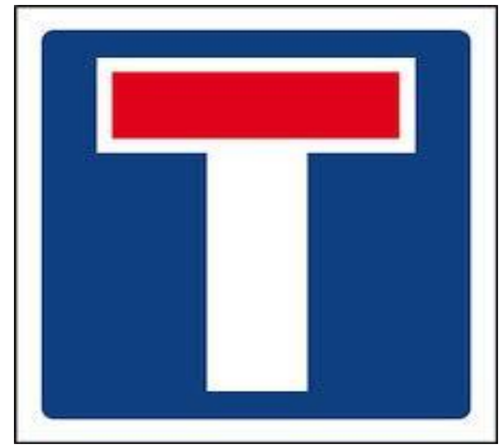
FISH VACCINES – A SHORT, BUT REMARKABLE,JOURNEY

Professor Patrick Smith
Tethys Aquaculture Ltd

Veterinary Vaccinology Network Meeting
Birmingham ICC
February 16th-17th 2015

Aquatic Animal Health Research



An exciting new highway **OR** A ‘blind alley’

Patrick Smith

Tethys Aquaculture Ltd

Fish Vaccines and Fish Vaccine Research

“From Zero to Hero”

The Aquaculture Industry

- **65 million tonnes**
- **US$ 150 billion**
- **40% of whole fish consumption**
- **Crossover (50/50) predicted between 2025 and 2030 already exceeded wild catch in Mediterranean**
- **Growth at 10-12% per annum (1% = capture fish; 2.3% = other animal production**
- **Fin-fish (30+ species), shellfish, crustaceans, algae**
- **Becoming a key component of worldwide Food Security Programmes**

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Commercially-Available Fish Vaccines

1982

1 Enteric Redmouth (ERM) vaccine
2 *Vibrio anguillarum* vaccine

TOTAL = 2

2014

1 Enteric Redmouth (ERM) vaccine
2 *Vibrio anguillarum* vaccine
3 Furunculosis vaccine
4 *Vibrio salmonicida* vaccine
5 Combined Vibriosis/Furunculosis vaccine
6 Combined Vibriosis/Furunculosis/Coldwater Vibriosis/*Moritella viscosa* vaccine
7 Combined Vibriosis/Furunculosis/Coldwater Vibriosis/*Moritella viscosa*/IPNV vaccine
8 IPN Virus vaccine
9 Pasteurella vaccine
10 Combined Pasteurella/Vibriosis vaccine
11 Vibriosis vaccine for cod
12 Shrimp Vibriosis vaccine
13 Warmwater *Vibrio* spp vaccine
14 SVC virus vaccine
15 *Lactococcus garvieae/Streptococcus iniae* vaccine
16 KHV vaccine
17 *Aeromonas hydrophila* vaccine
18 Carp Erythrodermatitis/Ulcer disease vaccine
19 *Piscirickettsia salmonis* vaccine
20 ISA virus vaccine
21 Gaffkaemia vaccine
22 *Flavobacterium psychrophilum* vaccine
23 Nodavirus vaccine
24 Pancreas disease virus vaccine
25 *Edwardsiella ictaluri* vaccine

TOTAL = 25 +

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Benefits of vaccination

- Economic benefits
- Environmental benefits
- Animal welfare
- Reduction in the use of antibiotics

Reduction in levels of disease

Reduction in losses due to disease

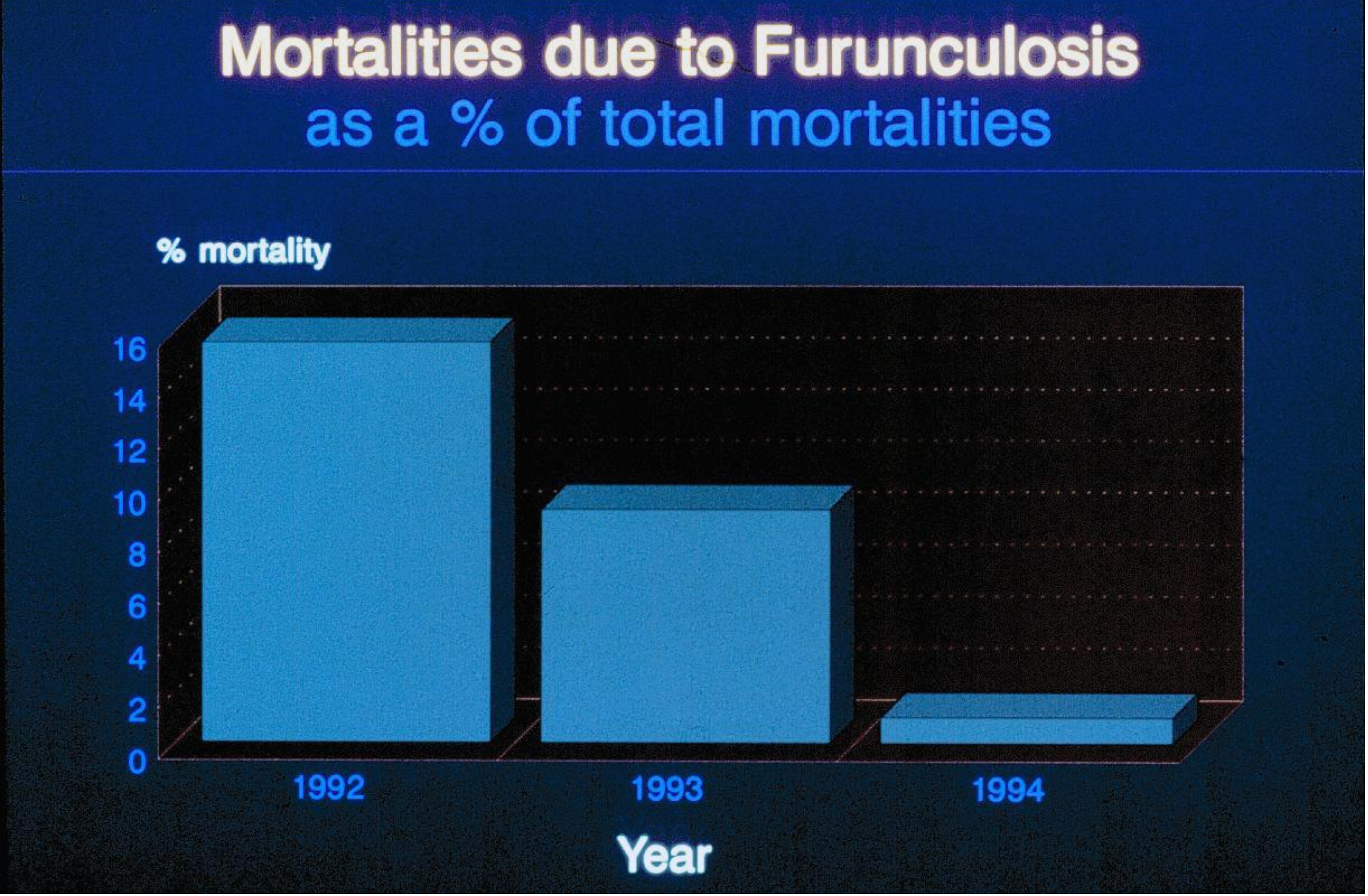
ERM VACCINE FIELD TRIALS

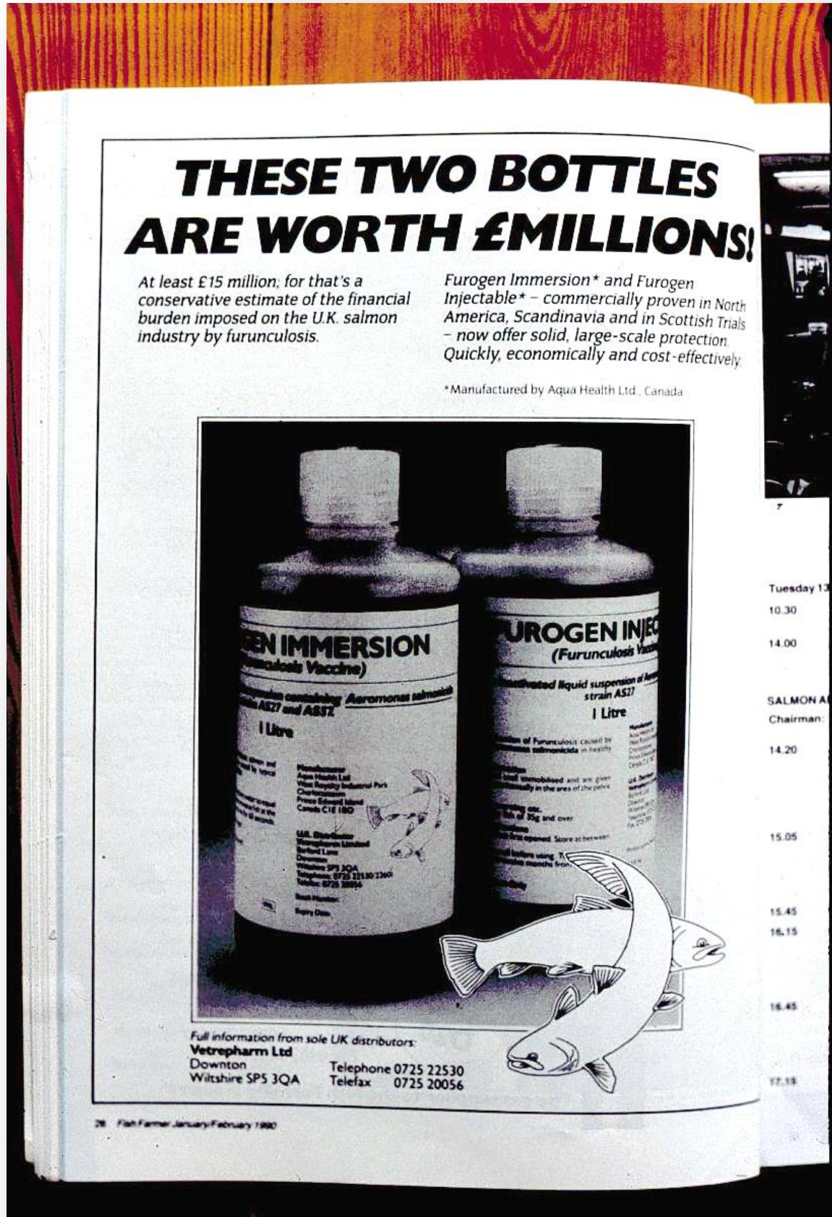
Field Performance of ERM Vaccines

		Number	Mortality	%	RPS
Trial 1	Vaccinated	10,095,793	119,568	1.18	67
	Control	1,869,524	67,298	3.60	
Trial 2	Vaccinated	9,727,850	135,139	1.39	89
	Control	1,521,516	184,840	12.48	
Trial 3	Vaccinated	3,135,686	45,108	1.44	86
	Control	831,688	85,504	10.28	
Trial 4	Vaccinated	82,500	3,878	4.7	78
	Control	26,400	5,755	21.8	
Trial 5	Vaccinated	129,600	0	0	100
	Control	101,805	45,252	44.45	

Weight at vaccination: 4.5g (100/lb)
Trials 1-3: weight vaccinated 103 tonnes

External Symptoms


Mortalities due to Furunculosis
as a % of total mortalities
% mortality
16
14
12
10
8
6
4
2
0
1992
1993
1994
Year


THESE TWO BOTTLES ARE WORTH £MILLIONS!
At least £15 million; for that's a conservative estimate of the financial burden imposed on the U.K. salmon industry by furunculosis.
Furogen Immersion* and Furogen Injectable* – commercially proven in North America, Scandinavia and in Scottish Trials – now offer solid, large-scale protection. Quickly, economically and cost-effectively.
*Manufactured by Aqua Health Ltd., Canada
IMMERSION
I Litre
UROGEN INJEC
(Furunculosis
I Litre
Full information from sole UK distributors:
Vetrepharm Ltd
Downton
Wiltshire SP5 3QA
Telephone 0725 22530
Telefax 0725 20056
28 Fish Farmer January/February 1990
Tuesday 13
10.30
14.00
SALMON A
Chairman:
14.20
15.05
15.45
16.15
16.45
17.15

Reduction in the use of antibiotics in the aquaculture industry following widespread adoption of vaccines

ESSENTIALLY A ‘GOOD NEWS’ STORY

ERM VACCINE FIELD TRIALS

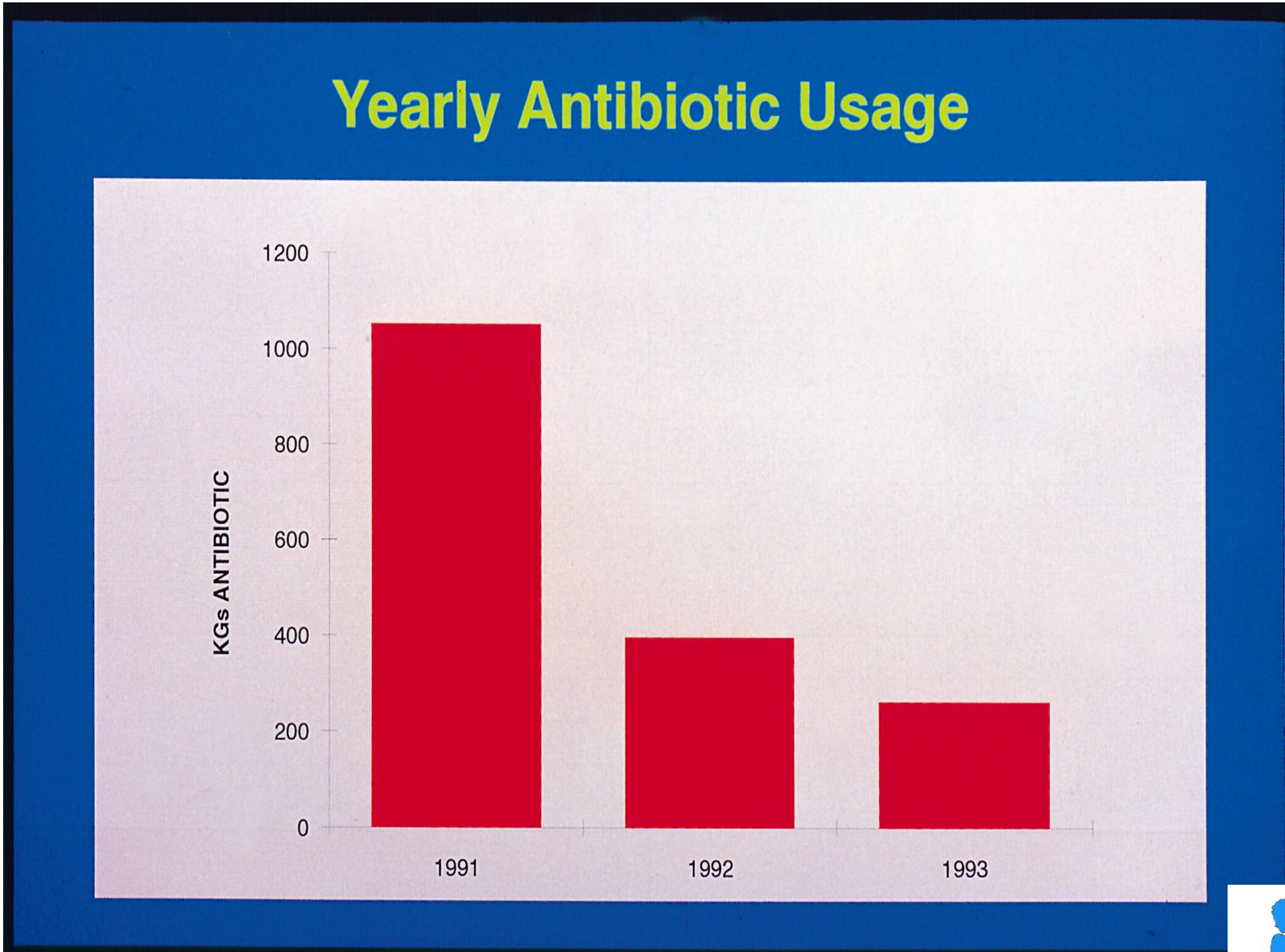
Medicated Feed Utilisation

	Medicated Feed (kg)/ 1000 Fish Vaccinates	Medicated Feed (kg)/ 1000 Fish Controls	% Reduction
Trial 1	4.5	16.8	73
Trial 2	2.27	15.9	86
Trial 3	6.3	22.0	72

ANTIBIOTIC USE IN EARLY DAYS OF FARMING ATLANTIC SALMON

- Introduction of a number of bacterial diseases- notably furunculosis.
- First response in absence of vaccine was to use a range of antibiotic treatments.
- “Dirty industry ?”
- Multiple resistance became commonplace.
- Effective vaccines developed and initial success led to widespread adoption of vaccination throughout industry.

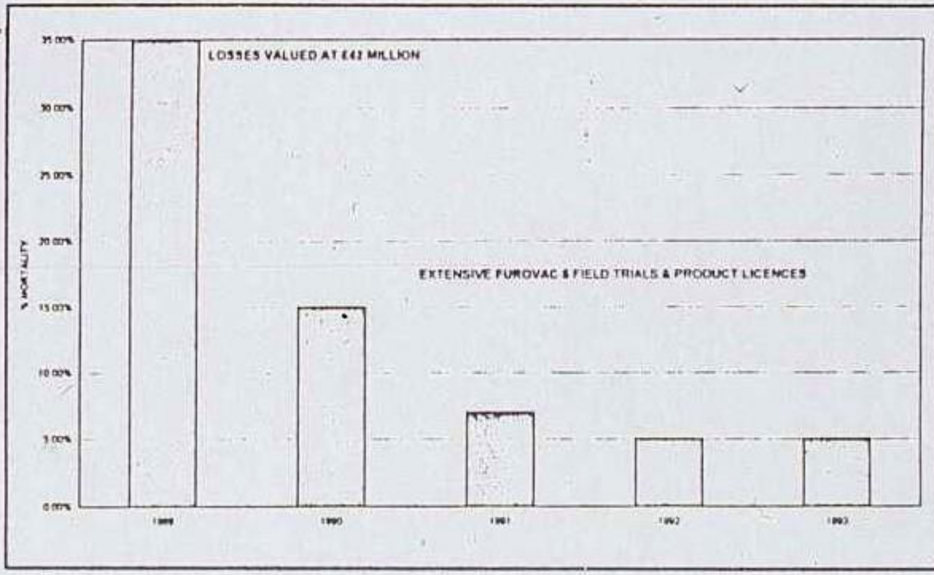
TETHYS AQUACULTURE



Yearly Antibiotic Usage
KGs ANTIBIOTIC
1200
1000
800
600
400
200
0
1991
1992
1993

SCOTLAND

AVL to show vaccines which saved Scottish farm salmon



Scottish salmon farm losses. In 1989, most of these were due to furunculosis.

ON ITS stand at the Scottish Fish Farming Exhibition in Aviemore next month, Aquaculture Vaccines Ltd will show products which have resulted from an ambitious research program to developed vaccines against new diseases of farmed fish.

AVL supports its wide range of health care products with a comprehensive technical back-up service.

Many of its vaccine projects are carried out in collaboration with various universities, research institutes and government laboratories specialising in aquaculture. Scottish Salmon Growers Association, the Institute of Aquaculture and University of Stirling, and the aquaculture team at the Marine Laboratory of the Scottish Office Agriculture and Fisheries Department.

"These vaccines employ new technology to produce novel and highly protective antigens," says Robin Wardle of AVL. "Field data collected over the past four years during the field testing and the widespread commercial use after licensing have shown that they produce high levels of protection of long dura-

AVL has developed a range of different formulations of the Furovac 5 range. The latest addition is an orally administered furunculosis vaccine, Aquavac Furovac 5 Oral, which has been granted an ATX Certificate by the UK licensing authorities.

Therefore combinations of immersion applied, injection applied and oral vaccines can be used to protect against furunculosis during the life cycle of the salmon.

ATX Certificates have been granted for the orally administered forms of AVL's furunculosis (Aquavac Furovac 5 -Oral) and ERM (Aquavac ERM -Oral) vaccines. As reported earlier in FFI, AVL has also been able to test the oral version of its vibrio vaccine in sea bass and sea bream in Greece, France stomach of the fish, thus making them available to stimulate immunity in the lower gut.

The vaccine is in the form of a sterile emulsion which can be added to any feed pellets irrespective of size of manufacturer, either at the farm site or the feed mill.

Booster

Trials have shown that the oral vaccine can be used to regularly booster vaccinate fish which have been primarily vaccinated by immersion or injection. But experiments are in progress to evaluate vaccination and re-vaccination using only orally administered vaccine.

AVL's product range includes the bacterial isolation and latex agglutination diagnostic kits, which it han- nostic package will be introduced at the the Aviemore show.

The Aquarapid kit employs enzyme-linked immunosorbent assay (ELISA) technology and is designed to rapidly identify bacterial pathogens in diseased fish tissues.

AVL explains that the test centres around specific antibody-coated "spoons" which are used for all the incubation and washing stages. A positive result is indicated by an easily observed red coloration in the "spoon".

"The kit is robust and simple to use," Mr Wardle adds, "without the requirement for sophisticated laboratory equipment, thus making it ideal for the small diagnostic laboratory or for pond-side diagnosis. Results can be obtained within one hour of sampling the fish with short hands-on time of only ten minutes."

Aquarapid kits are available for the detection of the common bacterial diseases of farmed fish, including furunculosis, bacterial kidney disease, vibriosis, pasteurellosis, and enteric redmouth disease.

AVL says it it has other research projects in an advanced stage for the development of pasteurella vaccines, streptococcal vaccines, testing of multivalent oral vaccines, and the evaluation of the pharmaceutical Fumagilin for the prevention and treatment of myxosporidian infections of salmon and trout.

