PC, M, I**	**F, M**	**IM, DV, SC, AS, RS, RT**	**122**	**70.1**	**63.1-77.1**	**1-251**	**6.48**	**4.09-15.01**	**2.5**	**2-3**
MFT 1	*Anisakis T1*	L3	C, L, Si, Sw,PC, M, I	F, M	IM, DV, SC, AS, RS, RT	85	48.9	41.2-56.5	0-187	5.72	3.22-17.2	2	2
MFT 2	*Hysteortilacium aduncum*	L3	C, L, Si, PC, I	F, M	IM, DV, SC, AS, RS, RT	50	28.7	22.1-36.1	0-59	2.82	1.58-7.76	1	1-2
MFT 3	*Anisakis T2*	L3	C, L, PC, I	F, M	DV, SC, RS, RT	40	23	17.0-30.0	0-6	1.55	1.27-2.08	1	1
MFT 4	*Hysteortilacium aduncum*	L4	Si, Sw,PC, M, I	F, M	DV, SC, AS, RS	11	6.3	3.2-11.0	0-4	1.91	1.27-2.73	1	1-4
MFT 5	NI2	-	Si, Sw,PC	F, M	DV, SC, RS	7	4	1.6-8.1	0-1	1	-	1	1
MFT 6	NI1	-	C, PC, Si	F, M	DV, SC, RS, RT	6	3.4	1.3-7.4	0-20	8.5	2.17-14.8	4.5	1-2
MFT 7	*Spinitectus sp.*	-	C	F, M	DV, SC	5	2.9	0.9-6.6	0-1	1	-	1	-
MFT 8	NI2	-	C, Si, I	F, M	SC, AS	4	2.3	0.6-5.8	0-3	1.5	1.0-2.0	1	-
MFT 9	NI3	-	C, Si	F, M	SC	3	1.7	0.4-5.0	0-2	1.33	1.0-1.67	1	-
MFT 10	NI4	-	Si	F	SC, RS	2	1.1	0.1-4.1	0-1	1	-	1	-
MFT 11	*Hysteortilacium aduncum*	A	Si	F	DV	1	0.6	0.0-3.2	0-1	1	-	1	-
MFT 12	*Contracaecum*	L3	Si	F	SC	1	0.6	0.0-3.2	0-1	1	-	1	-
MFT 13	*Camallanus*	A	I	M	DV	1	0.6	0.0-3.2	0-1	1	-	1	-
MFT 14	NI5	-	PC	M	DV	1	0.6	0.0-3.2	0-1	1	-	1	-
MFT 15	NI6	-	I	F	RT	1	0.6	0.0-3.2	0-1	1	-	1	-
	Hysteortilacium aduncum	L3+L4+A	C, L, Si, Sw,PC, M, I	F, M	IM, DV, SC, AS, RS, RT		32.8	25.8-40.3	0-62	2.86	1.67-7.29	1	1-2
Cestode													
MFT 16	Tetraphyllidean	P	C, L, Si, PC, I	F, M	IM, DV, SC, AS, RS, RT	72	41.4	34.0-49.1	0-500	70.89	51.5-98.7	28	15-50

Two different MFTs belonging to the genus *Anisakis* (Dujardin, 1984) were identified on the basis of their ventriculus length and the presence or absence of a mucron (Murata et al., 2011), i.e. *Anisakis* Type I (AT1) and *Anisakis* Type II (AT2) (see Annex I: Fig S5-S6). Based on this and on the review of anisakid host and distribution made by Mattiucci & Nascetti (2006). we regard this molecular identification outcome as also valid for our samples. A molecular analysis on fish from the same region performed previously by our laboratory (Ferrer-Maza et al., 2014) revealed that AT1 belongs to *Anisakis pegreffi* (Campana-Rouget and Biocca, 1955) and AT2 to *Anisakis physeteris* (Baylis, 1923). The overall dominant MFTs were AT1, *Hysterothylacium aduncum* (HA) third stage larvae (L3) and AT2, and the most intense infections were caused by NI1, AT1 and HA-L3 (Table 8). Despite our examinations only covered nematodes, the presence of one cestode which was tentatively identified as a tetraphyllidean plerocercoid (TEP) (Cherby 2002) following Khalil (1994), was also recorded because of its high occurrence (Table 8). Regarding the intensity of infection, IY_{AT1} correlated with IY_{AT2} ($\rho = 0.22$, $p = 0.0038$) and IY_{TEP} ($\rho = 0.18$, $p = 0.019$). For further analysis, MFTs corresponding to different HA stages (L3, L4 and adult) were pooled together.

No differences between sexes were found in P_{nem} nor in MFTs richness despite a few MFTs were only observed in females or in males (Table 8). However stochastic inequality of intensity distribution was found ($p = 0.006$) between females (mnIY ± SD = 5.35 ± 7.22; mdIY = 3) and males (mnIY ± SD = 8.90 ± 38.85; mdIY = 2). The P increased with TL up to $P \approx 90\%$ for nematodes and $P \approx 80\%$ for TEP (Fig. 29A). In average, the infected fish were significantly larger (TL: mean ± SD = 268.49 ± 48.48) than the non-infected (TL: mean ± SD = 237.84 ± 52.25). There was a positive relationship ($p = 0.0014$) between TL and IY_{nem} ($\rho = 0.37$), specifically for IY_{AT1} ($\rho = 0.35$), IY_{AT2} ($\rho = 0.43$) and IY_{NI1} ($\rho = 0.17$).

At a MFT level significant differences of prevalence aroused between MATs. In general, the most recurrent MFTs (AT1, AT2, TEP and NI1) showed similar P oscillation along the reproductive cycle, showing the lowest values in IM and AS and peaking just before and after de spawning, while P_{HA} increased from DV to RS hosts (Fig. 29B). The $mdIY_{TEP}$ was significantly different between MATs, being highest in DV hosts.

The P_{nem} differed between sites of infection ($p < 0.0001$) (Table 9), this was also true for the most common parasites (AT1, AT2, HA, NI1, TEP). The most recurrent sites of infection were the body cavity ($P_{nem} = 45.40\%$) and the lumen of the digestive tract (stomach, intestines and pyloric caecum) ($P_{nem} = 43.10\%$). On the second place, almost 30% of the examined livers were infected by at least one nematode. The AT1 was the most prevalent nematode in the cavity and in the liver while in the digestive tract it was the NI1 and the HA.

The most intense infections by nematodes were found in the liver and in the cavity, but no significant differences were found for mdIY. The P_{TEP} was highest in the digestive tract where severe infections were found in some hosts ($IY_{TEP} \approx 200\text{-}500$).

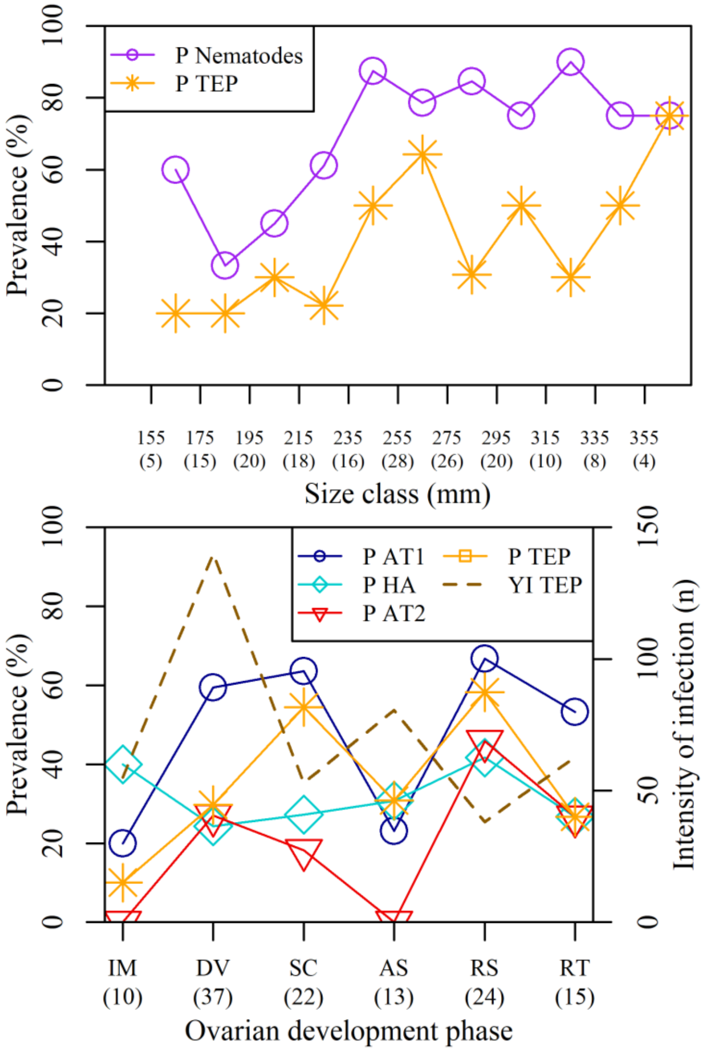


Fig 29. Relationship between prevalence of parasites and host body size (A) and host ovarian development phase (B) in *Micromesistius poutassou* from Gulf of Lion. Number of analysed samples in brackets. Ovarian development phases: immature (IM), developing (DV), spawning capable (SC), actively spawning (AS), regressing (RS), regenerating (RT). AT1: *Anisakis* type I, AT2: *Anisakis* type II, HA: *Hysterothylacium aduncum*, TEP: Tetraphyllidean plerocercoid. Intensity of infection (YI) of TEP shown in the second y axis of panel (B).

Table 9. Distribution of parasites in the body of *Micromesistius poutassou* from the Gulf of Lion. The prevalence of the main nematodes and the tetraphyllidean in the different tissues examined is shown.

	Prevalence (%)					
Site of infection	Nematodes	*A. pegreffi*	*A. physeteris*	*H. aduncum*	NI1	Tetraphyllidean
Digestive tract	43.10	9.20	5.17	27.59	32.76	39.08
Body cavity	45.40	32.76	16.67	7.47	0.00	2.30
Liver	29.31	27.01	3.45	1.72	0.00	4.60
Musculature	2.30	1.15	0.57	0.57	0.57	0.00
Gonads	0.00	0.00	0.00	0.00	0.00	0.00

On the comparison of the reproduction and condition variables (adjusted for TL, sex and/or MAT when required based on GLMs; see Annex I: Table S1) between hosts infected and non-infected by nematodes only BF showed significant differences being lower in infected fish (Table 10). The same results were obtained when hosts presenting infections by nematodes over and below the mdIY (mdIY = 2.5) were compared, while the GSI was higher in specimens infected over the median value. When we contrasted the same indices but between host highly and lowly-infected by nematodes, i.e. over and below the mnIY (mnIY = 6.48) respectively, highly infected hosts showed in average a higher HSI, higher SSI and, again, lower BF than lowly-infected hosts. All the other variables analysed showed no significant differences. At a MFT level, it became apparent that the results at a nematodes community level mostly reflect those of AT1 infection for HSI and SSI, however the differences of BF and GSI found were not detected at a MFT level, neither for the tetraphillidean infection which did not showed any significant result.

Table 10. Differences of condition and reproduction variables between levels of infection in *Micromesistius poutassou* from Gulf of Lion. Variables were adjusted for host body size (TL), sex and/or ovarian development phase (MAT). Pairwise contrasts were performed on the adjusted least square means; contrast statistic (z) and p-value (p) are reported. Only significant contrasts are shown. BF: batch fecundity, GSI: gonadosomatic index, HSI: hepatosomatic index, SSI: spleen-somatic index.

Variable (adjustment)	Grouping criteria	Adjusted mean	z	p
All nematodes				
BF (TL)	non-infected	9.76		
	Infected	8.64	3.176	0.0015
	Below-median	9.52		
	Over-median	7.80	2.063	0.0391
	Below-mean	9.52		
	Over-mean	7.80	2.063	0.0391
GSI (MAT)	Below-median	-0.08		
	Over-median	0.19	-2.008	0.0447
HSI (MAT)	Below-median	1.23		
	Over-median	1.46	-2.215	0.0268
	Below-mean	1.25		
	Over-mean	1.56	-2.432	0.015
SSI (TL)	Below-mean	-3.92		
	Over-mean	-3.55	-2.375	0.0175
AT1				
	non-infected	1.21		
	Infected	1.40	-2.31	0.0393
HSI (MAT)	Below-median	1.23		
	Over-median	1.60	-3.275	0.0011
	Below-mean	1.26		
	Over-mean	1.71	-2.696	0.007
SSI (TL)	Below-mean	-3.91		
	Over-mean	-3.30	-2.614	0.0089

4.2.5. CASE STUDY DISCUSSION

This study provides an assessment of the trade-offs of several life history traits such as parasitism, reproduction and condition of *M. poutassou* in the Mediterranean Sea in order to contribute to understand, from a biological point of view, the status of this exploited cold-water species in a climate change hot-spot (Lejeusne et al., 2010).

First of all, the results fill a knowledge gap on key aspects of *M. poutassou* health status and reproductive tactics. Our results showed that over 90% of the liver dry weight correspond to lipids, a higher percentage than in other species that use this organ as the main energy reservoir (Lambert & Dutil, 1997; Drazen, 2002; Hiddink et al., 2005; Lloret et al., 2008; Pavlov et al., 2009). We demonstrated that in blue whiting this organ has a main role as a supplier of energy to invest in reproduction, which matches with a capital breeding strategy, i.e. reproduction is financed using endogenous stored energy (Stephens et al., 2009). This finding is based on the decrease in the HSI concurrent with the increase in the GSI during the period of gonadal development, and the fact that SC and AS specimens showed lower HSI with respect to RT and DV. This is in accordance with reported observations of diminished feeding activity and decreased condition and liver weight during the spawning season (Bailey, 1982). Capital-breeding strategy may lead to a post-spawning increase of the fish vulnerability due to insufficient energetic reserves to escape predation, to face environmental pressures or to respond to diseases thus the environmental conditions in this phase is critical for adult survival (Lloret et al., 2014). It is expected for spawning time to be evolutionary fine-tuned for a successful reproduction (Cushing & Horwood, 1994). Actually, in the Atlantic sea a correlation was found for the spawning season of blue whiting and the period with highest secondary production (Bainbridge, V.; Cooper, 1973). In the NW Mediterranean Sea, a period of high primary production generally occurs in winter-spring followed by an oligotrophic summer (Bosc et al., 2004), thus a spawning season running from January to April should benefit the offspring survival and it also leaves a time window for a rapid build-up of the energy deposits once the spawning is over and before the productivity bloom ends, as reflected in our results by the steep increase of the HSI from March to May.

Temperature is one of the environmental factors influencing the reproduction of species either acting as a regulator or as a trigger signal (Kjesbu et al., 2010b), and here we have shown for the first time that gonadal development and maturation of blue whiting takes place during the cooling of waters, i.e. beginning with the sea water maximum temperature and ending with the minimum. Whether sea water temperature act as a direct cue or not, cannot be elucidated based on our data but it does exist a correlation with gonad development indicating that temperature may be playing a relevant role in regulating the reproduction in this species. In this wise, and because not all trophic levels respond equally to temperature, sea warming could induce a mismatch between the spawning and the optimal environmental conditions (Cushing, 1990) compromising the success of the reproduction and the survival of the larvae and the low-conditioned post-spawning mature fish.

