“Antimicrobial use in Chilean aquaculture”

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Salmon aquaculture in Chile

Atlantic salmon life history

Freshwater Ocean

smoltification

Parr → Young salmon

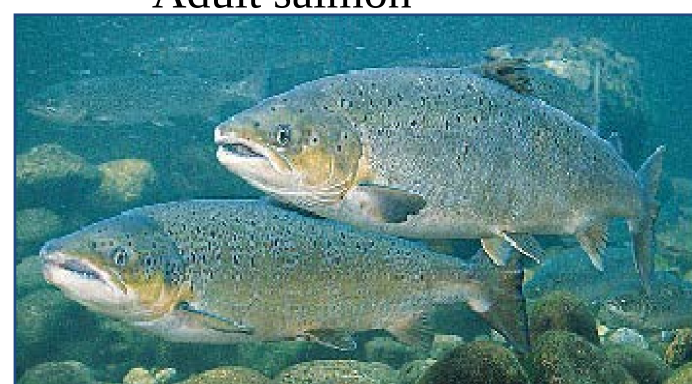
typically
2 to 3 years

Eggs

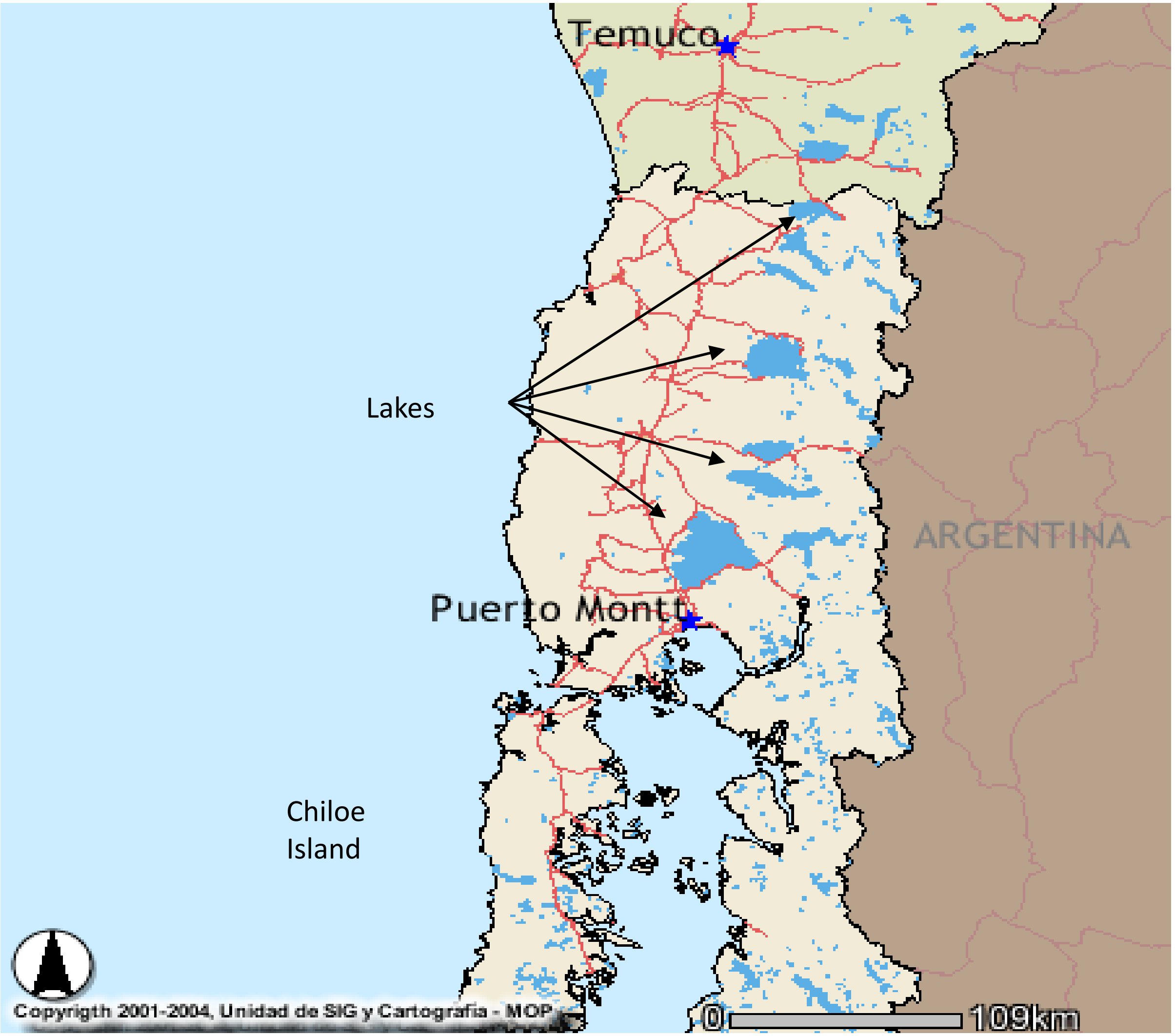
typically
2 years

desmoltification

Spawning ← Adult salmon



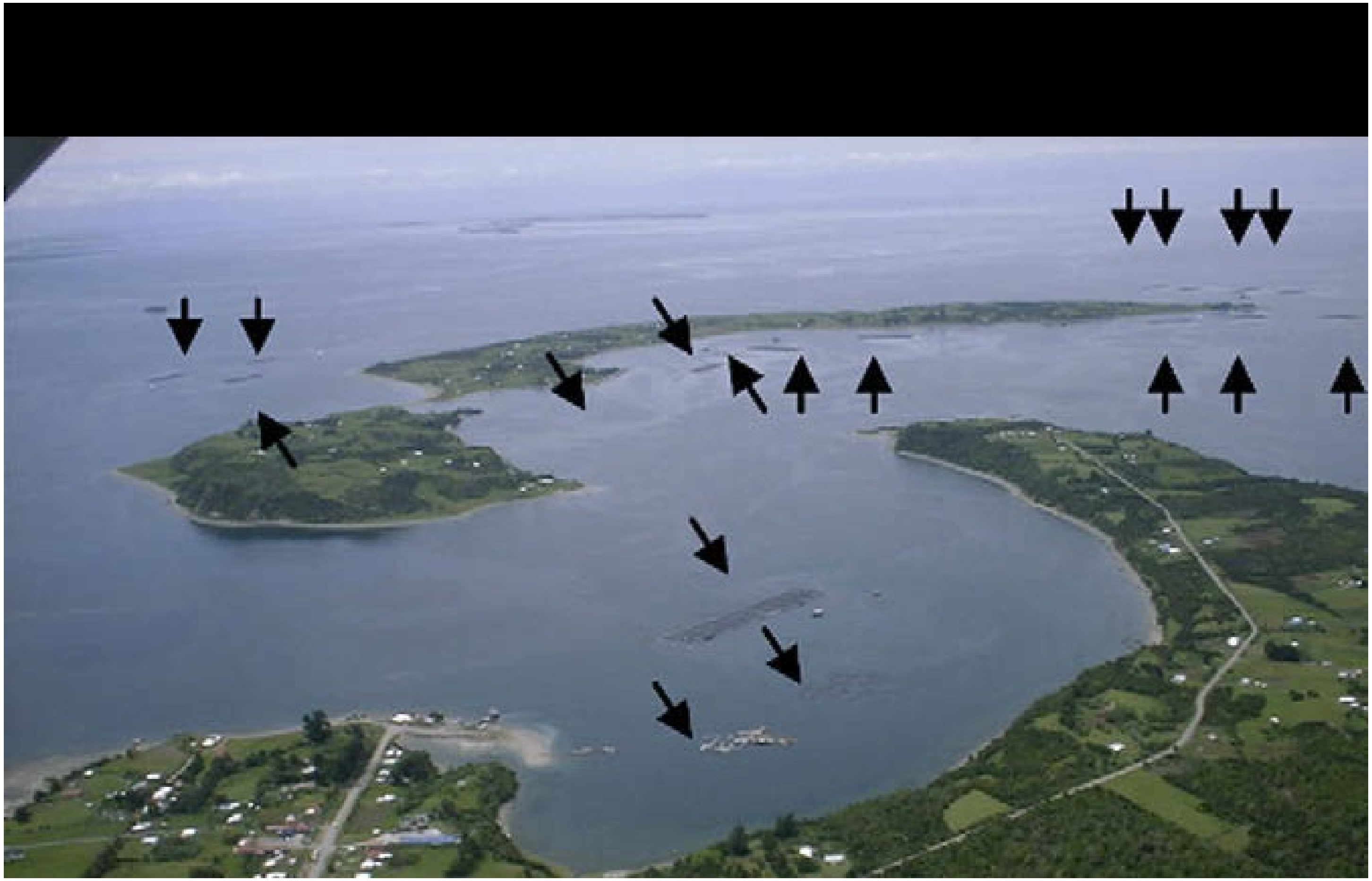
Chile Regions IX and X



Smolt transport

