Vaccini e risposta immunitaria nelle specie ittiche

Donatella Volpatti - Di4A UNIUD

INCONTRO TECNICO SCIENTIFICO SIPI - IN COLLABORAZIONE CON API

VERONA 10 NOVEMBRE 2023

Immunologia e vaccinazione nelle specie ittiche

Prime pubblicazioni su risposta immunitaria/vaccinazione nei pesci - 1935-1940

Primo vaccino brevettato ed approvato in USA per Bocca Rossa – 1976

Ad oggi disponibili 24 vaccini (approvati) a livello mondiale - utilizzabili in 17 specie ittiche

Sviluppati contro 22 specie batteriche e 6 virus

Non ci sono vaccini approvati contro i parassiti

La maggior parte dei vaccini commerciali includono adiuvanti e sono somministrati con **iniezione intra-celomatica**

Alcune formulazioni sono **multivalenti**, in particolare quelle per il salmone atlantico

(Adams, 2019. Fish and Shellfish Immunology, 90, 210-214)

Dall'immunità innata a quella adattativa … passando attraverso i pesci….

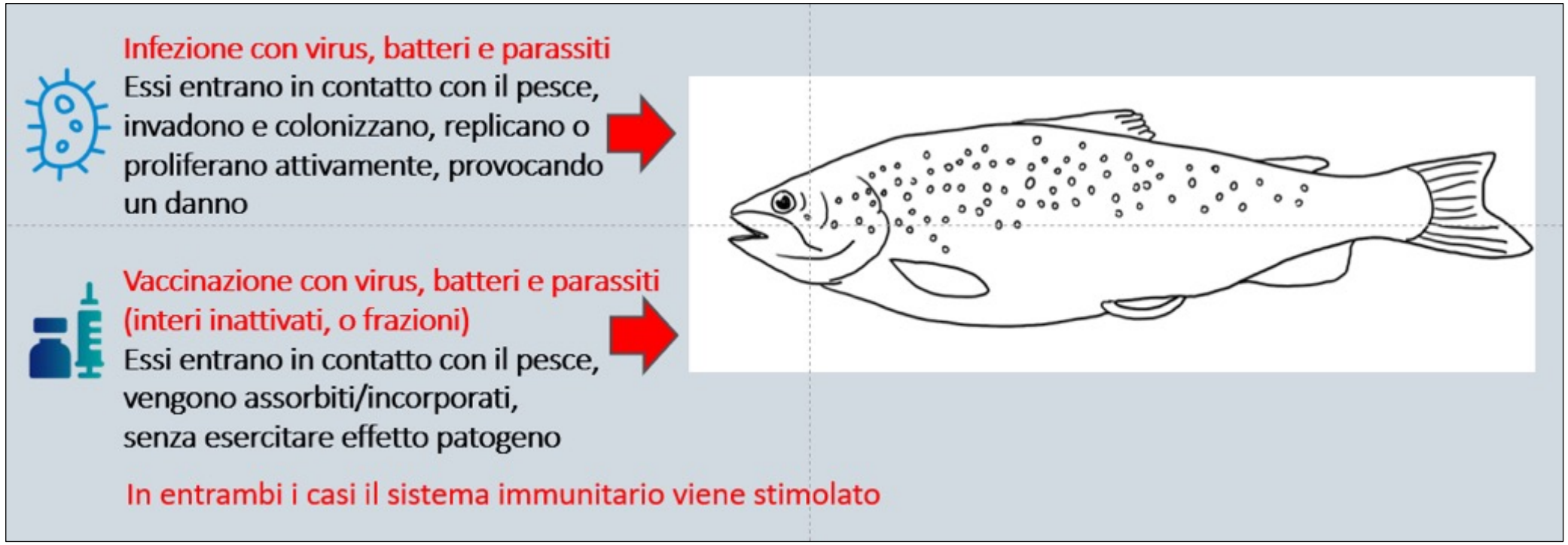




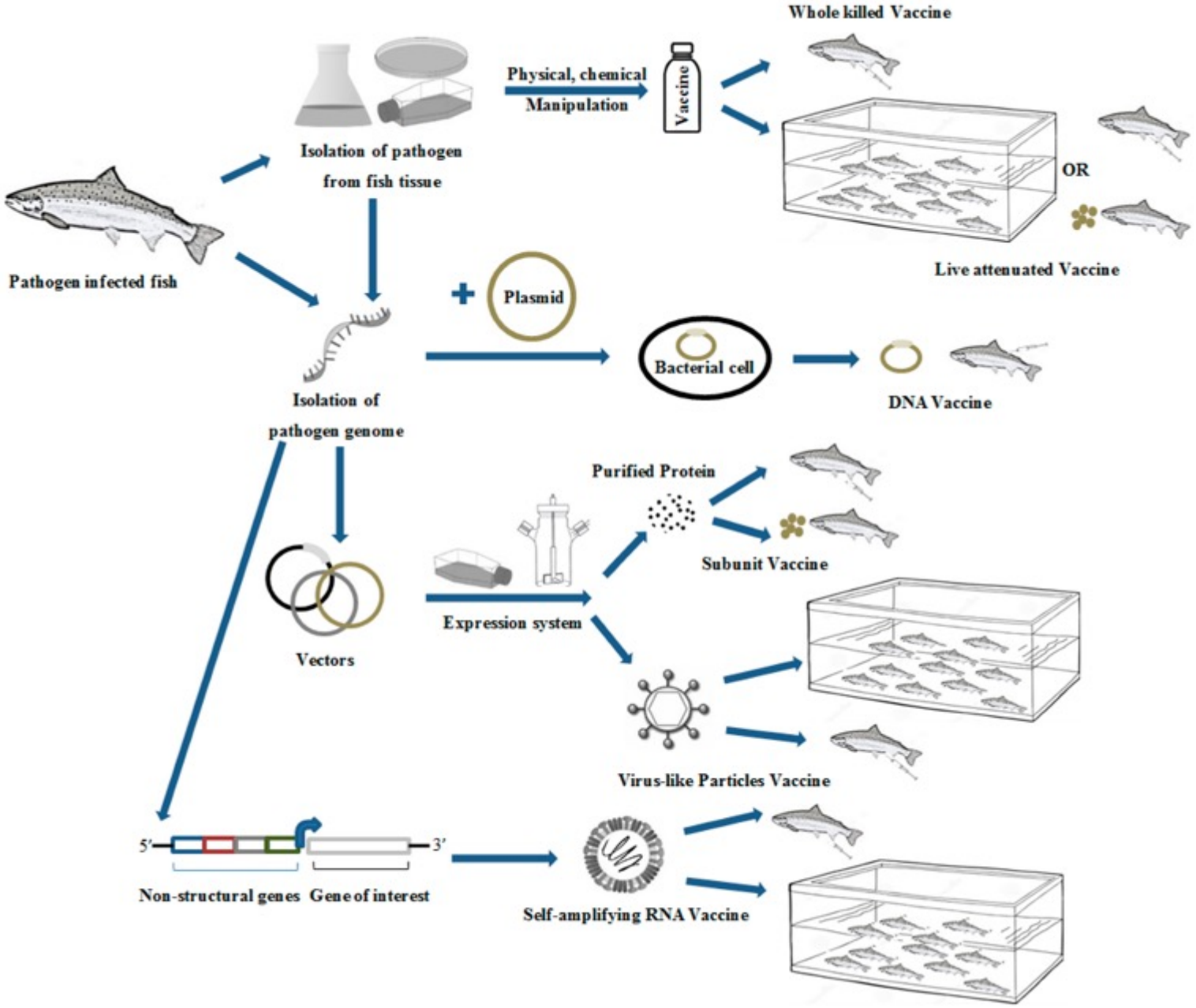
I pesci cartilaginei e ossei sono i primi vertebrati in grado di produrre ANTICORPI, perciò dal punto di vista evolutivo/biologico rappresentano un passaggio chiave dal sistema immunitario innato a quello adattativo (specifico) – questo è avvenuto 450 milioni di anni fa

NOTA: anticorpi, T cell receptor, B cell receptor e molecole MHC sono assenti negli invertebrati (molluschi, crustacei) e nelle lamprede (pesci senza mandibola)

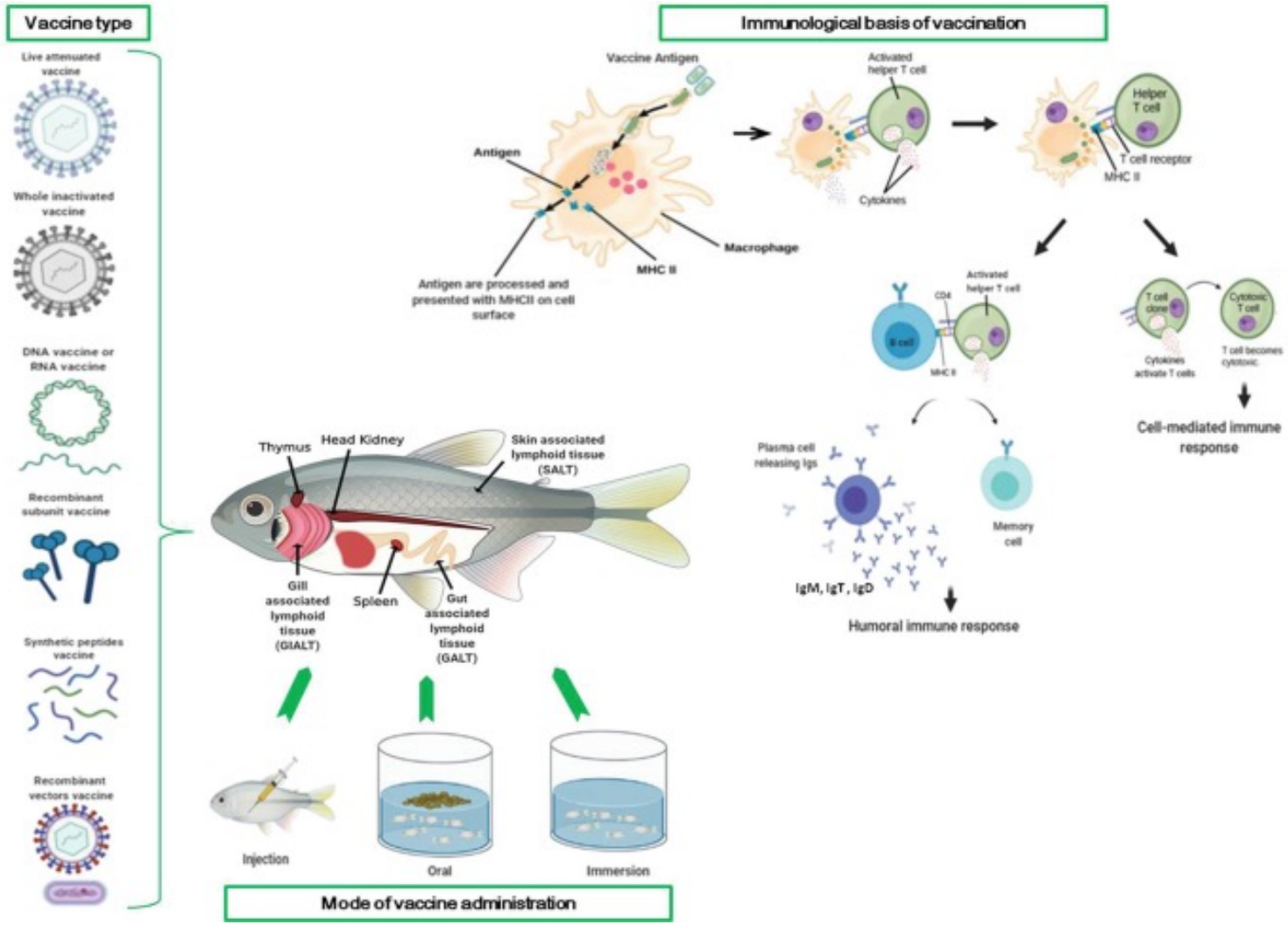
Interazione ospite-patogeno e interazione ospite-vaccino



