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A review of the effects of contamination and temperature in *Solea solea* larvae. Modeling perspectives in the context of climate change

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Abstract :

The flatfish species Solea solea has been the subject of research for supporting their management in fisheries, restocking natural populations, domestication in aquaculture, and ecotoxicology research. Soles undergo a metamorphosis with drastic morphological and physiological changes and settlement, processes that make them potentially more vulnerable to pollutants than other fish. Up to date, efforts made to develop its aquaculture production in Europe have been limited. In the context of climate change (CC), this review aims i) to gather research conducted in S. solea larvae that summarizes the effects of increased temperature and contaminant exposures during larval development; and ii) to provide a summarized and synergistic view about its larval development. The review consists of 4 sections. Section 1 justifies the selection of this species from ecological and economic perspectives. Section 2 focuses on larvae ontogenesis, metamorphosis, rearing challenges, and further aquaculture production. Section 3 reviews studies dealing with the effects of temperature change (due to CC) and pollutants on larval development. Finally, section 4 provides a “how to go forward on ecotoxicological research” guideline, in which we highlight the methods that we found promising as tools to study the combined effects of CC and pollution. The section includes a multidisciplinary framework that proposes how existing data coming from different scientific domains can be synthesized to be useful for risk assessors and ecotoxicologists. To benefit from such a framework, it is necessary to reach consensus and nurture team effort from players that operate in different research disciplines.

Highlights

► Flatfish are relevant within ecotoxicity and environmental risk assessment. ► We summarize the effects of temperature and chemical exposure on *S. solea* larvae. ► Effects on larval development are of interest for multiple disciplines. ► We present a multidisciplinary framework to improve collaboration between disciplines. ► Physiology based models are promising tools for predicting anthropogenic effects on this species.

Keywords : Flatfish, Climate change, Contamination, Larval stage, Multidisciplinary

Introduction: Working with flatfishes in ecotoxicology, why?

Fish are a prominent group within ecotoxicity evaluation guidelines and subsequently within environmental risk assessment for the aquatic environment. Up to date, there exist nine guidelines from the Organization for Economic Co-operation and Development (OECD) that acknowledge the importance of fish toxicology, which includes: the acute toxicity test (OECD 203, 1992), the short term toxicity test on embryo and sac-fry stages

(OECD 212, 1998), the fish juvenile growth test (OECD 215, 2000), the fish early life-stage toxicity test (OECD 210, 2004), the 21-day fish assay: a short-term screening for oestrogenic and androgenic activity, and vitellogenin and secondary sex characteristics (OECD 230, 2009), the fish sexual development test (OECD 234, 2011), the fish short term reproduction assay (OECD 229, 2012), bioaccumulation in fish: aqueous and dietary exposure (OECD 305, 2012), and the fish embryo acute toxicity (FET) test (OECD 236, 2013).

Current integrated toxicity testing strategies consider that fish are an indispensable component, and in general terms, this is mainly because:

- fish are an essential food resource for humans;
- the aquatic environment is the final reservoir for many chemicals;
- fish are critical in aquatic food webs by top-down and bottom-up regulation of nutrient and energy flow;
- fish have been and continue to be used as sentinels of water quality used for human consumption;
- fish kills following accidents are visible to the public and encourage them on the need to protect the aquatic environment from pollutants; and
- fishing has recreational value in many cultures (Lammer *et al.*, 2009).

Among fish, flatfishes have been the subject of research supporting their management in fisheries, restocking natural populations, and domestication in aquaculture. Also, they present unique ontogeny, metamorphosing from bilateral to asymmetric, which makes them a compelling target for evolution and developmental studies (Geffen *et al.*, 2015). Furthermore, flatfishes present similar gene regulation and consequently (except for few exceptions) similar physiological responses to pollutants (Lammer *et al.*, 2009).

Specific characteristics, like metamorphosis and settlement, make flatfishes respond in a potentially different manner when exposed to pollutants. To begin with, they undergo metamorphosis, with morphological and physiological changes associated with eye migration, a 90° rotation in posture, and asymmetrical pigmentation (Geffen *et al.*, 2007). Metamorphic larvae undergo drastic remodeling of tissues and physiological changes. The transition from larval to juvenile stage involves developing most organs and tissues, the maturation of different physiological functions, and the establishment of the immune system (Ferraresso *et al.*, 2013). Flatfish larvae also undergo settlement, a behavioral and ecological change associated with a transition from a pelagic to a benthic existence. As benthic animals feed on invertebrates, flatfishes are affected by sediment-associated pollutants; so, their life history and habitat also make flatfishes valuable models for ecotoxicology research (Geffen *et al.*, 2015). The benthic lifestyle potentially exposes flatfishes to a wide range of contaminants, including -but not limited- to endocrine disruptors, heavy metals, polycyclic aromatic hydrocarbons (PAH), and dioxins (Munschy *et al.*, 2010, 2011; Wessel *et al.*, 2010; Gilliers *et al.*, 2012; Geffen *et al.*, 2015). This fact makes them useful sentinels for monitoring the biological effects of contaminants in inshore and estuarine waters.

Besides physical changes, survival and growth after recruitment are often assumed to depend on the behavioral and ecological changes associated with metamorphosis (Geffen *et al.*, 2007). Optimal cues, such as sensory perception and interpretation, guarantee the survival of early juveniles, yet it is known that swimming activity, exploration ability, and place preferences can be affected by exposure to PCBs and PBDE (Péan *et al.*, 2012). Moreover, flatfish species are widely distributed, which facilitates conducting spatio-temporal comparisons.

The characteristics mentioned above (metamorphosis, benthic lifestyle, and behavioral adaptations) make flatfishes unique and potentially more vulnerable to anthropogenic activities and estuarine contamination. It is worth noting that the aquatic environment is the ultimate sink for many man-produced products, which are continuously being released at estuaries, either due to direct discharges or to hydrologic and atmospheric processes. The chemicals that end up in these habitats range from many thousands of organic trace pollutants (polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans (PCDFs), and dibenzo- p-dioxins (PCDDs)) to compounds of emerging interest, which include perfluoroalkyl substances (PFAS), nanoparticles, personal care products, detergents, estrogen-like products, and flame retardants. All these substances are potentially interacting and impacting the health of flatfish and represent a risk for the health of benthonic fish populations (van der Oost *et al.*, 2003).

Finally, Geffen *et al.* (2015) argued that one of the most rapidly developing areas that would benefit from the synergy of flatfish research in aquacultured and wild populations is the ecotoxicology domain. Unfortunately, research on flatfishes is distributed across journals in several (sub)disciplines, and because the reference lists of individual papers rarely extend across these disciplines, in order to gain a full benefit of knowledge exchange, it is necessary to read widely.

Objectives

This review aims to gather works primarily focused on ecotoxicological research conducted in the flatfish species *Solea solea* along with their larval development and early juvenile phase. We draw attention to this life period because it is critical for fisheries and aquaculture development and for understanding anthropogenic impacts on *S. solea* populations and ecosystem degradation. Additionally, a secondary objective is to provide a summarized and synergistic view on research conducted in *S. solea* larvae that serves different disciplines such as aquaculture, fisheries, marine ecology, and ecotoxicology.

We divided the review into four sections. The first section justifies the selection of this species and reviews *S. solea* ecologic and economic relevance.

Section two focuses on the main particularities of this species. Soles, like all flatfishes, undergo an extreme metamorphosis which translates into a susceptible larval stage. The section summarizes *S. solea* regular larvae development by citing the most recent literature describing the optimal conditions for *S. solea* laboratory rearing and growth. The main questions addressed in section 2 include: What are the main bottlenecks on the aquaculture production of *S. solea*? Furthermore, what have been the scientific advances that have been made to overcome these problems?

In the third section, we will focus on reviewing works studying the effects of climate change (CC, we only focus on temperature change) and contamination on larval development. This section comprises the main focus of the review and will include works from the molecular level until the population level, exploring and comparing these different "tools" employed to understand, estimate, and predict the effects of human activities on this species. The questions we intend to answer in section 3 include: What are the individual and combined effects of CC and environmental pollution on larval development of *S. solea*? Furthermore, can we predict or anticipate the effects of combined stresses?

Finally, in the last section, we will review the methods and efforts made to integrate different types of data to 1) have a holistic view on the subject and 2) to be able to extrapolate (if possible) to different levels of biological organization or other species. This section is meant as a "how to go forward on ecotoxicological research" guideline, in which we primarily highlight the methods that we found promising as tools to study the combined effects of CC and pollution. The main questions addressed in section 4 include: What methods allow us to understand the combined effects of CC and contamination? How can we reduce animal testing for new chemicals and improve risk assessment? And finally, how much can we rely on numerical models?

Section 1 - Why *Solea solea*?

1.1 Common sole life-cycle main events

The flatfish species, *Solea solea*, also known as the common or Dover sole, and from now on simply denoted as sole, is an important commercial species found in shallow waters (between 10 to 60 m depth but also down to 200 m) of the eastern Atlantic Ocean. Its distribution range extends from the north-western coast of Africa in the Mediterranean Sea to the southern coast of Norway (Zambonino-Infante *et al.*, 2013).

The common sole is a batch-spawner, and increases in temperature trigger spawning. Depending on the geographical location, spawning events might occur from late winter to spring. In the Mediterranean Sea, spawning occurs between January and April, with two peaks in February, while in the Bay of Biscay, spawning occurs between December and May (FAO, 2019), depending on the latitude. In the North Sea, spawning happens between April and June, and different sole stocks present differences in the average date of peak spawning, with spawns occurring from late March in the Bristol Channel to mid-May in the Irish Sea and in late May in the eastern-central North Sea (Fincham *et al.*, 2013). The optimal temperature for spawning ranges from 7 to 12 °C (Lacroix *et al.*, 2013).