Aquaculture region

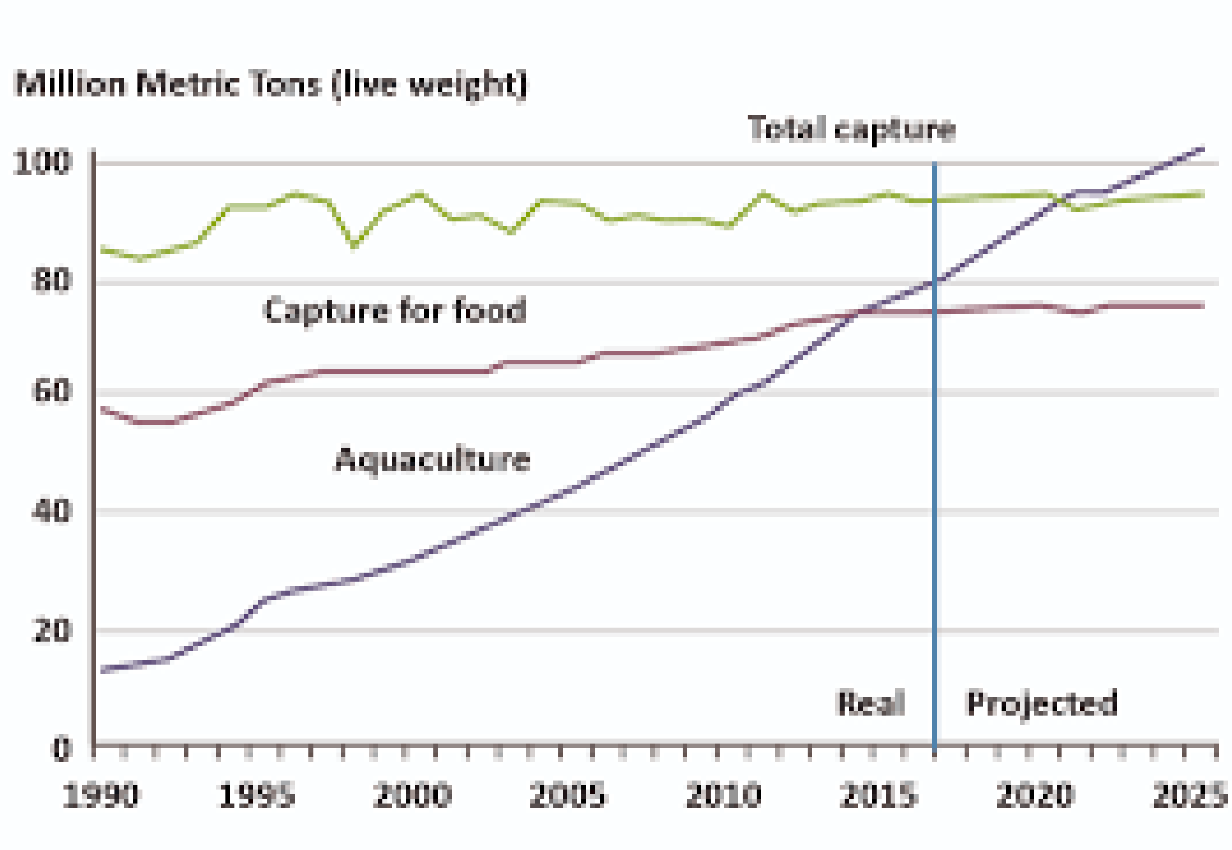
Salmon farms, Chiloe Island, Chile



Buschmann et al, 2009

Antimicrobial use in salmon aquaculture in Chile

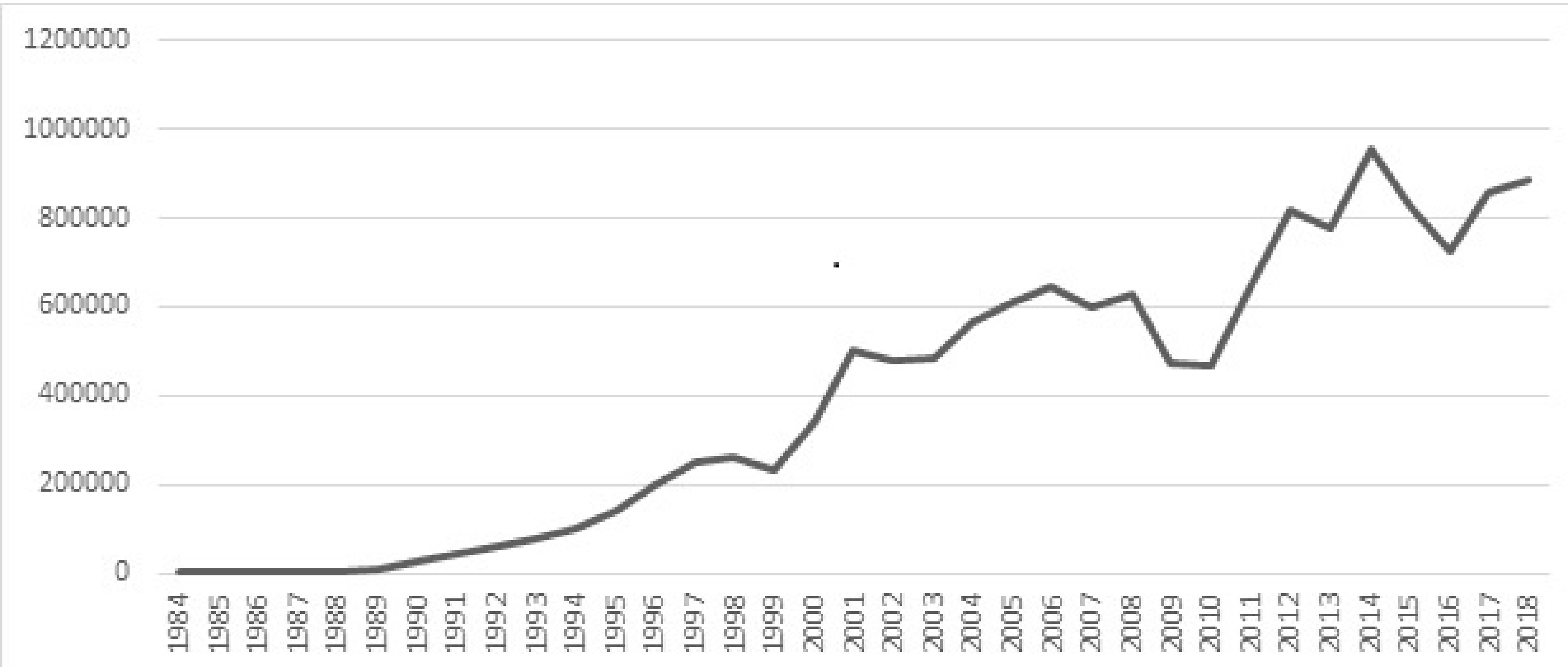
Growth of aquaculture



World aquaculture and wild capture fisheries production from 1990 to 2025 (real and projected). Total capture fisheries include harvests destined for food, fishmeal and industrial uses. Source: OECD-FAO Agricultural Outlook 2017-2026

