Fish-borne parasitic zoonoses

Anisakiasis / Anisakidosis

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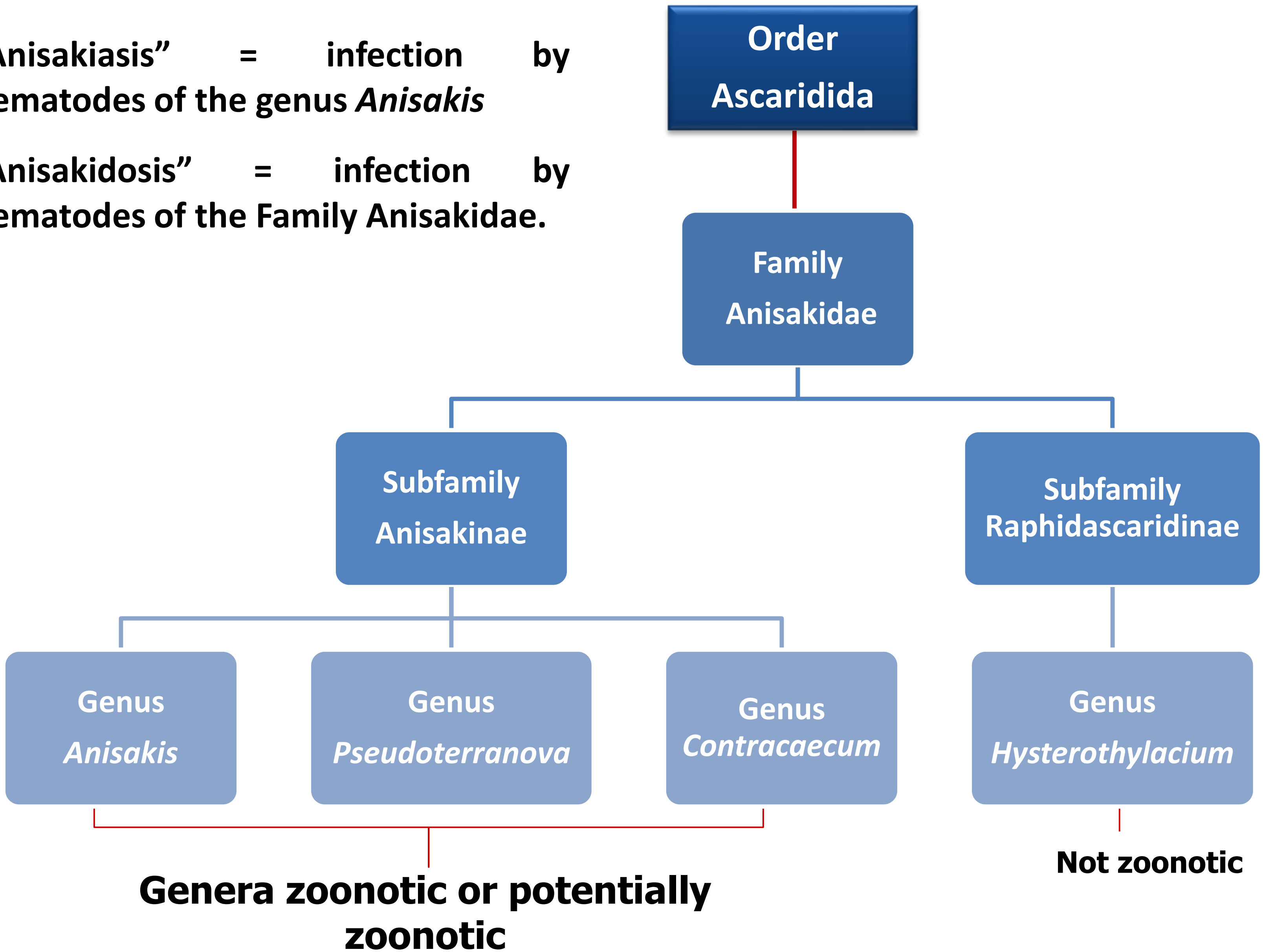
Nematodes Anisakidae and Anisakiasis

"Anisakiasis" = infection by nematodes of the genus *Anisakis*

"Anisakidosis" = infection by nematodes of the Family Anisakidae.

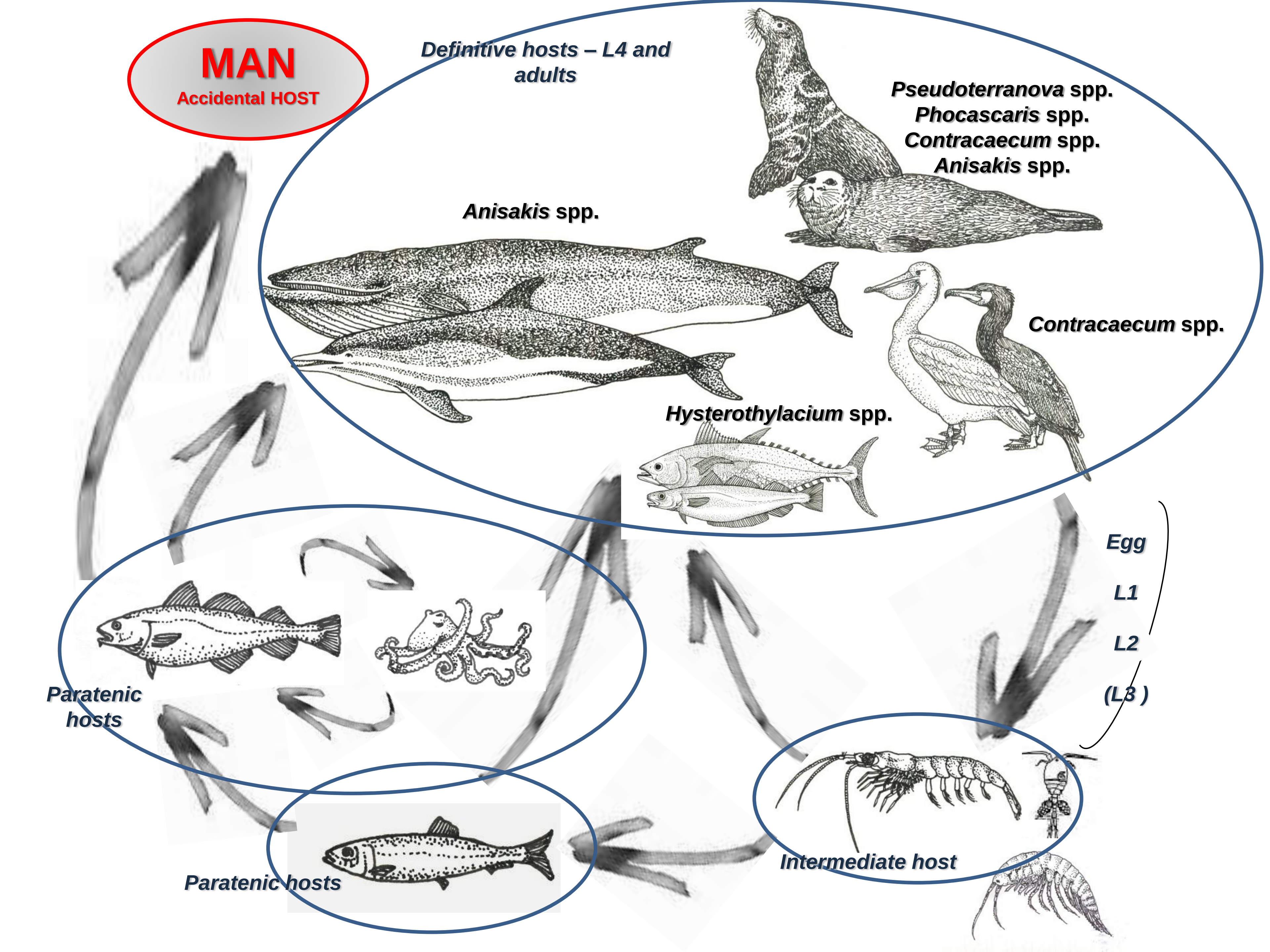


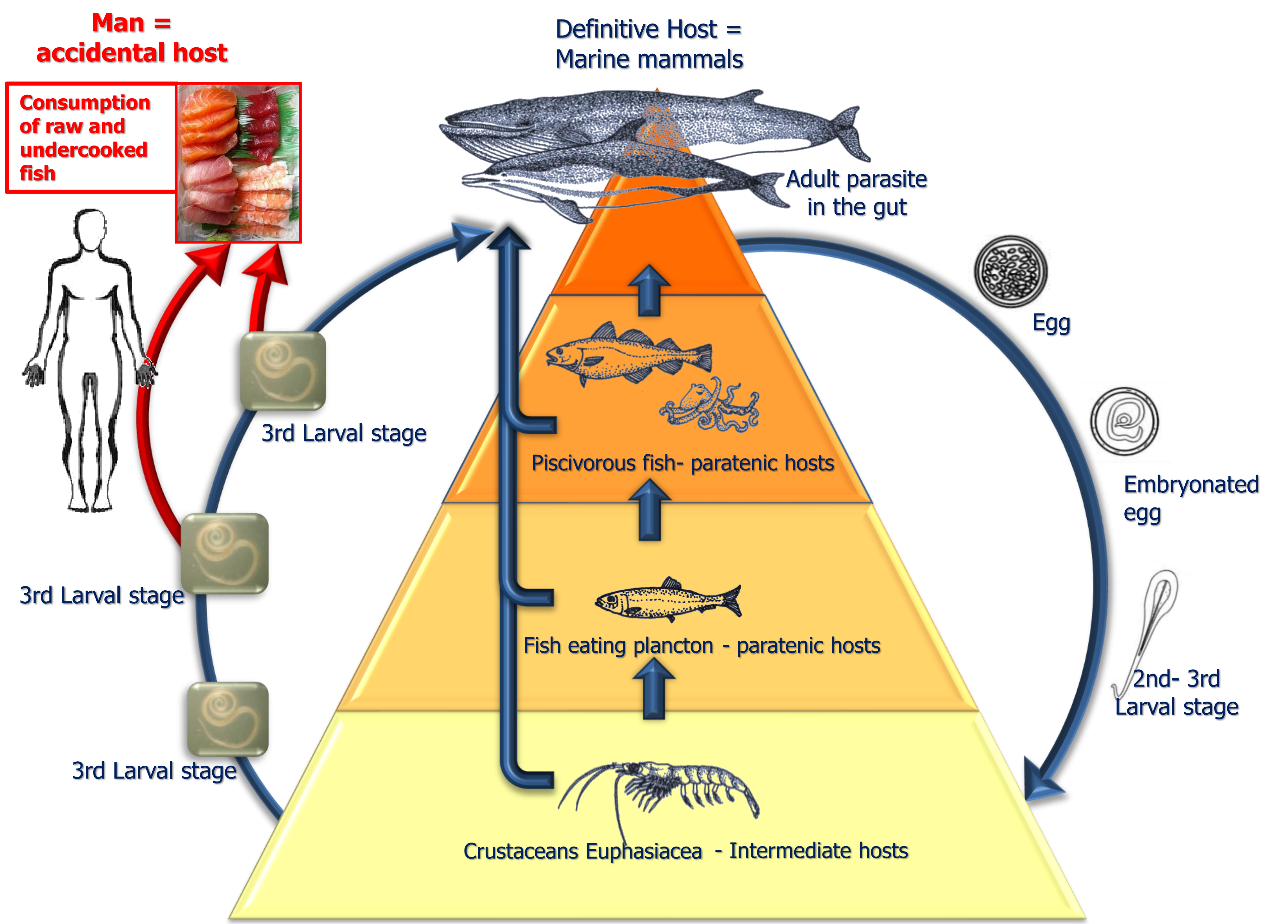
Nematodes Anisakidae

All Anisakidae nematodes have a complex life cycle (heteroxenous parasites) with different definitive hosts.

Anisakis and *Pseudoterranova* have marine mammals as defintive hosts (*Anisakis*: cetaceans and pinnipeds; *Pseudoterranova*: pinnipeds, *Phocascaris*: pinnipeds, *Hysterothylacium:* teleosts, *Contracaecum*: pinnipeds or fish eating birds.

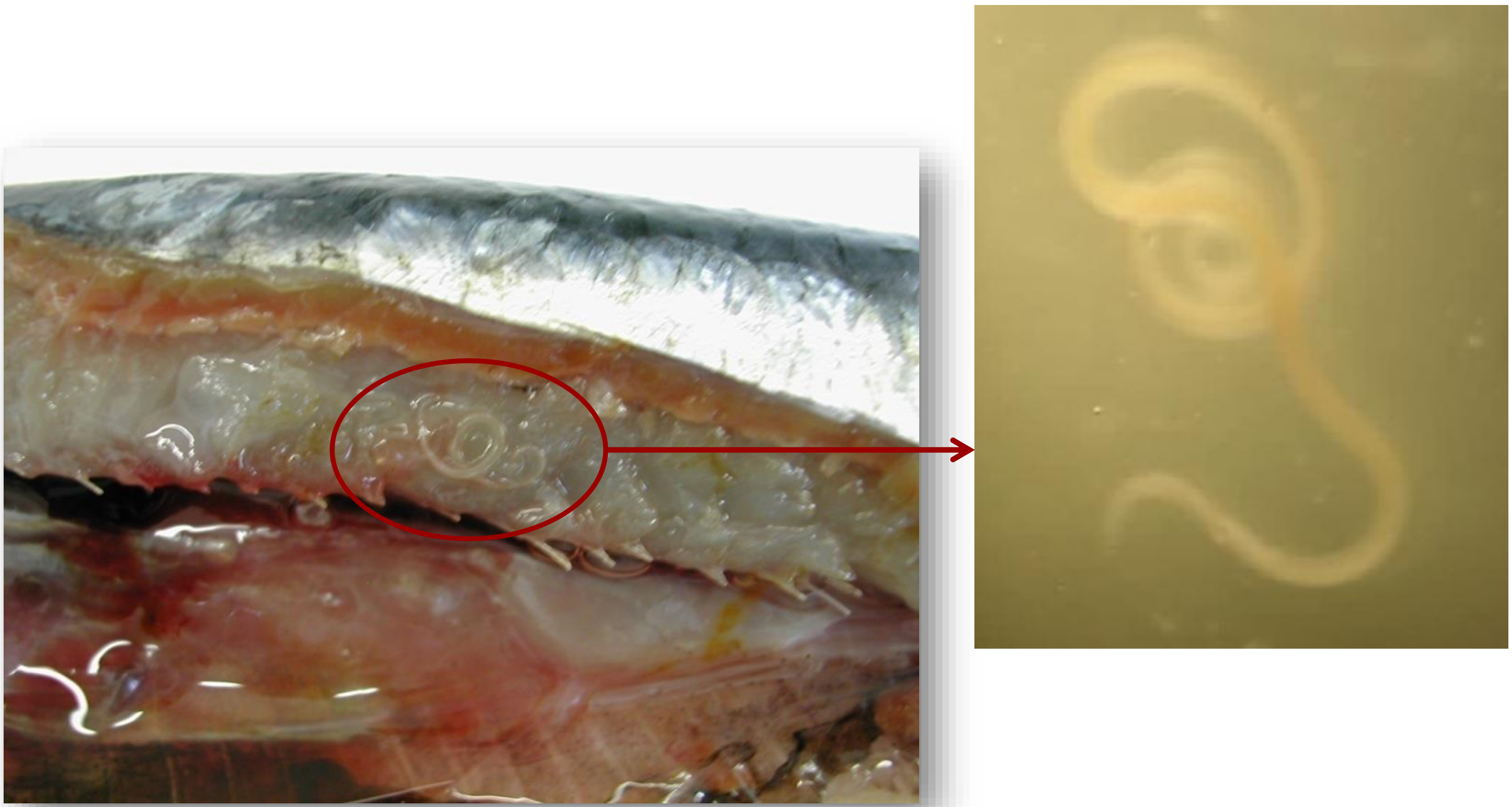
Intermediate hosts are small planctonic crustaceans: Euphasiacea (krill), Copepoda, Amphipoda, etc.

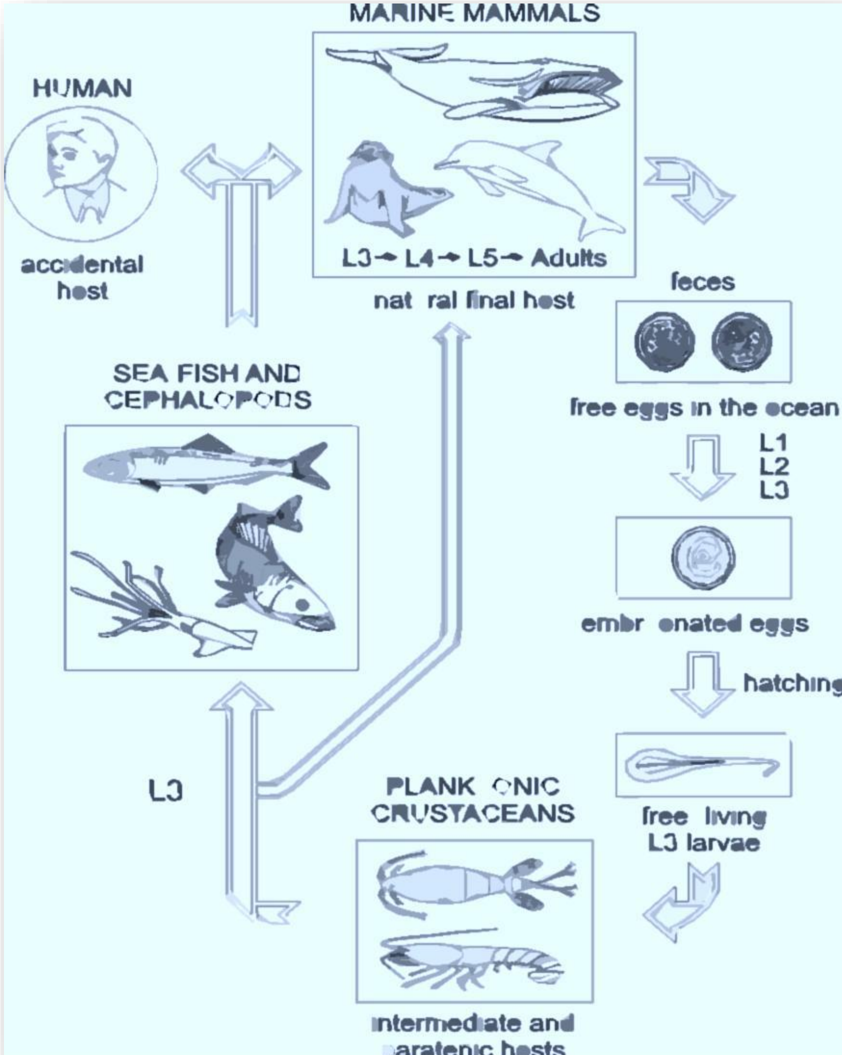

MAN
Accidental HOST
Definitive hosts – L4 and adults
Pseudoterranova spp.
Phocascaris spp.
Contracaecum spp.
Anisakis spp.
Anisakis spp.
Contracaecum spp.
Hysterothylacium spp.
Egg
L1
L2
(L3)
Paratenic hosts
Paratenic hosts
Intermediate host



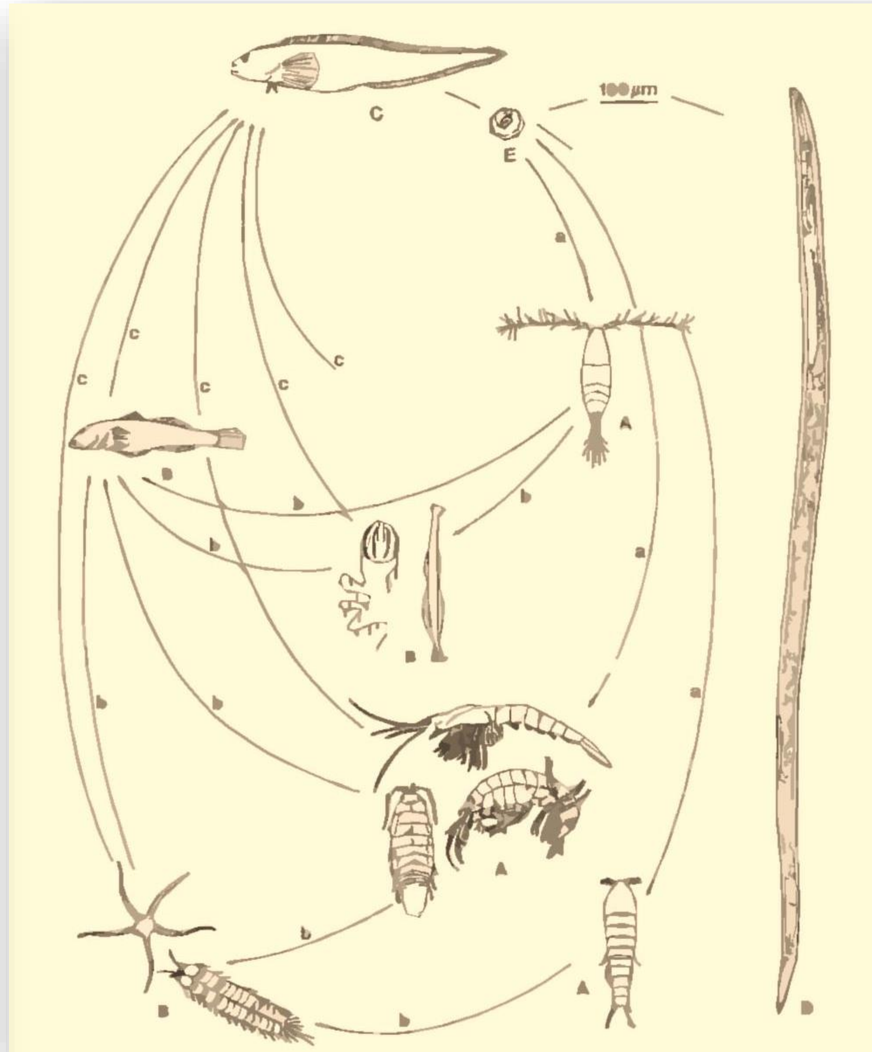
Life cycle of *Anisakis* spp. – transmission through the trophic chain

Fish eating planctonic crustaceans and piscivorous fish and cephalopods are an important link of Anisakidae as reservoir.