I vaccini sono in grado di stimolare la risposta immunitaria locale (mucosale) e la risposta immunitaria sistemica

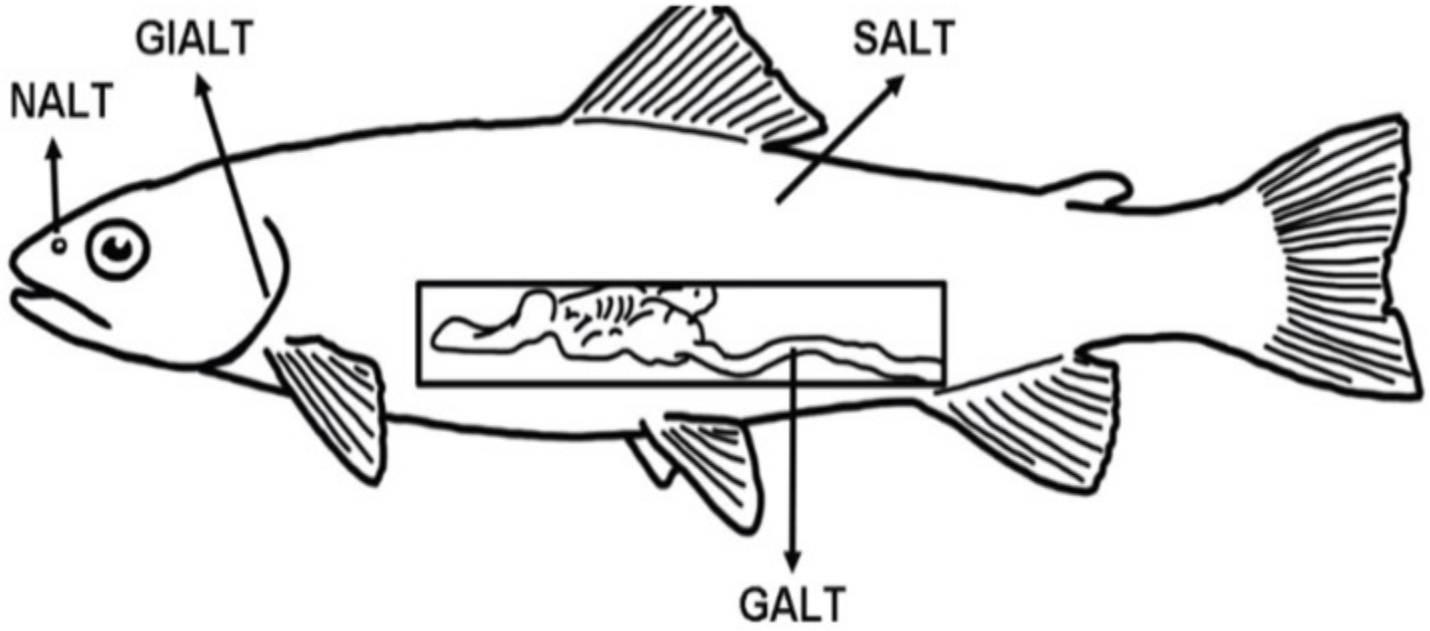


Microorganisms 2019, 7, 569;
doi:10.3390/microorganisms7110569



Bedekar, M.K., Kole, S. (2022). Fundamentals of Fish Vaccination. In: Thomas, S. (eds) Vaccine Design. Methods in Molecular Biology, vol 2411. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1888-2_9

Principali sedi di immunità associata alle mucose - MALT

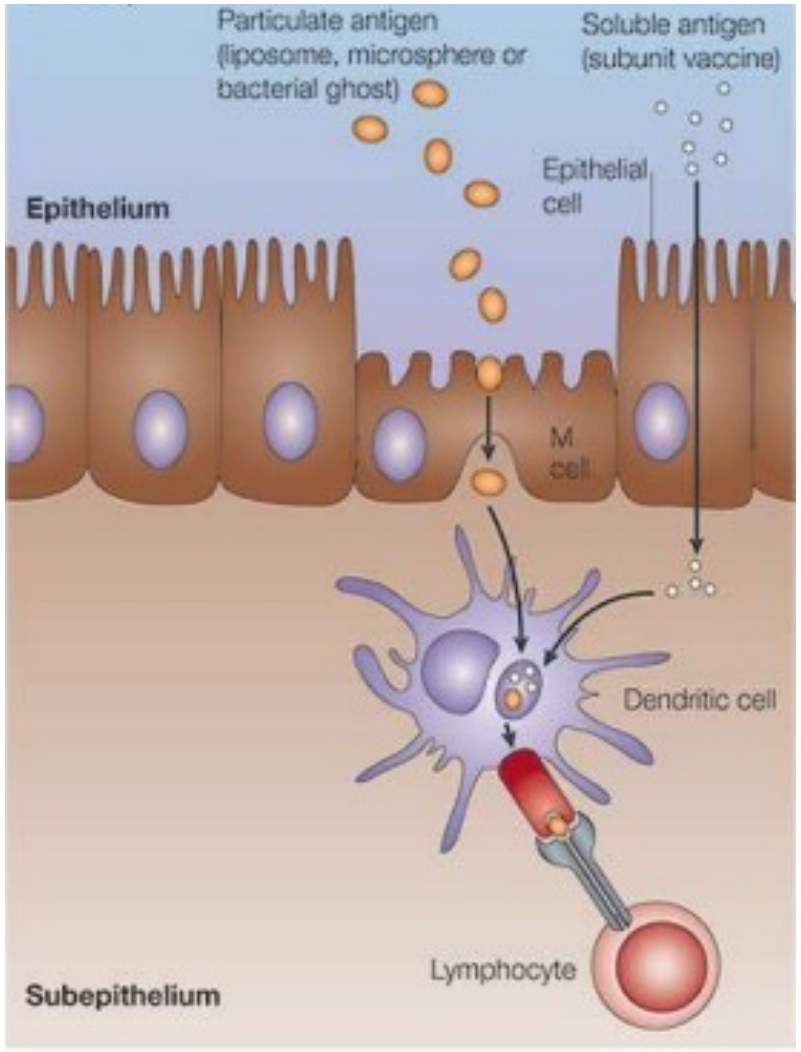


Questi siti sono le principali vie di entrata degli antigeni vaccinali

Sedi di "uptake/processazione" per i vaccini somministrati per bagno e oralmente

Salinas, 2015

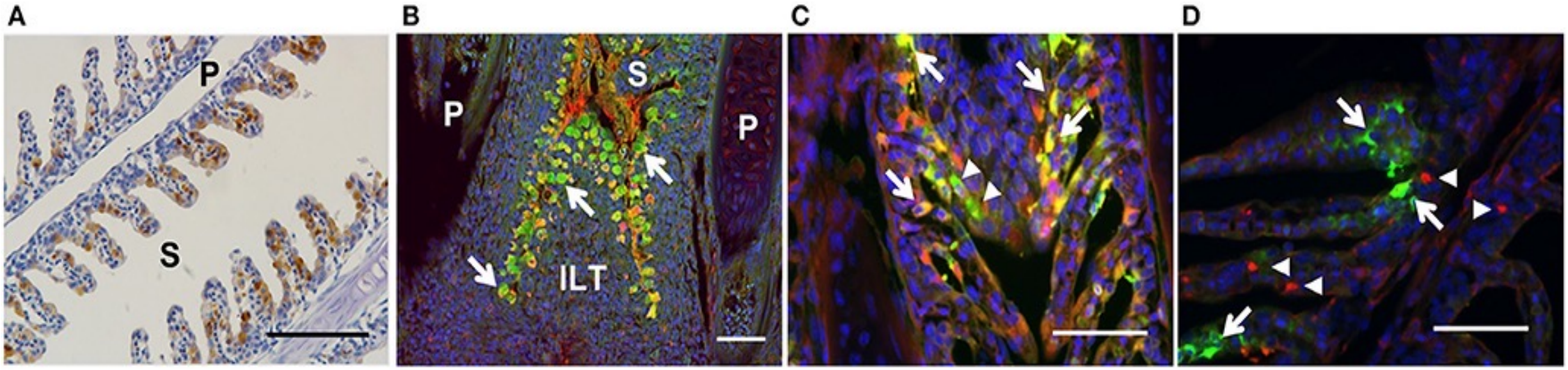
Mucosal M cells
(antigen sampling cells)
and
antigen presenting cells