Spawning behavior in *S. solea* involves three stages, courtship, spawning rise, and a post-spawning return to the seafloor. During courtship, the male follows a female on the bottom and then swims below the female. If courtship is successful, the pair swims vertically towards the water surface with closely synchronized body movements, and gametes are released at the apex of the rise, followed by a return to the seafloor (Baynes *et al.*, 1994).

Fertilized eggs and larvae are both pelagic (Fig. 1), which allows for wide dispersion and exposes them to environmental variability, notably temperature and food abundance. Fertilized eggs hatch after few days (5 days at 16 ºC, Imsland *et al.*, 2003), and the larval stage -from hatching until complete metamorphosis- can last between 26 and 15 days, at temperatures of 16 ºC and 19 ºC, respectively (De Swaef *et al.*, 2017; Richard *et al.*, 1991).

The larval stage comprises a metamorphic period, where significant ontogenic, morphological, physiological, and behavioral changes occur (Geffen *et al.*, 2007). The length of metamorphosis depends on temperature and food availability, and by the end of it, juvenile soles are ready to change from pelagic to benthic lifestyle to colonize shallow coastal waters in estuaries and bays (Zambonino-Infante *et al.*, 2013).

Newly metamorphosed juveniles settle in coastal areas for around two years until they reach maturity (Post *et al.*, 2017). These areas serve as nursery grounds, where abundant food (epi and endobenthic prey) favors growth. Further, the pelagic larval and the juvenile stage on nursery grounds are critical for common sole recruitment, *i.e.,* the number of fish surviving to enter a fishery and contribute to the next generation (Zambonino-Infante *et al.*, 2013). After settlement on estuarine nursery areas, *S. solea* individuals undergo further ontogenic habitat shift (in some populations, juvenile soles progressively migrate tens of kilometers from shallow estuarine waters to deeper coastal water).

At the adult stage, they migrate offshore (few or up to hundreds of kilometers depending on location) in particular for reaching spawning grounds, and they eventually come back closer to the shore out of reproduction season to reach feeding grounds (Vasconcelos *et al.*, 2014). Regardless of this migration, Diopere and collaborators (2017) argue that the wide geographical area at which this species is found and the fact that they do not conduct long migrations makes it reasonable to assume that sole in European waters belongs to more than one population. However, the precise habitat range of this species remains unclear (Post *et al.*, 2017). Moreover, and because spawning grounds are distant from the nursery areas, the planktonic larvae have a drifting period which ideally should end at an area that is suitable as a nursery for the juvenile stage. The question is whether they do anything to maximize their probability of arriving there or whether it is a random process (Duffy-Anderson *et al.*, 2015). Factors such as release time, location, stage duration, climate, and hydrodynamic circulation play a crucial role in larval mortality rates and determine the transport of eggs, larvae and the population renewal (Vinagre, 2007; Duffy-Anderson *et al.*, 2015).

Such a complex life cycle (Fig. 1) with progressive habitat shifts along *S. solea* ontogenesis makes it challenging to determine how population dynamics are affected by habitat quality. In this regard, Vasconcelos and collaborators (2014) showed in their review how little is known about habitat-specific demographic rates on species of commercial interest, including the common sole. Knowledge about the relationship between habitat characteristics and population dynamics is particularly valuable from a conservation and management perspective since the vulnerability of coastal habitats to anthropogenic stressors is increasing (Rochette *et al.*, 2013). Specific recommendations include emphasizing the spatiotemporal scales in studies addressing the reproduction, mortality, immigration, and emigration dynamics of *S. solea*. By this means, it will be possible to define settlement habitats, the degree of connectivity between primary and secondary settlement habitats, and the level of dependence of juvenile and adult populations on these essential coastal habitats (Vasconcelos *et al.*, 2014).

1.2 Ecological importance and habitat selection

Solea solea is an r strategist species, characterized by a strong fecundity -with hundreds of thousands of eggs spawned (per kg of female)- and low survival rates. The environmental fluctuations strongly influence survival rates, egg and larval life history along the larval dispersion process, and the transition from a pelagic to a benthic lifestyle (Savina *et al.*, 2016). At first, eggs derive towards nursery areas thanks to the action of winds, tides, and currents (Savina *et al*., 2010). After hatching, larvae start showing active vertical migration in the water column triggered when feeding starts (Lagardère *et al.*, 1999). Larvae use vertical migration to enter, leave, or remain in estuaries by either selecting the seaward outflow at the surface or the inflow near the bottom (Duffy-Anderson *et al.*, 2015). Therefore, the successful arrival in nursery areas is dependent on hydroclimatic conditions (Lagardère *et al.*, 1999).

Recently metamorphosed individuals settle in nursery areas and switch to a benthic lifestyle starting their critical juvenile stage when their movements are limited, which will last for up to 2 years (Eichinger *et al.*, 2010). According to the "nursery size hypothesis" (Van der Veer *et al.*, 2000), juvenile survival depends on the surface area of nurseries; as each nursery can only sustain a population in proportion to the size available (Duffy-Anderson *et al.*, 2015), and it is during this period that soles become top predators (Amara *et al.*, 2001). Indeed, early juvenile diet is mainly based on copepods, progressively changing to include short-lived, highly productive benthic organisms, such as polychaete worms, mollusks, and small crustaceans (Amara *et al.*, 2001; Rijnsdorp *et al.*, 2004).

Juvenile fish distribution is influenced, among other factors, by sediment type, food availability, and movement ability, and the trade-off outcome of these factors determines their habitat selection. For *S. solea*, estuarine and shallow coastal systems act as nurseries where the trade-off between growth and survival is optimized due to abundant food resources, few predators, and favorable environmental conditions (Freitas *et al.*, 2012). This fish-habitat interaction is of utmost importance at early-life stages when successful recruitment and development of fish from larval to juvenile stages depends on the quality and the characteristics of nursery areas (Post *et al.*, 2017).

For flatfishes, the type of sediment is also a decisive factor, as it can inhibit burial behavior or affect prey availability (Post *et al.*, 2017). That is why habitat degradation, such as beach sand nourishment, directly impacts recruitment success and species fitness. Coarsening the sediment in the coastal zone would increase the energy requirement for juvenile sole through prolonged digging behavior, consequently impacting individual growth and increasing their vulnerability to predation due to their smaller size (Post *et al.*, 2017). Post and collaborators (2017) measured the sediment preference of soles by determining their relative distribution over the sediment classes. Preference -defined as selecting a specific sediment type through their presence at the end of the experimental period (4 days)- was tested in a circular preference chamber. Their results demonstrated that juvenile soles prefer fine grains, and they suggest that such marked preference could be a consequence of higher food availability and lower energy to bury (Howell and Canario, 1987; Post *et al.*, 2017).

The juvenile stage, where soles have a benthic lifestyle and inhabit coastal nursery areas, is the period at which individuals are exposed to a broader range of contaminants, which includes not only hydrophilic compounds dissolved in the water column but also hydrophobic contaminants that can be either directly absorbed from the sediment or ingested from contaminated food (Rochette, 2011).

Given its characteristics and ecological importance, *S. solea* has been included within the EU Marine Strategy Framework Directive (MSFD) as a model and indicator species for evaluating the good environmental status in the marine environment in 2020.

Finally, readers seeking for more detailed information on niche ecology and habitat selection of *S. solea* are encouraged to revise the works from Vinagre *et al.* (2006) that evaluates the species distribution and determines the habitat quality using habitat suitability index models or the study by Vinagre and collaborators (2005), which evaluates the niche overlap between European flounder and *S. solea*. Furthermore, there is an important body of research that focuses on comparing *Solea senegalensis* and *S. solea* and their differential use of estuaries (Cabral and Costa 1999), species distribution (Vinagre *et al.*, 2009), prey consumption (Cabral 2000; Vinagre and Cabral 2008) and habitat-specific growth rates (Vinagre *et al.*, 2008) from populations inhabiting the Tagus estuary, Portugal. The list of publications on related topics goes on, including Martinho *et al.* (2008), where the authors compared the timing of estuarine colonization, the population structure, growth, and diet composition of flatfish juveniles from the species *Dicentrarchus labrax*, *Platichthys flesus*, *S. solea*.

1.3 Economic importance of soles

The sole species, *Solea solea,* and *Solea senegalensis,* are characterized by high flesh quality and high market value making them very promising candidates for European aquaculture (Howel *et al.*, 1997; Bonaldo *et al.*, 2011). Also, fisheries landings for *Solea spp.* have shown a reduction of over 43%, and the average size of wild sole has also declined (Morais *et al.*, 2014). This reduction in the offer has promoted average prices of different sole products (fresh, frozen, and farmed) to increase and consistently attain high prices in European markets (Morais *et al.*, 2014). For example, in 2013, farmed products attained 12.25 € kg^{-1} in MercaMadrid (Morais *et al.*, 2014), and in May 2019, *S. solea* reached an average price of 23.8 € kg^{-1} in France (FranceAgriMer, https://rnm.franceagrimer.fr/prix?SOLE&12MOIS). Their high commercial value, the growing market demand for adults and juveniles, and the increasing fishing pressure -not restricted to the coast but also present in the estuary- make sole fisheries an attractive business from both the socio and economic perspectives. These reasons have reinforced the interest and promise of developing commercial aquaculture with these species. In fact, *S. solea* and *S. senegalensis* have been repeatedly identified as the most promising species for aquaculture diversification in Europe for at least four decades now (Morais *et al.*, 2014).