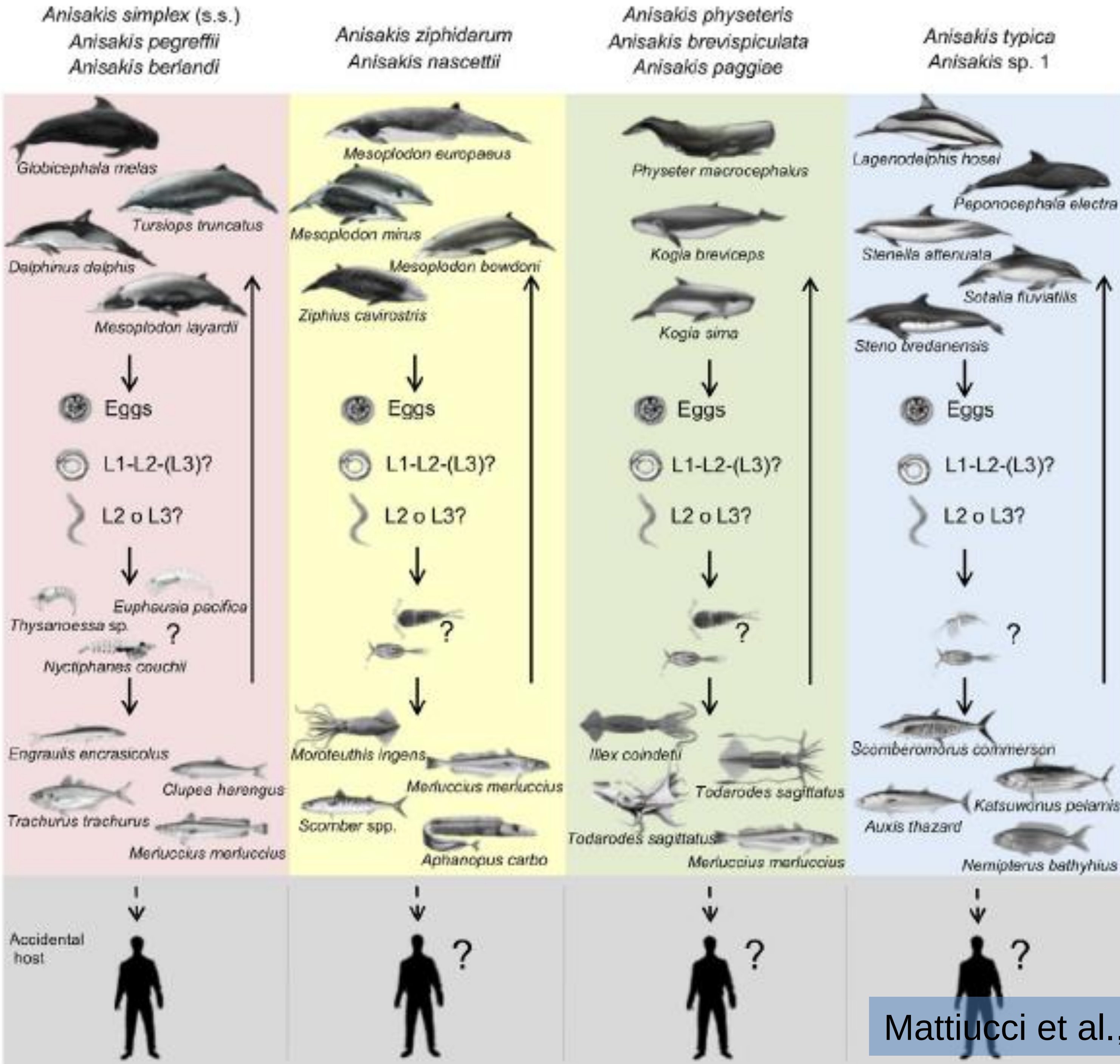




Life cycle of *Anisakis* sp. (Audicana and Kennedy, 2008)



Life cycle of *Hysterothylacium aduncum* (Koie, 1993)



Mattiucci et al., 2018

Transmission through the trophic chain

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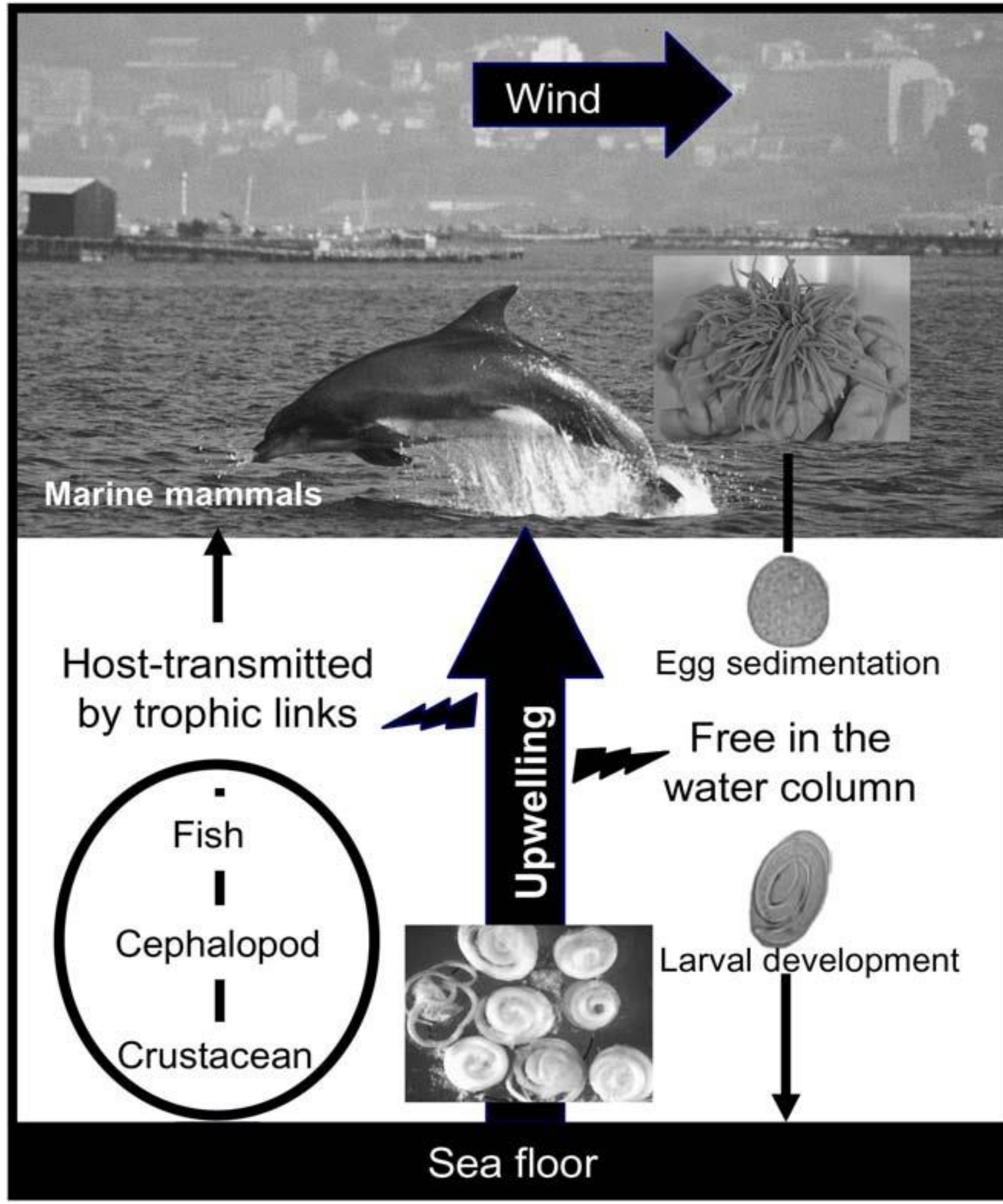
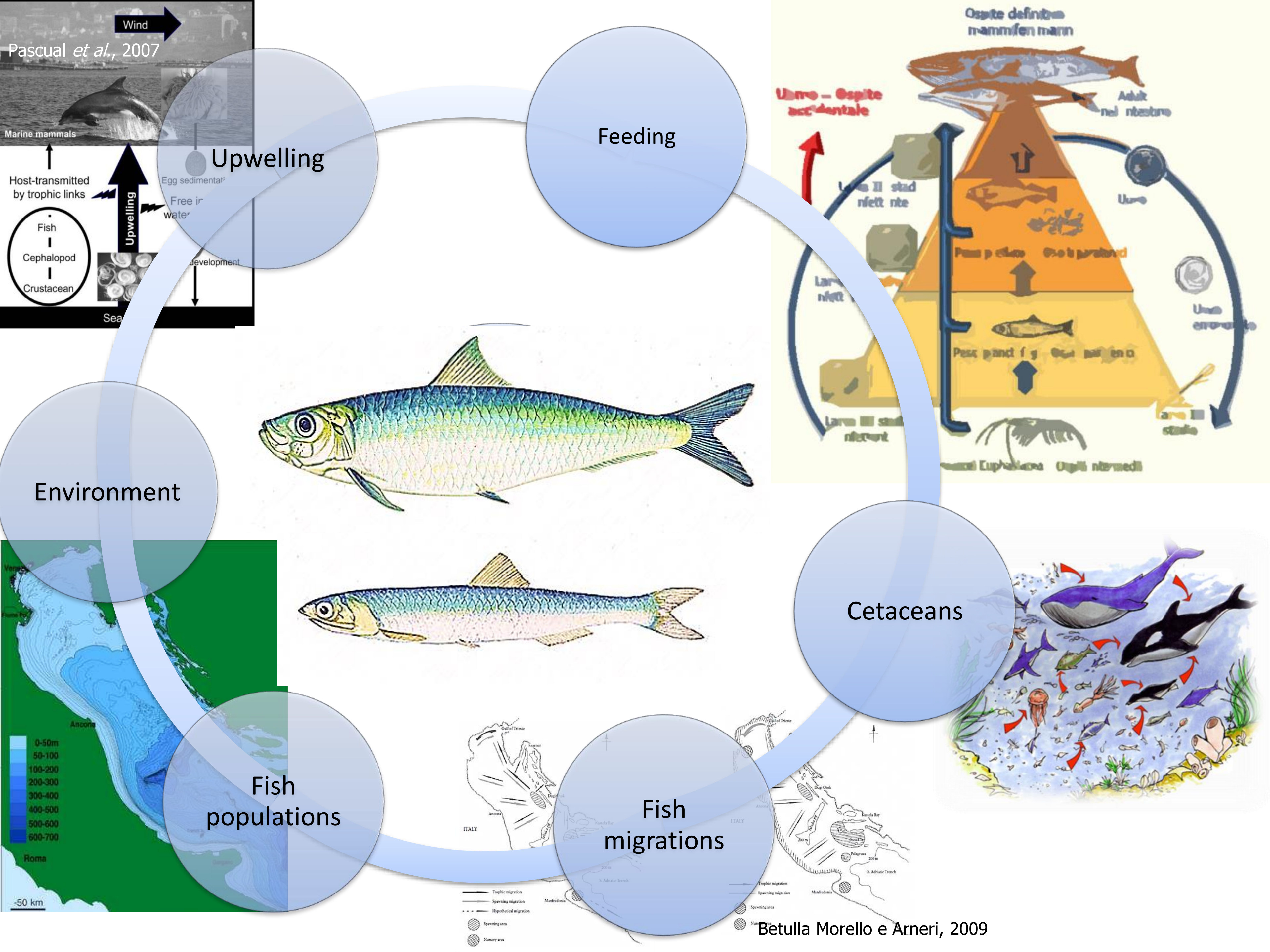
Hydrogeographical factors such as upwelling antropic influence and population dynamics

Upwelling = environmental stressor on life cycle strategies (dispersal and low host specificity) of food transmitted heteroxenous parasites with a broad host range (Pascual *et al.*, 2007)





Wind
Pascual et al., 2007
Marine mammals
Host-transmitted by trophic links
Fish
Cephalopod
Crustacean
Upwelling
Upwelling
Feeding
Environment
Cetaceans
Fish populations
Fish migrations
0-50m
50-100
100-200
200-300
300-400
400-500
500-600
600-700
Ancona
Roma
-50 km
Betulla Morello e Arneri, 2009

Prevalence values of Anisakidae in mediterranean fish (Pozio, 2004)

Species	Prevalence
Lepidopus caudatus	100
Trachurus trachurus	95
Micromesistius poutassou	95
Merlangius merlangius	76
Scomber japonicus	75
Scomber scombrus	71
Conger conger	44
Merluccius merluccius	40
Boops boops	35
Zeus faber	33
Lophius piscatorius	32
Todarodes sagittatus	22
Trachinus dracho	21
Phycis phycis	20
Trisopterus minutus	19
Engraulis encrasicolus	17
Diplodus annularis	16
Trigla lyra	16
Pagellus erythrinus	10
Mullus barbatus	10
Mugil cephalus	9
Cepola rubescens	9
Sardina pilchardus	1

Indagini condotte su alici e sardine commercializzate presso Mercati Ittici della costa adriatica

2003-2005

- 2636 alici esaminate ⇒ 700 (26,6%) positive per larve Anisakidae
- 1314 sardine esaminate ⇒ 544 (41,4%) positive per larve Anisakidae

⇓

7,7% alici positive per larve di *Anisakis* e 98,6% per *Hysterothylacium* (6,3% coinfezione)

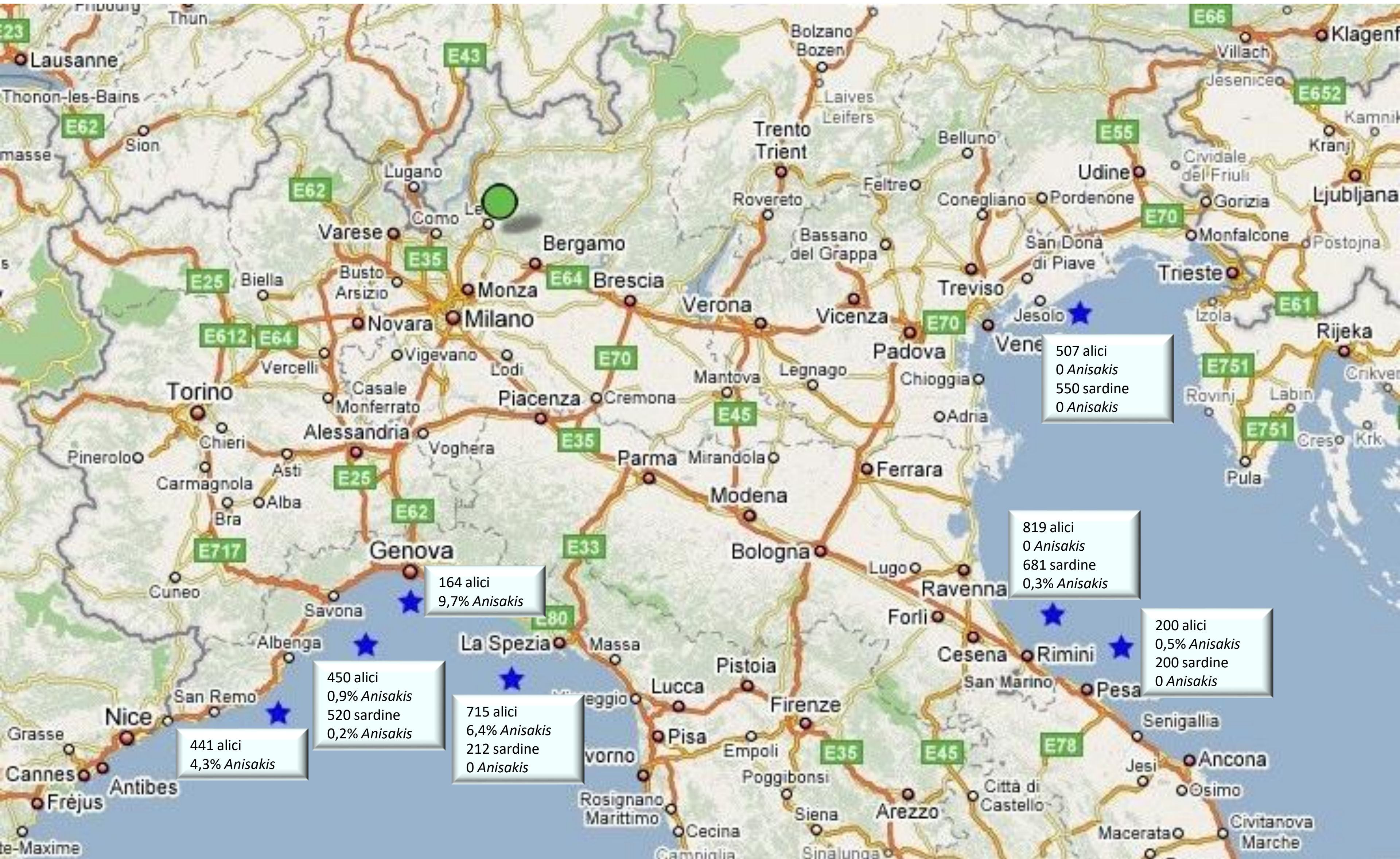
0,4% sardine positive per larve di *Anisakis* e 99,6% per *Hysterothylacium*

Survey carried out on anchovies and sardines from fish markets along the Adriatic coast

(Fioravanti *et al.*, 2006)

			Cesenatico	Ancona	Giulianova	Vasto	Manfredonia
Specie ittica	Alice	*Anisakis*	0.2%	7.8%	3.9%	3.5%	0%
		Hysterothylacium	22.9%	56%	24.9%	38.9%	9.8%
		TOTALE	***23.1%***	***56.5%***	***24.9%***	***40%***	***9.8%***
	Sardina	*Anisakis*	0%	0.3%	0%	-	0.2%
		Hysterothylacium	58.5%	53.7%	22.9%	-	29.3%
		TOTALE	***58.5%***	***54,0%***	***22.9%***	**-**	***29.5%***
Prevalenza per sito di campionamento			***32.2%***	***55.4%***	***23.9%***	***40%***	***16.6%***

Indagini condotte nel 2010-2011 nell'ambito di un Progetto di Ricerca Corrente del Ministero della Salute coordinato da IZS TO (Dr. Prearo) con UNIBO su Anisakis in alici e sardine del Mar Ligure ed Alto Adriatico x mappatura del rischio



Costantini et al., 2016
Anisakis infection in fish: an ecoparasitological study in different fishing grounds of the central-southern Adriatic Sea

Provenienza	N. soggetti esaminati	N. soggetti positivi	P Anisakidae (%)	P *Anisakis* (%)
Ancona	340	82	24,1	2,4
San Benedetto del Tronto	820	283	34,5	2,9
Manfredonia	614	195	31,8	12,9
Molfetta	400	148	37	12,5
Lecce	158	53	33,5	17,7
Totale	**2332**	**761**	**32,6**	**8,1**

Alici					
Provenienza	N. soggetti esaminati	N. soggetti positivi	Prevalenza Anisakidae (%)	*Anisakis* (%)	*Hysterothylacium* (%)
Ancona	340	82	24,1	2,4	22,9
San Benedetto	400	169	42,3	2,8	41
Manfredonia	89	41	46,1	7,8	42,7
Molfetta	200	111	55,5	24	42
Lecce	158	53	33,5	17,7	18,3
Totale	**1187**	**456**	**38,4**	**8,6**	**33,1**

Sardine					
Provenienza	N. soggetti esaminati	N. soggetti positivi	Prevalenza Anisakidae (%)	*Anisakis* (%)	*Hysterothylacium* (%)
Ancona	-	-	-	-	-
San Benedetto	400	109	27,25	2,5	26
Manfredonia	200	76	38,0	1,5	37,5
Molfetta	200	37	18,5	1,0	17,5
Lecce	-	-	-	-	-
Totale	**800**	**222**	**27,25**	**1,87**	**26,75**

Costantini et al., 2016
Anisakis infection in fish: an ecoparasitological study in different fishing grounds of the central-southern Adriatic Sea

Lanzardi					
Provenienza	N. soggetti esaminati	N. soggetti positivi	Prevalenza Anisakidae (%)	Anisakis (%)	Hysterothylacium (%)
San Benedetto	20	5	25	15	10
Manfredonia	36	33	91,66	86,1	27,7
Totale	**56**	**38**	**67,85**	**60,71**	**21,42**

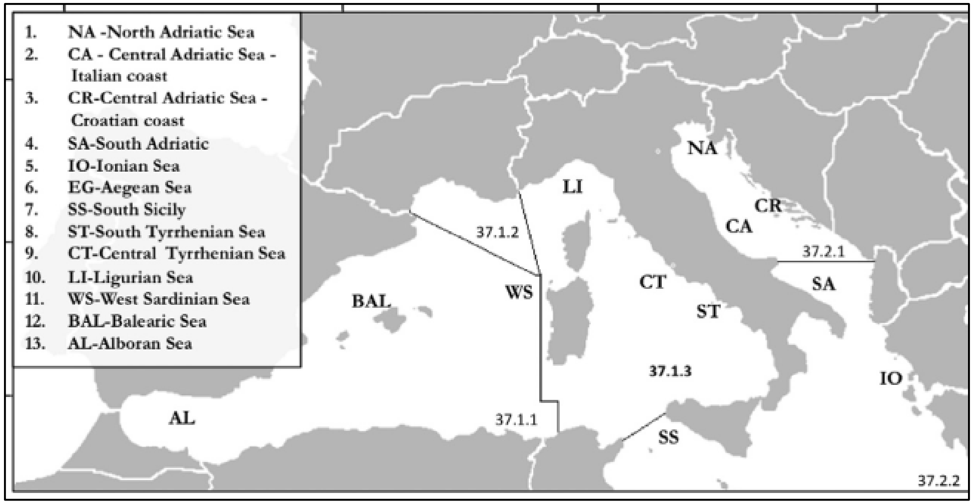
Specie	N. soggetti esaminati	N. soggetti positivi	Prevalenza Anisakidae (%)	*Anisakis* (%)	*Hysterothylacium* (%)
Alaccia	167	0	0	0	0
Nasello	70	17	24,28	17,14	10
Suro	36	17	47,2	44,4	16,6
Sgombro	15	11	73,33	66,6	13,3
Alosa	1	0	0	0	0
Totale	**289**	**45**	**15,57**	**8,26**	**5,19**

15 esemplari di lanzardo esaminati a settembre 2017
100% positivi per *Anisakis* spp.

Cipriani et al., 2017

Anisakis pegreffii (Nematoda: Anisakidae) in European anchovy *Engraulis encrasicolus* from the Mediterranean Sea: fishing ground as a predictor of parasite distribution

	N fish	Viscera	Flesh	Total
		P (%)	*P* (%)	*P* (%)
NA	645	2.8 0.017–0.04	0	2.8 0.017–0.04
CA	528	69.5 0.65–0.73	14.6 0.12–0.18	70.8 0.67–0.75
CR	518	38.4 0.34–0.43	4.8 0.03–0.07	39.8 0.35–0.44
SA	120	53.3 0.44–0.62	12.5 0.07–0.20	55.8 0.46–0.65
IO	160	0	0	0
EG	108	0.009 0–0.05	0	0.009 0–0.05
SS	200	0	0	0
ST	554	1.4 0.006–0.028	0.2 0.00–0.01	1.6 0.007–0.03
CT	336	2.4	0	2.4
LI	433	7.2 0.05–0.10	0.5 0.001–0.02	7.6 0.05–0.11
WS	200	11.5 0.07–0.17	1.00 0.001–0.04	11.5 0.07–0.17
BAL	100	0.10 0.00–0.05	0	0.10 0.00–0.05
AL	250	0	0	0



All the examined larvae (547 of 3202) were identified as *Anisakis pegreffi*

Mladineo and Poljakb (2014).
Ecology and Genetic Structure of Zoonotic Anisakis spp. from Adriatic Commercial Fish Species

TABLE 2 Larval ecological parameters of infection of *Anisakis* sp. parasites isolated from six fish species over a 1-year period

Fish species	Mean host length ± SE (mm)	Mean host wt ± SE (g)	Prevalence (CI)[a] (%)	Mean *I* (CI), range[b]	Mean *A* (CI)[c]	Mean *J* (range)[d]	v/x[e]	k	D[g]
E. encrasicolus	142.29 ± 7.74	17.73 ± 0.3	81.7 (73.81–87.76)	8.44 (7.26–9.57), 1–23	6.89 (5.83–8.07)	1.25 (1–2)	5.84	0.889[f]	0.507
S. pilchardus	149.53 ± 12.95	29.8 ± 0.41	3.3 (1.15–8.21)	1.25 (1.18–2.12), 1–5	0.04 (0.01–0.09)		1.37	0.227[f]	0.890
M. merluccius	342.92 ± 39.71	322.12 ± 6.54	70.8 (62,12–78,41)	9.69 (8,39–11,25), 1–32	6.87 (5,57–8,20)	1.33 (1–2)	7.80	0.566	0.567
M. merlangus	279.54 ± 18.23	140.3 ± 1.68	65.8 (56.69–73.80)	4,34 (2.32–3.47), 1–13	2,86 (2.32–3.47)	1 (1–1)	3.59	0.790	0.580
S. japonicus	300.725 ± 8.81	222.63 ± 1.62	100 (96.87–100)	10,48 (9.80–11.28), 1–22	10.48 (9.8–11.28)	2.13 (1–4)	1.60		0.211
T. thynnus	904.66 ± 99.37	1643 ± 49.1	23.3 (16.59–31.66)	6.5 (5.0–10.00), 1–6	1.78 (1.15–2.67)		10.06	0.091	0.851

[a] Total mean prevalence with Stern's exact 95% confidence limits (CI).
[b] *I*, intensity.
[c] *A*, abundance, with bootstrap 95% CI.
[d] *J*, intensity in fillets.
[e] *v/x*, variance-to-mean ratio.
[f] The exponent of the negative binomial (*k*) showed no statistical difference between observed and expected frequencies at $P = 0.05$.
[g] *D*, discrepancy index ($D = 0$ to 1).

Anisakis pegreffii (95.95%) and *Anisakis simplex sensu stricto* (4.05%)

Mladineo et al. (2012). Prevalence of *Anisakis pegreffi* in anchovies was 76.1%

Rello *et al.* (2009). International Journal of Food Microbiology, 129: 277–281

Eastern Atlantic
(Gulf of Cádiz and Strait of Gibraltar)