Consistently with previous studies (Mazhirina, 1978), we have found that blue whiting is a batch spawner with a discrete polymodal development of oocytes following Ganias & Lowerre-Barbieri (2018). However, discrepancy concerning the fecundity type of *M. poutassou* can be found in the little literature available (Mazhirina, 1978; Bailey, 1982). Some authors describe it as a determinate species (Bailey, 1982; ICES, 2013) whereas some report observations associated to an indeterminate type (Mazhirina, 1978; Murua et al., 2006a). At this point, one should take into account that how the assessments of the fecundity type should be addressed has been a controversial question because several criteria were published and, recently efforts are being made to standardize it (Ganias & Lowerre-Barbieri, 2018). Following Domínguez-Petit et al.(2015) and, based on our results, still cannot be stated with certainty which type of fecundity displays this species in the Mediterranean. In one hand, the lack of the hiatus between the previtellogenic and vitellogenic oocyte stocks and the increase in the intensity and prevalence of αATR at the end of the spawning season would point to indeterminate fecundity, i.e. continuous recruitment of oocytes into the vitellogenic standing stock; while, in the other hand, the decrease in the abundance of early developing oocytes and advanced developing oocytes suggests that there is no continuous replacement of the standing stock of oocyte during the spawning season, which is interpreted as an evidence of determinate fecundity. Additionally, the capital breeding strategy and the short spawning season support the determinacy as these are characteristics that have been related to species that set their potential number of vitellogenic oocytes well ahead the spawning (McBride et al., 2015). In the Southern blue whiting (*M. australis*), a closely-related species from the southern-hemisphere, a determinate fecundity type has been described (Pajaro & Macchi, 2001). However, regional variability or geographic gradients can also be expected due to the phenotypic plasticity of these traits (Morgan & Rideout, 2008). Moreover, there is no snapshot of secondary growth development which can definitively predict the fecundity type (Ganias & Lowerre-Barbieri, 2018) and evidences of intermediate traits for both the breeding strategy and the fecundity type, are sound because these are not anymore considered binary concepts but continuums (Ganias, 2013). In this sense, a better understanding of the oocyte recruitment dynamics and the cues operating may help to clarify the fecundity pattern.

Despite in this work we have focused on secondary growth oocytes, the use of the stereology has provided us with valuable data concerning the amount (Vv) of atresia, POF, blood and tissue, as well as, precise estimations of the numbers (OPDi and NOi) of three primary growth oocytes stages (EPVO1, EPVO2, CNRO) that in future studies will help to enlighten these issues. We verified here that the circumnuclear ring (CNR), which has been identified as a key stage on the oocyte recruitment (McPherson & Kjesbu, 2012), is clearly noticeable in the blue whiting as already noted by (Mazhirina, 1978) and (Kjesbu, 1987), and we demonstrated that this stage can be easily quantified applying the later methodology.

Although no previous data is available from the NW Mediterranean, compared to the NE Atlantic blue whiting (Mazhirina, 1978; Bailey, 1982; Tsoukali et al., 2016) our estimations of BF showed a wider range. From a quantitative point of view, Mazirinha (1978) counted 71000 mature oocytes in AS ovaries (equivalent to BF) of females caught in the NE Atlantic and stated that each female spawns no less than 3 batches per season. Bailey (1982), roughly estimated a total fecundity of 48000 eggs for a standard female of 30 cm from the same Atlantic region. More recently, based on this data and assuming determinate fecundity, Tsoukali et al. (2016) estimated a potential fecundity ranging from 22600 to 106000 eggs for individuals sizing 25-37 cm, which, using the batch interval of Mazirinha, would result in a batch fecundity of 7500-35300. Our estimations of batch fecundity using the gravimetric method ranged from 493 to 78379 oocytes per batch in 19.2-30.4 cm females The cNO_{MAB} should be equivalent to the BF but, due to technical differences during the processing of the subsamples, the absolute numbers may differ. Despite the estimated cNO_{MAB} (35814 ±45178) in average doubled the BF values (13570 ± 18248), the correlation we found between estimated cNO_{MAB} and BF values denote that both could be used in relative or qualitative assessments of egg production. Both variables increased with TL, supporting that in general larger fish spawn more eggs per batch than smaller ones (De Roos et al., 2003; Barneche et al., 2018). Here we should note that the L50 was estimated to be at about 19 cm, in agreement with old data (Bailey, 1982). However, the minimum landing size (MLS) for blue whiting in the Mediterranean is currently set at 15 cm (Council Regulation (EC) No 1967/2006) that is far below the maturity length found in our study. Thus, based on the estimated L_{50} and the higher reproductive potential of larger fish we found, it would be advisable to increase this MLS to 19 cm to guarantee a good status of the stocks of blue whiting in the Mediterranean.

As reproduction is energetically demanding, traits trade-offs may occur as a response to low energy reserves (Stearns, 1989). Our results show that better conditioned (high HSI) spawning females of blue whiting invested more energy on reproduction (higher GSI) but produced eggs of poorer quality (lower ODM). Consequently, to meet the increase of GSI at a lower ODM, a higher fecundity would be expected for greater HSI, resulting in condition-mediated compensations between the egg quality and quantity. This higher fecundity could be either achieved by increasing the BF or increasing the potential fecundity by spawning more batches. In fact, the preovulatory maternal condition effects on the egg quality and quantity have been reported in other species, e.g. *Engraulis encrasicolus and Sardina pilchardus* (Brosset et al., 2016) and *Pomadasys incisus* (Villegas-Hernández et al., 2015a). Here we couldn't find any correlation between the BF and the somatic indices. Thus, whether the compensation is made on the number of batches or on the BF is not clear because in the first case the total potential fecundity and the number of batches can only be estimated in population studies, and in the second case the correlation may have not been detected due to a small sample size. Regarding skipped spawning it is suggested to happen generally in 20-50% of the mature fish (Rideout et al., 2005), especially in group-synchronous capital breeders (McBride et al., 2015) and usually linked to environmental conditions (Rideout & Tomkiewicz, 2011) or fish age (Skjaeraasen et al., 2012). In blue whiting from the NE Atlantic retarded maturation (evidenced by 17-24 cm immature females with degenerated developing oocytes) and skipped breeding events (regular in >30-31 cm females) were observed and related to unfavourable sea water temperature (Ushakov & Mazhirina, 1972). Here we observed 7 mature females showing resting or massive atresia at the beginning of the spawning season, these could be cases of delayed or aborted maturation of gonads (sizes 192-260 cm) or age-related skipped spawning (female of 335 cm) although only two of them showed especially low HSI values.

The study of nematode parasites in blue whiting has provided original results that complement the existing literature. To our knowledge this study is a first host record for *Camallanus sp*. While there is no previous record of tetraphyllidean plerocercoids in the blue whiting from the Mediterranean, we found similar prevalence to the ones reported in the NE Atlantic (MacKenzie, 1979). After comparing Anisakids of blue whiting in the NW Mediterranean (this study) with the Anisakids found elsewhere (Molina-Fernández et al., 2018), it is seen that the fishing area is a potential indicator of the infection by Anisakids (Rello et al., 2009; Molina-Fernández et al., 2015). Comparatively, our results for *Anisakis spp* prevalence are in-between the highest (from Sardinia and Ligurian Sea) and the lowest (south-west) reported for the Mediterranean and are similar to some Atlantic surveys (e.g. MacKenzie, 1979). In accordance with Mediterranean results, *Anisakis pegreffii* (AT1) was the most common nematode and also the one reaching highest intensities. The *Hysterothylacium aduncum* was the second most recurrent parasite in this survey and although in previous Mediterranean studies it was not frequent (Valero et al., 2000; Ruiz-Valero et al., 2014), our results were close to Atlantic Spanish coast reports (Sanmartin Duran et al., 1989; Ruiz-Valero et al., 2014). This parasite is acquired through the consumption of crustaceans, chaetognaths and small fish infected with the L3 larvae; if the ingested larvae is longer than 3mm it will moult into L4 in the intestine lumen of the fish where it will remain free, while if it is ingested at a length lower than 3mm it will penetrate the intestinal wall where it will become encapsulated and eventually die or it will enter the body cavity where it grows free (Køie, 1993). Following (Adroher et al., 2004), our NI1 morphotype could correspond to the small or recently hatched L3 larvae of this species that become encapsulated and doesn't grow. Actually, some of the NI1 were observed emerging from an egg or capsule embedded in the digestive wall. All the mentioned differences of parasitism may partially relate to the local abundance of definitive hosts and to the abundance of the intermediate host in the diet of the blue whiting (Macpherson, 1978). In fact, the Gulf of Lion has a high richness and abundance of cetaceans which are the final hosts of several parasitic nematodes (UNEP/MAP/RAC/SPA, 2013). Small crustaceans such as euphasiids, which are one of the main preys of blue whiting (Cabral & Murta, 2002) and the first intermediate hosts of both Anisakis and *H. aduncum* (Hall, 1929; Hays et al., 1998). The Tetraphyllideans plerocercoids are acquired from infected copepods (Mudry & Dailey, 1971). The final hosts of adults of *Anisakis sp.* (Mattiucci & Nascetti, 2006) and *H. aduncum* (Køie, 1993) are marine mammals (the latter can also use marine birds as definitive hosts) and elasmobranchs are those of adult Tetraphyllideans (Mudry & Dailey, 1971).

In accordance with Valero *et al.* (2000b) larger specimens showed higher parasitism due to an accumulation of parasites on the fish body across its life-time. The differences of infection between MATs may come from reproduction-related behavioural changes (e.g. Gómez-Mateos *et al.*, 2016) or seasonal hydrodynamics (e.g. Klimpel and Rückert, 2005) which are particularly complex in the Gulf of Lion (Millot, 1990). Because fish become infected through the consumption of infected prey (Marcogliese, 2002) reduced feeding during the AS phase may explain a lower percentage of infected fish during this phase, as seen for *Anisakis spp*, NI2 and tetraphyllideans. The tetraphyllideans were mostly found in the digestive lumen, thus an increase of IY_{TEP} during the host gonads DV phase should result from the balance between a higher ingestion rate and the occasionally expulsion/dead rate. The distribution of the parasitism between the body organs partially reflects the preferences of the *A. pegreffi* larvae for the body cavity and the liver, in accordance with other studies (e.g. Cruz *et al.*, 2007). The blue whiting is a paratenic intermediate host for *Anisakis spp.,* thus the fate of this larvae is to remain embedded in the host tissues until the fish is ingested by the final host (Anderson, 2000). The digestive tract is also one of the main infection sites were relevant prevalence of NI1 and *H. aduncum* was found. The 3% of muscles was infected with nematodes, this is lower than the *Anisakis* prevalence of 7-39% from bibliography (e.g. Gómez-Mateos *et al.*, 2016). Anisakids larvae may migrate from the viscera to the musculature once the host is dead (Smith & Wootten, 1975). Thus, immediate evisceration of fishes after capture should reduce the potential risk for the public health.

Most of the Anisakids were encapsulated, thus they were not actively draining energy from the host tissues but the encapsulation itself implies some host immune response (Elarifi, 1982) which does have an indirect energetic cost especially in high infections (Lochmiller & Deerenberg, 2000). We found a lower production of eggs (BF) in infected fish compared to non-infected ones. This may suggest that the infected fishes are allocating the energy differently, e.g. a bigger fraction of energy being invested to maintenance or survival and consequently less energy being available for reproduction. Other variables didn't show any significant contrast between infected and non-infected fishes but differences aroused between highly and lowly-infected host. The higher condition of highly infected fish may just reflect the fact that fish that feed more have higher energetic reserves but also have higher chances of acquiring parasites. Taking into consideration the higher reserves on parasite fish, the observed reduced fecundity is not a matter of stored energy but may be of energy allocation pattern, e.g. redirection of energy to immune response. In fact, based on our results and following the traditional assumption that relates larger spleen with higher immune activity (Smith & Hunt, 2004), high parasitism by nematodes is associated with higher immune response, in accordance to other studies (e.g. (Seppänen et al., 2009)). However, the use of SSI as an indicator of immune activity in fish should be made with caution because even though we have adjusted it for TL, sex or MAT when required, there may be many other sources of noise (Marteinson et al., 2017) that we can't control for in this kind of studies (Smith & Hunt, 2004), e.g. stress (Marteinson et al., 2017), exposure to contaminants (Marcogliese & Pietrock, 2011), activity or co-occurrence of other infections.

Although the results of this study contribute to better understand the complex trade-offs between parasitism, reproduction and condition in a cold-water species such as blue whiting in the context of global change, it is necessary to continue with further research because as demonstrated here the interrelationships are complex and species-dependent and seem to be linked with other biotic and abiotic factors that make it hard to isolate them.

4.2.6. FUTURE WORK/PROSPECTS

Advanced theories such as the Dynamic Energy Budget (DEB) allow to trace over time the physiological state of a living organism making mass and energy balances covering the full life cycle of an individual (Koojiman, 2000; Nisbet et al., 2000). The DEB models are powerful tools with simple mechanistic bases that describe the dynamics and rates of the energy flow from the assimilation to the storing and allocation to reproduction, growth, development and maintenance following the K-rule (Koojiman, 2000). Schematically, the DEB model is composed by two sub-models: (i) the somatic submodel in which the energy is allocated to growth and there are energetic costs associated to the maintenance of the structure, and (ii) the maturity/reproduction submodel in which the energy is allocated to the production of reproductive output and there are maturity energetic costs (Fig. 30).

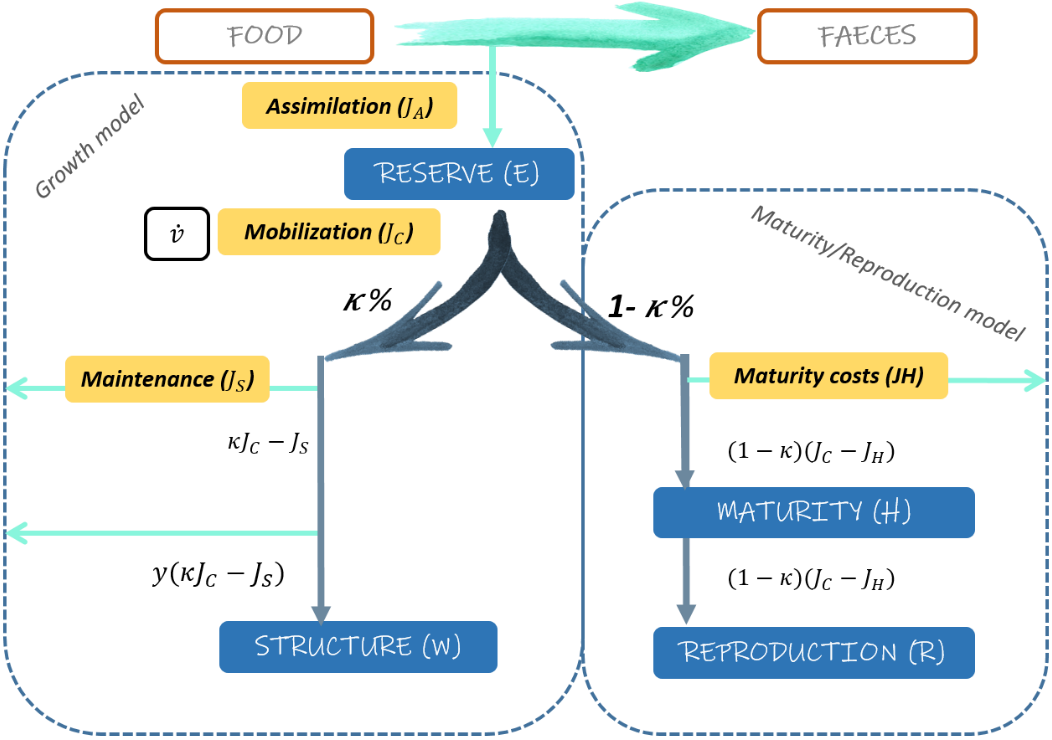


Fig 30. Schematic Dynamic Energy Budget (DEB) model diagram. The state variables (given as energy) and energy fluxes through an adult individual are shown (adapted from Van der Meer, 2006; Ledder, 2014). Author: Andrea Campos-Candela.