As a result, during the last 30 years, sole species have attracted considerable research attention, and significant investments have been made to develop rearing methodologies (Howell 1997; Dinis *et al.*, 1999). Nevertheless, the production of *Solea spp.* has failed to reach successful commercial development until recently, and this success is limited to *S. senegalensis*. The three major bottlenecks for *Solea spp.* production are 1) high larval mortality rates related to nutrition and growth dispersion, 2) sub-optimal larval weaning strategies that result in weaning onto formulated diets and lead to low growth and high juvenile mortalities; and 3) inadequate control measures for common diseases. Additionally, the techniques for intensive rearing of the sole are yet to be optimized, which limits the final volume of fish production (FAO, 2019; Morais *et al.*, 2014). Other problems include a decreased growth rate at high stocking densities, pigmentation abnormalities, malformations related to eye migration (Dinis *et al.*, 1999). Finally, the reproduction of cultured specimens is complicated in F1 and later generations (especially in *S. senegalensis*, Ibarra-Zatarain *et al.*, 2020). In the next section, we will summarize some of the research advances made to solve the above-mentioned problems.

Section 2 - Importance of studying *Solea solea* larvae: development, the cost, and challenge of metamorphosis

External biotic and abiotic factors affecting larval development have consequences at later life stages (Howel and Baynes, 2004). Fish larval stages represent a transitional period at which both differentiation and growth lead to substantial changes in structure, physiology, size, and morphology. Compared to any other life stages of a marine fish species, individuals at their larval stage will have the highest potential for growth, weight-specific metabolic rates, natural mortality rates, and the highest sensitivity to environmental stressors (Hutchinson *et al.*, 1998; Belanger *et al.*, 2010).

These factors highlight the importance of studying fish larvae. Early stages are usually characterized by strict environmental requirements, where factors such as nutrition, microbial environment, and physical/chemical conditions determine the healthy development and the survival rate. Moreover, all organs develop during the embryonic and larval stages, and often, causes of permanent abnormal development occur during this early stage, ultimately influencing how fish will perform later in life (The Research Council of Norway, 2009).

Up to date, only a few works have focused on reviewing research conducted on this species. Such works have mainly focused on the potential of *Solea solea* and the closely related species *S. senegalensis* for commercial aquaculture. These include the work by Baynes *et al.* (1993), which reviews the egg production by sole in captivity, and Howel (1997) summarizes the challenges and benefits of cultivation of sole. However, the information provided is limited to assessing the technical rather than the economic feasibility of farming sole. More recently, the works by Imsland *et al.* (2003), Morais *et al.* (2014), and Munoz-Cueto *et al.* (2019) focused on presenting the biology and discussing the potential use of *Solea spp. -S. solea and S. senegalensis* for aquaculture. In this last reference, a chapter by Solé (2019) walks the reader through the use of these species within ecotoxicological studies, particularly by measuring biomarkers in sole tissues.

To our knowledge, this is the first review that exclusively focuses on *S. solea* larval biology and ecotoxicology. In our opinion, this could be partly explained given that only until recently, advances in culturing marine fish species and in developmental biology -notably on model species such as zebrafish- have allowed for the entire understanding of many biological processes and have made the field of larval ontogenesis more accessible for experimental exploration (The Research Council of Norway, 2009). Added to this, the threats imposed by climate change and pollution justify the need for summarizing those research works devoted to understanding the individual or combined effects of CC and anthropogenic activities on larvae.

For this review, we searched various academic search services (Thomson Reuters, Web of Knowledge, and Web of Science) for works published in the period between 2003 and 2020 using combinations of the following topics: *Solea solea*, flatfish, larvae, larviculture, ontogenesis, chemical contamination, and climate change. We choose to start our searching from 2003 onwards to avoid duplicating information already available in Imsland *et al.* (2003).

2.1 Why does fundamental research on larval biology matter?

Fundamental research in larval biology is essential for assuring the ecosystem functioning of nursery areas. Larvae biology is also crucial for marine fish farming. Because all organs and biological systems develop during the embryonic and larval stages, early development strongly influences how the fish performs later in life (Howel and Baynes, 2004). Therefore, knowledge about optimal larval development can substantially impact further stages and make a big difference in economic and sustainable terms. An appropriate example was reducing halibut discards due to incomplete eye migration, which accounted for up to 40-60% of halibut fry. Such a problem was solved by only applying photoperiods during larval rearing (Solbakken and Pittman, 2004).

There is potential for improvements in aquaculture cost efficiency by increasing growth and survival rates and reducing the prevalence of deformities, and the key for such improvements might be related to larval development. Moreover, for wild stocks, knowledge about early life stages provides insight into how the fish survive under various conditions by adapting to their environment. Several reasons pinpoint fish early-life-stages (ELS) as more sensitive to pollutants than fully developed individuals from the same species (Hutchinson *et al.*, 1998). These reasons are related to a combination of critical developments occurring during

ELS, the limited possibilities to compensate for the impact of physiological defects, and significant changes in the composition of the developing tissue (Foekema *et al.*, 2012).

2.2 Advances made on larvae survival during rearing

Before standardizing a massive farming production of the sole, several problems are to be overcome, including larval mortality, feeding behavior, susceptibility to disease, stocking density, and reproduction. Besides, there are high mortality rates during juvenile production around metamorphosis and weaning (Bonaldo *et al.*, 2011).

For successful larvae rearing, the first factor to consider is related to the quality of the eggs, which can be defined as the egg's ability to be fertilized, survive, and develop into a normal embryo (Lund e*t al.*, 2008; Parma *et al.*, 2015). As the egg must satisfy the nutritional needs of embryonic and larvae stages, and because healthy larvae depend largely but not exclusively (hormones and other molecules transferred in eggs are also crucial for embryonic and larval development), on the amount of fatty acids (FAs), the quality of eggs is usually assessed by studying its lipid and FAs composition (Lund *et al.*, 2008; Parma *et al.*, 2015). Besides, broodstocks' nutrition and life history has been shown to affect egg nutrients qualitatively and quantitatively (Foekema *et al.*, 2012; Parma *et al.*, 2015).

In order to study the relationship between hatching rate, survival, and growth of larvae and egg quality, Parma *et al.* (2015) analyzed and characterized the FAs composition of *S. solea* eggs obtained from domesticated broodstock during an entire spawning season. According to their results, the authors conclude that the feed supplied to broodstock before and during vitellogenesis has a significant role in determining the eggs' FA profile in sole. Another objective of their work included defining the relationship between FAs and egg viability parameters and larval survival as a means to provide information for improving -if necessary- broodstock feed in common sole. Regarding this last objective, the authors observed that, on the one hand, saturated and monounsaturated (MUFA) fatty acids were positively correlated with the hatching rate. On the other hand, TL, 22:6n-3 (DHA), 20:4n-6 (ARA), polyunsaturated FAs of the (n-3) series (n-3 PUFA), and polyunsaturated FAs of the (n-6) series were negatively correlated with the hatching rate. MUFA, 20:5n-3 (EPA), n-6/n-3 were positively correlated with survival rate, while DHA, n-3 PUFA, DHA/EPA were negatively correlated. The relationships found between total lipids and FAs with egg and larval viability parameters showed a positive correlation with egg quality, while n-3 PUFA, DHA, DHA/EPA, and ARA content were inversely correlated with egg larval survival. This is typically different from many other farmed marine fish species, suggesting the need for a specific broodstock feed for this species.

In order to elucidate if offspring quality was affected by culture-related conditions, Lund *et al.* (2008) compared the eggs' quality -via FAs composition- and its relationship to egg size, fertilization, and hatching rate in eggs coming from cultured and wild broodstock. The FAs profile allowed discriminating between culture and wild origin. Eggs from wild broodstock were bigger, had higher levels of C16:1(n-7), C20:4(n-6), and C20:5(n-3), and larval survival was significantly higher than that from cultured broodstock. Cultured broodstock eggs had high levels of C20:1(n-9), C18:2(n- 6), and C18:3(n-3), and their fertilization and hatching rates were generally lower than eggs from wild broodstock but not related to FAs eggs composition (Lund *et al.*, 2008).

In a more recent series of experiments, De Swaef *et al.* (2017, 2019) aimed to achieve a more sustainable sole larviculture. They developed several research tools designed for tackling some key issues hampering commercial sole aquaculture. These tools include the design of a 24-well microplate rearing system (De Swaef *et al.*, 2017), the evaluation of several probiotic and prebiotic candidates to increase sole larval health (De Swaef, 2019), and the evaluation of disinfection protocols to control the bacterial disease vibriosis (De Swaef *et al.*, 2018).

For the development of a 24 well microplate rearing system, the authors argue that by rearing larvae individually, problems related to group housing, such as variation between replicates and changes in the water quality of the system after the death of one larva, can be avoided (De Swaef *et al.*, 2017; De Swaef, 2019) and consequently larvae mortality during rearing decreased. Consequently, developing a system for rearing fish larvae individually guarantees that a dead individual will not affect the health of an adjacent one and facilitates the monitoring of their health status, growth, and behavior. It also improves the experiment's reproducibility and finally (because each well is considered as a replicate), it reduces the number of experimental animals needed (De Swaef *et al.*, 2017).

Many of the problems faced during larval sole rearing relate to the fact that the molecular mechanisms underlying physiological and morphological changes during the larval-to-juvenile transition are still unknown. Because of this, efforts to sequence and characterize the transcriptome of *S. solea* have been done (Ferraresso *et al.*, 2013; Benzekri *et al.*, 2014). Ferraresso et al. (2013) revealed that pre-metamorphic larvae display a distinctive transcriptomic landscape compared to previous and later stages. The highest number of differentially expressed genes was found between 1 day-post-hatching (dph) and 4 dph, with a total of 1,539 significant genes (974 over and 565 under-expressed in 4 dph larvae), while only 120 genes (81 up- and 39 down-regulated) displayed a change in expression between 11 and 13 dph. In detail, genes related to the development of the visual system and neuronal processes are up-regulated during the early stages of larval development, while muscle development and anaerobic energy pathways increase in expression over time. Moreover, the expression profile of genes within the thyroid hormones cascade, the growth hormone, and the insulin-like growth factor genes revealed their crucial role in fish growth and initiation of metamorphosis (Ferraresso *et al.*, 2013).