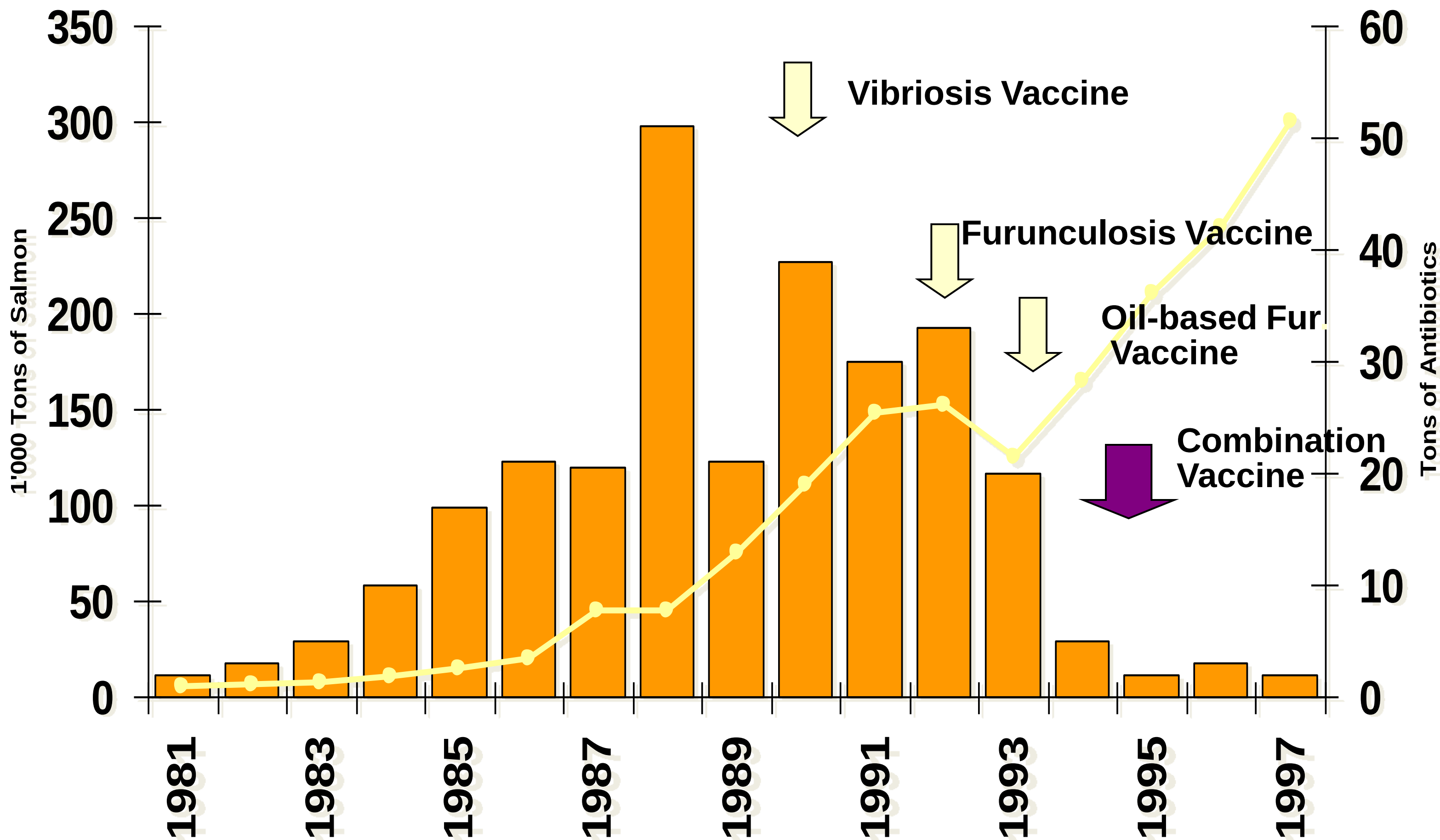
There has been a recent focus on the use of antibiotics in animal husbandry throughout Europe

- **"Over-use of antibiotics is an issue in animal husbandry, agriculture and fish farming"**
- **"The development of germs that are resistant to even the strongest of our current antibodies is one of the biggest health threats in the world."**
- **" We continue to use them (antibiotics) in agriculture, fish farming and myriad other areas of life."**
- **"......the typical farmed American salmon eats its own weight in antibiotics before it is sold."**

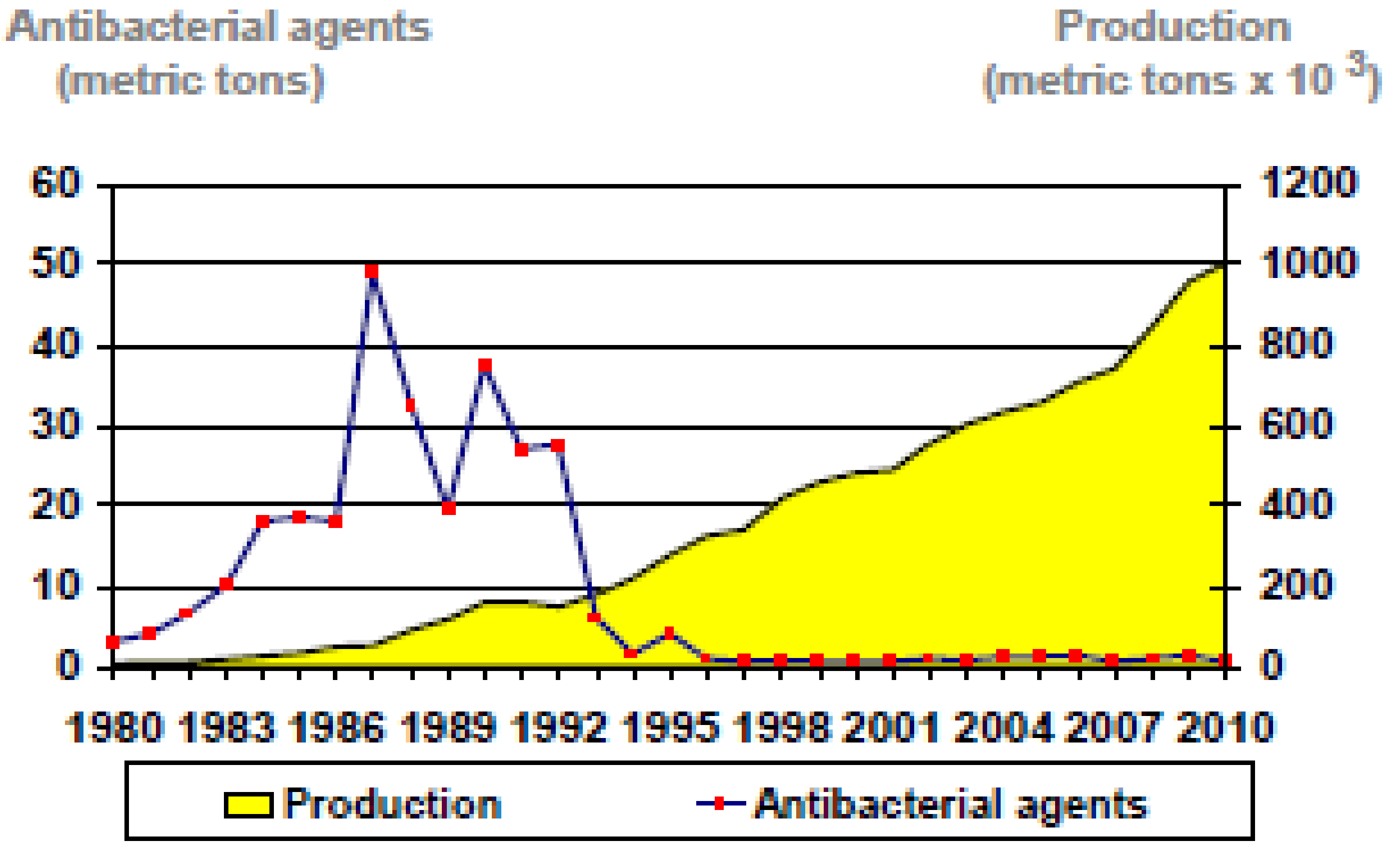
Dame Sally Davies, UK Chief Medical Officer

WHAT ARE THE FACTS ?

Norwegian Salmon Production
Consumption of Pure Antibiotics and Effect of Vaccines



Antibacterial agents



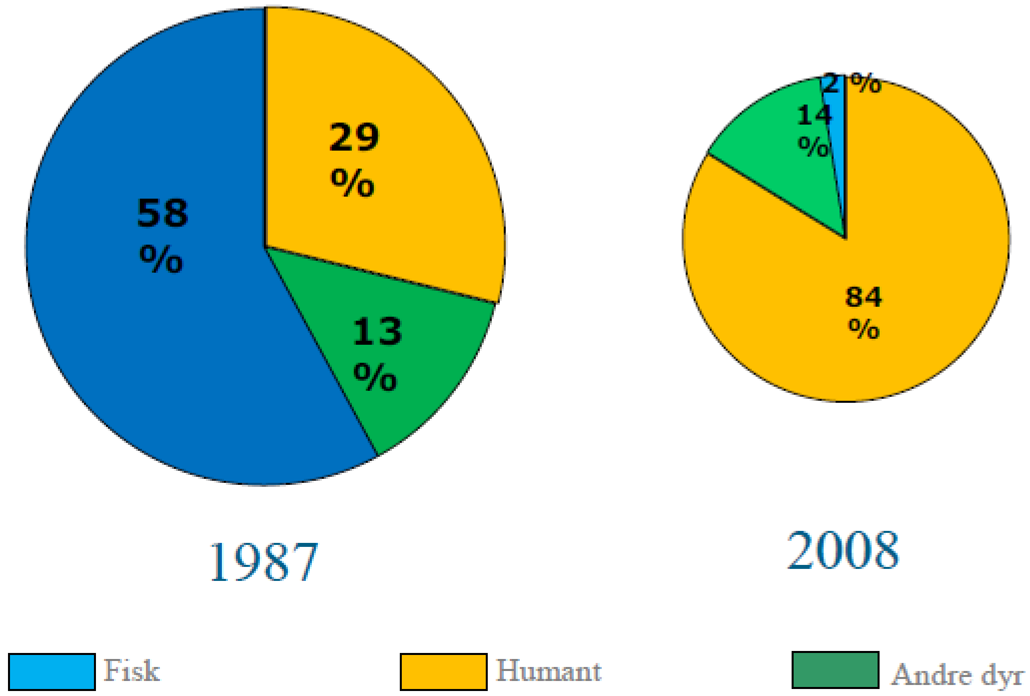
Norway

- **In 2013, the total sales of antimicrobial agents for therapeutic use in farmed fish were 972 kg of active substance of which quinolones accounted for 69%. The sales of antimicrobial VMPs in Norwegian aquaculture declined by approximately 99% from 1987 – 1996 and have thereafter remained relatively constant. This reduction is mainly attributed to the introduction of effective vaccines in salmonids.**

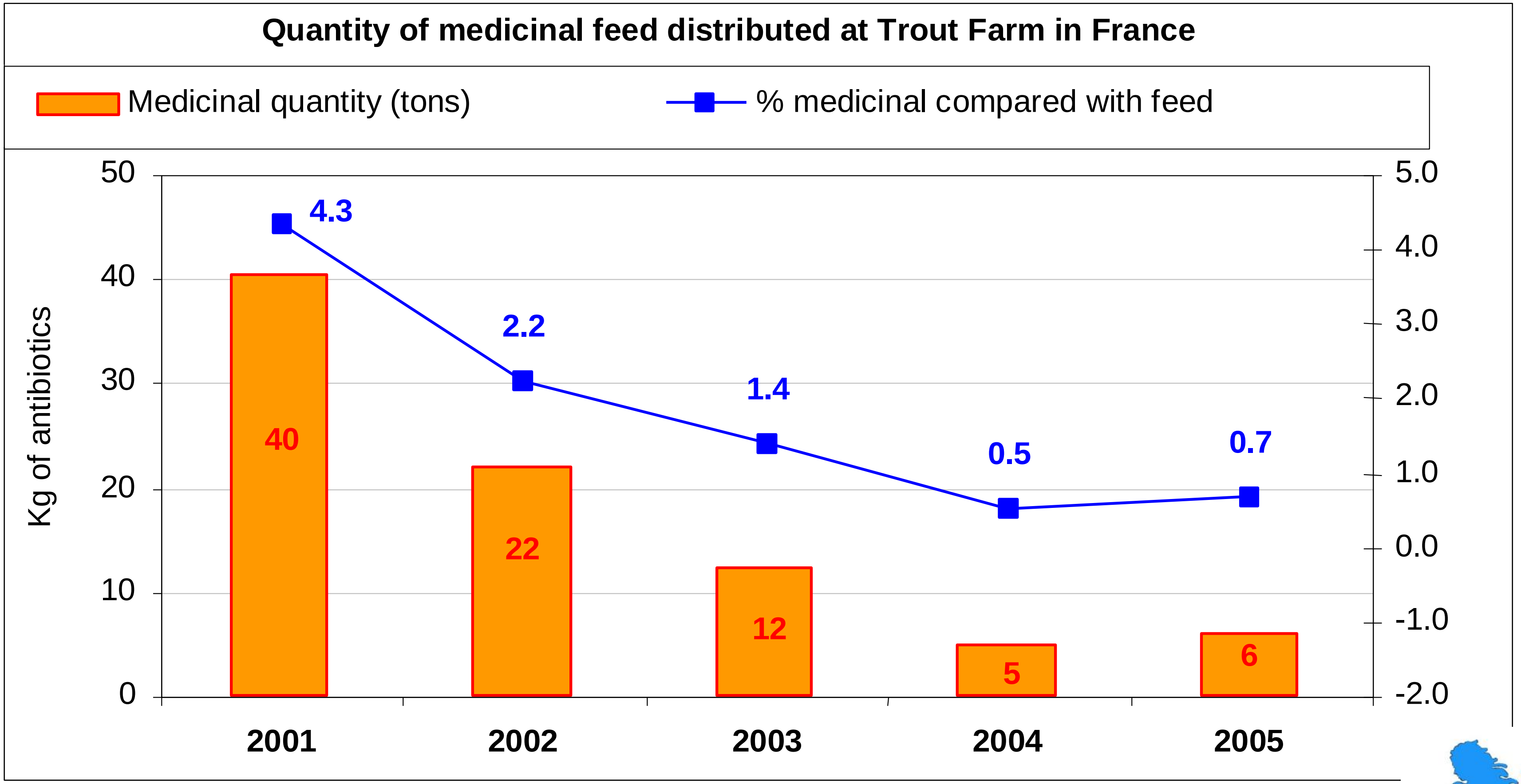
NORM/NORMVET 2013

“Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway”

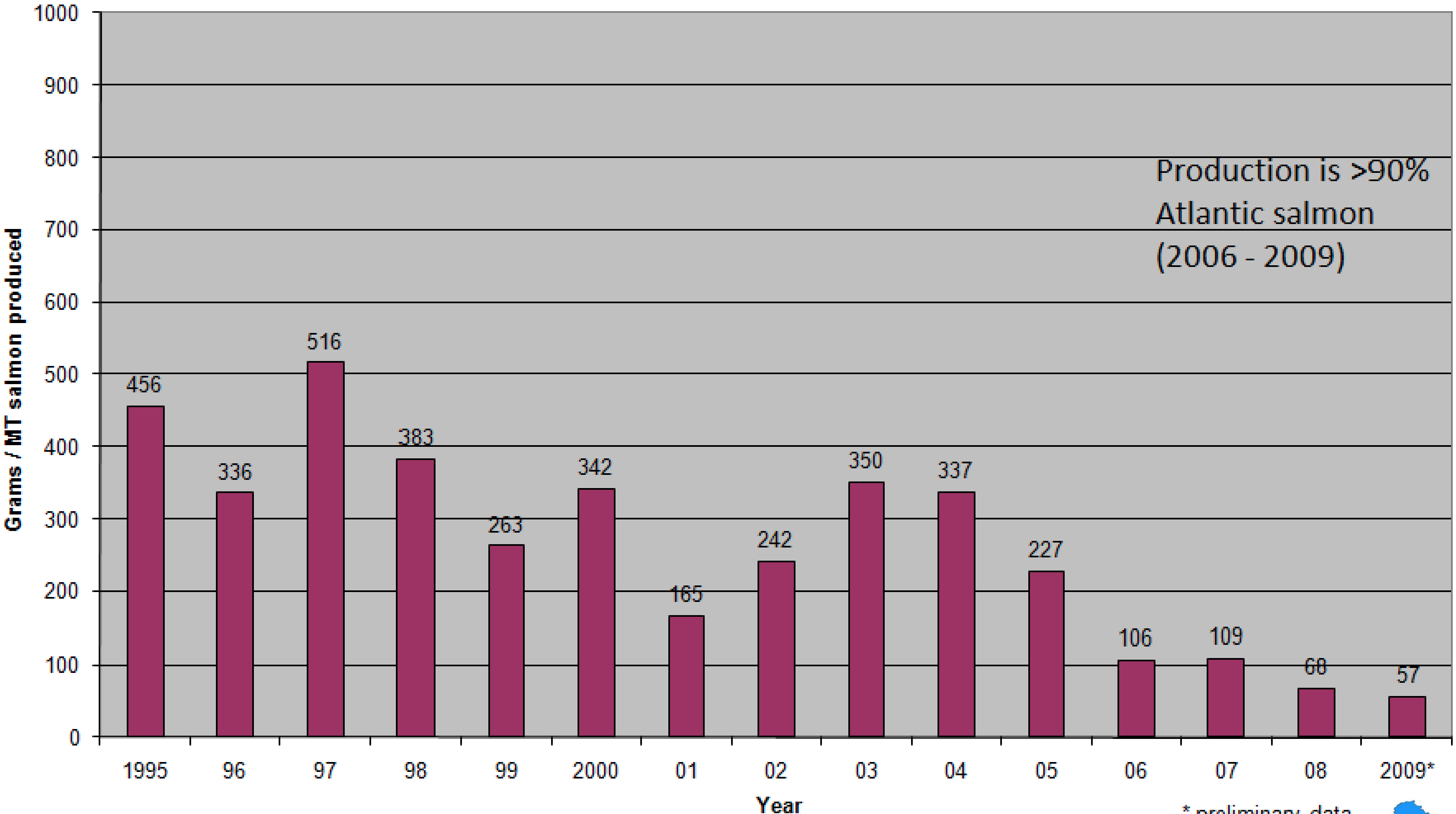
Bruk av antibiotika i Norge



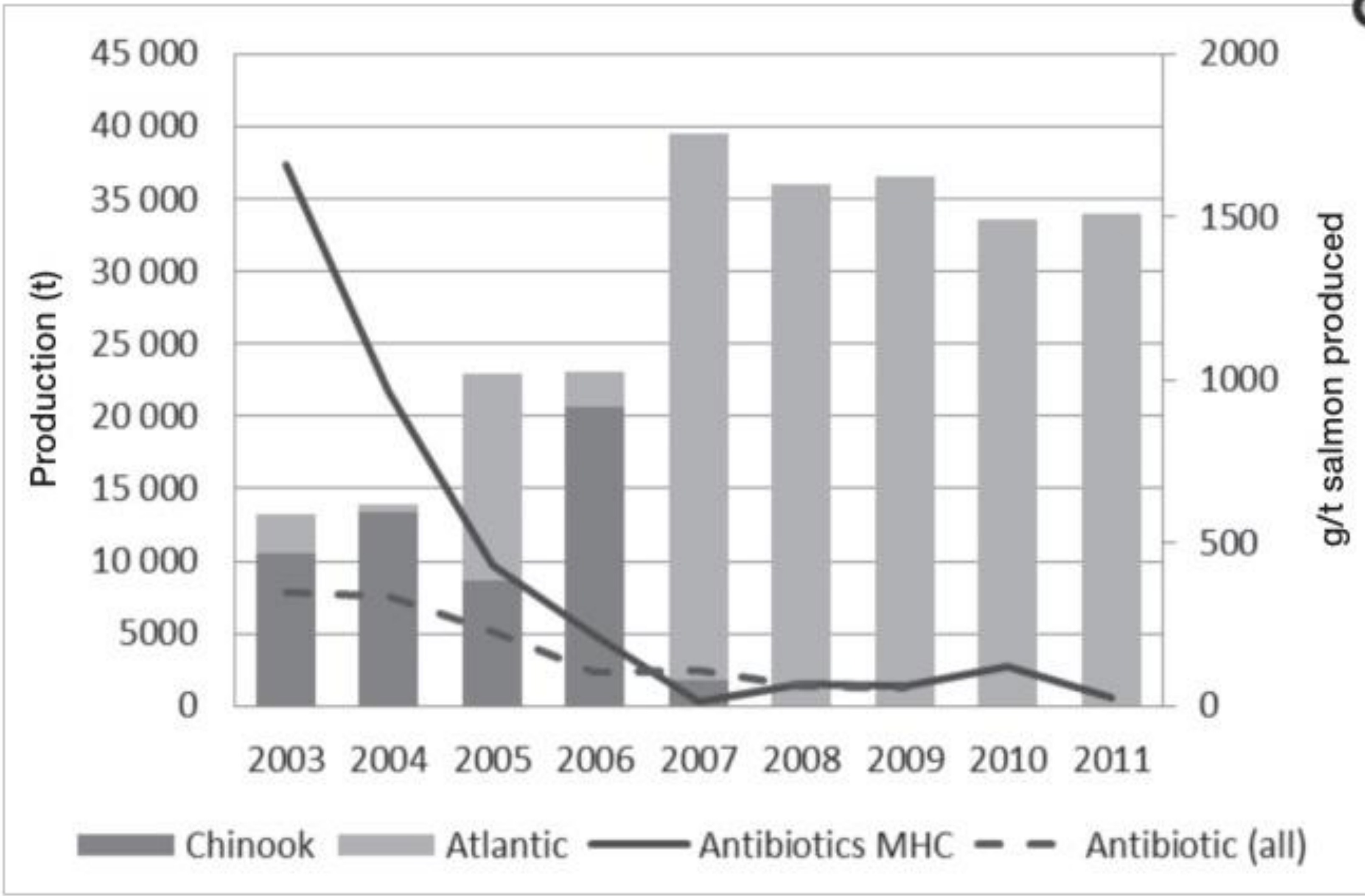
Example: Use of Antibiotics on a French trout farm as vaccine use has increased over time.