Once calibrated from a given species these models have been successfully used in different taxa to predict growth and reproduction under given environmental conditions, e.g. Pecquerie et al. (2009). The application of these models to parasitised hosts has been little explored (Hall et al., 2007; Flye-Sainte-Marie et al., 2009; Aylward, 2012).

Aylward (2012) modelled the full life cycle of a *Fasciola hepatica* by coupling the parasites model to its hosts models (*Lymnaea stagnalis* and *Rattus norvegicus*) and got a picture of the energy dynamics trough the different stages of its life cycle and how it is affected by changing environmental conditions; other authors focused the attention to the host energetic dynamics to develop a model that predicts the impacts of infection i.e. (Hall et al., 2007) modelled the infection of *Daphnia sp.* by two theoretical parasites (one consumer and one castrator) and (Flye-Sainte-Marie et al., 2009) modelled the infection of *Ruditapes philippinarum* by *Vibro tapetis.*

In a rather simplified and challenging way, DEB theory provide great prospects to predict the outcomes of the trade-offs between host reproduction and condition under different hypothesis of the effects of parasitism and under changing environmental conditions. Anyhow, previous to setting up the model for specific host-parasite relationships, descriptive studies such as the one presented here are required because the results should be the base to formulate the hypothesis and calibrate the model. For instance, based on our results and focusing on the effects of *A. pegreffi* for being the most common parasite, the following hypothesis could derive: (i) the immune response increases the maturity costs with consequent effects on reproduction outputs, this increase will grow with the intensity of infection and (ii) in high infections the physical damage of the affected tissue increases the maintenance costs. From this starting point the parameters in the DEB model could be re-estimated and the flow of energy in the host could be simulated under the given hypothesis. For the sake of argument, we illustrate here two very preliminary simulations at a nematode community level and based on hypothesis that we roughly formulated (note that these simulations are in the frame of a recently started collaboration with A. Campos-Candela). On the first simulation we hypothesized that the assimilation efficiency (J_A) could be decreased because the nematodes were highly prevalent in the digestive tract, and that the cysts surrounding the nematodes must imply some degree of immunological response which is related to the maturity cost (JH) because we detected immune response at high intensities of parasitism. On the second testing simulation we hypothesized that the high prevalence of nematodes encysted in the cavity and in the liver may be physically damaging the tissues increasing the maintenance cost (JS) due to reparation of structures.

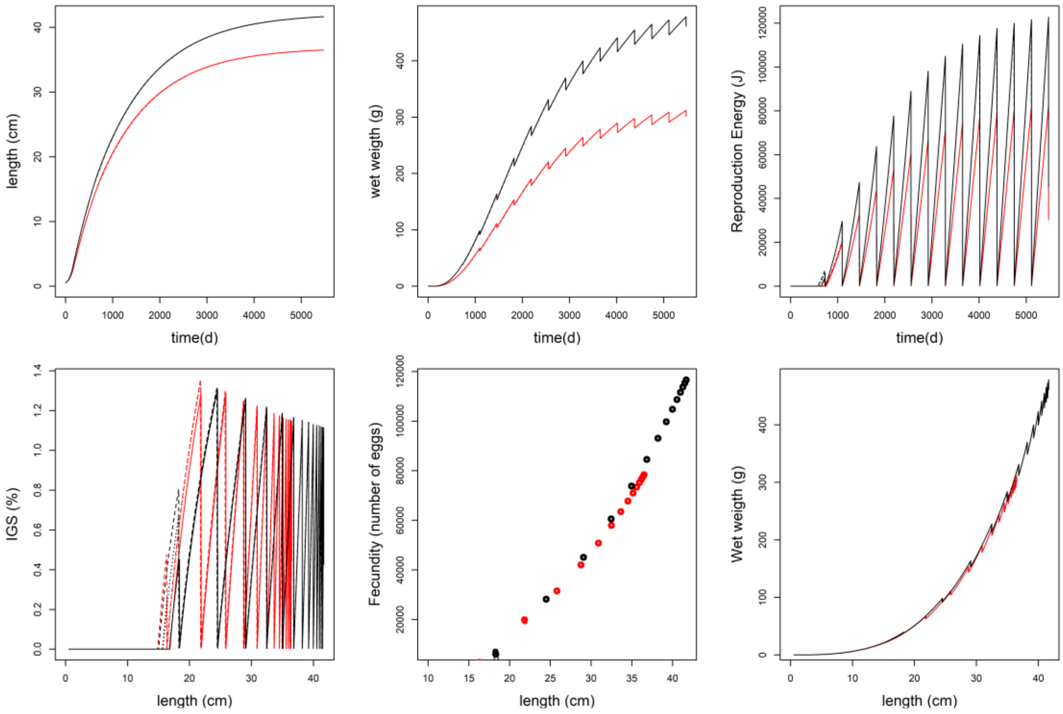


Fig 31. Simulation 1, under decreased assimilation efficiency and increased maturation costs. Evolution over time of (A) growth in length, (B) growth in weight, (C) energy invested to reproduction, (D) gonadosomatic index, (E) number of eggs (fecundity), (D) length-weight relationship; on a hypothetical individual.

The plots derived from the simulations (Figures 31 and 32) suggest a decrease on the maximum size related either to the lower assimilation efficiency (red) or the higher maintenance costs (blue). The major maturity costs (dashed or dot line) are implying a delate in the ripening of gonads at small sizes, more evident when the assimilation efficiency is low (in red). However, changes on assimilation efficiency (red) or in the maintenance costs (blue) have much bigger effects in all state variable dynamics than changes on maturity cost.

Despite this was just a testing simulation on how this models could perform, the application of this framework might help to elucidate the effects of parasites on the fish biologic performance and in a further step the calibrated DEB models could be coupled to bioclimatic envelops to explore the evolution of the host-parasite relationship under climate change scenarios.

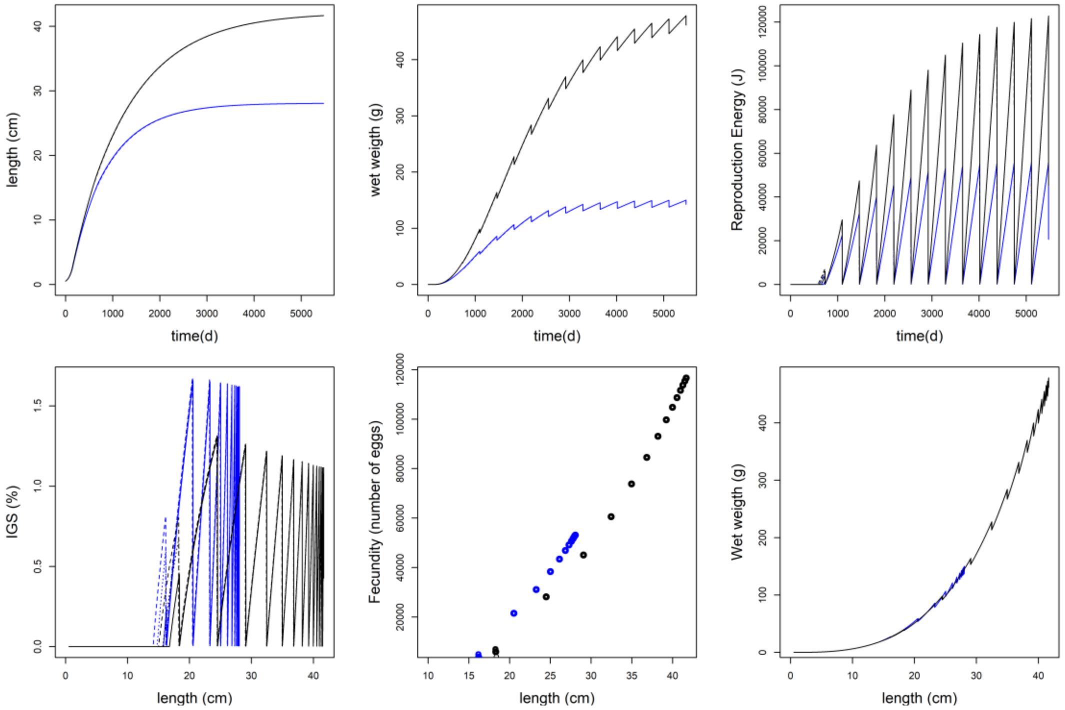


Fig 32. Simulation 2, under extra maintenance costs and increased maturation costs. Evolution over time of (A) growth in length, (B) growth in weight, (C) energy invested to reproduction, (D) gonadosomatic index, (E) number of eggs (fecundity), (D) length-weight relationship; on a hypothetical individual.

4.2.7. SUPPLEMENTARY MATERIAL

The following supplementary material can be consulted in the Annex I at the end of this thesis: Supplementary Figures (Figures S1-S14) and Supplementary Tables (Table S1- S2), accompanied with their respective captions.

4. 3. CASE STUDY III

The **European hake** (*Merluccius merluccius*) from the Galician shelf[4]

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[4] Serrat A, Saborido-Rey F, Garcia-Fernandez C, Muñoz M, Lloret J, Thorsen A & Kjesbu O S. **New insights in early oocyte dynamics unravel the complexities associated to fish productivity and reproductive strategies:** *Lightening up the "black box" in fish productivity fundamentals.* Manuscript under review.

New insights in early oocyte dynamics unravel the complexities associated to fish productivity and reproductive strategies

Lightening up the "black box" in fish productivity fundamentals

4.3.1. OVERVIEW

Egg production methods are often used to estimate spawning stock biomass (SSB), a proxy of reproductive potential. Thus the estimation of fish productivity and the understanding of the drivers affecting it, are essential in fisheries management to support sustainability of the stocks, but are also essential to investigate population resilience and adaptation to climate change, both very much depending on the species reproductive strategy. Despite its relevance, fundamental understanding of egg production and its variability remains unclear due to the high complexity and the methodological shortcomings involved in tracking and quantifying the fluctuations in oocyte recruitment, here hypothesized to affect the resulting fecundity. Here we applied advanced oocyte packing density theory to get in-depth quantitative insights across oocyte stages and seasons, selecting the commercially valuable European hake (*Merluccius merluccius*) as a case study. Our work evidenced sophisticated seasonal oocyte recruitment dynamics and the environmental cues involved, i.e. photoperiod and strength of oceanic upwelling, but also suggests that this species performs a low-cost predefinition of fecundity as a function of body size. This happens at a stage of oocyte development much earlier than previously thought for any other species, which implies a quasi-determinate fecundity type, altering the assumed reproductive strategy for this and, very likely, many other species as well. Spawning dynamics varied between seasons, reflecting environmentally modulated plasticity. Altogether these findings question today's simple classification of the fecundity style being either determinate or indeterminate, and states that the current practices on potential fecundity estimation should be complemented with studies on primary oocyte growth. We have shown that the methodology and approach taken in this study are useful tools to unravel some of the complexities associated to fish productivity.

SUBMITTED PAPER. EMBARGO UNTIL PUBLICATION DATE

5. GENERAL DISCUSSION

This section provides a summary of the main findings, a broader discussion with a global perspective that integrates all the results from the case studies, their implications and other relevant aspects.

In general terms, the risk of impacts on populations is a function of their exposure to hazards (e.g. fishing pressure or altered physical or chemical variables such as temperature, oxygen concentration, pH or food limitation) and their vulnerability. Vulnerability can be understood as species' adaptive capacity, which is mainly driven by genetic diversity (for long-term evolutionary responses) and species' sensitivity (short-term responses) (Field, 2014). In turn, sensitivity is firstly determined by the physiological tolerance and the biological and ecological traits of the species, and further mediated by its plasticity. The increasing awareness for the sustainability of many stocks of either targeted or bycatch species (Pauly et al., 2002) impel to assess their vulnerability to fishing and climate change. Recently some efforts have been done to develop models that may help to evaluate species' vulnerability (Jennings et al., 1998; Cheung et al., 2005; Jones & Cheung, 2018). These frameworks contemplate that the variations in species' responses to extrinsic stressors also depend on species-specific attributes, e.g. Hare et al. (2016), and for instance the species reproductive output or the length at maturity can be directly incorporated into vulnerability evaluations, e.g. Jennings et al. (1998); Williams et al. (2008). Despite theoretical advancements, the scarce updated data on biological and ecological traits for many marine species is limiting these kind of assessments.

In this context, the present thesis has generated novel knowledge on the main biological traits of exploited fish species with an affinity for cold waters, inhabiting close to the warmer edge of their distribution area (the NW Mediterranean and Galician waters in the northern hemisphere), and which are here hypothesized to be suffering stress from climate change and fishing. With this, we aimed to provide a scientific basis which could help in the future to evaluate their sensitivity to the aforementioned extrinsic pressures and their risk of impacts. Each of the case studies was focused in one "cold water species" and examined different aspects of its biological traits, from the factors regulating early oogenesis to the distinct reproductive tactics, the condition, the parasitism and the energy allocation trade-offs. The results obtained in each of the case studies are summed up in the next paragraphs. Subsequently, the main outcomes are discussed and interpreted from an integrated and ecological point of view with connections to the current context of climate change and intensive exploitation of fish stocks.

Firstly, in case study I, *Argentina sphyraena* was described as an iteroparous batch spawner species with discrete oocyte recruitment, determinate fecundity style and capital breeding strategy. Its major lipid deposit appeared to be the fat accumulated in the mesenteries which is allocated to reproduction and depleted during the spawning season, leaving the fish in a vulnerable post-spawning situation in front of unpredictable environmental changes. Larger fish showed enhanced acquisition of energy resources and increased batch fecundity. The spawning season in the Gulf of Lion takes place from December to May, which is earlier than the reported for northern Atlantic populations. However, in the Balearic Sea spawning activity extends until June. The samples from the Gulf of Lion showed higher condition, higher egg quality and lower egg quantity in relation to the Balearic Sea, where the waters are warmer and more oligotrophic apparently making of it a less favourable environment. Thus, the existence of environmentally-mediated trade-offs suggest some degree of plasticity that may partly buffer the effects of non-optimal habitats.

