

# The sea bass *Dicentrarchus labrax* as a marine model species in immunology: Insights from basic and applied research

Andrea Miccoli, Francesco Buonocore, Simona Picchietti, Giuseppe Scapigliati \*

Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Viterbo, 01100, Italy

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## ABSTRACT

This review summarizes the current knowledge on immune defence activities of the European sea bass *Dicentrarchus labrax* by reporting the consistent amount of work done on this economically-important species. A draft genome sequence is available for this species, together with whole transcriptomes from lymphoid and non-lymphoid tissues. Available full-length coding sequences of many immunoregulatory and immune-related genes allow for targeted quantitative PCR analysis, nowadays needed for -omics data verification, *ex vivo* and *in vitro*. The first anti-T cells monoclonal antibody teleost-wise was obtained in sea bass, followed by several monoclonal and polyclonal markers of lymphocyte populations, namely T cells (pan-T, CD3e, TcR $\gamma$ , CD45), and B cells (IgM, IgT, IgD). The combined use of molecular and biochemical tools enabled investigations on innate and acquired immune responses of sea bass in unstimulated/stimulated fish, along the development and under variable environmental conditions and food regimes. An overview of sea bass viral and bacterial pathogens and available vaccines against these microorganisms is also provided. The knowledge accumulated in the past 25 years validates the European sea bass as a reference marine model in the field of fish immunology.

## 1. Introduction

Since 1994, when the first reliable reagent to study fish T cells became available for the European sea bass (at the 1994 ISDCI meeting, Wageningen, NL), the knowledge on the immune system of this species has increased to an extent that *Dicentrarchus labrax* can now be considered as a reference marine species for immunobiology studies. The tools available are polyclonal and monoclonal antibodies for leukocyte populations and immunoregulatory molecules, as well as nucleotide probes for genes involved in immune defences, array platforms (Peñaloza et al., 2021) and a draft genome sequence. In addition, continuous cell lines and protocols for culturing of leukocytes are known for this species, allowing studies on *in vitro* reactions of lymphocytes following stimulation. In this review we describe a synthesis of the available knowledge on the immune system of the European sea bass in unstimulated conditions and following infections/vaccinations. As a key Mediterranean species distributed in the Mediterranean and Black Seas as well as in the eastern Atlantic Ocean which is caught from 6 FAO major fishing areas (i.e. 1, 4, 5, 27, 34 and 37) but whose value originates mainly from the over 190,000 tonnes produced by the aquaculture industry (Vandeputte et al., 2019), an in-depth understanding of its

immune system is critical both from a biological and an economics standpoint.

## 2. General asset of sea bass immune system

The morphological and physiological characteristics of the sea bass immune system are remarkably similar to those found in most of the Teleost species investigated (Chistiakov et al., 2007; Flajnik, 2018), with differences due to evolutionary traits, such as the absence of well-developed intestinal diverticula and of an evident interbranchial lymphoid tissue, as observed in salmonids. The leukocytes present in sea bass are thrombocytes (Esteban et al., 2000), macrophages (Esteban & Meseguer, 1997), melanomacrophages (Arciuli et al., 2017), neutrophils (Do Vale et al., 2003), eosinophils (Picchietti, Fausto, et al., 2009), basophils (Meseguer et al., 1990), dendritic cells (Picchietti et al., 2015), Natural Killer cells (Deschaux et al., 1983), and lymphocytes (Miccoli, Guerra, et al., 2021; Scapigliati et al., 2018). In addition to main cell populations, whole transcriptomes of sea bass lymphoid tissues revealed the expression of all the genes coding for master activities of lymphocyte populations and subpopulations as they are known in mammals (Nuñez Ortiz et al., 2014). Table 1 provides a summary of the cellular and molecular tools that are currently available to investigate the

\* Corresponding author.

E-mail address: [scapigg@unitus.it](mailto:scapigg@unitus.it) (G. Scapigliati).