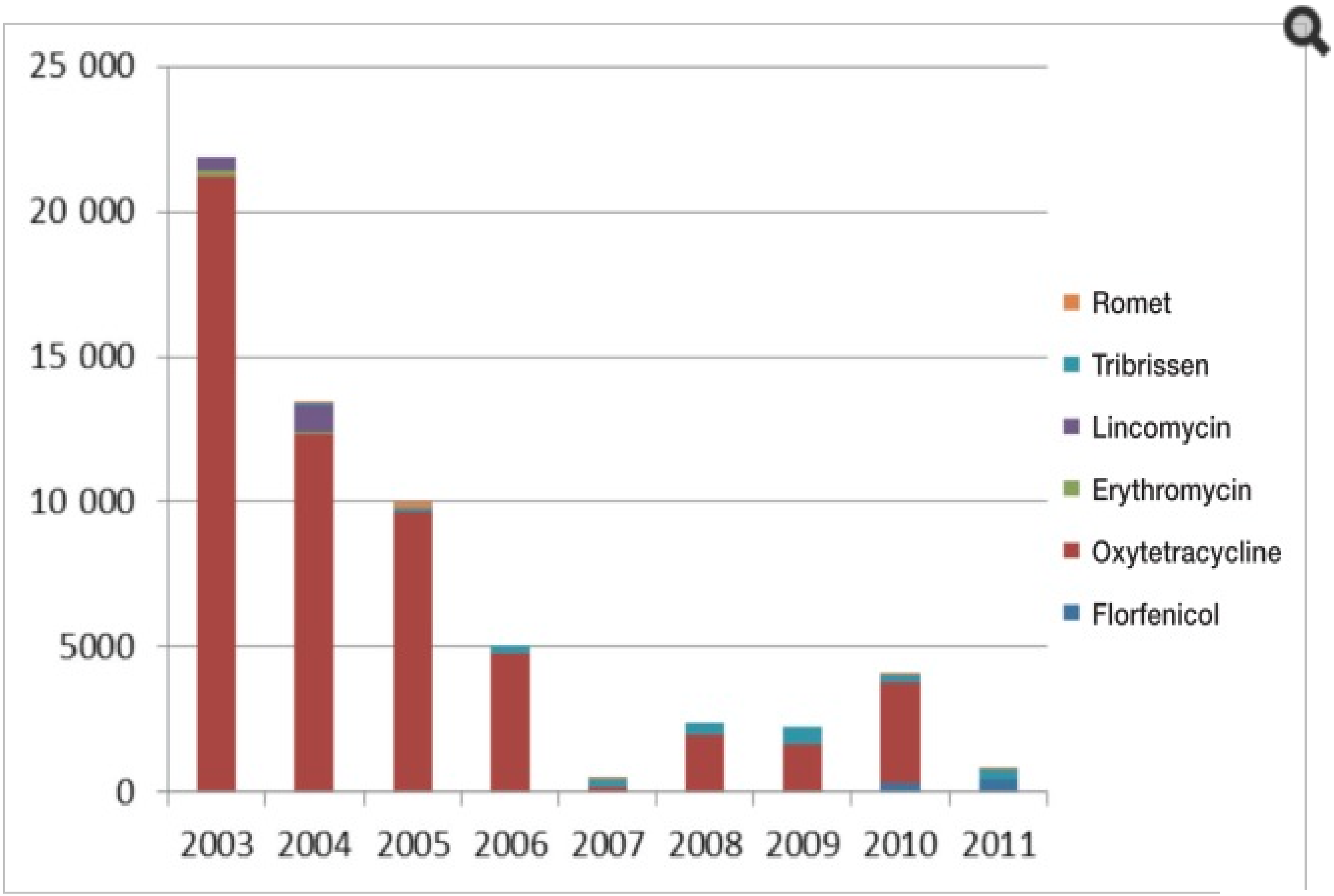


Antibiotic Use in BC Salmon Aquaculture 1995 -2009



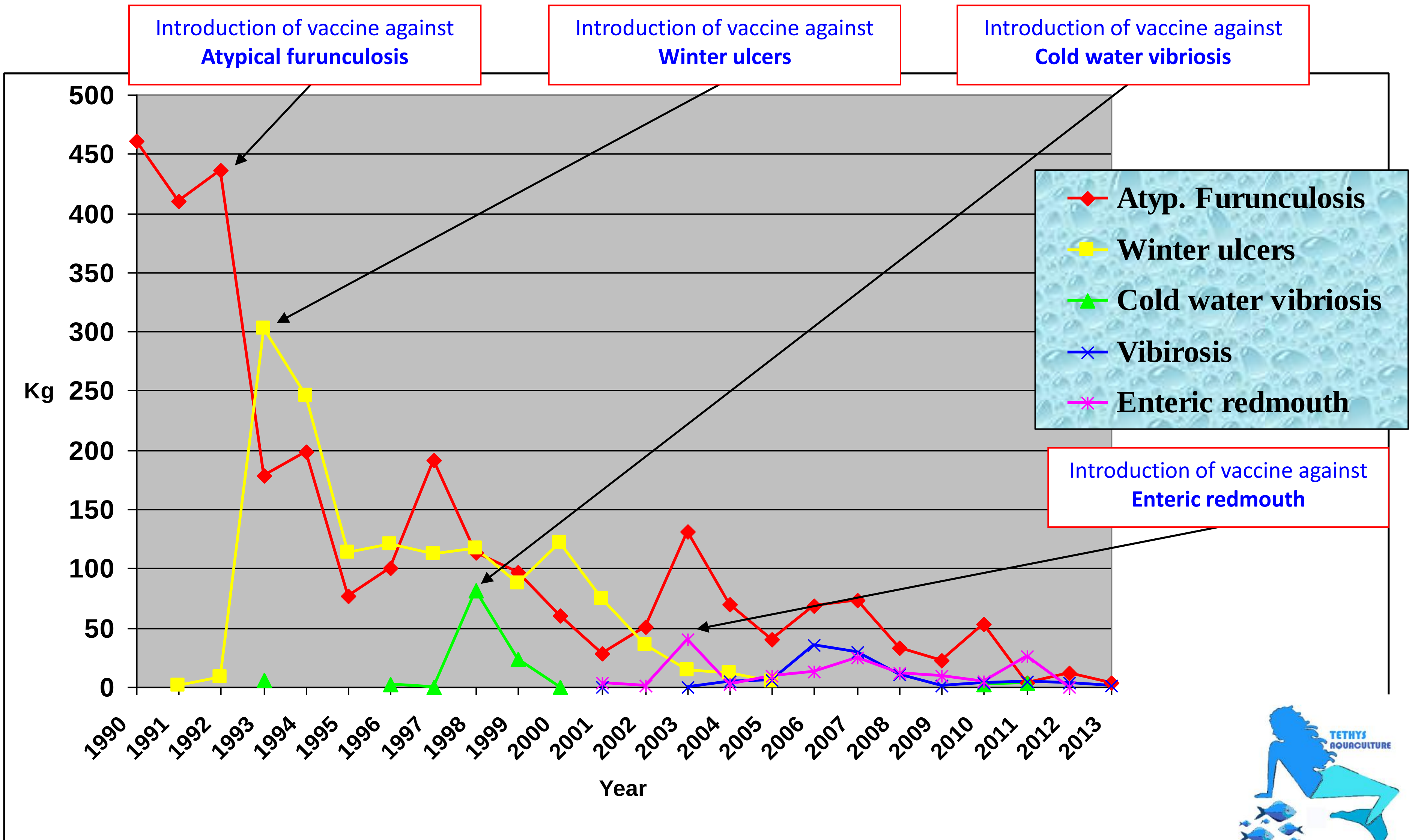
* preliminary data


45 000
40 000
35 000
30 000
25 000
20 000
15 000
10 000
5000
0
Production (t)
2000
1500
1000
500
0
g/t salmon produced
2003 2004 2005 2006 2007 2008 2009 2010 2011
Chinook
Atlantic
Antibiotics MHC
Antibiotic (all)
TETHYS
AQUACULTURE

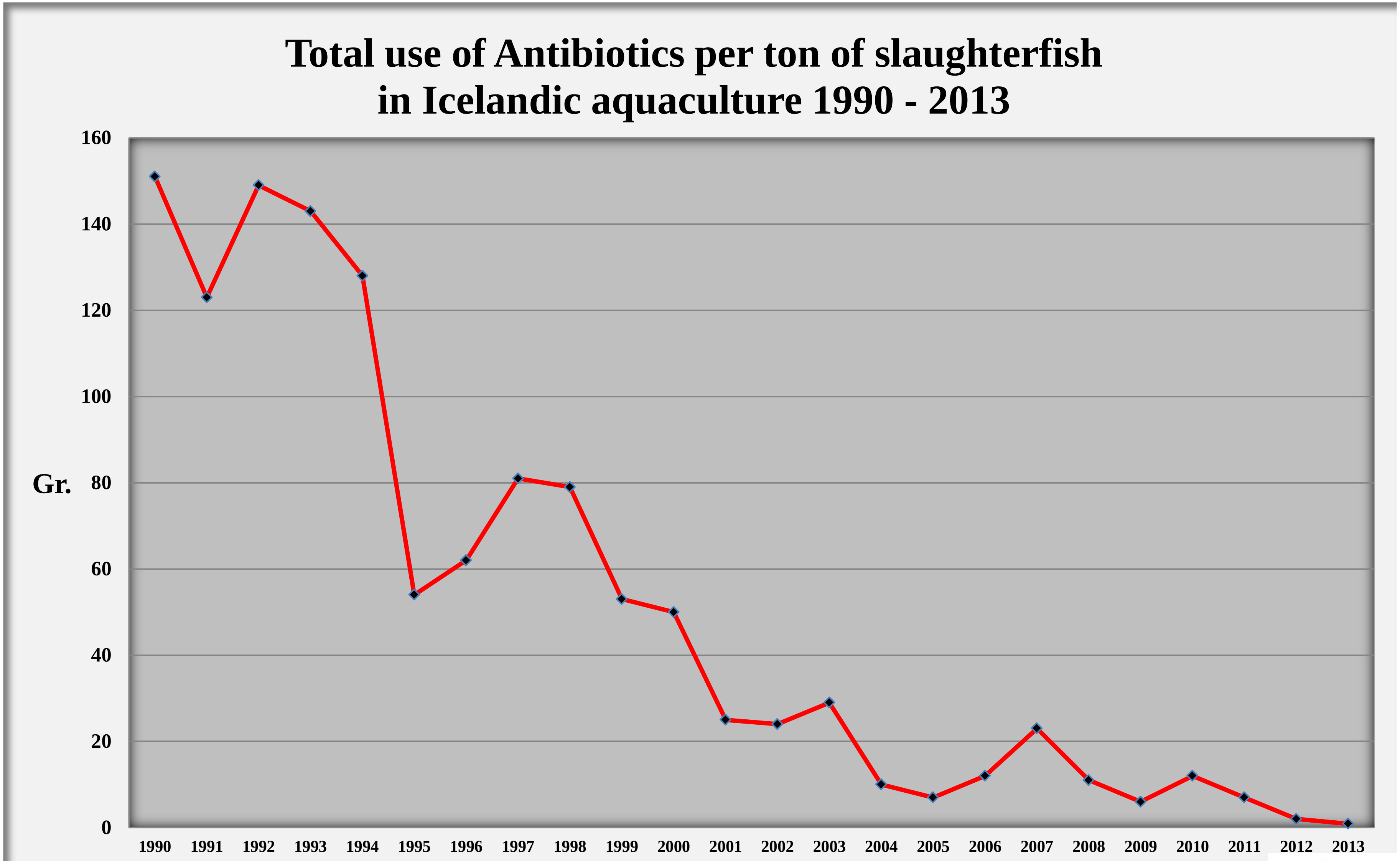


Total volume (kg) of active antimicrobial by year, 2003–2011.

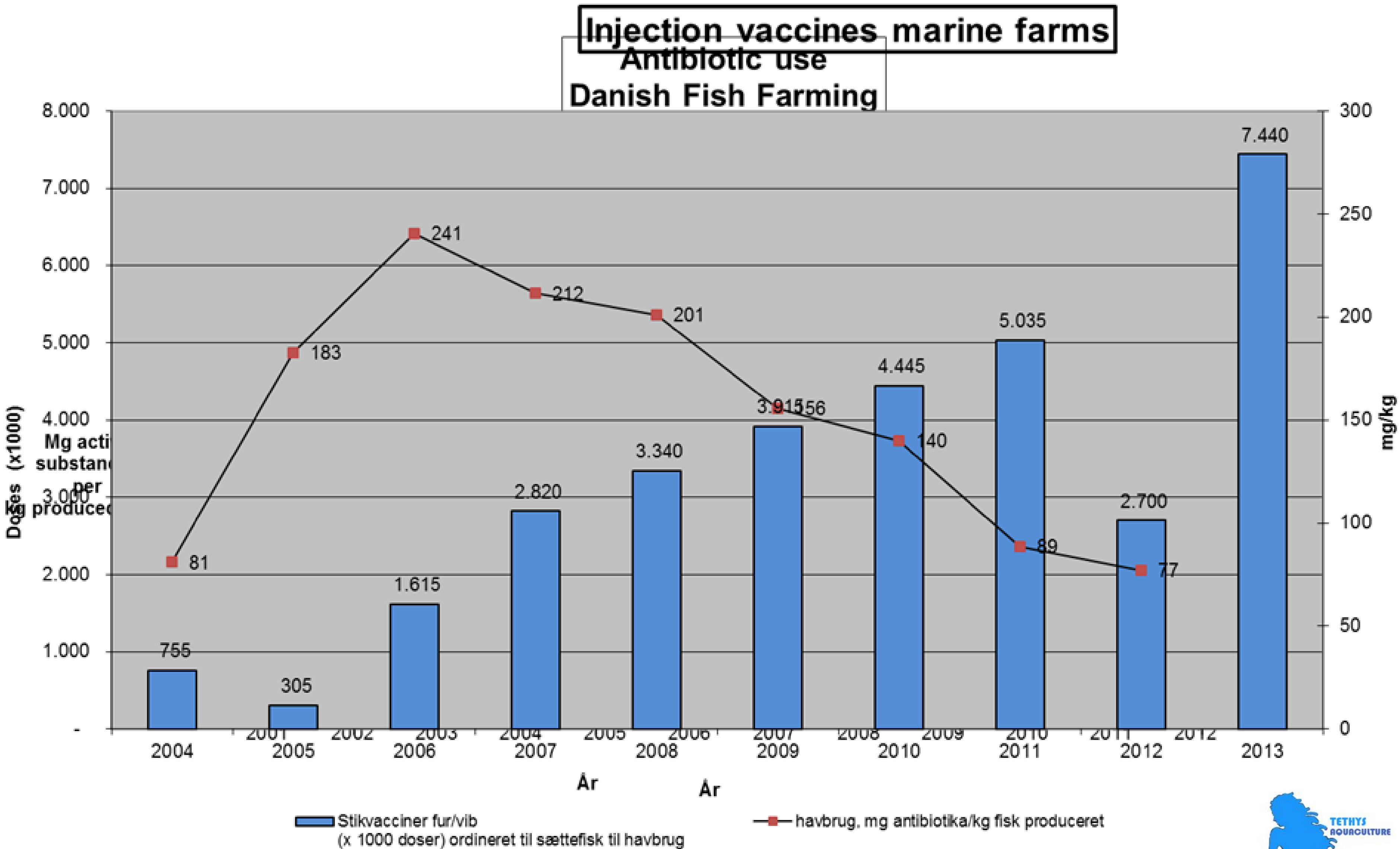
Use of antibiotics against fish diseases in Iceland 1990 - 2014



GJ 2014



Total use of Antibiotics per ton of slaughterfish
in Icelandic aquaculture 1990 - 2013
Gr.
160
140
120
100
80
60
40
20
0
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013



Injection vaccines marine farms
Antibiotic use
Danish Fish Farming
Doses (x1000)
Mg active substance per kg produced
mg/kg
8.000
7.000
6.000
5.000
4.000
3.000
2.000
1.000
-
300
250
200
150
100
50
0
755
305
1.615
2.820
3.340
3.915
4.445
5.035
2.700
7.440
81
183
241
212
201
156
140
89
77
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
År
År
Stikvacciner fur/vib
(x 1000 doser) ordineret til sættefisk til havbrug
havbrug, mg antibiotika/kg fisk produceret
TETHYS AQUACULTURE

Exceptions to the Rule ?

- Ornamental fish industry
- SE Asia
- Latin America (mainly Chile)

Latter two must be regarded as ‘work in progress’- new industries, new emerging diseases and lateness in developing/adopting vaccination.

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What about the use of other chemicals and anti-parasiticides?

- Possibly not such a ‘good news story’
- Use being reduced by integrated management systems.
- Much research into vaccine development
- Amoebic Gill Disease

This report marks the beginning of the second year of reporting fish health management information, specifically looking at sea lice.

During this quarter 179 sites were stocked with salmon, approximately 75% of the active sites in Scotland. All the production sites were stocked with just one year class of fish, as required by the Code of Good Practice for Scottish Finfish Aquaculture.

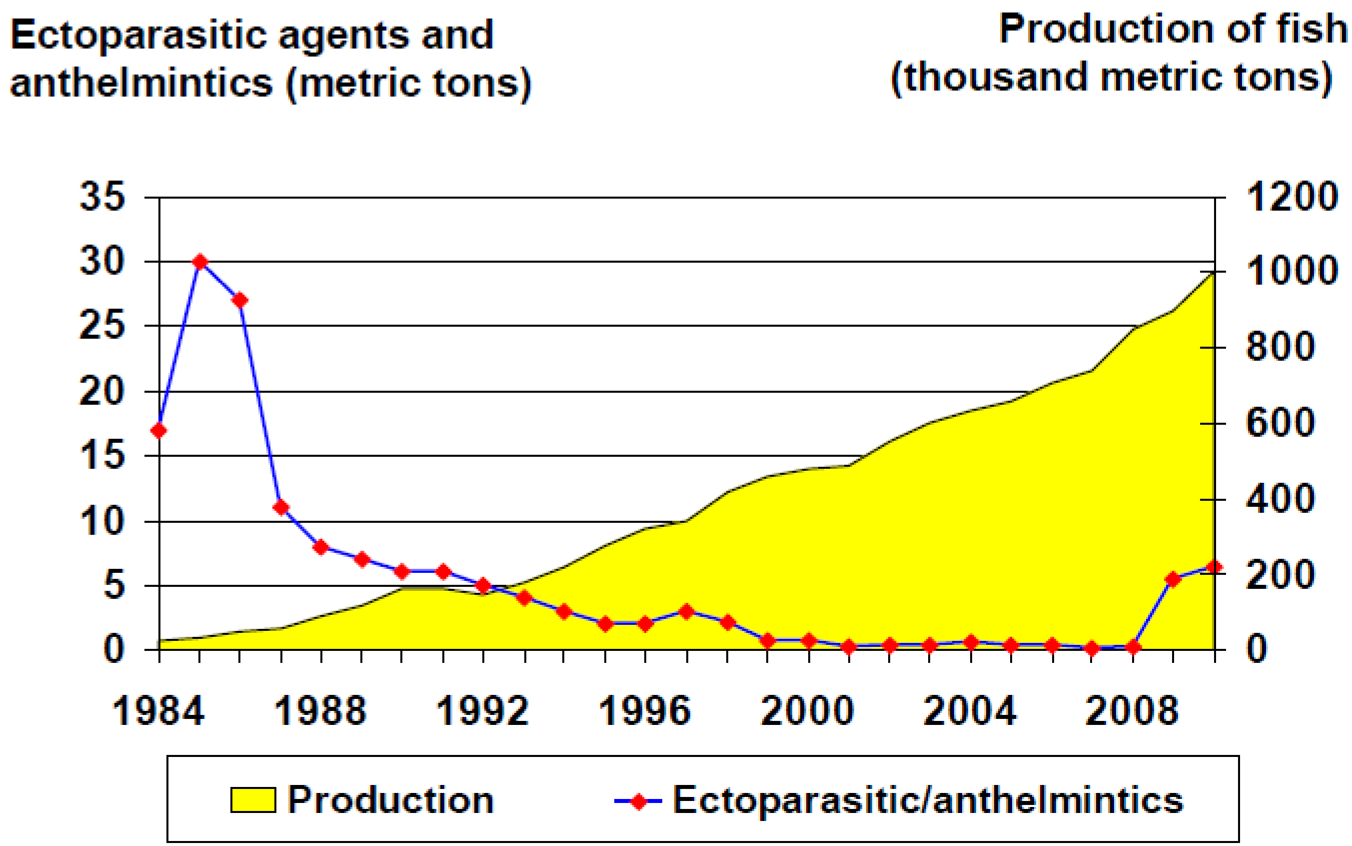
The Code of Good Practice requirements on sea lice management reflect Integrated Sea Lice Management protocols which were pioneered in Scotland and are now used internationally.

"All the production sites were stocked with just one year class of fish."