The development of visual perception is essential for larval feeding. At hatching, the eyes are slightly pigmented or non-pigmented and most likely non-functional. Therefore, successful first-feeding depends on a well-developed eye and visual system since fish larvae are essentially visual feeders. Light intensity is among the variables that could affect the survival of larvae during rearing. Bonvini *et al.* (2016) studied the effect of different light regimes on *S. solea* larvae reared in the laboratory. They prepared 4 different rearing treatments with the following light intensities 1000 Lux, 500 Lux, 50 Lux, and 3 Lux. Larvae were exposed to the different light intensities from fertilization until metamorphosis, and effects on development, metamorphosis success, and growth were evaluated. The authors conclude that sole larvae require different light intensities along ontogenesis. High light intensity (between 50-500 Lux) is recommended during the pelagic stage (4–12 dph), in which first feeding larvae would benefit from light to initiate feeding. Also, histological examination revealed the importance of vision and light in the first feeding of this species (Bonvini *et al.*, 2016). After metamorphosis, the full development of other sensory organs indicates that feeding activity is also mediated by chemosensory perception, and thus low light intensity (3-50 Lux) is recommended to ensure better growth from the onset of metamorphosis to the benthic phase (Bonvini *et al.*, 2016).

2.3 Advances made on larvae weaning, nutrition, and susceptibility to disease

Nutrition plays a key role in sustainable aquaculture development, where fish health and wellness are of prime importance. Problems such as reduced growth, low survival, malpigmentation, and malformations are often encountered when fish are subjected to stress or deficient diet (Piccinetti *et al.*, 2012). Moreover, standard feeding regimes during larval and early juvenile stages represent a challenge for fish farmers due to the required administration of live feed, which is usually characterized by variable availability, high price fluctuations, poor hygienic conditions, and high levels of pathogenic bacteria (Bonaldo *et al.*, 2011). Thus, the feasibility of early weaning in common sole is a key factor in the industrialization of farming processes of this promising aquaculture species. In this context, Bonaldo and collaborators (2011) experimented with different feeding regimes and evaluated the effects that weaning *Solea solea* larvae as early as 13 days post-hatching (dph) has over metamorphosis success, survival, and fish size. The experiment lasted until 29 dph and consisted of two groups, a control one fed with live feed from 4 dph until 27 dph; and a second one weaned after 13 dph using three different microdiets (MDs), AgloNorse, Ewos (FR-A), Gemma, Skretting (FR-G) and O.range Large, INVE (FR-O) (Bonaldo *et al.*, 2011). The authors did not find significant differences in survival nor the metamorphosis development between control and the early-weaned group. However, the larvae that were weaned earlier showed lower weight and length than the control ones (Bonaldo *et al.*, 2011). Besides, they obtained size variations regarding the type of MD administrated, with FR-G showing the lowest standard growth rate. The authors conclude that it is feasible to conduct very early weaning on common sole and that this practice is advantageous as it reduces the number of *Vibrio spp*. in the water, preventing disease outbreaks (Bonaldo *et al.*, 2011). Finally, because larvae length and fatty acid body composition were significantly influenced (reduced) by MDs, growth was negatively affected by the early introduction of MDs. Thus, the authors hinted at the need for further research linked to nutrient utilization and the digestive capability of sole larvae as a means to further improve larvae performances.

On this last note, Parma *et al.* (2013) studied the molecular ontogeny and expression of digestive enzyme precursors on early-weaned larvae. They aimed to gain further knowledge of the effects of early weaning on the performances and physiological mechanisms during larval development of common sole, optimize weaning protocols further and reduce mortality around this period (Parma *et al.*, 2013). For that, the authors cloned partial sequences of the enzymes trypsin (trypsinogen 1 (tryp1), trypsinogen 3 (tryp3), trypsinogen Y (trypY), chymotrypsin (chymotrypsinogen (chymt)), amylase (amy), and hepatic lipase (HL), which are among the most important pancreatic enzymes in teleost fish (Parma *et al.*, 2013). The expression of the former genes was assessed from the onset of exogenous feeding until the end of metamorphosis. Four weaning protocols (WPs) were evaluated, a control one with weaning occurring only at 27 dph, two intermediate treatments at which live food was provided until 13 and 18 dph, and a final treatment with larvae weaned to micro diets at 4 dph. Based on their results, Parma and collaborators conclude that the different WPs did not greatly influence the expression of selected genes. On the contrary, survival, growth rate, and metamorphosis success were influenced by the WP, notably for larvae weaned at 4 dph, which showed the lowest survival rate (18.6% vs. a mean survival rate of 52.8% ± 3.1 for the 3 remaining protocols) and the lowest growth rate for the first 13 days (SGR of 4.4 vs. a mean specific growth rate of 17.3 ± 0.4 for the 3 remaining protocols). Finally, regardless that most larvae started metamorphosis on day 19 (71% of larvae), none had reached complete metamorphosis by the end of the experiment in the early-weaned group (Parma *et al.*, 2013).

Regarding nutrient utilization, Piccineti *et al.* (2012) tested the effects of a total or partial replacement of live feed (mainly consisting of rotifers and *Artemia nauplii*, group A/control group) with a copepod-based diet (mixed diet of live feed and dry copepods, named group B) on common sole growth, survival, lipid composition, lipid assimilation, defense to stress and malpigmentation (Piccineti *et al.*, 2012). Their motivation was based on the observation that traditionally used live preys like rotifers and Artemia have nutritional deficiencies that result in a general decrease in fish health, development, growth, and pigmentation (Piccinetti *et al.*, 2012 and references therein). To develop rearing schedules suitable for market production, the authors conducted an experiment in which live preys were replaced with preserved copepods - the natural food of larvae- (Piccinetti *et al.*, 2012). For that, starting from day 9 post-hatching, group B larvae were fed a mixed diet (co-feeding), while group A and C were fed on live feed and copepod exclusive diets, respectively. Their results showed that including a complement of dry copepods (group B) within the diet increased survival by over 8% compared with group A (fed with rotifers and *Artemia nauplii*). In addition, larvae fed with a mixed diet started and ended metamorphosis faster than the group fed with live prey (group A: 16 dph- 25 dph vs. group B: 13 dph - 20 dph). Malpigmentation was significantly lower in group B than group A. Finally, larvae from group B had a higher standard growth rate both in terms of body weight (BW) and total length (TL) than individuals from group A. The authors explain that live prey motion is an essential attribute in stimulating larval predatory activity, which explains why the best results, in terms of survival, growth, and pigmentation, were achieved when the preserved copepods were administered together with live preys (rotifers and Artemia). Diet with only preserved copepods (group C) resulted in 100% mortality after 7 dph (Piccineti *et al.*, 2012).

The outbreak of infectious diseases can impact survival in marine fish larviculture (De Swaef *et al.*, 2018; Dinis *et al.*, 1999; Morais *et al.*, 2014; FAO, 2019). Microbiota can help to prevent and control diseases. However, knowledge about the establishment and action of microflora in fish's gut during larval stages is still scarce (Vino *et al.*, 2006). The most common members of the microflora of healthy marine fish are *Vibrio* *spp.*, *Pseudomonas spp.*, and *Acinetobacter spp.* (Vino *et al.*, 2006). Vibriosis, a bacterial infection caused by abnormal/unbalanced colonization of Vibrio spp., is among the diseases of primary concern for *S. solea*, being particularly challenging to tackle during early life stages (Austin and Austin, 2016; Novriadi, 2016).

Thus, another research subject in fish nutrition that has been primarily studied as an alternative for improving disease resistance and nutrient absorption is the effects that dietary manipulation can have over fish gut microbiota, mainly by the administration of prebiotics and probiotics (Ringø *et al.*, 2014; De Swaef, 2019).

During the last decade, many works have studied the effects of prebiotics and probiotics, not only for their role in improving disease resistance (as an immunostimulant) but also as a functional dietary supplement. Details on this subject escape from the scope of this work, and we refer the interested reader to consult the many other reviews available on the subject (Bricknell and Dalmo, 2005; Avella *et al.*, 2011; Ringø *et al.*, 2014; Torrecillas *et al.*, 2014; Vine *et al.*, 2006). However, in general lines, manipulation of gut microbiota has proven beneficial in adult fish, enhancing growth and feed efficiency and immunity and disease resistance (Ringø *et al.*, 2014). Regardless of many studies describing such beneficial effects, literature on the effects of prebiotics and probiotics administration to fish larvae is somewhat limited (De Swaef, 2019). In this regard, and to reduce losses in early stages of larval development and maximize production of captive common sole, Avella *et al.* (2011) studied the effects of administrating *Enterococcus faecium* IMC 511, isolated from sole broodstocks, as a probiotic candidate during *S. solea* larval development. During the first 50 days post-hatching, the probiotic was administrated daily through live feeds. The authors evaluated the intestinal presence of *E. faecium* IMC 511 during the experiment and the effects on intestinal microbial load, fish survival, welfare, and growth. For that, morphometric and molecular approaches were conducted. As markers of animal welfare, the authors monitored gene expression of cortisol and the 70 kDa Heat Shock Protein (HSP70). Their results showed reduced *Vibrio* populations in *S. solea* larvae intestines treated with the probiotic *E. faecium* IMC 511, but survival was not affected. The expression of stress markers (cortisol and HSP70) was significantly lower in probiotic-fed larvae than control larvae. By the end of the experiment, sole juveniles treated with probiotics had significantly higher body weight (BW) and total length (TL). Based on their results, the authors conclude that 50 days of probiotic treatment can improve common sole larval growth (Avella *et al.*, 2011).