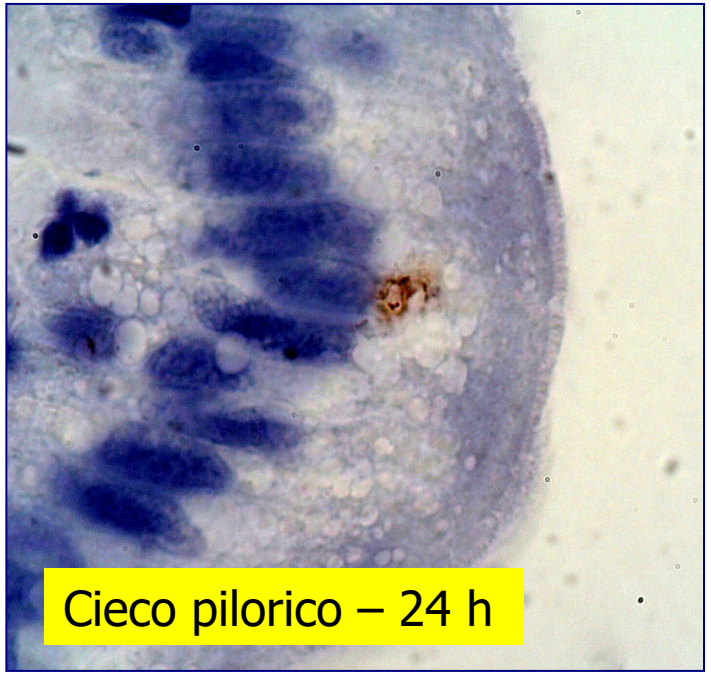


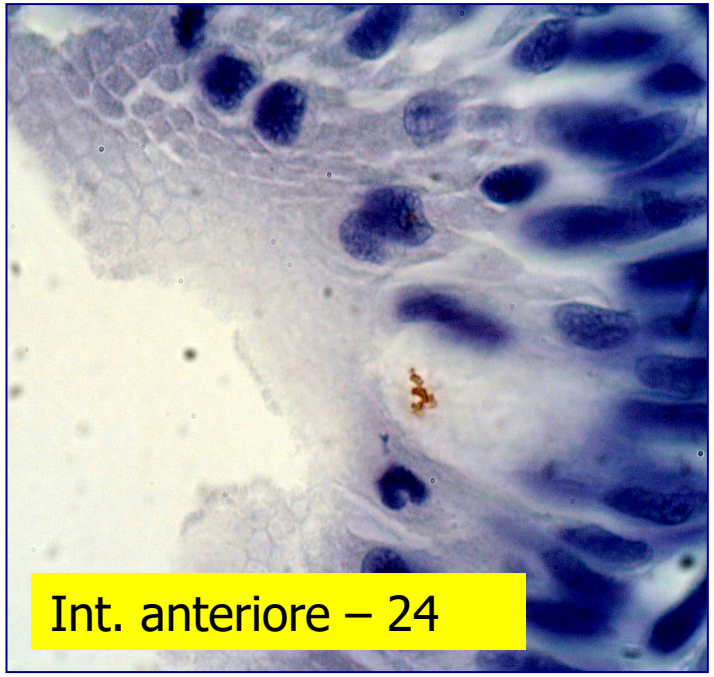
Nature Reviews | Immunology

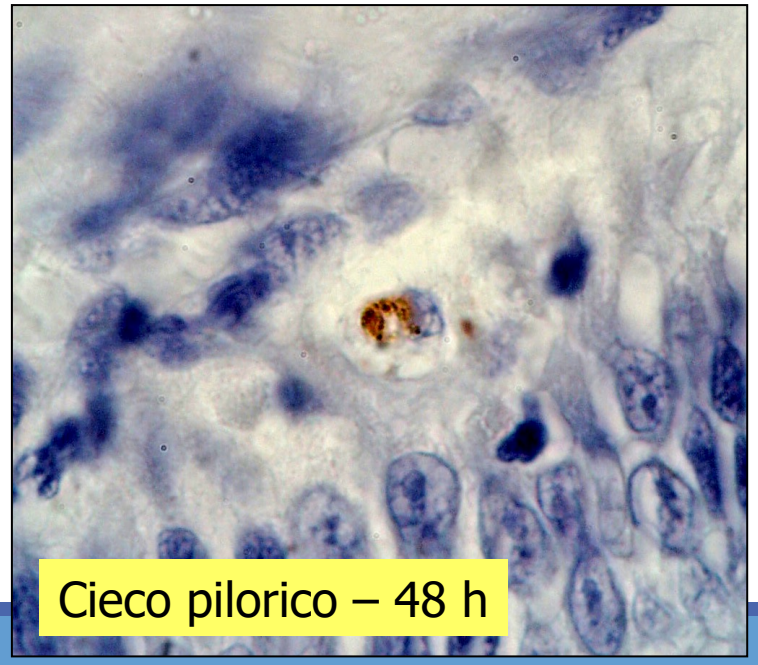
Antigen sampling cells (APCs)in sede mucosale

Kato *et al.* Front. Immunol., 20 September 2018 hanno identificato nelle branchie della trota iridea due fenotipi di ANTIGEN SAMPLING CELLS in grado di «incorporare» l'antigene vaccinale. Esprimono MHC-II, CD83, IL-1β. Morfologicamente riferibili a monociti-macrofagi-cellule dendritiche.









Vaccinazione orale in trota iridea con bacterin *L. garvieae*
rivelazione IHC dell'antigene nel tratto gastro enterico

L’ uptake mucosale
può essere promosso
anche dall’impiego
di sistemi di veicolazione

SVILUPPO E PRIMA VALIDAZIONE DI SISTEMI VACCINALI ORALI HI-TECH CONTRO *LACTOCOCCUS GARVIEAE* IN *ONCORHYNCHUS MYKISS*

XVII Convegno Nazionale
19-21 maggio 2011
Ostuni (BR)

Volpatti D.*, Cocchietto M., Galeotti M.*, Bulfon C.*, Zorzin L.**, Ballestrazzi R.*, Bassignana D.*, Voinovich D.***, Gallo D.**, Prearo M.****, Tesei E.*, Sava G.**/*******

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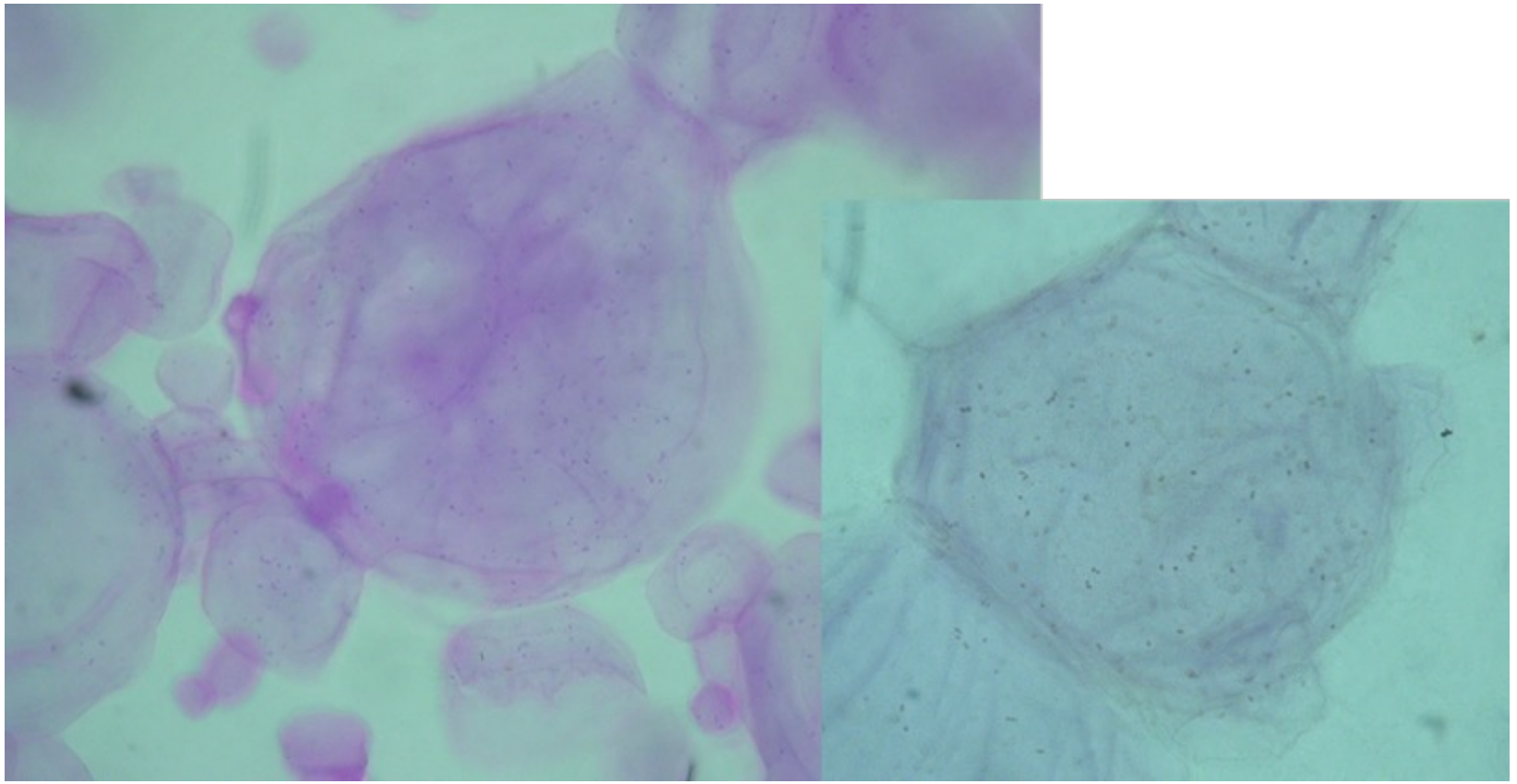
*****Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta;*
******Dipartimento di Scienze della Vita, Università degli Studi di Trieste.*

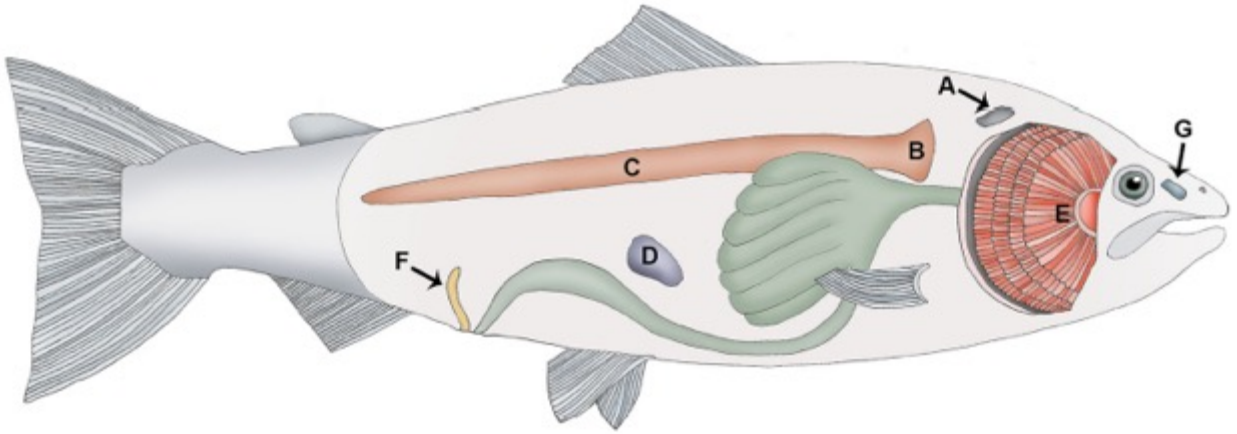


Immunità sistemica

Consiste nel coinvolgimento di cellule immunitarie presenti a livello degli organi principali del sistema immunitario: **timo, rene anteriore, milza**

Antigeni somministrati in cavità addominale (tessuto adiposo) sono processati in questa area e anche veicolati agli organi centrali al fine di promuovere la risposta sistemica **(T and B cells activation, cytokines synthesis, specific IgM/IgT synthesis)**

