NATIONAL REFERENCE CENTRE FOR ANISAKIASIS (C.Re.N.A.)- ITALY: 2020-2021 activity

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The National Reference Centre for Anisakiasis (C.Re.N.A.) has been established at the Istituto Zooprofilattico Sperimentale of Sicily by a Health Ministry decree on August 27 2004 (G.U. N° 43 on February 22 2005) and designated NRL for Anisakiasis since November 14 2006.

Main objectives

- Development and standardization of diagnostic methods
- Confirmation of diagnosis from other laboratories
- Organization, in collaboration with the ISS, of periodic “ring tests” among Italian IZSs
- Provision of reference material
- Research activities funded by Italian Ministry of Health
- Training courses for staff of other IZSs

And also…

- Network with human health centers that can detect anisakiasis to collect epidemiological data (human cases and allergic manifestation)
- Study of allergenic potential of *Anisakis*
- Risk analysis

2020 activity

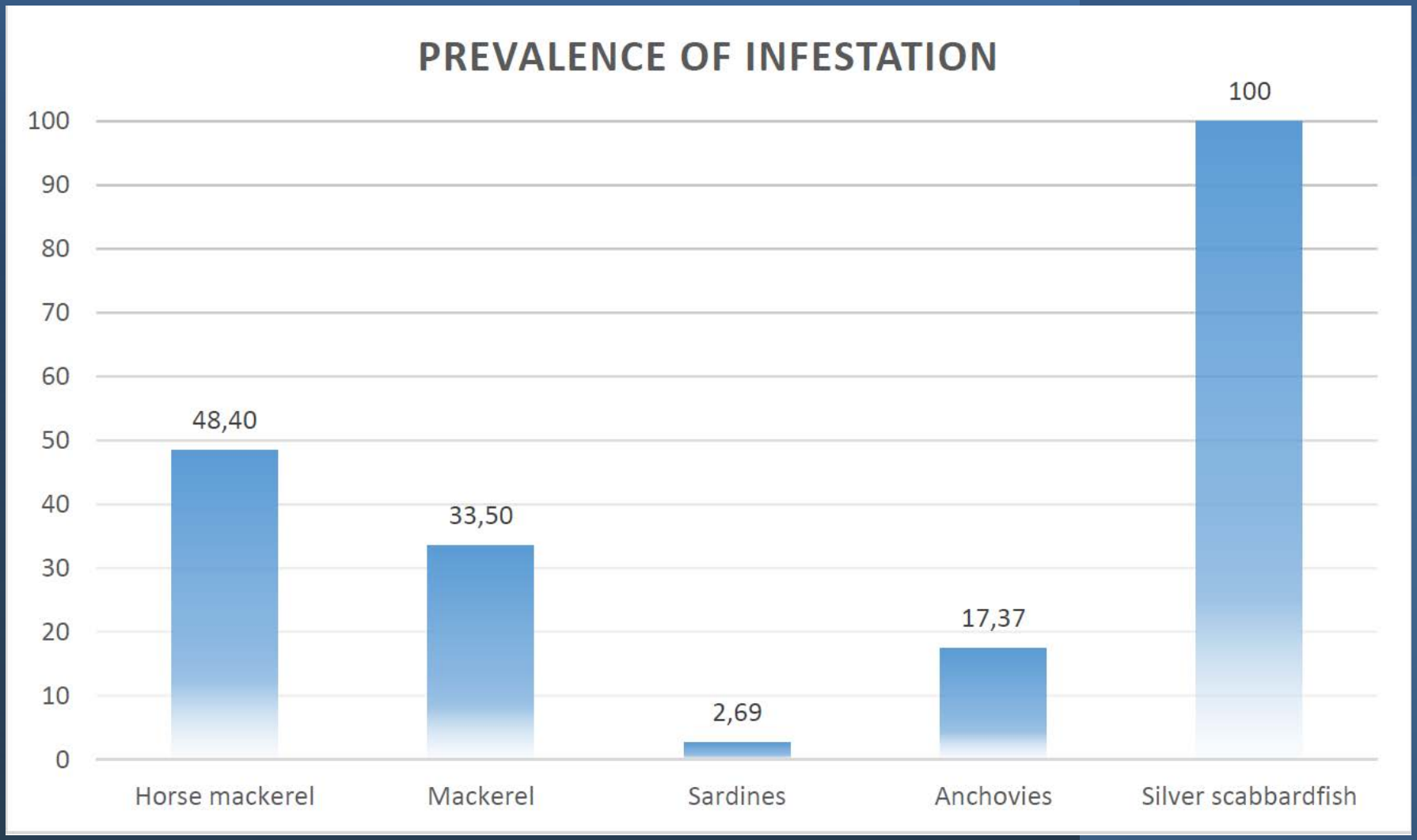
Inspection method

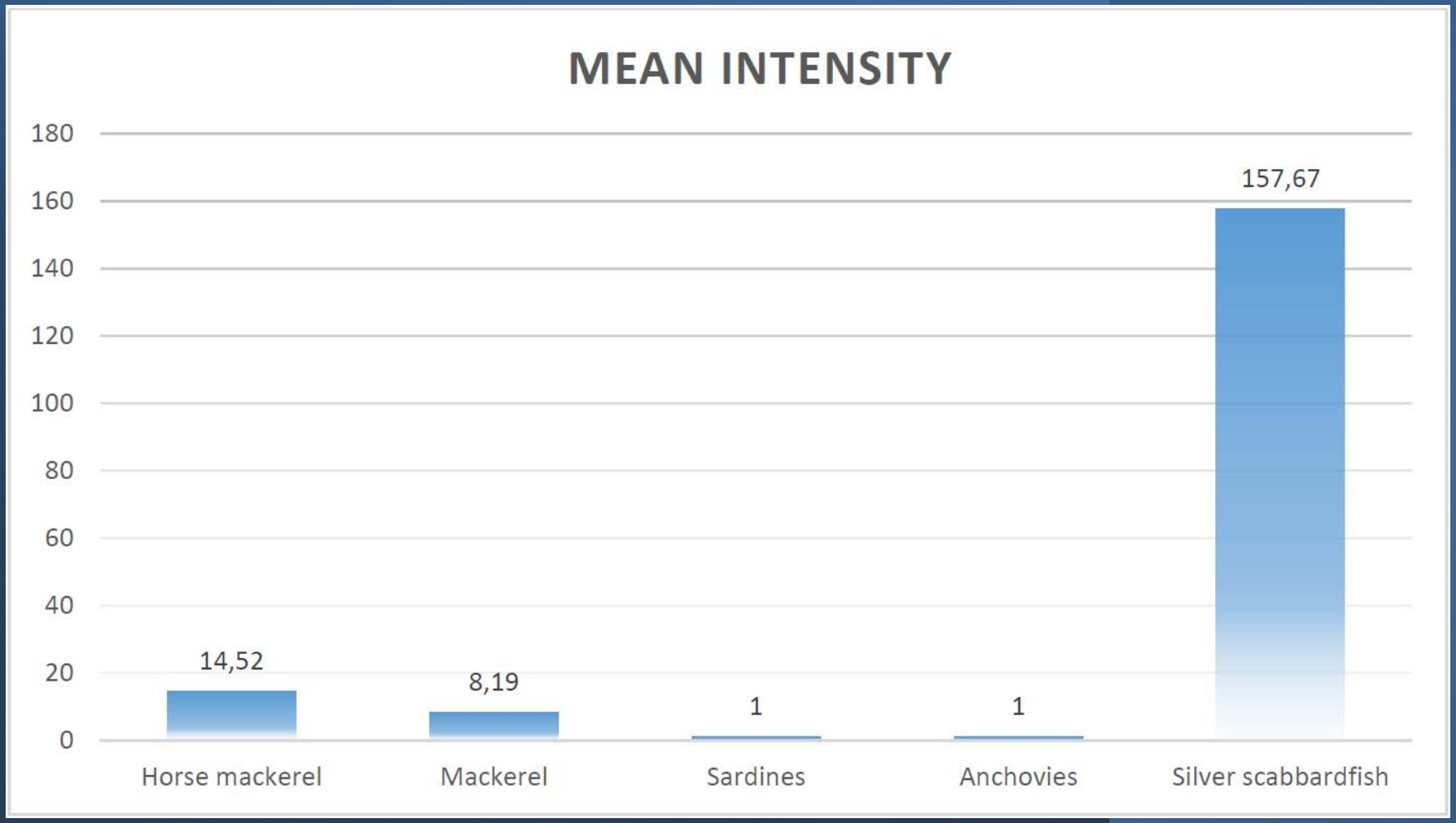
- Visual inspection
- Artificial digestion