Finally, De Swaef (2019) assessed the impact of dietary supplementation in *S. solea* larvae of two prebiotic candidates derived from the aquatic environment, the mannanoligosaccharide and alginic acid. Contrary to the results previously cited, the tested prebiotics did not increase larval growth or survival and did not shift the proliferation and apoptosis rate of the intestinal epithelium (De Swaef, 2019). On the contrary, the administration of alginic acid had a negative effect on standard body length and the larvae supplied with 0.2% alginic acid followed by a challenge test (consisting of infection by *V. anguillarum* WT at a final concentration of 1 × 107 colony-forming units/mL at 10 DAH) displayed a lower survival in comparison with the challenged larvae without any supplementation. These results reveal a possible negative impact of alginic acid supplementation on common sole larvae (De Swaef, 2019) and highlight the need for further research on the benefits of prebiotics, probiotics, or synbiotics (nutritional supplements combining a mixture of probiotics and prebiotics in the form of synergism, Ringø *et al.*, 2014) in *S. solea* larvae.

Because fish are free-living organisms since their initial stages, they are exposed to numerous pathogens even before their lymphoid organs, and consequently, their adaptive immunity has fully developed (Ferraresso *et al.*, 2016). Therefore, to defend the host from infection, newly hatched fish rely on their innate immune repertoire that acts in a non-specific manner. Ferraresso *et al.* (2016) argued that for developing effective approaches for disease management at early life stages of *S. solea* is necessary to understand the mechanism related to innate immune responses. For that, in their study, the authors characterized and assessed the transcriptional onset of unexplored relevant genes of both innate and adaptive immune systems during the *S. solea* ontogenesis, from hatching to the accomplishment of the juvenile form (Ferrareso *et al.*, 2016). Their results showed that several genes involved in the innate immune repertoire are up-regulated during the first stages (such as lysozyme, AMPs (hepcidin, b-defensin), PPRs, and complement components), supporting the hypothesis of protection from environmental pathogens during early development. Moreover, the onset of the expression of adaptive immune genes (i.e., Class I and class II MHC, TCRs) coincides with metamorphosis and larvae-to-juvenile transition. Finally, data collected suggest that full maturation of the cell-mediated immune system of *S. solea* is not completely mature by the end of metamorphosis (Ferraresso *et al.*, 2016).

2.4 Pigmentation and eye migration

Other developmental problems related to the successful commercialization of soles are problems related to the appearance of the fish product, which can be insufficient for the market standards, such as malpigmentation and incomplete eye migration.

Pigmentation problems are known to be related to vitamin A, fatty acid composition, and thyroid hormones. However, in most cases, it is an unbalanced fatty acid composition of the feed the main reason for malpigmentation of farmed flatfish, and the thresholds for concentrations of these essential fatty acids are species-specific (The Research Council of Norway, 2009). In this regard, Lund and collaborators (2008) studied the effects that diets supplemented with arachidonic acid (ARA) and eicosapentaenoic acid (EPA) have on sole survival, metamorphic success, and notably pigmentation. They hypothesized that the induction of malpigmentation (*i.e.*, hypomelanosis) was linked to a specific larval period or “a pigmentation window” at which larvae sensitivity to ARA increases. Their results confirmed their hypothesis of the presence of an early pigmentation window at pre-metamorphosis. Also, they revealed that in common sole larvae, ARA induced malpigmentation, but no effect was associated with diets enriched with EPA (Lund *et al.*, 2008). Regarding metamorphosis success, the initiation or advance of eye migration was not related to dietary ARA, EPA, but the size of the individuals (Lund *et al.*, 2008).

Section 3 - Effects of temperature and contamination on *Solea solea* larvae

Temperature is the main factor regulating flatfishes' species range. Human-induced climate change has played a central role in larval dispersal variability, leading to species losses and invasions, biogeographical and regime shifts, and changes in the adaptation capabilities of organisms (Lacroix *et al.*, 2016). Also, temperature modulates the duration of the egg and larval stages and, therefore, determines the distance between spawning and nursery grounds (Gibson, 2015). Further, the species range and the average level and the annual variability in recruitment regulate sole populations. The variability in recruitment is governed by two distinct processes, the effect of food availability on the adult condition at spawning time and the density-dependent mortality of juveniles on the nursery grounds (Gibson, 2015). Finally, temperature also affects fish growth rate and ultimate size.

Chemical exposure from anthropogenic activities negatively impacts marine habitats, notably on coastal habitats, where most of the human population is established (60% at 100 km from the water; Brown, 2006). Moreover, several species of economic interest expend part of their life cycle on coastal habitats and estuaries, where specific characteristics in temperature and physicochemical conditions guarantee their healthy development (Seitz *et al.*, 2014). However, estuaries can accumulate and stock several pollutants in the sediment compartment (Ridgway and Shimmield, 2002). Previous works that conducted inter-estuarine comparisons showed that the chemical contamination status notably affects individuals' growth and survival and thus the quality of the nursery area (Gilliers *et al.*, 2006; Amara *et al.*, 2007). In the case of *Solea solea*, unwanted changes in nursery ecosystems could affect (i) juvenile settlement, (ii) survival of juvenile specimens, and (iii) healthy development and growth, which in the long term could impact the renewal capacity of the population –recruitment-, and impact the species fitness.

3.1 Changes in spawning, larval development and survival, and recruitment related to temperature

In recent years, fish spawning patterns and growth rates have been changing. Increased temperatures are believed to have various effects on fish populations, including effects on their phenology by altering growth, the survival of juveniles and adults, and reproductive traits, such as the timing of spawning (Alix *et al.*, 2020). Shifts in the timing of spawning will have both direct and indirect consequences for fishery management and could have significant implications on species recruitment and population size. These effects include –among other things– mismatches between the timing of larval development and the availability of planktonic food sources, and a reduction in the effectiveness of fishery closures during spawning seasons (Fincham *et al.*, 2013; Alix *et al.*, 2020).

In their work, Fincham *et al.* (2013) evaluated temperature data from coastal monitoring sites situated close to spawning grounds, together with data on *Solea solea* timing of spawning. The last data came from market sampling programs in ports in England and the Netherlands, where random samples of fish landed by commercial fishing vessels are taken, and biological data, such as the proportion of spawning females amongst the total number of sampled adult females, was calculated for each week of the year for a time series of 40 years (Fincham *et al.*, 2013). Their results showed that four out of the seven stocks they studied had a significant shift in the time of spawning in response to increased sea surface temperature (SST). In detail, the stocks in the Irish Sea, east-central North Sea, southern North Sea, and the eastern English Channel spawned in earlier weeks of the year at a rate of 1.5 weeks per decade (Fincham *et al.*, 2013). They observed both spatial and temporal differences, which were strongly correlated to temperature. They also found that winter SST is inversely correlated with the timing of spawning, which is caused by the faster maturing of gonads due to warmer temperatures during the winter. The authors conclude that in a warmer world, *S. solea* populations from the English Channel will spawn earlier (Fincham *et al.*, 2013). However, as recruitment depends on food availability, changes in the phenology can have important implications for the population dynamics (Gibson, 2015). Evidence suggests that the timing of peaks in larval food abundance may not necessarily advance with warming temperatures (Wiltshire *et al.*, 2008). A mismatch between the timing of spawning and hatching periods with the peak of the phytoplankton or copepods will reduce the recruitment success and abundance of the species.

Although temperature seems to be the main driver to alter sole spawning phenology from 1970 until 2010, the authors do not dismiss the effect of other factors, including population density, growth, and maturity at age. However, and because the temperature has a positive influence on the growth of age-0 sole (Teal *et al.*, 2008), if phenology changes were due to changes in growth rate, then the rising temperature should delay the timing of spawning, resulting in bigger but younger fish at first spawning (Fincham *et al.*, 2013). Nevertheless, there is evidence that sole size and age at first spawning have decreased (Mollet *et al.*, 2007), while the timing of maturation may be delayed in smaller, relatively more abundant fish in their first year of maturation (Ramsay and Witthames, 1996). Thus, if growth affects phenology, the rising temperature would lead to the reverse effect of that observed by the authors, *i.e.*, delayed timing of spawning. This reasoning strongly suggests that the observed phenological shifts are less likely to be the indirect result of changes in the size-age composition of spawning fish but a more direct effect of winter temperatures on gonad maturation (Fincham *et al.*, 2013).

After the spawning, the transport of eggs and larvae from spawning grounds to nursery areas is primarily driven by hydrodynamic processes. However, behavioral and environmental factors might also influence the final dispersal pattern as well as the larval survival and abundance (Lacroix *et al.*, 2013). The survival of the early life stages is dependent on environmental constraints -notably temperature- the availability of food, and the presence of predators (Lacroix *et al.*, 2013). After that, fish recruitment is dependent on larval abundance and success of metamorphosis at nursery areas. Therefore, understanding larval dispersal is of utmost importance as it might explain recruitment patterns and has direct consequences for the conservation of fish, stock management, the design of marine reserves, the management of non-indigenous invasive species, and the prediction of climate change effects (Lacroix *et al.*, 2016).

On the question about larval dispersal, Lacroix and collaborators have notably contributed with a series of works (Savina *et al.*, 2010; Lacroix *et al.*, 2013, 2016; Barbut *et al.*, 2019) for which the main focus has been to understand the impact of interannual variability of hydrodynamics and temperature on larval dispersal and supply to the nurseries. The works also aim to predict the potential changes in *S. solea* dispersal patterns under warmer scenarios due to climate change in the English Channel and the North Sea. Other relevant works related to sole larval dispersal in other regions include the works by Vinagre *et al.* (2007) in the Tagus Estuary in Portugal and the study by Tanner *et al.* (2017) in the Iberian coast.

For evaluating larval dispersal, a larval transport model for sole coupled with a 3D hydrodynamic model of the North Sea was implemented, and interannual variability of the transport of fish larvae during different periods was investigated (Savina *et al.*, 2010; Lacroix *et al.*, 2013, 2016). Shortly, the study of 2013 showed important interannual variability (coefficient of variation of 29.2%) of the total larval abundance at the nurseries. The larvae dispersal patterns were broader than those reported in the first generation model by Savina *et al.* (2010). In their work from 2016, the inclusion of wind speed and directions as factors in the model together with simulations based on predicted scenarios from the "Warm+" IPCC showed an increase in the dispersal distance (+70%) and an increase in the pelagic larval duration (+22%) which was in response to reduced temperature (-9%) endured by larvae that hatched earlier (Lacroix *et al.*, 2016).