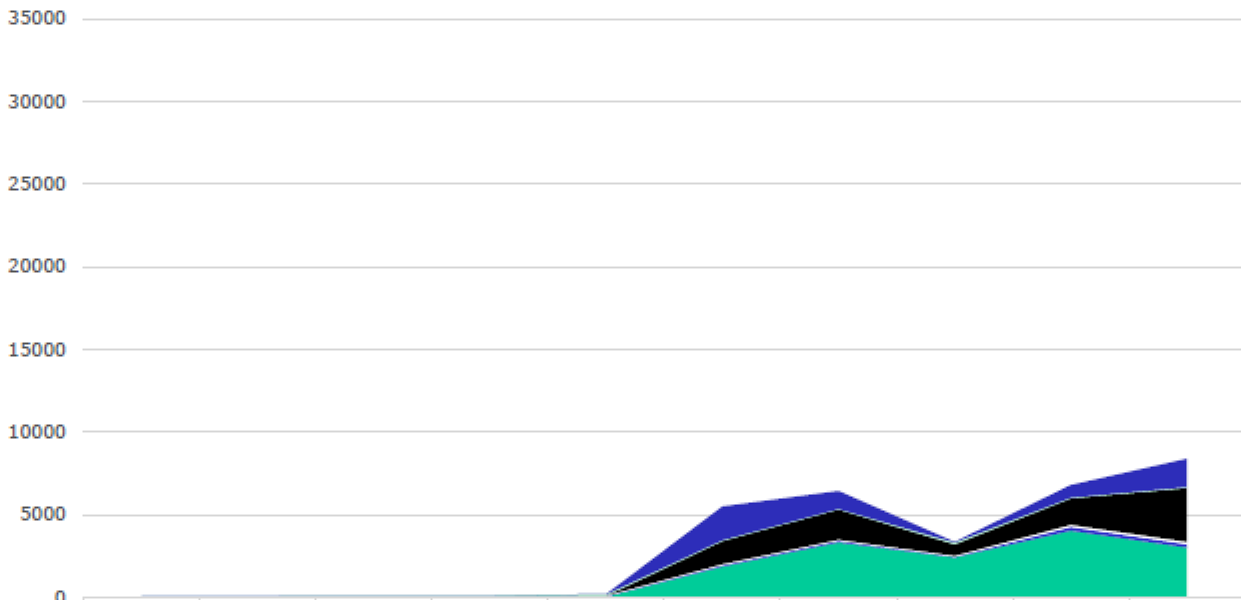
Integrated Sea Lice Management

All in/all out stocking
Weekly monitoring
Cleaner fish
Fallowing
Physical barrier
Lice filtration
Synchronisation
Single year class stocking
Area management
Information
Treatment using licensed medicines
Research and innovation
exchange

Bruk av lusemiddel i oppdrett



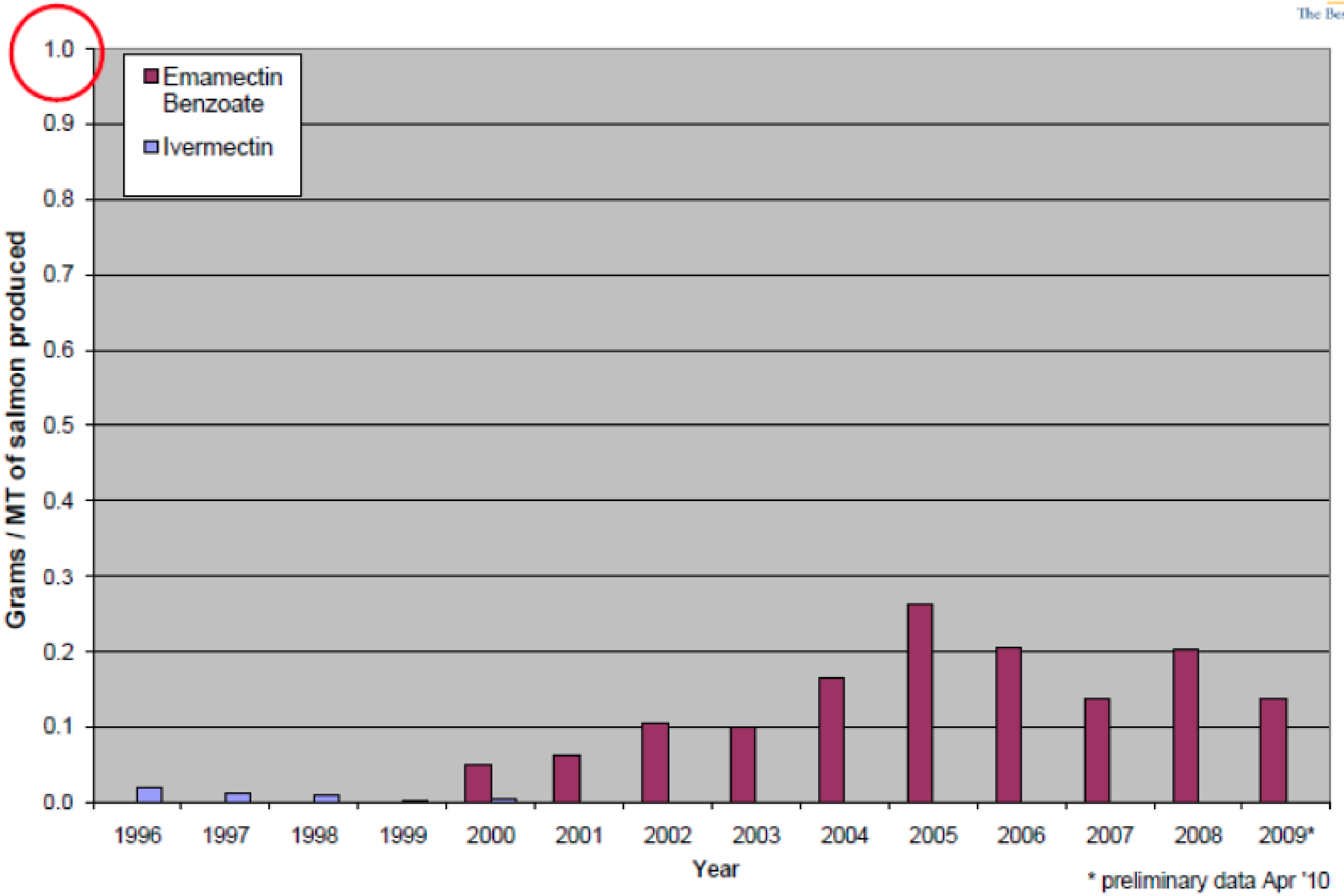
Bruk av lusemidlar



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
teflubenzuron	0	0	0	0	0	2028	1080	26	751	1704
emamektin	32	39	60	73	81	41	22	105	36	51
diflubenzuron	0	0	0	0	0	1413	1839	704	1611	3264
deltametrin	17	16	23	29	39	62	61	54	121	136
cypermetrin	55	45	49	30	32	88	107	48	232	211
azametifos					66	1884	3346	2437	4059	3037

Kjelde: www.FHI.no

Use of In-Feed Sea Lice Therapeutants in British Columbia (1996-2009)



The ‘blind-alley’

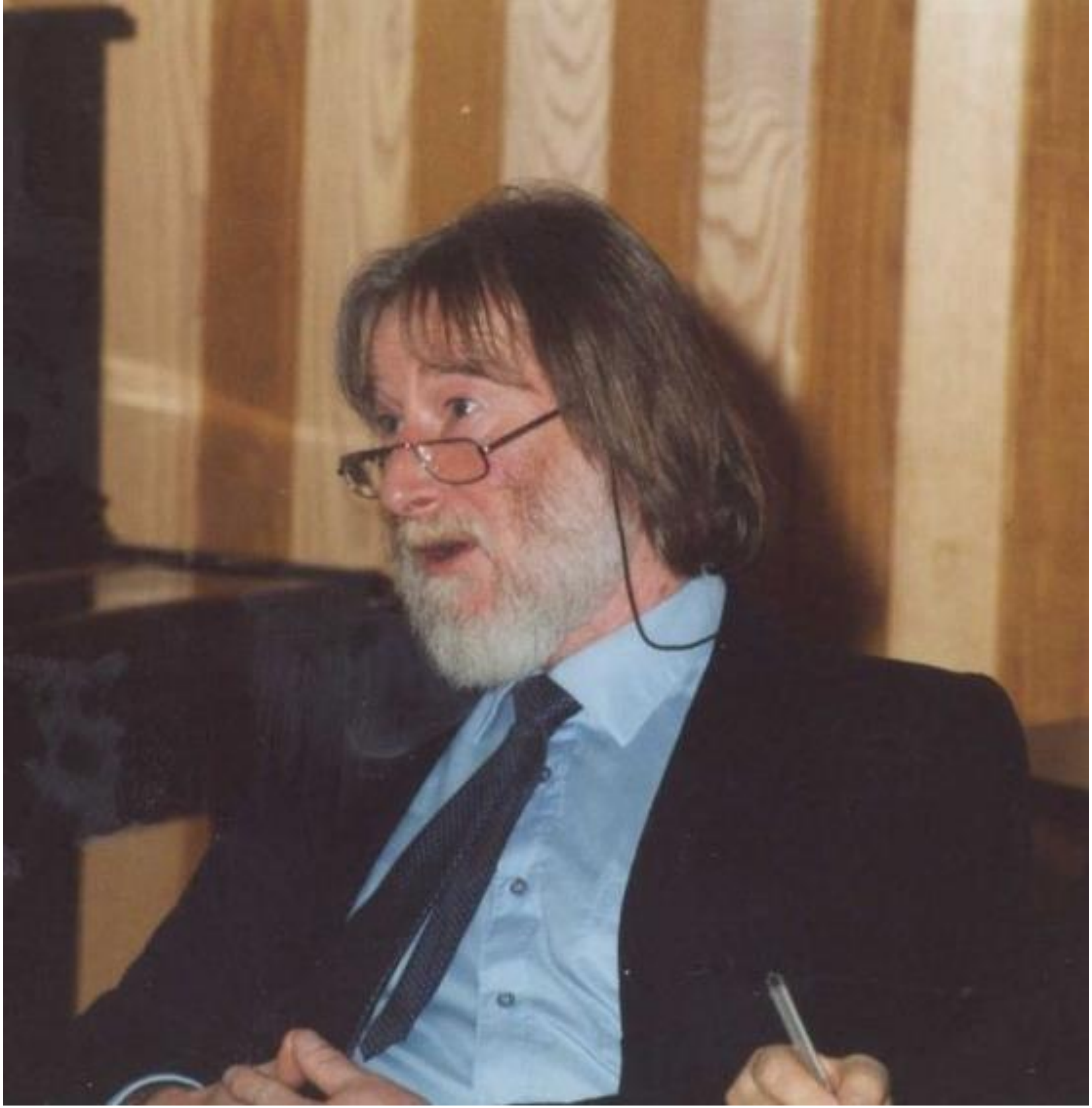
- **Most ‘easy’ vaccines have been developed leaving vaccines against the more-difficult pathogens to be focused upon e.g. viruses, slow-growing bacteria, fungi, parasites.**
- **We need to focus on new technologies etc. to make these vaccines available and thus remove a considerable constraint on the continued development of the aquaculture industry.**

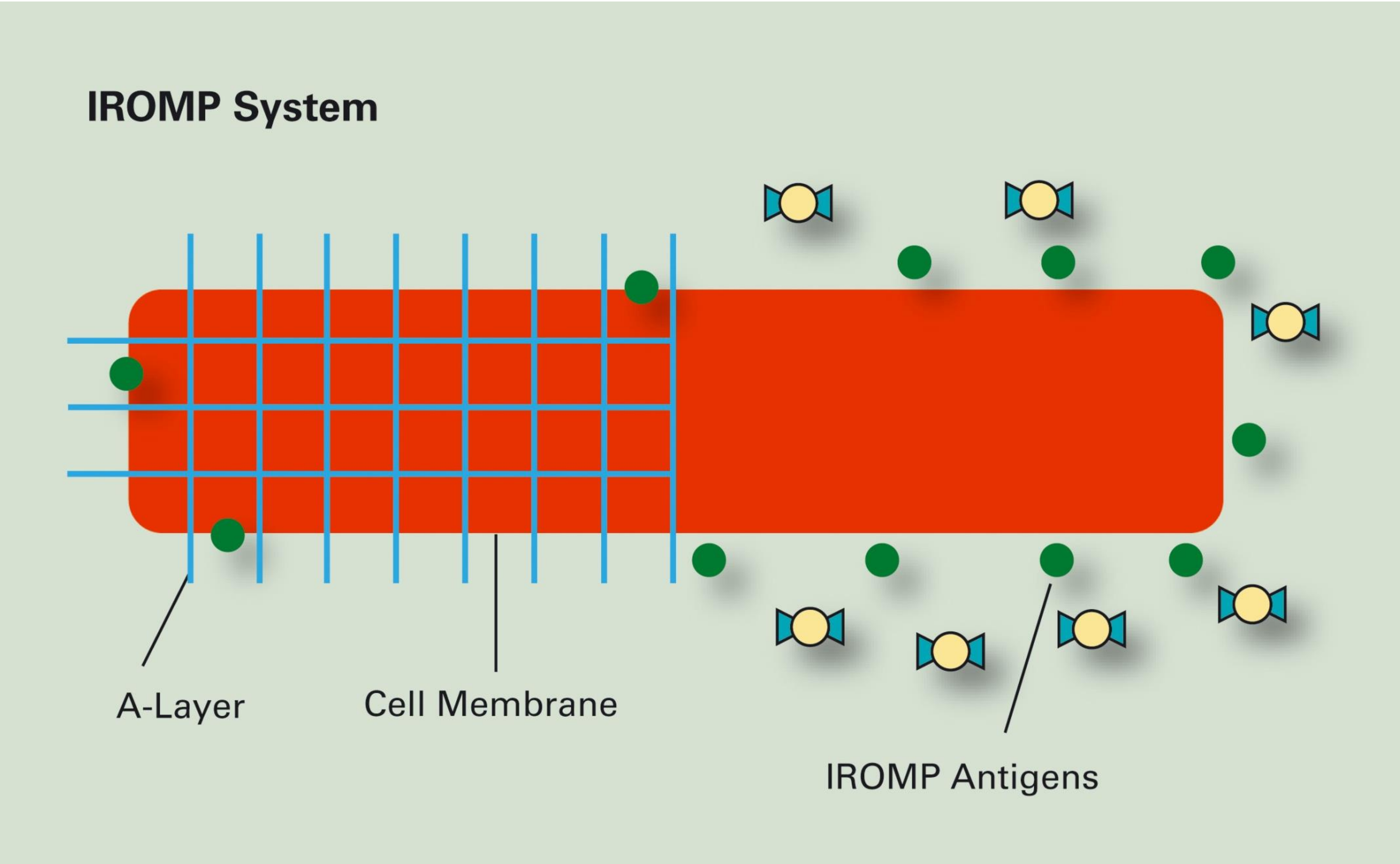
Despite these new approaches to fish vaccine development, there are still a range of fish disease pathogens against which it is difficult/impossible to develop effective vaccines using conventional techniques:

- Viruses
- Slow-growing bacteria (BKD, SRS)
- Parasites
- Fungi

Development and application of new technologies

Manipulation of the growth medium




IROMP System
A-Layer
Cell Membrane
IROMP Antigens

Attenuated Vaccines

Attenuated Vaccines

- **Viral**

  Channel Catfish Virus (CCV)
  Infectious Haematopoetic Necrosis Virus
  Infectious Pancreatic Necrosis (IPN) Virus
  Spring Viraemia of Carp (SVC) Virus
  Viral Haemorrhagic Septicaemia (VHS) Virus
  Koi herpes virus (KHV)

- **Bacterial**

  Flavobacterium psychrophilum
  Aeromonas salmonicida
  Aeromonas hydrophila
  Edwardsiella ictaluri
  Flexibacter columnarae
  Edwardsiella tarda

- **Parasite**

  Crytobia salmositica

However, it is the problems with licensing attenuated vaccines for use in the aquatic environment which has been a major constraint on their continued development.

Recombinant DNA Vaccines

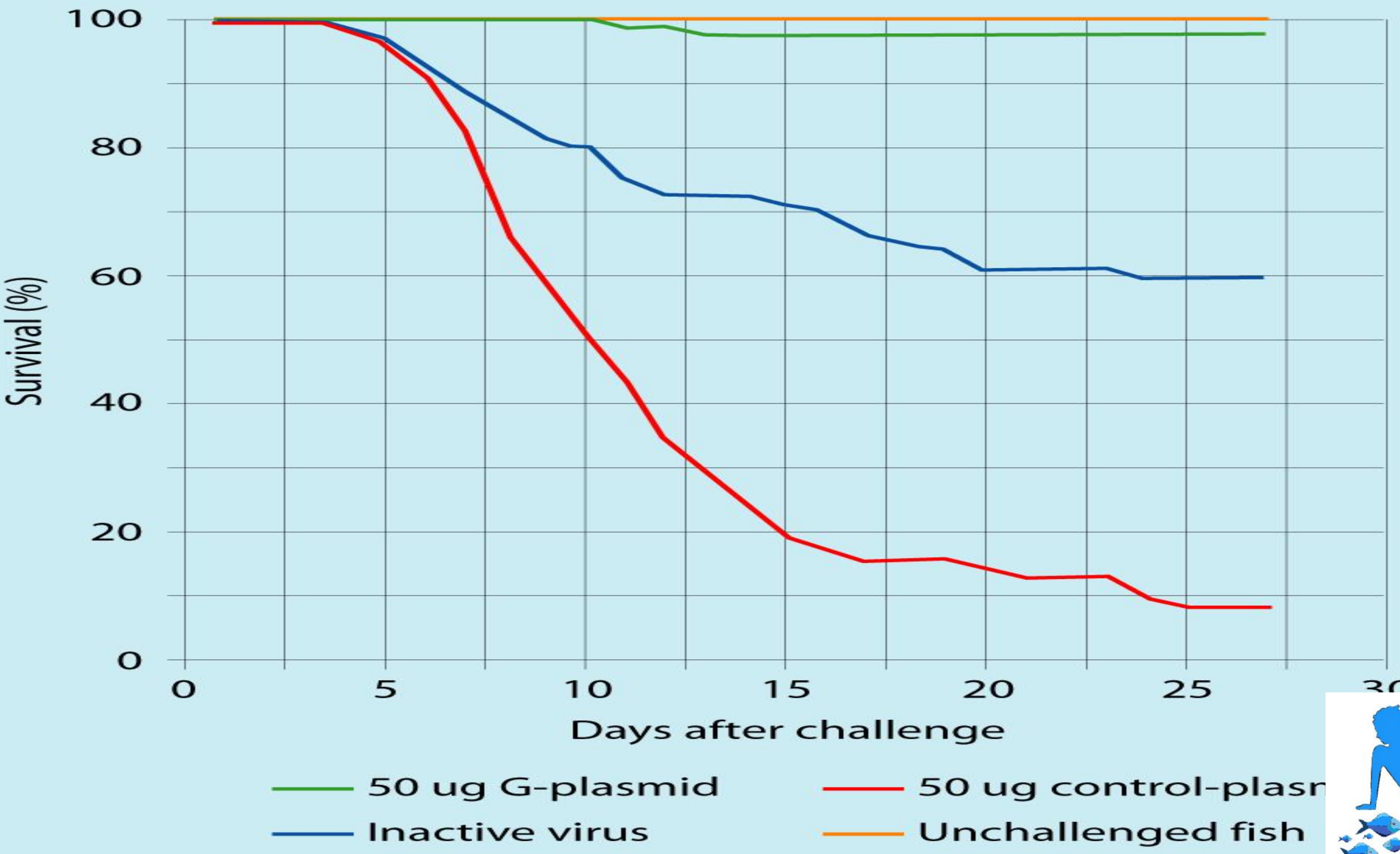
Recombinant Fish Vaccines

- **Viral**
  Channel Catfish Virus (CCV)
  Infectious Haematopoietic Necrosis (IHN) Virus
  Infectious Pancreatic Necrosis (IPN) Virus
  Spring Viraemia of Carp (SVC) Virus
  Viral Haemorrhagic Septicaemia (VHS) Virus
  Infectious Salmon Anaemia (ISA) Virus
  Whitespot Virus of Shrimp (WSV)
- **Bacterial**
  Bacterial Kidney Disease (BKD)
  Piscirickettsia salmonis (SRS)
- **Parasite**
  Ichthyophthirius multifiliis ("Ich")
  Lepeophthirius salmonis (Salmon louse)

DNA Vaccines

DNA-vaccination against VHS

Survival curves following challenge

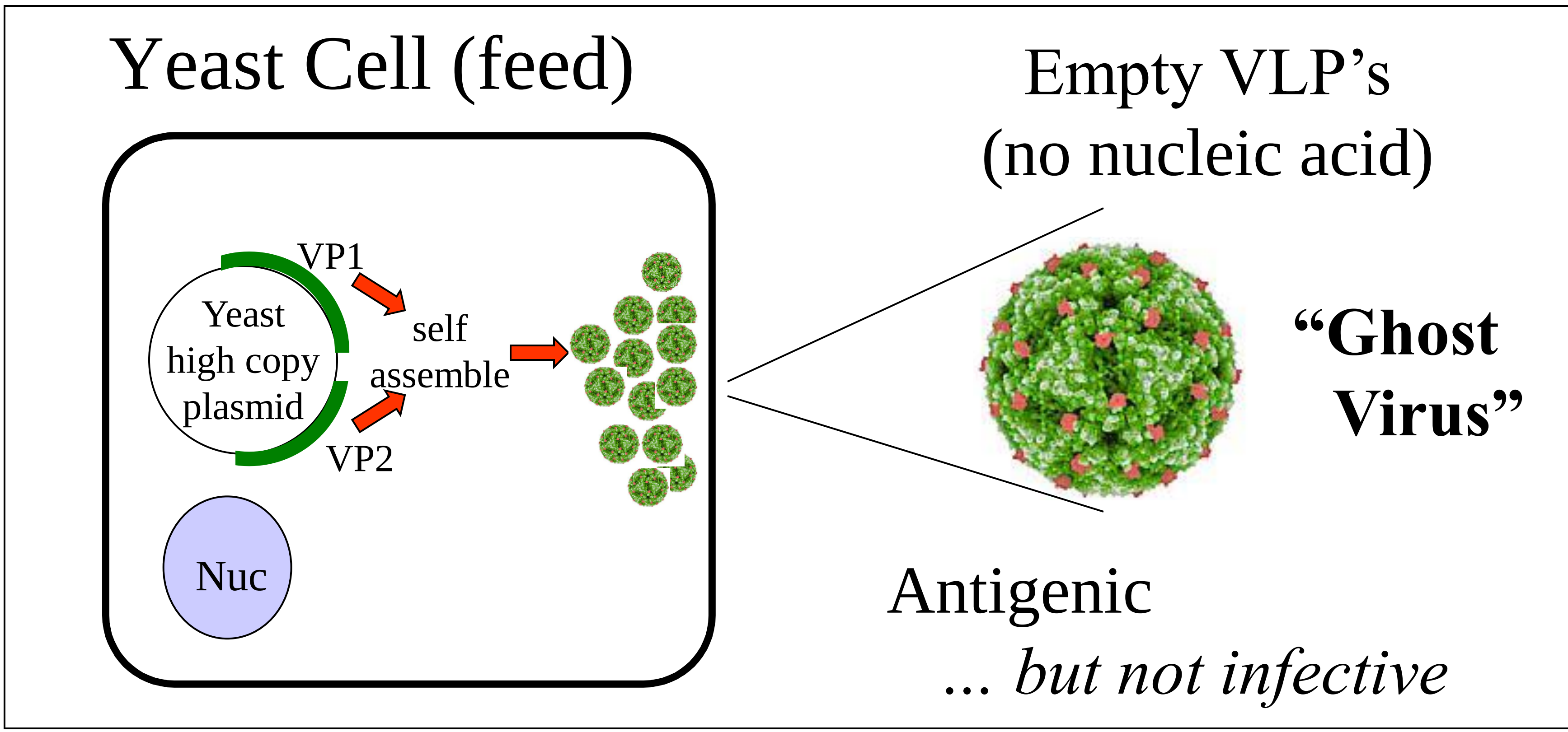


Other ‘second generation’ vaccines/technologies which have shown promise under experimental conditions

- Virus-like Particle (VLP) Vaccines
- Chimera vaccines
- Molecular Decoys
- Molecular sponges
- Fish-derived anti-microbial peptides (broad-spectrum antibiotic properties.(No anti-microbial resistanc problems ?)