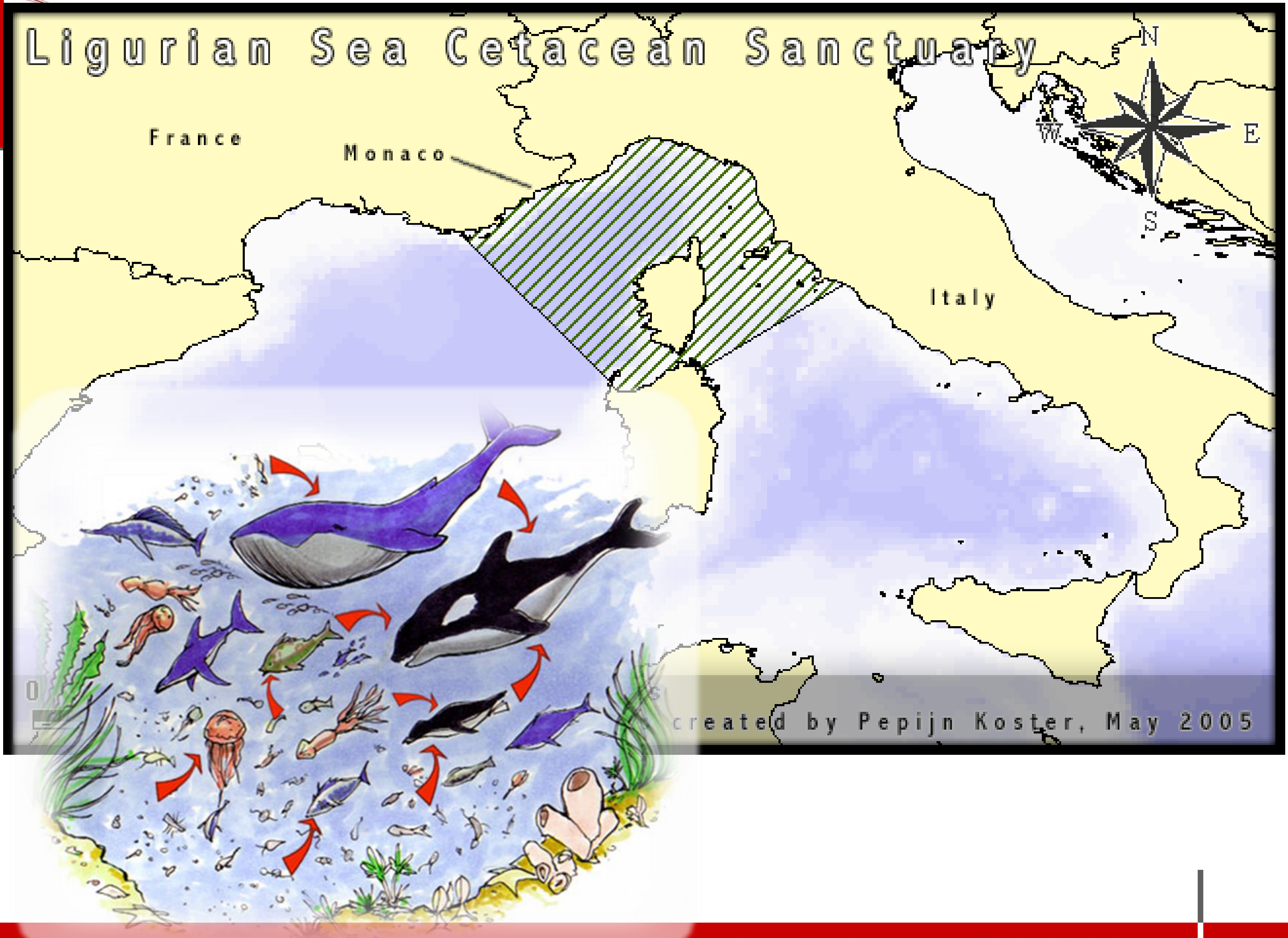
Western Mediterranean

(Ligurian Sea, Gulf of Lion, Catalonia coast and NWAlborán Sea

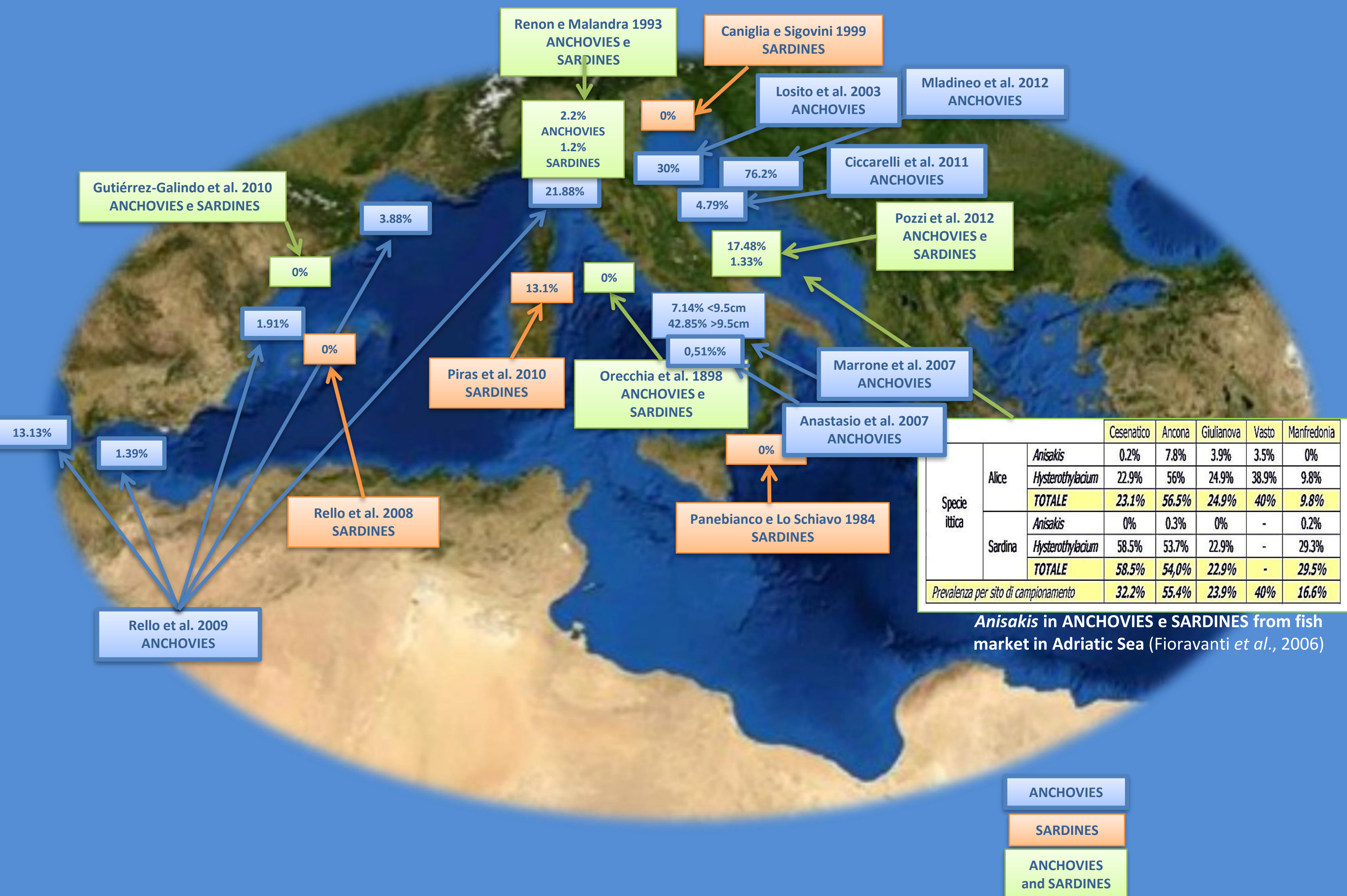
Prevalenze più alta per *Anisakis* che *H. aduncum* in alici pescate in Atlantico rispetto al Mediterraneo e viceversa

Prevalenza di *Anisakis* in alici del Mar Ligure 5 volte almeno più alta rispetto ad altre aree del Mediterraneo occidentale

Fishing area (number of fish)	Parasite	Prevalence 95%CI[a]	Mean abundance 95%CI	Mean intensity 95% CI (range)	Mean intensity in fish muscle 95% CI (range)
NW Alborán Sea (72)	*Anisakis* larva type I	1.39 0.08–7.40	0.01 0.00–0.04	1[uc] (1)	0[na] (–)
	H. aduncum	2.78 0.50–9.52	0.11 0.00–0.28	4[uc] (4)	1.50[uc] (1–2)
Catalonia Coast (157)	*Anisakis* larva type I	1.91 0.53–5.60	0.03 0.01–0.10	1.67 1.00–2.33 (1–5)	1.33 1.00–1.67 (1–2)
	H. aduncum	37.58 30.22–45.53	0.80 0.61–1.03	2.12 1.78–2.54 (1–7)	1.62 1.23–2.15 (1–4)
Gulf of Lion (103)	*Anisakis* larva type I	3.88 1.34–9.56	0.06 0.01–0.13	1.50 1.00–1.75 (1–6)	1[uc] (1)
	H. aduncum	68.93 59.26–77.27	11.74 9.03–15.20	17.03 13.61–21.34 (1–78)	4.51 3.47–5.98 (1–23)
Ligurian Sea (64)	*Anisakis* larva type I	21.88 13.06–33.51	0.78 0.41–1.27	3.57 2.64–4.79 (1–9)	3.67 2.44–5.56 (1–9)
	H. aduncum	70.31 57.86–80.66	4.03 2.88–5.95	5.73 4.29–8.18 (1–32)	4.90 3.38–7.21 (1–20)



Ligurian Sea Cetacean Sanctuary
France
Monaco
Italy
N
E
S
W
created by Pepijn Koster, May 2005



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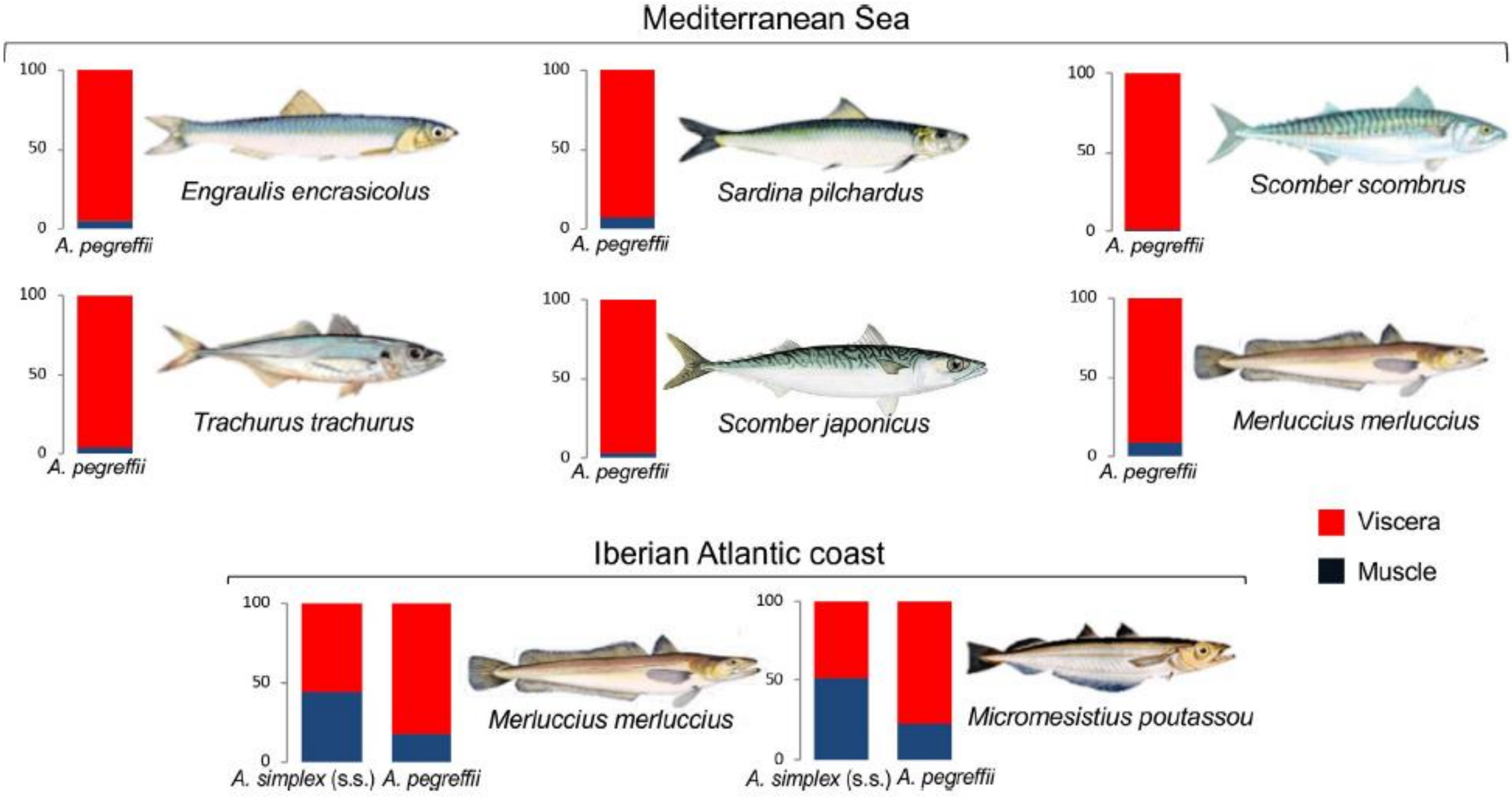
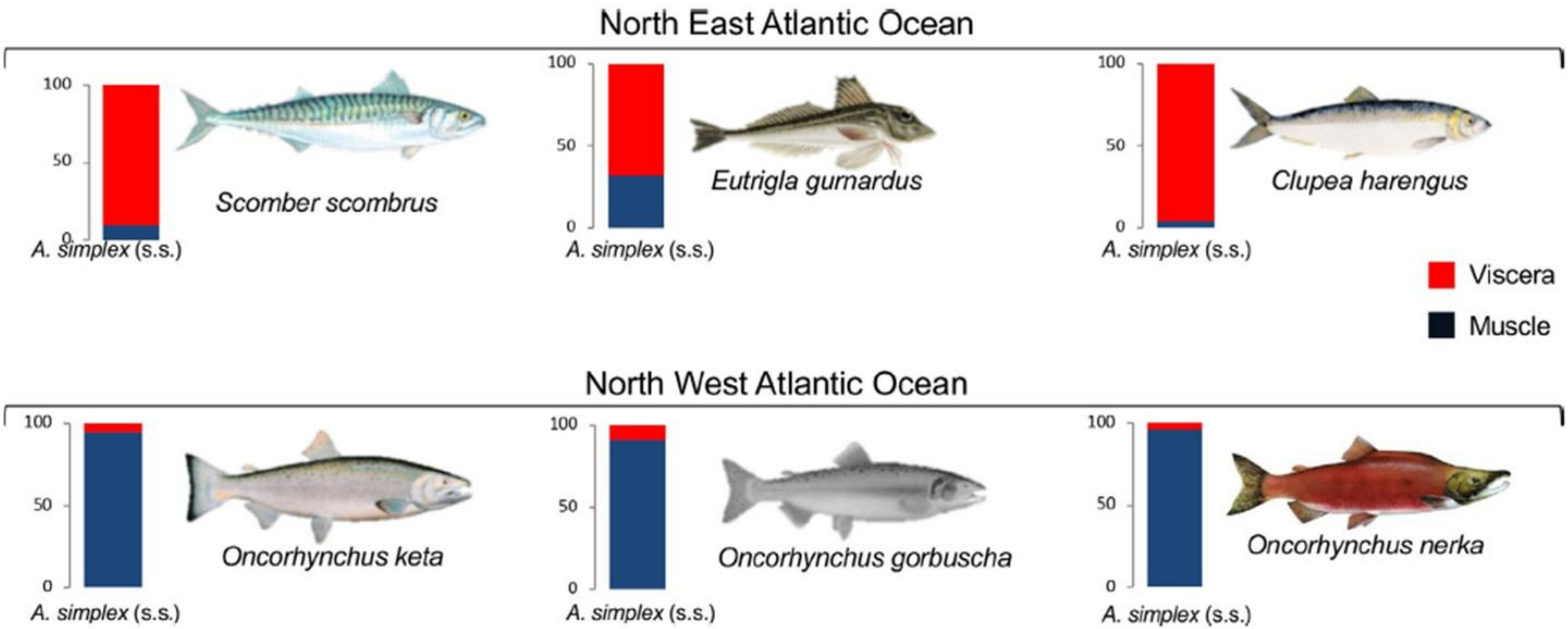
Prevalenza per sito di campionamento			*32.2%*	*55.4%*	*23.9%*	*40%*	*16.6%*

***Anisakis* in ANCHOVIES e SARDINES from fish market in Adriatic Sea** (Fioravanti *et al.*, 2006)



Mattiucci et al., 2018



Mattiucci et al., 2018

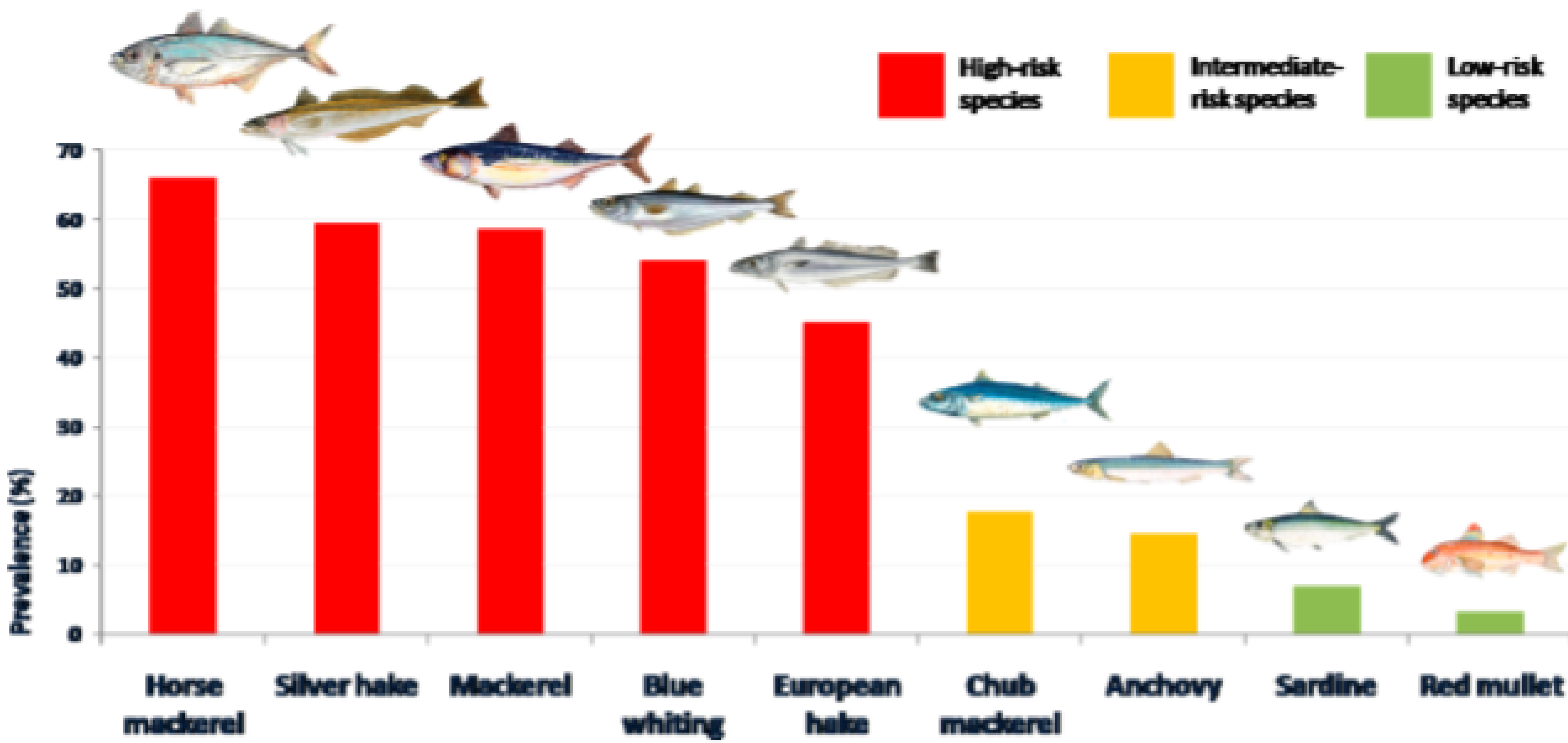


Figure 1. Classification of the fish species based on the prevalence (%) of *Anisakis* type I larvae.

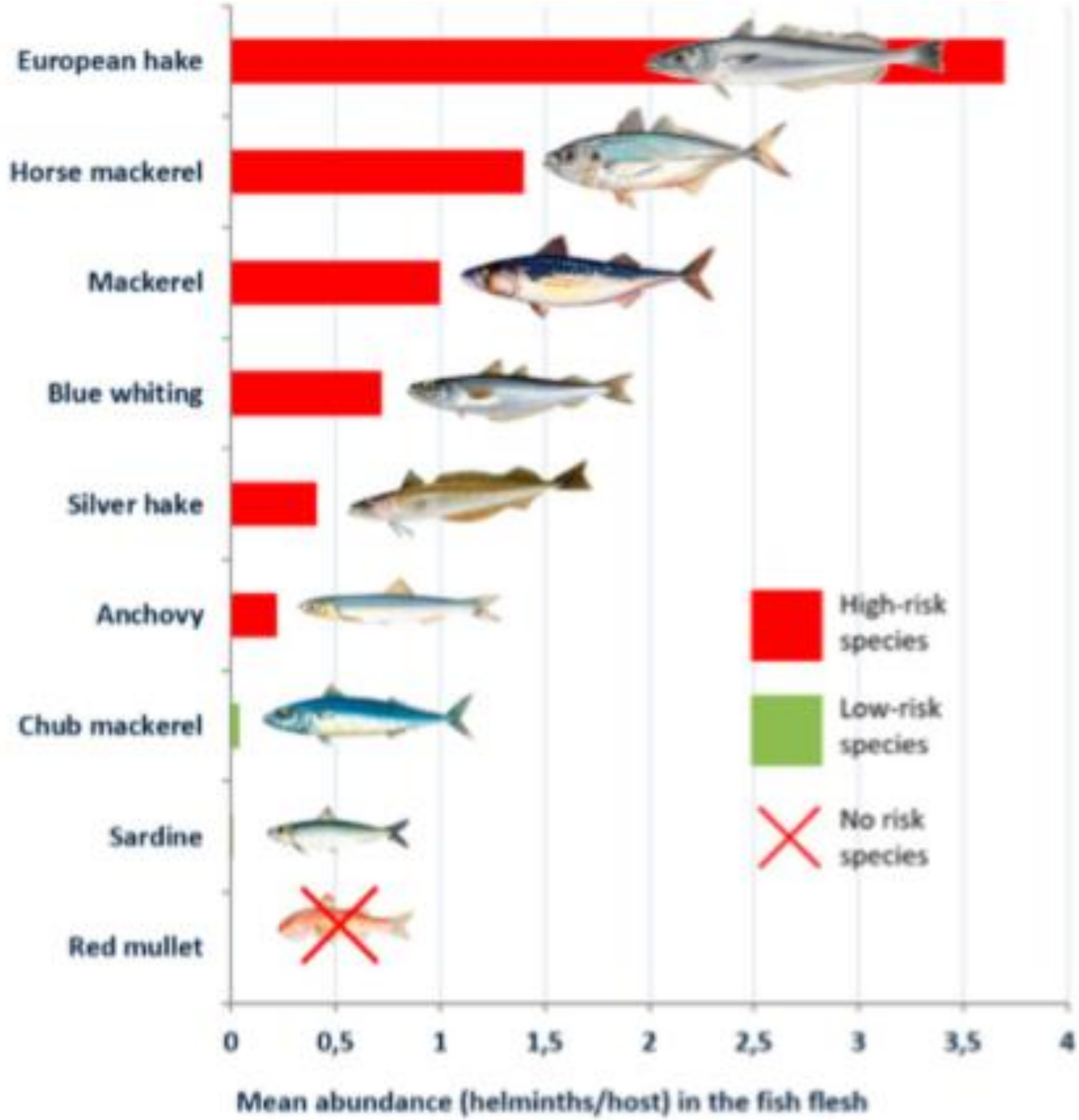
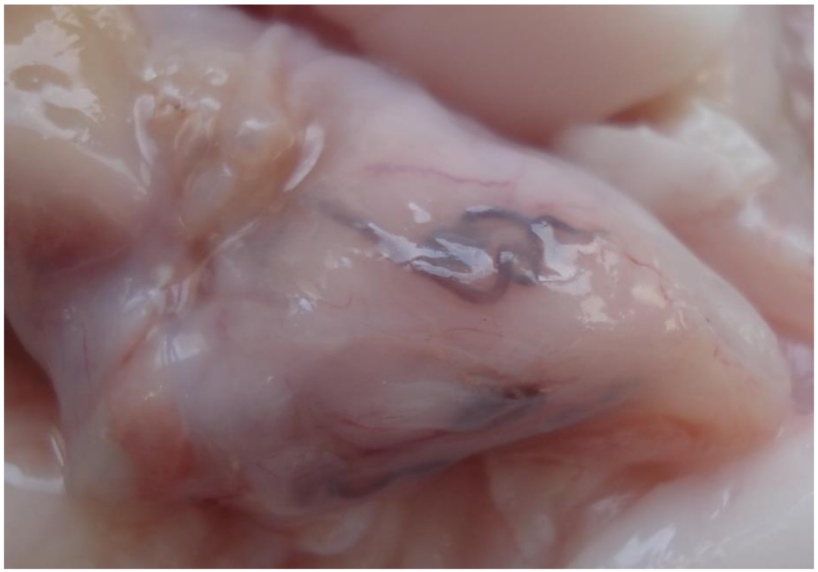


Figure 2. Classification of the fish species based on the mean abundance (helminths/host) of *Anisakis* type I larvae observed in the fish flesh.

Infection by L3 of *Anisakis simplex sensu stricto* in European sea bass *Dicentrarchus labrax* from FAO Area 27 (North-Eastern Atlantic Ocean)



(Bernardi, 2009)

	1-2 Kg	2-3 Kg	>3 Kg
March - June 2007 (viscera) Bernardi (2009)			
N examined fish	334	180	47
N positive fish	218	153	42
Prevalence (%)	65,27	85,00	89,36
September 2008 - March 2009 (viscera + muscles) Bernardi *et al*. (2009)			
N examined fish	44	9	5
N positive fish	39	8	5
Prevalence (%)	88.63	88.88	100.00
Mean Intensity	1-372 (64,2)	30-1029 (240,8)	15-169 (56,0)

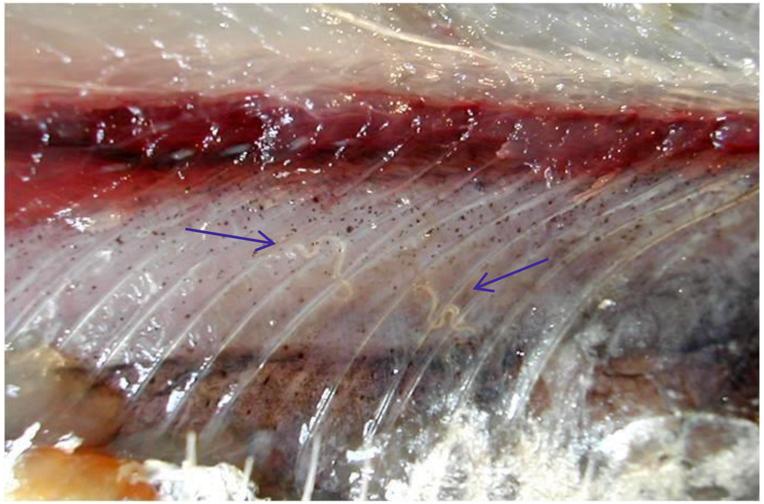
Positivity in muscles for *Anisakis* spp.: 15 su 58 (25,9%)

Intensity in muscle: 1-9 (2,7)

***Anisakis* sp. L3 in silver scabbardfish**

***Anisakis* sp. L3 in anchovies**

***Pseudoterranova* sp. L3 in angler fish**



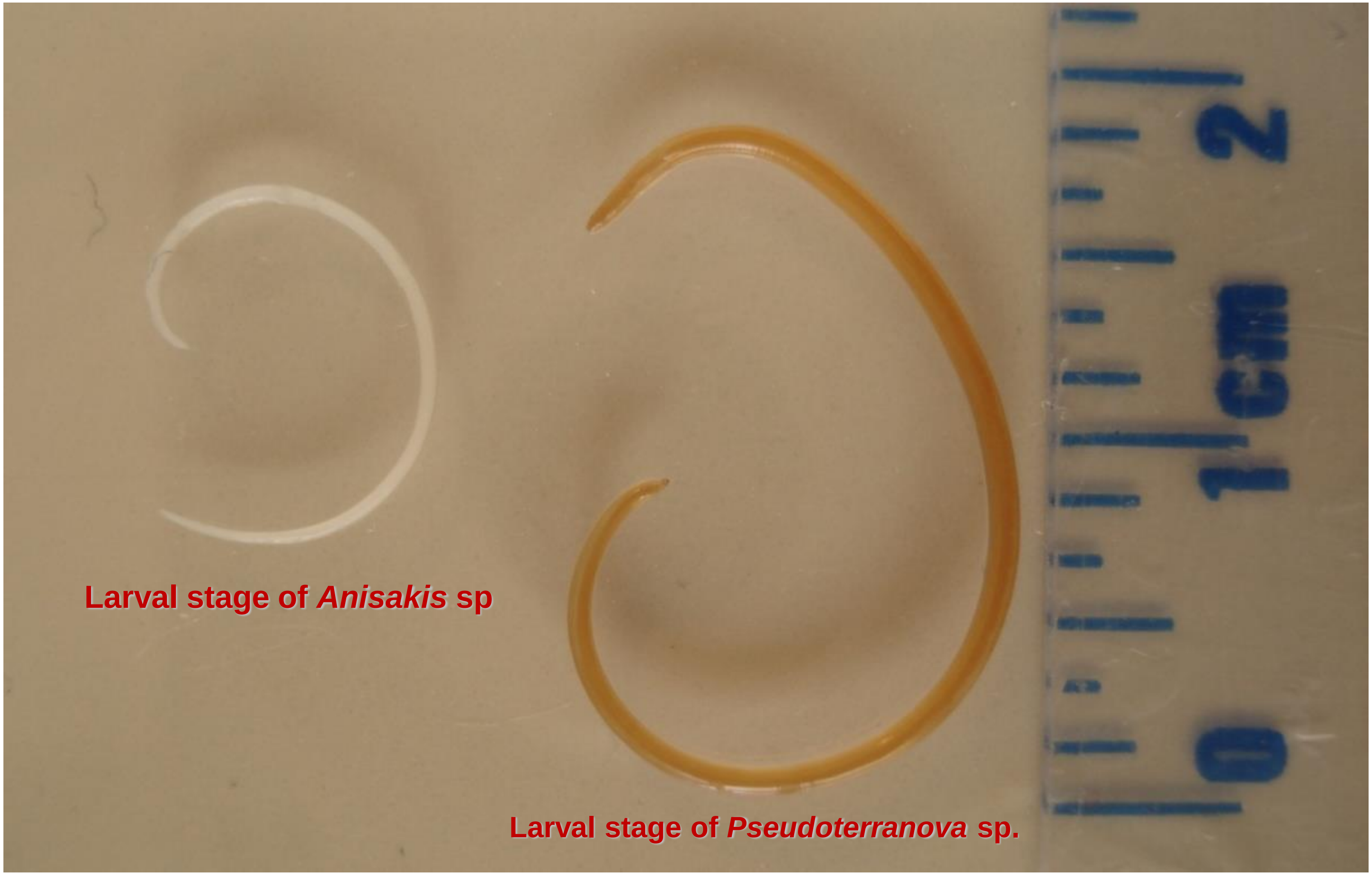
***Hysterothylacium* sp. L3 in sardine**

Larvae of Anisakidae try to migrate to the external environment after the death of the host (*post mortem* migration), penetrating the lateral muscle.

