UNIVERSITÀ
DEGLI STUDI DI TRIESTE

LAUREA MAGISTRALE IN SCIENZE PER L'AMBIENTE MARINO E COSTIERO

Biologia ed Ecologia della Pesca

Subtle and disregarded human impacts on the functioning of marine systems

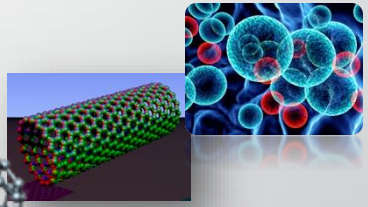
Antonio Terlizzi
Laboratorio di Zoologia e Biologia Marina
aterlizzi@units.it

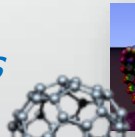
TRADITIONAL POLLUTANTS…

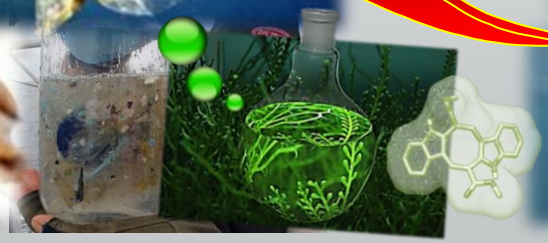
…EMERGING POLLUTANTS

Polycyclic aromatic hydrocarbon
Heavy metals
Pesticides, PCB, Dioxins

Endocrine Disruptors
Drugs
Natural Bioactive Molecules
Plastics e Microplastics
Nanoengineered particles