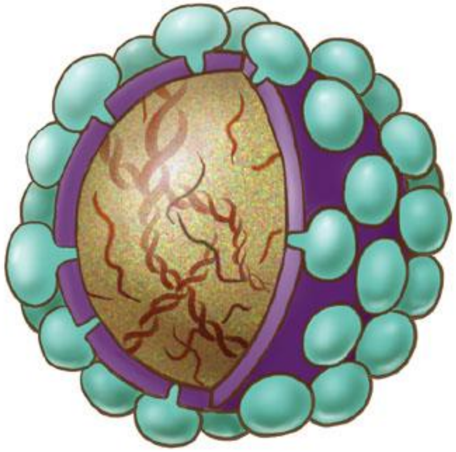
Virus-Like Particles (VLPs)

... The way it works – a "Ghost" virus

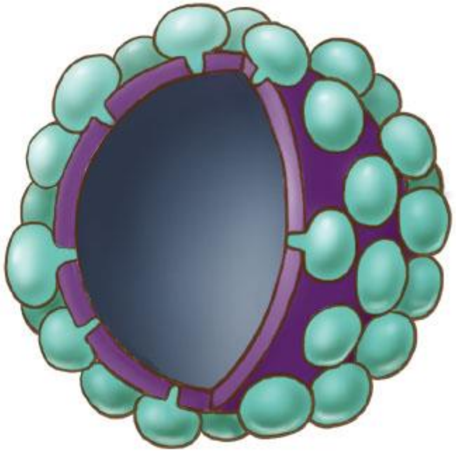


… The way it works – a "Ghost" virus

"Effective, but not Infective"



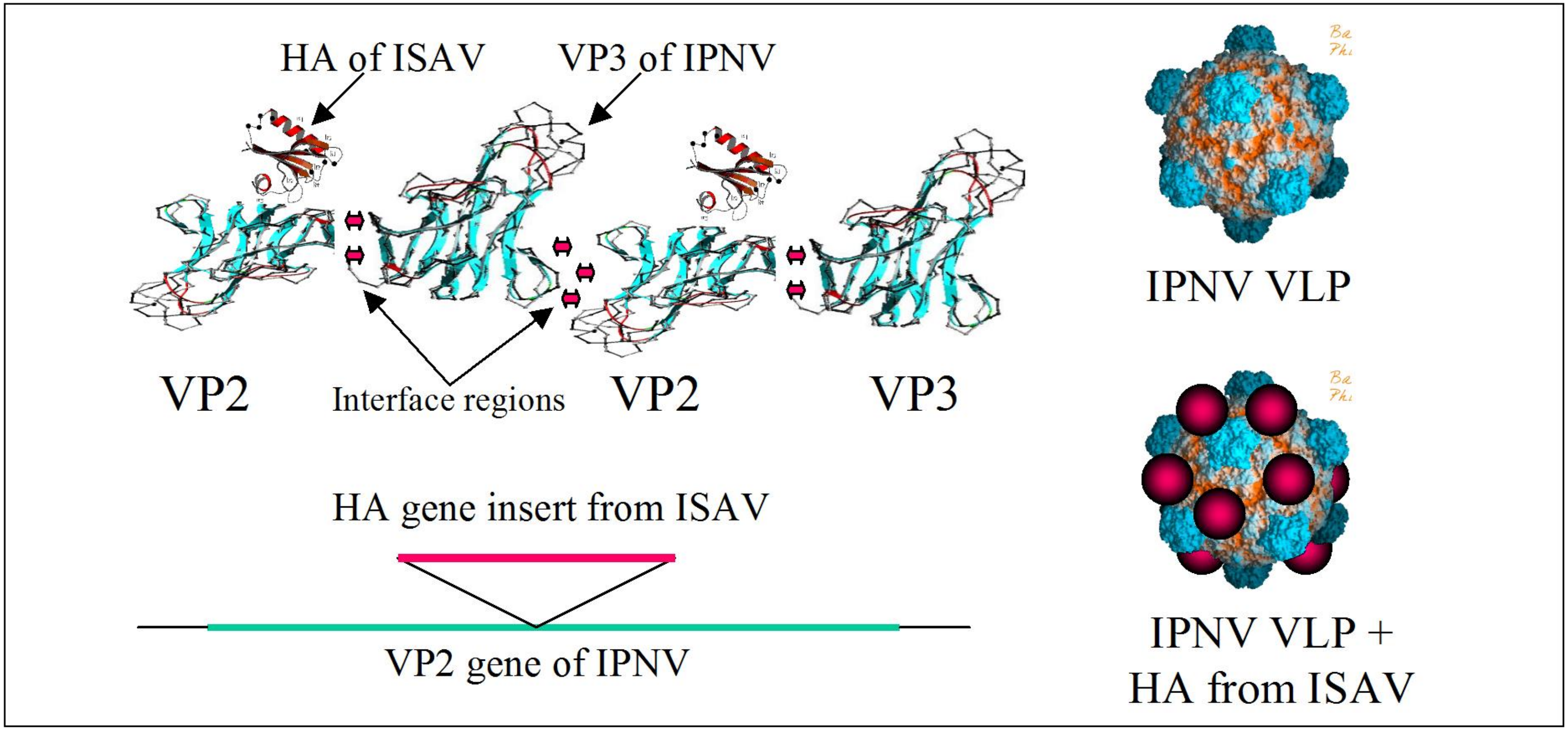
Infective virus



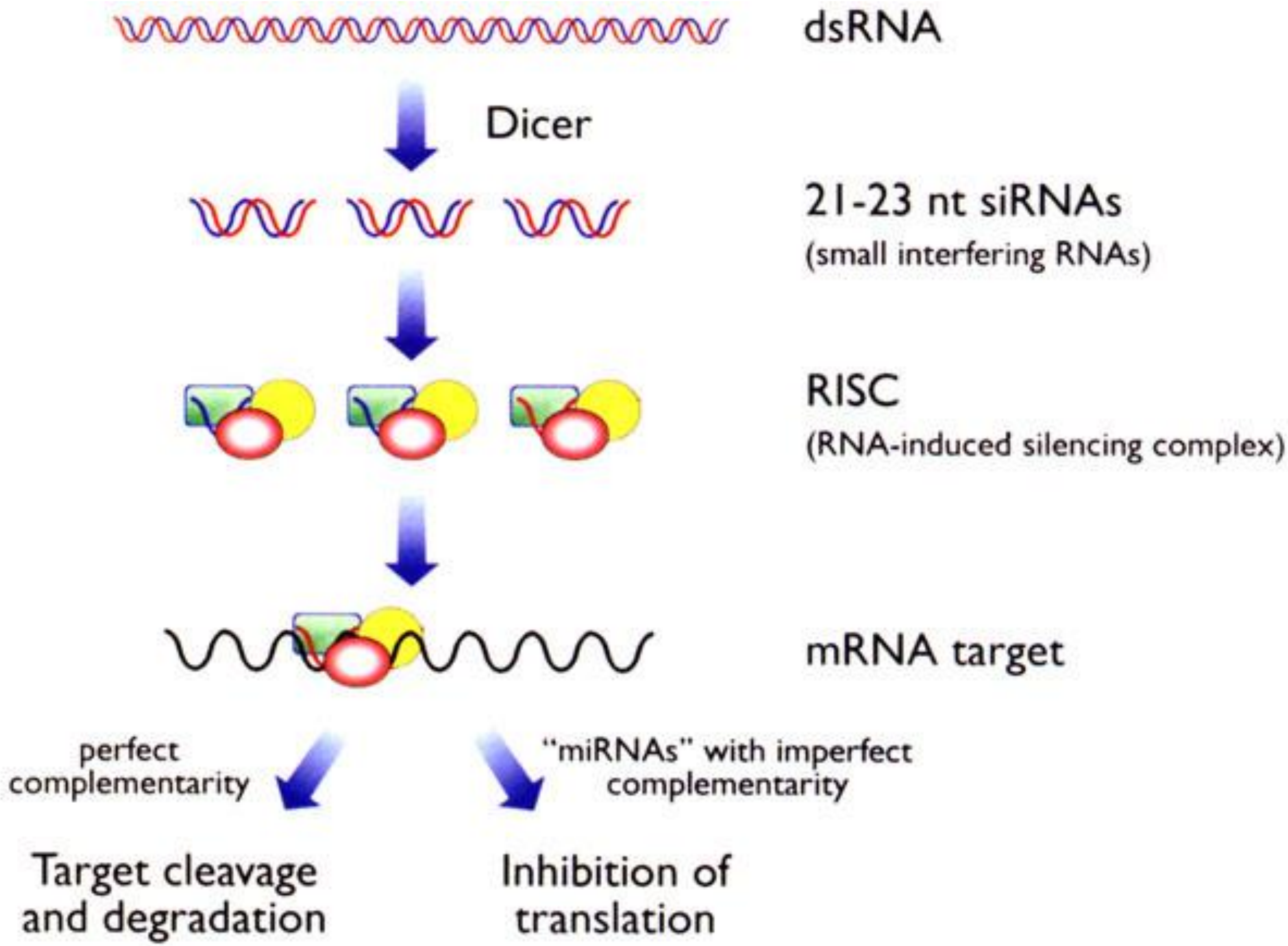
Empty virus-like particle

Chimera Vaccines

... *Making a bivalent vaccine using an IPNV VLP Platform*



Gene Silencing

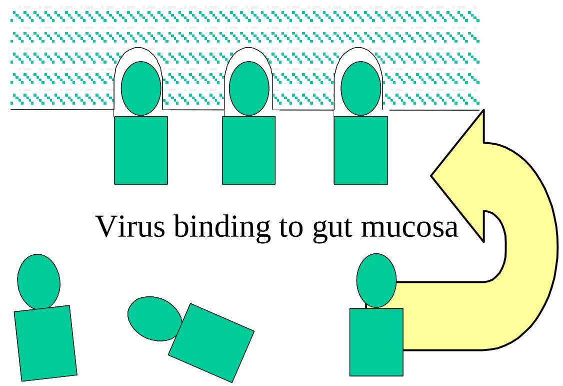

dsRNA
Dicer
21-23 nt siRNAs
(small interfering RNAs)
RISC
(RNA-induced silencing complex)
mRNA target
perfect complementarity
"miRNAs" with imperfect complementarity
Target cleavage and degradation
Inhibition of translation

Molecular Decoys

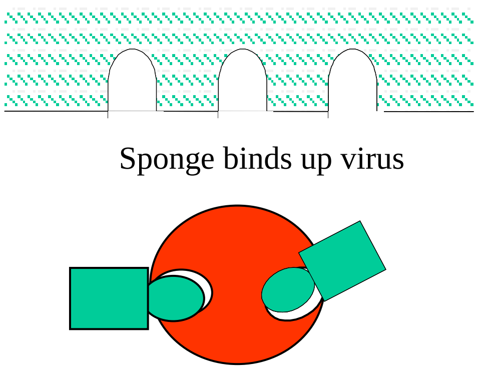
Edible Shrimp "Vaccines"

... *"Sponges" vs. "Decoys"*

Diseased



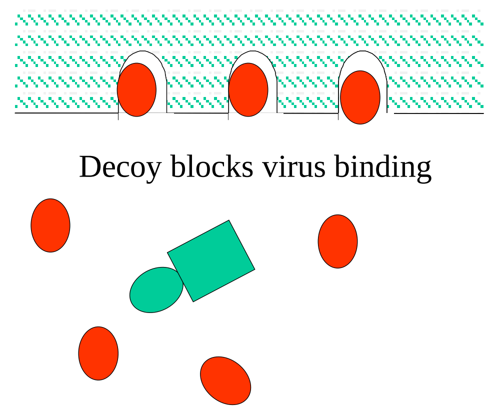
Healthy



Molecular Sponges

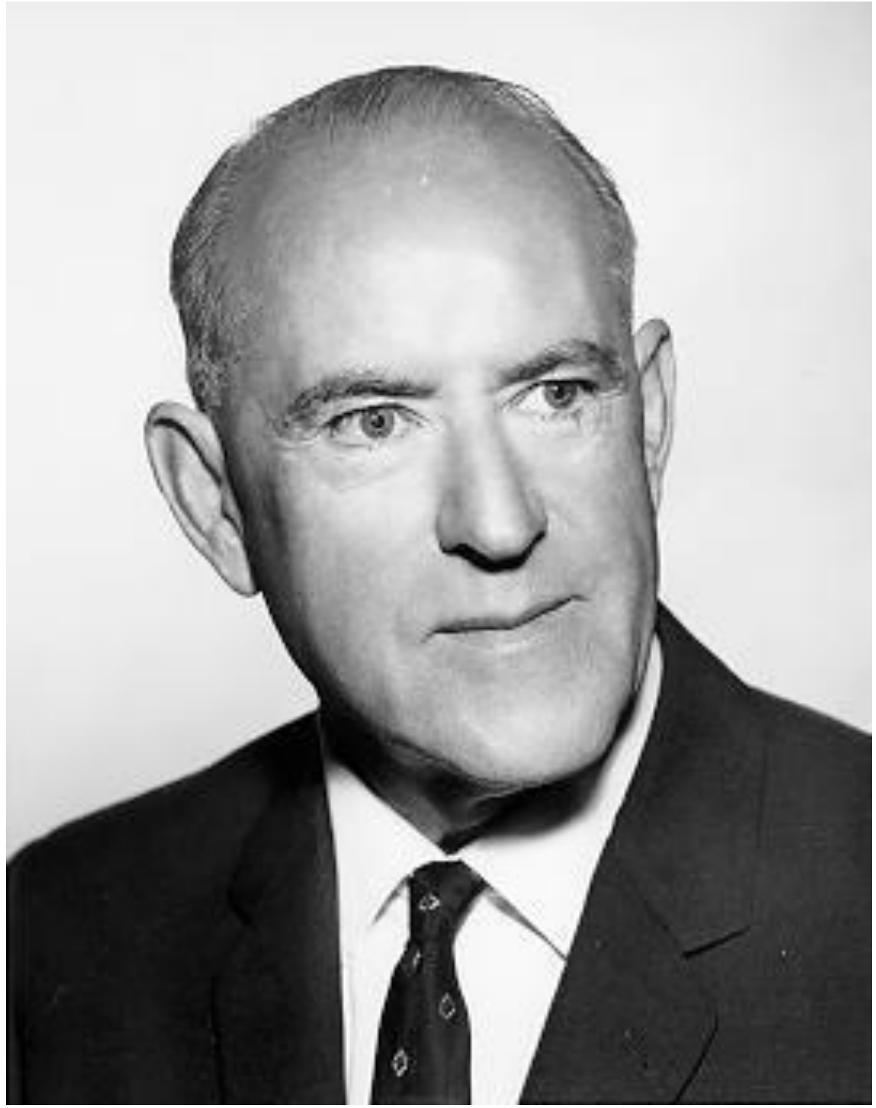
Both strategies presently in trials at SC-DNR

Healthy



Molecular Decoys

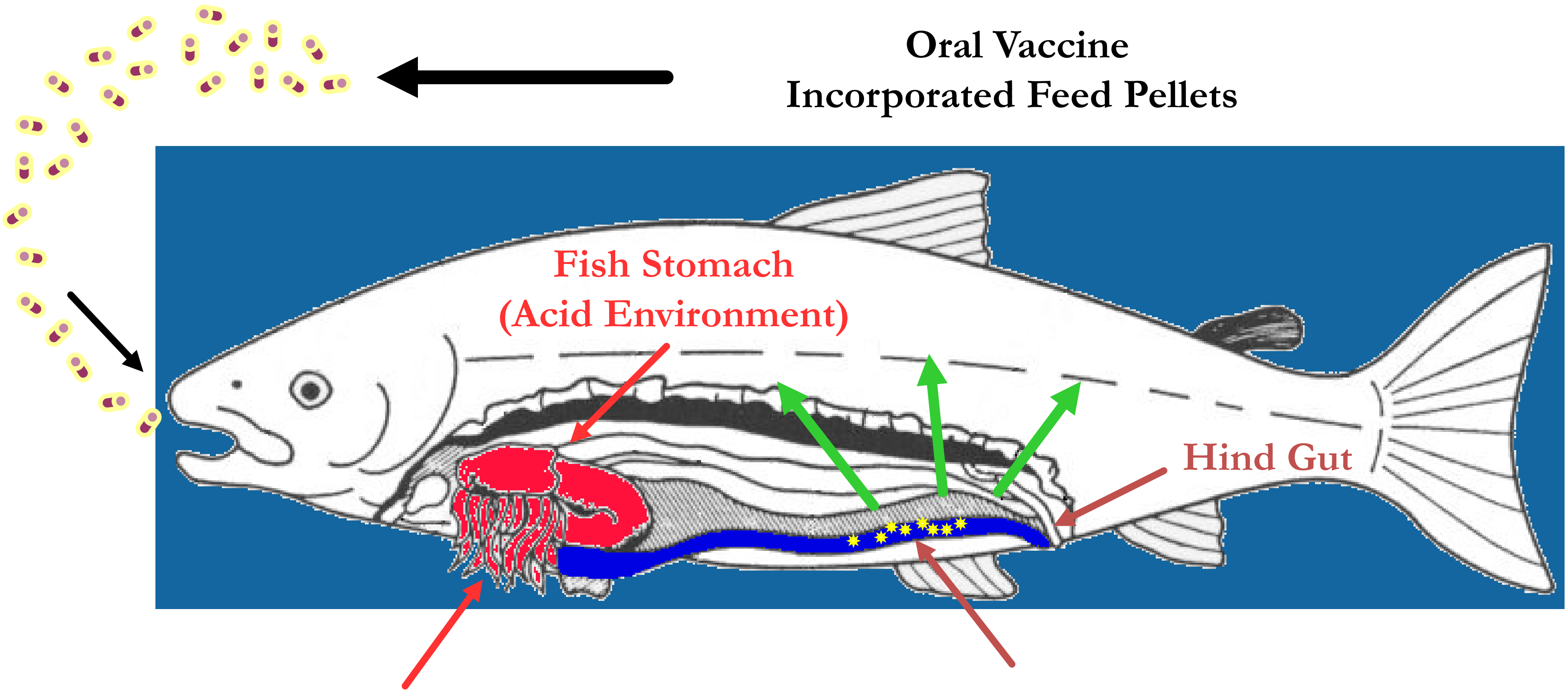
Oral Vaccines



Pathogen and Reference		Fish Species	Challenge Route	% Mortality vaccinated (control)	Protection
Aeromonas hydrophila					
Post	(1966)	*S. gairdeneri*	i.p	60-90 (80-100)	+
Aeromonas salmonicida					
Duff	(1942)	*S. clarkii*	bath	25 (75)	+
			i.p.	68 (90)	+
Snieszko & Friddle	(1949)	*S. fontinalis*	i.p.		-
Spence et al	(1965)	*O. kisutch*	scrape/bath		-
Overholsen	(1968)	*O. kisutch*	field	3 (22)	+
Klontz & Anderson	(1970)	*s. fontinalis*	field	0 (58)	+
Udey & Fryer	(1978)	*o. kisutch*	field		-
Michel	(1979)	*s. gairdneri*	i.m.		-
Smith et al.	(1980)	*s. trutta*	field	35-62 (86)	+
Flexibacter columnaris					
Fujihara & Nakatani	(1971)	*s. gairdeneri*	field	19 (27)	+
		O. kisutch	field	8 (48)	+
Vibrio anguillarum					
Hayashi et ak.	(1964)	*s. gairdneri*	field		-
Schreckenbach	(1974)	*Anguilldac salmonicida*	field		+
Qunnels et al.	(1976)	*Salmonidae*	field		
Braaten & Hodgins	(1976)	*S. gairdneri*	i.p.	7 (100)	+
Hastein et al.	(1977)	*S. gairdneri*	field		+
Prescott	(1977)	*Various marine tropical fish*	sub cutaneous	9 (62)	+
Sawyer & Strout	(1977)	*S. kisutch*	field	3 (24)	+
Fryer et al.	(1978)	*O. tshantyoria*	field	7 (80)	+
Kusuds et al.	(1978)	*P. altivelis*	cohabitation	10 (90)	+
Gould et al.	(1978)	*O. nerka*		23 (58)	+
Nakajima & Chikahata	(1979)	*F. altivelis*	cohabitation	22 (50)	+
Baudin-Laurencin & Tangtronpiros	(1980)	*S. gairdneri*	i.p.	32 (34)	-
Evelyn & Ketcheson	(1980)	*O. nerka*	field	18 (42)	+
Amend & Johnson	(1981)	*O. kisutch*	bath	27 (52)	+
Kawai et al.	(1981)	*P. altivelie*	cohabitation	2(100)	+
Horne et al.	(1982)	*s. gairdneri*	i.p.	94 (100)	-
Agius et al.	(1983)	*s. gairdneri*	i.p.	40-100 (100)	+
Johnson & Ahmed	(1983b)	*O. nerka*	bath	15-45 (55)[a]	+
				0 55[b]	
Yersinia ruckeri					
Ross & Klontz	(1965)	*S. gairdneri*	i.p.	10 (90)	+
Anderson & Ross	(1972)	*S. gairdneri*	sub cutaneous	10 (100)[c]	+
				90 (100)[d]	+
				100 (100)[e]	-
Anderson & Nelson	(1974)	*S. gairdneri*	i.p.		+
Johnson & Amend	(1983b)	*S. gairdneri*	bath	24 (65)[a]	+
				3 (65)[b]	+

a = oral intubation
b = anal intubation
c = fish were fed with chloroform killed cells
d = phenol killed cells
e = sonicated formlin killed cells

Antigen Protection System (APV)



1. In the acid environment of the fish stomach, the feed pellets are digested. The Antigens themselves are protected by the APV and pass through intact.
2. Antigens are delivered to the area of the hind gut where they are absorbed and activate an effective immune response in the fish.