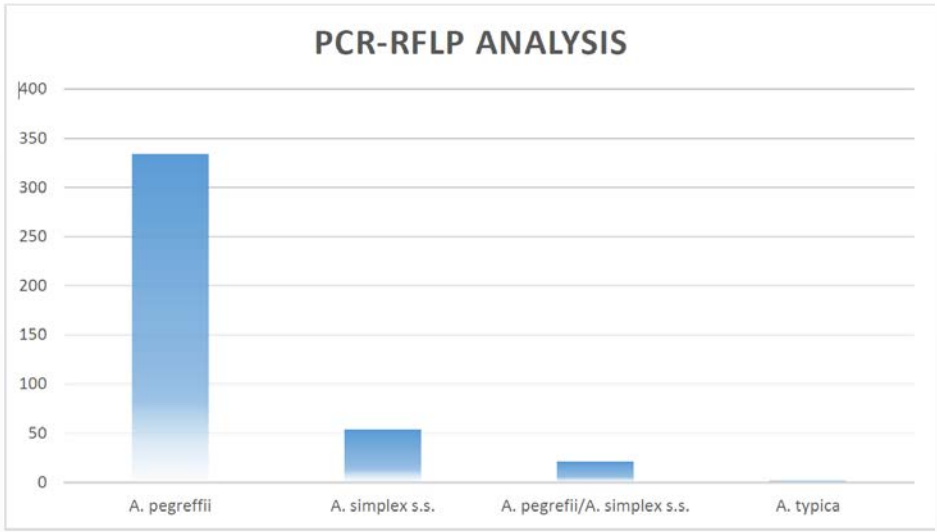
Anisakidae identification

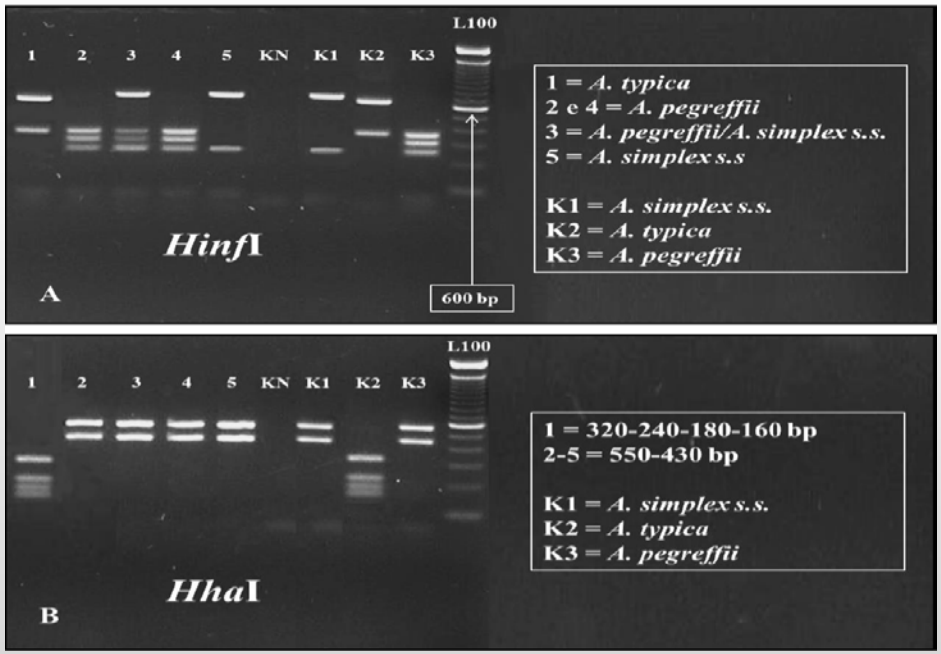
- Optical microscopy
- PCR-RFLP
- Multiplex PCR
- Real-time PCR assay

Specie	N. samples	N. samples infested	N. larvae	Visual inspection	Chloropeptic digestion
Engraulis encrasicolus	3373	586	586	586	-
Lepidopus caudatus	60	60	4888	60	-
Sardina pilchardus	3566	96	96	92	4
Scomber scombrus	269	87	671	72	15
Trachurus trachurus	275	133	1931	124	9


PREVALENCE OF INFESTATION
100
90
80
70
60
50
40
30
20
10
0
48,40
33,50
2,69
17,37
100
Horse mackerel
Mackerel
Sardines
Anchovies
Silver scabbardfish

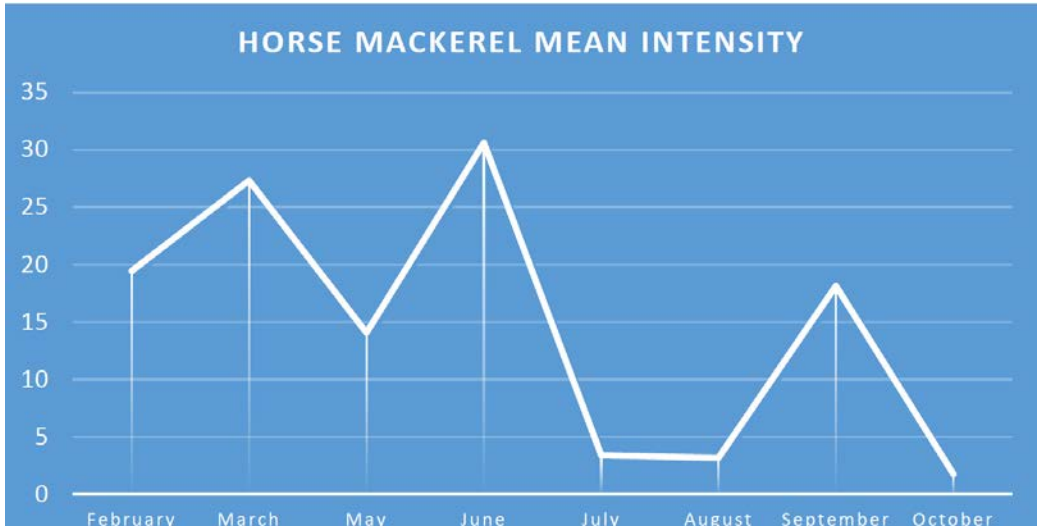

MEAN INTENSITY
180
160
140
120
100
80
60
40
20
0
14,52
8,19
1
1
157,67
Horse mackerel
Mackerel
Sardines
Anchovies
Silver scabbardfish