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List of abbreviations	
AMPs	Antimicrobial peptides
dpf	days post-fertilization
dph	days post-hatching
ELISA	Enzyme-linked immunosorbent assay
GIALT	Gill-associated lymphoid tissue
HMA	Heads of Medicines Agency
IIF	Indirect immunofluorescence
IHC	Immunohistochemistry
ISH	<i>In situ</i> hybridization
LPS	Lipopolysaccharide
mAb	Monoclonal antibody
MLR	Mixed leukocyte reaction
NKLPs	NK-lysin-derived peptides
pAb	Polyclonal antibody
RPS	Relative percentage of survival
TLR	Toll-like receptor
VLP	Virus-like particles

**Table 1**  
Cellular and molecular tools available for the European sea bass *Dicentrarchus labrax*. mAb = monoclonal antibody; pAb polyclonal antibody; GIALT = Gill-associated lymphoid tissue.

Name	Origin	Specificity	Reference
DLT15	mAb	Thymocytes, pan-T cells	Scapigliati et al. (1995)
DLT22	mAb	CD45-lymphocytes	Marozzi et al. (2012)
DLIg3	mAb	IgM-B cells	Scapigliati et al. (1996)
RaIgM	pAb	IgM	Scapigliati et al. (1997)
RaIgT1	pAb	IgT/IgT-B cells	Picchiatti et al. (2017)
RaIgD1	pAb	IgD-B cells	Presented in this work
RaCD3/1	pAb	CD3ε	Picchiatti, Buonocore, et al. (2021)
RaTRγ1	pAb	TcRγ-chain	Miccoli, Guerra, et al. (2021)
DLEC	Embryonic cells	Cell line	Buonocore et al. (2006)
DLB-1	Brain	Cell line	Morcillo et al. (2017)
P96A	q-PCR	PCR-array	Randelli et al. (2009)
Medfish	Microarray	30 K markers	Peñaloza et al. (2021)
–	GIALT	Transcriptome	Núñez Ortiz et al. (2014)
–	Gonad	Transcriptome	Ribas et al. (2019)
–	Brain/liver	Transcriptome	Magnanou et al. (2014)
–	–	Genome (scaffold)	www.ncbi.nlm.nih.gov/assembly/GCA_905237075.1/

immuno-physiology of sea bass.

3. Innate immunity

Teleost fish display potent innate immune activities characterized by a molecular and cellular system where most of its elements are conserved throughout the vertebrate lineage.

3.1. Humoral components

The complement cascade system was investigated, the alternative-pathway component C3 gene cloned (Mauri, Roher, et al., 2011) with its expression found to be modulated by both high density farming and *in vivo* stimulation with viral/bacterial antigens (Mauri, Romero, et al., 2011).

Among opsonins, an F-type Fucose-Binding Lectin (DIFBL) was reported and resulted specifically expressed in hepatocytes and intestinal cells; peritoneal macrophages showed an enhanced phagocytic activity

of inactivated *E. coli* exposed to DIFBL (Salerno et al., 2009).

Pattern recognition receptors of the TLR family have been identified in sea bass, namely TLR1, TLR2 and TLR9 which are specific for Gram-positive bacteria, peptidoglycans and phosphorylated lipoglycans, and unmethylated CpG sequences in DNA molecules of bacteria and viruses (Núñez Ortiz et al., 2014). Like in most teleosts, a TLR4-expressing gene recognizing Gram-negative LPS has not been identified, despite a strong sensitivity of leukocytes to LPS stimulation.

At present, no information is available about other components/activities of the vertebrate innate immune system such as neutrophils extracellular traps, scavenger receptors and chemotactic peptides.

Antimicrobial peptides (AMPs) are a heterogenous class of peptides differing in size, structure, activity and specificity characterized by antimicrobial and immunomodulatory activities against a wide spectrum of targets that are encoded by every Eukaryotic organism investigated. In sea bass, the identification of fish-specific AMPs remains poorly understood because a comprehensive list of the piscidin family members as well as a detailed study of their biological roles is missing. The first cDNA encoding for a 22 aa long piscidin-like peptide (dicentracin) was characterized in sea bass and found in granulocytes and monocytes of peripheral blood and head kidney as well as in macrophages from the peritoneal cavity (Salerno et al., 2007). One piscidin isoform was characterized biochemically and quantified immuno-enzymatically from mucosal tissues (Corrales et al., 2010). Recently, the piscidin family was more thoroughly characterized at genomic and transcriptional levels and the antimicrobial activity of synthetic analogues was tested against a range of aquaculture-relevant bacteria (both Gram-negative and Gram-positive) and two parasite species (Barroso et al., 2020).