Fig. 1 Schematic topography of immune organs in Atlantic salmon. **A** Thymus, **B** head kidney, **C** trunk kidney, **D** spleen, **E** gills with the interbranchial lymphoid tissue (ILT), **F** salmonid bursa, **G** olfactory organ with the nasopharynx-associated lymphoid tissue (NALT)



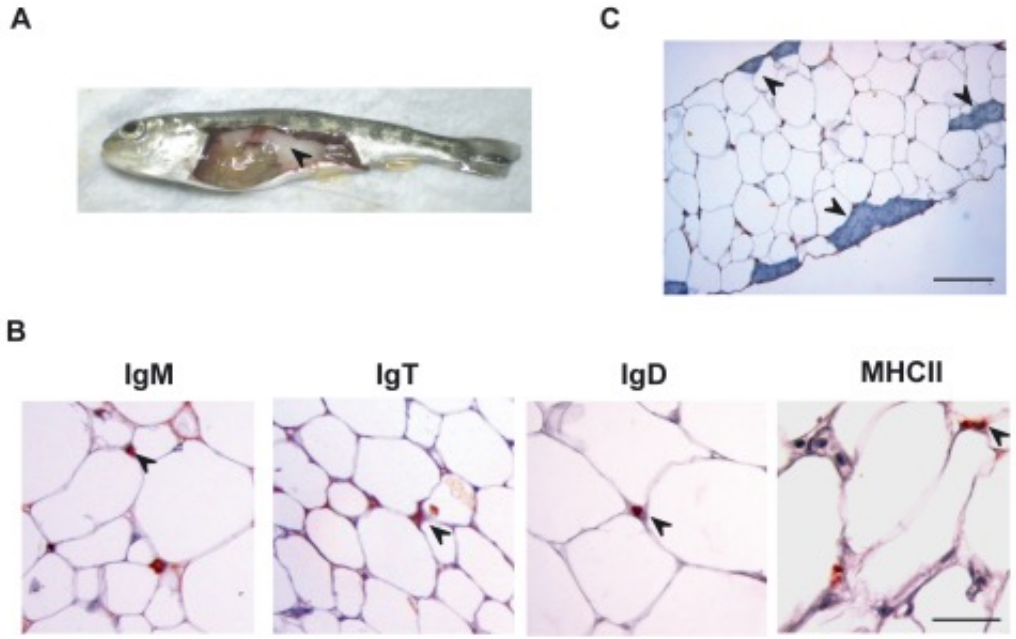
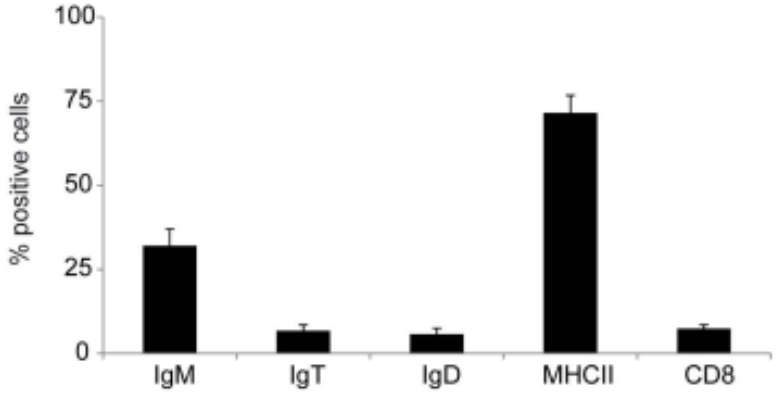


Figure 1. Immunohistological analysis of visceral rainbow trout AT. (A) The rainbow trout visceral AT (black arrow) was removed, fixed in Bouin's solution, embedded in paraffin and sectioned at 5 µm. After dewaxing and rehydration, sections were subjected to an indirect immunocytochemical method to detect trout IgM, IgT, IgD and MHC-II (B) Arrow heads point to representative positive staining. Scale bars, 50 µm. (C) Representative photomicrograph of an IgM immunostained section showing structures that resemble mammalian milky spots (arrow heads). Scale bars, 100 µm.
doi:10.1371/journal.pone.0110920.g001

Naïve fish adipose tissue found to contain APCs and T-cells which then increased in size, number and complexity following vaccination. Following peritoneal stimulation **the visceral adipose mass in fish likely plays an important role in vaccine antigen uptake and presentation** by APCs, as well as subsequent T-cell activation and differentiation.



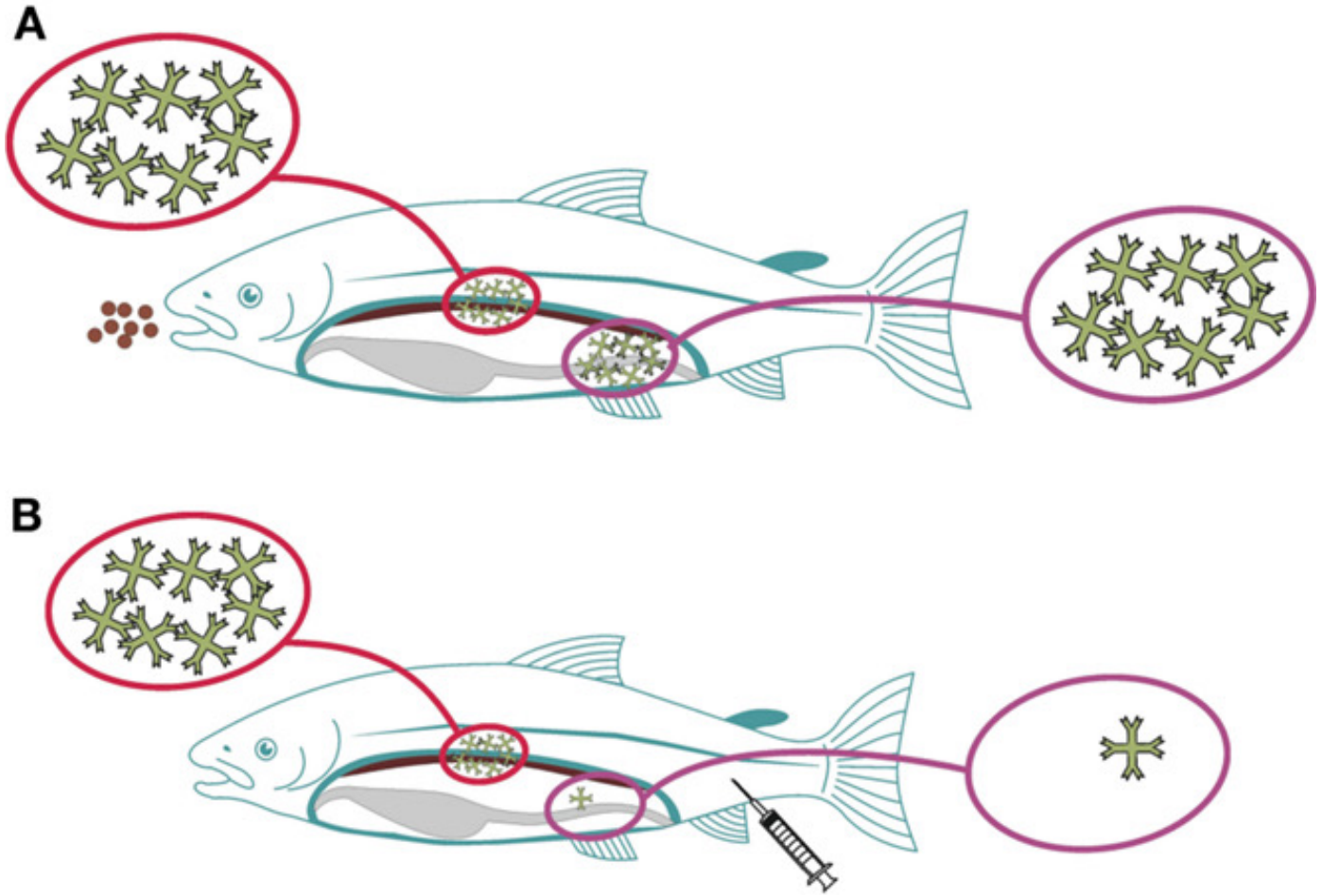
Pignatelli et al. (2014). PLoS ONE 9(10): e110920. doi:10.1371/journal.pone.0110920

Veenstra et al., 2019. Fish & Shellfish Immunology, Volume 87, https://doi.org/10.1016/j.fsi.2019.02.001.

PROPOSED ASYMMETRY FOR IMMUNE RESPONSES INDUCED VIA MUCOSAL (GUT) VERSUS PARENTERAL ROUTES IN FISH.

When antigens are delivered via the gut, local and systemic immune responses will be elicited, symbolized by high amounts of circulating IgM (A).

When the antigens are delivered parenterally, systemic responses will be strong, while local (gut) responses will be almost absent (B).



Mutoloki Stephen, Munang'andu Hetron Mweemba, Evensen Øystein. Oral Vaccination of Fish – Antigen Preparations, Uptake, and Immune Induction. Frontiers in Immunology
VOLUME=6 YEAR=2015