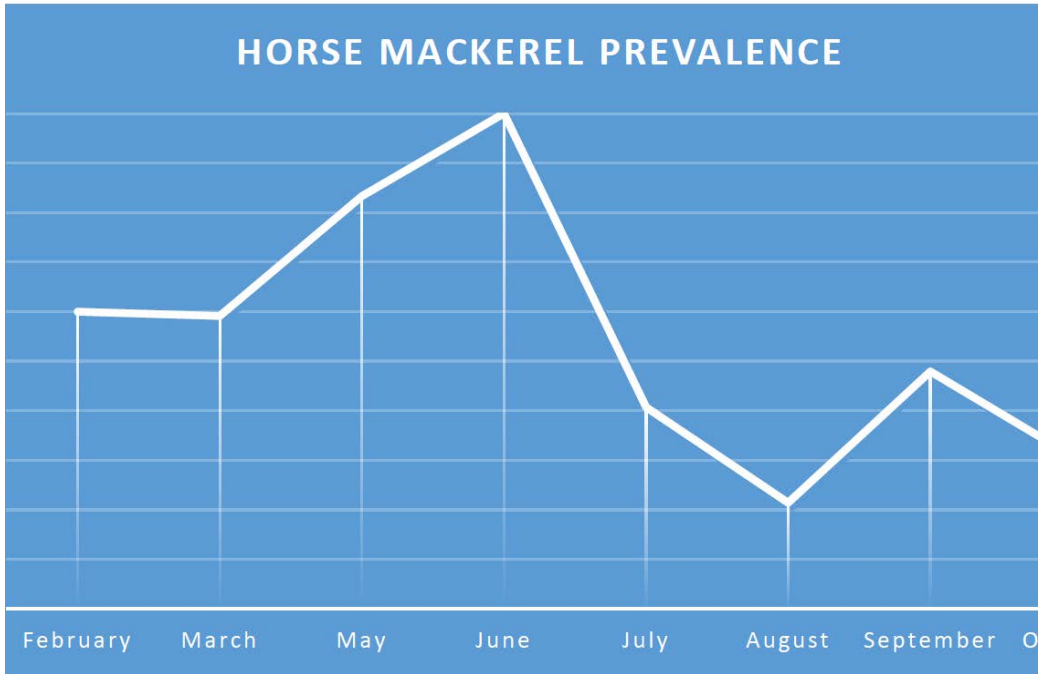




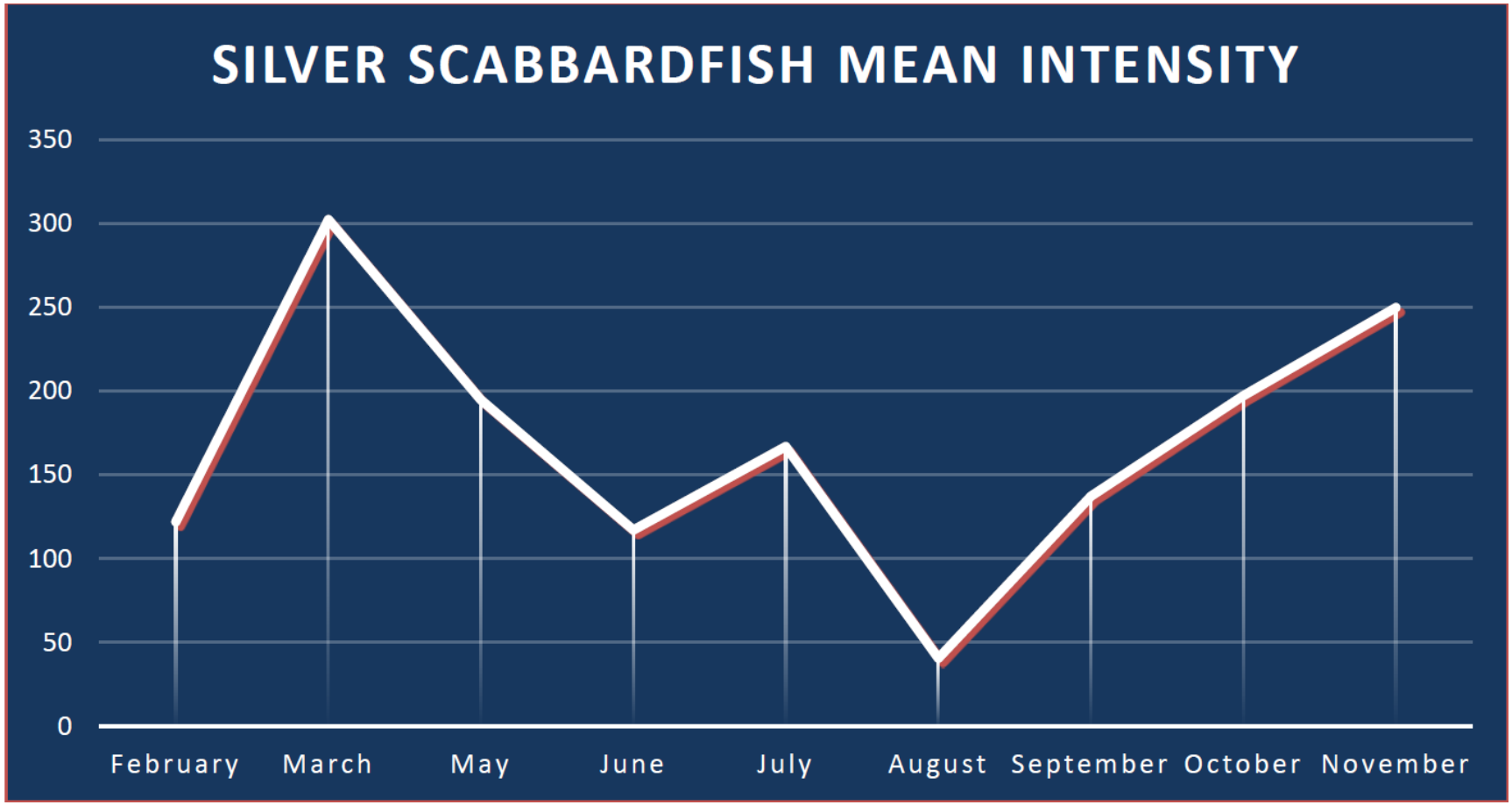
Molecular analysis

Seasonal trend





Seasonal trend



Natural Product Research ›
Formerly Natural Product Letters
Volume 34, 2020 - Issue 1: Ethnomedicine and Foods sources: novel perspectives. Guest Editor: Nicola Cicero