Microencapsulation

<u>Problems associated with microencapsulation:</u>

- Expensive
- Shelf-life/stability
- Difficulties with licensing
- Difficulty of incorporation into commercial feeds
- Palatability

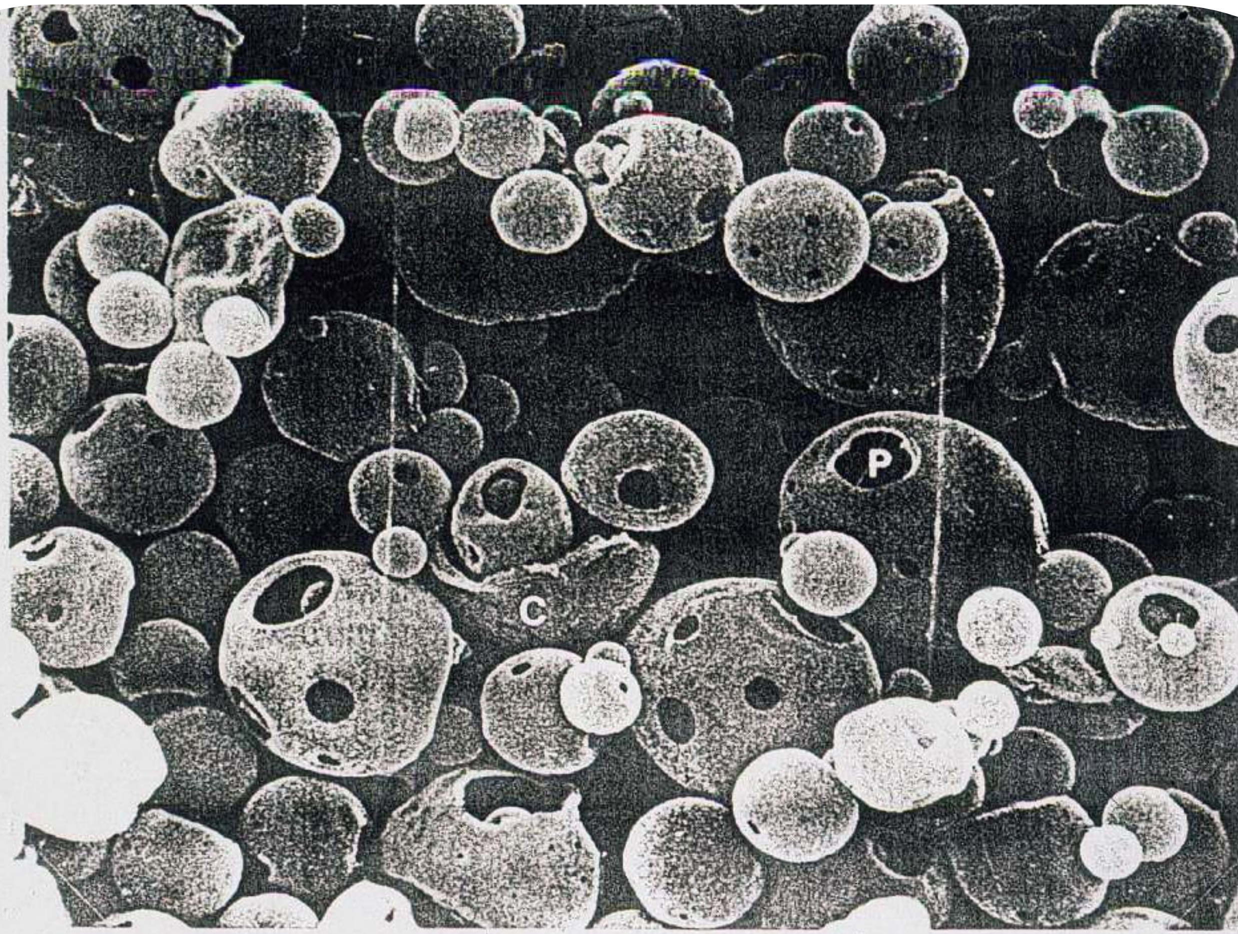
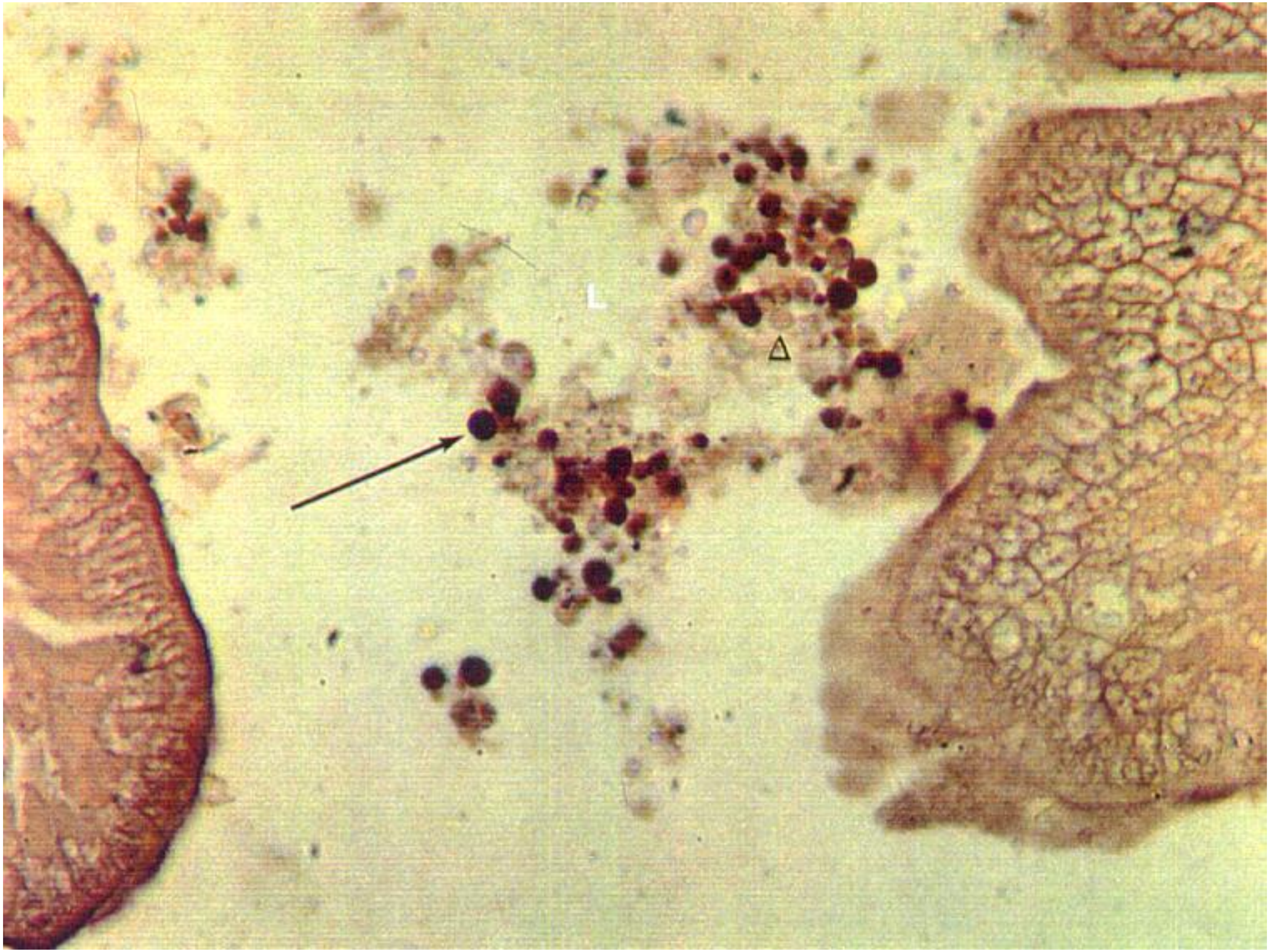
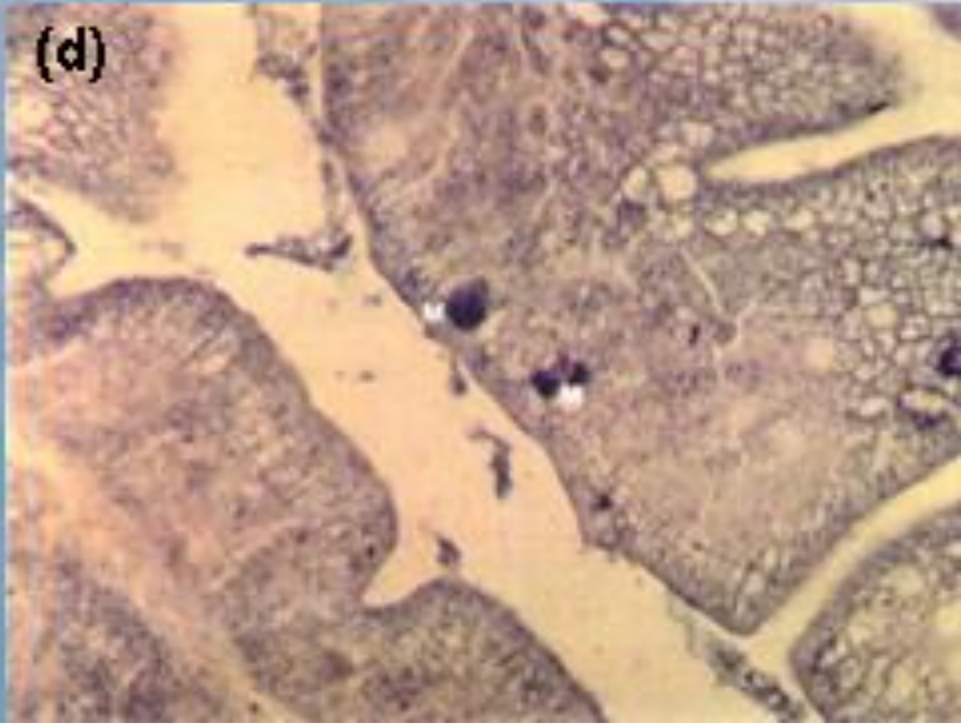


Fig. 1. Scanning electron micrograph of PLGA (50:50) microparticles, incorporating HGG, 18 weeks post-incubation at 15° C (× 2700). Note that many of the microparticles appear pitted (P) and some have collapsed (C).


L
Δ

(c) Day 56, epithelial surface, supranuclear vacuoles (V) intensely stained (x400). DAB peroxidase development.

(d) Day 1, macrophages (M) showing immunoreactivity (x400). $NiCl_2$ enhanced DAB peroxidase development.



VETERINARY PRACTICE MANAGEMENT

A SUPPLEMENT TO VETERINARY PRACTICE

● CONTENTS ●

HOW TO SUCCESSFULLY MARKET THE BOOSTER

Part 1: SOWING THE SEEDS

DAVID HIGGINSON of the Veterinary Health Centre, Blackburn

gives a step-by-step guide to promoting vaccinations and regular pet health care check-ups

Stress 1. The need for annual boosters.

and 2. The benefits of an annual health check. Show by your detailed examination of the pup/kitten that they can expect a *thorough* examination each year.

3. Explain that you will remind them by post.

4. Put on the front of your vaccine certificate a bold message that reads e.g. "*BEN IS PROTECTED UNTIL OCT 90*".

5. We also inform clients that they will be re-called for a full health check at nine months of age (more about this in the next issue).

Harvesting the crop – The recall

1. Large/attractive envelope with picture (preferably colour). Addressed to the PET by name.
2. Invitation to Annual Health Check and Booster (see Panel A). Also pictorial, and including a *health checklist*. This invites involvement, promises much more than just a 'jab', and brings the client in primed with problems to be solved.
3. **Gift Incentive** (e.g. a paw-print tea towel) if vaccinated within 14 days. (See Panel B). (Thanks to RMB for developing this idea).
4. **Information leaflet** on a selected topic – e.g. 'Identichip'. Only one or two leaflets, don't over do it. Overdue reminders are sent out to all boosters not done within three months of the date due.

Going through the mill (See Panel C) – Three grinding wheels to maximise your response

1. **What the client 'thinks' his pet needs**

Whatever else you do, you must deal with the problems uppermost in the client's mind – however trivial. Listen, enquire, read between the lines, and listen to your nurse. Nurses are often better placed to enquire into those initial concerns while he/she weighs the pet, finds out what food is being fed, and when the animal was last wormed. (All valuable ammunition to have before the consultation starts).

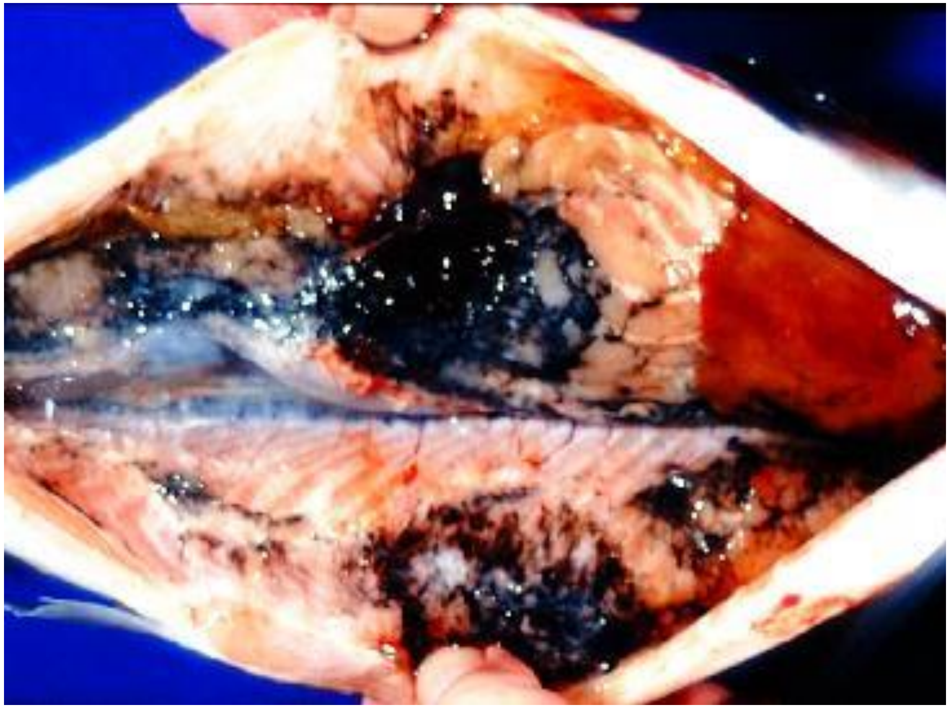
2. **What you discover that the pet needs**

Discover is the important word here. The *thrill* and excitement of finding hidden treasure. Be enthusiastic about your *thorough* examination. Be seen to be examining the whole body, and talk your way through it. Say "I am looking in here because . . . "I am checking this because . . . *Congratulate the normal. Accentuate the abnormal*. (pause, frown, be concerned, but

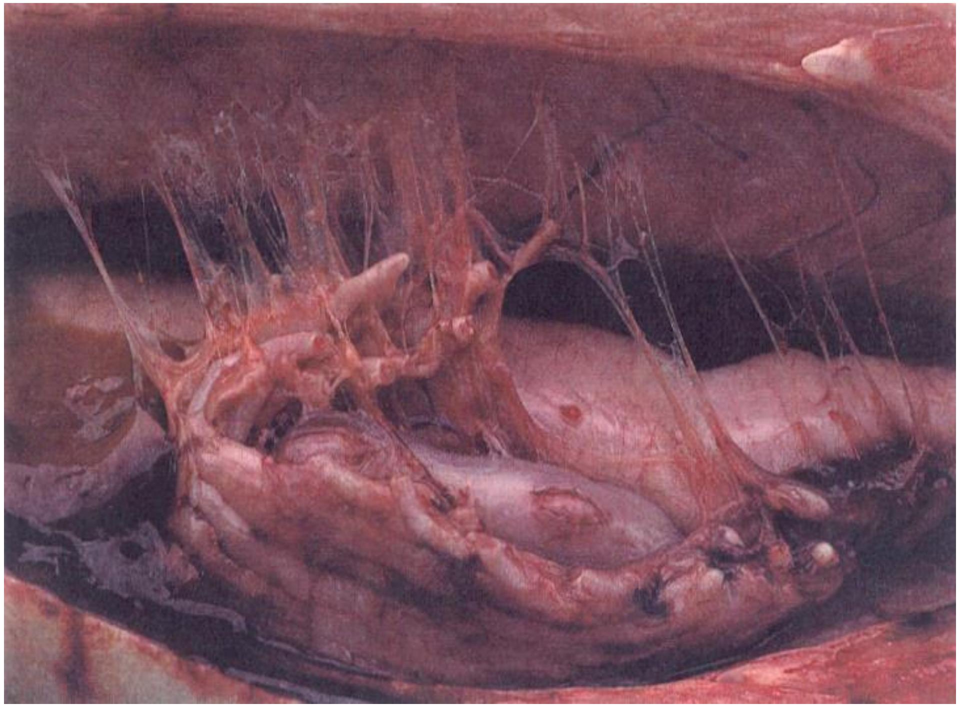
Continued overleaf ▶

Development of Improved Adjuvants

Speilberg Scale - Adhesion Scoring



GRADE 6



GRADE 6?

Adjuvants that specifically stimulate Th1 response in fish

Interleukins

Interleukins are produced by many cell types in response to damage and infection.

So far in mammals 15 interleukins have been discovered.

Of those Il-1 is the most important in the regulation and control of the immune response including:

- Killer-cell activity
- Polymorphonuclear leucocyte activity
- Activation of macrophages and macrophage killing
- B-cell proliferation
- Other functions

Recombinant Ovine Interleukin-1β as an Adjuvant for Multivalent Bacterial Vaccines

8

M. Elhay[1], G. Barcham[2], A. Cameron[3], A. Andrews[2] and A. Nash[2]

[1]TB Research Unit, Statens Seruminstitut, Artillerivej, 5, Copenhagen, 2300S, Denmark; [2]Centre for Animal Biotechnology, The University of Melbourne, Parkville, Victoria 3052, Australia; [3]CSL Ltd, 45 Poplar Road, Parkville, Victoria 3052, Australia

INTRODUCTION

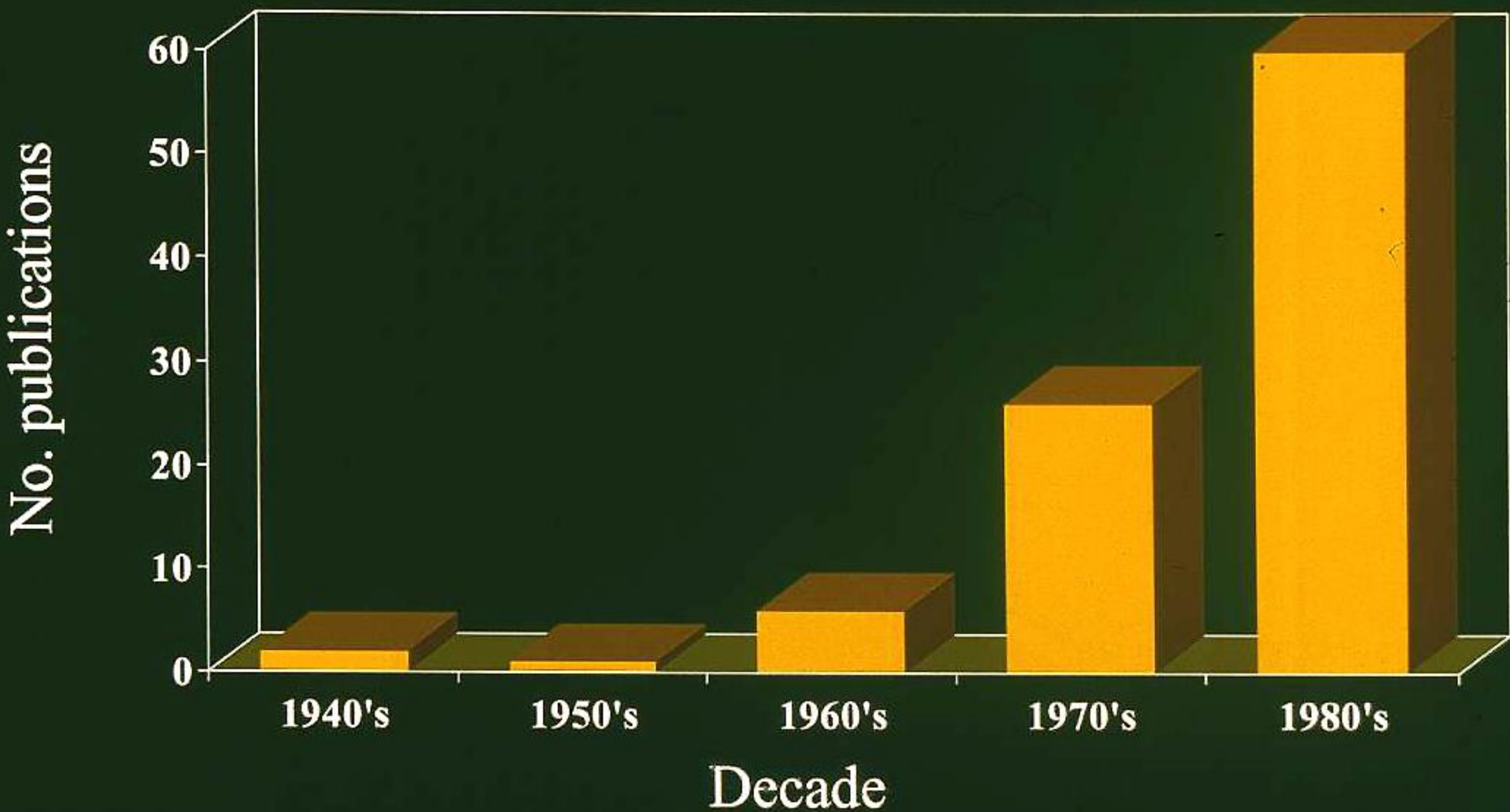
The multifaceted nature of the immune response allows for development of protective immunity against a variety of pathogens, each of which may use a different strategy to infect and multiply within the host. To be effective, vaccination must induce an immune response of both sufficient magnitude and of an effector phenotype appropriate for rejection of the pathogen in question. With the exception of attenuated live vaccines, and some whole cell formulations, the magnitude and phenotype of the response to vaccination is determined primarily by the choice of adjuvant. Currently, aluminium based adjuvants such as aluminium hydroxide [$Al(OH)_3$] gel are the only adjuvants licensed for use in man and the most commonly used adjuvants for animal vaccines. While these compounds have been shown to be relatively safe, i.m. administration can be associated with nodule or granuloma formation (Gupta and Relyveld, 1991) and there may be other less well characterized systemic effects related to the use of aluminium-based compounds. In addition, induction of humoral and particularly cell-mediated immunity (CMI) by aluminium adjuvants is poor compared with induction by more reactive oil-based adjuvants. The response induced by $Al(OH)_3$ is typical of the Th2 type (Grun and Maurer, 1989; Lise and Audibert, 1989) and while this may be appropriate for some pathogens this will not always be the case. Despite intense analysis of alternatives including mycobacterial fractions such as muramyl dipeptide, saponins and their derivatives, block-copolymer gels and hydrocarbon derivatives (reviewed in