Anticorpi nei pesci

Teleost IgM

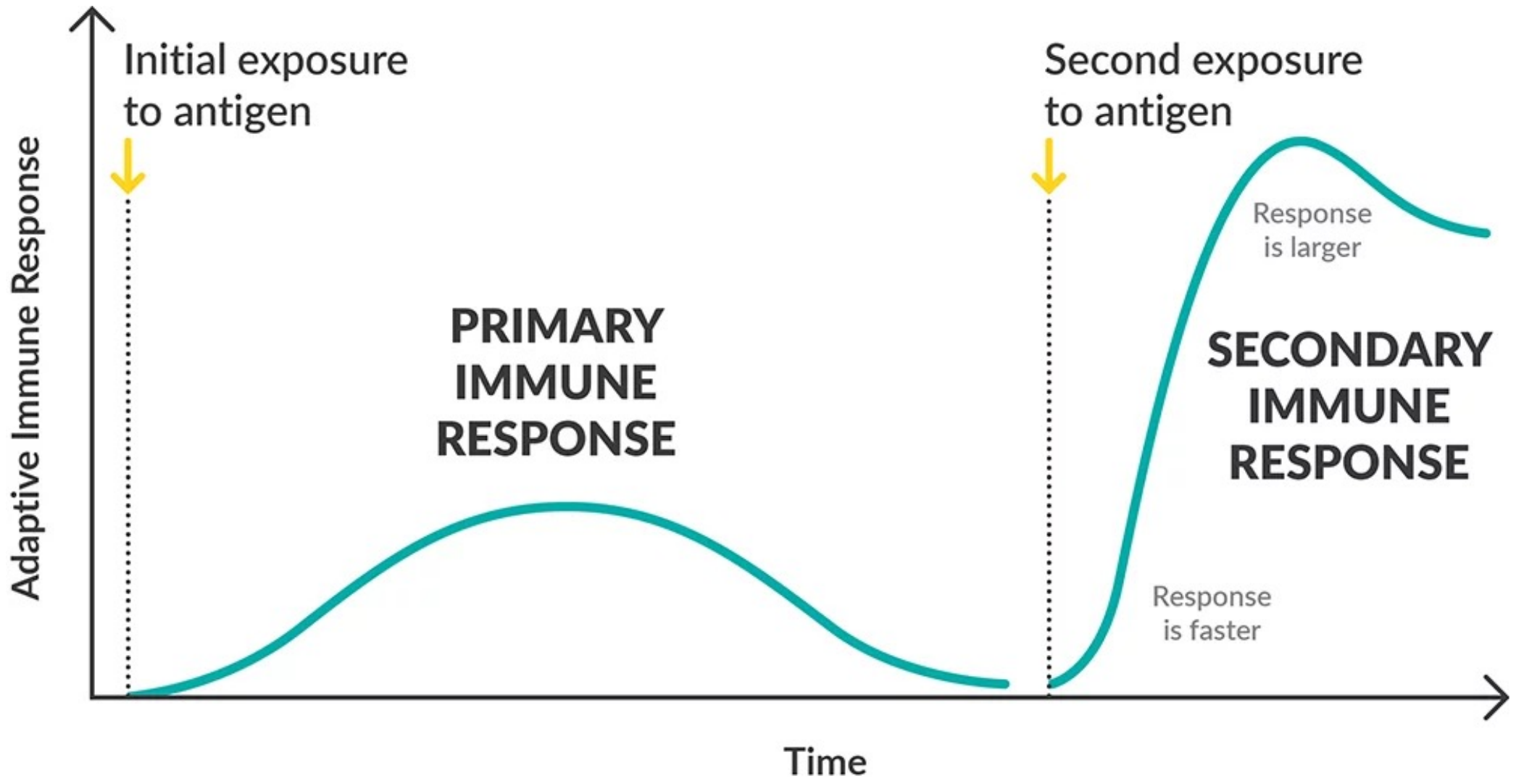
tetrameric molecule - most prevalent immunoglobulin in plasma - gut and skin mucus are reported to have very low concentrations of IgM - secreted mainly by plasma-like cells that are located mostly in the head kidney - after booster immunization teleost fish undergo a substantial increase in IgM titers (temperature dependent process)

Teleost IgT

specialized in gut mucosal immunity - present in serum as monomers, whereas in the gut mucus it forms mainly multimers similar in mass to those of IgM - most bacteria in the gut lumen of rainbow trout are coated with IgT, and IgT responses to gut parasites are measurable only in the gut, whereas IgM responses are detected only in serum - although it is suspected that IgT has a key role in other mucosal areas (such as the skin and gills)

Teleost IgD

variety of secreted IgD isoforms with different molecular masses (monomers in serum) - role remains obscure


Adaptive Immune Response
Initial exposure to antigen
Second exposure to antigen
PRIMARY IMMUNE RESPONSE
SECONDARY IMMUNE RESPONSE
Response is larger
Response is faster
Time

Trasferimento di fattori di immunità dai riproduttori alla progenie

Sviluppo del sistema immunitario negli avannotti

Comparsa linfociti B e anticorpi + tardiva nelle specie marine rispetto a quelle di acqua dolce (dati specie specifici)

Trasferimento di anticorpi “materni” a uova/embrioni dimostrato in varie specie ittiche.

IMPORTANTE per sviluppare protocolli di vaccinazione riproduttori

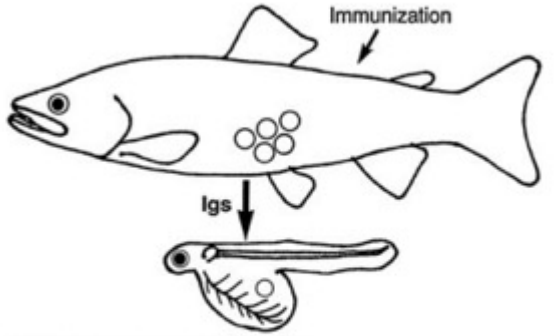


FIGURE 6.3 Maternal immunization provides offspring with protection via antibody. Transfer of maternal immunity has been demonstrated in various fish species.

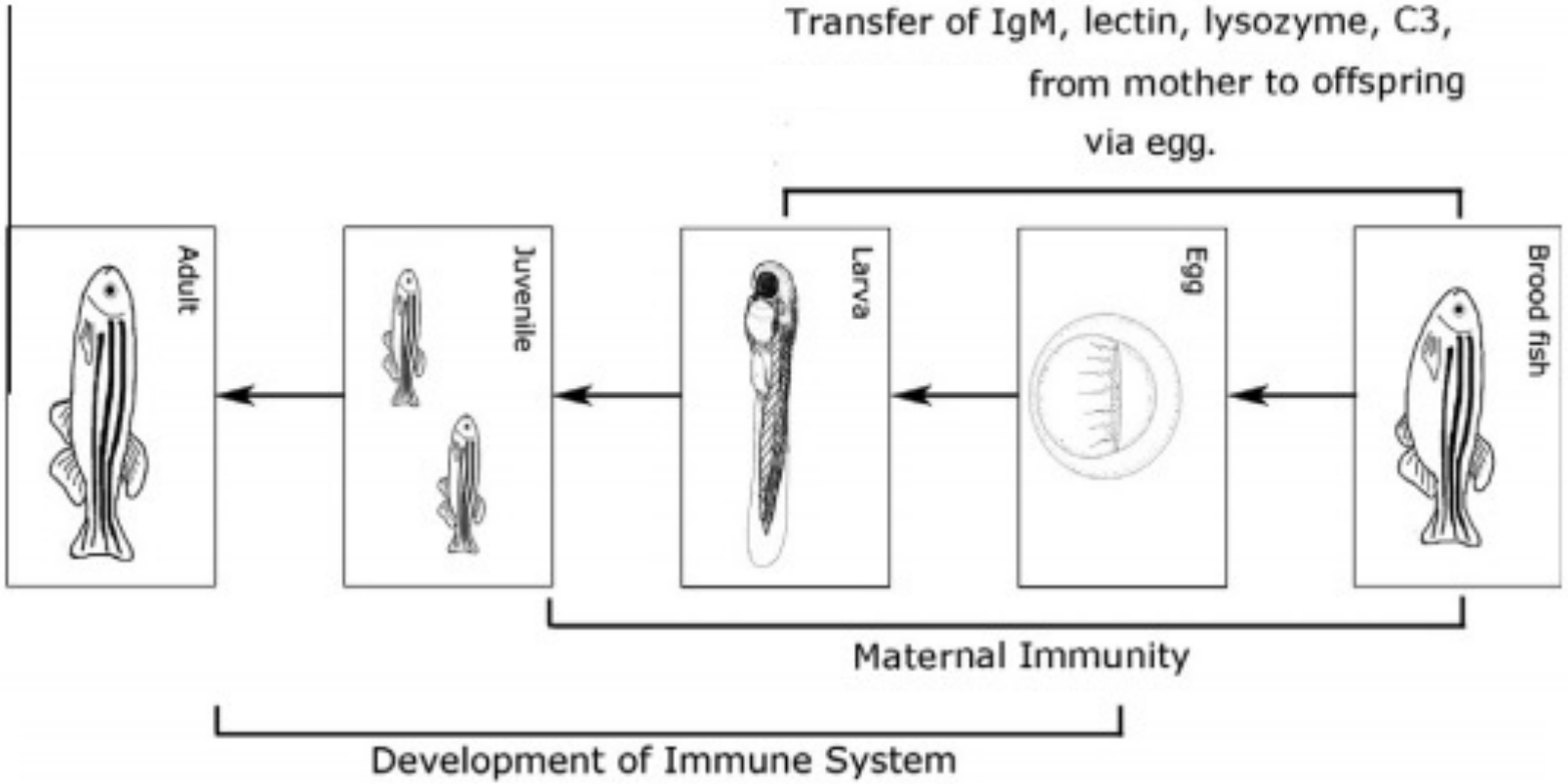


Fig. 1. A diagram showing the transfer and persistence of maternal immunity with respect to acquisition of immunocompetence at the different stages of development. Both innate and adaptive immune-relevant factors are transferred from mother to offspring, which play a critical role to protect the vulnerable offspring against pathogenic attacks before full development and maturation of immune system in fish.

TABLE 6.1 Maturation of lymphoid organs and adaptive immune responses for cell-mediated immunity and antibody production.

Fish species (temperature)	Appearance of lymphocytes (organ)	Appearance of Ig + lympho-cytes (organ)	Occurrence of allograft rejection	Antibody production (antigen: + response, −tolerance)	References
Carp (22°C)	3 dph (thymus)	1 mph (spleen)	16 dph (subacute)	4 wph (*A. salmonicida*: +, HGG: −, SRBS: −)	Botham and Manning (1981)
	6 dph (kidney)			2 mph (HGC + FCA: +, *A. salmonicida*: +)	van Loon et al. (1981)
	8 dph (spleen)				
Rainbow trout (14°C) 	3 dph (thymus)	4 dph (kidney)	14 dph (chronic)	3 wph (HGG + FCA: −, *A. salmonicida*: + nonmemory)	Grace and Manning (1980)
	4–5 dph (kidney)	1 mph (spleen)	21 dph (subacute)	2 mph (HGG + FCA: +, *A. salmonicida*: +)	Tatner and Manning (1983)
	6–14 dph (spleen)				Grace and Manning (1980)

dph, day posthatch; *HGG*, human gamma globulin; *mph*, month posthatch; *SRBC*, sheep red blood cells; *wph*, week posthatch.

In European sea bass (*Dicentrarchus labrax*), complete immunological maturity is achieved between 137- and 145-days post-hatching (dph), when adult levels of T and B lymphocytes are reached. Before then, fish immunity is based on innate responses.