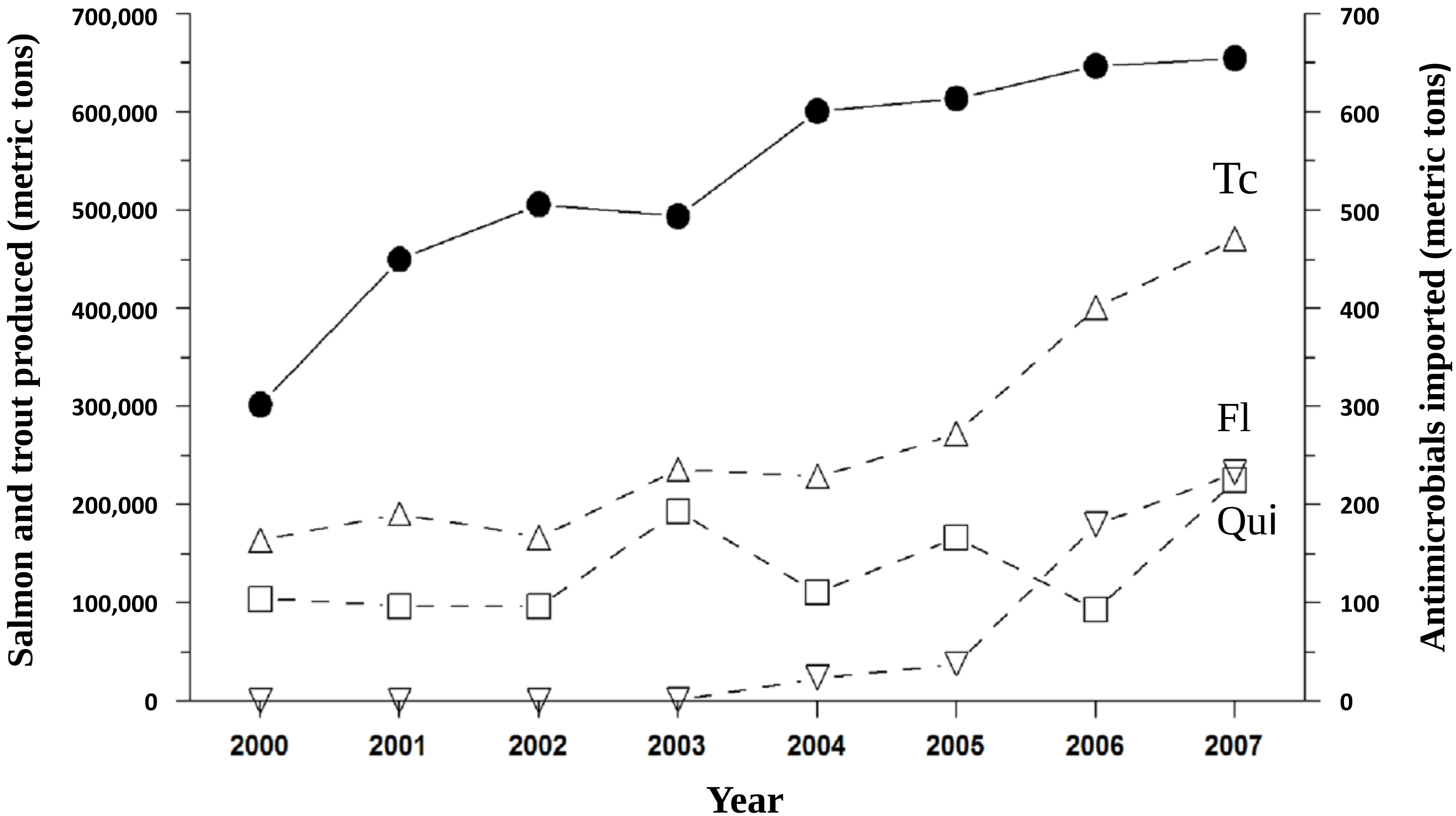
Lutz and Green, 2018

Annual growth of the production of salmon in Chile

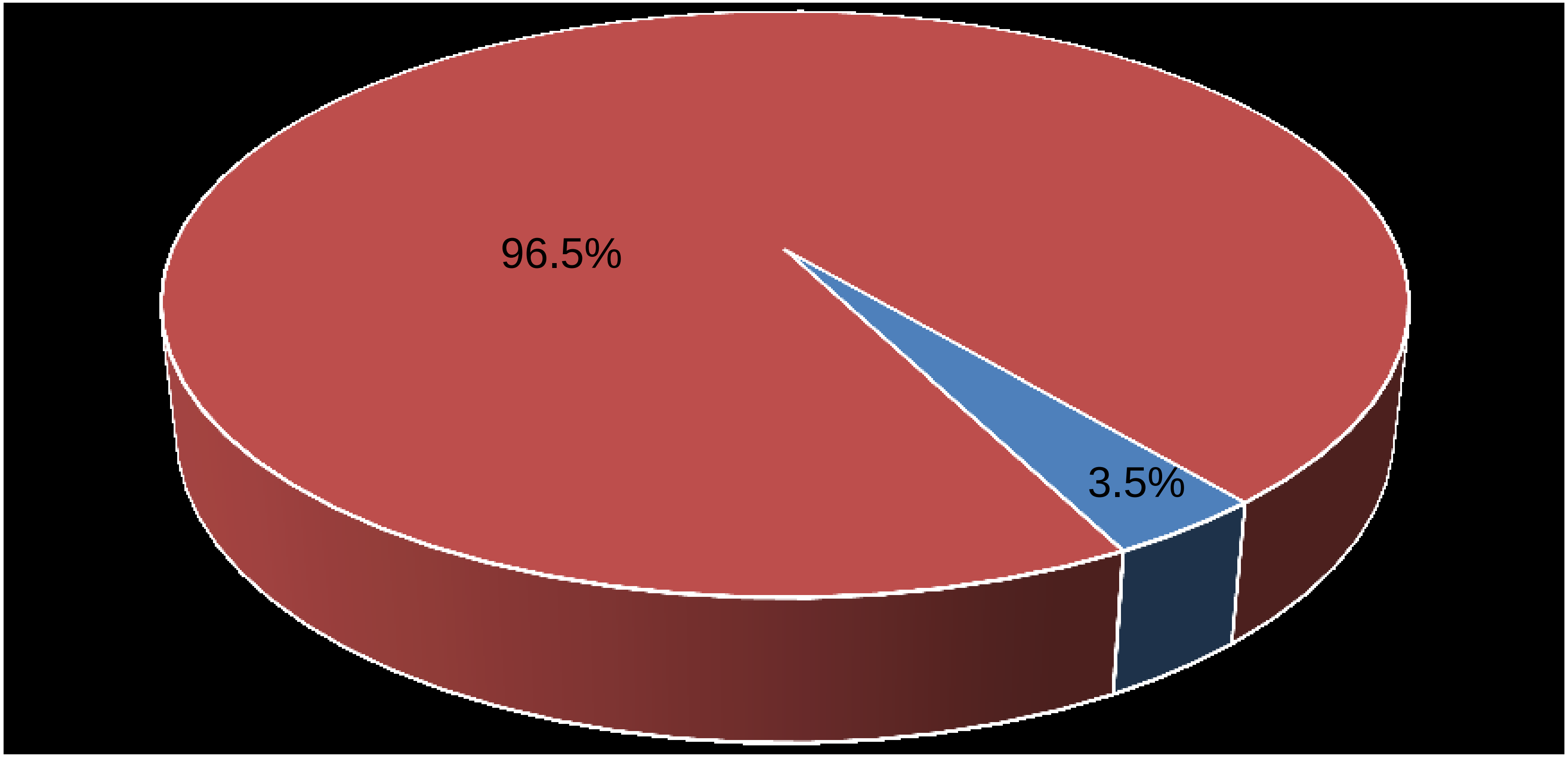


Bustos B, Chile, 2020

Production of salmon and trout in Chile and net imports of selected antimicrobials, 2000-2007

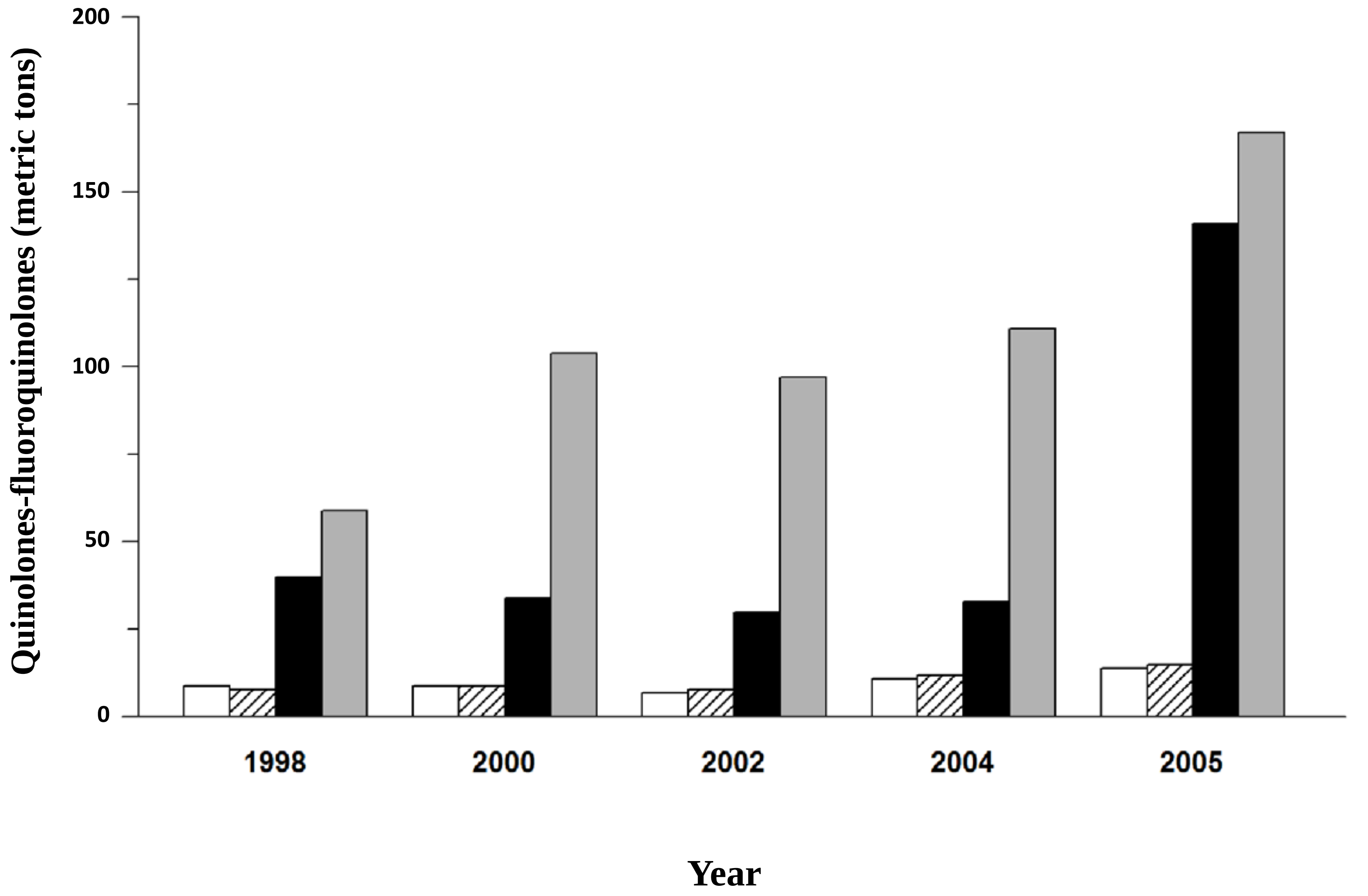


Distribution of the amounts of antimicrobials used in salmon aquaculture in the marine and fresh wáter environments in Chile



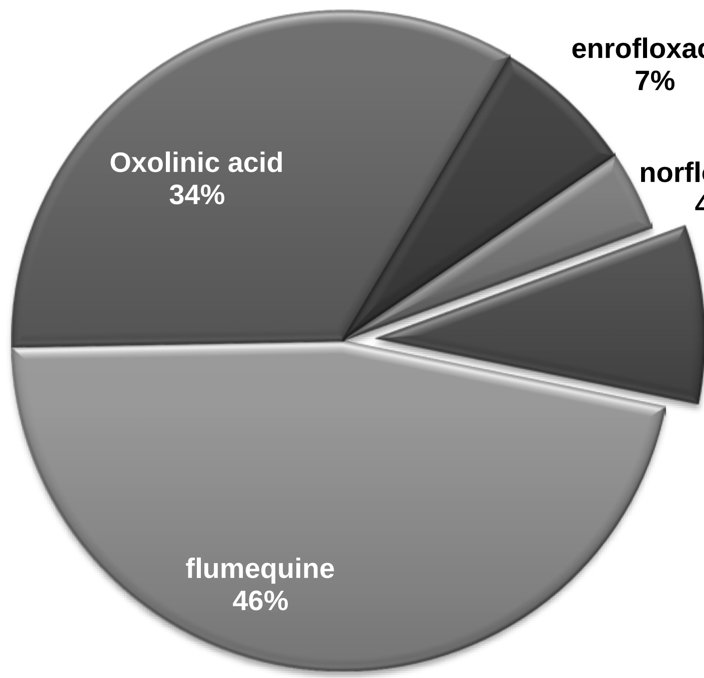
Sernapesca, Chile, 2019

Authorized and imported quantities of quinolones and fluorquinolones for human and veterinary medical use in Chile, 1998-2005



Cabello et al., 2013

Percent distributión of 1193 tons of quinolones y fluorquinolones used in human medicine and veterinary medicine in Chile during the períod 2000 a 2007



Millanao et al, 2010

Amounts of antimicrobials, salmon production and antimicrobial consumption index

Año	Cantidad antimicrobianos (t)	Biomasa cosechada de salmónidos (t)	ICA (%)*
2007	385,6	600,862	0,064
2008	325,6	630,647	0,052
2009	184,5	474,174	0,039
2010	143,2	466,857	0,031
2011	206,8	649,492	0,032
2012	337,9	826,949	0,041
2013	450,7	786,091	0,057
2014	563,2	955,179	0,059
2015	557,2	883,102	0,063
2016	382,5	727,812	0,053
2017	393,9	791,793	0,050
2018	322,7	842,679	0,038

**Índice de Consumo de Antibiótico (%) es, la cantidad de principio activo utilizado (ton) dividido por la cosecha anual de salmónidos por 100.*