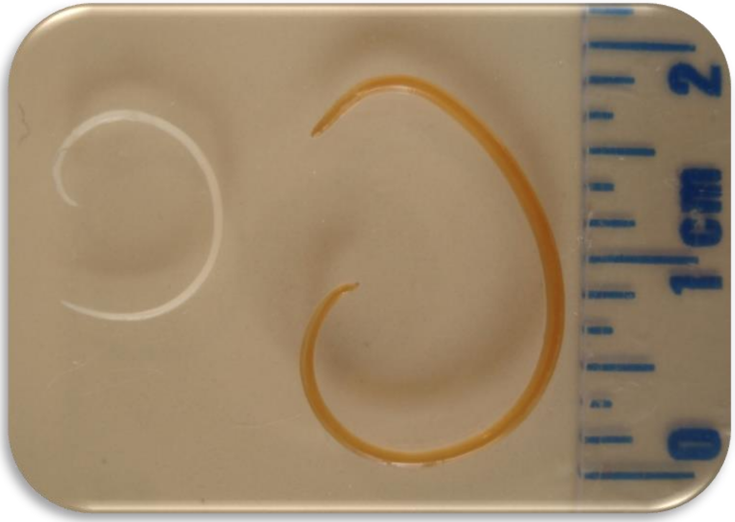
Larvae of *Pseudoterranova* in general are in the muscle *intra vitam.*

Identification of larval stages of Anisakidae nematodes on morphological approach allows to identify them at Genus level.

Molecular identification is mandatory



PARASSITI ITTICI ZOONOTICI: ORDINI DI GRANDEZZA



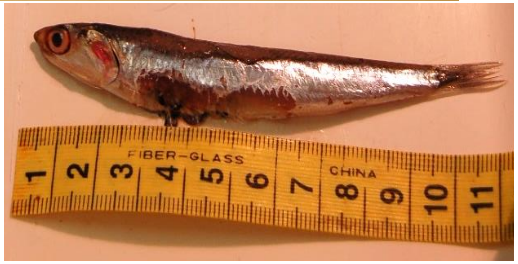
NEMATODI ANISAKIDAE

LARVE DI
DIPHYLLOBOTHRIUM LATUM

METACERCARIE DI DIGENEI OPISTHORCHIIDAE

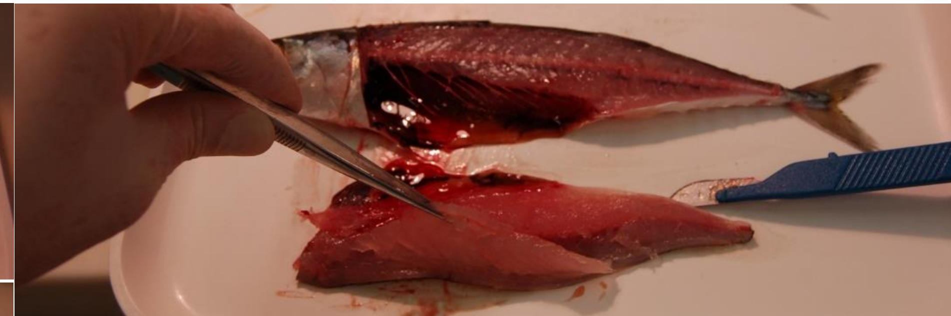

ANISAKIASI
DIAGNOSTICA

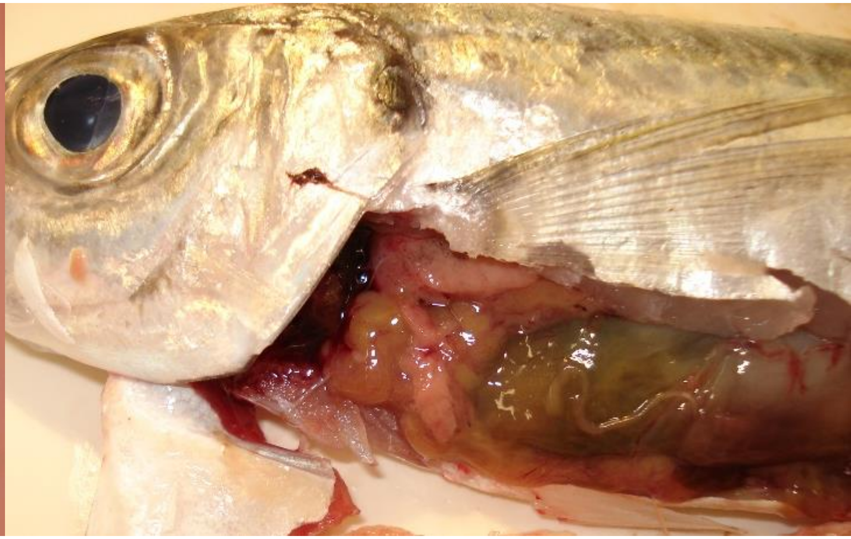
Protocollo per la ricerca di nematodi Anisakidae in specie ittiche

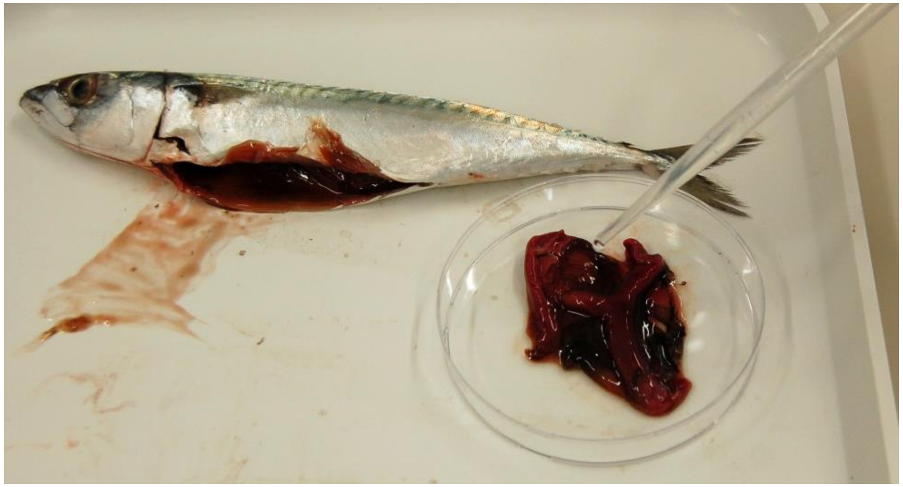


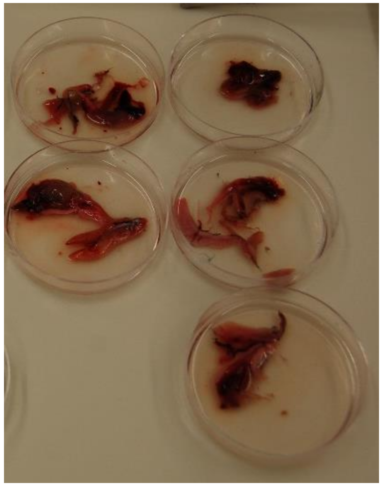








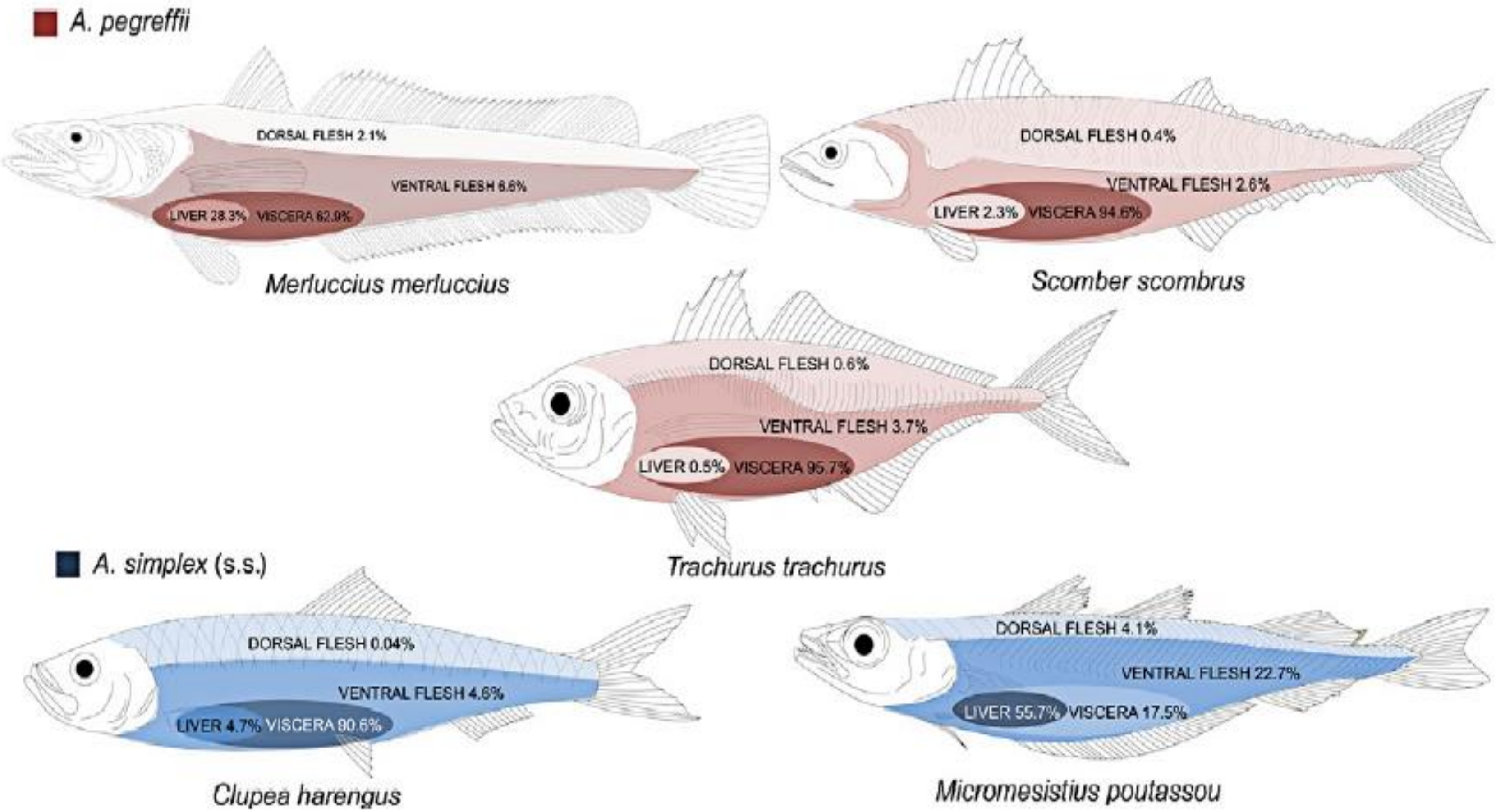



White spot

DIAGNOSIS

Viusal inspection

Transillumination or «candling»



Mattiucci et al., 2018

CANDLING

Fish fillets are positioned on the glass and observed on an artificial light. Anisakid larvae should be dark inside the fillet, so well visible.

Transillumination works better in a dark room

A smart fish inspector is able to examine 300 fillets/hour

In a research carried out by the European Food Safety Authority (EFSA, 2010) candling showed the biggest efficacy for fillets up to 2.5 mm of thickness.

Levsen *et al*. (2005) report an efficacy of candling alone of 7 to 10% of *Anisakis* larvae in fillets
This value greatly increase (no less of 75% of *Anisakis* larvae detected) when visual inspection and candling are combined together.

Compression and UV transillumination method

Receipt of samples

Samples may be received as whole fish or as pre-prepared fillets or tissue sections.
Samples should be immediately assessed for their condition.
Samples should be assigned a specific reference number.

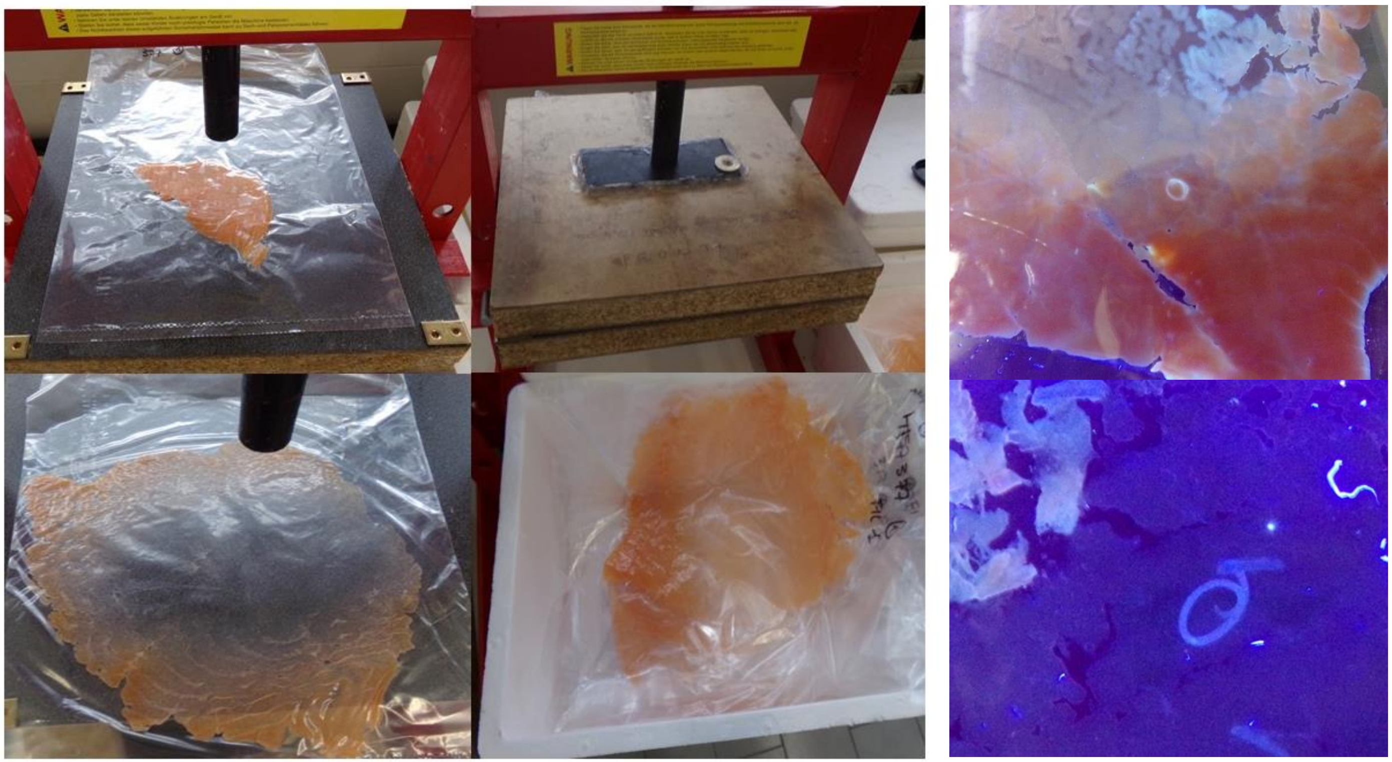
If immediate screening is not possible, samples can be stored in a fridge (approx. 4°C) for a maximum of 48 hours.

Sample Preparation

If fish samples have been received whole, carefully fillet using a suitable knife ensuring that muscle tissue is removed from the carcass as cleanly as possible. Fillet both sides of the fish. Place each fillet into a clear plastic bag ensuring there is sufficient space in the bag for compression. Using a suitable system, squeeze both fillets until they are approximately 1-2mm thick.
Freeze the fillets (at -20°C if possible).
The skin can be removed prior to compression or after freezing as required

(Wootten e Bron, 2008)

UV-press



[redacted]

Currently employed solutions

DIAGNOSTIC TOOLS: UV-PRESS METHOD

PARASITE, FP7 project: http://parasite-project.eu/

https://www.youtube.com/watch?v=W5CNmvFcbO8

PARAFISHCONTROL, H2020 project https://www.parafishcontrol.eu

https://www.youtube.com/watch?v=YCn4-zEOSZk&t=3s

	UV-Press	Artificial Digestion
Accuracy	100%	97%
Sensitivity	100%	96%
Specificity	100%	100%

http://parasite-project.eu/didactic/Workshop_UV-Press_en_web.pdf

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634429. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

Artificial Digestion by digestive solution (1000 ml of saline solution 0,85% NaCl + 10 g of pepsin + 6N di HCl (pH2)

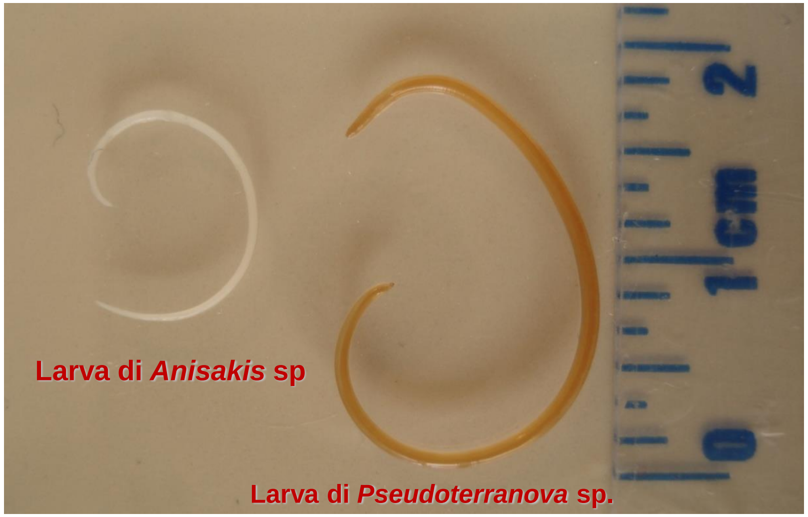
PROCEDURE FOR ANISAKIDAE

- Skin and eviscerate the fish, wash the carcass with tap water and collect the muscular tissues;
- Add to a glass beaker the Digestion solution
- Transfer the blended or chopped tissues into the beaker together with the digestion solution and add the stirring rod
- Place the beaker on the magnetic stirrer and set the heating plate at 40-42°C;
- Incubate the solution under stirring condition until the tissue disappear (approximately 15-20 min);
- Switch off the stirrer and pour carefully (to avoid overflow) the digestion solution through the sieve into a beaker;
- Anisakidae larvae can be detected on the sieve, collected and examined under the light microscope. The larvae can be transferred in a vial filled with 70% ethanol and stored at a temperature range between -20 °C and 10 °C up to 5 years.

L'identificazione degli stadi larvali dei nematodi Anisakidae può essere condotta a livello di genere su base morfologica, mentre per raggiungere un'identificazione a livello di specie bisogna condurre analisi molecolari.

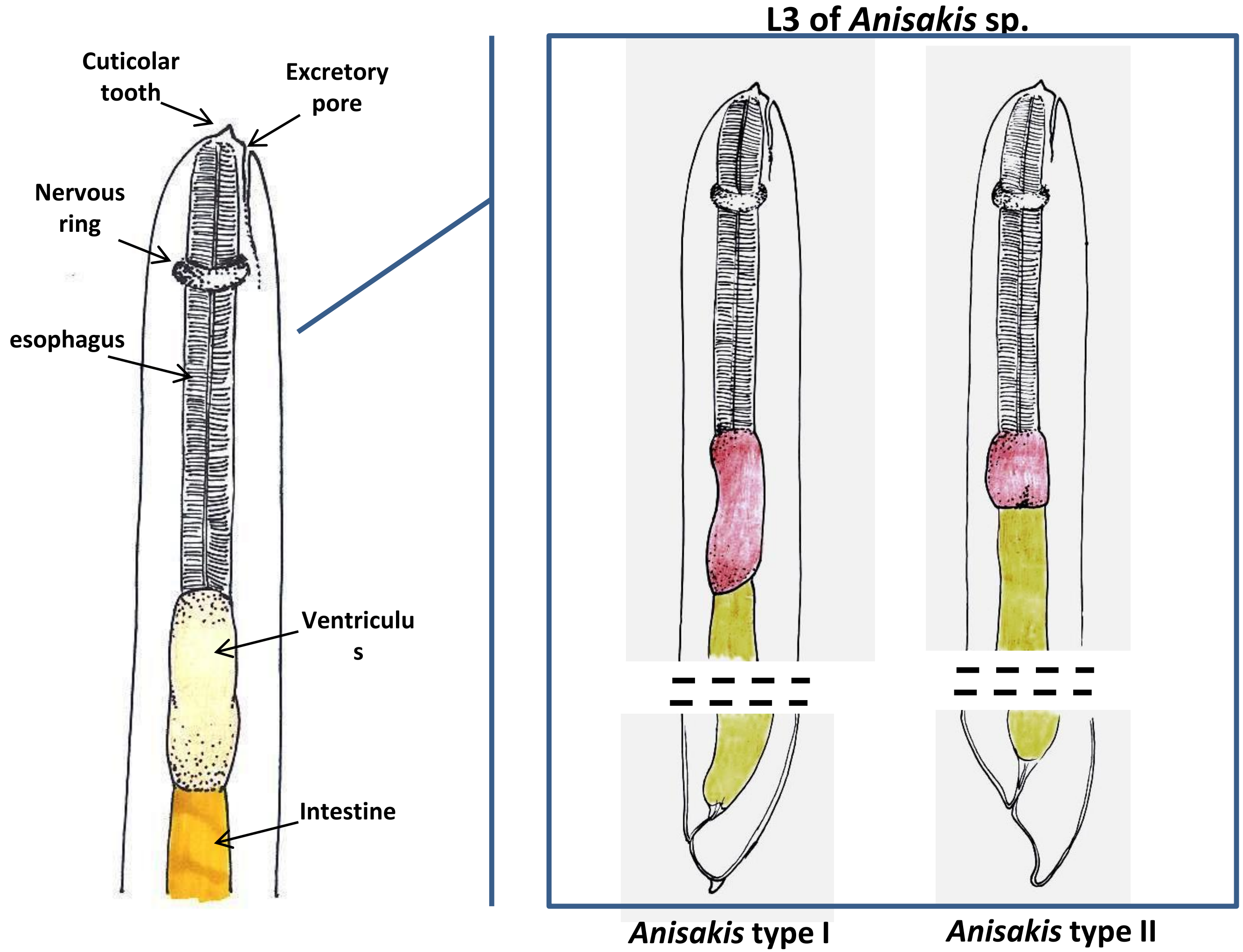
Per lo studio morfologico delle larve anisakidi gli elementi chiave da prendere in considerazione sono