In recent work, Barbut *et al.* (2019) evaluated the relationship between dispersal capacity and larval traits, such as behavior, pelagic larval duration, seasonality of reproduction, and structure of the water column and currents, from populations of six commercially exploited flatfish species in the North Sea. Their results demonstrate considerable variation in both connectivity and spatial distribution of all studied species, with two groups emerging with similar life-history traits and dispersal patterns features (Barbut *et al.*, 2019).

The set of papers from this group tell a coherent story in which knowledge about larval dispersal increases progressively. However, this group of researchers' methods and the model are common, and since they are based on a single model, the level of uncertainty was not included. On this last point, we direct the reader to Hufnagl *et al.* (2017 and references therein) work in which the variability that can be expected concerning connectivity, uncertainty, and the order of magnitude of variability resulting from choosing different physical models, was addressed by comparing a suite of 11 North Sea models (Hufnagl *et al.*, 2017).

On a final note, warmer scenarios may improve the survival of juveniles. Earlier spawning will prolong the growing season of 0-group fish and may result in an increase in the body size during the 1st winter (Teal *et al.*, 2008), and since winter survival is positively related to body size, 0-group cohorts with enhanced energy stores are more likely to survive (Post and Parkinson, 2001). However, evidence suggests that fish size may shrink in response to climate-induced changes in temperature and oxygen (Baudron *et al.*, 2014). The physiology behind the pattern is well established for aquatic ectotherms, higher temperatures decrease the aerobic capacity, and individuals with smaller body sizes have a reduced risk of oxygen deprivation (Baudron *et al.*, 2014). For *S. solea*, there is evidence showing that exposure to elevated temperatures during early developmental stages alters the environmental adaptation performance of fish at later stages, making juveniles more resistant to later in life hypoxia events (Zambonino *et al.*, 2013). However, and in contradiction to previous results, Zambonino *et al.'s* (2013) results showed that higher temperatures during early stages resulted in larger-bodied individuals.

3.2 Changes in spawning, larval development, and survival, related to chemical exposure

The presence of significant levels of pollutants in sole tissues can affect their physiological performance and harm the health of consumers. Given this, several studies assessing the bioaccumulation capacity and toxicity of different chemical contaminants (organic pollutants and metals) have been conducted using *Solea solea* as target species (Dierking *et al.*, 2009; Wessel *et al.*, 2010; Trisciani *et al.*, 2011; Cannas *et al.*, 2013; Sanchez-Nogué *et al.*, 2013; Sister *et al.*, 2013; Solé *et al.*, 2013; Cuevas and Zorita, 2018; Mounier *et al.*, 2020a, 2020b), some other study focused on differential mortality in age-0 juveniles (Guinand et al. 2011). However, all these studies have focused on the juvenile stage and the ecotoxicity of contaminants at the early life stages of *S. solea* it is still, up to date, understudied.

Although working with juvenile fish in laboratory conditions requires specific facilities and is logistically challenging, most ecotoxicity tests conducted with soles are designed at this developmental stage because it guarantees enough tissue for running tests in target organs (*i.e.*, biomarker and bioaccumulation analyses). Moreover, working with juveniles reduces sex-related endpoints variability and does not consider the influence of reproduction on bioaccumulation. Larval stages (*i.e.*, egg, yolk-sac larvae, and free-feeding pre-metamorphic larvae) are considered to be the most sensitive stages of soles life cycle (Ferraresso *et al.*, 2013). As discussed in the previous sections, mortality under control conditions still reaches up to 60% (Foekema *et al.*, 2008, 2014), and protocols for rearing larvae in the lab still need to be refined (De Swaef, 2019). At the same time, the fields of biology and toxicology have seen some important developments. Advances in measurement technologies, computational capabilities, fundamental toxicological understanding at the molecular level, and *in vitro* technologies have increased the amount of information produced following ecotoxicity tests (Ankley *et al.*, 2010). This fact, together with the refinement of analytical protocols (Olsson *et al.*, 1978; Thomson and Budzinski, 2000) and the proposal of easier to manipulate rearing methods (such as the microplate early life stages adapted protocol presented in De Swaef *et al.*, 2017) opens the door for conducting ecotoxicity tests with sole larvae.

There are three sources for contaminant exposure during the larval stages. The first is the result of parental exposure. During fish vitellogenesis, most of the lipid resources are stored in the yolk, and during this process, lipophilic pollutants are also transferred to the eggs (Foekema *et al.*, 2012). The second exposure route occurs after hatching when pelagic larvae start the uptake and elimination (through detoxification mechanisms) of pollutants from the water. Once the larvae deplete the yolk reserves, it will start feeding, and therefore, the third exposure route is activated through ingestion of large amounts of lipid-rich -and potentially contaminated-zooplankton.

As the first route of exposure involves maternally transferred contamination, there is the question of the effects of lipophilic persistent organic pollutants (POPs) on early life stages (ELS).. For predicting sole's bioaccumulation of POPs, such as polychlorinated biphenyls (PCBs), Foekema *et al.* (2012) adapted the bioaccumulation model OMEGA (Hendriks *et al.*, 2001) to the ELS of sole turning it to ELS-OMEGA. The model was validated with experimental data and proved effective in predicting the development of PCBs' concentrations in the tissue of developing soles. The authors measured and modeled the concentrations of PCBs (52, 110, 101, 118, 138, and 153) in water and tissue during the development of an embryo into a juvenile fish. The model showed good fits to the measured data, and according to the simulations, tissue concentrations of pollutants with log octanol-water partition ratio (K_{OW}) > 5 peaks when the lipid reserves are depleted at the moment that larvae become free-feeding, revealing that maternally transferred highly lipophilic compounds are barely excreted (Foekema *et al.*, 2012). Moreover, these peaks may exceed two to four times the concentration in the spawning parent, concentrations that could result in extreme body burdens during critical stages of larval development, with potentially severe consequences for larval survival. Once the fish starts feeding externally, fish increase rapidly in weight, and dilution by growth plays an essential role in tissue concentration decrease (Foekema *et al.*, 2012).

The authors highlight the risk of underestimating the effects of maternally transferred PCBs (especially those with $K_{OW} > 5$) at the early-life-stages, principally because of the exposure to waterborne substances of a fish embryo inside the egg is limited compared with the post-hatch situation (Foekema *et al.*, 2012). Unfortunately, the ELS toxicity test focus on exposing eggs via the water, and very often, the experiment will end just before the end of the yolk-sac stage, the moment at which the internal concentration will peak to its maximum (Foekema *et al.*, 2012). The authors argue that terminating ELS tests before the yolk-sac stage ends is valid for testing substances with log $K_{OW} < 5$. They, however underline that when testing more hydrophobic substances, mixtures of substances, or unknown mixtures (such as those present in effluents or contaminated sediments), the toxic effects of the lipophilic compounds will be seriously underestimated (Foekema *et al.*, 2012).

The previous statement is supported by data from two more works by Foekema *et al.* (2008, 2014), in which delayed effects after exposure during the egg stage were observed on *S. solea* larvae. In their earlier work, the authors exposed *S. solea* eggs to a concentration series of PCB 126 in seawater until 4, 8, 10, and 15 days post fertilization (dpf), and the development of the larvae was followed up under unexposed conditions until larvae were fully metamorphosed (up to 50 dpf) (Foekema *et al.*, 2008). Their second work aimed to recreate the effects of maternally transferred POPs in early larvae development (Foekema *et al.*, 2014). The results from both studies indicate that ELS fish tests underestimate the effect of highly lipophilic compounds, which continue to affect survival and metamorphosis success long after the exposure, showing that, at least for sole, the moment of observation has a significant impact on the determination of the effect concentration (approximated by the LC_{50} in Foekema's works), even without further exposure (Foekema *et al.*, 2008; 2014).

Section 4 - Methods that integrate the data -bridging the gap-

Studying the effects of temperature and contamination along *Solea solea* larvae ontogenesis is relevant for several disciplines (Fig. 2). Researchers in fisheries and ecology want to know if the stocks will be secured in future years and, what will be the species biogeographical distribution, the impact of invasive species (e.g., Marras *et al.*, 2015), and how spawning and larval dispersal might be affected by increased temperatures (Pankhurst & Munday, 2011). Aquaculture research is interested in understanding larvae's physiology and developing protocols for rearing them while minimizing the cost and maximizing survival. Environmental chemists develop analytical protocols to detect smaller and smaller concentrations of environmental pollutants that might be present in several matrixes (water, pore water, sediments, and biota). Their efforts orientate towards understanding and predicting bioaccumulation and transfer of pollutants in sole into the trophic web. Moreover, their results are input for fate and transport models. Finally, ecotoxicologists aim to predict and understand the effects of combined stress from exposure to a mixture of chemicals under future climate change scenarios to provide relevant data for chemical regulation.

Generating relevant data for risk assessment and chemical regulation is the major challenge that ecotoxicologists have and keep facing up to today. In short, the ecotoxicology domain aims to answer two impossible questions i) which of the millions of species are at risk from pollution? And ii) which of the tens of thousands of chemicals contribute most to the risk? As Ashauer and Jager (2018) stated, the great pitfall in ecotoxicology is that there is no theory to link the effects of toxicants at the molecular or cellular level to changes in life-history traits. Moreover, at the cellular level, there are plenty of examples that demonstrate that responses are cell-type and species-specific and conditioned by age, size, gender, season, and temperature, among many other biotic and abiotic variables (van der Oost *et al.*, 2003; Au, 2004; Martínez-Álvarez *et al.*, 2005; Hardman, 2006). Also, multiple types of effects depend on the pollutant or the mixture of pollutants, and that can vary depending on the exposure route (Hardman, 2006). Even with a fast pace of research production, there is no way that we can catch up with the speed at which the industry produces new chemicals. So, how can we catch up with xenobiotics production? Furthermore, which are the tools that we need to bring together to bear on climate change and pollution effects?