 Cytokines in Veterinary Medicine (eds V.E.C.J. Schijns and M.C. Horzinek)

Growth of Research

Increased interest in Finfish vaccination

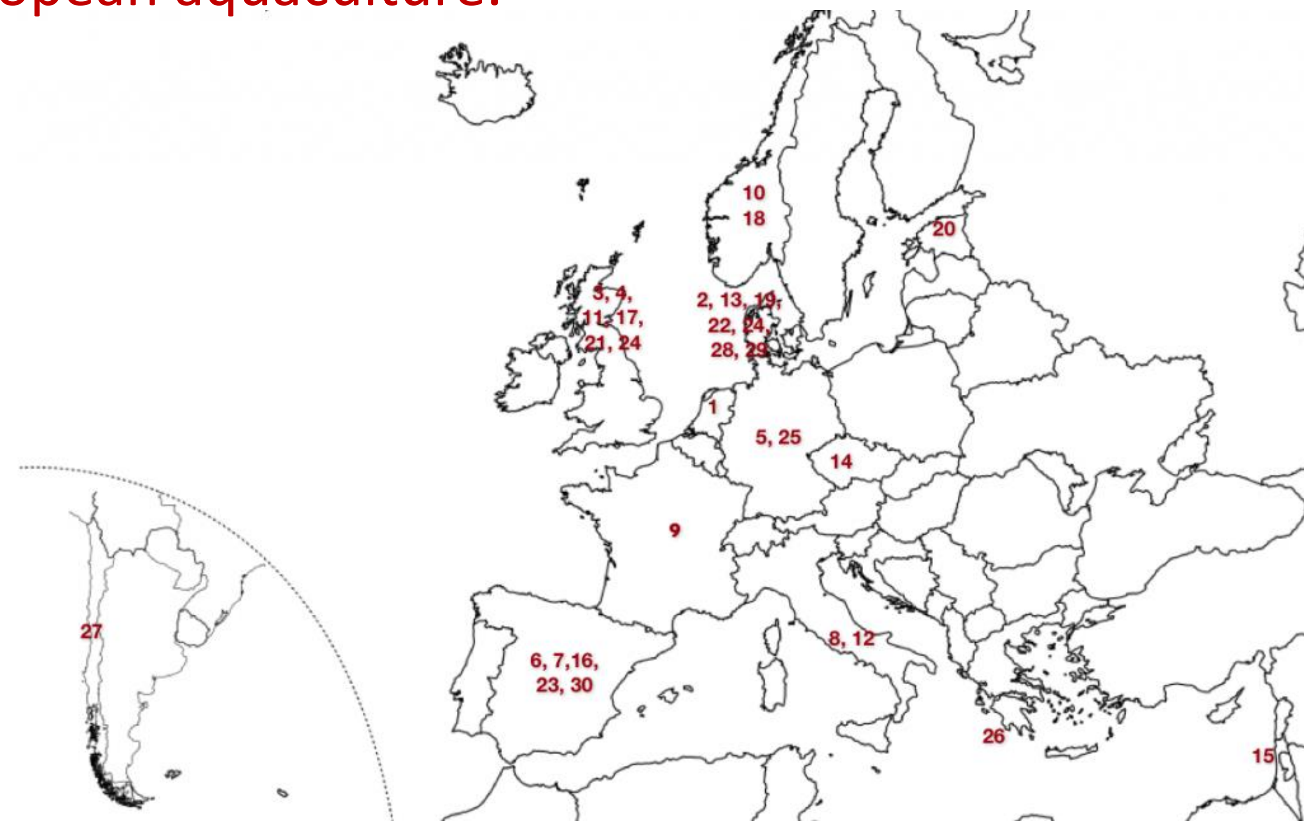
Based on publications on vaccination efficacy trials: 1940’s to 1980’s
(Data from Newman [6])



TargetFish

SEVENTH FRAMEWORK
PROGRAMME

TargetFish brings together a large number of leading European research groups that are experts on the fish immune system and enterprises from the Biotech and Veterinary sectors to advance the development of vaccines against important viral or bacterial pathogens in European aquaculture.



TargetFish is a large collaborative project funded by the European Commission under the 7th Framework Programme for Research and Technological Development.

SEVENTH FRAMEWORK PROGRAMME

The ‘blind-alley’

- **Most ‘easy’ vaccines have been developed leaving vaccines against the more-difficult pathogens to be focused upon.**
- **We need to focus on new technologies etc. to make these vaccines available and thus remove a considerable constraint on the continued development of the aquaculture industry.**

Registration of ‘second generation vaccines – a way forward or a ‘glass ceiling’ for aquatic animal health products?

- All veterinary vaccines for which the active ingredient has been derived by biotechnology must be registered through the Centralised Procedure
- Includes those containing genetically modified organisms (GMOs), recombinant but non-living organisms, virus-like particles, DNA vaccines, etc. (Definition in Directive 2001/18)

- Pros and cons:

- Compared to National Procedures the Centralised route is the most predictable in terms of timelines, administration and interaction with the Rapporteur and Co-rapporteurs.
- It can be expensive - > Euro 150,000 + Euro 32,000 maintenance fee. (but see later)

- The requirements for the registration dossier are fairly honerous
- An Environmental Risk Assessment (ERA) is required- expensive !!
- Potency test required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How can we utilize some of the incentives available for the registration of 'second generation' products ?

- Not an easy answer and 'level playing field' arguments come into play.
- MUMS and SMEs can command a reduction in registration fees – Fees reduced by 90-100% !
- Rainbow trout = MUMS

  Sea Bass and Sea Bream =MUMS
- If we use SME status, how can large pharmaceutical companies work with them.

Disease/ Research Prioritisation

With the increase of disease pressures in aquaculture and funding constraints there needs to be a robust and regularly-applied

Prioritisation Exercise

League table 1 - 2013

TROUT CONDITION	2013 v. 2012	5 year trend	Ranking/Cost ????	CONTROL	COMMENTS
Proliferative Kidney Disease (P.K.D.)	↑	↓	1 £??	Exposure programmes No treatment Resistant strains?	Failure of 2012 exposure programmes?
Red mark Syndrome (R.M.S.)	=	↑	2 £??	Untreated recovery Oxytetracycline Health diets	Increase on restocking farms and sport fisheries Now confirmed on brown trout Rejections on table farms
Enteric Redmouth (E.R.M.)	=	↓	3 £??	Immersion/oral /injection vaccination Oxolinic acid (Branzil)	5 year trend down. Properly executed vaccination is controlling impact. Some farms injecting – 100% effective
'Puffy Skin Condition'	↑	↑	4 £??	None? Fish health diets?	Mostly large triploid fish, increasing incidence and severity, CEFAS project
Rainbow Trout Fry Syndrome (R.T.F.S.)	=	=	5 £??	Florfenicol (Florocol), Amoxycillin (Vetremox) Preventative health diets,	Affecting larger fish. Resistance to florfenicol ??
White Spot	↑	↓	6 £??	Formalin, long term salt baths, increase water velocity	Increase v. 2012 Hot weather and low water levels Future availability of formalin?

DISEASE SCORECARD SEALICE	None (0)	Low (1)	Moderate (2)	High (3)	Weight	Weighted item score
1. RISK & IMPACTS						
1.1 Human health			(only one score per item)			
Is this a zoonotic disease? What is the risk?	0				5	-
Risk of food poisening?	0				5	-
Sum Human health						-
1.2 Aquatic animal health			(only one score per item)			
Risk of territorial spread				3	4	12.0
Risk of spread across species		1			4	4.0
Sum health risk						8.0
1.3 Environmental			(only one score per item)			
Effect on wild populations				3	4	12.0
Ecological effects			2		3	6.0
Sum environmental score						9.0
1.4 Financial			(only one score per item)			
Direct loss from mortality			2		5	10.0
Direct loss due to high FCR/reduced growth/lower quality			2		5	10.0
Trade loss		1			3	3.0
Loss due to cost of official control measures			2		4	8.0
Sum Financial						7.8
1.5 Customer view and societal impact			(only one score per item)			
Customer view		1			1	1.0
Societal impact			2		2	4.0
Security of supply	0				4	-
Sum customer view and societal impact						1.7
TOTAL RISK & IMPACT SCORE						26.4
2. KNOWLEDGE GAP			(only one score per item)			
Characterisation of agent / disease	0				4	-
Basic epidemiology			2		5	10.0
Immunology				3	5	15.0
Strain/species variation			2		5	10.0
Diagnostics	0				4	-
SUM GAP SCORE						7.0
3. REQUIREMENT OF CONTROL MEASURES			(only one score per item)			
Management/husbandry practices		1			5	5.0
Chemotherapeutants				3	3	9.0
Vaccines	0			3	5	15.0
SUM CONTROL SCORE						9.7
OVERALL RATING						**75.3**


INDUSTRY
$\pi x^3 + ab^2$
$+ 3dx^4 - xy$
$+ ab^2(2ab^2)$
$+ 3bc^3 - 2d$
$= 4x^2$
$3bc - 2d$
$3bx^3 -$
$2d^2 - ab^2$
$3d - b$
$3-d$
$x - b^2$
$3ad$
$- 42b -$
$+ 3ac/2$
$=$
$3ad$
$- 4bc$
$- b^2$
$2x$
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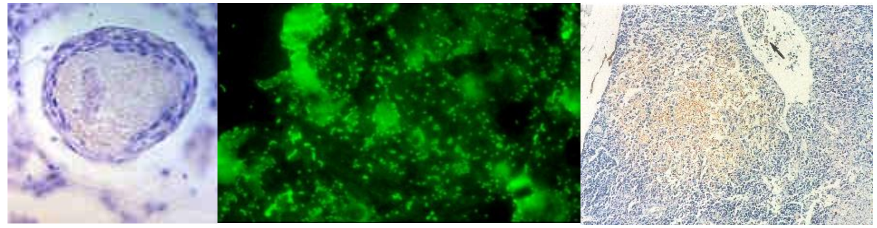
Graphic by John Joyce

Diagnostics

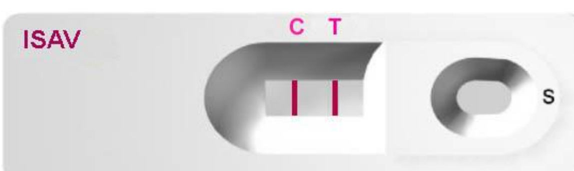
Reduction in the use of antibiotics,as well as relying on the use of vaccines, also depends on trained veterinary monitoring and the development and routine use of rapid diagnostic methods.

AQUATIC Diagnostics Ltd



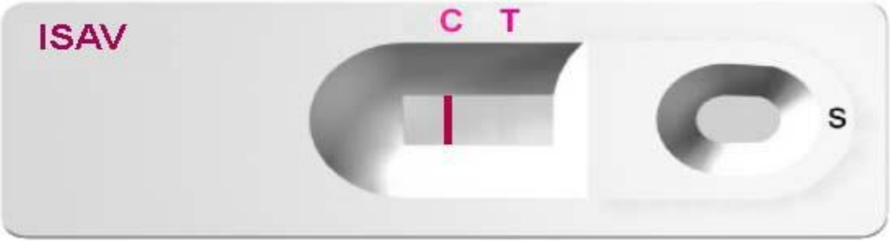
PRODUCTS

- AquaMab-P **Detect pathogens** IHC & IFAT
- AquaMab-F **Detect fish species IgM** ELISA
- Pre-coated ELISA plates
- HRP Conjugates
- Rapid Test Kits

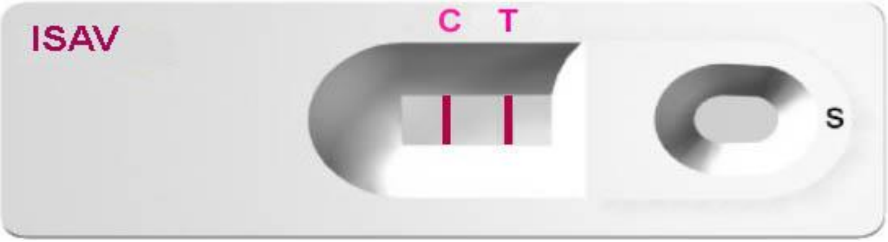


ISAV RAPID KITS

- **Negative Result**



- **Positive Result**



Thank you

There has been a recent focus on the use of antibiotics in animal husbandry throughout Europe

- “Over-use of antibiotics is an issue in animal husbandry, agriculture and fish farming”
- “The development of germs that are resistant to even the strongest of our current antibodies is one of the biggest health threats in the world.”
- “ We continue to use them (antibiotics) in agriculture, fish farming and myriad other areas of life.”
- “......the typical farmed American salmon eats its own weight in antibiotics before it is sold.”
-

Dame Sally Davies, UK Chief Medical Officer

WHAT ARE THE FACTS ?

TETHYS AQUACULTURE

(2008)

Les Burridge[1], Judith Weis[2], Felipe Cabello[3] and Jaime Pizarro[4]

Country	Salmon Production (Metric Ton)[a]	Therapeutant Type	Kg (active ingredient) Used	Kg Therapeutant/ Metric Ton produced
Norway	509544	Antibiotics	805	0.0016
		Anti-louse	98	0.0002
		Anaesthetics	1201	0.0023
Chile	280,481	Antibiotics	133800	0.477
		Anti-louse	136.25	0.0005
		Anaesthetics	3530	0.013
UK	145609	Antibiotics	662	0.0045
		Anti-louse	110	0.0007
		Anaesthetics	191	0.0013
		Disinfectants	1848	0.013
Canada (includes data from Maine, USA)	111,178[b]	Antibiotics	30,373[c]	0.273
		Anti-louse	12.1	0.00011

a Data accessed at FAO (http://www.fao.org/fi/website/FIRetrieveAction.do?dom=collection&xml=global-aquaculture-production.xml&xp_nav=1)

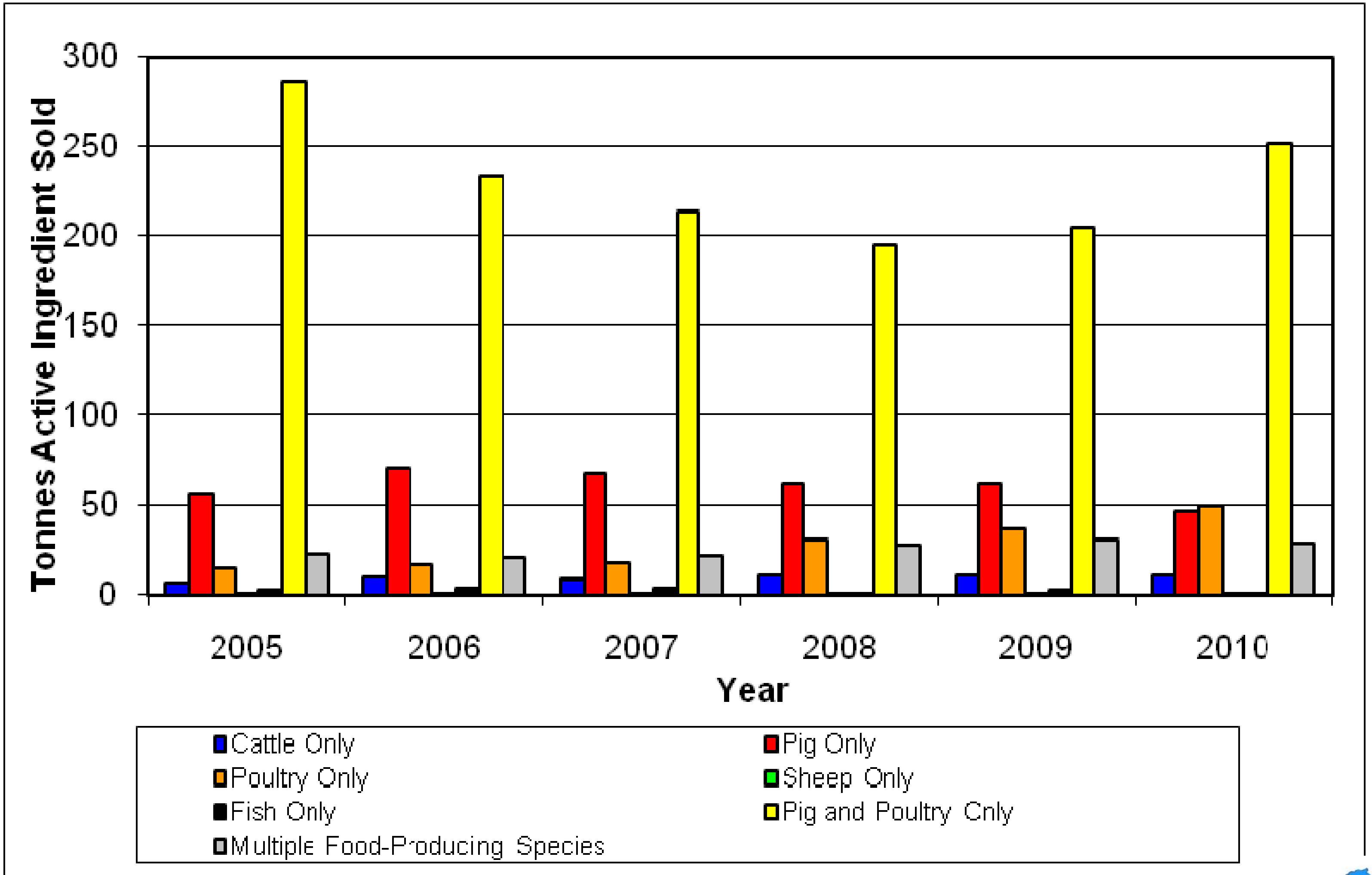
b Data accessed at http://www.dfo-mpo.gc.ca/communic/statistics/aqua/index_e.htm and New Brunswick Salmon Growers Association (personal communication).

c Source: Government of British Columbia (http://www.al.gov.bc.ca/ahc/fish_health/antibiotics.

ERM VACCINE FIELD TRIALS

Medicated Feed Utilisation

	Medicated Feed (kg)/ 1000 Fish		%
	Vaccinates	Controls	Reduction
Trial 1	4.5	16.8	73
Trial 2	2.27	15.9	86
Trial 3	6.3	22.0	72



Tonnes Active Ingredient Sold
300
250
200
150
100
50
0
2005
2006
2007
2008
2009
2010
Year
Cattle Only
Pig Only
Poultry Only
Sheep Only
Fish Only
Pig and Poultry Only
Multiple Food-Producing Species

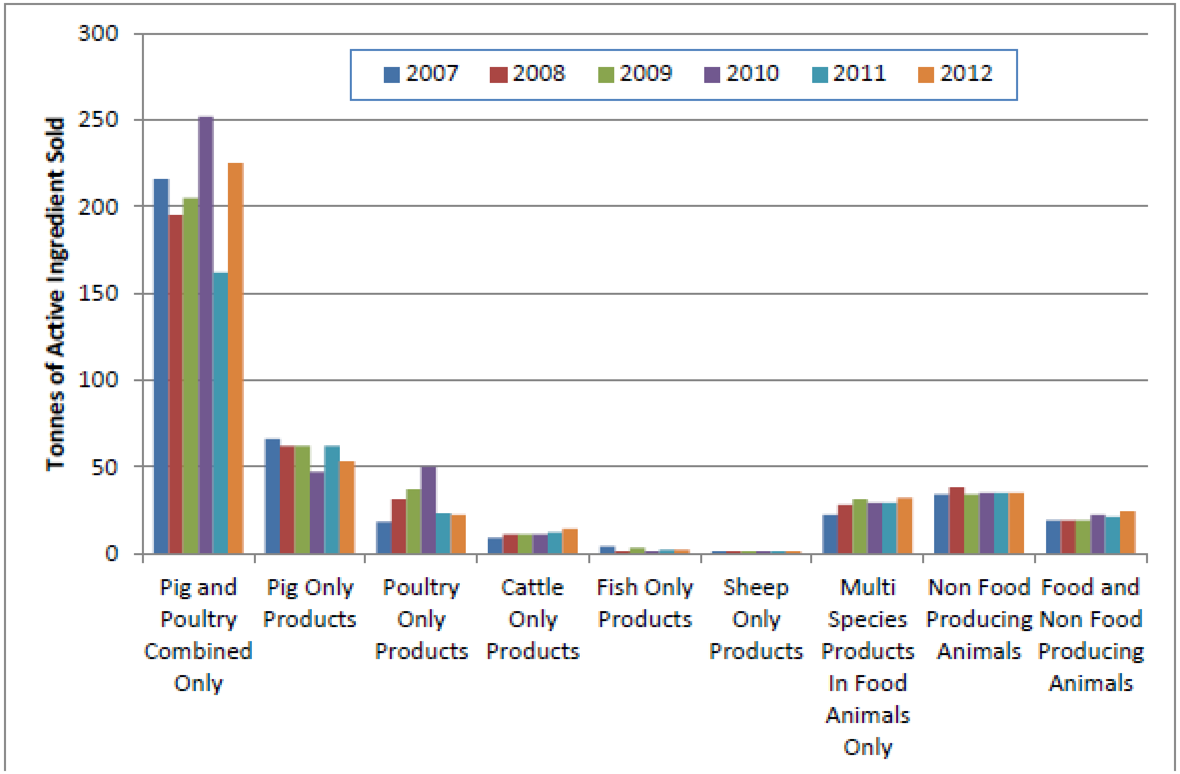
Table 2: Distribution of antibiotic sales by species

	2007	2008	2009	2010	2011
	Tonnes Active Ingredient				
Cattle Only Products	9	11	11	11	12
Pig Only Products	66	62	62	47	62
Poultry Only Products	18	31	37	50	23
Sheep Only Products	<1	<1	<1	<1	<1
Fish Only Products	4	1	3	1	2
Pig and Poultry Combined Only	216	195	205	252	162
Multi Species Products In Food Animals Only	22	28	31	29	29