Misura (lunghezza e larghezza) e colore

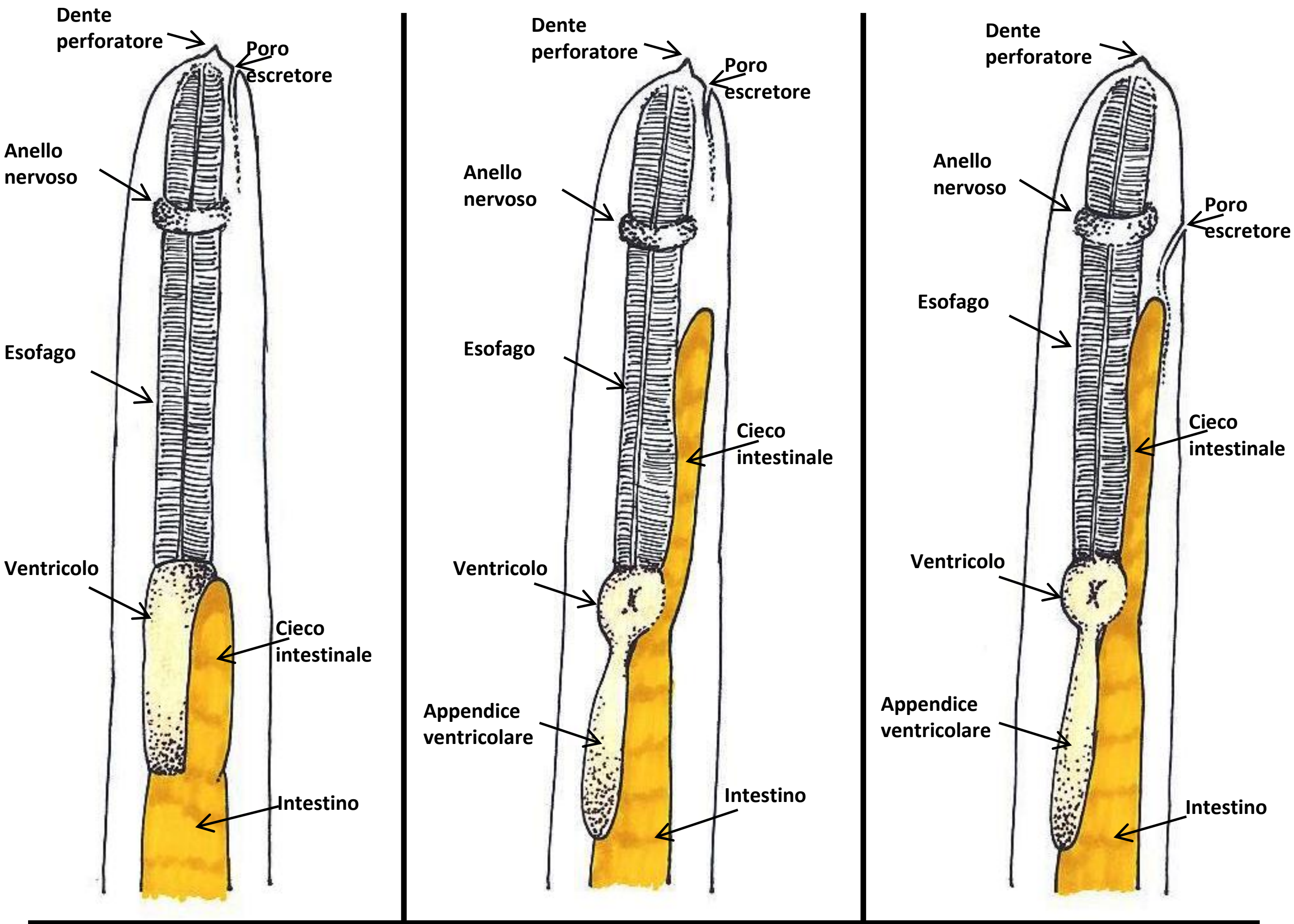


PROTOCOLLO PER L'IDENTIFICAZIONE MORFOLOGICA DI NEMATODI ANISAKIDAE

- Fissazione in etanolo 70° (100 ml di etanolo assoluto + 47,75 ml di H_2O) o 70% (70 ml di etanolo assoluto + 30 ml di H_2O)
- Chiarificazione in lattofenolo di Amman / glicerina
- Osservazione delle caratteristiche morfologiche utili all'identificazione a livello di genere:
  - Dimensione del corpo
  - Conformazione del ventricolo
  - Presenza/assenza appendice ventricolare
  - Presenza/assenza del cieco intestinale
  - Localizzazione del poro escretore (anteriore/posteriore)



Cuticolar tooth
Excretory pore
Nervous ring
esophagus
Ventriculu s
Intestine
L3 of *Anisakis* sp.
Anisakis type I
Anisakis type II



***Pseudoterranova* sp.**

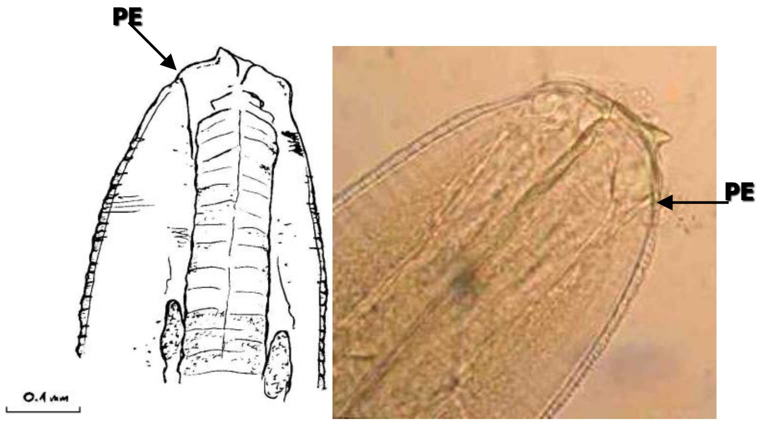
***Contracaecum* sp.**

***Hysterothylacium* sp.**

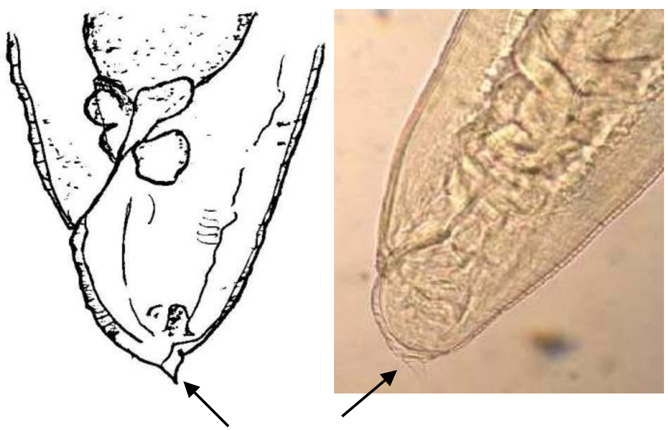
Larvae of *Hysterothylacium* sp.

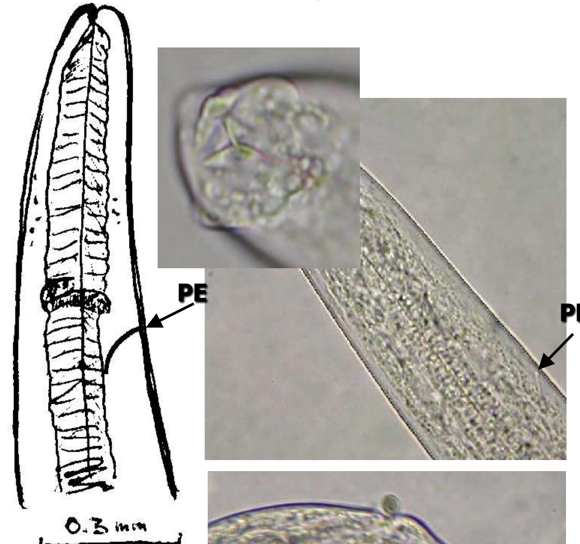
Larvae of *Anisakis* tipo I

Posizione del poro escretore e presenza/assenza di mucrone a livello dell’estremità posteriore

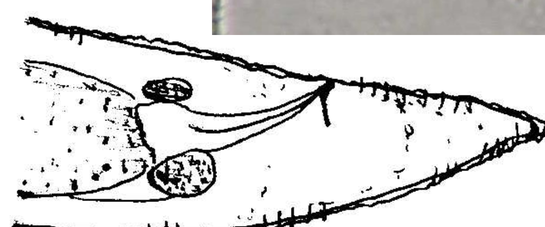


Larve di 3° stadio di *Anisakis*



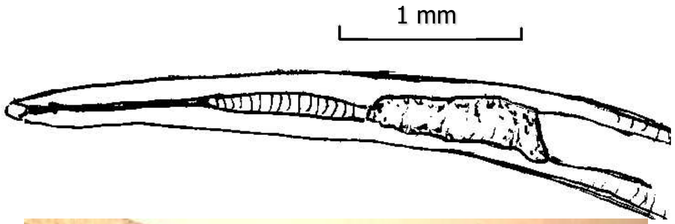


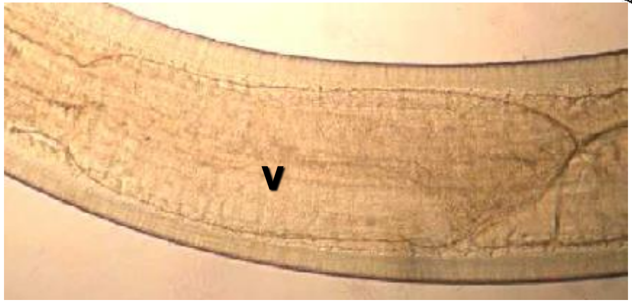
Larve di 3° stadio di *Hysterothylacium*



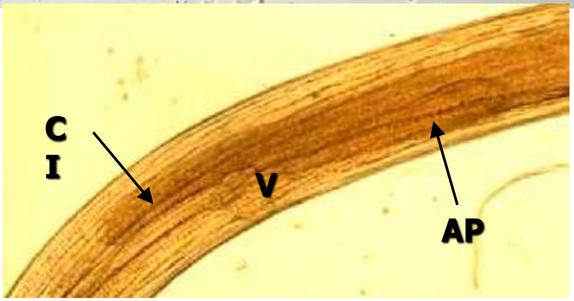
Struttura dell’esofago, con particolare riferimento al ventricolo (porzione ghiandolare sita tra esofago muscolare e intestino) ed alla presenza/assenza di appendici ventricolari e /o cechi intestinali

Struttura del ventricolo in larve di 3° stadio di *Anisakis*

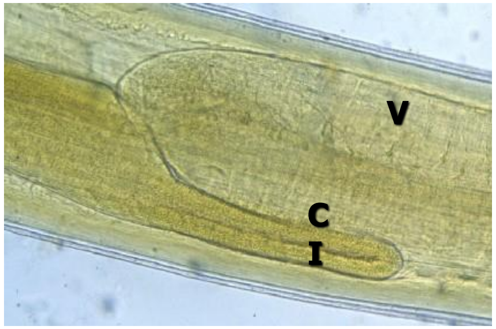


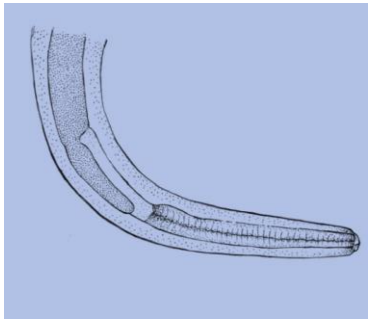


Struttura dell’apparato ventricolare in larve di 3° stadio di *Hysterothylacium*



Struttura del ventricolo+cieco intestinale in larve di 3° stadio di *Pseudoterranova*





IDENTIFICAZIONE MOLECOLARE DI NEMATODI ANISAKIDAE

- Estrazione DNA mediante kit del commercio (sistemi in colonnina)
- Amplificazione mediante PCR
- Geni target: ITS rDNA gene largamente impiegato per la diagnostica delle malattie parassitarie in acquacoltura
    - multicopia, *tandem repeat,* altamente conservato,
    - primers universali
    - zone variabili

- PCR-RFLP con enzimi *Hinf*I e *Hae*III

Parasite

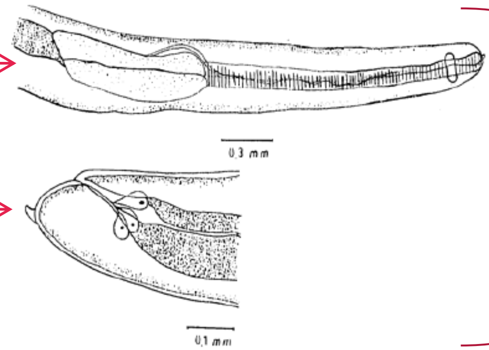
The use of hydrolysis probe in Real Time PCR for the detection of Anisakids in fish products

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 312068

Su base morfologica

Su base genetica

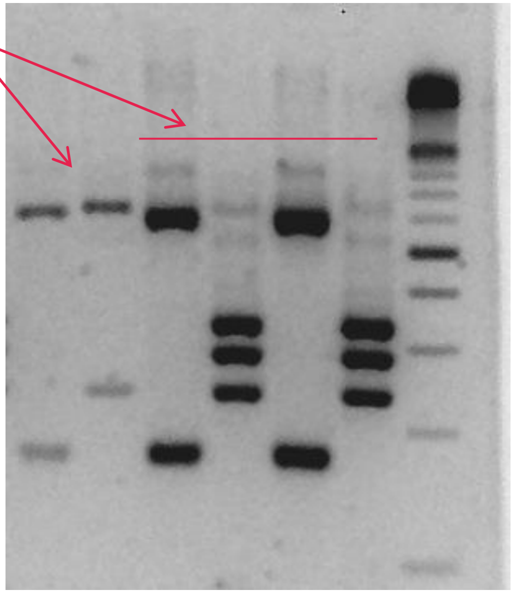
Larve di 3° stadio di *Anisakis* tipo I (Berland, 1961)



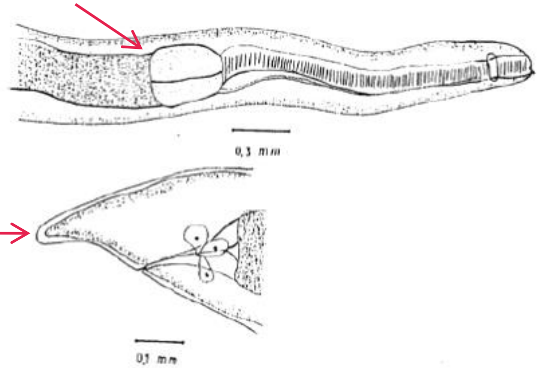
Anisakis simplex s.s.
A. pegreffii
A. berlandi

Anisakis simplex complex

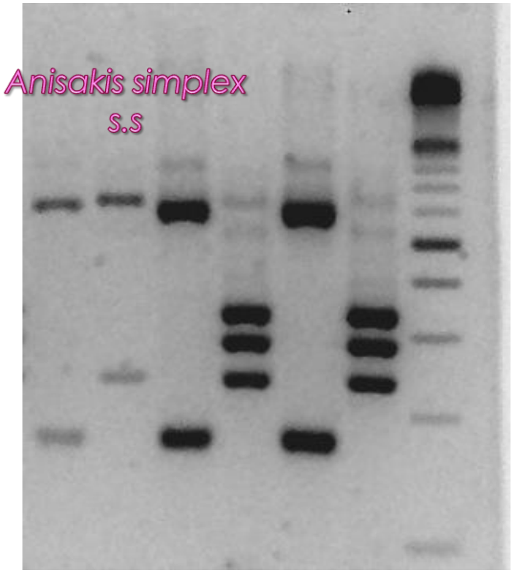
A. ziphidarum
A. typica
Anisakis nascetti

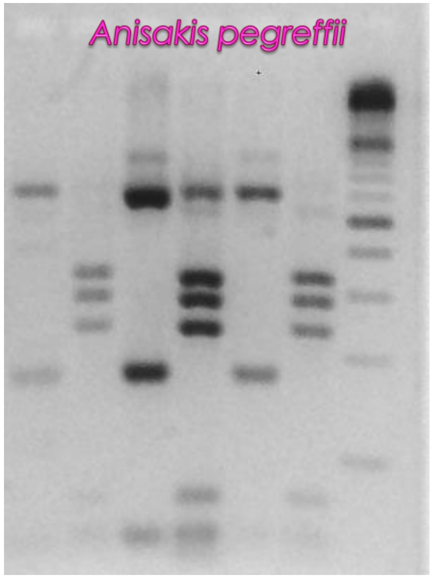


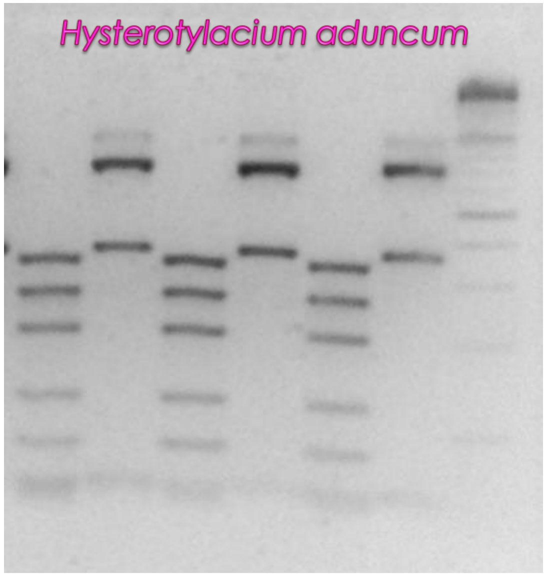
Larve di 3° stadio di *Anisakis* tipo II (Berland, 1961)



A. physeteris
A. brevispiculata
A. paggiae


Anisakis simplex
s.s


Anisakis pegreffii

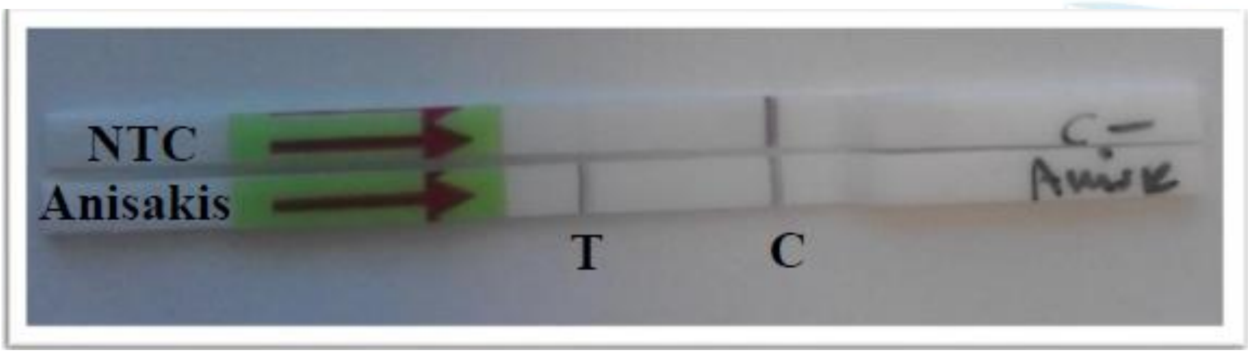

Hysterotylacium aduncum

ParaFishControl

DIAGNOSTIC TOOLS: RAPID AND PORTABLE *ANISAKIS* DETECTION METHOD

qPCR and/or Isothermal PCR (AZTI)

For the very first time it is possible to identify *Anisakis simplex* (excluding DNA isolation) within 15 minutes! This cheap, portable, and user-friendly kit could become a valuable tool in the future to certify that fish farmed in the EU is grown in an environment free of *Anisakis*.



LF RPA Assay for the detection of *Anisakis simplex*: Test line (T); Internal Control line (C). Negative Template control (NTC)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634429. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

Novel polymorphic microsatellite loci in *Anisakis pegreffii* and *A. simplex* (s. s.) (Nematoda: Anisakidae): implications for species recognition and population genetic analysis

Parasitology, 2019

Simonetta Mattiucci[1], Eleonora Bello[2], Michela Paoletti[2], Steve C. Webb[3], Juan T. Timi[4], Arne Levsen[5], Paolo Cipriani[5] and Giuseppe Nascetti[2]

[1]Department of Public Health and Infectious Diseases, Section of Parasitology, "Sapienza -University of Rome", Laboratory affiliated to "Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Italy" P.le Aldo Moro, 5 00185 Rome, Italy; [2]Department of Ecological and Biological Sciences, Tuscia University, Viale dell'Università s/n 01100 Viterbo, Italy; [3]Cawthron Institute, Nelson, New Zealand; [4]Instituto de Investigaciones Marinas y Costeras, Universidad Nacional de Mar del Plata-CONICET, Mar del Plata, Argentina and [5]Institute of Marine Research, Bergen, Norway

Comparative analysis of excretory-secretory antigens of *Anisakis simplex*, *Pseudoterranova decipiens* and *Contracaecum osculatum* regarding their applicability for specific serodiagnosis of human anisakidosis based on IgG-ELISA

Maciej Kochanowski[a], Miguel González-Muñoz[b], María Ángeles Gómez-Morales[c], Bruno Gottstein[d], Joanna Dąbrowska[a], Mirosław Różycki[a], Tomasz Cencek[a], Norbert Müller[d,*], Ghalia Boubaker[d]

[a] *Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Partyzantów Avenue 57, 24-100, Puławy, Poland*
[b] *University Hospital La Paz-FIBHULP, Paseo Castellana, 261, 28046, Madrid, Spain*
[c] *Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy*
[d] *Institute of Parasitology, Vetsuisse Faculty, University of Bern, Länggass-Strasse 122, 3012, Bern, Switzerland*

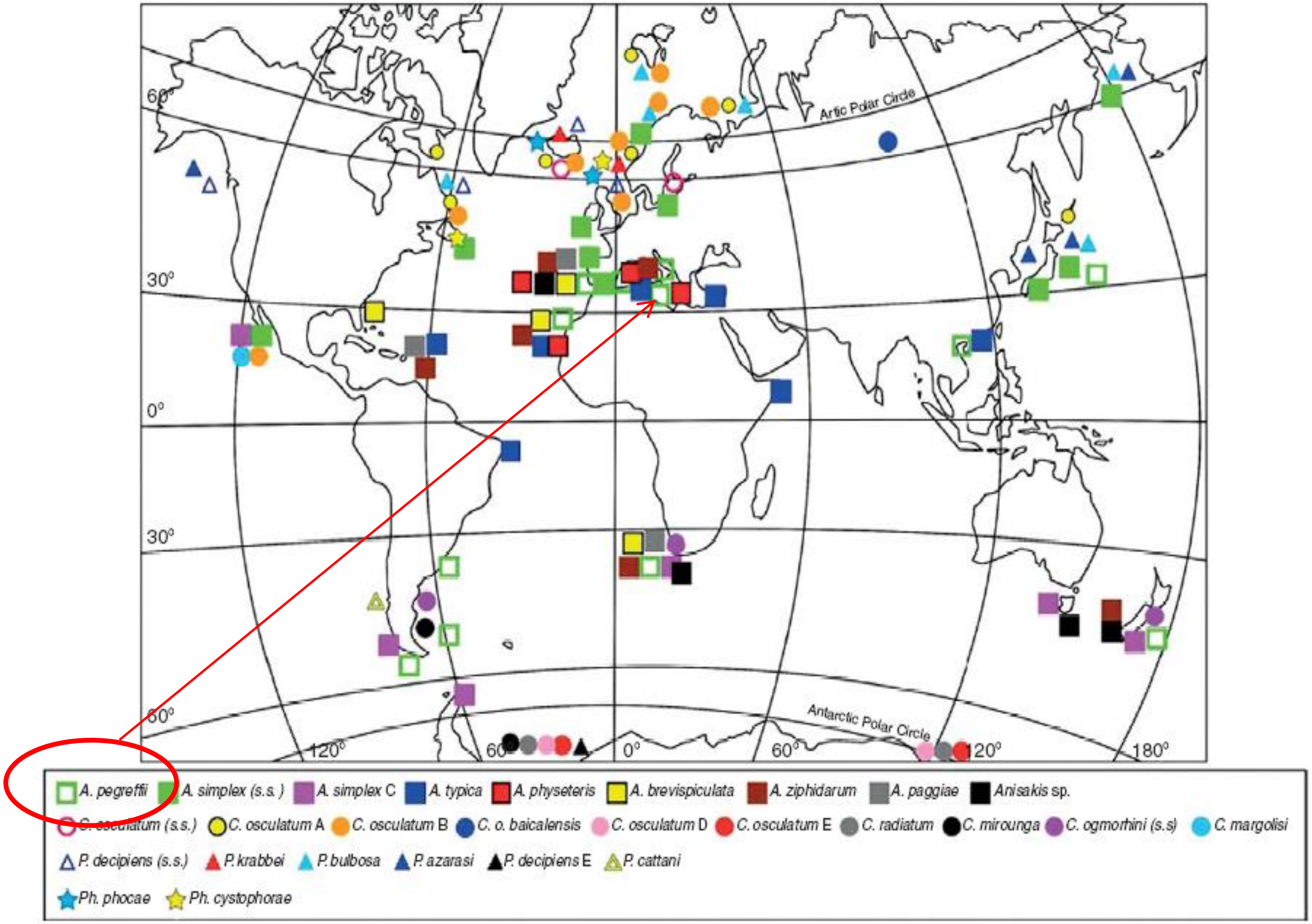


FIGURE 2.1 World map showing the so far known distribution areas of anisakid species of *Anisakis* (□), *Pseudoterranova* (△), *Contracaecum* (○) and *Phocascaris* (⋆). The geographical areas indicated are related to the sampling localities for their definitive and intermediate hosts.

From Mattiucci e Nascetti, *Advances in Parasitology*, 2008

Species of *Anisakis* more often involved in human cases are *Anisakis simplex* e *A. pegreffii*

Casi di Anisakiosi umana segnalati nel mondo:

- **Olanda:** dal 1955 al 1968 ⇒ 160 casi (sostenuti prevalentemente da *Anisakis*)

- **Giappone:** 16.090 casi diagnosticati fino al 1990 (sostenuti prevalentemente da *Anisakis* - solo circa 50 casi da *Pseudoterranova*) (circa 2000 casi / anno)

- **Usa:** circa 10 casi/anno diagnosticati (sostenuti frequentemente da larve di *Pseudoterranova*)

- **Francia:** si osservano circa 6-7 casi all'anno, dovuti soprattutto a larve di A*nisakis*.