Secondly, in case study II, reproduction, condition and parasitism of blue whiting (*Micromesistius poutassou*) was described. The results revealed that this species is a capital breeder that uses the energy stored in the liver to fuel the reproduction. It shows a mix of evidences of indeterminate and determinate fecundity type, e.g. the standing stock of oocytes seems to be defined at some early point but posteriorly down-regulated. Because temperature is apparently related to its gonad development, sea warming could potentially influence the timing of the spawning season threating the reproductive success. Moreover, there again seems to occur condition-mediated balances between the egg quality and quantity. The combination of histological (OPD) and gravimetric methods on the assessment of the reproductive tactics gives a more comprehensive overview than in the previous case study, e.g. the circumnuclear ring has potential to be targeted in oocyte development studies to elucidate the oocyte recruitment patterns in order to improve the estimation of its fecundity. We also found that relationships between reproduction, condition and parasitism appeared mainly on high infection. Batch fecundity seems to decrease as a consequence of high parasitism which is apparently causing an immune response on the fish (higher spleen relative size) while the higher hepatosomatic index may relate to the condition of the host that allowed a higher infection. Eventually, I note that further research regarding the ecology of the host-parasite interactions would help to understand the effects of parasitism, and in this line I consider DEB models as a promising tool to explore.

And finally, in case study III, new information about the oocyte recruitment variability and fecundity style regulation underlying egg production was provided for European hake (*Merluccius merluccius*) from Galician waters (North Atlantic). The findings indicated that oocyte recruitment of direct consequences for the resulting egg production occurs much earlier than previously thought, i.e. already during the gonadotropin-independent stage (most likely at PVO4b, but certainly at PVO4c) and that the standing stock of PVO4b-VTO stages reasonably well reflects the potential fecundity of European hake. Sticking to the normal definition of a determinate and an indeterminate style, the hake clearly falls into the last category as we found clear evidence of *de novo* oocyte recruitment during spawning (all the way from PVO4b,c to VTO). However, this conceptual thinking becomes blurred by our findings that the level of fecundity is set much earlier, as mentioned, meaning that hake shows much more of a determinate than indeterminate fecundity style at the "base line", provided we revise the currently accepted definitions. Further to this, early oocyte recruitment patterns clearly differed throughout the year. This work also pinpoints a complex picture of environmental cues involved. Although still many issues being unresolved, the outlined fecundity style may well occur in other teleosts. Thus, several well-established conceptions were questioned: the primary and secondary growth oocytes being separate categories rather a continuum, the determinate versus indeterminate fecundity strategies limited to the spawning season, and, finally, it was exemplified that at least two environmental cues (day length, temperature and upwelling strength) are likely operating but acting on different types of oocytes at different times of the year.

From here on, the discussion is oriented to the interpretation of the results with an ecological perspective to shed some light on which of the parameters can potentially influence species sensitivity to perturbations. But before proceeding, some remarks should be done regarding the limitations I encountered during the design and execution of this research. This was a thesis mostly focused on biological descriptions of the reproductive strategy and condition of this species, with some insights on parasites. The selected species because of being considered "cold-water species" in the Mediterranean, were here hypothesized to be suffering some physiological stress due to suboptimal environmental conditions and also facing fisheries pressures. Thus, we found interesting to keep an eye on the environmental variables which were only used as background and only assessed by visual analysis of plots. However, to firmly state the influence of the environment on any of these processes not only statistical analysis should have been performed to test the relationships with, for instance, temperature, food availability and fishing pressure, but ideally experimental work should have been carried out. However, our case studies are species which are very difficult to keep in tanks. Similarly, the study of the parasites should have gain of experimental work controlling for all the variables and comparing between infected and uninfected fish. Extending the analysis of nematodes to other parasites would give us a more global and realistic picture of the health status of the fish and would have allowed us to find interesting relationships for other parasites that may have a bigger effect on the fish energetics and reproduction. Molecular identification of parasites would have provided extra interesting data. Despite having bigger number of samples, some of the parameter were studied in smaller subsamples, but these were supposed to be representative. In the first case study a more extended sampling period in Balearic sea would have resulted in more conclusive results on the regional variability of reproduction and condition. Despite the mentioned constrains, the findings in this thesis not only generated knowledge on the biology of these marine exploited cold water species but also provided new insights on relevant aspects of fish reproduction and health that could apply to other species.

All the aforementioned results can have implications on the biology and ecology of the species and can help to evaluate the vulnerability of these exploited cold-water species in front of the synergistic effects of extrinsic pressures such as climate change and, particularly, fishing. As seen, condition reflects the nutritional status and mediates reproduction. The stored lipid energy seems to be widely applicable as a predictor of population dynamics at least for capital breeders, as for instance total lipid energy in *Gadus morhua* was proportional to total egg production (Marshall et al., 1999). In the case study I, *A. sphyraena* from the apparently more favourable region (Gulf of Lion, colder and more productive) showed higher condition and better quality eggs than in the warmer and more oligotrophic Balearic Sea, suggesting that environmental changes may be detrimental for the nutritional status and thereafter may compromise the reproductive output. In fact, direct and indirect effects of temperature and its variability on condition and reproduction have been reported for several species, e.g. herring (Engelhard & Heino, 2006), Atlantic salmon (Todd et al., 2008) or sole (Vasconcelos et al., 2009). Thus gathering of biological data on fish condition can help to detect situations of stress and to anticipate periods of lower reproductive performance. The decision of which indicator has to be used to monitor condition relays on the species-specific biological traits. Here we promote the exploration and use of easy and efficient condition predictor such as e.g. the visual assessment of mesenteric fat relative amount in *A. sphyraena* which can be utilised even in the field and incorporated in survey routines with no extra cost. But it should be noted that quantitative verification of lipid distribution is required to ensure the use of the proper indicator. To record the status of the biological traits and life history tactics can provide scientists and managers alike with valuable information about the vulnerability of the stocks and its level of affectation by extrinsic pressures. However, regarding the interpretation of trends and shifts in biological traits one should be aware that the time of response and the sensitivity may vary between traits, species and stressors. Whereas some responses can be very rapid, as in the case of the abrupt changes of length at maturity of sardine and anchovies in the NW Mediterranean (Brosset et al., 2016); other can only be detected after a time-lag, as for instance the level of fecundity in Atlantic herring which appeared to be influenced by the environmental conditions during the feeding season of three years before (dos Santos Schmidt et al., 2017).

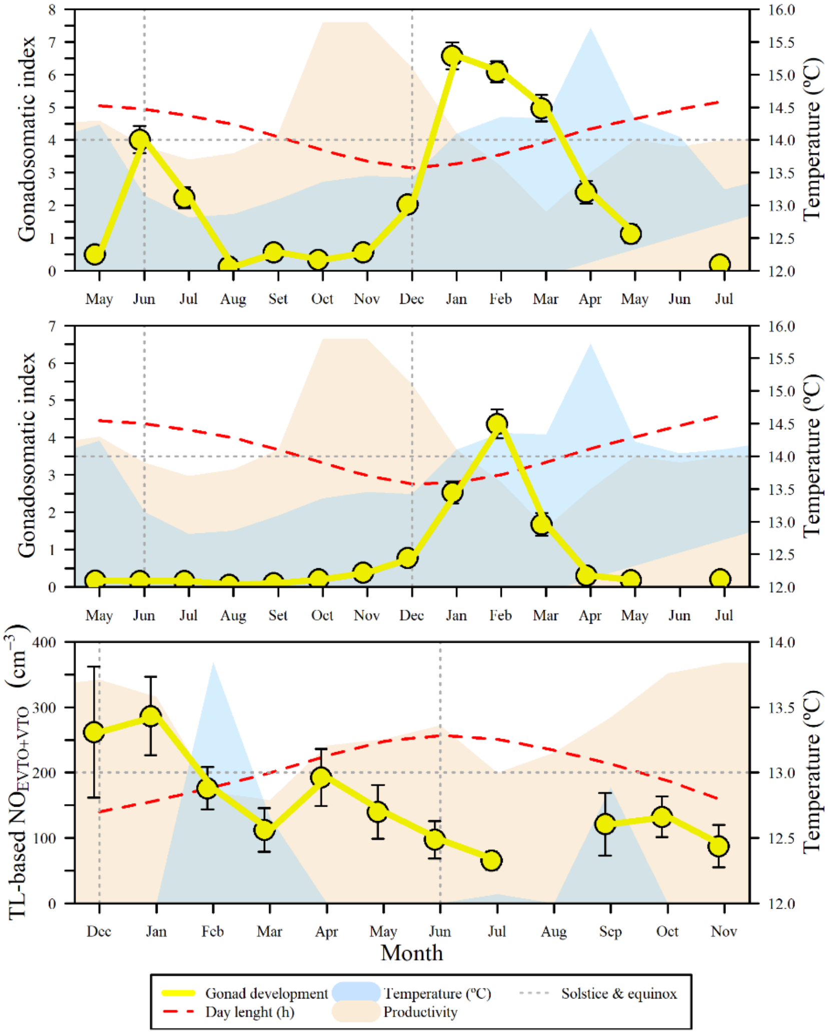

Fig 48. Simplified representation of the gonad development cycle and the main control factors for *Argentina sphyraena*, *Micromesisitus poutassou* and *Merluccius merluccius*. Note that for *A. sphyraena* (A) and *M. poutassou* (B) the gonadosomatic index is used to illustrate the evolution of the gonad development (yellow line), while for *M. merluccius* (C) this is represented by the relative number of vitellogenic oocytes (TL-based $NO_{EVTO\text{-}VTO}$) as a measure of vitellogenesis. As a proxy of primary productivity (blue shaded area) the concentration of Chlorophyll A (mg/m3) at 42.5ºN 3.5ºW (https://www.nodc.noaa.gov) was used in A and B, and up-welling index (www.indicedeafloramiento.ieo.es) in C. Sea water temperature (brown shaded area) in panels A and B was registered at -80m in l'Estartit (42.1 ºN 3.2ºW) (http://meteolestartit.cat) and in panel C is represented by the mean temperature from 50-350 m depth at 43.5ºN 9.5ºW (IBI_ REANALYSIS_PHYS_005_002, http://www.copernicus.eu) in C. The day length (dashed red line) represents the monthly mean daily hours of sunlight at 42.5ºN 3.5ºW (A, B) and at 43.5º N 9.5º W (C) (http://aa.usno.navy.mil/index.php).

Individuals risk of impact is higher if the environmental constrains occur during periods of increased sensitivity (Pankhurst & Munday, 2011). For instance, the nutritional status of an individual may exacerbate the stressors' negative impact on the biological performance because it is a determinant of organisms' survival and it is tightly coupled with the reproductive output (Lambert et al., 2000). Thereafter, one of these periods of increased sensitivity takes place at the end of the spawning season in capital breeder species because the fish energetic reserves have been diminished or exhausted reducing its availability to face unfavourable or unpredicted environmental conditions (Stephens et al., 2009). The results from this thesis showed that this is the case for *Micromesistius poutassou* and, especially of *Argentina sphyraena* whose main energetic deposits are fully depleted in post-spawner individuals. During this period, the fish fitness is highly dependent upon the availability of external resources and its sensitivity to hazards is heightened. Nevertheless, the timing and duration of the spawning season has been evolutionarily fine-tuned to match this period of fish impaired condition with the optimum environment and the required food availability (Cushing & Horwood, 1994; Durant et al., 2007; Marshall et al., 2009). In agreement, the three species here examined showed the main spawning season in winter-spring, thus the end of the breeding period occurred approximately at the same time as the primary productivity generally bloom in the study areas, i.e. the NW Mediterranean (Rodriguez, 1982) ant the Galician shelf in the North Atlantic (Bode et al., 1998), possibly enhancing in this way the chances of post-spawners survival and the success of the offspring.

Several factors interact on the regulation of gonadal development and spawning timing (Lowerre-Barbieri et al., 2011a). The results compiled in this thesis reinforce the idea that temperature, nutritional status and/or photoperiod are the main variables controlling reproduction (Fig. 49), alone or in distinct combinations depending on the species or populations (e.g. Pankhurst & Porter (2003), Wright & Trippel (2009)). As an example, increasing spring temperatures are required to cue maturation in several spring and early summer spawners (Colin, 1992; Scott & Pankhurst, 1992; Sadovy, 1996; Hilder & Pankhurst, 2003). This is in line with the observations from case studies I and II in which the allocation of energy to reproduction co-occurs with the positive trend on sea water temperature, i.e. the allocation of energy to gonadal development began after the temperature reached its minimum and ended when the waters were warmest. On the other hand, the first spawning capable females of *A. sphyraena* and *M. poutassou* appeared around the winter solstice, thus indicating that photoperiod could have a role on triggering the later stages of oogenesis, i.e. maturation of the advanced vitellogenic oocytes which will result in ovulation. A similar combination of temperature and photoperiod effect has already been reported in other species (Pankhurst & Porter, 2003). In fact, in capital breeders the gonad development and the spawning is not coupled to the food availability (McBride et al., 2015) and therefore other factors than the nutritional status should be at play as cues of reproduction processes.

However, this are no conclusive results and the influence of the nutritional status shouldn't be disregarded since for instance it could have had a role on the unexpected delay or protraction of the spawning season of *A. sphyraena* in the Balearic Sea in relation to the specimens from Gulf of Lion. In contrast to this, the European hake as an income breeder needs to meet the reproductive energetic requirements by immediate feeding, and in accordance with the case study III, vitellogenesis appeared to be related to the upwelling strength (i.e. food availability) and the temperature, whilst the photoperiod seemed to be involved in the early key stages of oogenesis particularly those that were here hypothesised to set the level of fecundity (appearance of the circumnuclear ring in the oocyte cytoplasm). In any case, to firmly pinpoint the factors and its roles on regulating the different stages of oogenesis, lengthy laboratory experiments would be needed where all factors could be controlled for. However, this kind of settings are highly challenging specially with such species, and moreover it is not feasible to fully replicate wild conditions as for instance behaviour and synergistic effects would surely be altered.

Potentially, capital breeders in which photoperiod is at play on inducing the reproduction as we here suggested for *M. poutassou* and *A. sphyraena*, are at greatest risk of impacts from sea warming and ocean currents alterations since they are less likely to adjust the timing of reproduction to suit the thermal environment and the availability of resources (Munday et al., 2008; Wright et al., 2017) what could lead to mismatches between breeding (partly cued by day length) and optimal conditions for the larvae (related to temperature and food availability) (Edwards et al., 2004). Moreover, capital breeders are energetically less efficient because they have to incur costs related to the energy storage and mobilization as well as the maintenance of these lipid stores (McBride et al., 2015), which makes them more susceptible to suffer from environmental stress as reported for instance with the steeper decline in growth and condition of *Sardina pilchardus* (capital breeder) in relation to *Engraulis encrasicolus* (income breeder) in the NW Mediterranean (Brosset et al., 2016). On the other hand, income species such as *M. merluccius* are predicted to have a greater capacity of adjusting its reproduction to the environmental conditions (Stephens et al., 2009), as evidenced in case study III by the environmentally-modulated plasticity of spawning dynamics and the aforementioned relation between the temperature and vitellogenesis. In fact, spatio-temporal variability of several reproductive traits has been reported in other studies of *M. merluccius*, e.g. the breeding strategy switches seasonally in the NW Mediterranean (Ferrer-Maza et al., 2014). Thus it is expected that at least at short term such a plastic species should be able to buffer up to some degree the effects of the environmental stress on the reproduction, while in this respect *A. sphyraena* and *M. poutassou* could be considered more sensitive species.