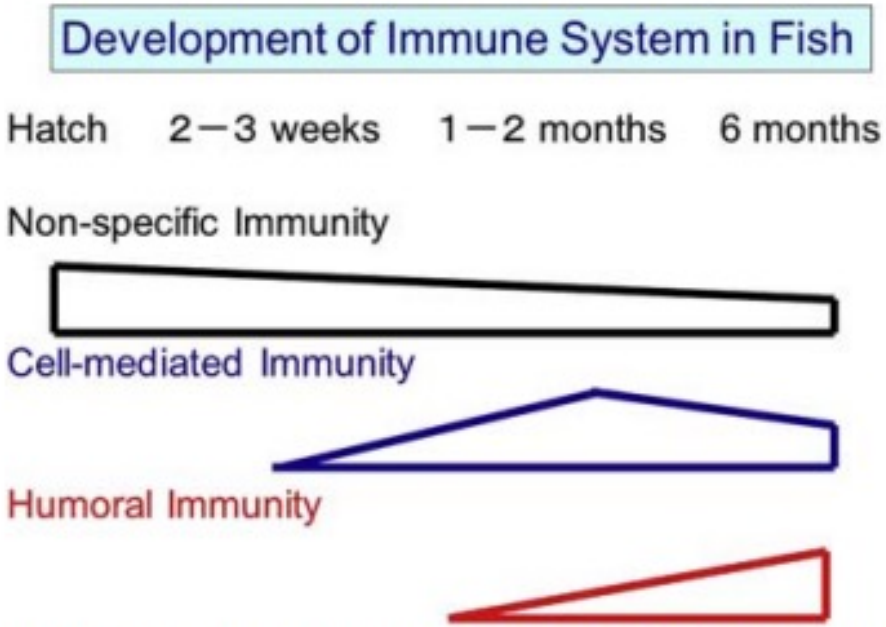
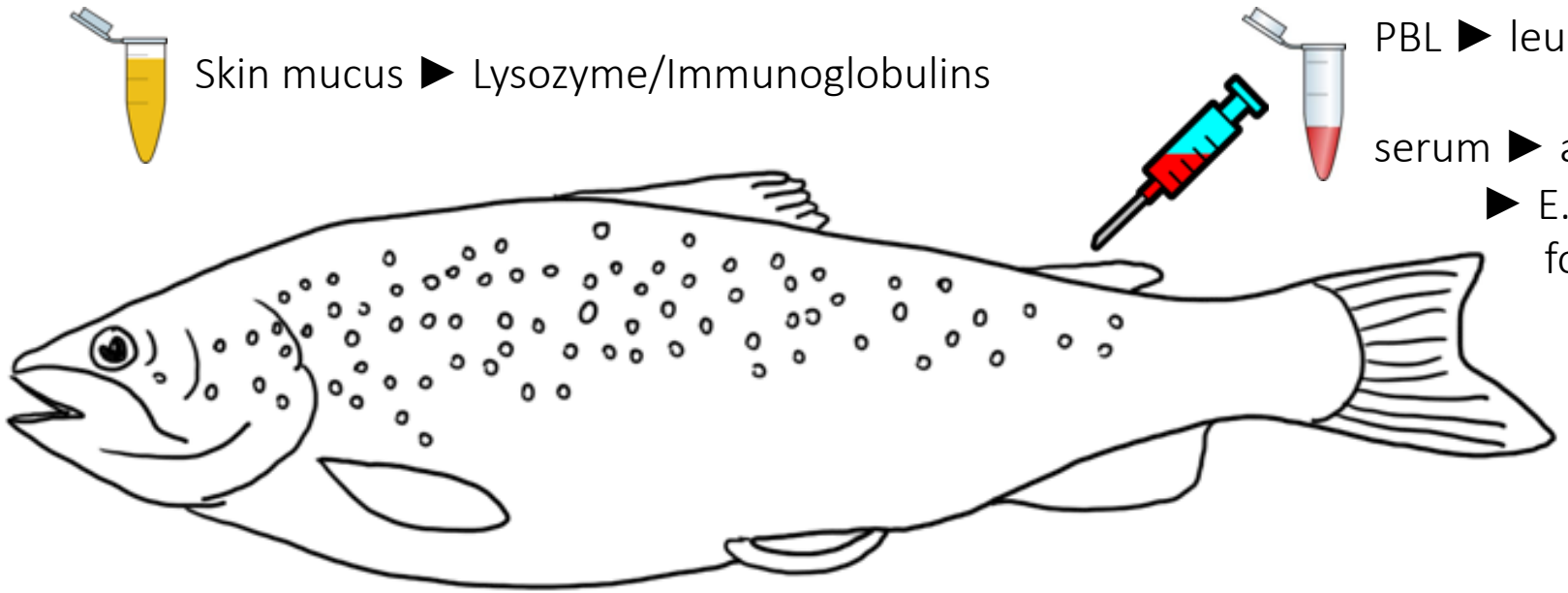


FIGURE 6.2 Schematic illustration of the development of the immune system in teleost fish. Nonspecific immunity is functional before hatch (*black* [*black* in print version]). Cell-mediated immunity (*blue* [*black* in print version]) develops earlier than humoral immunity (*red* [*gray* in print version]). Height of the diagrams indicates the relative importance of each immunity in host defense.

Come valutare la risposta immunitaria dopo la vaccinazione?

Raccolta di campioni biologici a diversi time points



Skin mucus ► Lysozyme/Immunoglobulins

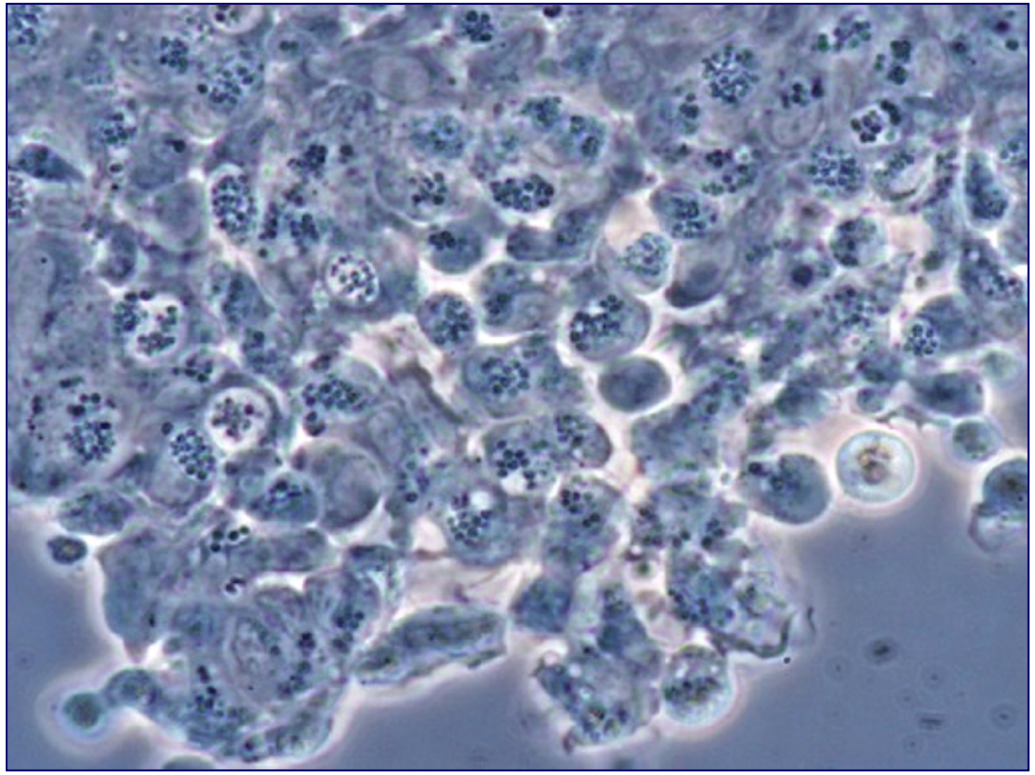
PBL ► leucogram

serum ► aspecific parameters
► E.L.I.S.A./agglutination
for specific IgM

HK/PBL for leukocytes respiratory burst/proliferation
HK/spleen/gut/gills ► qPCR for immune genes expression

Campioni in pool o individuali, per ottenere siero/plasma/leucociti

Valutazione *in vitro* delle attività biologiche dei leucociti (fagocitosi-burst-proliferazione) in seguito allo stimolo con “candidati” antigeni



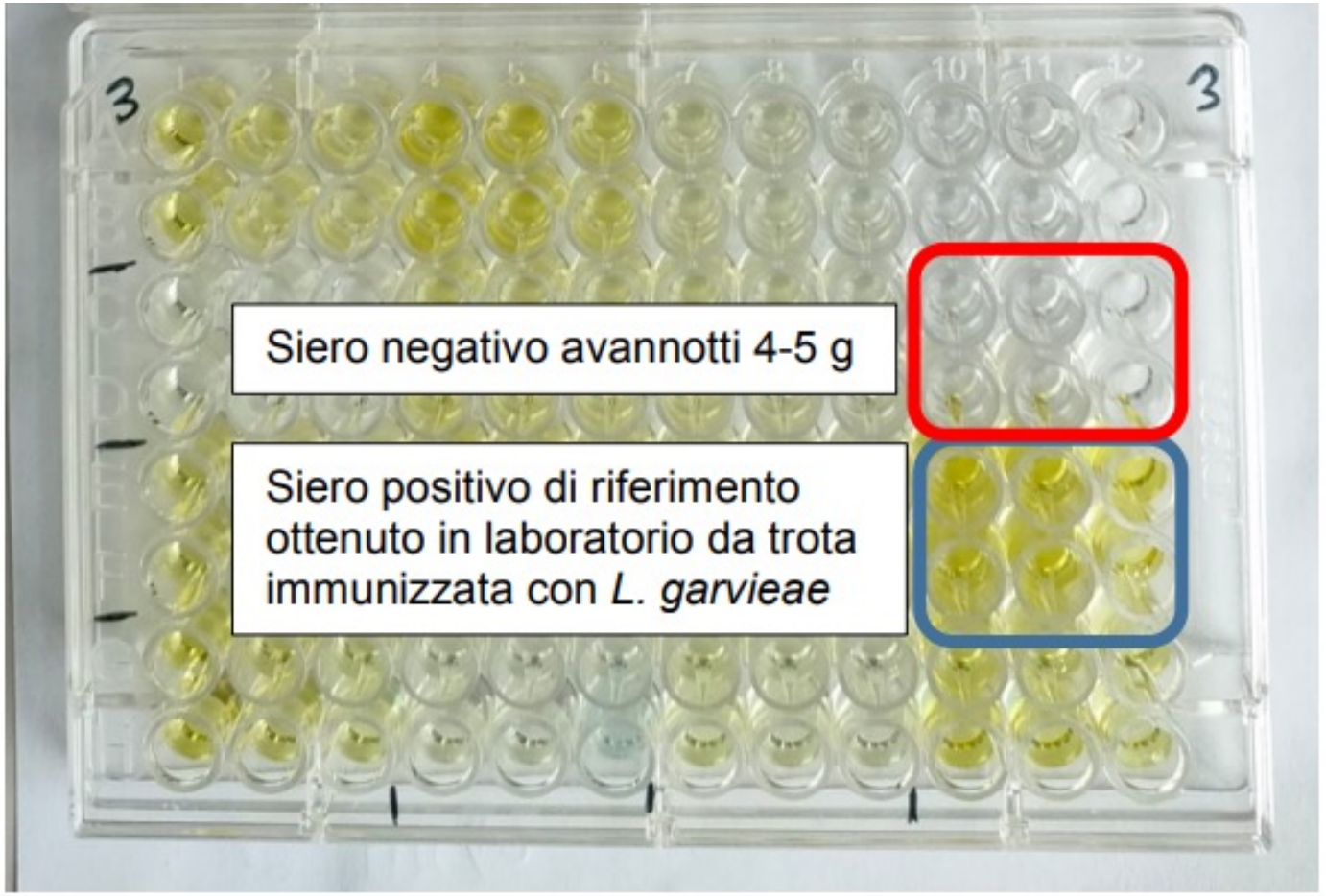


Fig. 6. Esempio di piastre usate per il test ELISA. Nel riquadro rosso è evidente la lettura ottenuta dai soggetti non vaccinati prelevati in avannotteria (controllo negativo). Nel riquadro azzurro è evidente la lettura ottenuta con un siero di trota disponibile in laboratorio (controllo positivo). Nel resto dei pozzetti sono contenuti i sieri oggetto di valutazione, con vari livelli di positività.