Membranolytic AMP-like molecules such as NK-lysin (orthologue to human granulysin) and NKLPs were described to take part in innate and cell-mediated cytotoxic responses (Andersson et al., 1996; Stenger et al., 1998). Their antimicrobial activities were demonstrated *in vitro* in several fish species. In sea bass, NK-lysin is maternally transferred to eggs and intermittently transcribed along the ontogeny, with the greatest expression occurring at 69 dpf and in response to NNV infections (Valero et al., 2020). Recently, an *in vivo* study found that synthetic NKLPs only slightly modulate immune markers (i.e. immunoglobulin μ heavy chain, macrophage colony-stimulating factor 1 receptor 1, CXCL chemokine receptor 3, T cell receptor beta chain, IL-1β and IL-8) related to inflammation, macrophage functions, activation of NK and T cells and antibody-mediated immunity at the mRNA level but are able to reduce the disease signs and mortality upon NNV infection (Valero et al., 2021).

With regards to cytokines, many transcripts were found by exploiting transcriptomics data on the basis of an *a priori* knowledge about most likely expression tissues. cDNA amplification by means of degenerate primers targeting evolutionary-conserved ILs regions or NGS data mining allowed the identification of many sequences codifying for immunological mediators, namely IL-1β (Scapigliati et al., 2001), IL-2 and IL-2L (Buonocore, Gerdol, et al., 2020), IL-4/13 (Stocchi et al., 2017), IL-8 (Sepulcre et al., 2007) (Sepulcre et al., 2007), IL-10 (Pinto et al., 2007), IL-13, IL-17, IL-21 and IL-22 (Núñez Ortiz et al., 2014).

3.2. Cellular components

The main leukocyte types shared by vertebrate classes were described in sea bass. The myeloid lineage is composed of professional phagocytes: macrophages (Esteban & Meseguer, 1997) and granulocytes (Do Vale et al., 2003; Meseguer et al., 1990; Picchiatti, Fausto, et al., 2009). These phagocytes display *in vitro* activation by beta-glucans (Vazzana et al., 2003), macrophage activating factor and LPS (Buonocore et al., 2010; Sarmiento et al., 2004) and *in vivo* activation by bacterial pathogens (Do Vale et al., 2003). Due to the importance of antigen delivery into the peritoneal cavity, it will be interesting to investigate phagocytes from such anatomical site as available data suggest active

phagocytosis and killing (Cammarata et al., 2000; Do Vale et al., 2002; Vazzana et al., 2003). No information is presently available on activities of sea bass thrombocytes in the homeostatic and immune responses.

Because fish rely mostly on innate immunity for non-specific defences, this subject is intensely investigated in all species of interest. Accordingly, many studies on innate defences are reported for sea bass, confirming the great similarities of this ancient defense among vertebrates. In addition to what reported above and in a brief summary, we redirect the readers to inflammatory activities (Poisa-Beiro et al., 2008), stress-related activities (Samaras et al., 2016), complement activities (Mauri, Romero, et al., 2011), humoral antibacterial activities (Barroso et al., 2020; Buonocore et al., 2014) and antiviral activities (Scapigliati et al., 2010).

## 4. Acquired immunity

### 4.1. Immunoglobulins and B cells

Lymphocytes are the evolutionary product of ancient immunocytes that are capable of performing somatic recombination of Ig genes as well as acquiring and maintaining exquisite antigen specificity (Scapigliati, 2013). The IgM is the predominant B-cell antigen receptor conserved among vertebrates and is presently considered to be the only class of immunoglobulin universally found in all jawed vertebrates (Bengtén et al., 2000). Other classes than IgM have been found in fish, namely IgD (Wilson et al., 1997) and IgT/Z (Danilova et al., 2005; Hansen et al., 2005). In sea bass all three Ig heavy chains were found to be expressed.