Short Communication

Seasonal trend of Anisakidae infestation in South Mediterranean bluefish

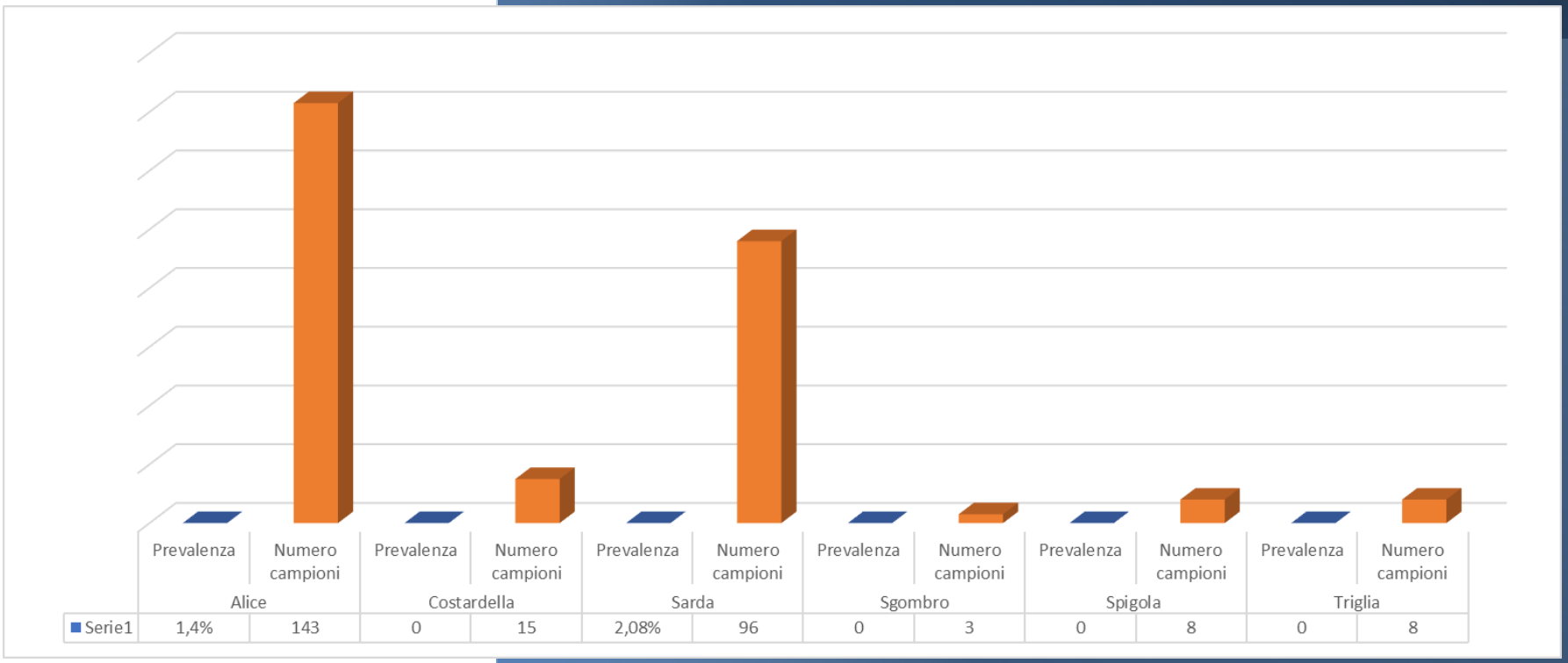
Gaetano Cammilleri, Andrea Pulvirenti, Antonella Costa, Stefania Graci, Rosaria Collura, Maria Drussilla Buscemi, ...show all

Pages 158-161 | Received 29 Oct 2018,Accepted 15 Jan 2019,Published online: 20 Feb 2019

2021 Activity

Inspection method
- Visual inspection
- Artificial digestion

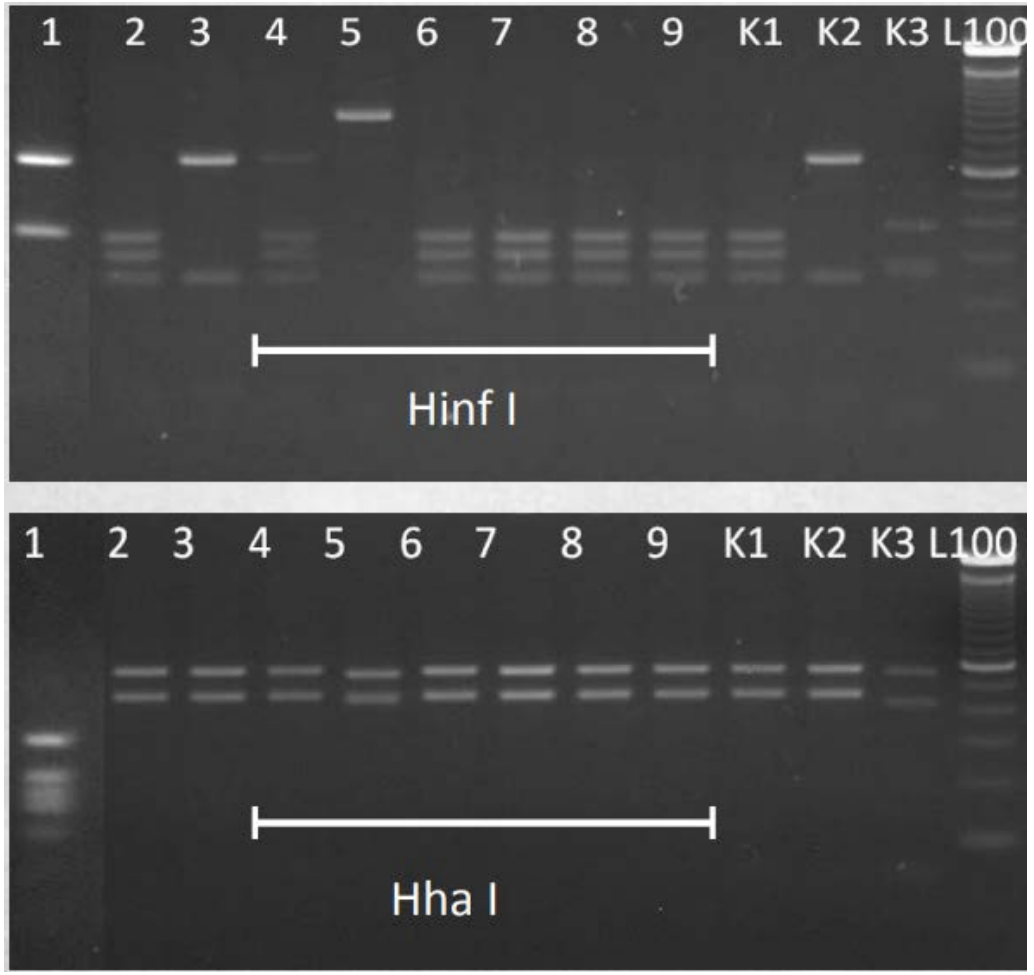
Anisakidae identification
- Optical microscopy
- PCR-RFLP
- Multiplex PCR
- Real-time PCR assay