- **Spagna:** decine di casi descritti negli ultimi anni, molti di natura allergica

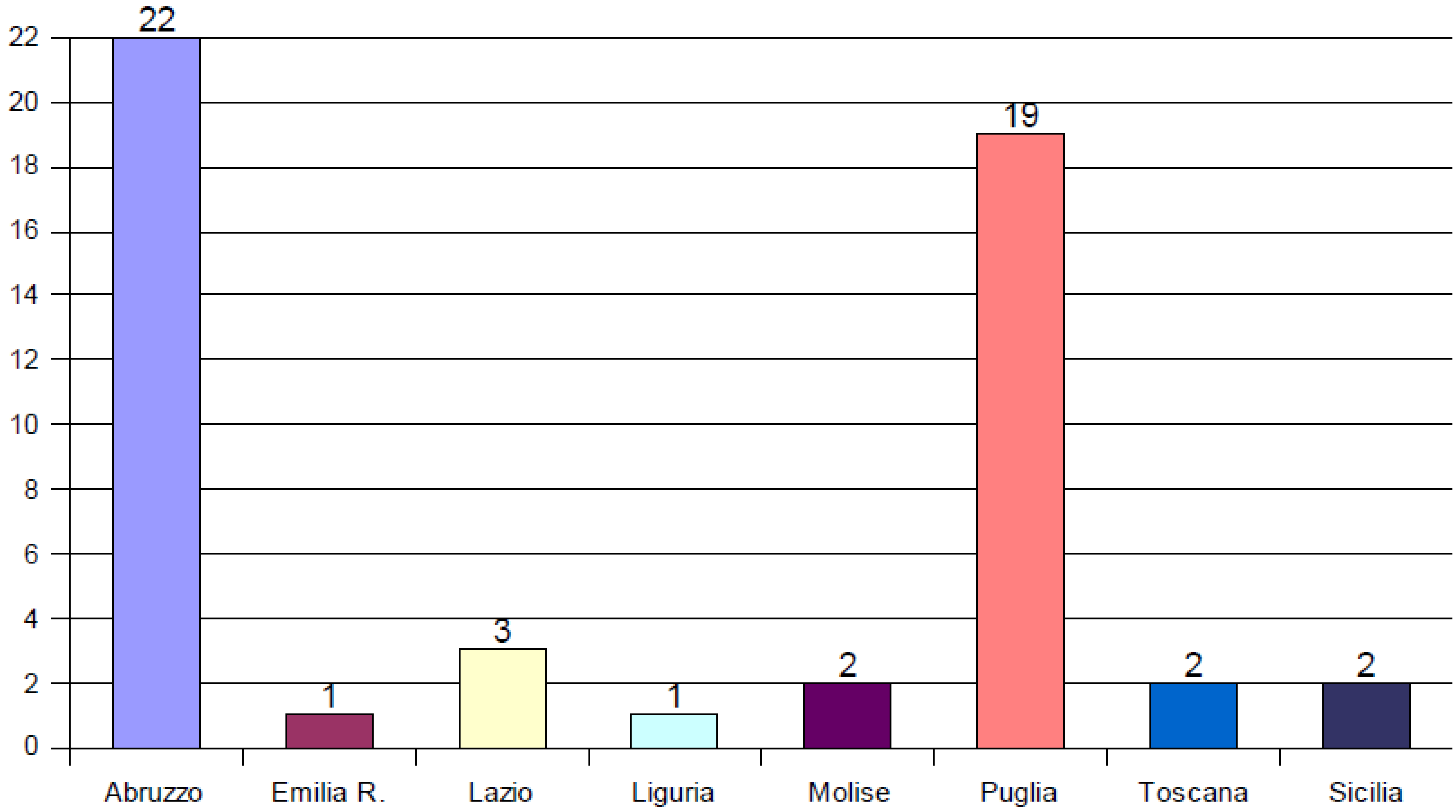
- + casi sporadici in Germania, Danimarca, Inghilterra, Norvegia, Belgio, Cile, Canada

Anisakiosi umana in Italia

- **Il 1° caso ad eziologia certa risale al 1996, la paziente presentava una violenta epigastralgia associata a vomito e diarrea.**
- **+ 28 casi diagnosticati su base istologica/morfologica o su base sierologica riportati in letteratura**
- **+ in Abruzzo dal 1998 al 2002: almeno 32 casi sospetti (oltre 20 confermati) osservati presso l'ospedale di Pescara**
- **1 caso di infestazione da *Pseudoterranova decipiens* complex segnalato nel 2002**
- **Dall'anamnesi di tutti i soggetti risultava il consumo di pesce crudo o poco cotto pochi giorni prima il manifestarsi dei sintomi**
- **Sono stati descritti alcuni casi di sindromi allergiche da *Anisakis* in seguito ad ingestione o inalazione (possibile malattia professionale immunoallergica?)**

Anisakiosi umana in Italia

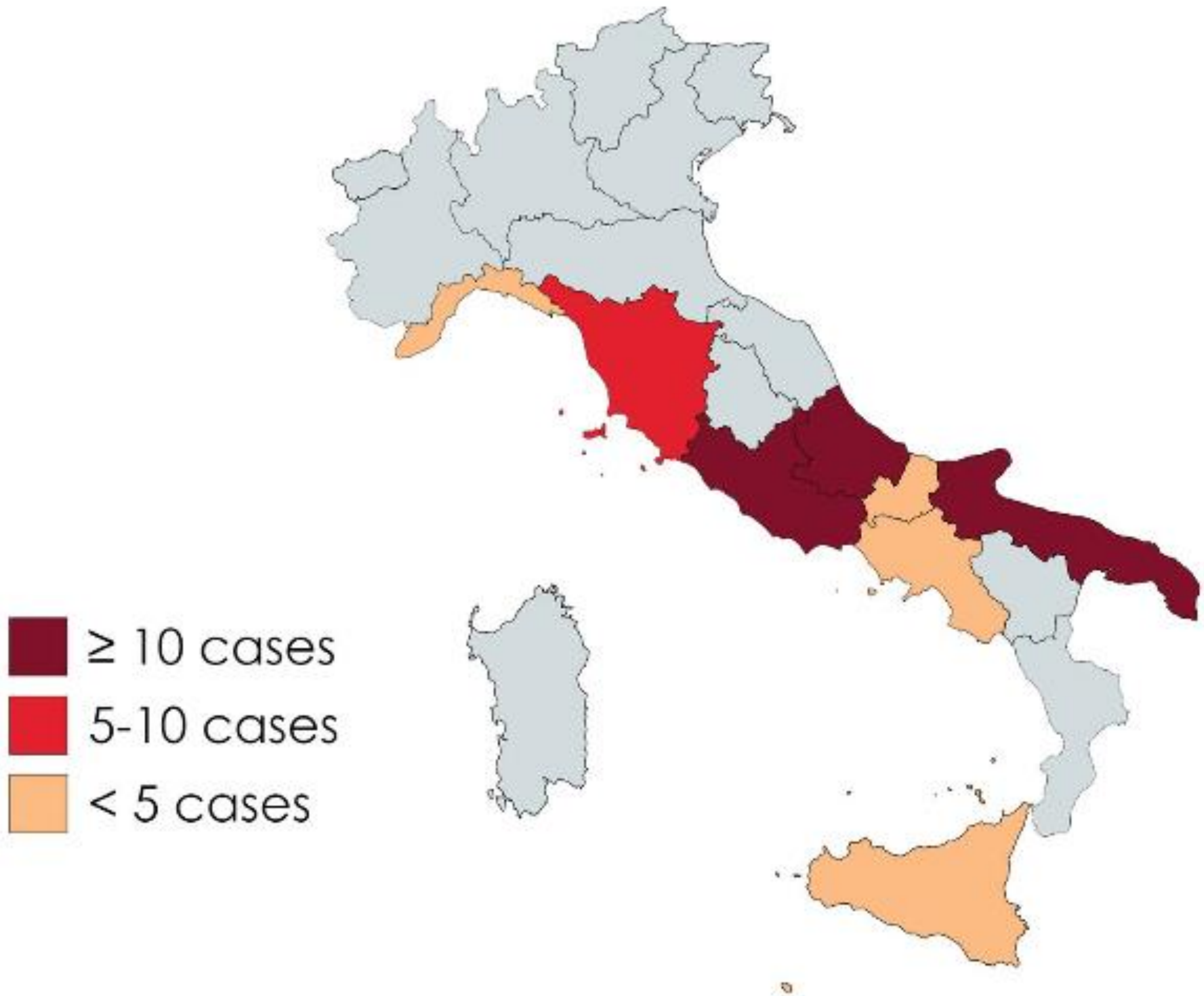
Il 1° caso ad eziologia certa risale al 1996, la paziente presentava una violenta epigastralgia associata a vomito e diarrea.



Casi clinici di Anisakiasi umana diagnosticati in Italia dal 1996 al 2011 (Di Rosa, 2011)

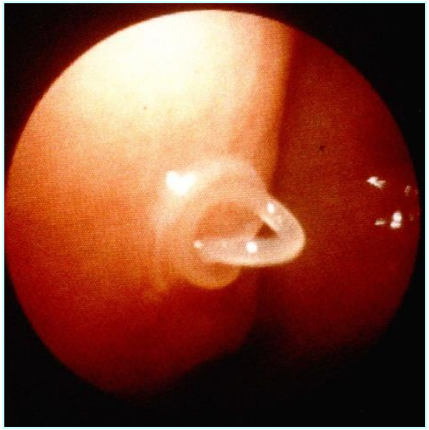
From 1996, 73 human cases of Anisakiasis are reported in Italy

L. Guardone et al.: Parasite 2018, **25**, 41



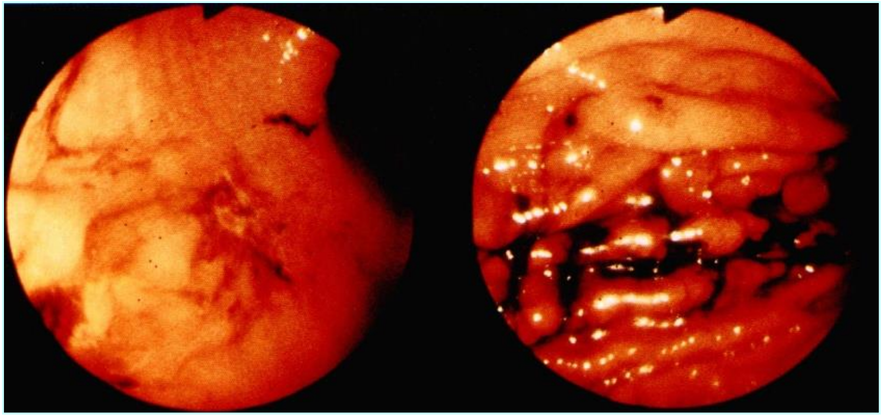
Pathology in man (invading form)

After penetration of the larva on the gastric/intestinal wall, causing edema, hyperemia and sometimes bleeding, they an inflammatory reaction with eosinophilic and lymphocytic infiltration, followed by connective tissue proliferation resulting in granuloma formation is frequently observed.



Invasion of the stomach wall is more frequently than the intestinal wall; In the intestine, the ileum seems to be more involved.

Most of them remain in the submucusal layer, but some can reach the abdominal cavity penetrating the entire wall thickness.



Gastro-intestinal invasive form

- During acute course strong abdominal pain, sickness, vomit, diarroea, are observed. Appendicitis-like.
- Symtomps occur after few hours in the gastric form and 6-7 days after consumption of parasitized fish in the intestinal one.
- During the chronic course usually 4 steps have been described:
  - Phlegmon
  - Abscessum
  - Abscessum-granuloma
  - Eosinophilic Granuloma

Pathology in man (allergic form)

Growing awareness for potentially life-threatening allergic reactions when the parasite penetrate the gastric mucosa.

These reactions are characterized by urticaria, occurring generally on the arms and abdomen, and by angioedema or anaphylaxis.

Anisakis antigens seem resistent to cooking and freezing process.

Probably a previous contact with *Anisakis* allergens is necessary for the allergic reaction

Prevention and control of Anisakiasis

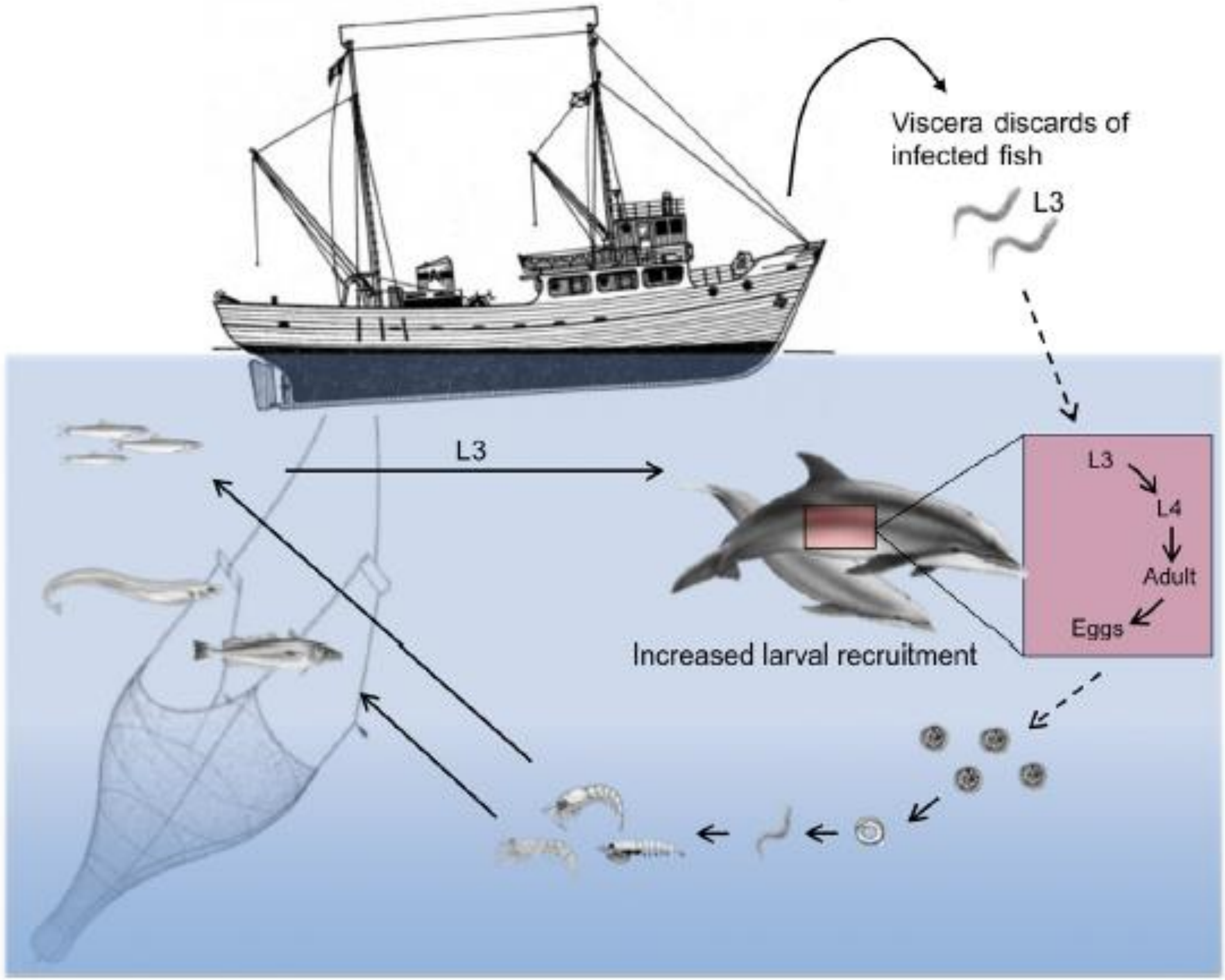
- Alimentary education

- Fish inspection at the market

- Cooking and freezing at the proper temperature



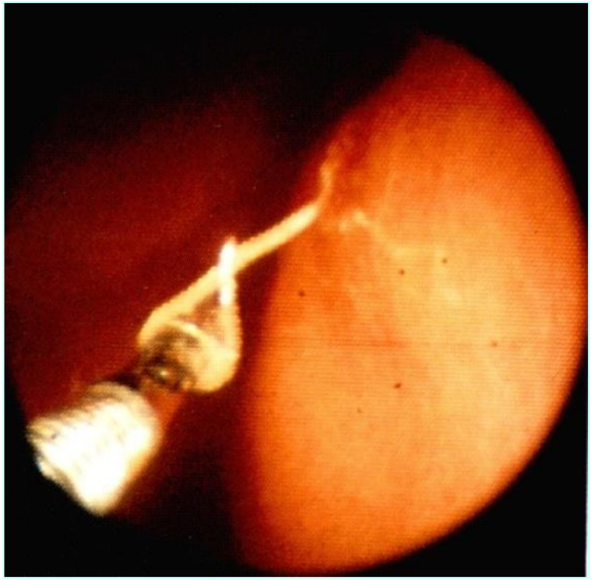
- Removal and destruction of fish viscera immediately after fishery to avoid larval migration in the muscle (when feasible)

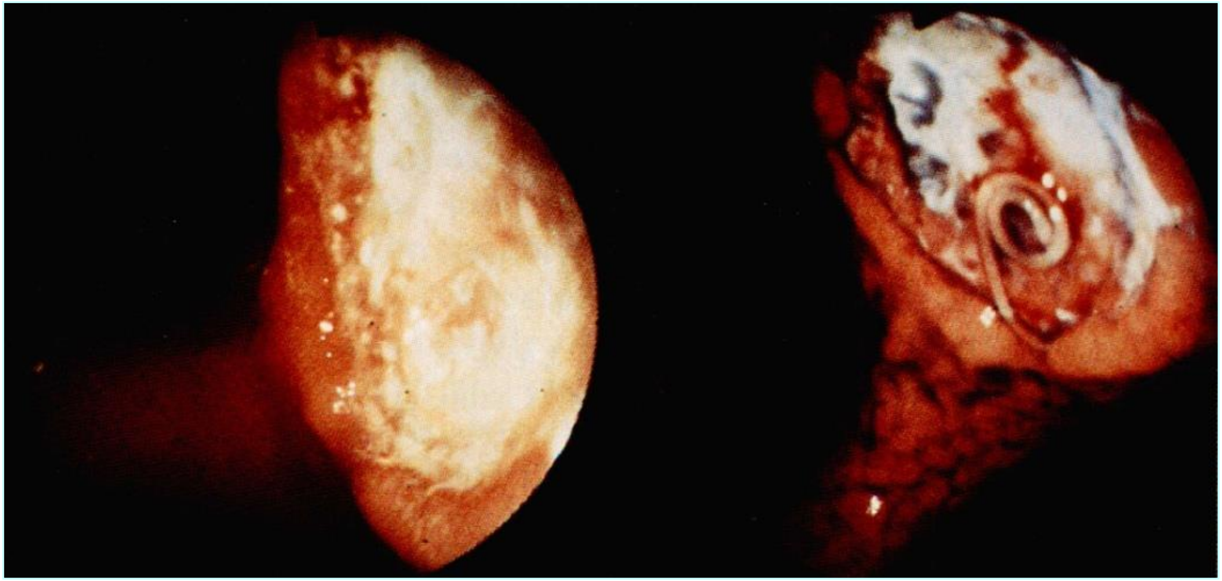

Viscera discards of infected fish
L3
L3
L3
L4
Adult
Eggs
Increased larval recruitment

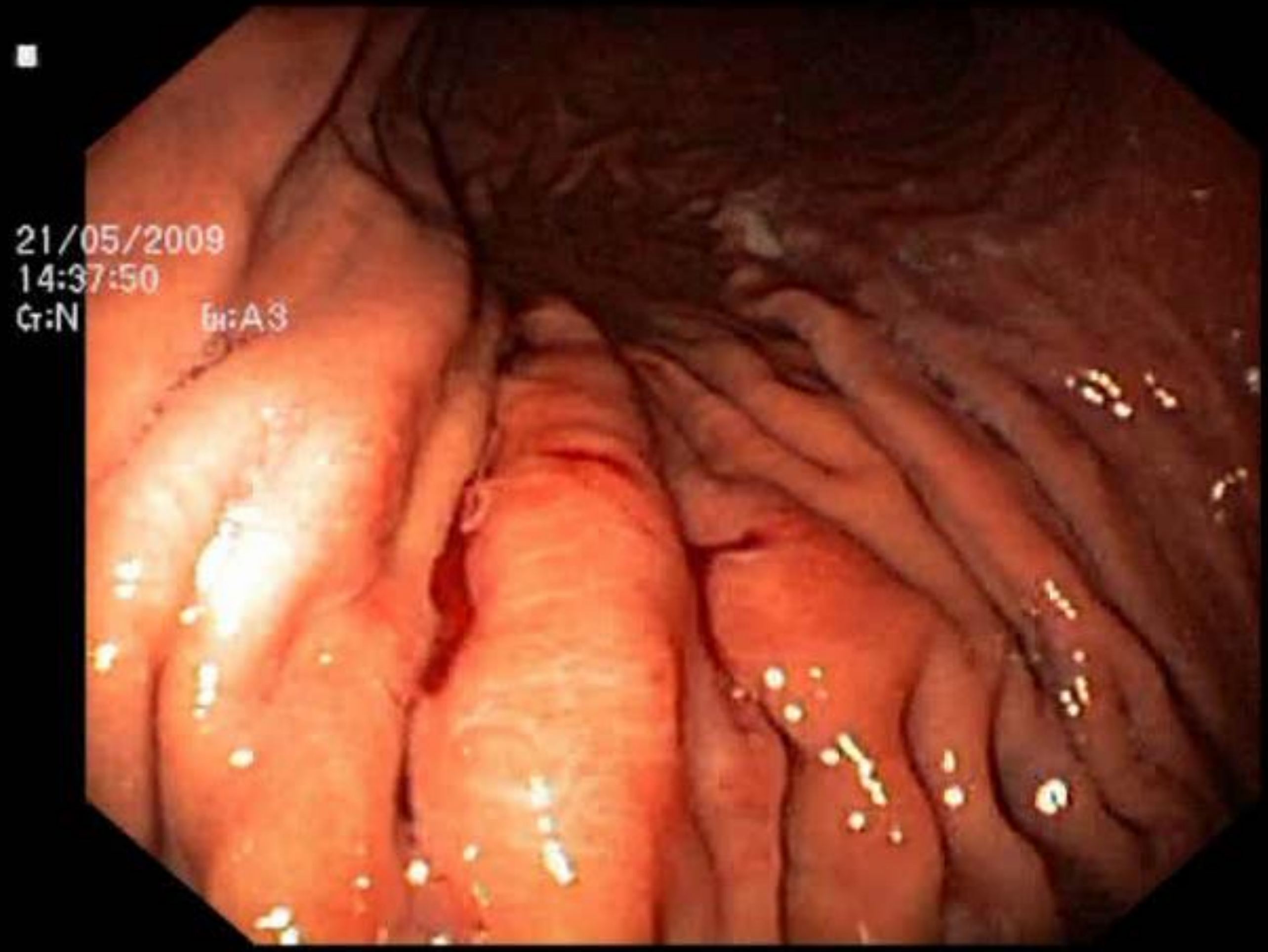
Therapy

Surgery

Antihelminthics (albendazole)

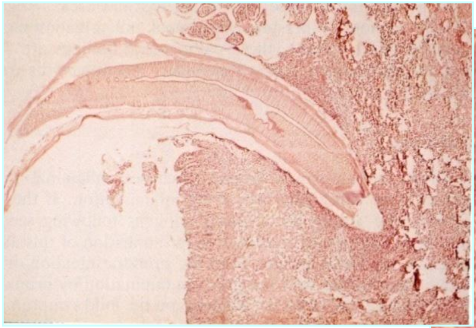


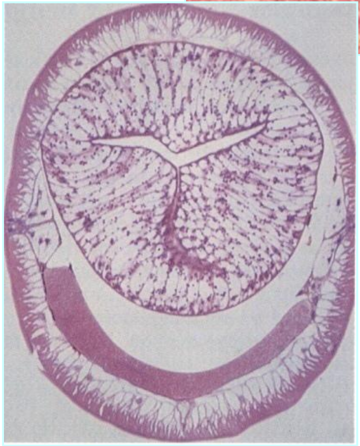



21/05/2009
14:37:50
Ct:N
Eh:A3

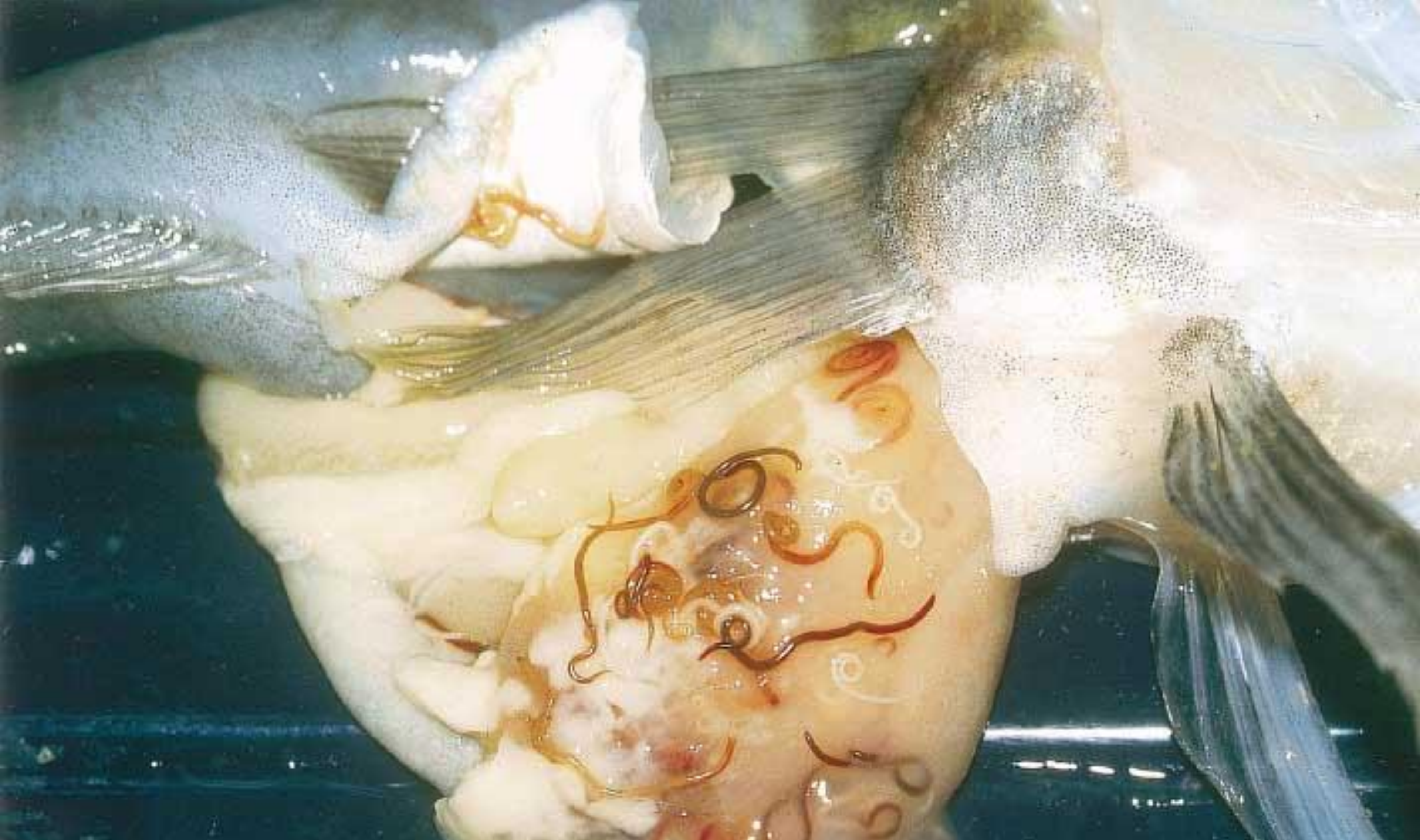
Diagnosis of human anisakiasis

- **X-rays**
- **Endoscopy**
- **Sierology**
- **Histology**





- For allergic syndromes by *Anisakis*:
  - IgE
  - IgE specific (anti-Ani s 1)
  - *Skin prick* test
- ANAMNESIS !