Fish IgM is composed of heavy  $\mu$  polypeptide chains associated with four different isotypes of Ig light genes (Edholm et al., 2009), each with an antigen binding (variable) region and a more constant class-specific region. These chains combine in equimolar amounts to give a complex polymeric molecule, usually a tetramer in teleosts, although it can also be monomeric (Elcombe et al., 1985). Both membrane-bound and secreted forms of IgM exist in fish (Mashoof & Criscitiello, 2016), and their schematic representation is available in Bilal et al. (2021). Worthy of note, IgM structure and function is conserved across all vertebrate classes.

The IgM has been investigated in tens of fish species and the antibody production mostly related to stimulation and vaccination responses. In sea bass the knowledge on IgM structure and antibody production is also extensive, and the main findings can be resumed in the first purification of the IgM molecule (Bourmaud et al., 1995) and the subsequent preparation of monoclonal antibodies (Scapigliati et al., 1996) and antisera (Scapigliati et al., 1997). These antibodies allowed a detailed characterization of IgM-bearing cells in lymphoid and non-lymphoid tissues in unstimulated conditions (Scapigliati et al., 2002) and the purification of IgM-B cells (Scapigliati et al., 2003).

IgM-B cells have physiological activities related to *in vivo* effects of immunostimulants (Bagni et al., 2005), viral infections (Scapigliati et al., 2010), *in vitro* stimulations and changes in age and environmental conditions (Scapigliati et al., 1999).

Monoclonal and polyclonal anti-sea bass IgM led to the production of very sensitive immunoenzymatic assays to monitor the presence of both antigen-specific IgM and antigens from complex biological sources (Nuñez-Ortiz et al., 2016a, 2016b). In this respect, an innovative cell-based ELISA assay representing a simplified alternative to classical ELISPOT assay was developed in sea bass to monitor antigen-specific IgM produced *in vitro* by plasma cells of immunized fish (Meloni & Scapigliati, 2000).

The mucosal IgT, precursor of mammalian IgA, was identified and characterized in sea bass by analysing in detail its 3D structure and by investigating its basal and virus-stimulated expression and distribution in mucosal and non mucosal tissues (Buonocore et al., 2017; Picchiatti et al., 2017). A preponderance of IgT-B cells was observed in gut and gills by IHC employing a specific antiserum, and ISH revealed a differential expression along the intestinal tract, with a major expression in

the posterior intestine, suggesting the hindgut as a site for the recruitment of IgT-B cells in sea bass. Interestingly, IgT transcripts increased in gills and spleen following an infection with nodavirus, highlighting the importance of IgT in antiviral responses.

IgD-B cell subset was described as resident in the European sea bass. Synthetic peptides were derived from the full length sea bass IgD sequence and employed as antigen in the production of a polyclonal antiserum in rabbit. Immunoreactive cells with a lymphocytic morphology were evidenced in gut and kidney (Fig. 1). This preliminary observation suggests that, like in other fish species (Perdiguero et al., 2019), the IgD gene is actually translated into a functional antibody produced by IgD-B cells.