Sernapesca, Chile, 2019

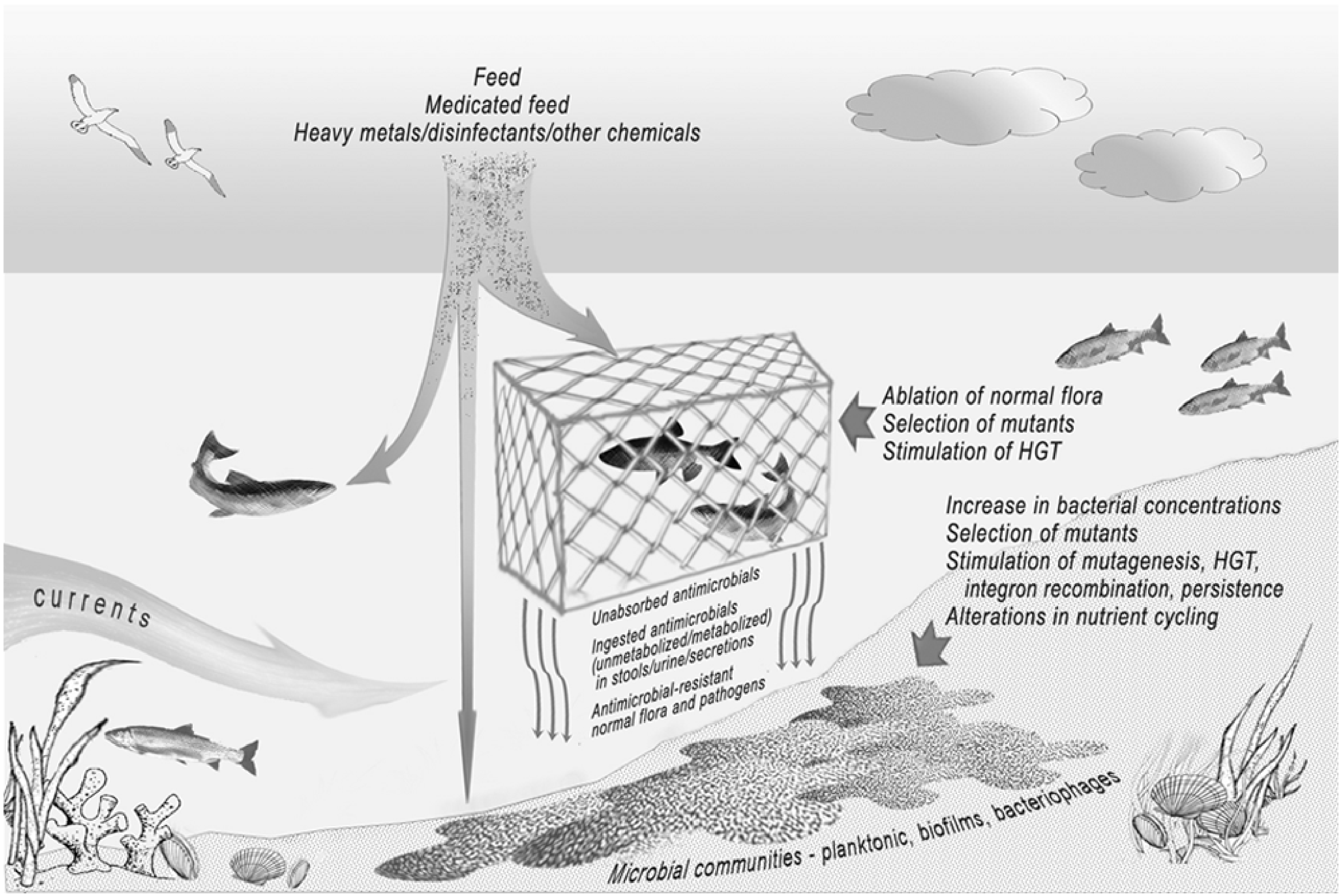
Antimicrobial use indexes of Chilean companies compared to Norway (0.7 g/ton). Red above Norway, Green below

Empresa	Índice de consumo de antibióticos (g/ton)
Cultivos Yadran	799,39
Cooke Aquaculture Chile	623,43
Salmones Camanchaca	532,08
Marine Farm	396,05
Salmones Blumar	381,45
Salmones Multiexport	354,83
Salmones Austral (Trusal y Pacific Star)	351,32
Marine Harvest Chile (Mowi)	296,34
Productos del Mar Ventisqueros	289,11
Salmones de Chile	282,68
Australis Mar	258,05
Salmones Antartica	235,12
Salmones Aysén	94,07
Cermaq Chile	83,36
Salmones Caleta Bay	10,79
Aquagen Chile (*)	10,74
Salmones ICE VAL	5,09
Salmones Humboldt	4,80
Nova Austral	0
Invermar	s/i
Aqua Chile	s/i
Aguas Claras	s/i
Acuimag	s/i
Los Fiordos	s/i

*Es un centro reproductor.

Oceana. 2020

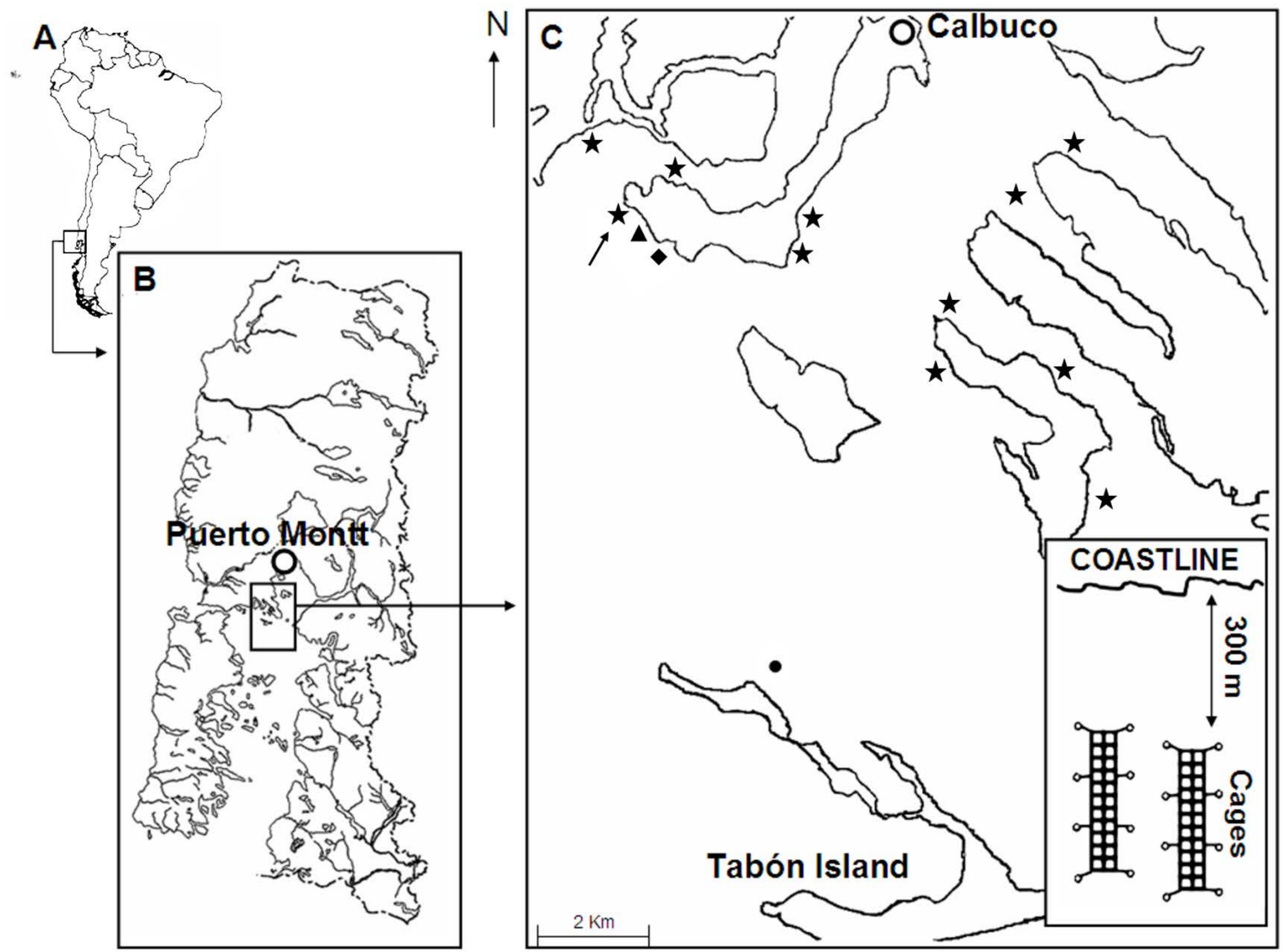
Pathways and effects of administration of antimicrobials in salmon farming



Cabello et al., 2016

Does this excessive antimicrobial use has any effect?