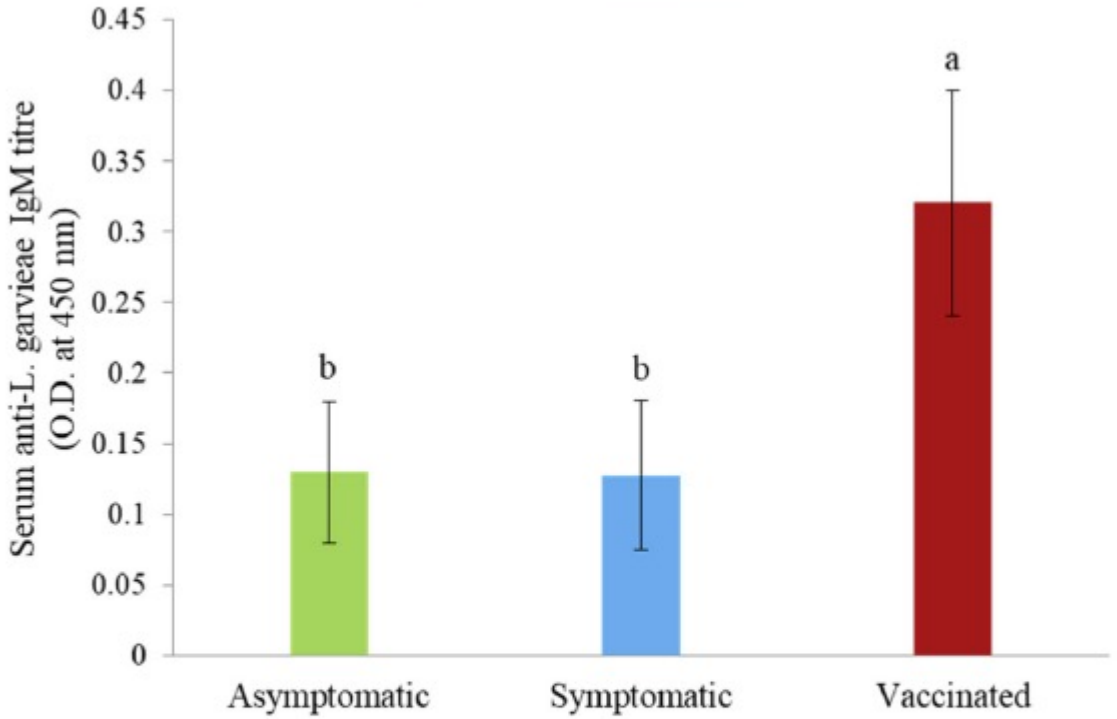


FIGURE 6 Serum IgM titres against *Lactococcus garvieae* detected by ELISA (O.D. at 450 nm) in unvaccinated asymptomatic, unvaccinated symptomatic and vaccinated rainbow trout. Data are expressed as mean ± SD ($n = 10$ for symptomatic and vaccinated; $n = 20$ for asymptomatic). Different letters indicate significant differences among groups ($p \leq .05$).

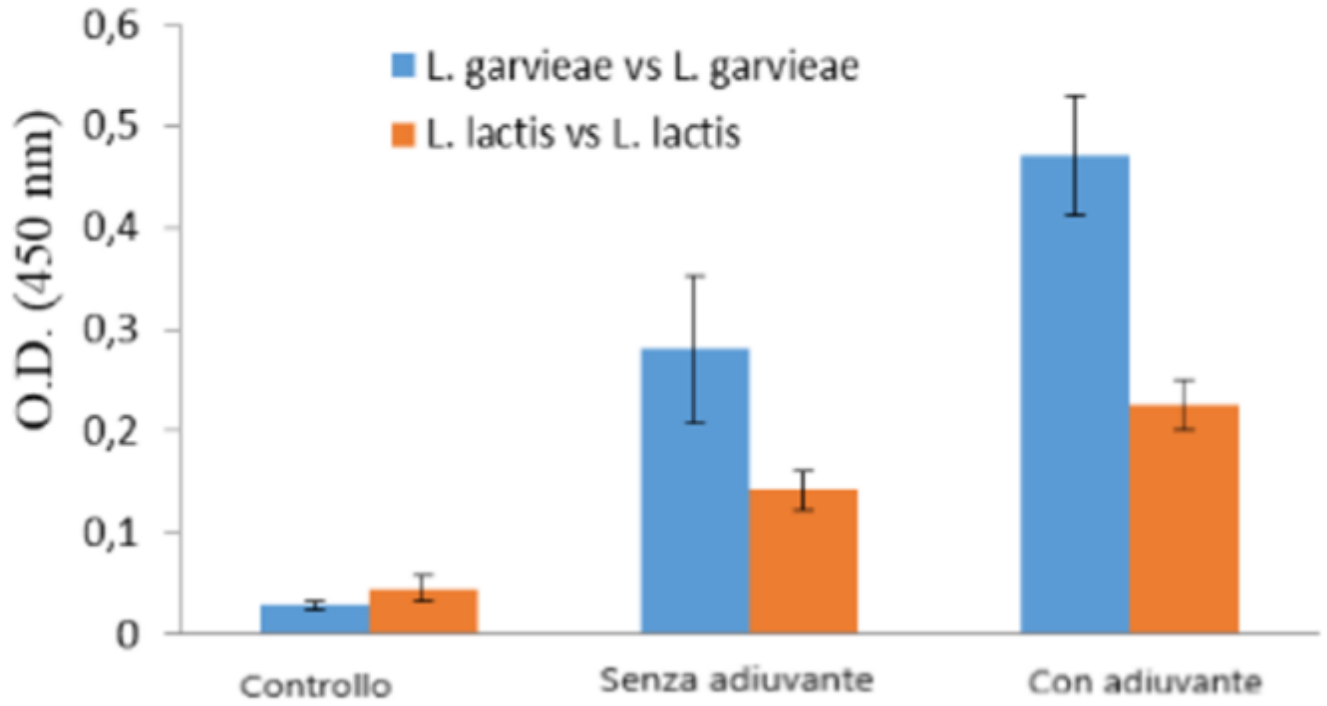


Grafico 1 - Risposta anticorpale specifica (IgM) dei soggetti vaccinati con bacterin *L. garvieae* o *L. lactis*, con e senza aggiunta di adiuvante. Nel grafico sono riportati anche i risultati ottenuti per i soggetti di controllo. Il coating è stato effettuato con il medesimo antigene usato per immunizzare i pesci. I sieri sono stati analizzati previa inattivazione al calore. Ogni gruppo sperimentale era composto da un numero di soggetti variabile da 3 a 11.

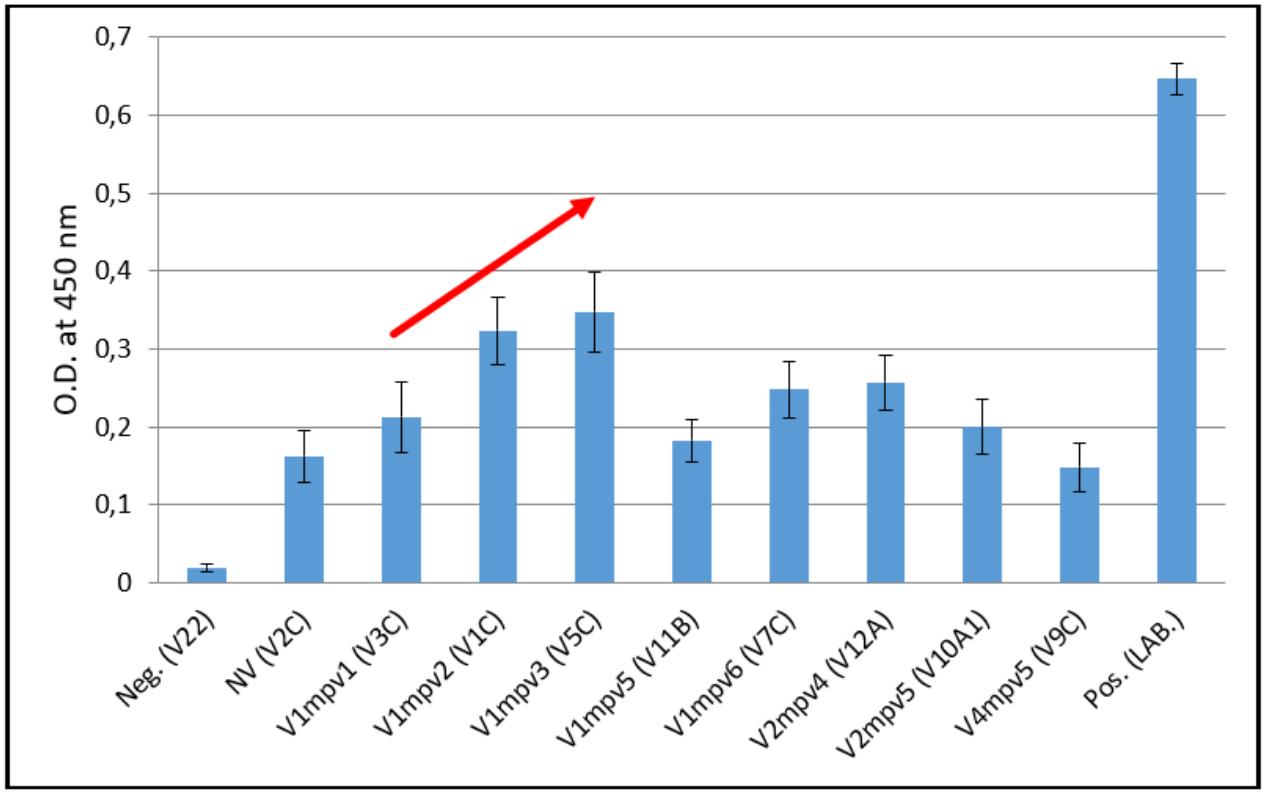


Fig. 7. Esito delle analisi relative al test ELISA. Ciascuna colonna rappresenta la lettura ottenuta come media di 10 soggetti per ciascun bacino, ad esclusione dei campioni di riferimento negativo e positivo (prima e ultima colonna) che sono singoli. In base alla nostra esperienza, i campioni possono essere considerati negativi per *L. garvieae* quando il valore di O.D. è inferiore a 0,2.