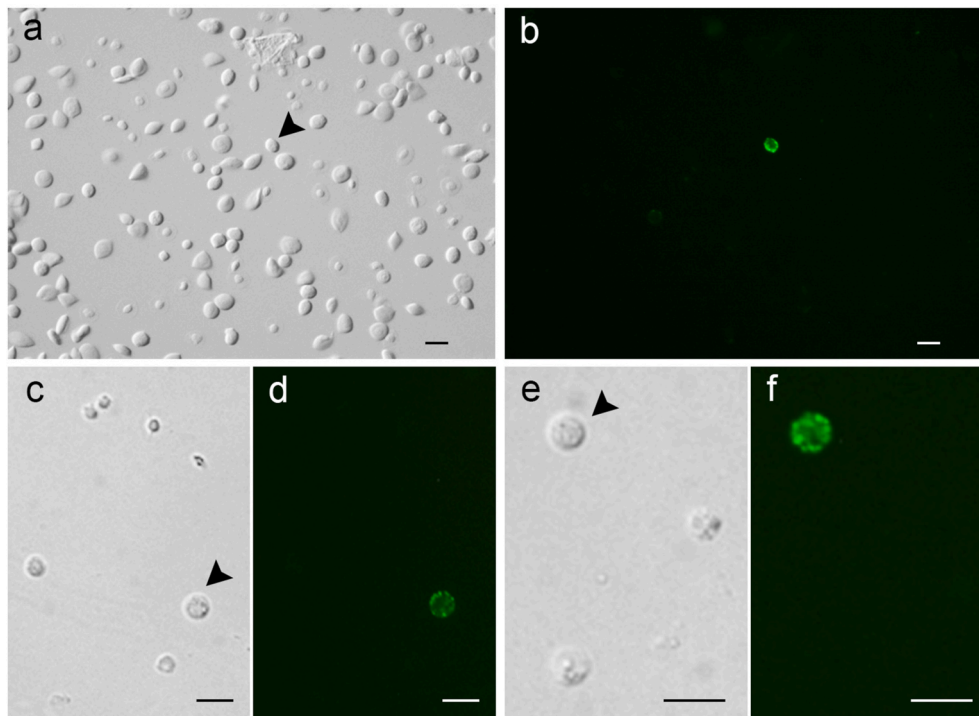
Recently, a chimeric IgT/IgD gene was found to be expressed in sea bass (Buonocore, Scapigliati, et al., 2020) and high transcription levels were observed in gills, gut and head kidney. IgT/IgD chimeric genes have been found in few teleost species so far, probably as a result of an ancestral duplication of the entire IgH locus followed by a chimerization of C $\delta$ /C $\tau$  in one of the two loci. It is unclear whether such chimeric Ig be functional but it may be related to as yet physiological activities as IgT/IgD gene transcription is stimulated by LPS (Buonocore, Scapigliati, et al., 2020). More work is required to better investigate such peculiar feature.

### 4.2. T cells

The presence of functional T cells in teleost fish was established almost 40 years ago (Miller et al., 1985) and sea bass was the first teleost species where a clear and typical *in vivo* activity of T cells, i.e. an allograft rejection, was demonstrated (Abelli et al., 1999). This was made possible by the mAb DLT15, the first in fish immunology, which recognizes a thymocyte antigen and peripheral T cells. The antibody, together with nucleotide probes for T cell subpopulations, enabled a precise quantification of T cells in lymphoid and non-lymphoid tissues in unstimulated and stimulated conditions, including viral/bacterial infection and vaccination (Buonocore et al., 2019; Moreno et al., 2018), as well as in the modulation of intestinal microbiome (Picchiatti, Fausto, et al., 2009), and also permitted immunopurification of T cells from lymphoid tissues (Scapigliati et al., 2000). Importantly and as expected, a preponderance of T cells was confirmed in mucosal tissues by means of DLT15 (IIF) and through the identification of CD4/CD8 subpopulations by ISH, and interestingly, a density gradient was shown along the intestinal tract (Picchiatti et al., 2011, 2021b). A sea bass gills transcriptome showed the expression of all the master genes coding for T cell subpopulations known in mammals (Nuñez Ortiz et al., 2014).

The TcR complex is composed of  $\alpha\beta/\gamma\delta$  peptide chains interacting with CD3, CD4 or CD8 co-receptors to achieve functional features. These molecules can be identified *in situ* in sea bass by using antibodies and/or ribonucleotide probes (Miccoli, Guerra, et al., 2021; Picchiatti et al., 2009b, 2011, 2021a), and their use revealed a modulation of T cell subpopulations in the intestine upon dietary changes (Miccoli, Guerra, et al., 2021; Picchiatti, Buonocore, et al., 2021). These are valuable observations for future development of oral vaccination protocols and for deepening the understanding of the effects of diet supplements on the intestinal mucosa. We call the attention on the fact that the  $\gamma\delta$ -TcR undergo spontaneous somatic recombination in the intestine in the absence of antigenic stimulation (Buonocore et al., 2012); this stimulates the debate on an extra-thymic origin of T cell subpopulations and, on this basis, the sea bass intestine can be considered a lymphoid tissue for T cells. The *in vitro* measurable activities of sea bass leukocytes attributable to T cells and monitored by cellular/molecular probes are cell proliferation induced by MLR (Meloni et al., 2006), lectins (Nuñez Ortiz et al., 2014), T-dependent immunization antigens (Picchiatti et al., 2017), as well as cytokine-induced, receptor-mediated intracellular Ca<sup>++</sup>-mobilization (Benedetti et al., 2006).