The maintenance of the reproductive potential, using most often spawning stock biomass (SSB) as a proxy, is a key issue in fisheries management as it should be kept above a certain level to support the production of new generations. In cases where egg production methods are applied to get indications of SSB, species are split based on its fecundity style. Despite its relevance, the traditional assessments of fecundity style are currently being questioned. Dos Santos Schmidt et al. (2017) documented that the standard focus on secondary oocytes needs to be supplemented with examinations of early oocyte recruitment. In the same line, (Ganias & Lowerre-Barbieri, 2018) argued that there is no snapshot of secondary growth oocytes development which can definitively predict the fecundity style. Moreover, because this is not anymore considered a binary concept but a continuum, intermediate fecundity styles can also be found (Ganias, 2013). Indeed, in the case study II difficulties were met for describing the fecundity type of *M. poutassou* because evidences of both styles (i.e. determinate and indeterminate) aroused when following the traditional criteria mostly focused on the secondary growth and on the spawning season (Hunter & Macewicz, 1985). With this regard, the case study III was focused on the early oocyte dynamics of *M. merluccius*, a well-documented species firmly assumed to show indeterminate fecundity. However, when these early stages of oogenesis were included in the general picture, signs of determinate fecundity arouse and it became evident that oocyte recruitment occurs in an earlier stage than thought and that potential fecundity could be estimated from the standing stock of oocytes including some primary growth stages. Whether this "base line" determinacy applies only for this species or it can be generalized to other indeterminate species with protracted season cannot be stated yet. But overall, the results suggest a revision of the definitions of indeterminate and determinate, and primary and secondary growth. Hopefully, these results would stimulate new discussions within the scientific community to address teleost reproductive biology, particularly fecundity estimation, differently than done today. This may have direct consequences in fisheries management regarding estimation of stock reproductive potential and SSB, and thereby potentially help improving sustainability.

One of the outcomes emerging from the case studies I and II refers to the so-called preovulatory maternal effects resulting in egg production trade-offs between quality and quantity, e.g. higher fecundity in detriment of egg quality or vice versa. These kind of balances can have relevant impacts on populations dynamics since egg quality is closely linked to hatching-larvae survival (e.g. Riveiro et al. (2000)). Apparently this is a condition-mediated response to environmental stress widely documented in several species, however there is more disparity on the direction of the trade-off. For instance, in the Gulf of Lion the better-conditioned specimens of *Pomadasys incisus* spawn better quality eggs but less numerous (Villegas-Hernández et al., 2015a). The same authors in the same region again observed that the better environmentally-suited *Sphyraena sphyraena* which shows an income strategy and has higher condition, spawn larger eggs at the expenses of quantity in contrast to the thermophilic capital breeder *Sphyraena viridensis* (Villegas-Hernández et al., 2014). The findings emerging from this thesis showed evidences of this plasticity at intra an interpopulational level but the output varied between species. In case study II, the better-conditioned *M. poutassou* females invested more energy on reproduction but produced eggs of lower quality. Thus one would expect that if as a result of environmental changes the food becomes limited or the energetic expenditure (energetic physiological requirements) increases, this species will respond lowering the fecundity but increasing the chances of off-spring survival by doting the eggs with higher energetic content. Contrastingly, in case study I, females of *Argentina sphyraena* from Balearic Sea where the condition was lowered, produced lower quality eggs but apparently compensated it by higher quantities, compared to the specimens from Gulf of Lion. It should be noted that attributing these compensations only to condition status is merely tentative because other factors may be at play. In this sense, in the case of *A. sphyraena* the fishing pressure could be ruled out as the main driver of the trade-off in egg production since this is not a fisheries target-species and moreover the fishing pressure that may be suffering as a by-catch should be higher in the Gulf of Lion (Ordinas & Massutí, 2009). Taking into account the Balearic warmer waters (André et al., 2005) and the higher primary production from Gulf of Lion (Siokou-Frangou et al., 2010), food-availability together with energetic expenditure are likely the major factors underlying the egg production trade-off in this case study. Thus it cannot be asserted whether the direction of the balance between egg quality and quantity is species-specific or stressor-specific but this is indeed a common response in front extrinsic pressures and it implies that the level of egg production is a rather plastic trait.

It is well known that the larger fish have a higher biological performance, e.g. higher egg production. This is in agreement with our results which showed for all the case studies a positive relation between the fish size and the number of eggs produced per spawning batch. Moreover, in *A. sphyraena* the relationship of batch fecundity with both, fish body size and mass, fitted reasonably well to a logarithmic function in line with the conclusions from a recent meta-analysis which suggested that larger females contribute disproportionally to populations' offspring production (Barneche et al., 2018). Larger females of *A. sphyraena* also showed greater capacity of energy acquisition, which could explain their increased fecundity as it has been suggested for other species such as European hake, e.g. Mehault et al. (2010), Ferrer-Maza et al. (2014). As the present examinations on European hake, were mostly focused on oogenesis-related dynamics and based on samples from the same region as the latter authors, I any relation with body condition was tested here. Nonetheless, the results which again showed higher potential fecundity in larger females, did have rather interesting and novel implications for the relation between egg production, fish body size and environmental conditions. As seen, this species shows three spawning periods in a year, each one encountering different environmental conditions. The potential fecundity did not vary much between periods but instead the batch fecundity and the number of batches (and thereafter probably the length of the spawning period or the spawning frequency) did show periodicity and this variation differed between size classes. In other words, it can be stated that this species changes its reproductive strategy across the year probably as a function of the environmental variables (mainly temperature and food availability) and distinctly according to females' body size.

Larger specimens also generally show higher parasitism due to life-time accumulation (Valero et al., 2000; Marcogliese, 2002). This is supported by our results from case study II where prevalence and intensity of parasitic infestations increased with *M. poutassou* body size. In addition, specimens infested by nematodes showed reduced batch fecundity. Thus parasitism may be also compromising the aforementioned expected higher contribution of larger specimens to the reproductive output, which is already threatened by climate change and fishing pressure. In terms of condition and energetic trade-offs, the influence of parasites on their hosts could also be expected to differ between breeding strategies. While income breeders may compensate the cost of the immunological response by feeding more, in capital breeders the energetic drain associated to the parasites may compromise the energy stores on which other vital functions rely. However, if the energetic costs of responding to the infection outweigh the benefits, low-intensity infections may be allowed, especially in front of resource limitations (food or energy) (Sheldon & Verhulst, 1996). Probably due to its potential influence on public health, there is a growing public awareness about the potential increase on the prevalence of nematode parasitism in fish. In fact, a survey carried out by the Government of Catalonia (Informe de resultats del Programa de vigilància i control del grau d'infestiació per nematodes de la família Anisakidae en peixos de la costa catalana, 2006) in the Mediterranean Catalan ports during the period 2000-2007 showed indications of a positive trend on the prevalence of nematodes in *M. poutassou*. However, whether parasitism is becoming more frequent and its relation to climate change and fishing practices is under debate (Marcogliese, 2008). Our results evidenced links between high intensities of infection and spleen relative size (here used as a proxy of immune activity). Thus in this context, based on the latter results and assuming that sustained physiological stress derived from extrinsic pressures may be impairing the immune system, it could be persuasive to predict an increase on the frequency and virulence of parasitic nematodes which ultimately may be influencing population dynamics.

Altogether, it is plausible to expect that larger species with longer life-span such as *M. merluccius* may have evolved to a greater phenotypic plasticity to counteract the slower evolutionary rate, therefore they may have more capacity to apparently maintain individuals' fitness in front of short-term impacts of environmental anomalies, but moresensitivity to long-term extrinsic pressures (Chirgwin et al., 2018); whereas species with lower generation time, as *A. sphyraena* and *M. poutassou,* despite supposedly not showing as much plasticity, they seem to be more unstable but less vulnerable to sustained environmental stress (Chirgwin et al., 2018). These thoughts need to be checked in the future with more research. In addition to the sensitivity to sustained pressures, the susceptibility to fishing mortality is higher in populations of larger and late maturing species (Jennings et al., 1998). Thus, under this hypothesis these larger species are expected to be under higher pressure from multiple stressors. This is in line with the predicted decrease on the maximum size and the size at maturity as a response to both, the sea warming (Stearns & Koella, 1986) and the fishing pressure (Enberg et al., 2012). Furthermore, many larger fish tend to an earlier and longer spawning season due to the positive relationship between energetic state and age in many fish, e.g. *Gadus morhua* (Hutchings & Myers, 1993); thereafter, the size-truncation resulting from unbalanced harvesting with a target for the bigger sizes is expected to lead to induce shifts on demography but also on the reproductive phenology (timing and duration of spawning) which it is also influenced by sea warming, e.g. as reported for *Gadus chalcogrammus* (Rogers & Dougherty, 2018).

All the above-mentioned considerations take on particular importance for fisheries assessment and management because reproductive output drives the replenishment of stocks, and in addition to fish body size, seasonality on the spawning and on the reproductive strategies, condition status, and parasitism should also be considered. Our outcomes do not just support the requirement of reconsidering the minimum landing size for those species whose length at maturity do not exceed the minimum landing sizes, as was reported here for *M. poutassou*, but it is also in agreement with the concerns regarding the potentially dramatic consequences for the sustainability of populations due to the predicted decline in fish body size. Although the conclusions and implications emerged from this thesis apply particularly to each of the local case study species, some of them are of general interest in the field of fish ecology and fisheries management worldwide, as they have implications regarding the status and sensitivity to environmental changes and fishing pressure of exploited species with an affinity for temperate or cold-waters.

6. CONCLUSIONS

This sections compiles the main conclusions of this thesis.

The main conclusions of this PhD Thesis are:

→ *Argentina sphyraena* in the north-western Mediterranean presents a discrete polymodal oocyte recruitment, determinate fecundity and a spawning season that lasts from December to May. Its primary energy source is mesenteric fat which is invested in reproduction, in line with a capital breeding strategy.

→ *Argentina sphyraena* performs better (healthier condition and higher quality eggs) in the colder and more productive waters of the Gulf of Lion than in the apparently less favourable Balearic Sea, where waters are warmer and more oligotrophic.

→ *Micromesisitus poutassou* in the north-western Mediterranean shows evidence of both indeterminate and determinate fecundity, and reproduces from January to April. Fat in the liver is the primary energy source and is invested in reproduction, in line with a capital breeding strategy.

→ Parasitism by nematodes reduces batch fecundity in *Micromesisitus poutassou*. Any relations to health variables appeared only in highly-infected fish which, in addition to the lower batch fecundity, also presented higher energy reserves and lower spleen size. Understanding whether these observations reflect the cause or the consequence of the infestation by parasites will need further research (e.g., via development of Dynamic Energy Budget models).

→ Oocyte recruitment in *Merluccius merluccius* occurs during the gonadotropin-independent stage (likely PVO4b), much earlier than previously thought.

→ When early oocyte stages are included in the oocyte recruitment examinations, a species that was previously firmly considered to have indeterminate fecundity (in this case, *Merluccius merluccius* from Galician waters) turns out to have a “quasi-determinate” type of fecundity (i.e. performs an early predefinition of fecundity). Thus potential fecundity, which is a function of body size, could be estimated from the standing stock of PVO4b-VTO stages. This could apply to other apparently indeterminate species

→ *Merluccius merluccius* from Galician waters presents three spawning peaks per year, with similar potential fecundity but probably distinct spawning patterns in relation to environmental variations. Photoperiod, food availability (nutritional status) and temperature may have a role on oocyte recruitment, development and spawning.

→ The oocyte packing density theory was found to be an accurate and efficient tool for evaluating oocyte recruitment independently of the type of fecundity, as well as for providing additional information on other relevant ovarian features.

→ Condition-mediated trade-offs between egg quality and quantity are expressed differently between species and/or environments.

→ The breeding strategy, the egg production and their plasticity can give some insights on the species' sensitivity to extrinsic pressures, e.g. climate change and fishing.

→ Temperature and food availability are apparently influencing condition and reproductive timing and strategy. Thus, these biological traits are potentially susceptible to climate change.

→ Traits such as length at maturity, body size, spawning pattern and seasonality, fecundity, condition and parasitism should be regularly monitored using standardized indicators in order to assess the vulnerability of stocks to fishing and climate change.

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ANNEX I

This annex contains supplementary information for the case study II and III.

SUPPORTING INFORMATION CASE STUDY II:

Trade-offs between life-history traits in a cold-water fish in the Mediterranean: the case of blue whiting (*Micromesistius poutassou*).

This supporting information includes

Supplementary figures: Figure S1 to S14

Supplementary Tables: Table S1- S2

References for Supplementary material citations

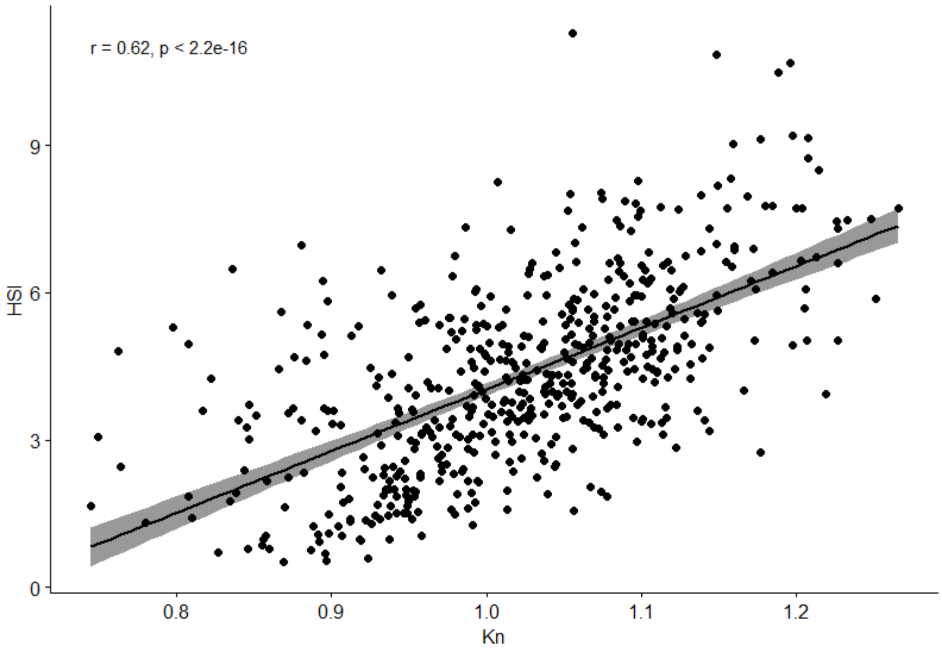


Fig S 1. Relationship between condition factor (Kn) and hepatosomatic index (HIS) in *Micromesistius poutassou*.