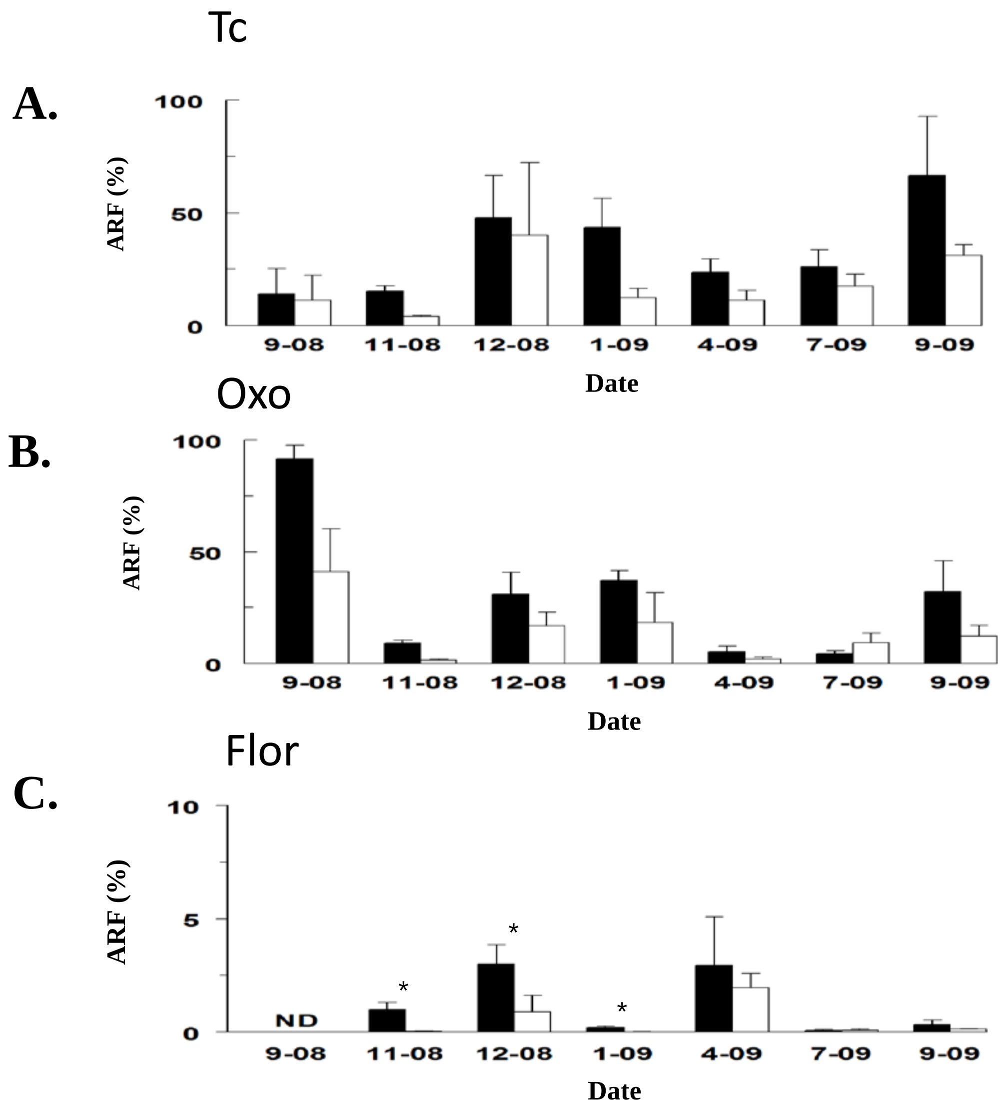
Geographical location of the study



Buschmann et al, 2012

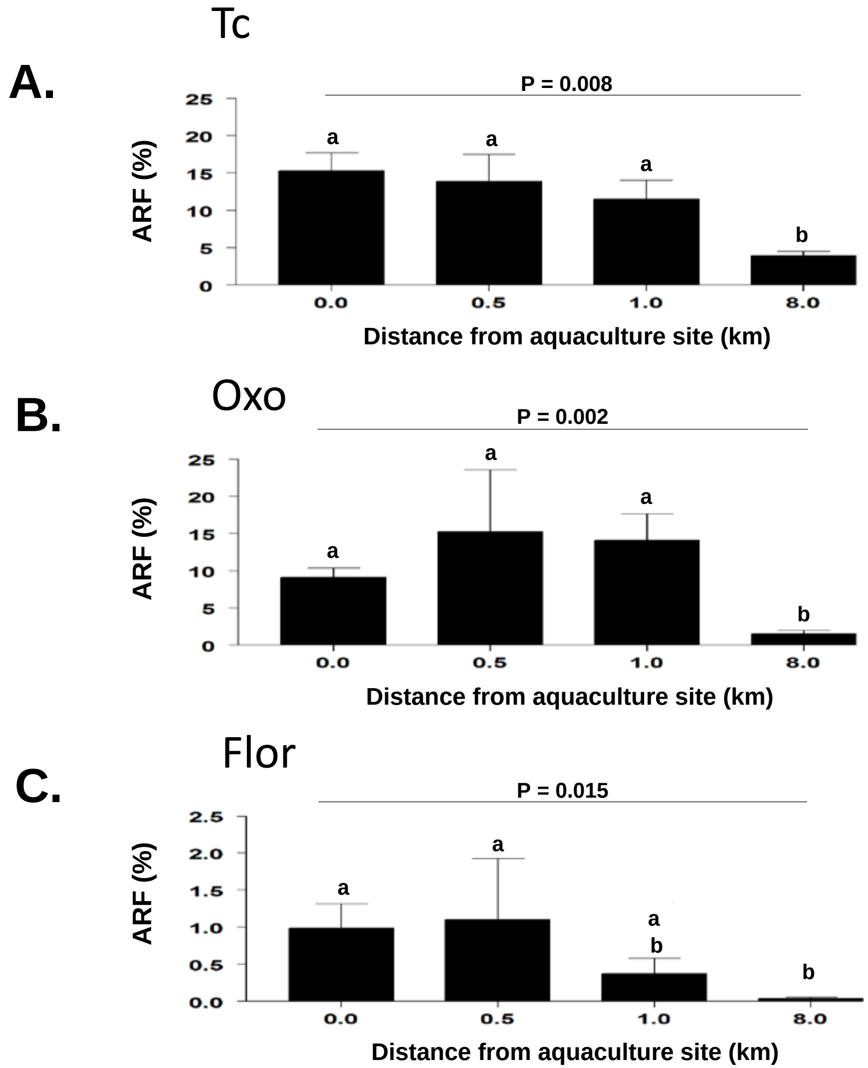
Impact and control site

Antimicrobial resistant bacteria in sediment samples from aquaculture and control sites



Buschmann et al, 2012

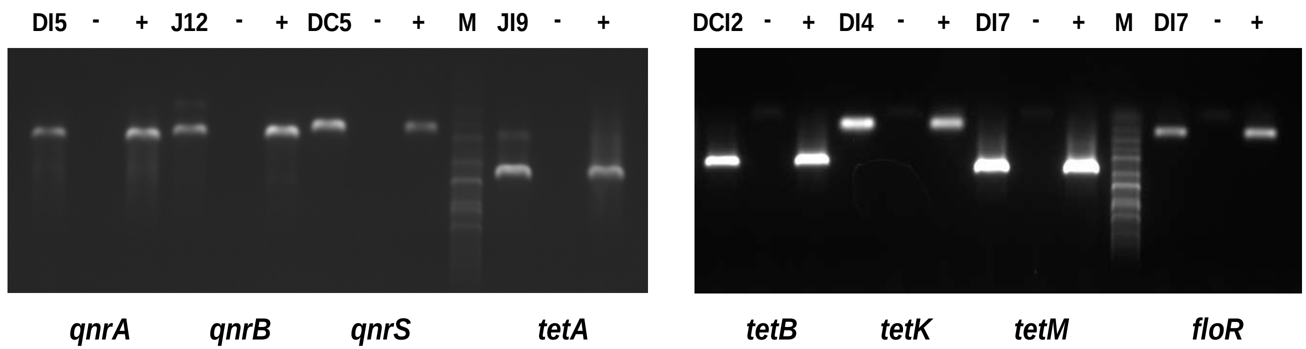
Variation in antimicrobial resistance fraction (ARF) to selected antimicrobials with distance from aquaculture site



Buschmann et al, 2012

Detection by PCR of PMQR and tetracycline resistance genes in bacteria of the marine sediment

Bacterial isolates and controls



Antibiotic resistance genes

Buschmann et al, 2012

Antibiotic resistance genes present in marine sediment bacteria from aquaculture and control sites in Chile

		Antibiotic resistance genes to												
Site	**No. of Strains**	**Tetracycline**			**Quinolones**								**Florfenicol**	
		tetA	*tetB*	*tetK*	*qnrA*	*qnrB*	*qnrC*	*qnrD*	*qnrS*	*qepA*	*oqxA*	*aac* (6') *Ib-cr*	*florR*	*Int1*
Aquaculture	24	4	5	5	2	1	0	0	3	0	3	4	4	3
Control	24	4	5	4	2	1	0	0	5	0	3	1	2	1

Buschmann et al, 2012

.

Bacterial species identified by 16S rDNA sequences

Site	Bacteria	Similarity to GeneBank (%)	Antibiotic resistances found against (N) number of antibiotics
Aquaculture (16)	Agarivorans albus	97	5
	Arthrobacter bergeri (isolate 1)	99	6
	Arthrobacter bergeri (isolate 2)	99	8
	Bacillus aryabhattai	98	2
	Exiguobacterium sibiricum	99	7
	Halomonas venusta	99	4
	Marinobacter litoralis	99	9
	Pectobacterium carotovorum	99	9
	Pseudomonas stutzeri	99	5
	Psychrobacter pulmonis	99	0
	Psychrobacter marincola	99	1
	Shewanella algidipiscicola	99	5
	Sporosarcina saromensis	99	0
	Stenotrophomonas maltophilia	99	9
	Vibrio kanaloae (isolate 1)	99	8
	Vibrio kanaloae (isolate 2)	99	9
Non-aquaculture (11)	*Exiguobacterium aurantiacum*	97	0
	Providencia spp.	99	3
	Pseudoalteromonas mariniglutinosa	99	2
	Psychrobacter submarinus	98	5
	Serratia grimesii	99	8
	Serratia proteamaculans	99	4
	Thalassospira xiamenensis (isolate 1)	97	0
	Thalassospira xiamenensis (isolate 2)	97	2
	Vibrio cyclitrophicus	99	4
	Vibrio lentus	99	5
	Vibrio splendidus	99	9