The knowledge accumulated on sea bass and fish lymphocytes and their observed activities allow important evolutionary considerations,



**Fig. 1.** Indirect immunofluorescence. The figure shows a typical indirect immunofluorescence staining of head kidney leukocytes (1a, 1 b) and GALT (1c–1f) employing a rabbit antiserum (diluted 1:400) against sea bass IgD constant region and a secondary fluorescein-labeled antibody. Immunoreactive cells display a surface membrane staining (1 b, 1 d, 1f) and the typical lymphocyte size (bars = 10  $\mu$ m). DIC image of same IIF fields are shown (1a, 1c, 1e).

since it can be speculated that most of peripheral fish lymphocytes display features that are remarkably similar to those of mammalian innate-like lymphocytes, namely B1–B cells and Tc $\gamma\delta$ -T cells (Scapigliati et al., 2018).

## 5. Ontogenesis of immune components

In contrast to higher vertebrates, most fish species are free-living organisms already at the embryonic stage of life and, therefore, it is fundamental to provide information on the development of immune components and reactions. Much work has been done exploiting the reagents listed in Table 1, and we can now assert that T-cells develop much earlier than B-cells in sea bass (Abelli et al., 1996; Picchiatti et al., 1997), at approx. 30 and 49 dph, respectively. Also, T cell subpopulations are located in different zones of the thymus during development (Picchiatti et al., 2008). It is still difficult to establish whether the first DLT15-immunoreactive T cells originate in thymus or in the intestine. However, an extra-thymic origin of T cells, mostly of the  $\gamma\delta$  phenotype, was reported in mammals, and data obtained on sea bass support the idea that a similar process may take place in bony fish since thymus and GALT are evolutionary related and GALT possibly precedes thymus during evolution (Matsunaga & Rahman, 2001). It should be noted that, due to the extensive availability of specific markers, the knowledge on the development of the European sea bass immune system in its cellular and molecular components is substantial, compared to other marine fish species (see reference list). Recent data also showed the importance of stress conditions on the development of sea bass thymus (Moreira et al., 2021).

## 6. Sea bass pathogens and vaccines

Sea bass is the most heavily farmed marine finfish species within the Mediterranean aquaculture, followed by sea bream *Sparus aurata*, turbot *Psetta maxima* and meagre *Argyrosomus regius* (data from FAO fisheries as of July 2021). Inevitably, any outbreak affecting sea bass, especially

of bacterial origin, has a biological significance and an enormous economic relevance (Barnes et al., 2005; Ina-Salwany et al., 2019).

*Dicentrarchus labrax* is susceptible to a wide range of diseases such as epitheliocystis, mycobacteriosis, myxobacteriosis, photobacteriosis, tenacibaculosis, vibriosis and viral nervous necrosis diseases caused by *Chlamydia* spp. (bacteria), *Mycobacterium marinum* (bacterium), *Flexibacter maritimus* (bacterium), *Photobacterium damsela* subsp. *piscicida* (formerly *Pasteurella piscicida*) (bacterium), *Tenacibaculum maritimum* (bacterium), *Vibrio* spp. (bacteria) and nodavirus, respectively (Miccoli, Manni, et al., 2021; Muniesa et al., 2020). This is especially true under intensive rearing conditions. Additional diseases caused by dinoflagellates, ciliates, myxosporidia, platyhelminthes, nematodes and isopods were reported and for some no treatment is currently available.

Bacterial outbreaks are most commonly treated with antibiotics but their use has become strongly regulated in many countries due to the onset and transfer of resistance mechanisms among bacteria species as well as to the bioaccumulation/biomagnification and the uncertainty of long-term effects to the environment (Lulijwa et al., 2020; Sanseverino et al., 2018). New EU legislation will prohibit all forms of routine antibiotic use in farming as of January 28th 2022 when Regulation (EU) 2019/6 on Veterinary Medicines and Regulation (EU) 2019/4 on Medicated Feed will come into force. The focus has therefore shifted towards preventive measures based on biocontainment (e.g. Jia et al., 2017), water treatment (e.g. Ben-Asher et al., 2019), dietary supplementation of immune- and growth-stimulating bioactive substances (e.g. Piccolo et al., 2015; Sorroza et al., 2012) and vaccination practices, with the overall aim of improving animal husbandry and welfare conditions.