Human infections produced by larval stages of *Psudoterranova decipiens* are commonly named codworm anisakiasis or Pseudoterranovosis according to standardized nomenclature of animal parasitic diseases.

Third or fourth stage larvae of *P. decipiens* can produce human infections, the latter are only rarely found.

According to EFSA Scientific Opinion on risk assessment of parasites in fishery products (European Food Safety Authority, 2010, EFSA J., 8, 1543) all wild fish should be considered at risk of containing any viable zoonotic parasites if these products are to be eaten raw or almost raw, pointing out the need to carry out epidemiological surveys on presence/diffusion of zoonotic parasites in all fishery grounds.

Respect to infections due to Anisakid larvae in marine fish, until recently in Italy a lower attention has been devoted to the study of zoonotic helminths in freshwater fish populations, such as *in primis* the cestode *Diphyllobothrium latum* and the digenean *Opisthorchis felineus*.

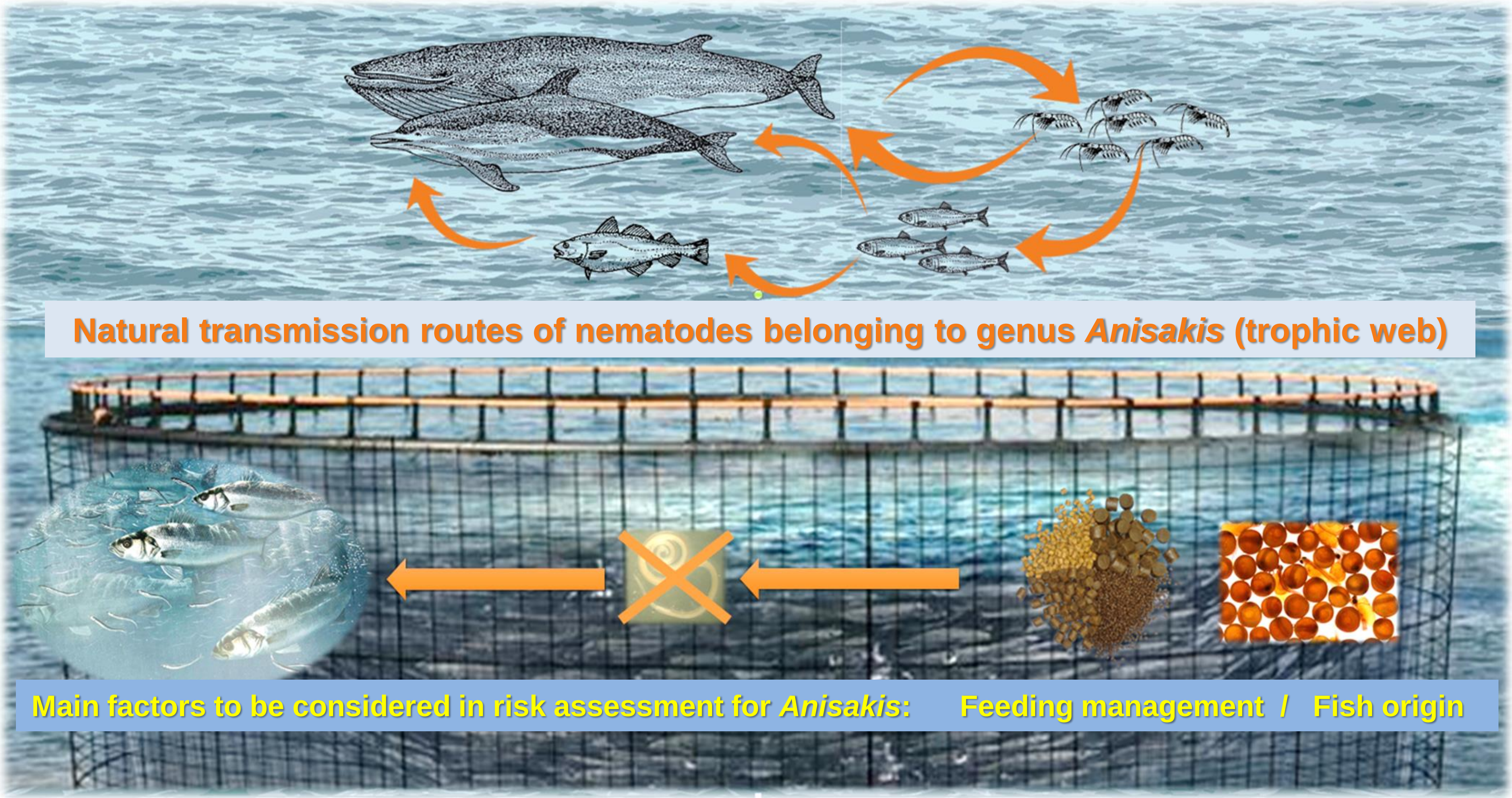
Is Anisakis in farmed fish a risk?

Norwegian Salmon Seminar 2018 - Milan

05.06.2018 15:00 - 05.06.2018 18:45
Milan, Italy

Andrea Gustinelli – Department of Veterinary Medical Sciences

Zoonotic parasites in aquaculture



Zoonotic parasites in aquaculture

Which chance of transmission of fish-borne parasites to human?

Zoonotic risk are often strictly connected with management problems/difficulties:

Feeding programs not well tailored to the farm/fish population

Creation of different size classes

Lack of continous surveillance plans specifically aimed at assessing the presence/absence of zoonotic helminths

PARASITIC ZOONOSES IN AQUACULTURE

EFSA Panel on Biological Hazards (BIOHAZ)

European Food Safety Authority

EFSA Journal 2010; 8(4):1543

Scientific Opinion on risk assessment of parasites in fishery products

All wild caught seawater and freshwater fish must be considered at risk of containing any viable parasites of human health concern if these products are to be eaten raw or almost raw. For wild catch fish, no sea fishing grounds can be considered free of *A. simplex* larvae. For farmed Atlantic salmon, if reared in floating cages or onshore tanks and fed on compound feedstuffs, which are unlikely to contain live parasites, the risk of infection with larval anisakids is negligible unless changes in farming practices occur. Apart from farmed Atlantic salmon, sufficient monitoring data are not available for any other farmed fish therefore it is not possible to identify which farmed fish species do not present a health hazard with respect to the presence of parasites.

EFSA recommends that co-ordinated studies to improve surveillance and diagnostic awareness of allergic reactions to parasites in fishery products should be implemented, and encourage epidemiological studies on a European scale to assessing the impact of *A. simplex* parasitized fish on human associated disease, including all allergic forms.

Controllo e trattamenti di bonifica dei prodotti ittici: quadro normativo

Regolamento CE n. 853 del 2004: congelamento preventivo a -20°C per almeno 24 ore a tutti *i prodotti della pesca che vanno consumati crudi o praticamente crudi* (es. sottoposti a trattamenti di affumicatura a freddo, di marinatura o di salatura).

Recentemente il Ministero della Salute ha emanato la Circolare n. 4379 del 17/02/2011 su: "Chiarimenti concernenti alcuni aspetti applicativi del Regolamento CE n. 853/2004 in materia di vendita e somministrazione di preparazioni gastronomiche contenenti prodotti della pesca destinati ad essere consumati crudi o praticamente crudi".
Nella circolare viene evidenziato l'obbligo previsto dal regolamento europeo di congelare (trattamento di bonifica preventiva) ad una temperatura non superiore a -20 °C per almeno 24 ore, il pesce (anche di acqua dolce) destinato ad essere somministrato crudo.

Regolamento UE n. 1276/2011 dell'8 dicembre 2011, che modifica l'allegato III del Regolamento (CE) n.853/2004: per i parassiti diversi dai trematodi, il congelamento deve consistere in un abbassamento della temperatura in ogni parte della massa del prodotto fino ad almeno -20°C per almeno 24 ore oppure -35°C per almeno 15 ore.

Gli OSA non sono tenuti a praticare i trattamenti di congelamento che uccidano i parassiti vivi potenzialmente rischiosi per il consumatore qualora i prodotti della pesca:

1. siano sottoposti o destinati ad essere sottoposti ad un trattamento termico che uccida il parassita vivo prima del consumo. Caso in cui trattasi di parassiti diversi dai trematodi, il prodotto è riscaldato ad una temperatura al centro del prodotto superiore o uguale a 60°C per almeno un minuto;
2. siano stati conservati congelati per un periodo di tempo sufficiente ad uccidere i parassiti vivi;
3. derivino da catture in zona di pesca non di allevamento, ma in tal caso devono esistere dati epidemiologici indicanti che le zone di pesca d'origine non presentino rischi sanitari con riguardo alla presenza di parassiti e le autorità competenti lo autorizzino;
4. derivino da piscicoltura, da colture di embrioni e nutriti esclusivamente secondo una dieta priva di parassiti vivi che rappresentano un rischio sanitario, purché uno dei seguenti requisiti sia soddisfatto:

a. sono stati allevati esclusivamente in un ambiente privo di parassiti vivi, oppure

b. l'operatore del settore alimentare verifica mediante procedure approvate dall'autorità competente che i prodotti della pesca non rappresentano un rischio sanitario con riguardo alla presenza di parassiti vivi.

EU Regulation 1276/2011

3. Food business operators need not carry out the freezing treatment set out in point 1 for fishery products:

(a) that have undergone, or are intended to undergo before consumption a heat treatment that kills the viable parasite. In the case of parasites other than trematodes the product is heated to a core temperature of 60 °C or more for at least one minute;

(b) that have been preserved as frozen fishery products for a sufficiently long period to kill the viable parasites;

(c) from wild catches, provided that:

(i) there are epidemiological data available indicating that the fishing grounds of origin do not present a health hazard with regard to the presence of parasites; and

(ii) the competent authority so authorises;

(d) derived from fish farming, cultured from embryos and have been fed exclusively on a diet that cannot contain viable parasites that present a health hazard, and one of the following requirements is complied with:

(i) have been exclusively reared in an environment that is free from viable parasites; or

(ii) the food business operator verifies through procedures, approved by the competent authority, that the fishery products do not represent a health hazard with regard to the presence of viable parasites.

Regolamento CE 2074/05 Allegato II, sezione I: "parassita visibile" = parassita o gruppo di parassiti che per dimensioni, colore o struttura è chiaramente distinguibile nei tessuti dei pesci - "controllo visivo" = esame non distruttivo di pesci o prodotti della pesca, effettuato ad occhio nudo, in buone condizioni di illuminazione e, in casi specifici, anche mediante speratura.

In fase di produzione il controllo visivo del pesce eviscerato deve essere effettuato da persone qualificate che dovranno osservare la cavità addominale, il fegato e le gonadi destinati al consumo umano. Nel caso di pesci piatti o di filetti di pesce il controllo visivo verrà effettuato mediante "speratura".

Nota prot. n. 4379 del 17/02/2011 del Ministero della Salute: come prevede il Regolamento (CE) n. 1020/2008, anche nella vendita al dettaglio devono essere rispettati i requisiti specifici relativi ai parassiti e quelli concernenti l'esame visivo per la loro ricerca in conformità a quanto previsto nel Regolamento (CE) 853/2004.

INFORMAZIONI AL CONSUMATORE

per un corretto impiego di

pesce e cefalopodi freschi

Decreto del Ministro della salute 17 luglio 2013 (GU n.187 del 10.8.2013)

IN CASO DI CONSUMO CRUDO, MARINATO

O NON COMPLETAMENTE COTTO

IL PRODOTTO DEVE ESSERE PREVENTIVAMENTE CONGELATO

PER ALMENO 96 ORE A – 18 °C

in congelatore domestico

contrassegnato con tre o più stelle

Ministero della Salute

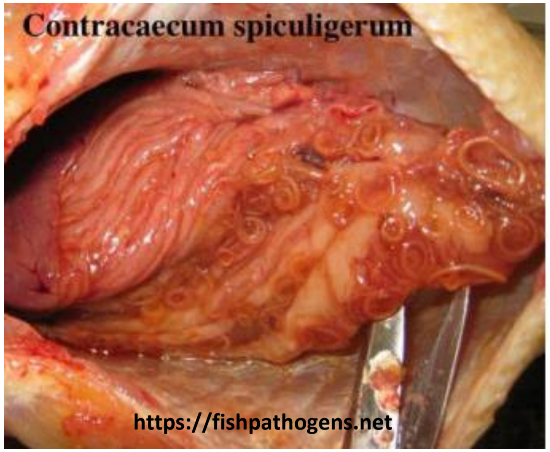
Direzione generale per l'igiene e la sicurezza degli alimenti e la nutrizione
www.salute.gov.it

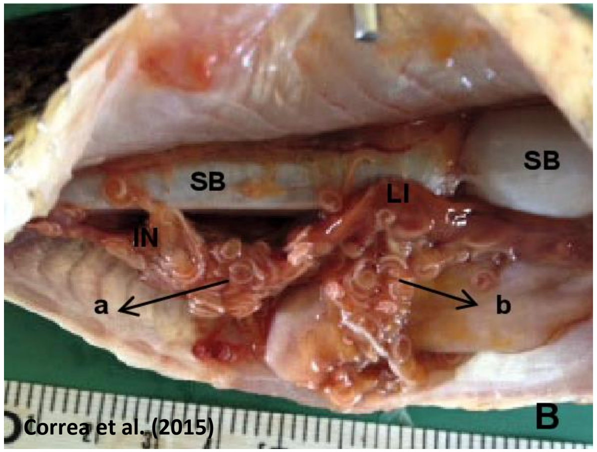
CONTRACAECUM SPP.

Taxonomy, description and diagnosis (http://www.fao.org/3/v9551e/v9551e16.htm)

Larval stage of nematodes frequently reported in African fish are representatives of:
Anisakidae: genera *Amplicaecum, Contracaecum* and *Porrocaecum;*
Dioctophymidae: the genus *Eustrongylides;*
Rhabdochonidae: the genera *Rhabdochona* and *Spinitectus*, also infecting fish at an adult stage.
Species of *Contracaecum* are among the most prevalent fish nematodes in Africa (Khalil and Polling 1997), with several studies having investigated infection levels in fish within southern Africa and other African countries.

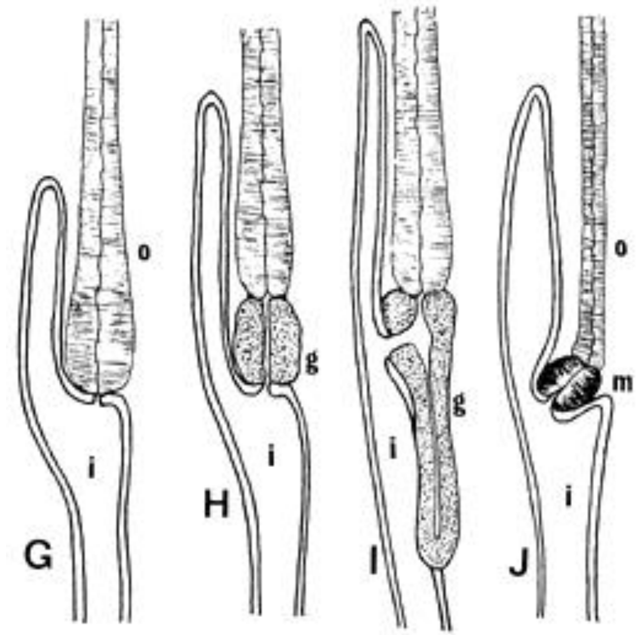
Larval nematodes occur either encysted in tissues or free in body cavities, most often in the abdominal or pericardial cavity. Larvae of *Contracaecum* tend to escape from their cysts and crawl out of their host body after its death.





Identification of larval nematodes, particularly to species level is not usually feasible, since the larvae lack genital systems and several other features of adult stages which are utilized as taxonomic criteria.

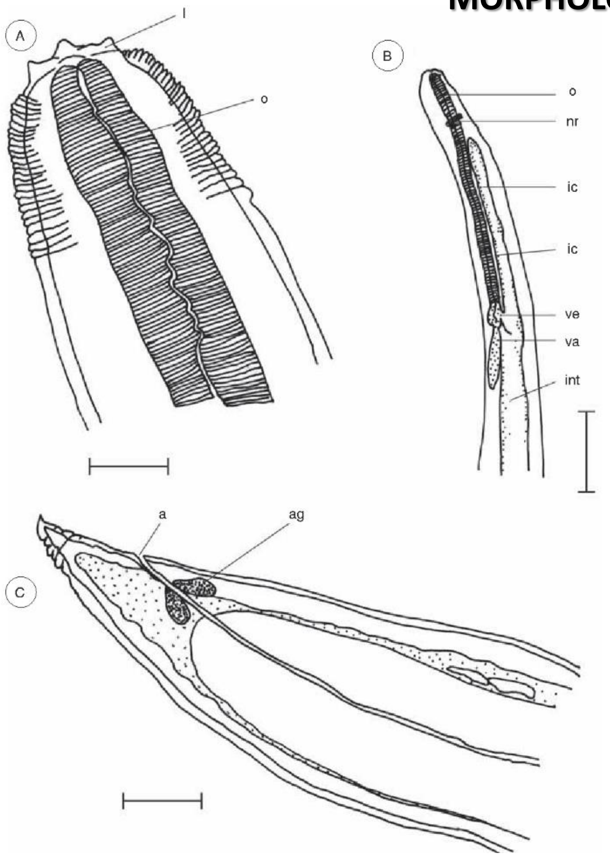
Anisakiid larvae are variable in size, often very large and thick, up to 60 mm long and 3 mm in diameter, with characteristic outgrowths (appendices) of either the anterior end of the intestine or the posterior end of the oesophagus (the ventriculum) or both: in *Contracaecum* appendices are formed (in opposite directions) from both the ventriculum and the intestine, while *Porrocaecum* has only one appendix of the intestine present and the ventriculum is separated from the oesophagus.



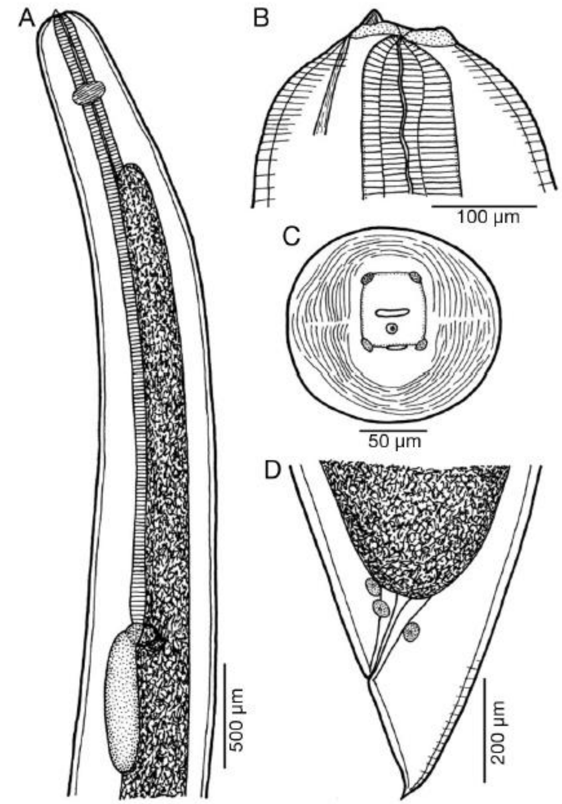
G–J. Position of ventricular (g) and intestinal (i) coecca in larval Heterocheilidae: G. *Amplicaecum*, H. *Porrocaecum*, I. ***Contracaecum***, J. *Dujardinascaris* with muscular ventriculus (m). o, oesophagus. (A – D, after Moravec, 1974a.)

Methodologies of identification of larval stages (of Anisakidae) by biochemical (isoenzyme) methodology utilising multilocus electrophoresis analysis has been developed (Orecchia *et al.*, 1986), so far substituted by molecular analyses.
DNA and allozyme data are particularly valuable in studies requiring species level identification of larval stages or in investigations of cryptic species where morphological criteria alone do not permit identification to species level. For example, some species of *Anisakis*, *Pseudoterranova* and *Contracaecum* appear to be composites of cryptic species that show differences in geographical range and final hosts (Mattiucci et al. 1998; Paggi et al. 2000; Mattiucci & Nascetti 2007, 2008).

MORPHOLOGY OF THIRD STAGE LARVAE



Contracaecum sp. larvae from *Claria gariepinus*. (A) head. Scale bar = 200 µm; (B) anterior end showing ventricular region. Scale bar = 500 µm; (C) posterior end. Scale bar = 200 µm. a = anus, ag = anal glands, ic = intestinal caecum, int = intestine, l = lip (labium), nr = nerve ring, o = oesopha-gus, va = ventricular appendix, ve = ventriculus (Barson & Avenant-oldewage, 2006)



Contracaecum sp. third-stage larva from *Sandelia capensis*. (A) Anterior end of body, lateral view; (B,C) cephalic end, lateral and apical views, respectively; (D) tail, lateral view (Moravec et al., 2016)

Life history and biology

Definitive hosts of *Contracaecum* are pelicans, cormorants and herons. Pelicans (*Pelecanus onocrotalus*), incriminated as the definitive hosts of *Contracaecum* (found in the pericardial cavity of farmed tilapia, *Oreochromis* hybrids), were found to be infected by two species, *C. multipapillatum* and *C. micropapillatum*, but only the former appears to be implicated in infections of tilapia (L. Paggi and colleagues, unpublished).

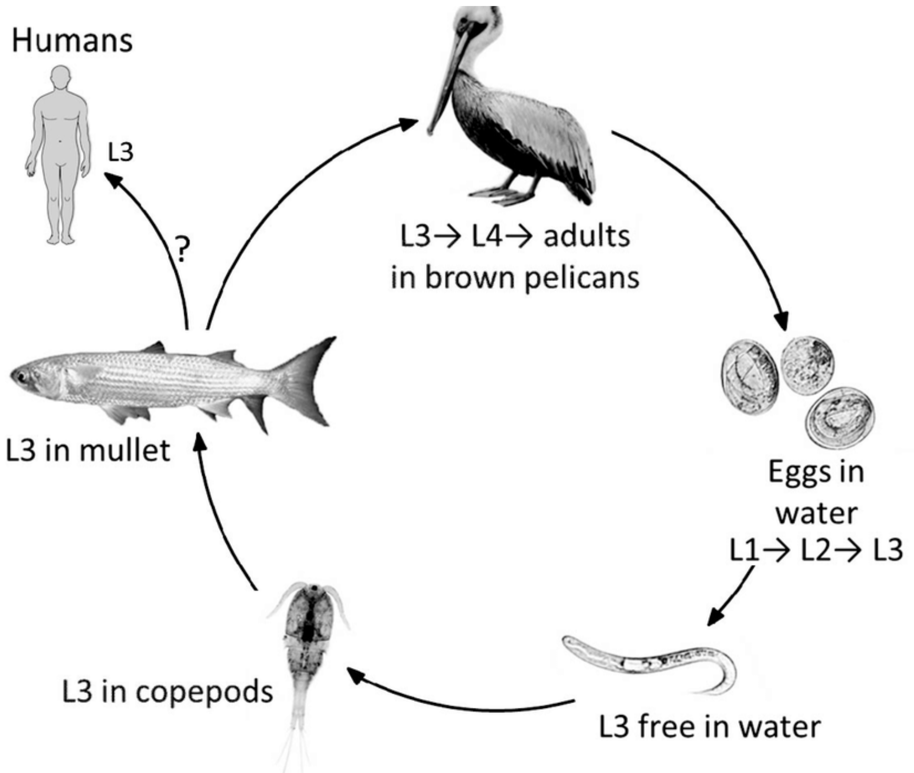
Eggs are released via defecation. They are also released into water when whole nematodes are vomited from the stomach by regurgitation. Eggs are released from such discharged nematodes by oviposition or after death, following their decomposition. Eggs hatch within 2–3 days at 24°C, 5–7 days at 21°C; hatching is not simultaneous and is further delayed in some of the eggs.

Free living infective (second) stage larvae can survive in water for several months. Larvae become firmly attached by their posterior end to a substrate in the aquatic habitat. Small crustaceans are the first intermediate hosts of anisakid nematodes. In Israeli fish ponds, copepods of the genus *Cyclops* were the first intermediate hosts to *C. multipapillatum* and *C. micropapillatum* obtained from pelicans and to *C. rudolphi* released from cormorants (*Phalacrocorax carbo*).

Consumed larvae entered the haemocoel of the copepods while transforming into a subsequent developmental stage. Infection was retained in copepods for over 40 days. In those fish which became infected after consuming the infected copepods, larvae (third stage) migrated into the viscera, entered the swimbladder and finally accumulated in the pericardium.

Within 2–4 months worms grew from 0.5 mm to 60 mm. They then persisted in the pericardium for up to 15 months (Landsberg, 1988), or throughout the second year after infection.