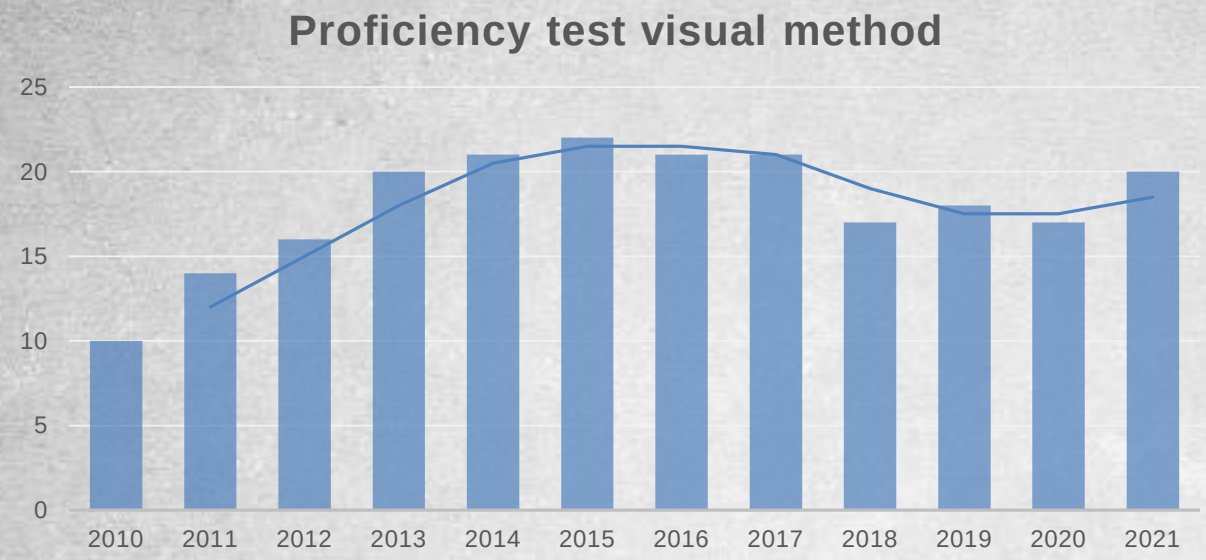
	Alice		Costardella		Sarda		Sgombro		Spigola		Triglia	
	Prevalenza	Numero campioni	Prevalenza	Numero campioni	Prevalenza	Numero campioni	Prevalenza	Numero campioni	Prevalenza	Numero campioni	Prevalenza	Numero campioni
Serie1	1,4%	143	0	15	2,08%	96	0	3	0	8	0	8

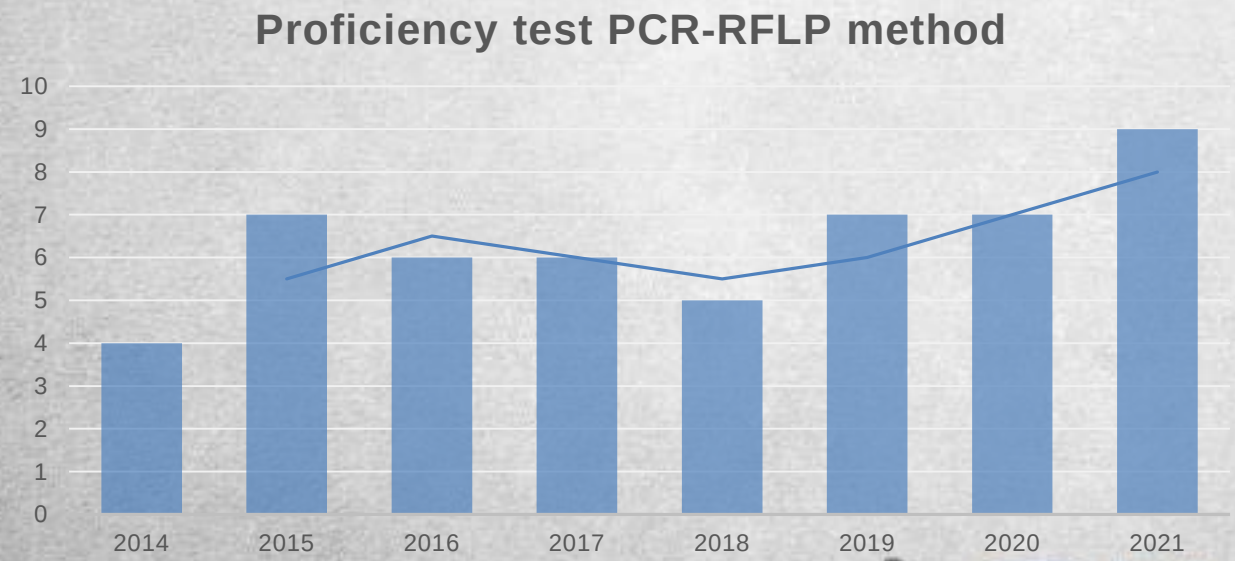
Molecular analysis

- 83,3% *A. simplex s.s.*
- 16,7% *A. pegreffii*



Organization of Proficiency tests





C.Re.N.A. organized three proficiency tests in November 2020 and 2021:

- Qualitative detection (absence-presence) of *Anisakis* larvae in fish fillets (visual method)
- Detection for *Anisakis* larvae by artificial digestion method - ISO 23036-2/2021: dispatch n. 3 fish fillet samples
- Identification of Anisakis larvae by PCR-RFLP: dispatch n. 3 DNA samples

The graphs show the number of participants per year

Validation of ISO 23036-2:2021

- Detection of L3 Anisakidae larvae in fish products.
- Fresh or processed
- Chloro-peptic digestion of muscle and viscera
- Determination of the vitality (37 °C)

POS CRENA 04 Comparison

POS CRENA 04

2 L water46-48 °C

- ❑Start weight 100 g
- ❑16 mL HCl 25%
- ❑ vortexing
- ❑10g of pepsin (1:10.000 NF)
- ❑44 - 46°C
- ❑Filtering after 30 – 45 min

ISO 23036-2:2021

2 L of water 37± 2°C

- ❑Start weight 25-200g
- ❑16 mL HCl 25%
- ❑vortexing
- ❑10g of pepsin (1:10.000 NF)
- ❑ 37°C
- ❑Filtering after 45 min

Optimization of the protocol

- **temperature: 37°C** (alive larvae) for 15-30’ (no more than 45’)
- weight 25-200 g
- Digestion fluid: HCl 16 ml in 2 l water, pepsin (30 ml), 10 g for powder.

- **Over 30 trials at different conditions**
  - **Farmed Sea bream muscle**
  - **Superior part / ventral part**
  - **epiaxial muscles**
  - **Tail muscles**

Optimal conditions

- **2 L of water**
- **11 ml of HCl≥35%**
- **10 g pepsin 1:10.000**
- **50±5 g of sample**
- **Digestion time 40 min**
- **Temperature 36±1°C**

Validation of the method

Larvae vitality: acid solution up to 30 min from the digestion

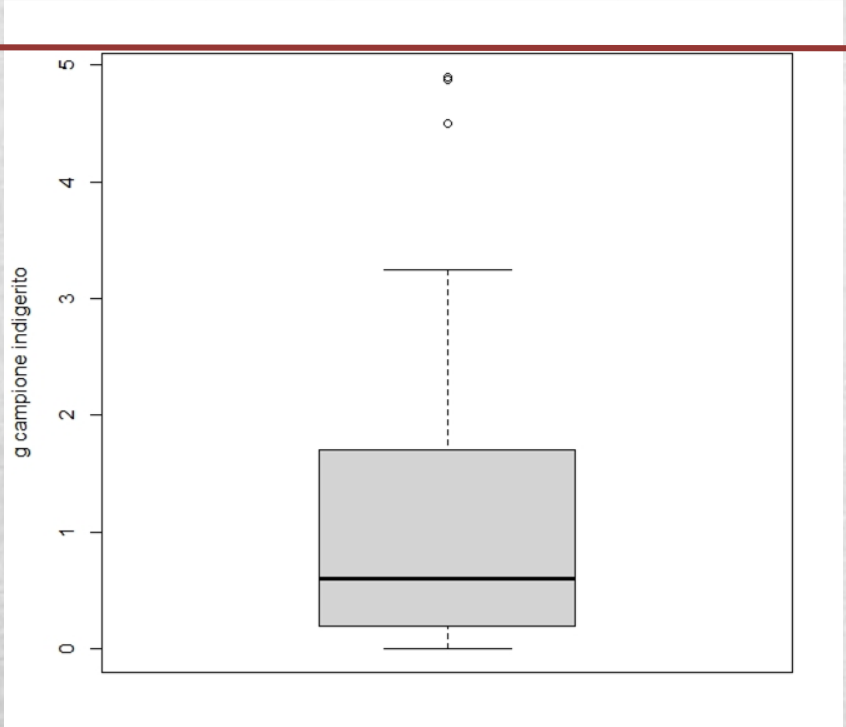
Motility examination by stereomicroscope