Shah et al, 2014

Antimicrobial resistant bacteria in salmon intestines

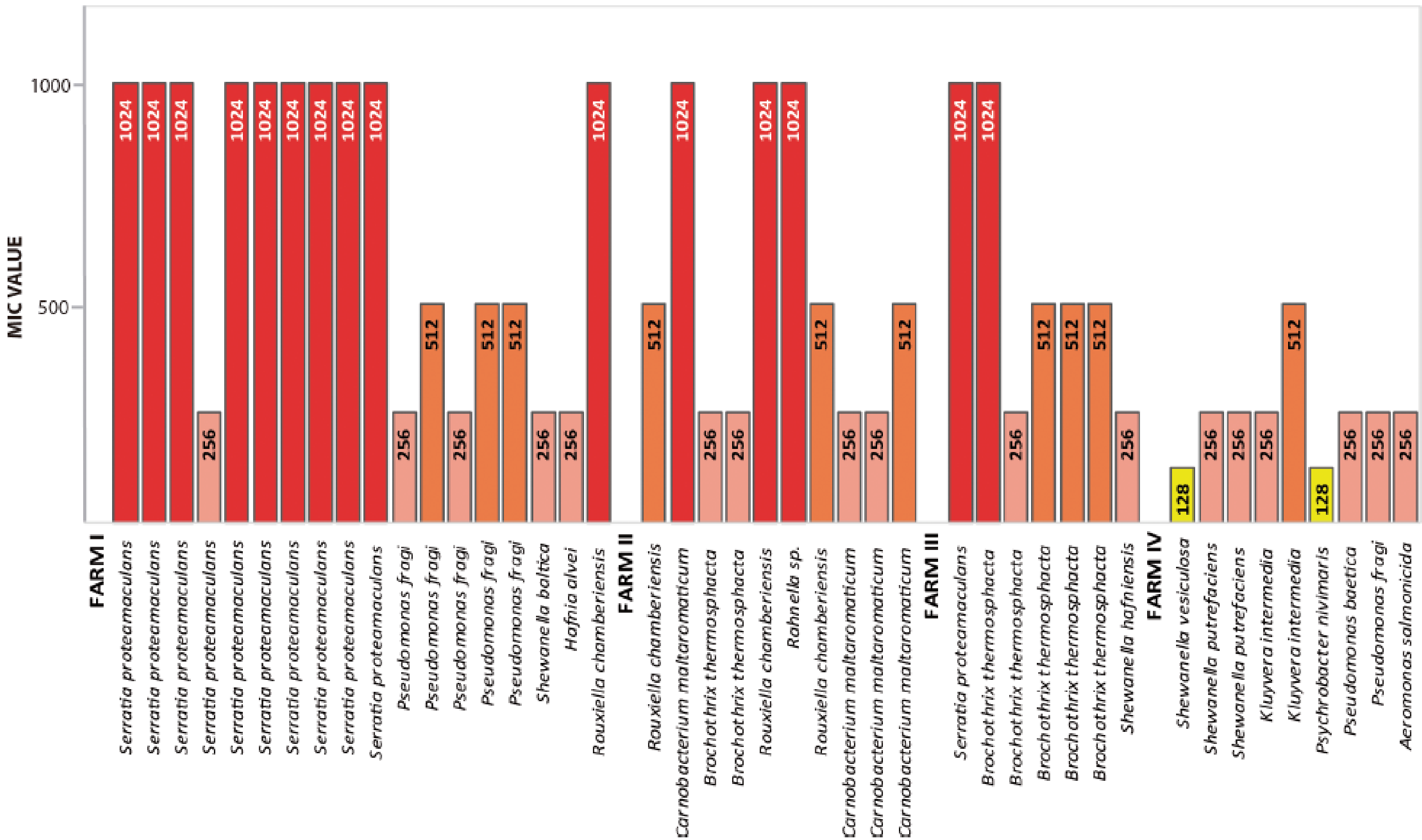


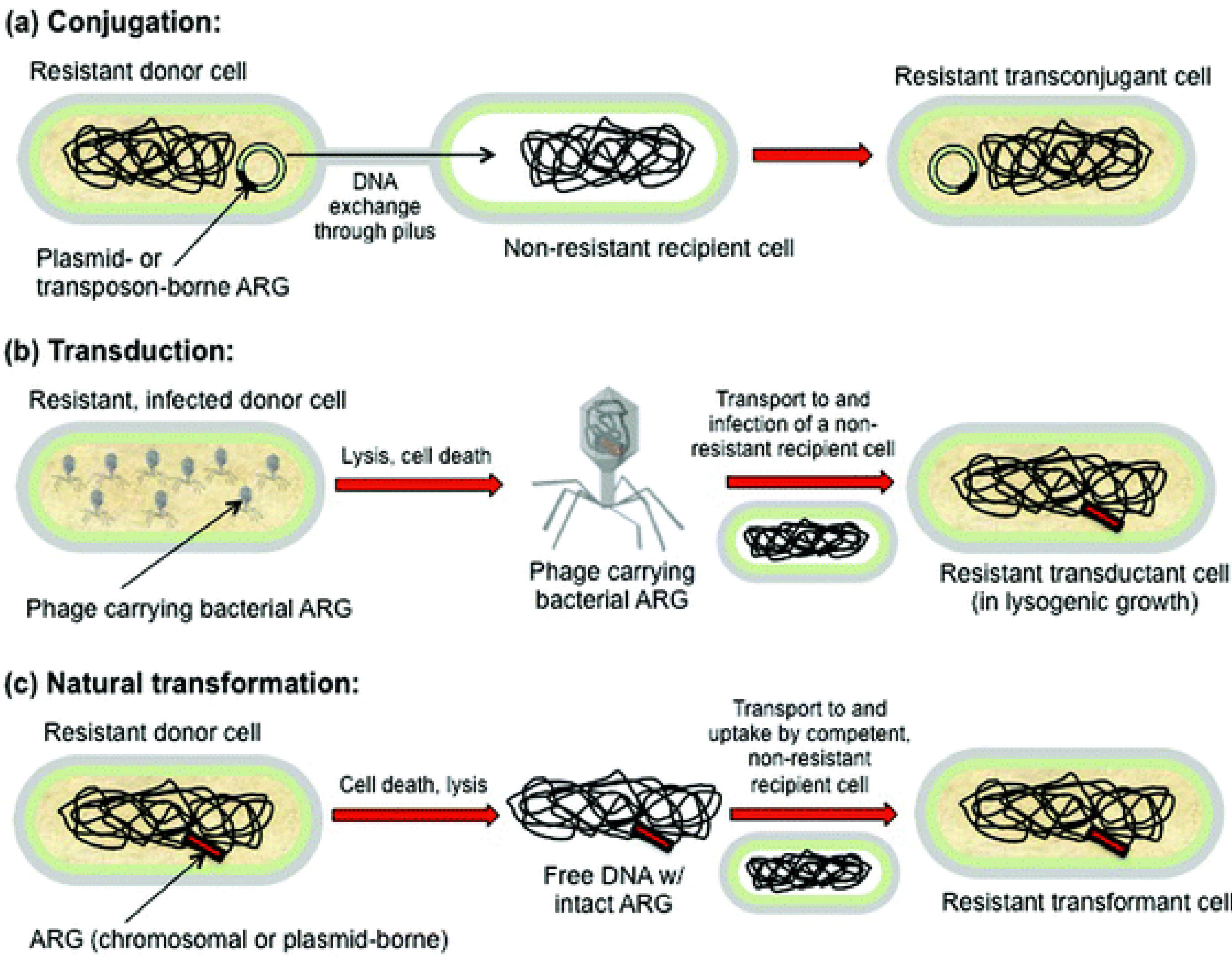
Fig 2. Minimum inhibitory concentrations (MIC) in bacteria resistant to oxytetracycline. (μg/mL).

https://doi.org/10.1371/journal.pone.0203641.g002

Higuera;Llanten et al., 2018

Is there horizontal gene transfer between marine bacteria and animals and human pathogens?

Antimicrobial resistance mobilization by HGT



Phenotypic and genotypic characteristics of donor marine bacteria from aquaculture and control sites tested in conjugation

Site	Use	Strain	Phenotypic resistance						Genotypic resistance				
			Tet	Smt	Tmp	Amo	Str	Oxo	Tet	Smt	Tmp	Amo	Str
Aquaculture	Donor	A-1	R	R	S	R	R	S	–	*sul1*	–	–	*aadA9*
Aquaculture	Donor	B-1	R	R	R	S	R	S	–	*sul1*	–	–	*aadA9*
Aquaculture	Donor	F-7	S	S	R	R	S	S	–	–	*dfrA12*	*bla*$_{TEM}$	–
Aquaculture	Donor	F-12[b]	R	R	R	R	R	S	*tetA* *tetG*	*sul2*	–	–	*strA-B*
Aquaculture	Donor	G-9	R	R	R	R	S	R	–	–	*dfrA1,5,7,12*	*bla*$_{TEM}$	–
Aquaculture	Donor	G-23[b]	R	R	R	R	S	R	*tetA*	*sul2*	–	–	–
Control	Donor	B-21	S	S	R	R	S	R	–	–	*dfrA1,5,12*	*bla*$_{TEM}$	–
Control	Donor	C-19	S	R	R	S	S	R	–	–	*dfrA1,5,12*	–	*strA-B*
Control	Donor	D-15	S	S	R	S	S	S	–	–	*dfrA1,5,12*	–	–
–	Recipient	*E. coli* DH5α	S	S	S	S	S	R	–				
–	Recipient	*E. coli* HB101	S	S	S	S	R	S	–				

a Bacterial growth inhibited (S) or not inhibited (R) by: tetracycline (Tet), 80 μg; sulfamethizol (Smt), 240 μg; trimethoprim (Tmp), 5.2 μg; amoxicillin (Amo), 30 μg; oxolinic acid (Oxo), 10 μg; and streptomycin (Str), 10 μg.

b Bacterial isolates able to transfer resistance genes.

Shah et al, 2014

Class 1 and 2 integrons and gene cassettes in marine bacteria from aquaculture and control sites in Chile

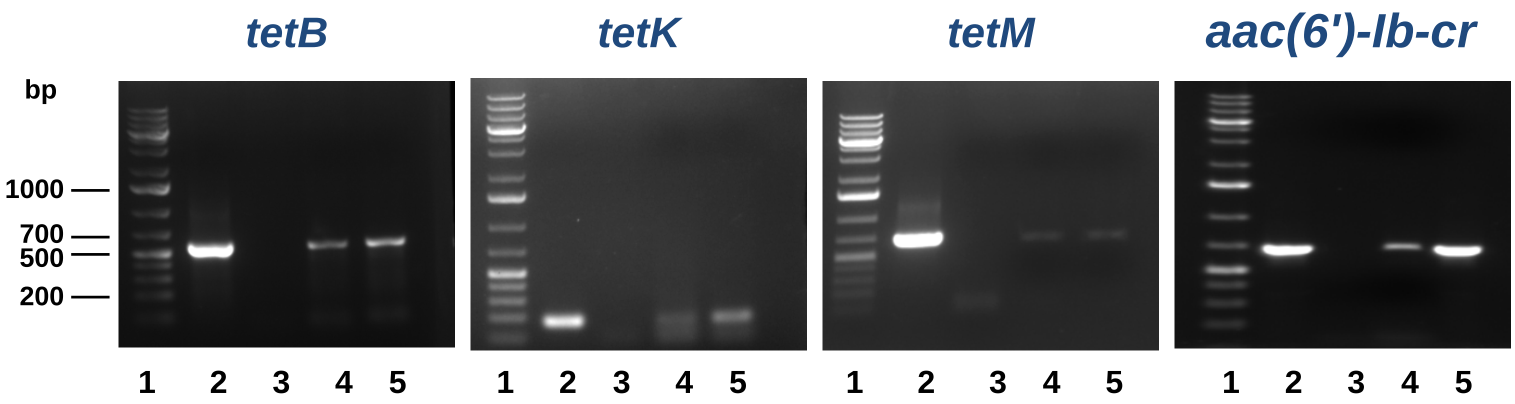
Site	Gene	Integron	Gene cassette	Species
Aquaculture[a]	*sul1*	*intl1*	*aadA9*	*Arthrobacter bergeri* isolate 1
	sul1	*intl1*	*aadA9*	*Arthrobacter bergeri* isolate 2
	sul1	*int1*	–	Unknown
	sul1	*int1*	–	*Bacillus aryabhattai*
	sul1	*int1*	–	*Exiguobacterium sibiricum*
	sul1	*int1*	–	*Marinobacter litoralis*
	sul1	*int1*	–	*Psychrobacter pulmonis*
	sul1	*int1*	–	*Stenotrophomonas maltophilia*
	sul1	*int1*	–	*Thalassospiro xiamenensis*
	sul1	*int1*	–	Unknown
	sul1	*int1*	–	Unknown
	sul1	*int1*	–	Unknown
	dfrA1	*int2*	–	*Agarivorans albus*
	dfrA*1*	*int2*	–	*Vibrio kanaloae*
	dfrA1	*int2*	–	Unknown
Control[b]	*sul1*	*int1*	–	*Pseudoalteromonas mariniglutosa*
	sul1	*int1*	–	Unknown

a Twelve *sul1*-containing isolates were tested for *intl1*, 10/12 were positive for *intl1* (92% positive), and 2/10 *intl1*-containing isolates were also positive for *aadA9* gene cassette (20% positive). Ten *dfrA1*-containing isolates were tested for *intl2*, 3/10 were positive (30% positive).

b Two *sul1*-containing isolates were tested for *intl1*, both were positive (100% positive); 8 *dfrA1*-containing isolates were tested for *intl2*, none was positive (0% positive).