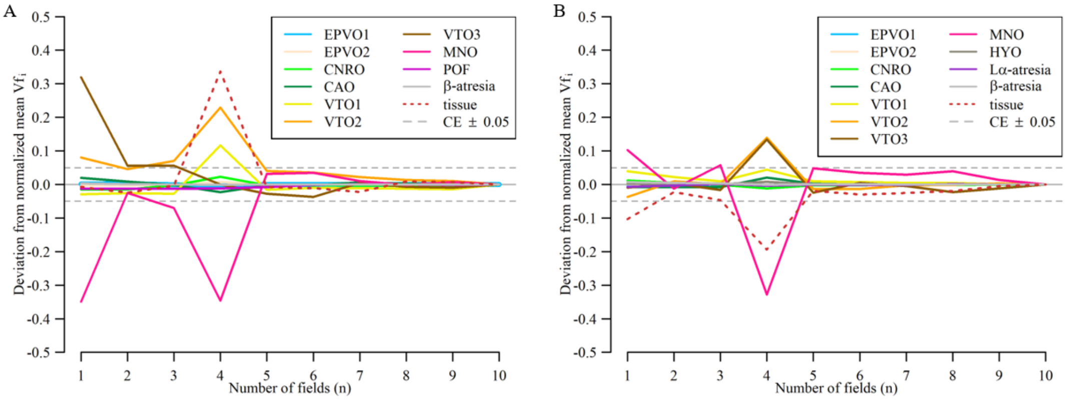


Fig S 2. Required counting fields. Deviation from normalized grand mean of volume fraction (V_{fi}), estimated by grid counting as a function of increasing number of counting fields (from 1 to 15) for one female in actively spawning phase (A) and one in spawning capable phase (B). Oocyte stages and additional ovary elements considered: EPVO1 (early previtellogenic 1 oocytes), EPVO2 (early previtellogenic 2 oocytes), CNRO (previtellogenic oocytes showing the circumnuclear ring), CAO (cortical alveoli oocytes), VTO1 (vitellogenic 1 oocytes), VTO2 (vitellogenic 2 oocytes), VTO3 (vitellogenic 3 oocytes), MNO (migratory nucleus oocytes), HYO (hydrated oocytes), α-atresia (alpha atresia), β-atresia (beta-atresia), POF (postovulatory follicles) and tissue (other organic elements).

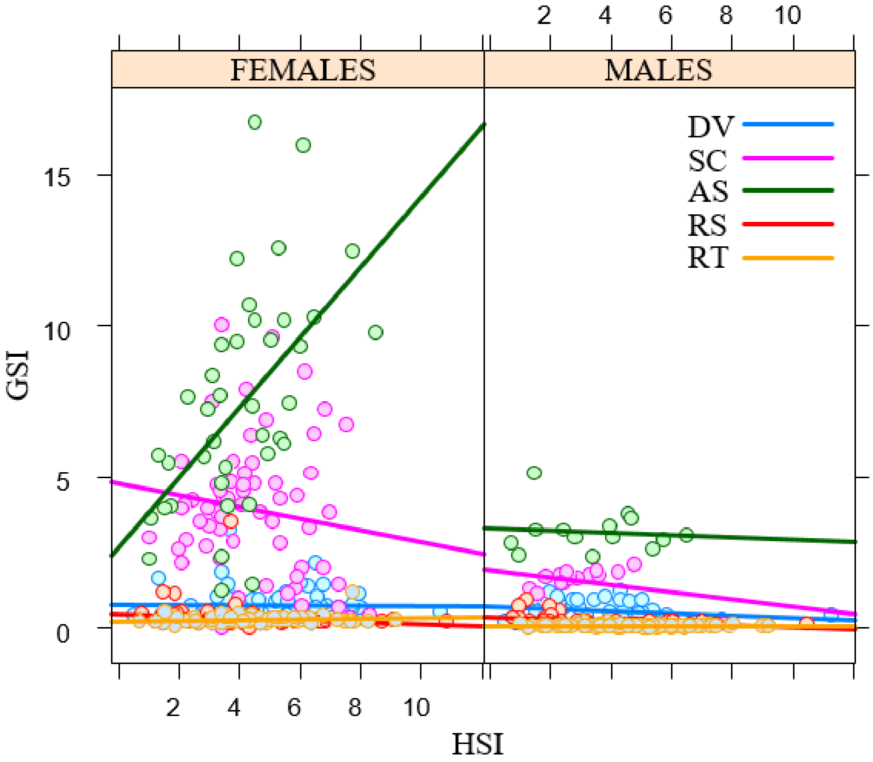


Fig S 3. Relationship between the gonadosomatic index (GSI) and the hepatosomatic index (HSI) in blue whiting (*Micromesistius poutassou*) from the Gulf of Lion. The figure displays the GSI as a function of HSI at different gonad development phases (MAT) for females (1) and males (2) given from generalized linear model runs. MAT: developing (DV), spawning capable (SC), actively spawning (AS), regressing (RS) and regenerating (RT).

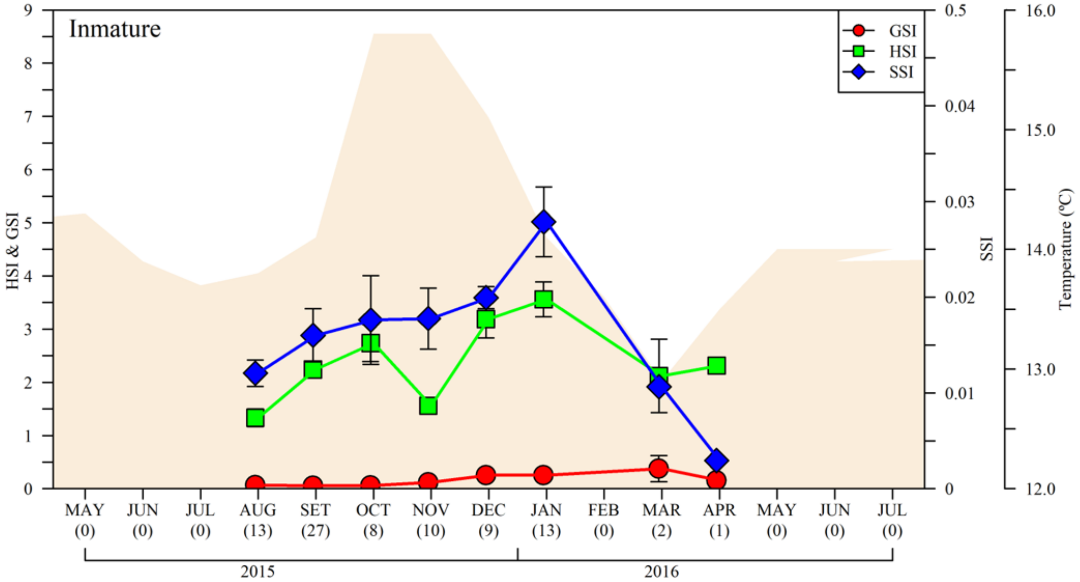
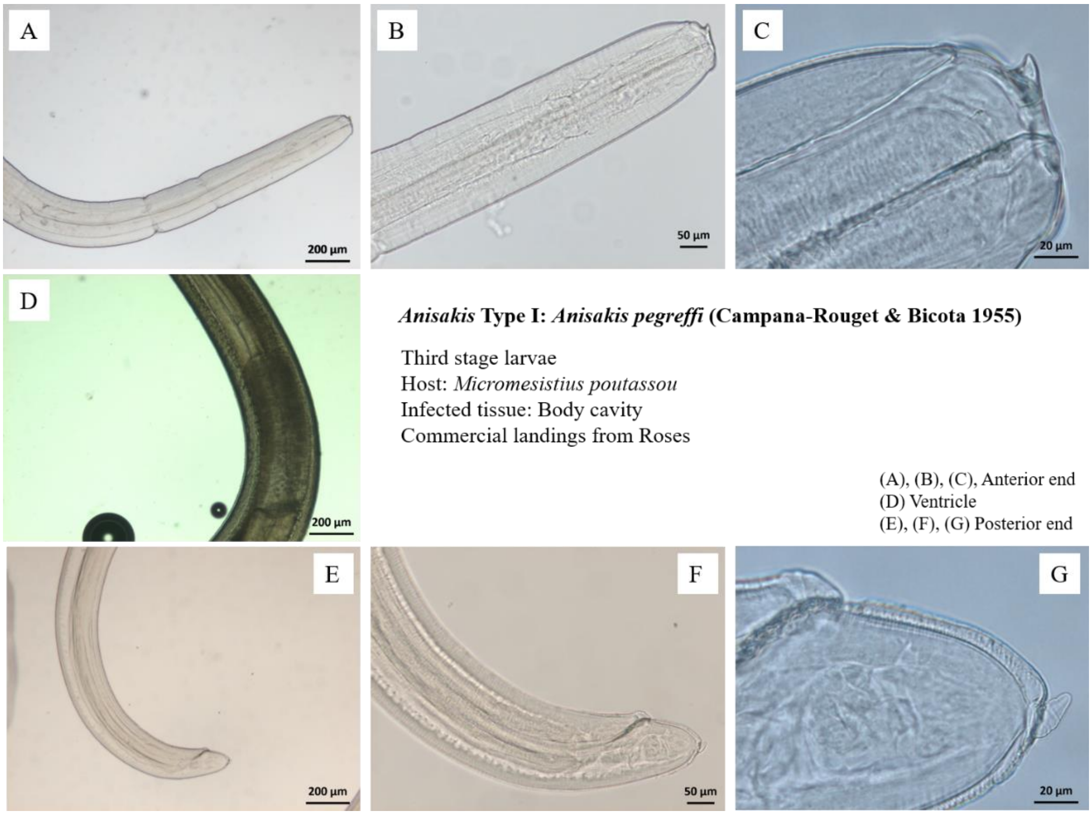


Fig S 4. Monthly variation in somatic indices in immature blue whiting (*Micromesistius poutassou*) from the Gulf of Lion. Variation of the gonadosomatic (GSI), hepatosomatic (HSI) and spleen-somatic (SSI) indices are shown.



***Anisakis* Type I: *Anisakis pegreffi* (Campana-Rouget & Bicota 1955)**

Third stage larvae
Host: *Micromesistius poutassou*
Infected tissue: Body cavity
Commercial landings from Roses

(A), (B), (C), Anterior end
(D) Ventricle
(E), (F), (G) Posterior end

Fig S 5. Photomicrographs of *Anisakis* Type I observed under the light microscope.

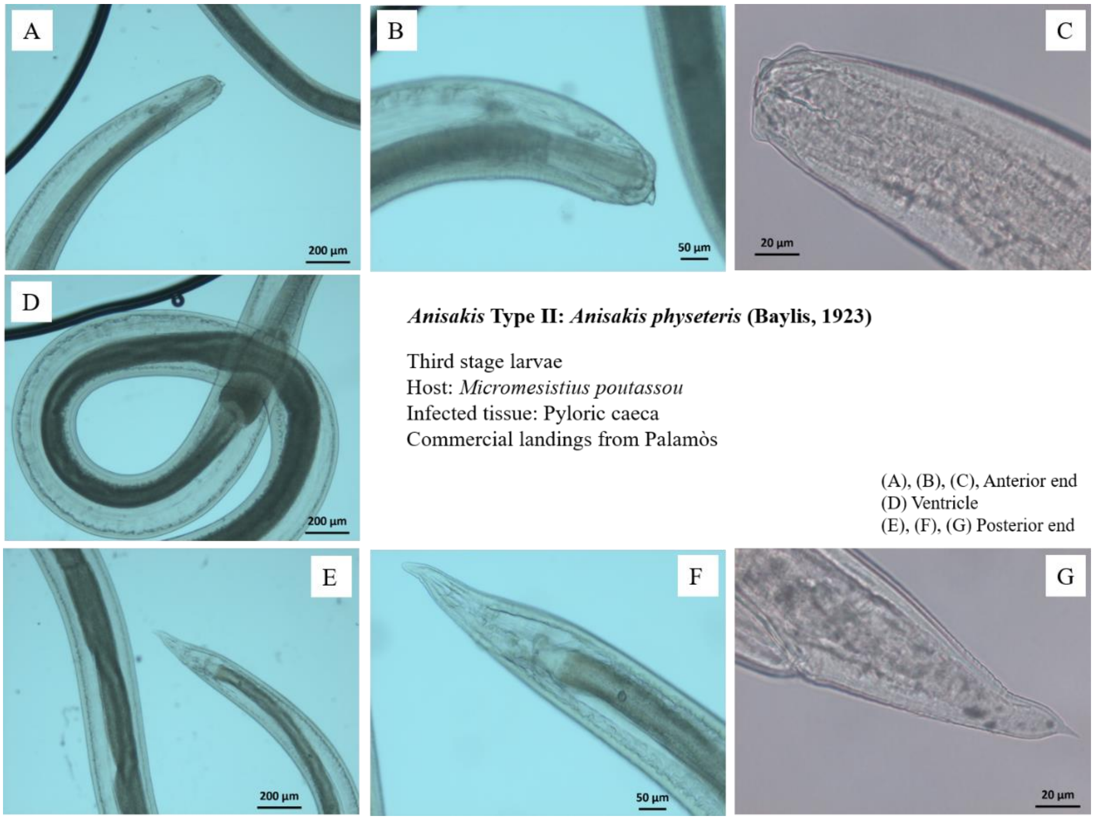
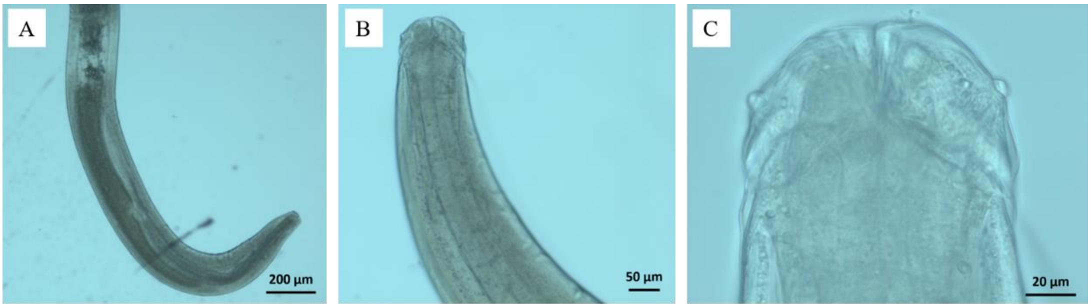


Fig S 6. Photomicrographs of *Anisakis* Type II observed under the light microscope.



***Hysterothylacium aduncum* (Rudolphi, 1802)**

Third stage larvae
Host: *Micromesistius poutassou*
Infected tissue: Pyloric caeca
Commercial landings from Llançà

(A), (B), (C), Anterior end
(D), (E), (F) Posterior end

Fig S 7. Photomicrographs of *Hysterothylacium aduncum* L3 observed under the light microscope.