52,5% of the samples examined

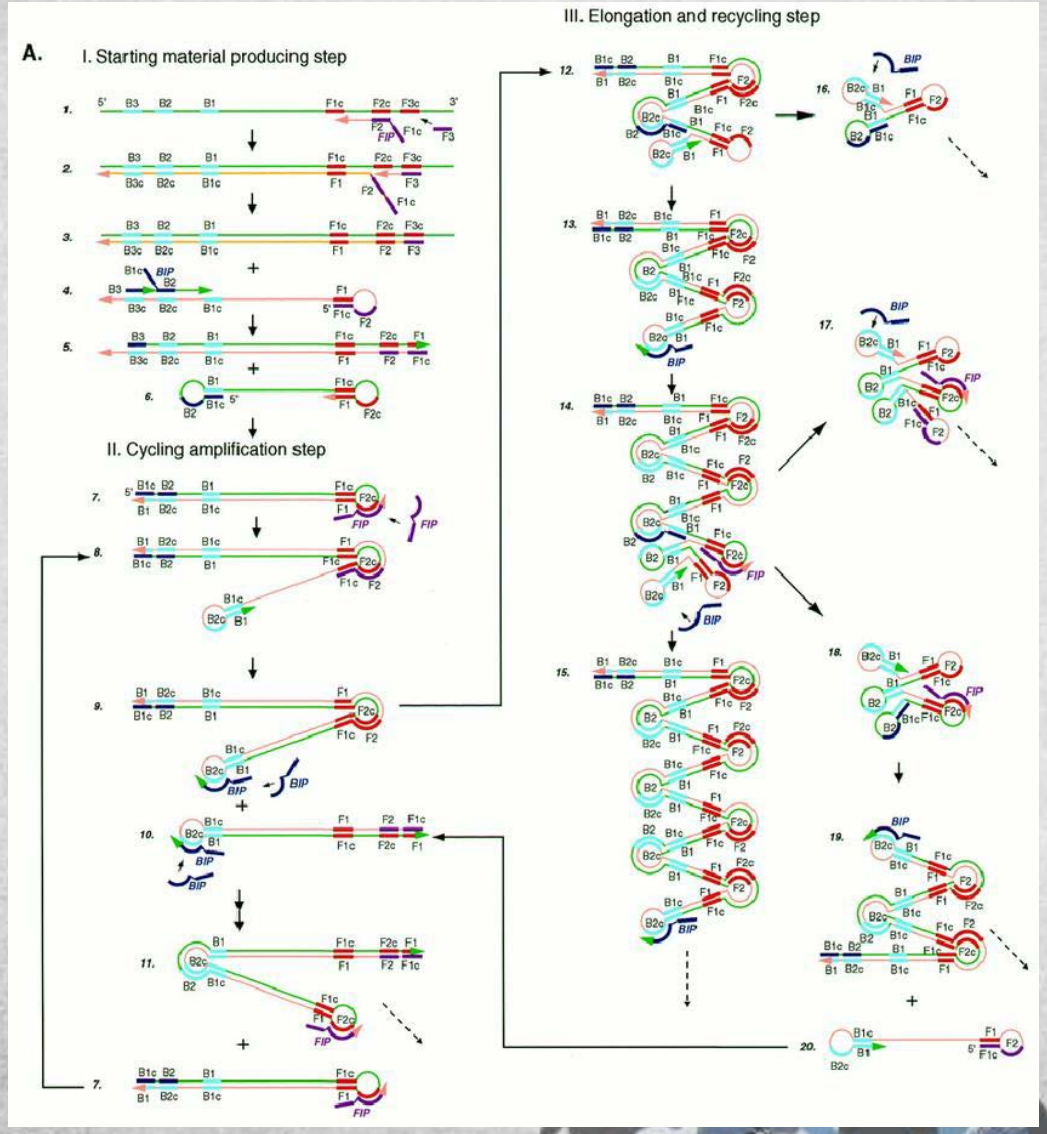
Validation of the method

Undigested samples

Mean of 1,19 grams



LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)



LAMP assay: Primer design

Anisakis sequences reported in gene bank for the primers design

Lehgth between primeds, GC content, melting temperature, absence of complementarity. secondary structures. TTR

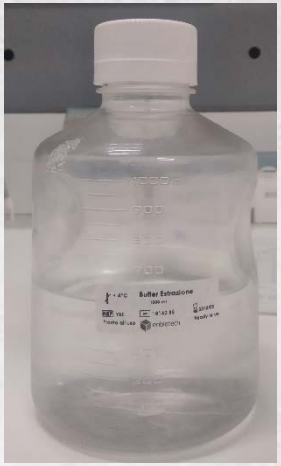
A set of 6 primers (2 outer (F3 and B3), 2 inner (FIP and BIP) and two loop (LF and LB)) using the Primer Explorer software