Tentative life cycle of *Contracaecum multipapillatum*



Although some authors (e.g. Tavakol et al. 2015) still consider copepods and fishes to be the first and second intermediate hosts, respectively, of *Contracaecum* spp., the available data show that the third-stage larvae of these nematodes develop already inside eggs in the external environment (water) and are already infective for the definitive host (Thomas1937, Køie & Fagerholm 1993, 1995, Dziekonska-Rynko & Rokicki 2007, Moravec 2009). However, avariety of invertebrate (copepods, larvae of aquaticinsects) and vertebrate (fish, less often amphibians and reptiles) paratenic hosts usually participate in the transmission of *Contracaecum* species to the definitive host (Moravec 2009).

Epizootiology

Epizootiology of the pericardium inhabiting *Contracaecum* is linked with migration of piscivorous birds, particularly (or even only) pelicans, between Europe and tropical East Africa. Infection of ponds in Israel occurs after they have been visited by pelicans during spring migration. Definitive hosts of the other forms of *Contracaecum* (piscivorous birds), *Amplicaecum* (aquatic reptiles) or *Eustrongylides* (cormorants), are apparently sedentary as infection is geographically localised.

Anhinga melanogaster

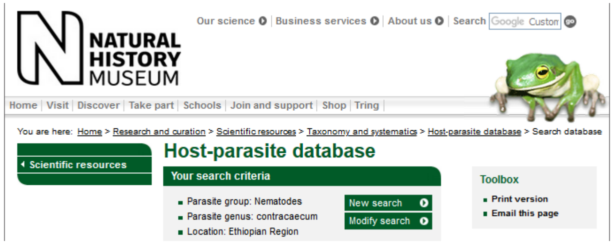
Phalacrocorax carbo, P. africanus

Pelecanus spp.

In South Africa, where encysted *Contracaecum* are common in a wide variety of fish of diverse families (Boomker, 1982; Mashego, 1982; Mashego & Saayman, 1980; Van As & Basson, 1984), *C. micropapillatum, C. microcephalum* and *C. spiculigerum* are found in cormorants and pelicans, and the latter also in herons (Prudoe & Hussey, 1977).
Cormorants (*P. africanus*) also harboured *C. carlislei* (Boomker, 1982).

CONTRACAECUM (NEMATODA: ANISAKIDAE)

Species from birds of Ethiopian region listed in Natural History Museum Database



Contracaecum species	Bird species
Contracaecum carlislei Ortlepp, 1938	*Anhinga melanogaster, Phalacrocorax africanus, Phalacrocorax carbo*
Contracaecum jokli Mokgalong, 1996	*Anhinga melanogaster, Phalacrocorax africanus, Phalacrocorax carbo*
Contracaecum lawrencei Bisseru, 1955	*Anhinga melanogaster, Phalacrocorax africanus, Phalacrocorax carbo*
Contracaecum microcephalum (Rudolphi, 1819)	*Anhinga melanogaster, Phalacrocorax africanus, Phalacrocorax carbo*
Contracaecum micropapillatum (Stossich, 1890)	*Pelecanus onocrotalus*
Contracaecum rodhaini (Gedoelst, 1916)	*Anhinga melanogaster, Phalacrocorax africanus, Phalacrocorax carbo*
Contracaecum rudolphii Hartwich, 1964	*Anhinga melanogaster, Phalacrocorax africanus, Phalacrocorax carbo*
Contracaecum tricuspis (Gedoelst, 1916)	*Anhinga melanogaster, Phalacrocorax africanus, Phalacrocorax carbo*
Contracaecum variegatum (Rudolphi, 1809)	*Diomedea cauta*
Contracaecum sp.	*Anhinga melanogaster*

CONTRACAECUM (NEMATODA: ANISAKIDAE) LARVAE

Species from fish of Ethiopian region listed in Natural History Museum Database

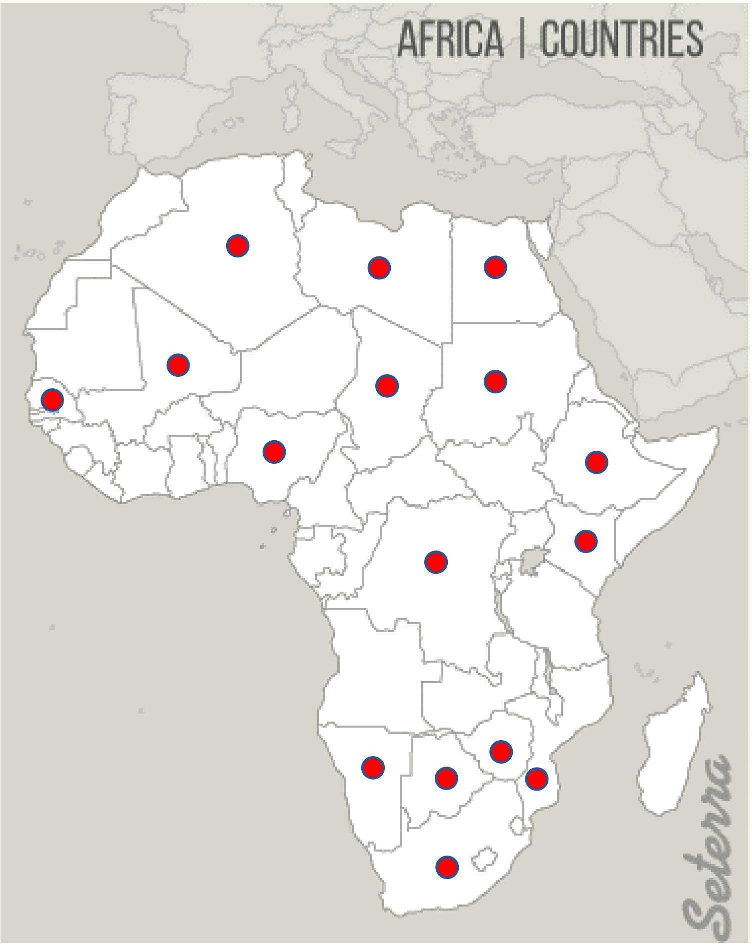
Eutropius depressirostris, Oreochromis leucostictus, Tilapia galilea, O. niloticus, Tilapia zillii, Clarias gariepinus, C. lazera, C. liocephalus, C. platycephalus, C. theodorae, Brycinus imberi, Barbus altianalis, B. intermedius, B. marequensis, B mattozoi, B. paludinosus, B. trimaculatus, B. unitaeniatus, Barbus sp., Hydrocynus vittatus, Micropterus salmoides, Schilbe intermedius, Bagrus bayad, B. docmac

Geographic range of *Contracaecum* (larvae) in Africa

Contracaecum occurs in Israel (Paperna, 1964), Egypt, Mali, most large and small East African (Rift Valley) lakes (including lakes Kivu, Edward and Albert—Campana-Rouget, 1961), Zaire, Mali (Niger) (Khalil, 1971) and South Africa, where it was also reported from brackish water hosts (Boomker, 1982; Van As & Basson, 1983).

Infections of the pericardia in cichlid fish occur in Israel (Landsberg, 1988) and in lakes Victoria, George, Nakuru, Naivasha, Baringo and Magadi (Paperna, 1974a; Malvestuto & Ogambo Ongoma, 1978). Reports in African countries on the South, indicating *Contracaecum* spp. as the most prevalent species.

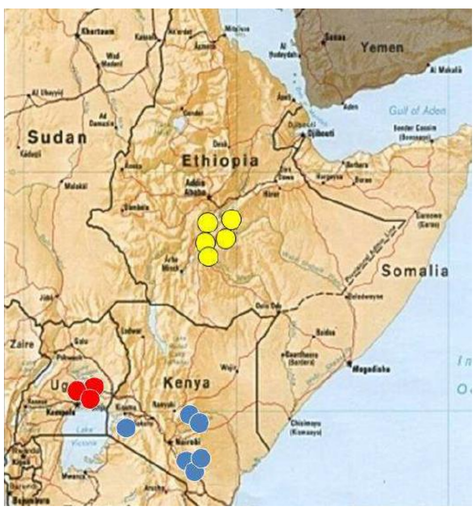
To be considered almost cosmopolitan in Africa



BOMOSA SURVEY – *CONTRACAECUM* LARVAE

The BOMOSA project is based on the application of cage-based fish farming systems, especially prototyped for East African conditions, as a small-scale productive model integrating rural resources, technological optimization, socio-economic aspects and capacity building in order to furnish a know-how indispensable for the sustainable and permanent developing of Eastern Africa aquaculture.

Diseases could represent an important constraint to aquaculture production, causing both production losses and public health problems. Therefore, the knowledge of the diseases of major concern in a fish farming system is necessary in order to assess the risk factors influencing their introduction/spreading and define the measures useful to their prophylaxis and control



SAMPLING SITES

Kenya

1. Sagana Fish Farm
2. Ruthagati Dam
3. Ngeki Dam
4. Ngei Dam
5. Lukenya Dam
6. Sangoro Farm

Uganda

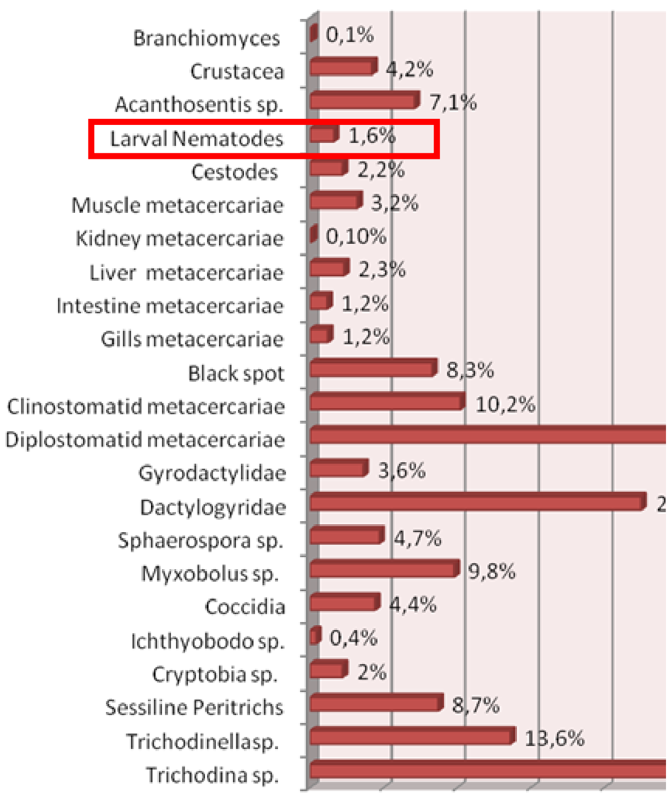
7. Kasolwe Dam
8. Ndolwe Dam
9. Busoga Dam

Ethiopia

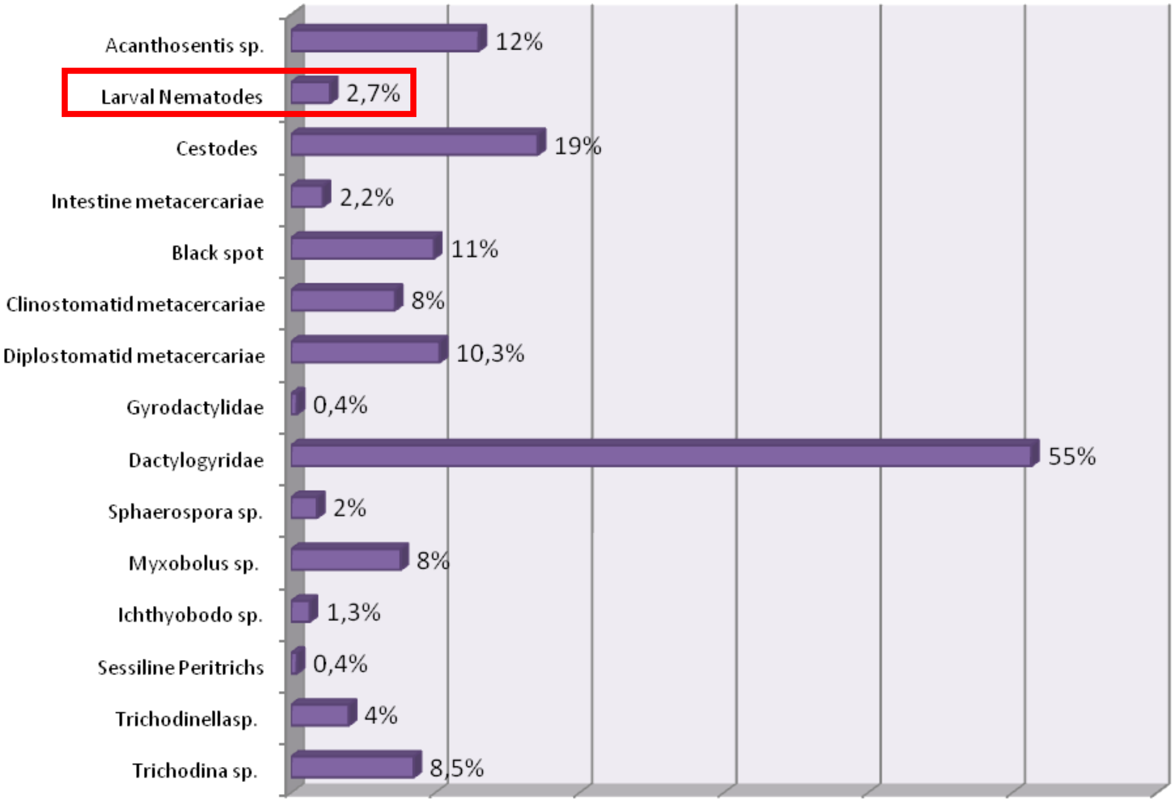
9\. Babogaya Lake
10\. Hora Lake
11\. Alagae Dam
12\. Wonji Dam
13\. Awassa and Chamo Lake

From March 2007 to October 2008 a total of 982 tilapias (*Oreochromis niloticus niloticus*) were examined, 685 from Kenya, 222 from Uganda and 75 from Ethiopia

BOMOSA SURVEY – KENYA & UGANDA



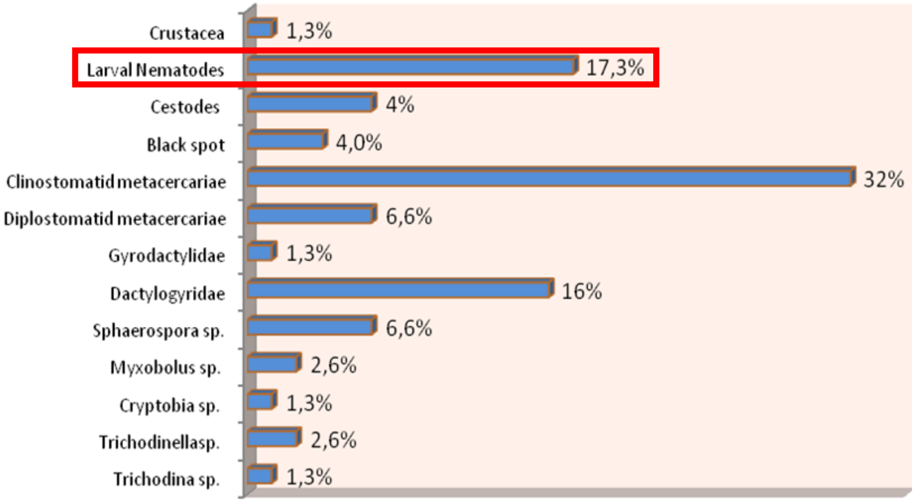
Kenya: prevalence of parasites in tilapia



Uganda: prevalence of parasites in tilapia

BOMOSA SURVEY - ETHIOPIA

Contracaecum infections were observed mainly in wild tilapia from Ethiopia, with an overall prevalence of 17.3% and a mean intensity of 4.1 parasites/host. In the other countries both prevalence and intensity showed low values. The larvae were always found in pericardial cavity, as already reported by other authors. The body condition of the infected fish seemed to be good, and the migration of larvae in muscle was never observed. The low prevalence observed in caged fish may be related to their different feeding behavior as pelleted feed is given by the farmer and fish don't feed actively on zooplankton, where infective stages could be present.

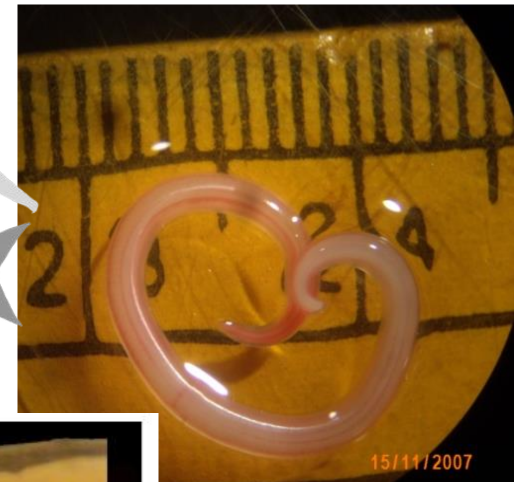


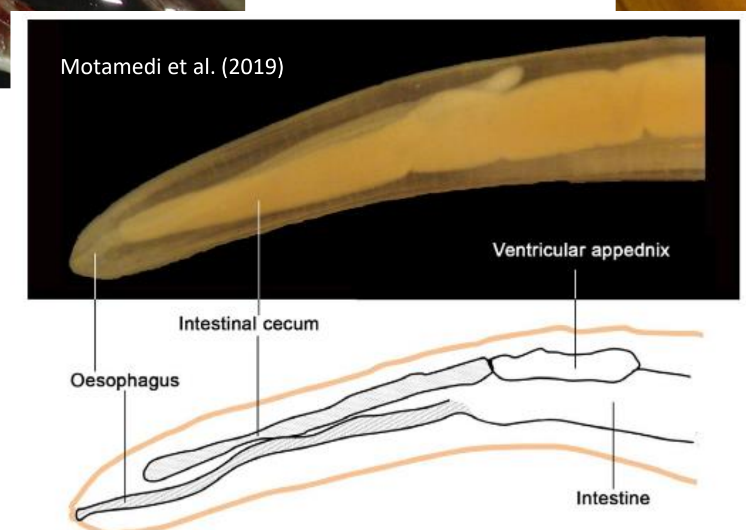
Ethiopia: prevalence of parasites in tilapia

Parasite	Prevalence		Mean Intensity		Abundance	
	Caged	Wild	Caged	Wild	Caged	Wild
Trichodina sp.	–	1.5%	–	very low	–	–
Trichodinella sp.	–	3.1%	–	low	–	–
Cryptobia sp.	–	1.5%	–	low	–	–
Myxobolus sp.	–	3.1%	–	low	–	–
Sphaerospora sp.	–	7.7%	–	low	–	–
Dactylogyridea	–	18.5%	–	4.4	–	0.8
Gyrodactylidae	–	1.5%	–	5	–	1.4
Diplostomatid metacercariae	–	7.7%	–	5.2	–	0.4
Clinostomid metacercariae	30.0%	32.3%	1	3.9	0.3	0.9
Black spot	–	4.6%	–	3	–	0.1
Cestodes	10.0%	6.1%	1	3	0.1	0.2
Larval Nematodes	30.0%	15.4%	6	3.6	1.8	0.5
Crustacea	–	1.5%	–	1	–	0.01

Table 5 - Ethiopia: prevalence, mean intensity and abundance of the parasites found in caged and wild tilapia

All the *Contracaecum* larvae found in the pericardial cavity of tilapia from Ethiopia were identified as *C. multipapillatum* by morphology





Parasites of fish at Lake Tana, Ethiopia

Eshetu Yimer, Mulualem Enyew

Parasites of fish from a total of 1766 fish of different species sampled during 1998–1999. ***Contracaecum*** spp. were the most common larval nematode parasite that were identified from the mesentery of 52 (41.94%) Catfish (*Clarias gariepinus*), 17(24.64%) *Barbus acutirostris*, 102 (9.44%) *Barbus tsanensis*, 14(12.84%) *Barbus brevicephalus*, and from the pericardial cavity of 52 (59.8%) Nile tilapia (*Oreochromis niloticus*).

The other larval nematodes identified from the mesentery included *Amplicaecum* spp. and *Eustrongylides* spp. *Cuccullanus* spp. (new geographic record) were recovered from the intestinal lumen of 2(0.19%) *B. tsanensis*.

Among the digeneans, *Clinostomum* spp. were identified from the branchial cavity and skin of 50 (57.47%) and 4 (1.15%) *O. niloticus*, respectively. The *Euclinostomum* spp. were recovered from the kidney of 4 (4.6%) and branchial cavity of 2 (2.3%) *O. niloticus*.
Mixed infection by *Clinostomum* spp. in their branchial cavity and *Contracaecum* spp. in the pericardial cavity was observed in 35 (40.23%) *O. niloticus.*

Ligula intestinalis was the most prevalent cestode affecting 88 (8.14%) of *B. tsanensis*, 20 (40.0%) other small barbs and 32 (54.24%) of *B. trispilopleura* and it was considered as a significant pathogen.
Proteocephalus spp. (new geographic record) was recovered from the intestinal lumen of 9 (0.83%) *B. tsanensis*.

SINET: Ethiop. J. Sci Vol.26(1) 2003: 31-36

A study of Clinostomum (Trematode) and Contracaecum (Nematode) parasites affecting Oreochromis Niloticus in Small Abaya Lake, Silite Zone, Ethiopia

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J Aquac Res Development ISSN: 2155-9546 JARD,

Study conducted at Lake Small Abaya Ethiopia
A total of 384 *O. niloticus* sampled during November 2013- April 2014.
Of the 384 samples collected, 138 (35.9%) were infested with nematode of ***Contracaecum*** species and 72 (18.8%) were infected with trematode of *Clinostomum* species.
The intensity of infestation by ***Contracaecum*** and *Clinostomum* was 1-19 worms per fish (mean intensity=4.47) and 1-12 worms per fish (mean intensity=3.56) respectively.
There was no significant difference (p>0.05) in the prevalence of infestation among host sex, host size and host weight. There was no any statistically significant (p>0.05) correlation between the number of *Clinostomum*, ***Contracaecum*** and the mixed number of parasites and the fish's condition.

Mitiku et al., Ethiop. Vet. J., 2018, 22 (2), 65-80 Ethiopian Veterinary Journal
DOI https://dx.doi.org/10.4314/evj.v22i2.6

Parasites of Nile tilapia (*Oreochromis niloticus*) from selected fish farms and Lake Koftu in central Ethiopia

Study was conducted from October 2016 to January 2017 at Lake Koftu, Sebeta ponds and selected private fish farms in Wonchi area, Ethiopia.

A total of 302 *O. niloticus* (101 from Lake Koftu, 127 from Sebeta pond and 72 from selected small-scale fish farms) were collected and examined for the presence of parasites

Table 2. Total prevalence (P), mean intensities (MI), mean abundance (MA) of *O. Niloticus* parasites at Lake Koftu, Sebeta ponds and private farms (n=302)

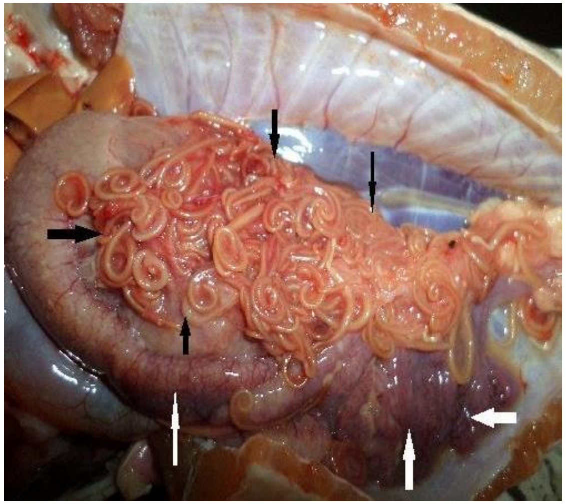
Parasite Taxa	Location Host	Developmental stage	P (%)	MI	MA
Ectoparasites					
*Trichodina*spp.	Gills	Adult	52.9	-	-
*Cichlidogyrus*spp.	Gills	Adult	52.6	10.4	5.5
Dolops	Skin	Adult	3.3	0.2	0.01
Endo parasites					
*Clinostomum*spp.	Brachial cavity	Larvae	20.1	3.51	0.7
*Euclinostomum*spp.	Gills/Kidney	Larvae	16.8	5.8	0.9
*Tylodelphys*spp.	Gill cavity	Larvae	36.5	7.3	2.7
Blackspotmetacercariae	Skin/gills	Larvae	19.1	-	-
Cestoda larvae	Gut wall	Larvae	9.9	-	-
*Contracaecum*spp.	Pericardial cavity	Larvae	25.3	5.5	1.4
Unidentified Nematode	Body cavity	Larvae	0.99	5.0	0.1
Acanthogyrustilapiae	Intestine	Adult	5.3	7.0	0.4

- Data not available

Contracaecum larvae were previously recorded from many Ethiopian water bodies such as Koka reservoir, Yemlo ponds, Lake Babogaya, Lake Tana and Lake Small Abaya (Yimer and Eniyewu, 2003; Tadesse, 2009; Florio *et al*., 2009; Gulilat *et al*., 2013; Reshid *et al*, 2014).

Pathology

Neither encysted nor free *Contracaecum* larvae will severely affect fish.
Tissue reaction, inflammation, epitheloid formation and fibrous encapsulation around encysted larvae is localized.
Multiple infection of the mesenteries resulting in extensive inflammation, fibrosis and even some visceral adhesions, were seen only in large fish, with no apparent impact on their body condition (Mbahinzireki, 1980).



Worms inhabiting the pericardial cavity do not induce any visible damage. Large (200–350 g) tilapia can accommodate up to 12 worms, which may reach a length of 6 cm and 2–3 mm in diameter. However, these infections, which affect the large fisheries of L. Naivasha in Kenya and intensive tilapia pond cultures in Israel, cause significant loss of income to these enterprises.
As worms tend to migrate to the surface once fish die, such “wormy” fish deter customers. Fish have to be de-gutted and fileted in order to be sold for consumption, the cost of which has to be paid by the producers.

Prevention and Control

The prevention and control of the infection by *Contracaecum* spp. should be based on avoiding the presence of the host involved in the life cycle of the parasite. Prevention by keeping away piscivorous birds is impractical not only in natural habitats or reservoirs but even in fish ponds. Similarly the elimination of copepod crustaceans, first intermediate hosts, from the farming environment is not feasible.

ZOONOTIC POTENTIAL OF *CONTRACAECUM* LARVAE TO BE DISCUSSED


THANKS