Fish immunobiology has been investigated actively in terms of pathogen antigen components, immunization strategies and delivery methods stimulating the mounting of specific and prolonged responses for efficient vaccination programmes. Twelve vaccine formulations are currently available and authorized in Europe by HMA for sea bass (Table 2). The table builds on Miccoli et al. (2019) and it is updated with data extracted from the latest HMA's "Veterinary Medicinal Products



**Table 2**

HMA authorized and commercially-available vaccines optimized for sea bass.

Vaccine name	Company	Active substance	Onset of immunity (degree days)
Alpha Dip Vib	Pharmaq AS	Inactivated <i>Listonella</i> ( <i>Vibrio</i> ) <i>anguillarum</i> serotype O1 strain AL 112	600
Alpha Dip 2000	Pharmaq AS	Inactivated <i>L. anguillarum</i> serotype O1 and inactivated <i>Photobacterium damsela</i> subsp. <i>piscicida</i>	NA
Alpha Ject 2000	Pharmaq AS	Inactivated <i>L. anguillarum</i> serotype O1 strain AL 112RPS1 and inactivated <i>P. damsela</i> subsp. <i>piscicida</i> strain AL 5051	560
Alpha Ject Micro 1 Noda	Pharmaq AS	Inactivated red-spotted Grouper Nervous Necrosis Virus (RGNNV)	NA
Ichtiovac VR	Laboratorios Hipra S.A.	Inactivated <i>L. anguillarum</i>	NA
Ichtiovac VR/PD	Laboratorios Hipra S.A.	Inactivated <i>P. damsela</i> subsp. <i>piscicida</i> strain DI 21 and inactivated <i>L. anguillarum</i> serotypes O1, O2 $\alpha$ and O2 $\beta$	798–882
Ichtiovac VNN	Laboratorios Hipra S.A.	Inactivated Betanodavirus strain 1103	924
AQUAVAC Photobac Prime/Boost	Intervet International B. V.	Inactivated <i>P. damsela</i> subsp. <i>piscicida</i> strain Pr85	NA
AQUAVAC Vibrio Oral	Intervet International B. V.	Inactivated <i>L. anguillarum</i> serotypes O1 and O2 $\alpha$	NA
AQUAVAC Vibrio Pasteurella	Intervet International B. V.	Inactivated <i>L. anguillarum</i> biotype I and II and <i>P. damsela</i> subsp. <i>piscicida</i>	NA
AQUAVAC Vibrio Vab	Intervet International B. V.	Inactivated <i>L. anguillarum</i> strain 78 SKID and <i>Vibrio ordalii</i> strain MSC 275	NA
Vibri-Fishvax	Fatro S.p.A.	Inactivated <i>L. anguillarum</i> and <i>Vibrio ordalii</i>	NA

intended for fish” published in April 2021 ([https://www.hma.eu/fileadmin/dateien/Veterinary\\_medicines/Miscellaneous/VMPs\\_in\\_EU\\_for\\_fish\\_April21.xlsx](https://www.hma.eu/fileadmin/dateien/Veterinary_medicines/Miscellaneous/VMPs_in_EU_for_fish_April21.xlsx)). These products are authorized in Croatia, Cyprus, France, Greece, Italy, Poland, Portugal and Spain, while uncertainty exists with regards to product authorization in extra-EU countries. Attention is called on the fact that only a small fraction of the seven most common diseases (see above) are currently addressed with varying efficacy. These products have the whole inactivated pathogens as main antigen, whose effectiveness has been demonstrated. On one side, such a complex antigenic mixture is relatively easy to produce; on the other, the immunity they induce was found to be limited in time, with the longest reported duration of 12 months.