PrimerExplorer V4 Software

LAMP : DNA extraction

250±50 mg

Extraction buffer



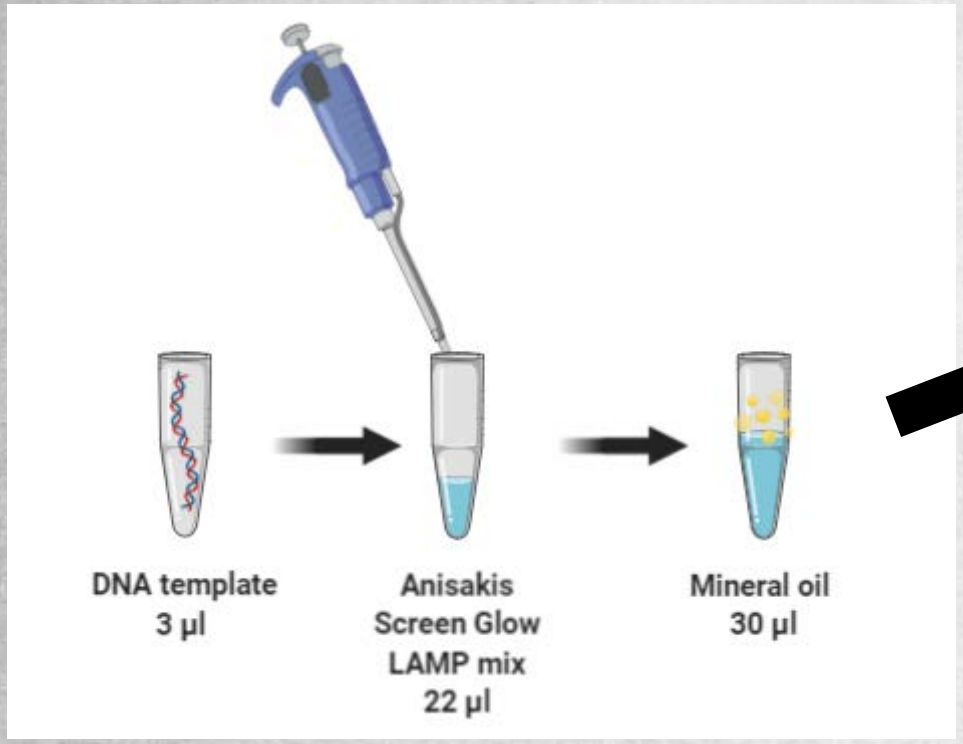
4 ml

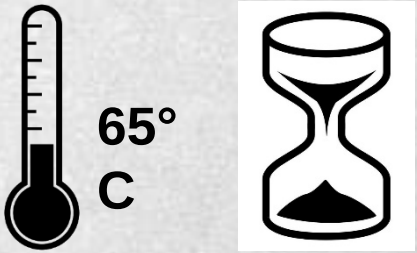
Incubation 40±5 min

1:5 dilution

LAMP assay

- **Real-time monitoring of time and temperature**

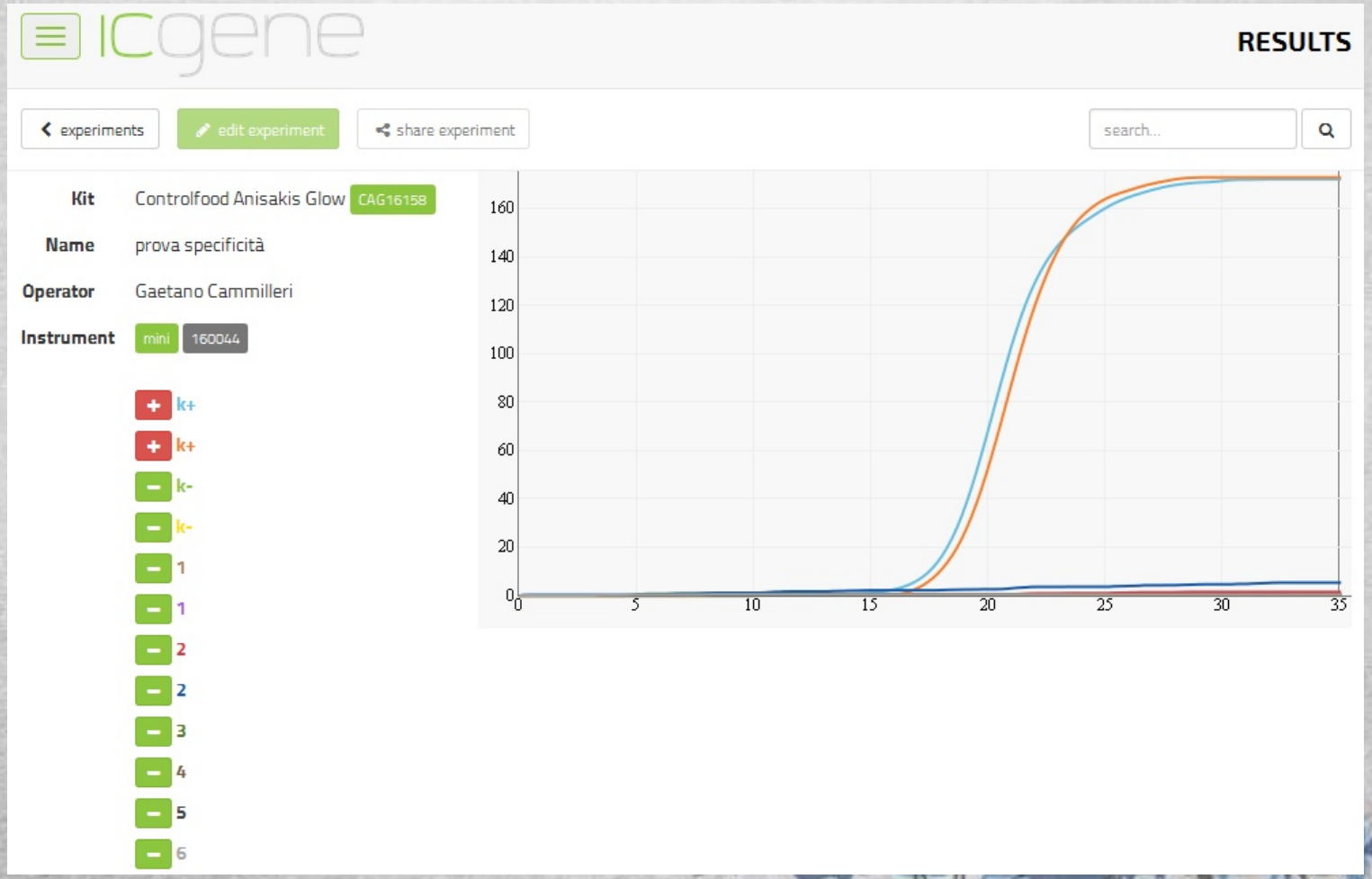




RESULTS

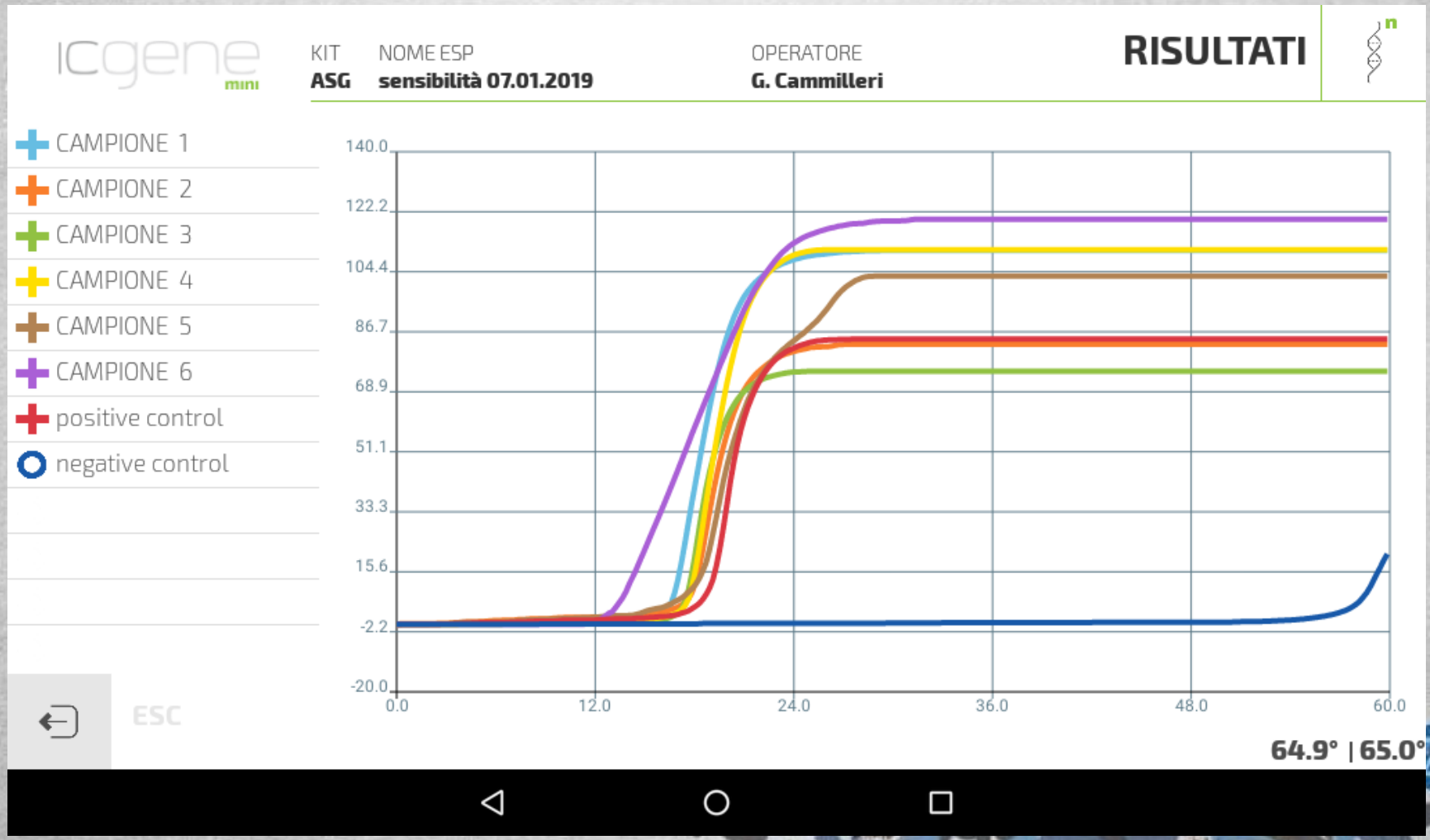
- No amplification was found specificity =100%.