Our poor knowledge of physiological modes of action (pMoA) and how they vary across species and toxicants has been recently identified as a major knowledge gap (Ashauer and Jager, 2018). Physiological MoA refers to how chemical stress (single compound or mixture) affects an organism's energy allocation and, consequently, life-history traits. It can be determined using Dynamic Energy Budgets (DEB) models, which have strong theoretical foundations (Kooijman, 2010). In their paper, Ashauer and Jager (2018) proposed pMoA as a key element to extrapolate from small to larger biological levels. We believe that the authors' proposition is correct and that researching pMoA is a promising venture.

According to the authors, knowing pMoAs is an excellent way to boost and complete the information that the two main approaches in ecotoxicology -data from chemical stress ecology and high-throughput bioassays of cellular or molecular markers ELS- offer to ecotoxicologists (Ashauer and Jager, 2018). Shortly, chemical stress ecology treats pollution as one of the many factors that influence individuals, populations, communities, and ecosystems. On the one hand, this approach aims to provide ecological relevance and realism to environmental risk assessments, but it fails to discriminate if the observed effects are due to a single pollutant or a mixture of them, giving little relevant information from the regulatory point of view. On the other hand, high-throughput bioassays orbits on the promises to upscale results from bioassay to organisms and beyond, rallying around the idea of quantitative adverse outcome pathways (AOPs) first proposed by Ankley *et al.* in 2010. The AOP framework aims to provide a useful structure within which existing knowledge can be organized, from which key uncertainties and research priorities can be identified, and through which we can improve predictive approaches needed to advance regulatory ecotoxicology. It integrates evidence from *in vitro* assays linking a molecular initiating event (e.g., a molecular interaction between a xenobiotic and a specific biomolecule) and an adverse outcome in the context of a pathway that leads to an adverse outcome at a biological level of organization relevant to risk assessment (Ankley *et al.*, 2010). However, to answer which of the millions of species are at risk from pollution, it is necessary to extrapolate quantitative AOPs across species. For that, we need to assume that molecular pathways are qualitatively and quantitatively conserved, and data suggest that a significant fraction of receptors and target sites are not conserved between species (Gunnarsson *et al.*, 2008; Rand-Weaver *et al.*, 2013).

For covering the present needs in ecotoxicology, focusing on determining the pMoA would be a strong tool as it would represent a middle point between AOPs and stress ecology. As organisms require resources to grow, develop, and reproduce, it is these traits that we ultimately require to link AOPs to ecological theory and upscale to the population level and higher (Ashauer and Jager, 2018). Changes due to toxicant exposure or environmental stress in energy-demanding traits -such as growth and reproduction- logically imply changes in the energy budget and model parameters. By using energy-budget models and linking the related parameters to AOPs, we will be able to 1) identify where energy allocation has changed due to a stressor -the physiological mode of action- and by how much, and 2) justify the observed changes -if any- with data from AOPs, which will therefore allow us to interpolate from the individual to the subcellular level. Further, pMoA acts at the individual level, and there are theories available in ecology that allow extrapolating from individual to population and community level (*e.g.,* Physiologically Structured Population model (PSP), Caswell *et al.*, 1997; Ghwila and Willms 2019). Thus, pMoAs could be the missing link between subcellular levels and higher levels of biological organization. Nevertheless, it is important to highlight that, on the possible types of pMoAs within a CC and contaminated scenario, one important dichotomy involves toxicant-induced changes that alter the ability of an organism to respond to CC stressors (toxicant-induced climate susceptibility [TICS]), in contrast to climate-induced toxicant sensitivities (CITS), which is the scenario where CC affects the toxicity of chemicals (Hooper *et al.*, 2013). We invite the reader to seek further details regarding pMoAs and AOPs in Ashauer and Jager (2018) and Perkins *et al.* (2019), respectively.

Finally, the reason why studying combined effects of temperature and chemical exposure is challenging is that, as previously mentioned, the main approaches followed by ecotoxicologists are incapable to either discriminate the separate effect of each stressor (as in chemical stress ecology) or measuring the mixed effect and translate it to a relevant level of biological organization (when using high-throughput bioassays). The implementation of DEB models provides the means to overcome this issue while taking advantage (or making use) of the information provided by these two ecotoxicological approaches. The ecological theory is indispensable to extrapolate from the individual to the population level, and subcellular data complements the information from model parameters, allowing us to understand the molecular mechanisms behind the pMoA. Further, concentrating efforts from different disciplines into a common modeling goal directly translate in reducing animal testing (by reusing data from tests already conducted), and since DEB models offer a real opportunity for species extrapolation (see Kooijman, 2010 for examples of model parameters variation related to body size; Baas *et al.*, 2015; Sardi *et al.*, 2019), it also translates in reducing costs for research associated to risk assessment.

We hope that within this section, we have managed to convey the following message: existing data can be synthesized in a useful way to risk assessors and ecotoxicologists, but it demands consensus and team effort from players that operate in different ways disciplines of research.

Further research recommendations and conclusions

Working with DEB and derived models (GUTS see Jager and Ashauer, 2018a and b, and DEBtox, Kooijman and Bedeaux, 1996 or Jager and Zimmer, 2012) offers an excellent opportunity to answer (individually or by coupling them to other models) to several -if not all- the research questions asked in many different disciplines, and shortly stated at the beginning of the previous section (Fig. 2).

In DEB models, temperature and food availability are forcing variables (Kooijman, 2010). The last means that we could run simulations for higher temperatures and provide insights about the effects of sea warming on energy budgets allocation, growth, maturation, and reproduction success. Besides, DEB models can be coupled with larval dispersal and hydrodynamic models (Huret *et al.*, 2012), which will provide information about the time and size of fish at spawning for a given temperature and the dispersal of hatched larvae. Also, by combining DEB models with ecosystem models, it is possible to build habitat quality maps (*e.g.*, Teal *et al.*, 2012) at which the effect of climate change on the spatio-temporal fish dynamics can be explored. Moreover, this type of approach can be conducted in similar species with different temperature optima (such as it is the case between *S. solea* and *S. senegalensis*) and provide insights about changes in species biogeographical range or native species displacement by invasive ones (see Solé, 2019).

In ecotoxicology, coupling DEB with TKTD models (DEBtox model, Kooijman and Bedeaux, 1996; Billoir *et al.*, 2007; Jager *et al.*, 2004; Augustine *et al.,* 2012; Sadoul *et al.*, 2019) allows for depicting the pMoAs which are potentially the missing link between subcellular processes and effects on higher levels of biological organization. DEB and DEBtox models are so far the most powerful available tool that could allow for species and chemical extrapolation, and their standard implementation could be the necessary information for improving environmental risk assessments and chemical regulation.

In aquaculture research, larvae rearing methods could benefit from the information coming from simulations done with DEB models, in which growth is fitted for different temperature and food conditions. Also, DEBtox or GUTS models can be applied to evaluate the effect of antibiotics, probiotics, and disinfection methods often applied for reducing larvae mortality. This practice will potentially reduce the experimental cost and render the business profitable. This last point is not only crucial from a commercial perspective. Improving rearing systems and developing the aquaculture on this species also impact the food security and human health (by preventing humans from consuming fish that have bioaccumulated pollutants), and decrease the fishing pressure over natural populations, which might face difficulties in their renewal owing to the changes in temperature and pollution projected for future years (Fig. 2).

Finally, DEB models coupled to bioaccumulation models would provide the means to predict contaminant dilution by growth effect and to estimate the concentration of chemicals ingested by individuals along ontogenesis, both common questions in environmental chemistry research (Arnot and Gobas, 2006).

In a nutshell, even though the development of DEB models requires significant effort and investment -as it requires the user to understand DEB theory, its mathematical foundation, notation, and interpretation of model parameters- its application is potentially beneficial for many disciplines, opening the door for interdisciplinary research. In this line of thought, we would like to propose an interdisciplinary framework that aims to bring together and take full advantage of already existing data (often coming from different research fields) and tools (often operating at different levels of biological organization) to bear on climate change and pollution effects on *S. solea* larvae ontogenesis. Figure 3 illustrates such a framework. At least five different disciplines have an interest in studying the combined effects of temperature and chemical contamination in sole larvae, though their research questions (outer blue circle) differ. Answering those questions requires collecting/generating different types of data (purple circle), which are often used as input for calibrating models (dark pink circle) that operate at different levels of biological organization. Finally, all these models can be used either individually or in combination, but we can only achieve full understanding regarding the effects of contamination or temperature stress at the individual level after determining the pMoAs (light pink circle) and linking these to processes at the sub-cellular level with AoPs (yellow circle, Fig. 3).

To conclude, models are simply tools to deduce quantitative conclusions from a set of assumptions and data. In this case, the DEB family of models offers an incredible opportunity to ecotoxicologists searching for the missing link between effects at subcellular level and effects at the population and community level and, consequently, producing and presenting data in a way that is useful to environmental risk assessors.

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Figure 1. *Solea solea* life cycle. Soles have a complex life cycle that depends on biotic and abiotic variables. In the continental shelf, temperature triggers spawning from benthonic adults. After hatching, the pelagic larvae undergo extreme metamorphosis and migrate towards the nursery and coastal areas, all while undergoing metamorphosis. Migration and metamorphosis success depends on hydrodynamics and food availability. The length of metamorphosis varies depending on temperature and food availability, but it can last up to 15 days at 16 ºC. The abbreviation dph stands for days-post-hatching.