TETHYS AQUACULTURE

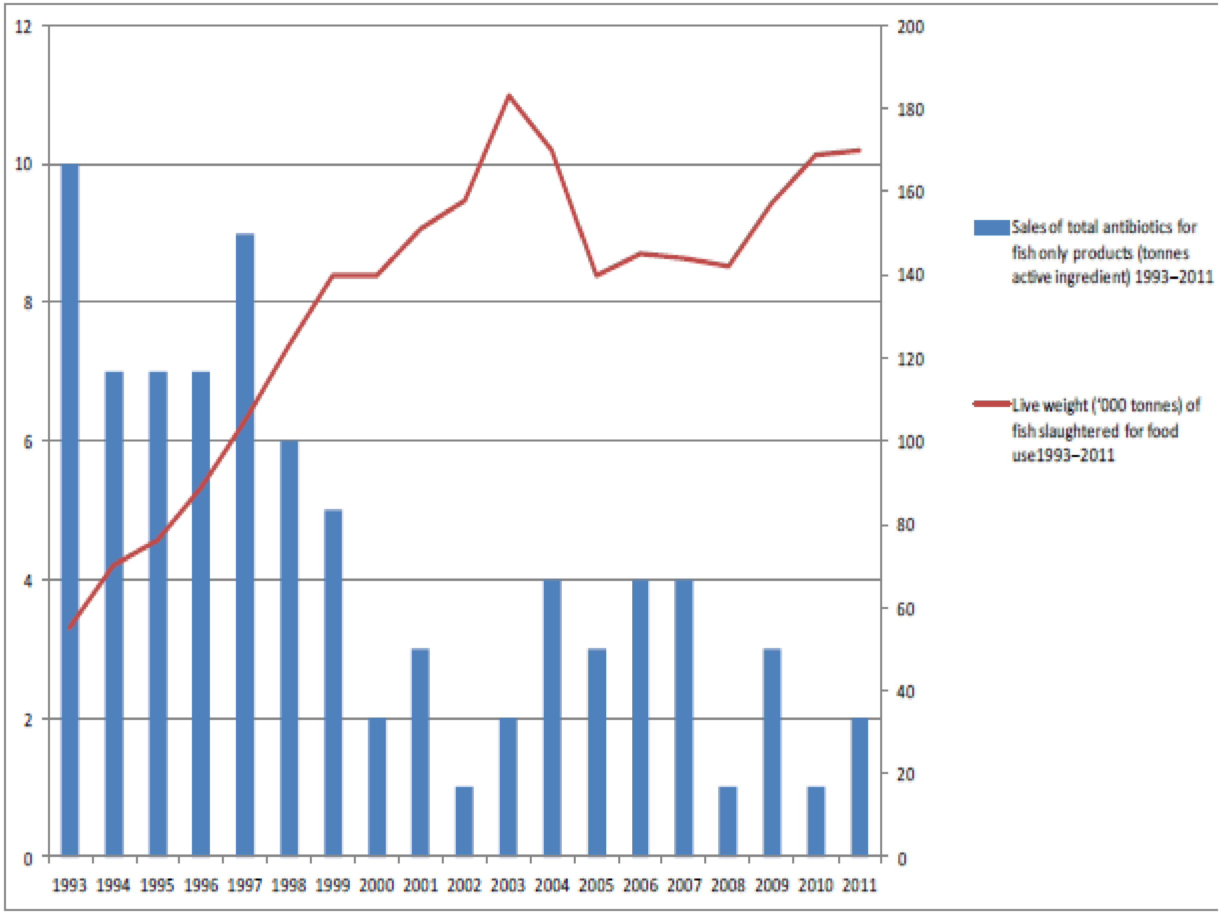
UK

Figure 2: Distribution of antibiotics sold by species



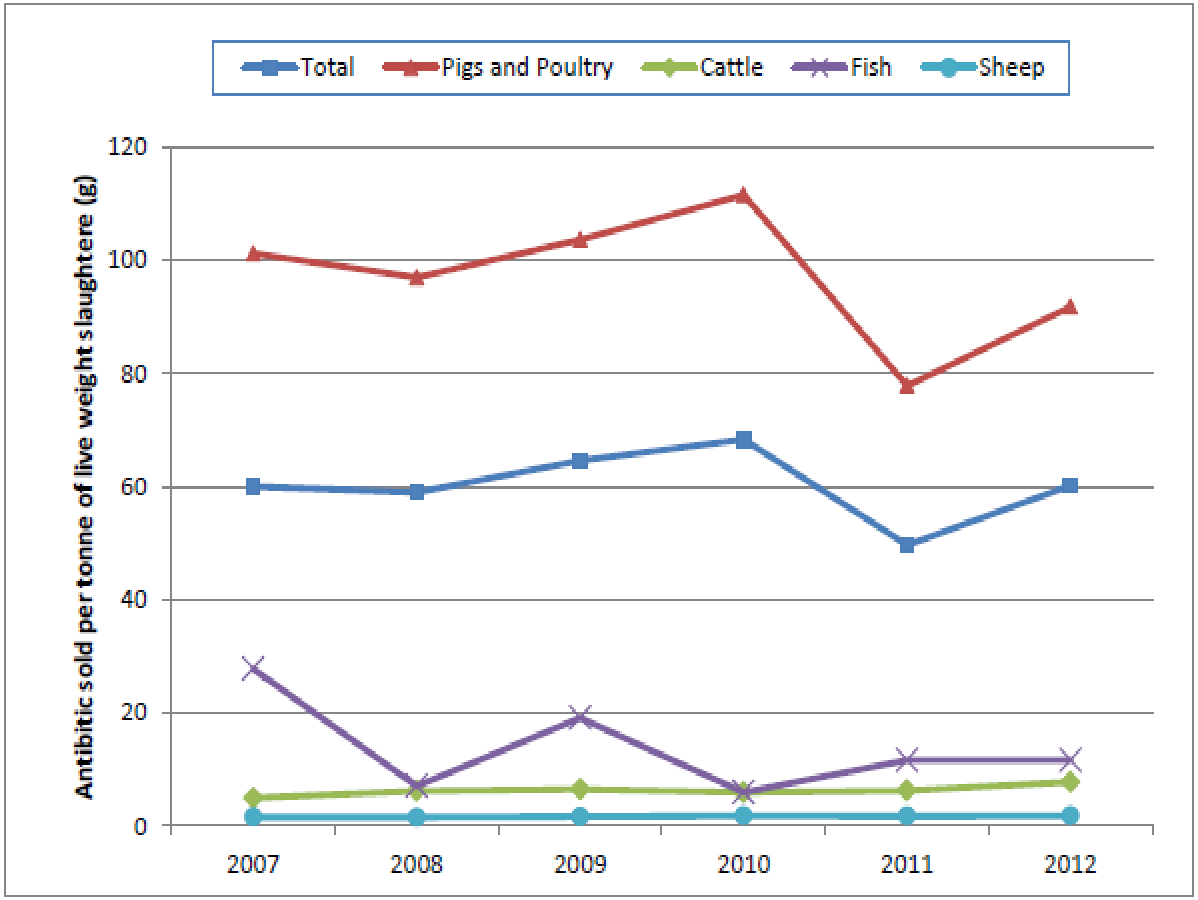
UK

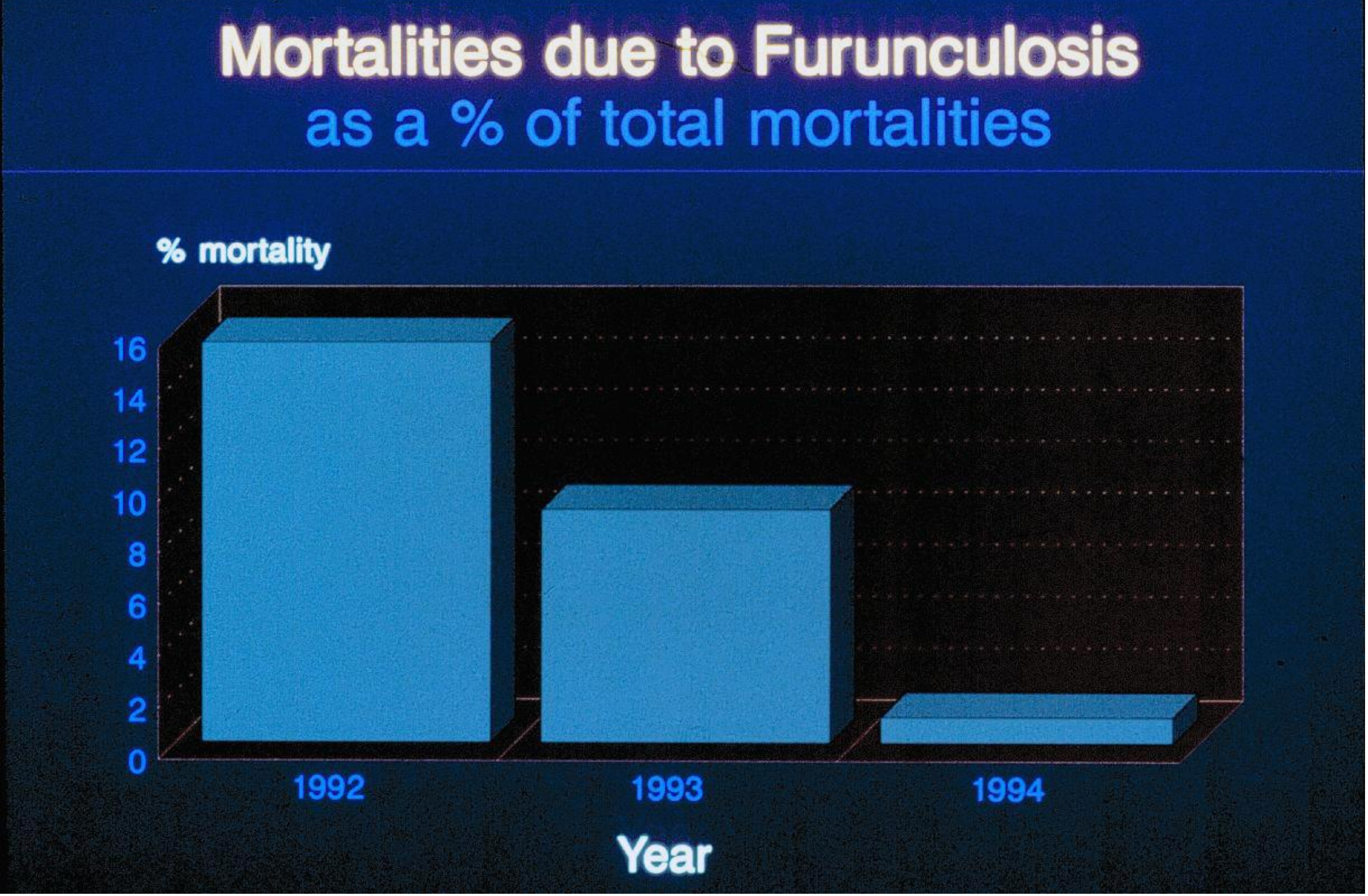
Figure 3: Sales of total antibiotics for fish only (tonnes active ingredient) by live weight ('000 tonnes) of animals (fish) slaughtered for food use 1993–2011



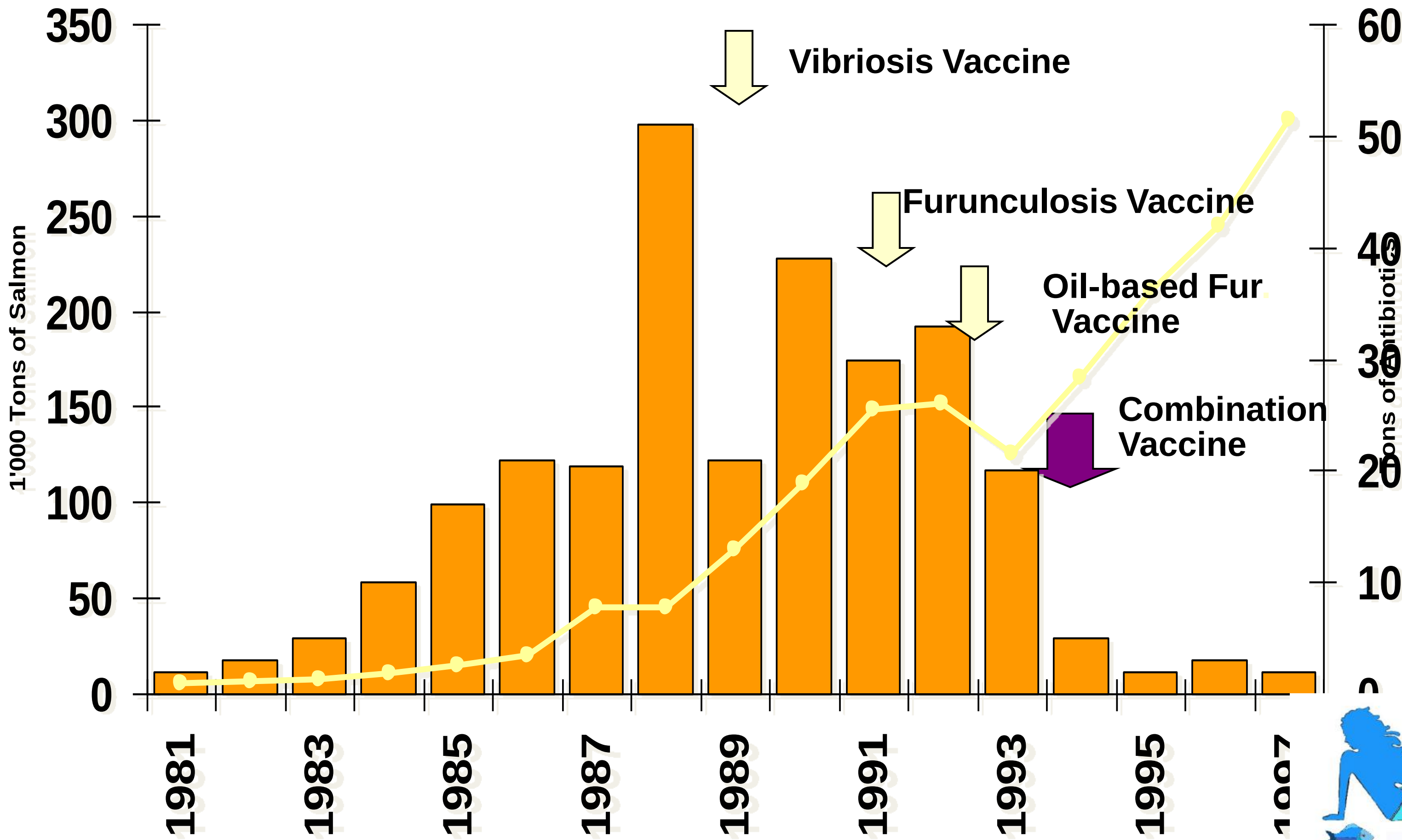
- UK

Figure 4: Distribution of antibiotics sold (grams active ingredient) per tonne of live weight of animals slaughtered for food

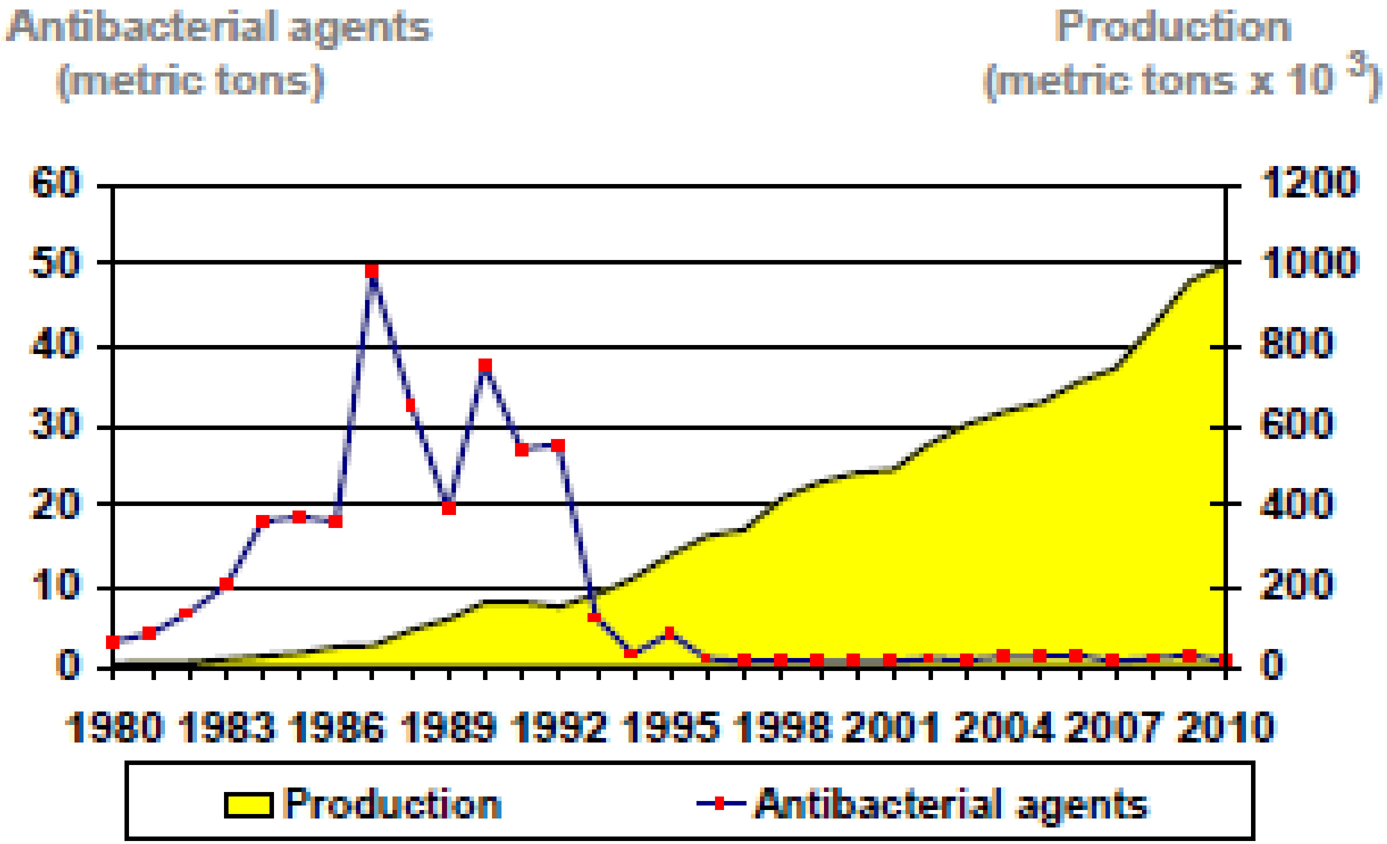



Mortalities due to Furunculosis
as a % of total mortalities
% mortality
16
14
12
10
8
6
4
2
0
1992
1993
1994
Year

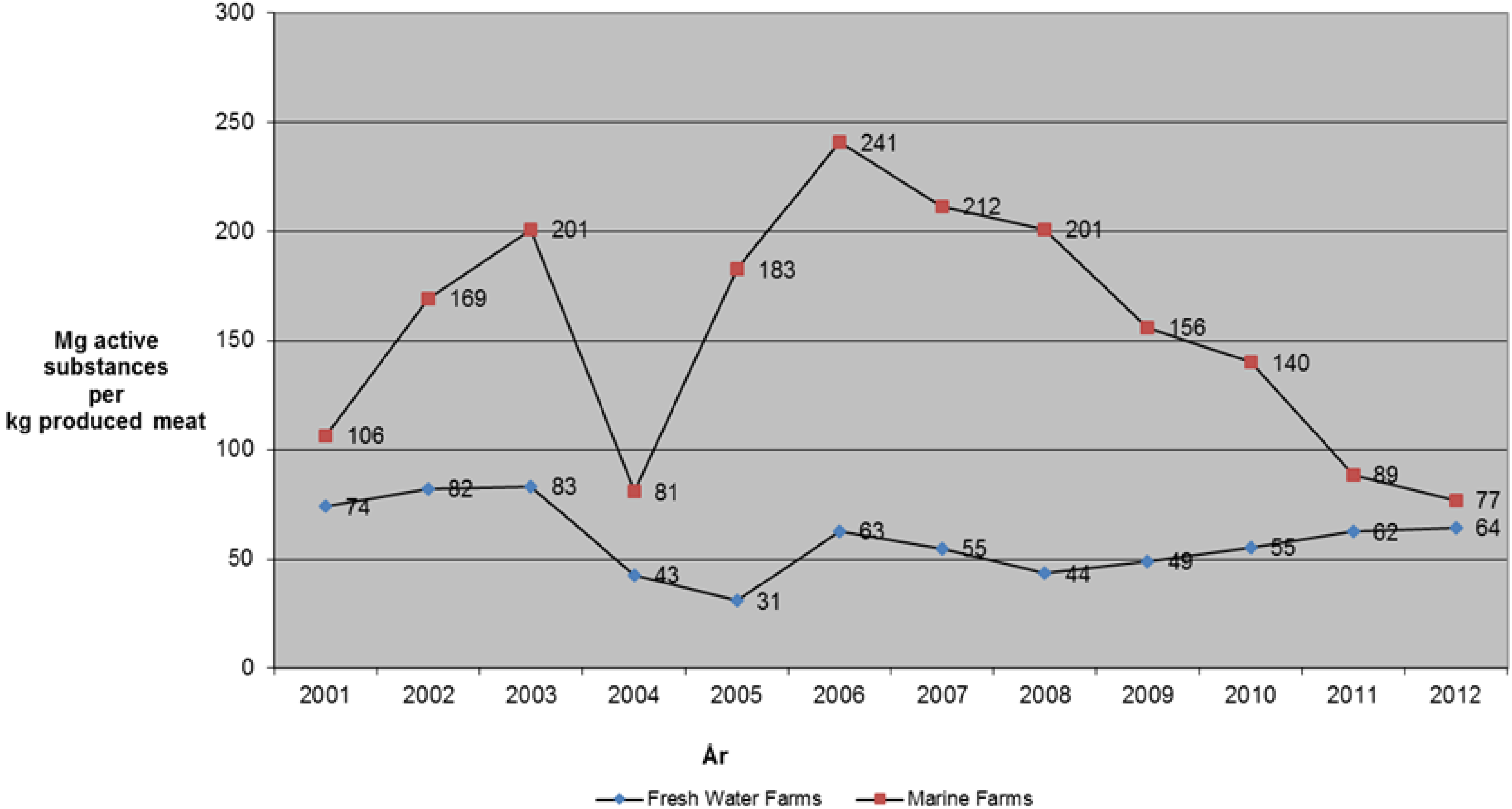
Norwegian Salmon Production
Consumption of Pure Antibiotics and Effect of Vaccines



Antibacterial agents



Antibiotic use
Danish Fish Farming



Exceptions to the Rule ?

- Ornamental fish industry

- SE Asia

- Latin America (mainly Chile)

Latter two must be regarded as ‘work in progress’- new industries, new emerging diseases and lateness in developing/adopting vaccination.