No amplification was found with samples spiked with DNA and larvae of *Contracaecum* sp. *Pseudoterranova* sp. and *Hysterothylacium* sp.



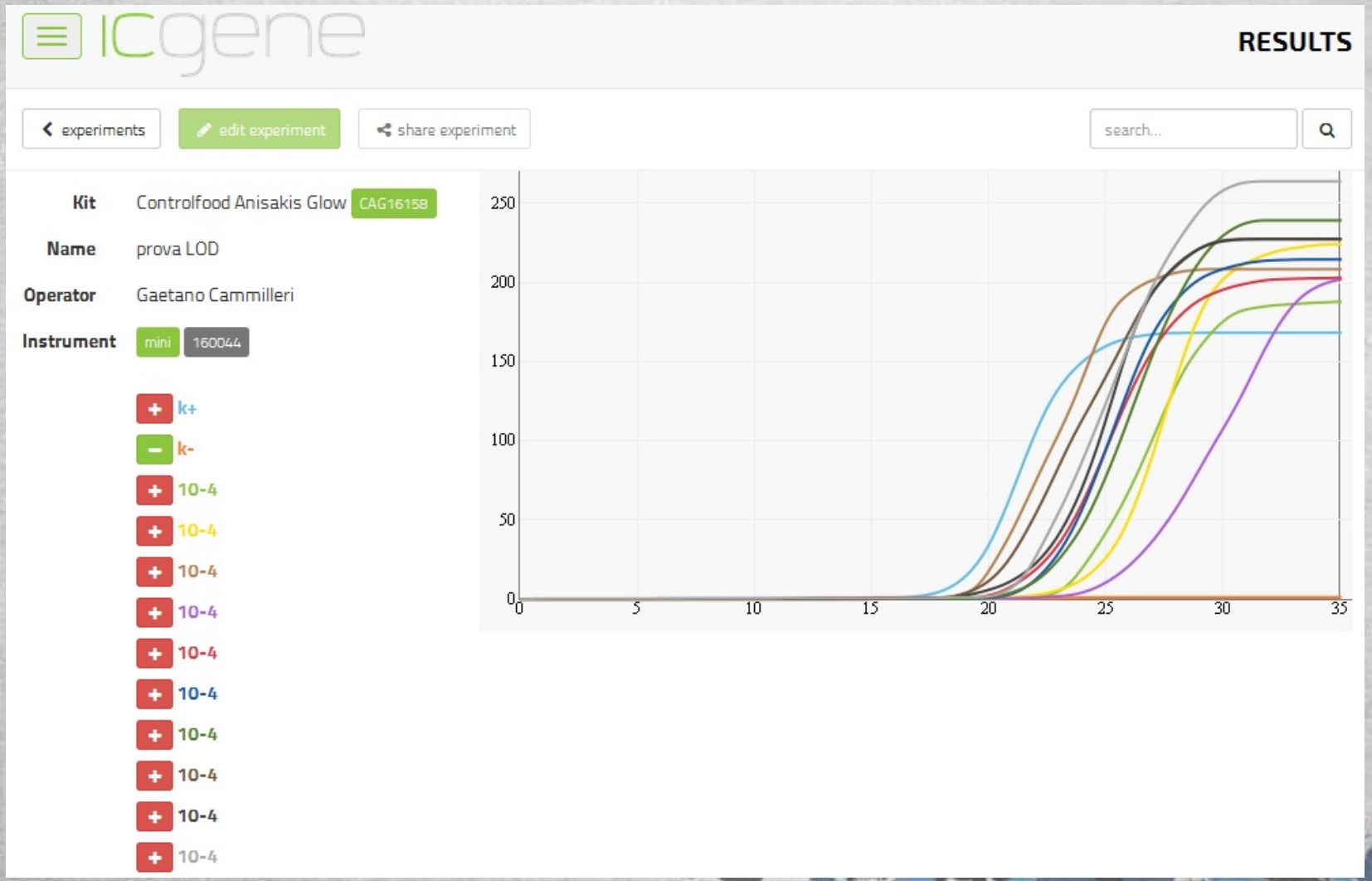
RESULTS

- Sensibility 100% for every sample examined – Amplification for *A. simplex* s.s., *A. pegreffii*, *A. physeteris*, *A. ziphidarum* and *A. typica* DNA.



RESULTS

- *Anisakis* spp. DNA detected with a dilution up to 10^{-4} (0.00022 ng μl^{-1}), with amplification of all the replicates.



foods

MDPI

Article

Validation of a Commercial Loop-Mediated Isothermal Amplification (LAMP) Assay for the Rapid Detection of *Anisakis* spp. DNA in Processed Fish Products

Gaetano Cammilleri [1,2,*], Vincenzo Ferrantelli [1], Andrea Pulvirenti [2], Chiara Drago [3], Giuseppe Stampone [3], Gema Del Rocio Quintero Macias [3], Sandro Drago [3], Giuseppe Arcoleo [3], Antonella Costa [1], Francesco Geraci [1] and Calogero Di Bella [1]

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Another publication in 2020

ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 52, N.3, 131-141, 2020

I. Brusca[1], S. Graci[2], M. Barrale[1], G. Cammilleri[2], M. Zarcone[3], R. Onida[1], A. Costa[2], V. Ferrantelli[2], M.D. Buscemi[2], C.G. Uasuf[4], M. Gjomarkaj[4], M. Vazzana[5], S.M. La Chiusa[1], G. Iacolino[1], F. Vitale[3], W. Mazzucco[3]

Use of a comprehensive diagnostic algorithm for Anisakis allergy in a high seroprevalence Mediterranean setting

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