Hysterothylacium aduncum **(Rudolphi, 1802)**

Third stage larvae
Host: *Micromesistius poutassou*
Infected tissue: Pyloric caeca
Commercial landings from Llançà

(A), (B), (C), Anterior end
(D), (E), (F) Posterior end

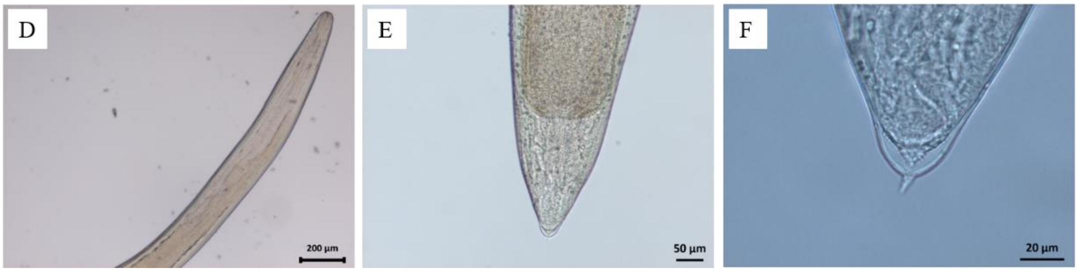
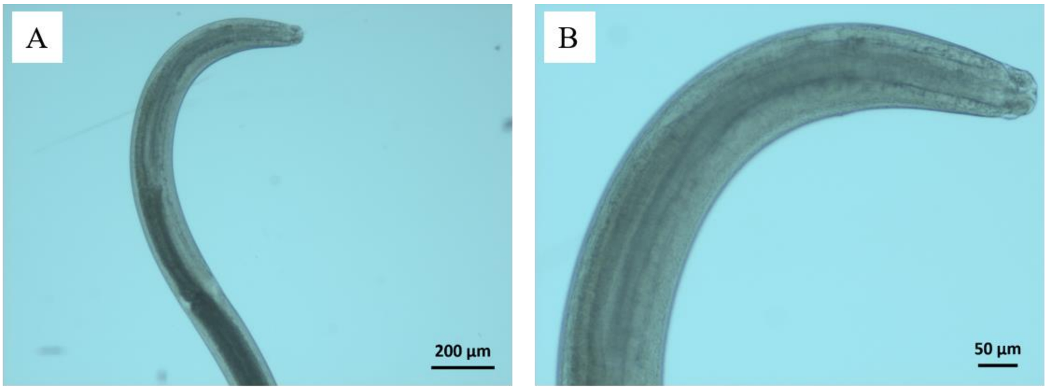


Fig S 8. Photomicrographs of *Hysterothylacium aduncum* L3-L4 observed under the light microscope.



***Hysterothylacium aduncum* (Rudolphi, 1802)**

Fourth stage larvae
Host: *Micromesistius poutassou*
Infected tissue: Pyloric caeca
Commercial landings from Llançà

(A), (B), Anterior end
(C), (D), Posterior end

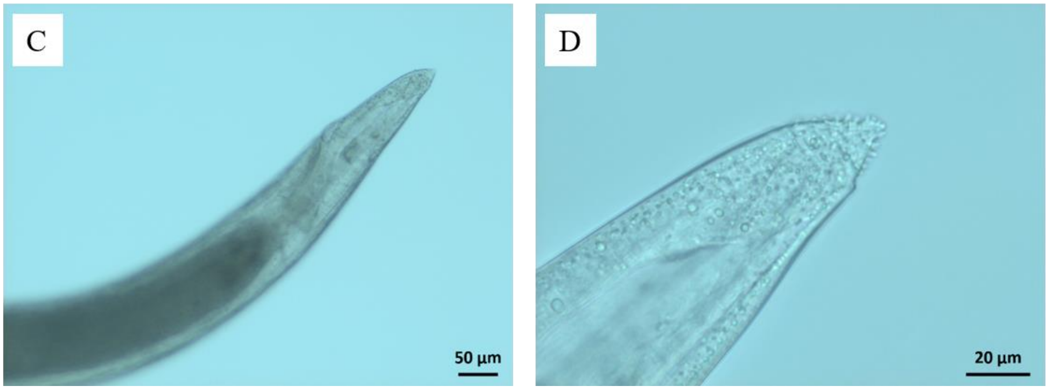
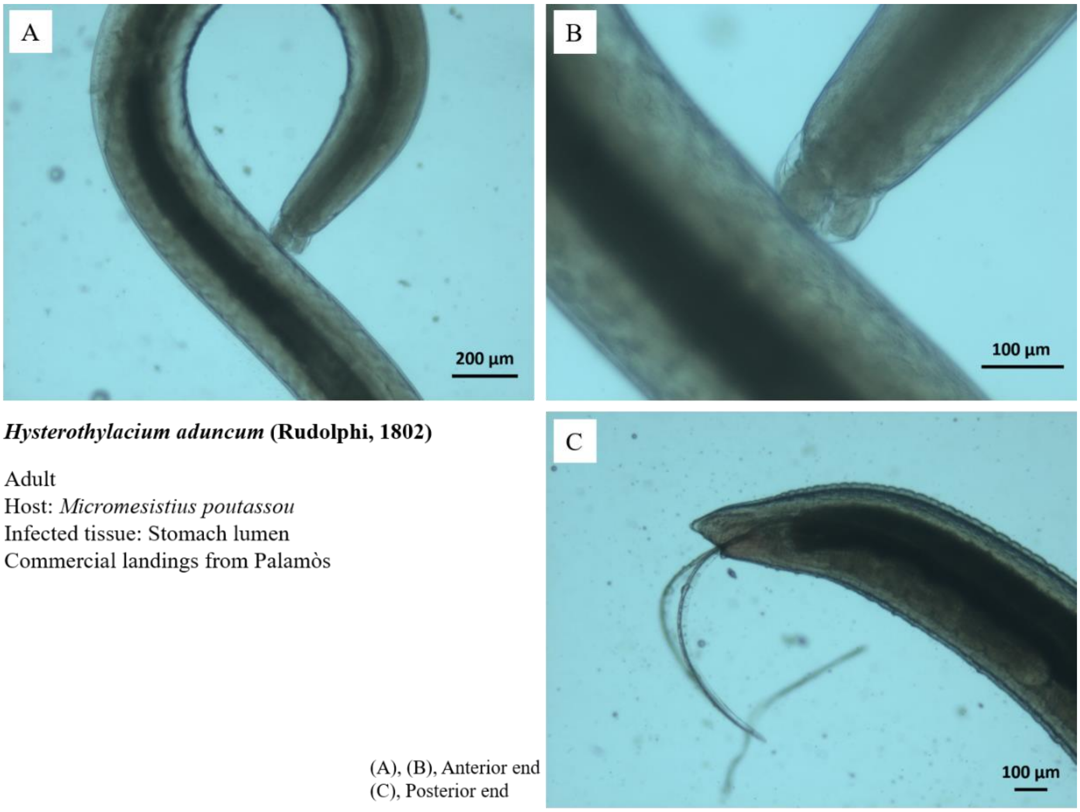


Fig S 9. Photomicrographs of *Hysterothylacium aduncum* L4 observed under the light microscope.



Hysterothylacium aduncum **(Rudolphi, 1802)**

Adult
Host: *Micromesistius poutassou*
Infected tissue: Stomach lumen
Commercial landings from Palamòs

(A), (B), Anterior end
(C), Posterior end

Fig S 10. Photomicrographs of an adult of *Hysterothylacium aduncum* observed under the light microscope.

Camallanus sp.

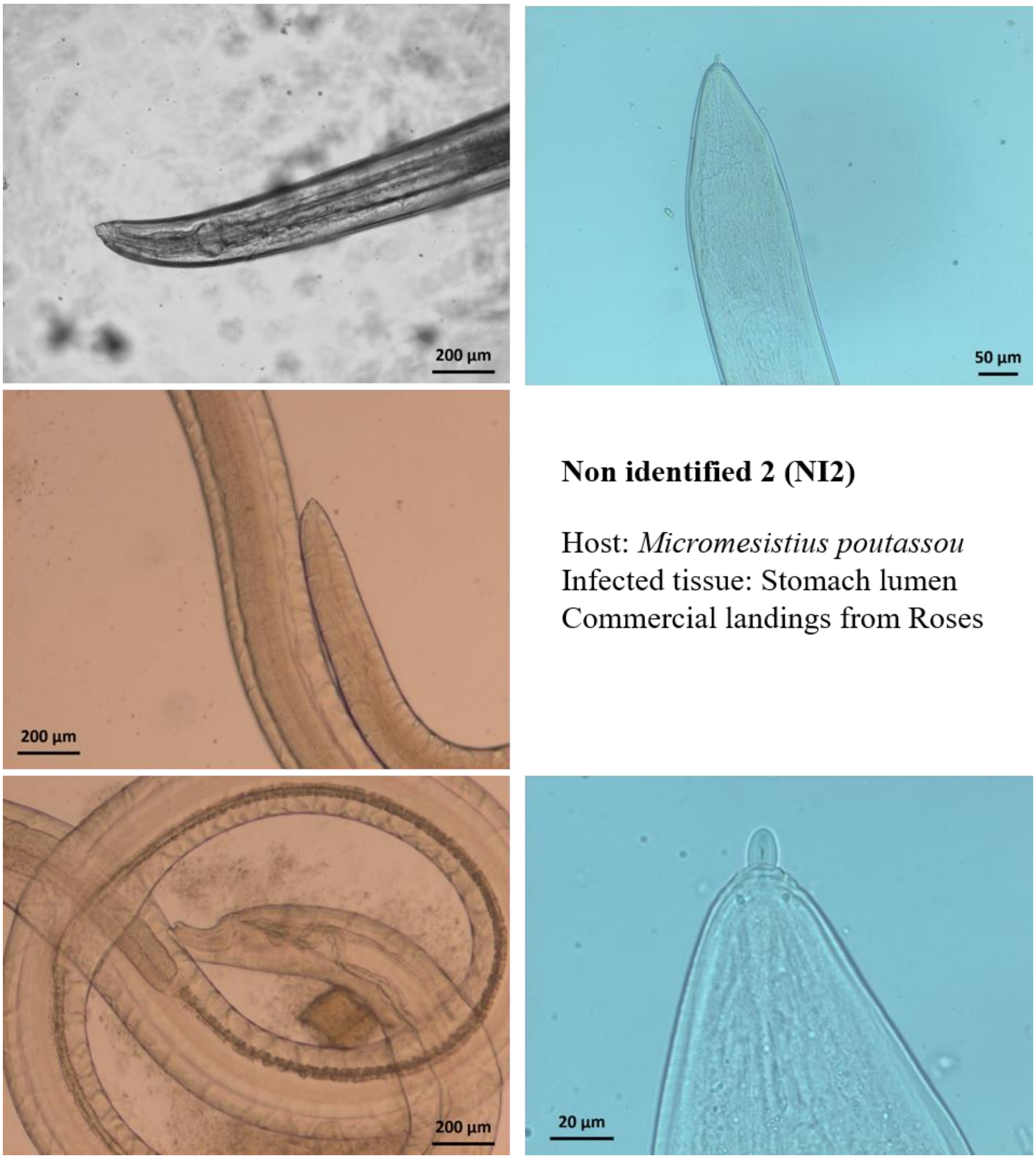
Host: *Micromesistius poutassou*
Infected tissue: Intestine lumen
Commercial landings from Palamòs

Fig S 11. Photomicrographs of *Camallanus* sp. observed under the light microscope.

Non identified 1 (NI1)

Larvae
Host: *Micromesistius poutassou*
Infected tissue: Pyloric caecum
Commercial landings from Palamòs

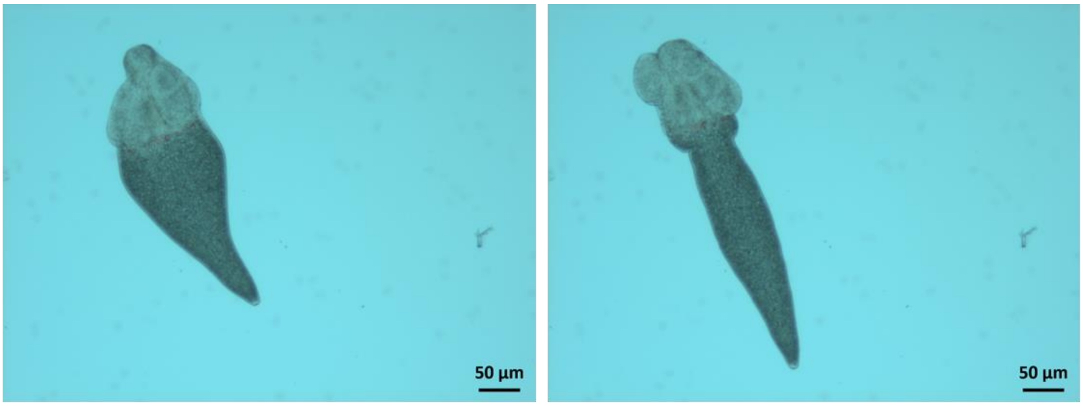
Fig S 12. Photomicrographs of non-identified nematode (NI1) observed under the light microscope.



Non identified 2 (NI2)

Host: *Micromesistius poutassou*
Infected tissue: Stomach lumen
Commercial landings from Roses

Fig S 13. Photomicrographs of non-identified nematode (NI2) observed under the light microscope.



Tetraphyllidean (Cherby 2002)

Plerocercoid
Host: *Micromesistius poutassou*
Infected tissue: Stomach lumen
Commercial landings from Llançà

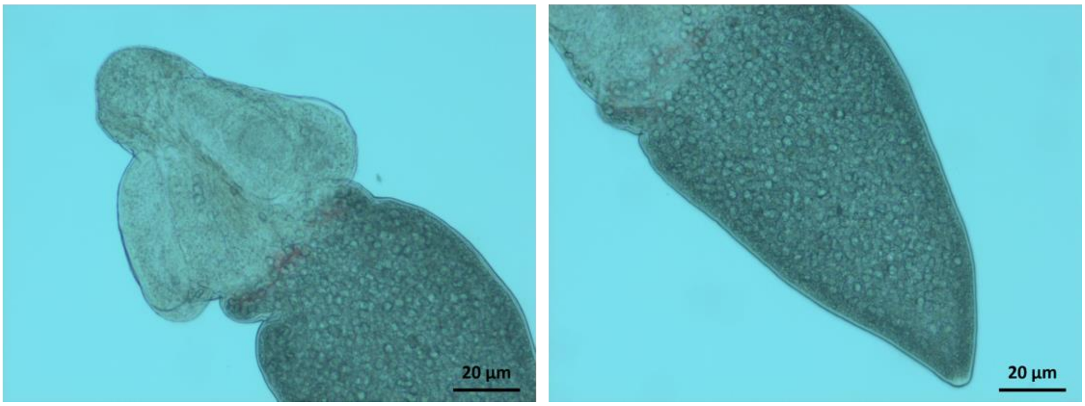


Fig S 14. Photomicrographs of cestode plerocercoid observed under the light microscope.

SUPPORTING INFORMATION CASE STUDY III:

New insights in early oocyte dynamics unravel the complexities associated to fish productivity and reproductive strategies

Lightening up the “black box” in fish productivity fundamentals.