Many promising experimental formulations against bacterial and viral diseases have been recently tested for sea bass. They employ inactivated pathogens, extracellular products, LPS and the recombinant technology as well as potentially immunostimulatory feed supplements (e.g. plant essential oils) or adjuvants produced by means of heterologous systems (e.g. recombinant Tumor Necrosis Factor  $\alpha$ , NKLPs) added to either experimental or already-established commercial formulations, respectively (e.g. Galindo-Villegas et al., 2013; Gonzalez-Silvera et al., 2019; Khalil et al., 2018; Marsian et al., 2019; Valero et al., 2016). All of these studies produced encouraging results: statistically higher RPSs, increased phagocytic activity and biochemical/hematological parameters (e.g. total protein, globulin, and lysozyme activity) were observed. Relevant innate and adaptive immune responses at mRNA and protein levels consisted of upregulation of *ifn*, *mx*, and *ifng*, *IL-1 $\beta$* , *IL-10* and *lysozyme*, promotion of T-cell (*tcr $\beta$* , *cd8 $\alpha$* , *MHC-I $\alpha$* ) markers and immunoglobulin (*IgM*, *IgT*) transcription, increased abundance of

intraepithelial DLT15<sup>+</sup> leukocytes. In some cases, correlation between antibody quantity/functional characteristics and induced immunity was poor and improved resistance to pathogens seemed independent of pathogen-specific serum IgM titers (Galindo-Villegas et al., 2013). This suggests that cellular immunity can be as protective to bacterial or viral pathogens as humoral immunity and warns that a vaccine efficacy should never be investigated solely by means of serological assays.

It must be noted that the great majority of formulations for sea bass, either commercial or experimental, are intended for delivery through injection or immersion. The injection route is the most effective in eliciting immune responses but also the most distressing for the animal and limiting for the aquaculture industry as it is unfeasible on larvae and small juvenile fish. On the contrary, oral vaccination is effortless to operate, cost-effective, suitable for all fish sizes and stress-free compared to parental delivery; these features make it an attractive delivery method (Mutoloki et al., 2015), although currently under-explored not only in sea bass but in all farmed species (Ma et al., 2019). One of the main challenges related to oral immunization consists of a thorough understanding of oral tolerance in fish, i.e. the usual hypo-responsiveness of fish to fed antigens that consequently demands high antigen quantities for an immune response to be mounted (Mutoloki et al., 2015). The molecular mechanisms of oral tolerance are known in detail in higher vertebrates (Tordesillas & Berin, 2018) and only recently Chen et al. (2014) demonstrated the key role of homologous transcriptional factors and cytokines in the induction of regulatory T cells in fish. Further challenges related to oral immunization are the lack of an adequate duration of the protection, the uncertainty regarding the exact antigen dose up-taken by each specimen, the need of protecting the antigen(s) via encapsulation to avoid digestion and the requirement to stimulate an effective immune response both locally and systemically (Embregts & Forlenza, 2016; Soto et al., 2015). The subject of mucosal immunity has received growing interest in the last decade from academia and industry (e.g. Yu et al., 2020).

At last, while societal and legislative acceptability of standard inactivated vaccines has already been certified, greater concern exists for attenuated and, particularly, next-generation vaccines that are based on live pathogens and on the recombinant-technology/mRNA/DNA, respectively. The employment of recombinant viral-like particles (VLPs)-based formulations (e.g. Marsian et al., 2019) or may simplify the regulation and approval processes because, while being efficacious in mounting an immune response, they are also operationally safe as they lack any viral genetic material. For this reason, they are unable to replicate in fish and do not require the use of live virus during production stages.

## 7. Conclusions

From the information herein presented it is clear that the European sea bass *Dicentrarchus labrax* can be established as a marine model species for studies on fish immunophysiology. Together with Atlantic salmon *Salmo salar*, it is the only marine species for which much information is available immunology-wise. Many molecular and genomics data and tools such as antibodies, transcriptomes and a full-genebuild genome have been generated along the years: on one hand, they allowed for the elucidation of important immunophysiological features (e.g. evolution of T lymphocytes); on the other, we expect that future knowledge on basic and applied research topics will build upon such resources for designing and controlling vaccination strategies and stimulating important questions on the evolution of immune responses among vertebrates, with potential applications also in mammalian immunology. At last, it is hoped that the aquaculture industry will be able to fully exploit such resources for maximizing benefits for multiple stakeholders as well as ensuring a sustainable use of finite resources.

## Declaration of competing interest

The authors have no conflict of interest to declare.

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