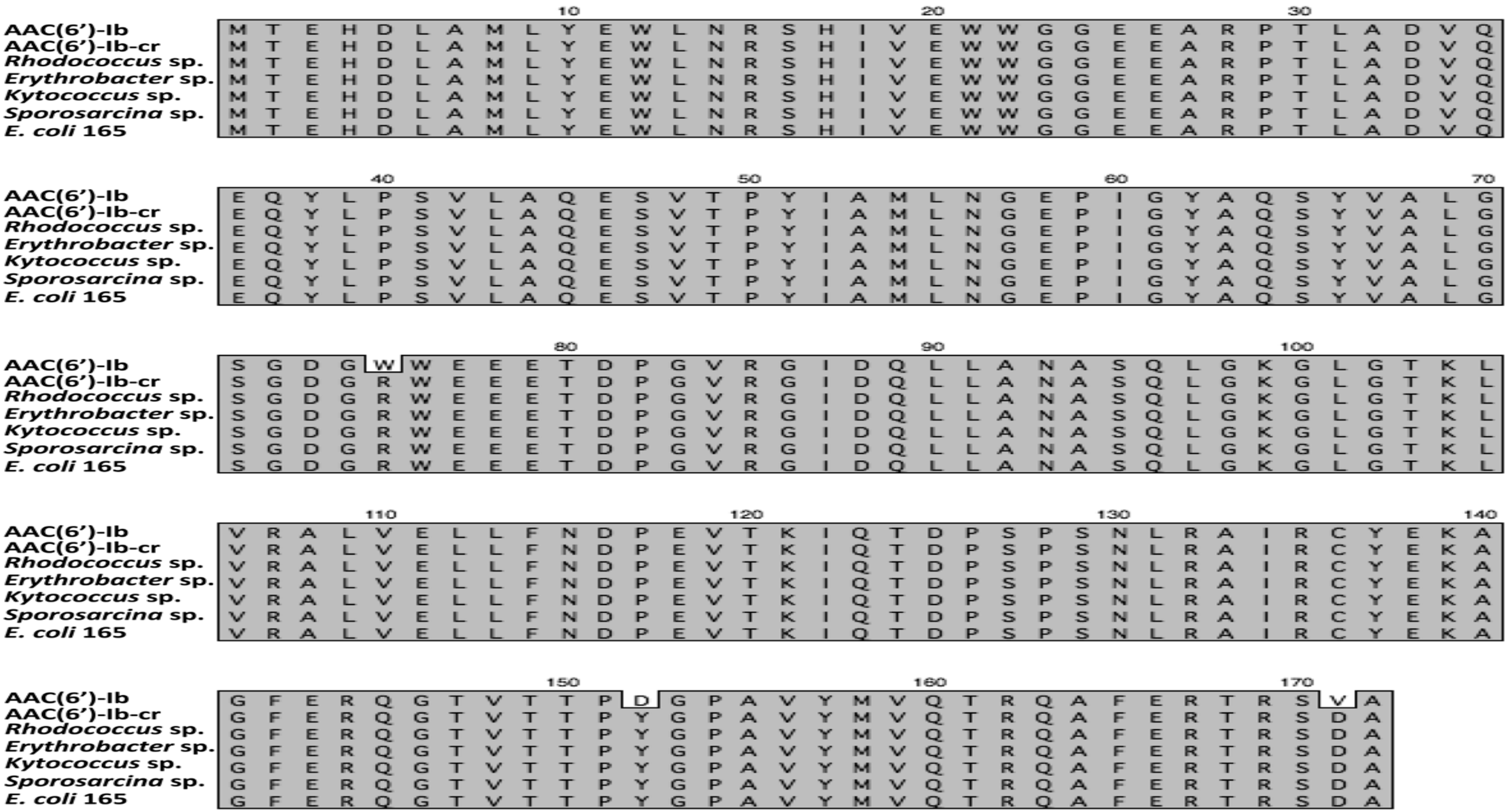
Shah et al, 2014

Sharing of tetracycline resistance genes and PMQR gene *aac(6')-Ib-cr* between marine bacteria and four urinary tract *Escherichia coli*



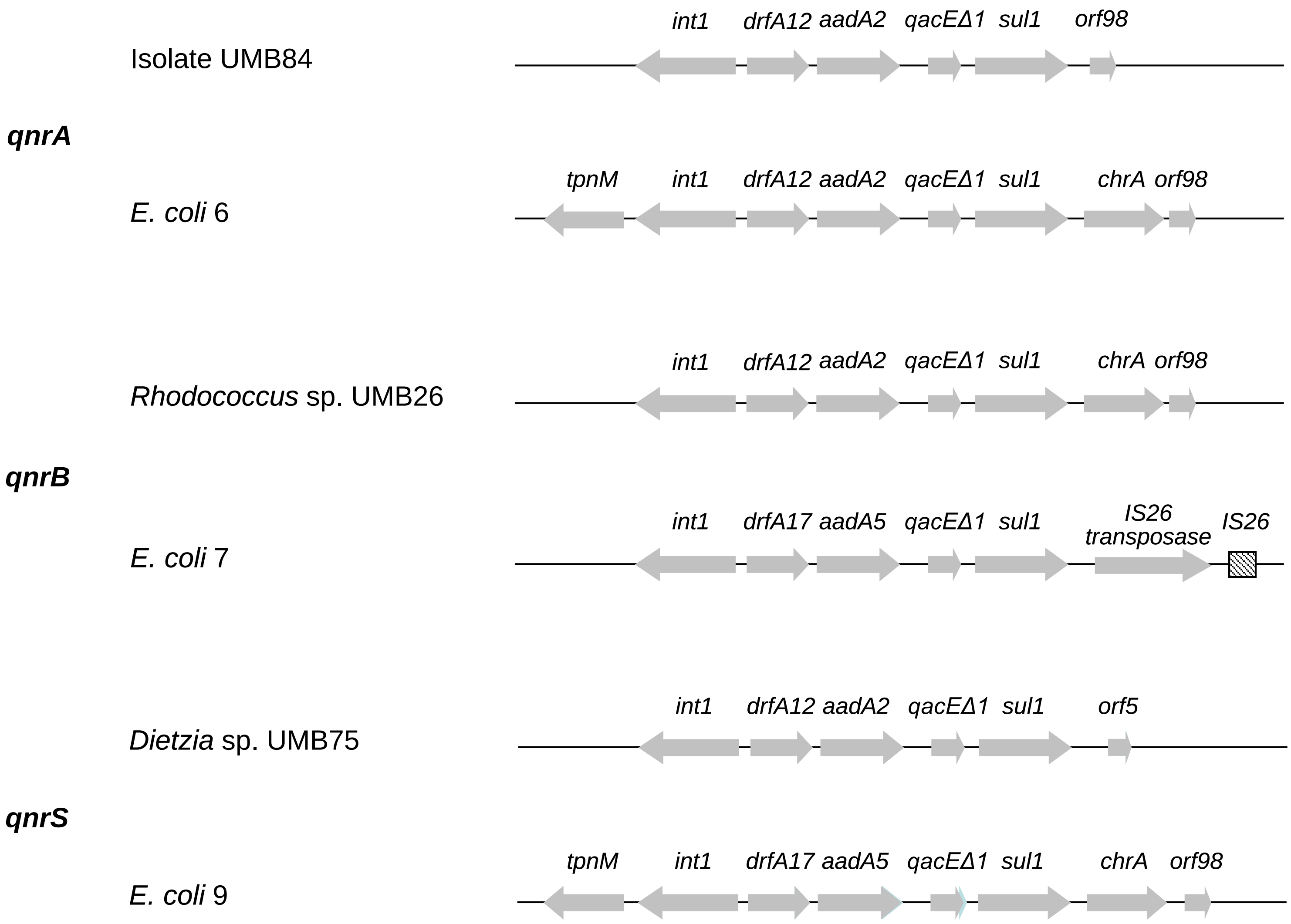
Cabello et al, 2013

Identity of the Aac(6')-1b-cr enzyme protein from marine bacteria and uropathogenic *E. coli* that inactivates kanamycin and fluoroquinolones



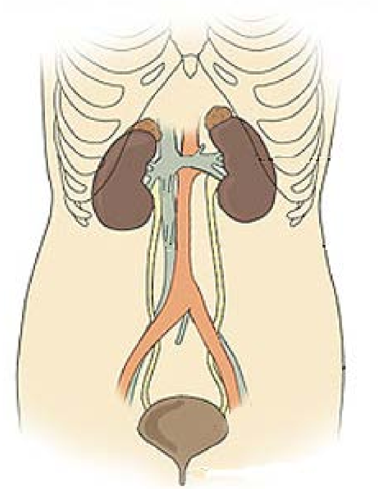
Aedo et al, 2014

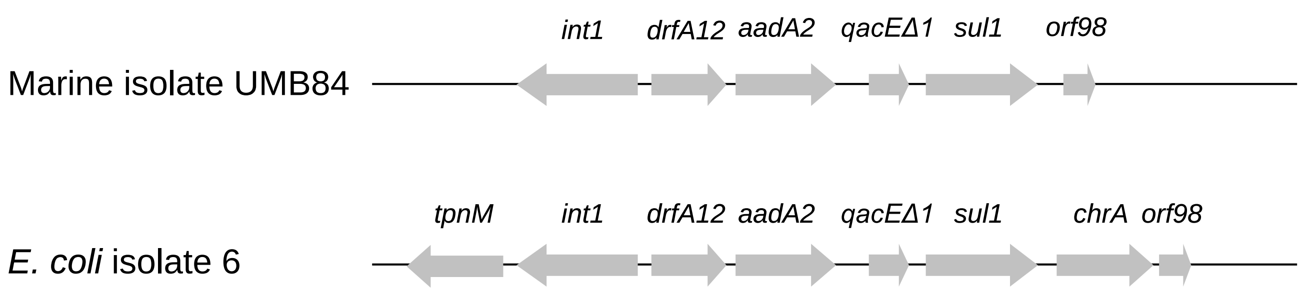
Integron structure in marine bacteria and uropathogenic *E. coli*



Tomova et al, 2016

Sharing of antimicrobial resistance genes between bacteria of marine sediments. salmon intestine and human pathogens





Quinolone-resistant *qnrA*⁺ marine isolates from an area of intensive salmon aquaculture in the Calbuco Archipelago, Región de los Lagos (Región X), Chile (left), and *Escherichia coli* isolated from patients with urinary tract infections (right) in an adjacent coastal region contain structurally similar class I integrons and PMQR genes .