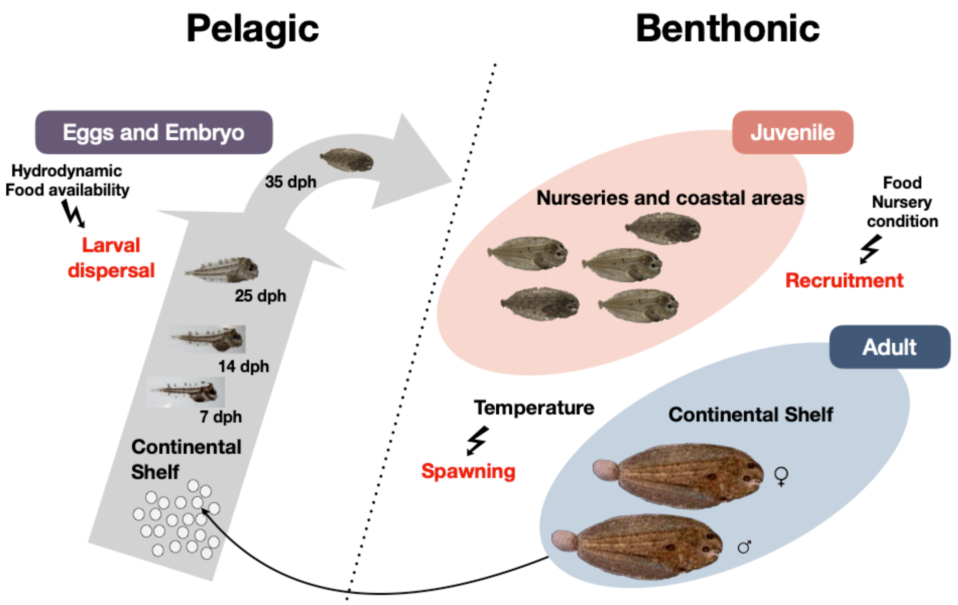


Figure 2. Scheme illustrating some of the research questions related to sole and larval ontogenesis for different research disciplines. See the text for proposed tools and solutions.

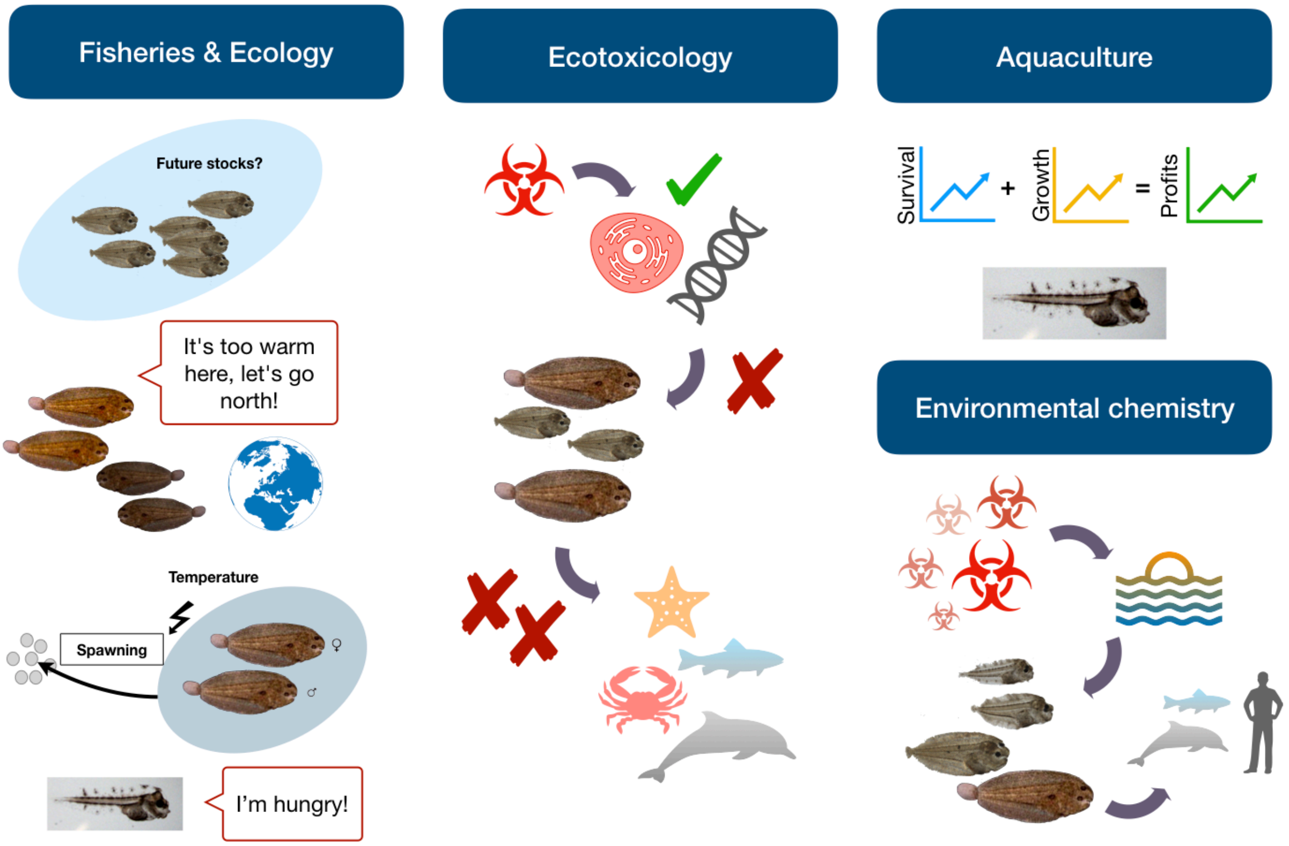
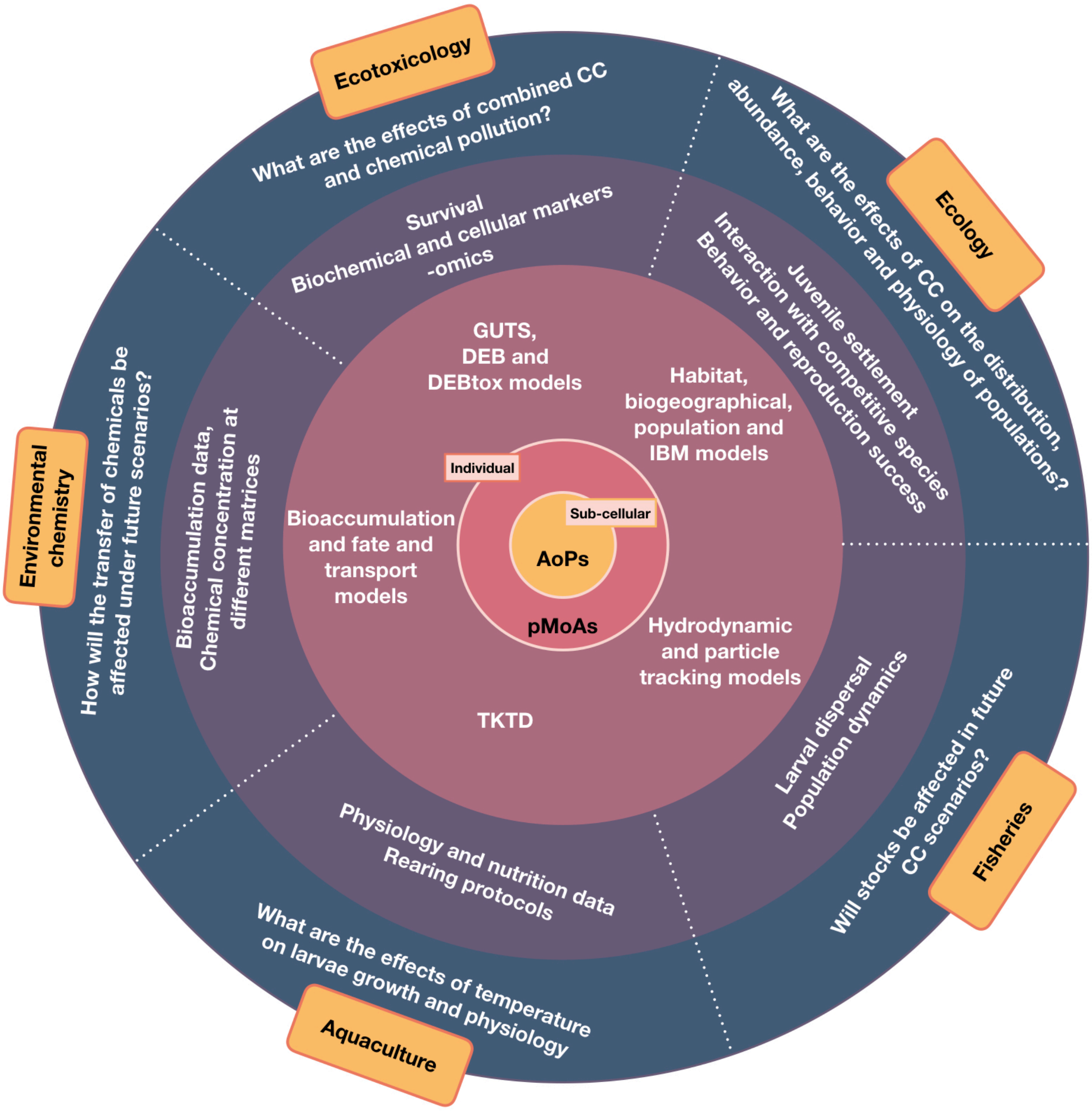


Figure 3. Interdisciplinary framework to enhance ecotoxicology and climate change-related research in fish larvae. Abbreviations stand for Climate Change (CC), GUTS (General Unified Threshold model of Survival), DEB (Dynamic Energy Budgets), TKTD (Toxico-kinetic toxico-dynamics), IBM (Individual-Based Model), AoPs (Adverse outcome pathways), and pMoAs (physiological modes of actions).



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