Legenda:

Neg, siero avannotti 4-5 g prelevati prima di uscire dall'avannotteria;
Pos, siero trota positivo per *L. garvieae* disponibile in laboratorio UNIUD;
NV, non vaccinato;
V1, V2, V4, vaccinati 1, 2 o 4 volte;
mpv, mesi post vaccinazione.

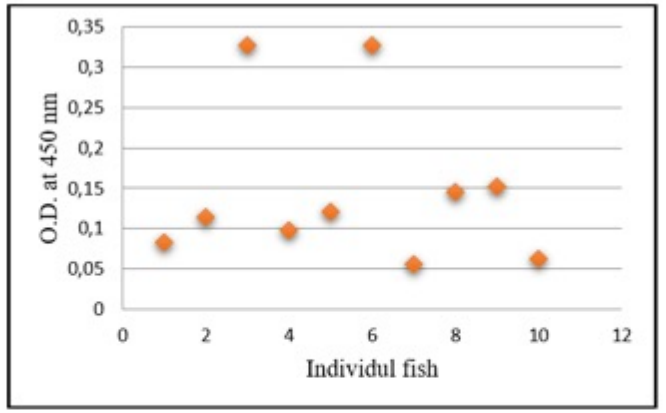


Fig. 8. Risultato di lettura ottenuto per i singoli individui nella vasca V9C, il cui valore medio è 0,15. Due soggetti su dieci hanno valore superiore a 0,3, mentre 8 su 10 hanno valore uguale o inferiore a 0,15, per cui la media totale della vasca risulta al di sotto del valore di riferimento 0,2. I pesci sono stati analizzati individualmente per tutte le vasche, quindi è possibile disporre di tutti i valori individuali. Abbiamo riportato la vasca V9C come esempio.

Indagine svolta presso
AZIENDA AGRICOLA ITTICA RIO SELVA S.R.L

IMPACT OF WATER TEMPERATURE ON THE SEA BASS (*D. LABRAX*) RESPONSIVENESS TO IN FIELD VACCINATION AGAINST VIBRIOSIS/PHOTOBACTERIOSIS

Torino 11-13 ottobre 2018

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XXIV Convegno Nazionale S.I.P.I.

AUTUMN/WINTER SEASON

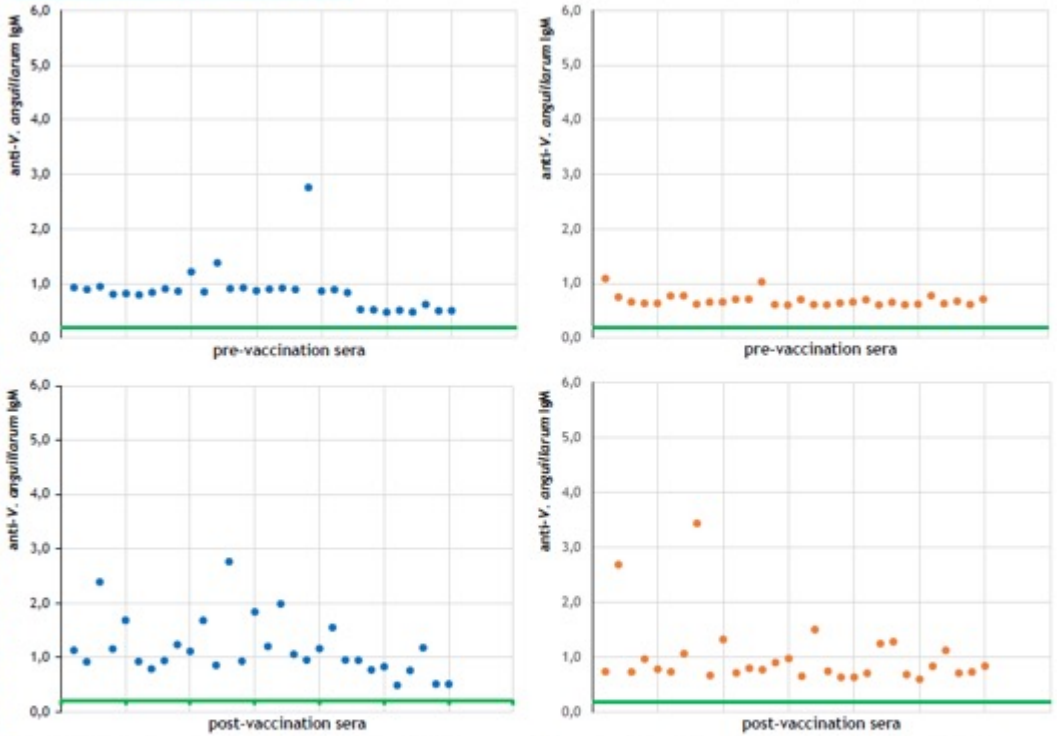

Fig. 3a (above) - 3b (below). Specific IgM in sera collected from sea bass allocated to cage LADL17 SK2 before vaccination (27/11/2017, 15.6°C) and after vaccination (04/01/2018, 13.7°C). The green line indicates the blank mean O.D. + 3 standard deviations of the blank O.D.

Fig. 4a (above) - 4b (below). Specific IgM in sera collected from sea bass allocated to cage LADL17 K9 before vaccination (18/11/2017, 16.4°C) and after vaccination (20/12/2017, 14.1°C.). The green line indicates the blank mean O.D. + 3 standard deviations of the blank O.D.

SPRING SEASON

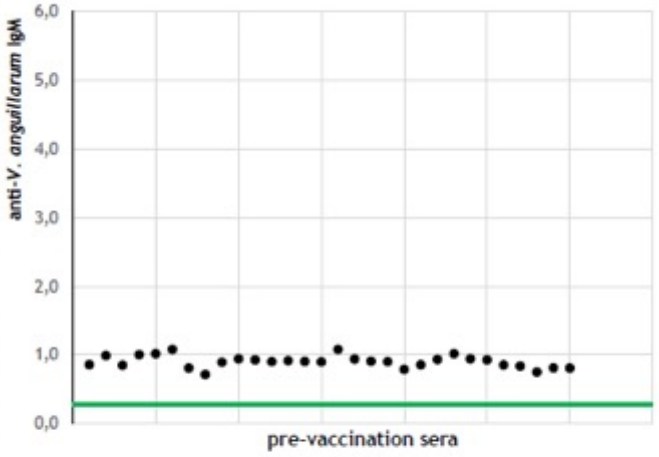

Fig. 7a. Specific IgM in sera collected from sea bass allocated to cage LADL17 K21 before vaccination (30/05/2018, 21.7°C). The green line indicates the blank mean O.D. + 3 standard deviations of the blank O.D.

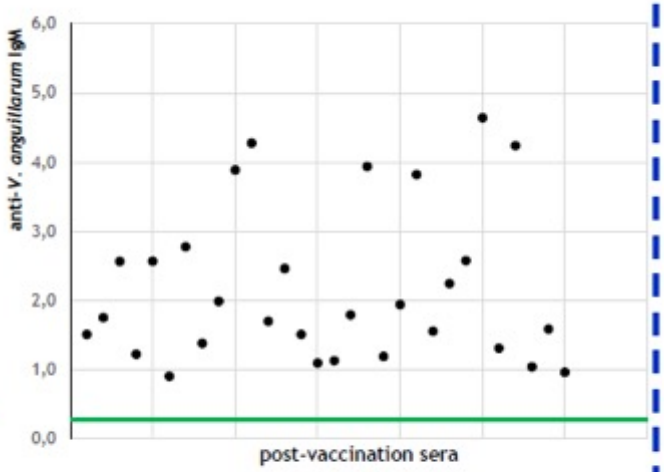

Fig. 7b. Specific IgM in sera collected from sea bass allocated to cage LADL17 K21 after vaccination (21/06/2018, 22.5° C). The green line indicates the blank mean O.D. + 3 standard deviations of the blank O.D.

Jose Priya and Kappalli
Vaccine 40 (2022)
5873–5881

Table 1
The immunological perspective of vaccine administration methods.

Criteria	Injection delivery		Immersion delivery		Oral delivery	
	Intraperitoneal	Intramuscular	Direct immersion	Spray vaccination	Vaccine through feed	Encapsulated vaccines
Vaccination process	Time-consuming		Less time-consuming		Requires no extra time than routine farm husbandry	
Manual labour	labour intensive		Less labour		Requires no extra labour than routine farm husbandry	
Mass vaccination	Difficult to administer a large number of animals		A large number of animals can be vaccinated at the same time			
Stress to the immunised animal	Stress due to several steps, including fasting, weighing, anaesthetising, injection, and recovery		Do not cause stress	Stress due to spray pressure	Do not cause stress	
Immune response	Sub-optimal and short-term protection		Less chance of antigen uptake	Elicits both local and systemic innate and adaptive immune responses	Provoke poor and not long-lasting immunity	Elicit long-lasting and robust immunity
Antigen dosage	Low amounts of antigen are sufficient		A large dose of antigen is required for effective uptake	A large dose of antigen is required	A large dose of antigen is required for effective immunity	Low amounts of antigen are sufficient
Antigen delivery to immune responsive sites	Fast and complete absorption of antigen into the systemic circulation via capillary and lymphatic transport	Antigen find its way between muscle fibres, but slow release into the target tissue	Antigens are taken up by the skin, gills or gut and processed by the immune system		Low pH and high enzymatic activity in the foregut tend to destroy the vaccine and cause poor antigen delivery to the hindgut and other lymphoid organs	Encapsulation material helps to resist the vaccine destruction in the foregut, favouring better antigen delivery to immune responsive sites in the hindgut
Target animal	Cultured fish should be of a reasonable size. Fry cannot be vaccinated by i.p injection	Enable vaccinating fry of any size above the critical size of immune responsiveness	Cultured fish of any size can be vaccinated			
Specific target immunity	Systemic immunity	Local inflammatory responses	Mucosal immunity		Elicits both local and systemic innate and adaptive immunity	
Key sites of antigen uptake	Peritoneal cavity	Inflammatory cells	Olfactory organ, skin and gills		Hindgut and other lymphoid organs	
Need for an adjuvant	Oil-based adjuvants are required to prolong the protection					Adjuvants are not required

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Systems biology

Computational modeling of immune system of the fish for a more effective vaccination in aquaculture

lent formulation against La and Psp. In this work, the immunological model C-ImmSim (Bernaschi and Castiglione, 2001; Castiglione, 2006; Castiglione *et al.*, 2005; Motta *et al*, 2005; Pappalardo *et al.*, 2005) was applied to simulate the sea bass immune response against two pathogens. To test the model's ability to properly reproduce the main immunological parameters and fish survival under different vaccination conditions, the model results were compared with the *in vivo* findings with single or double pathogens. The work was divided into three steps: (i) set up of the optimal vaccine/infectious doses; (ii) comparison of the *in silico* and *in vivo* specific immune parameters; (iii) comparison of the cumulative mortality rates during the challenge infections. The present study represents an innovation in fish immunology research and supports the *in silico* approach as a supplementary tool to optimize the design of vaccination treatments in aquaculture prior to large-scale application.

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