This supporting information contains

Supplementary figures: Figure S15 to S19

Supplementary Tables: Table S3

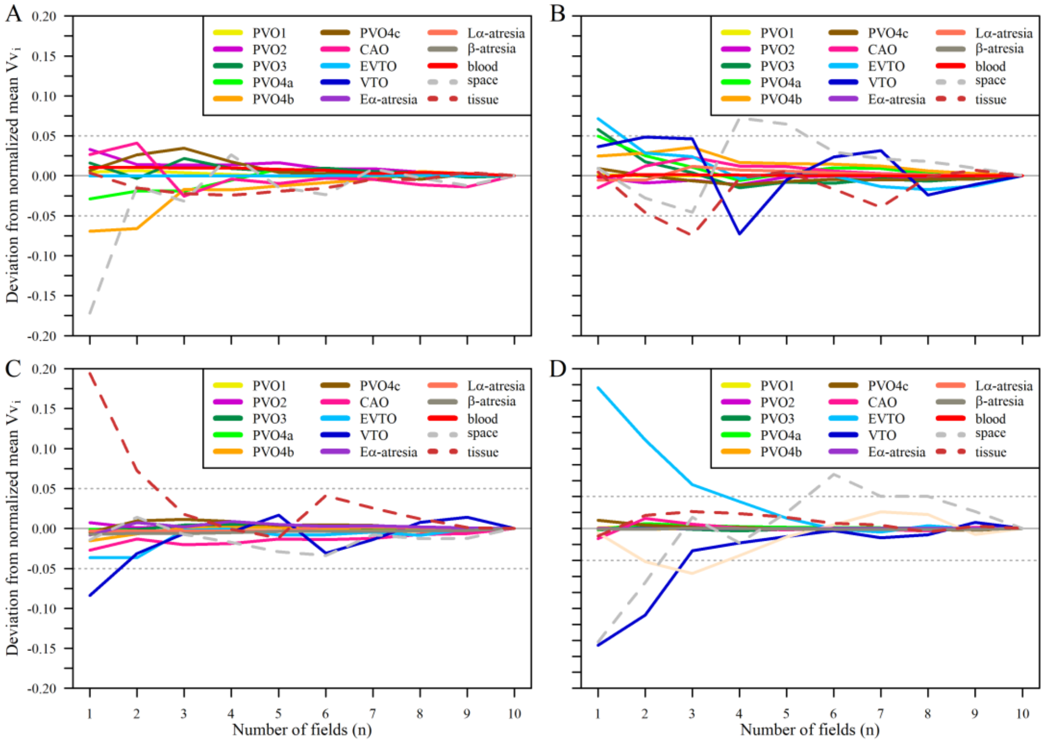


Fig S 15. Counting fields. Deviation from normalized grand mean of volume fraction (Vv_i), estimated by grid counting as a function of increasing number of counting fields (from 1 to 10) for four females in different ovarian phases (see SI Materials and Methods). Ovarian oocytes and additional elements considered: PVO1 (previtellogenic 1 oocytes), PVO2 (previtellogenic 2 oocytes), PVO3 (previtellogenic 3 oocytes), PVO4a (previtellogenic 4a oocytes), PVO4b (previtellogenic 4b oocytes), PVO4b (previtellogenic 4b oocytes), PVO4c (previtellogenic 4c oocytes), CAO (cortical alveoli oocytes), EVTO (early vitellogenic oocytes), VTO (medium and late vitellogenic oocytes), Eα-atresia (early-alpha atresia), Lα-atresia (late-alpha atresia), β-atresia (beta atresia), blood (blood capillaries), space (naturally empty space) and tissue (other organic elements).

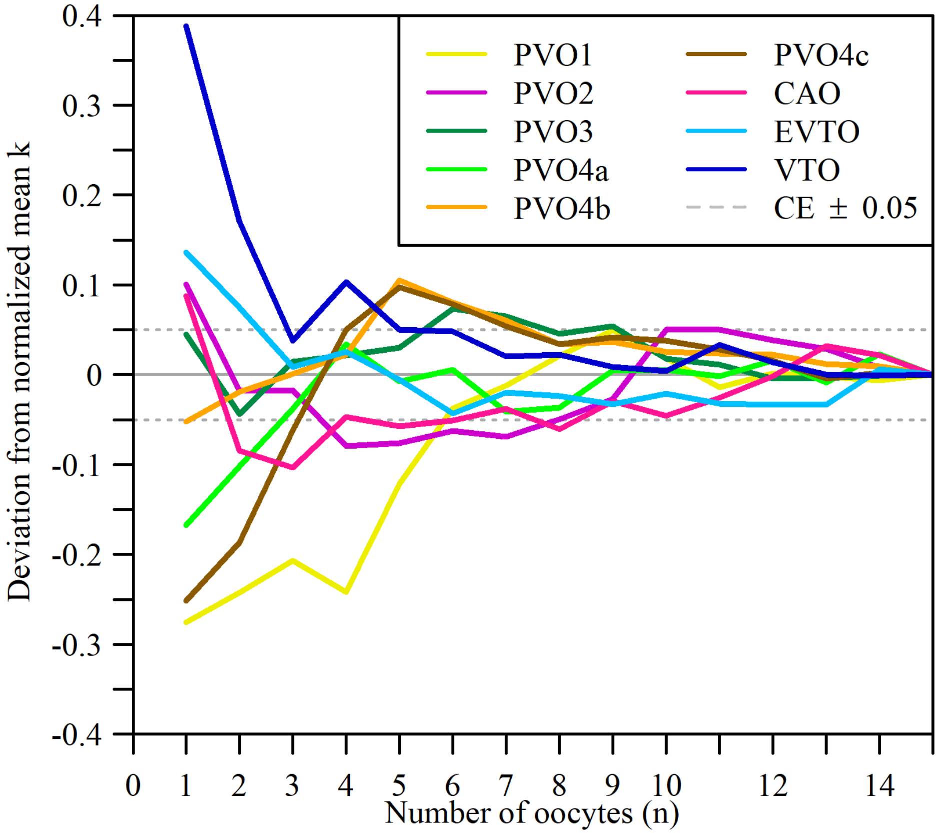


Fig S 16. Number of oocyte measurements. Deviation from normalized mean of stage$_i$ mean oocyte shape factor (ki), estimated by considering increasing number of measurements (from 1 to 15) of single oocytes in different stages. Oocytes considered: PVO1 (previtellogenic 1 oocytes), PVO2 (previtellogenic 2 oocytes), PVO3 (previtellogenic 3 oocytes), PVO4a (previtellogenic 4a oocytes), PVO4b (previtellogenic 4b oocytes), PVO4b (previtellogenic 4b oocytes), PVO4c (previtellogenic 4c oocytes), CAO (cortical alveoli oocytes), EVTO (early vitellogenic oocytes), and VTO (medium and late vitellogenic oocytes). CE is coefficient of error.

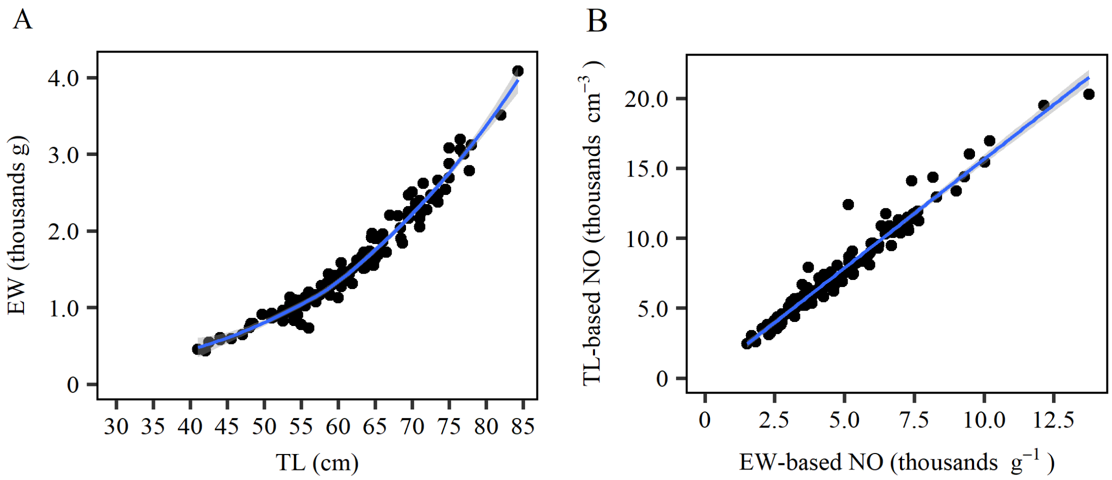


Fig S 17. Relationships between total length (TL) and eviscerated body weight (EW) (A) and TL-based and EW-based oocyte number (NO) (B) of southern European hake. In (B) EW-based NO equals NO/EW, while TL-based NO equals NO/TL3. The solid line is either the fitted power (A), i.e. EW = 0.004 × TL3.103, or the linear regression line (B), where the shaded area in both cases reflects the 95% confidence band.

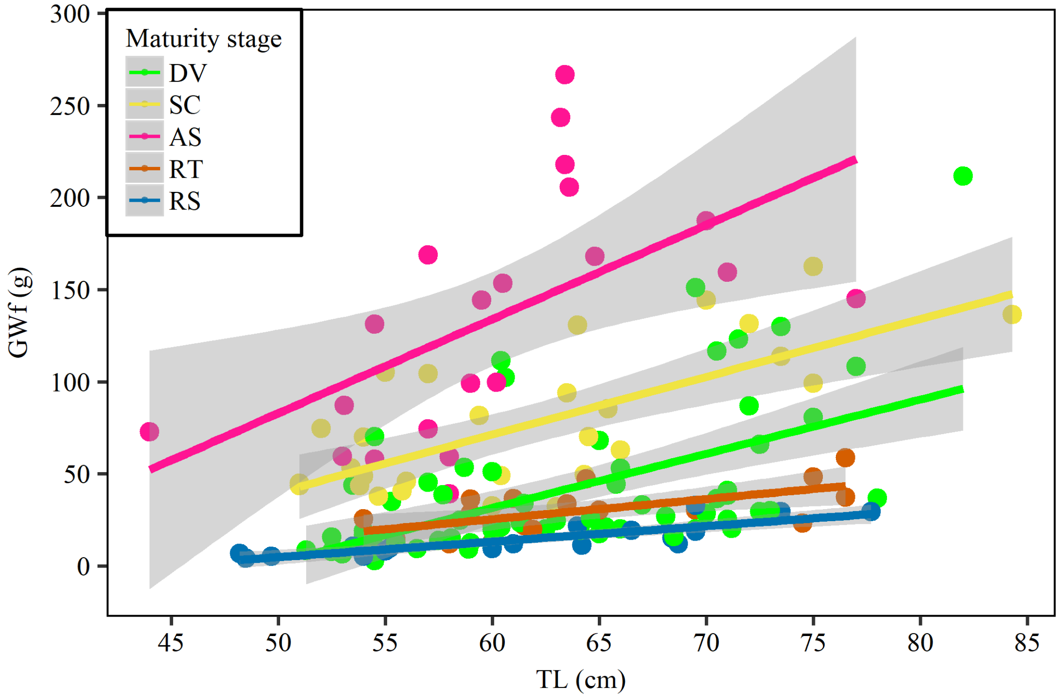


Fig S 18. Relationship between gonad weight and fish size. Formalin-fixed gonad weight (GW$_f$) as a function of total length (TL) at different maturity stages (ovarian phases; MAT) in southern European hake, given from Generalized linear model runs. Shaded region refers to the 95% confidence band. DV: developing; SC: spawning capable; AS: actively spawning; RT: regenerating; RS: regressing females.

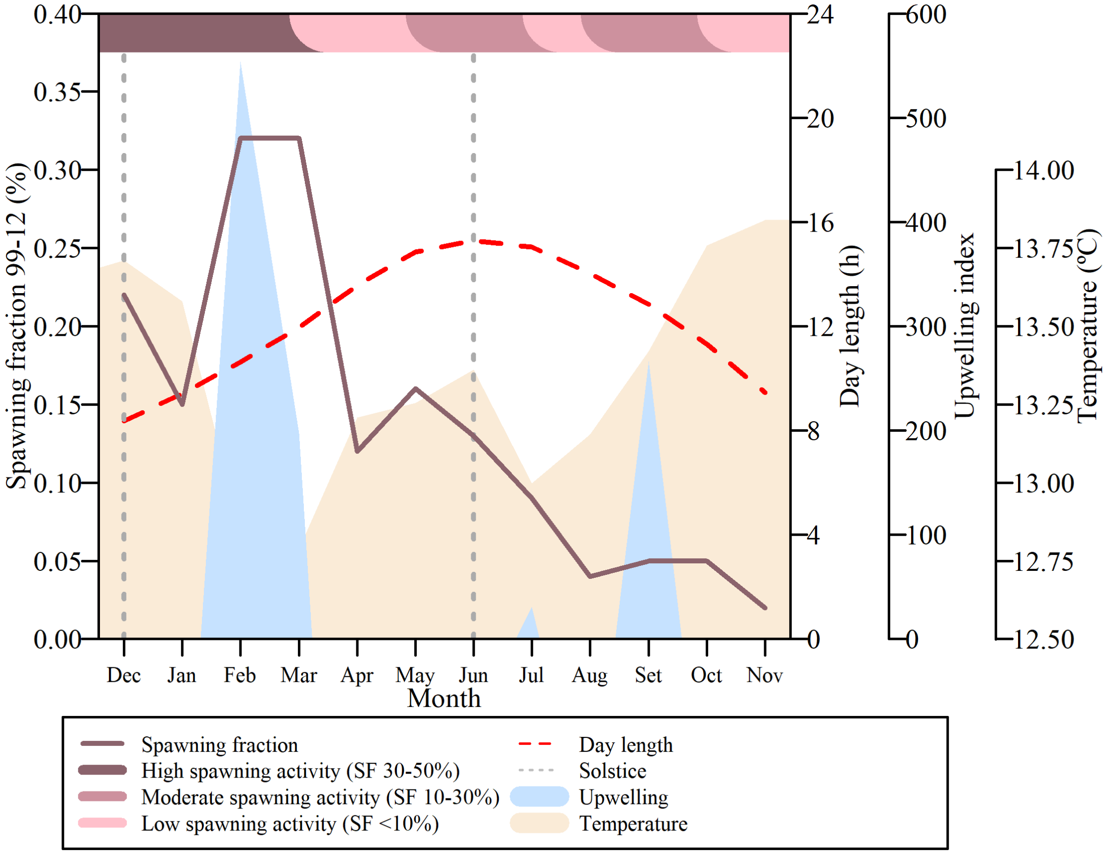


Fig S 19. Environmental variables. Monthly variation in mean values of the presently considered environmental variables along with spawning fraction of southern European hake (December 2011 – November 2012). Brown-shaded area shows sea water temperature from 50 to 350 m depth at 43.5ºN 9.5ºW, blue-shaded area upwelling events (upwelling index > 0), dashed line day length, and dotted line winter and summer solstice. The upper box of the plot summarizes presently noticed spawning activity based on spawning fraction (SF) estimations: dark, medium and light purple corresponds to 30-50, 10-30 and <10% SF, respectively. Solid line depicts overall SF in the same waters from 1999 to 2012.

ANNEX II

Protocol for embedding ovaries subsamples in paraffin:

Day 1:

1. Ethanol 50º
2. Ethanol 70º (overnight)

Day 2:

1. Ethanol 96º (1h 30')
2. Ethanol 96º (1h)
3. Ethanol 100º (1h)
4. Ethanol 100º (1h)
5. Ethanol 100º (1h)
6. Eucaliptol essence (12-224h)

Day 3:

1. Histoclear II (to replace the use of Xylol) (1h)
2. Histoclear II (1h)
3. Paraffin (heated at 56-58º into the oven) (5h 30')
4. Paraffin (>3h)

Day 4

1. Mounting blocs in the paraffin dispensing unit Kunz instruments WD-4

Day 5

2. Sectioning at 4 µm using the microtome Shandon Finesse Me Thermo Electron

Illustration: Alba Serrat Llinàs