Cabello et al., unpublished

Number of days in water and average weight per smolt of salmon

Year	**2003**	**2004**	**2005**	**2006**	**2007**
Kg/smolt	3.71	3.66	3.57	3.34	3.14
Number of days in water	487	497	484	488	543

Katz, Lizuka and Munoz, 20011,
Bustos and Irarrazabal, 2016

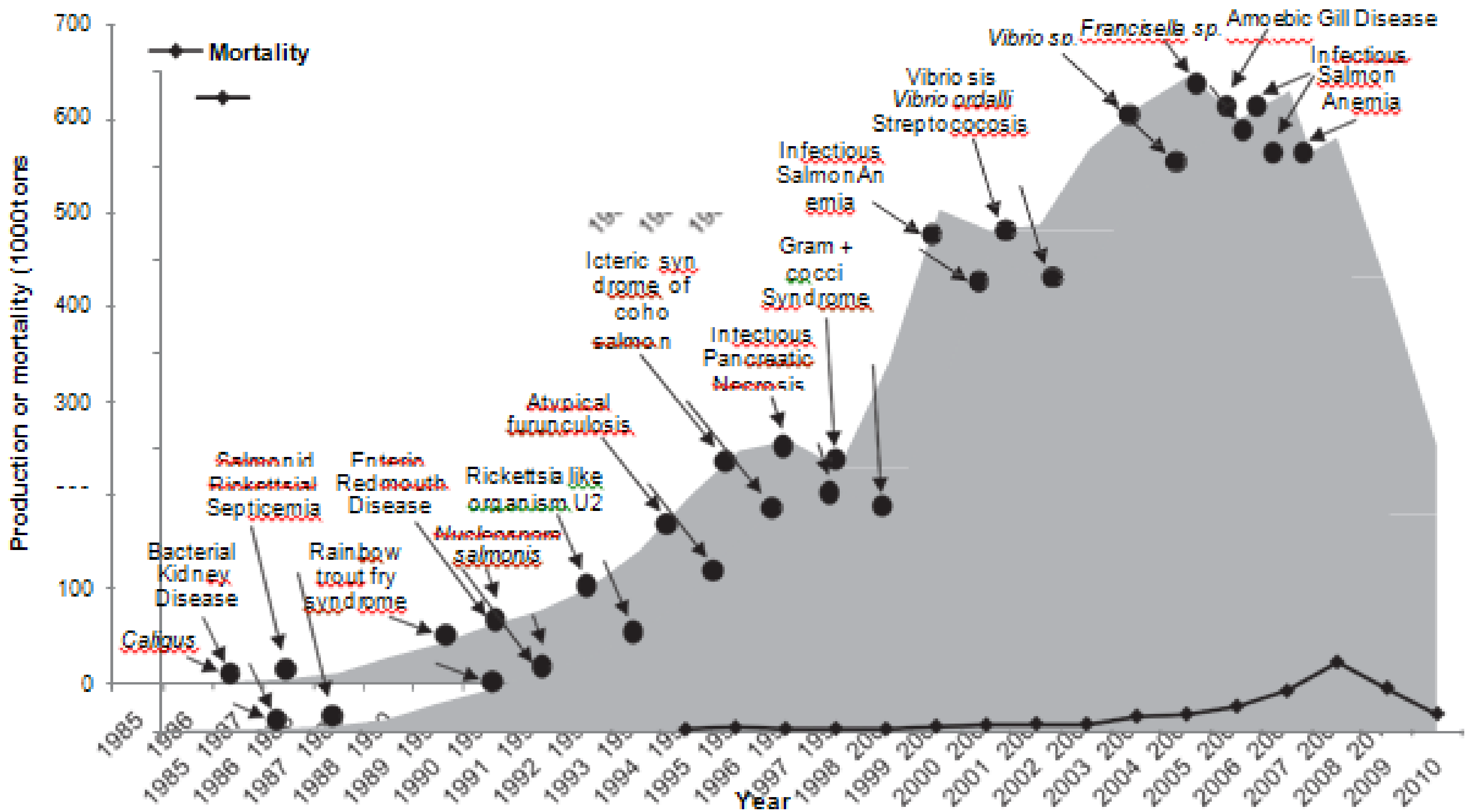
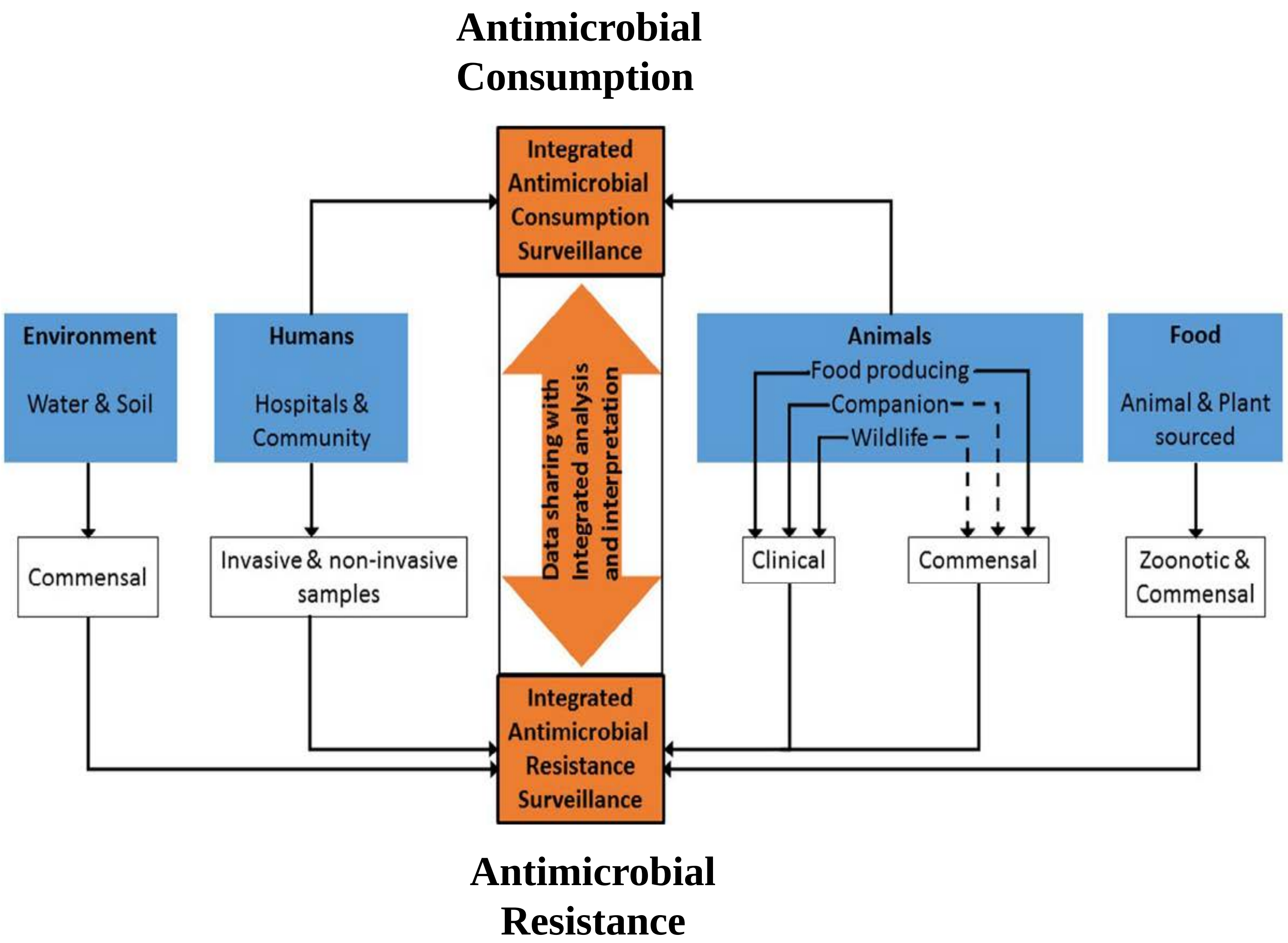


Fig. 7. Time line of production, disease emergence and mortality in salmon farming in Chile (period 1985-2010). Source: Sernapesca, 2011b; TechnoPress, 2010.

One Health surveillance framework to prevent antimicrobial resistance



Queenan et al., 2016

Conclusions

- **Salmon aquaculture activities increase the number of bacteria in marine sediments**
- **Salmon aquaculture activities generates residual concentrations of antimicrobials in the sediment**
- **Salmon aquaculture activities increase antimicrobial resistant bacteria in the marine sediment for many antimicrobials, including resistance to tetracycline, oxolinic acid florfenicol, sulfas, trimethoprim, streptomycin and amoxacillin**
- **The increase in antimicrobial resistant bacteria is the result of their selection by antimicrobials used in salmon aquaculture activities**
- **Increased antimicrobial use results in increased antimicrobial resistance in sediments**
- **Marine sediment bacteria share with fish, animal and human pathogens antimicrobial resistance (ARG) genes and mobile genetic elements (MGE) able to recombine and capable of horizontal gene transfer (HGT)**
- **This antimicrobial use affects negatively human, animal and environmental health**