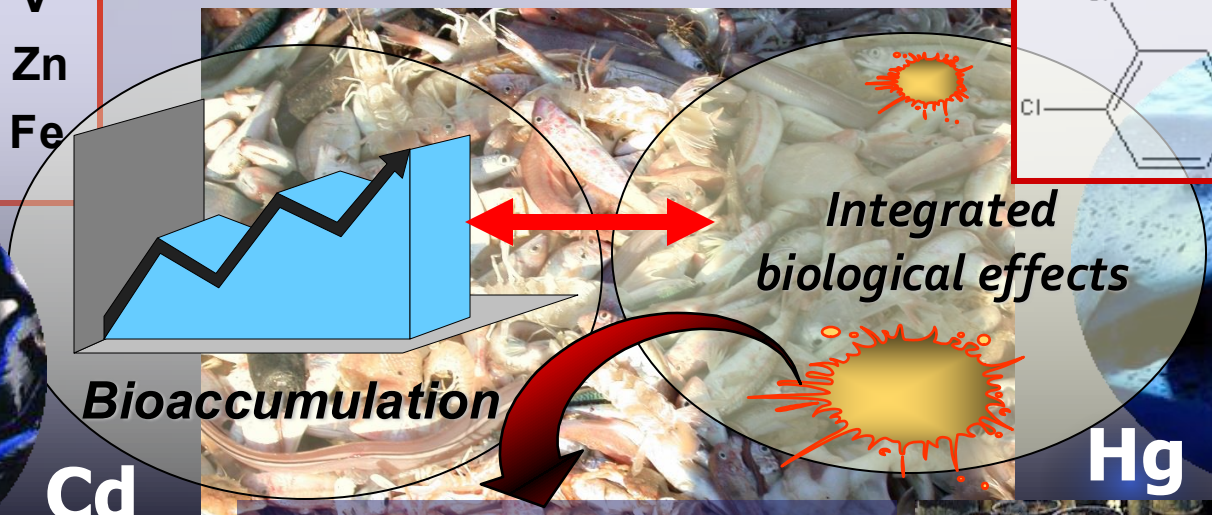


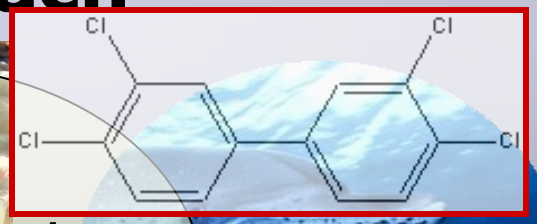


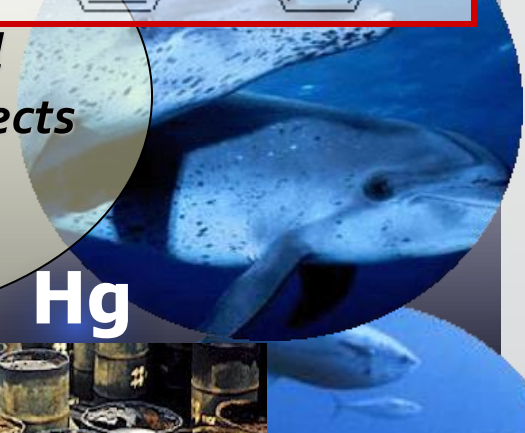


Use of bioindicator organisms and ecotoxicological approach

Hg Cd Cu V
Pb As Cr Zn
Mn Be Co Fe

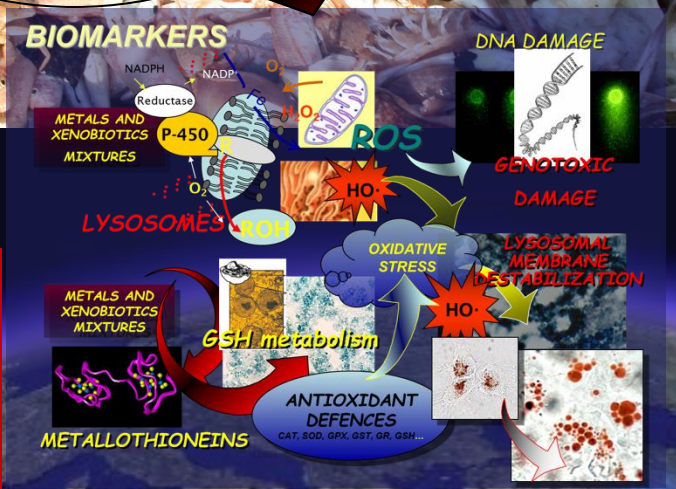


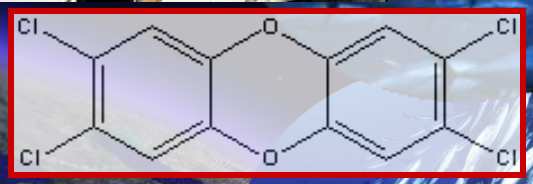




Cd

Hg





As

Cr

Mn

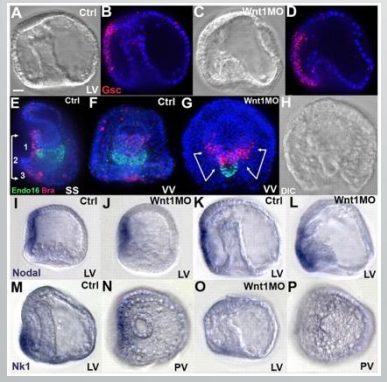
Cu

Pb

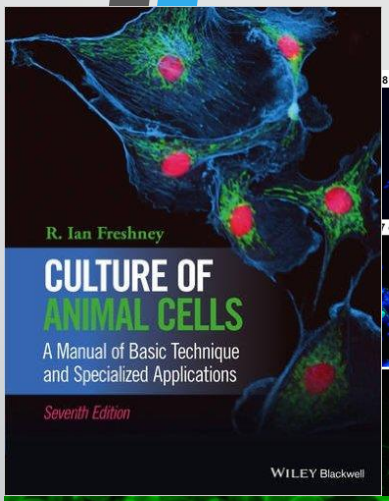
Bioindicator organisms

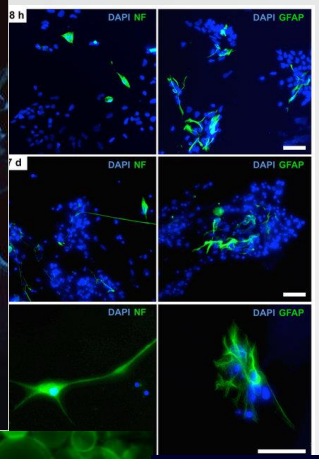
From plankton….
….to top predators..

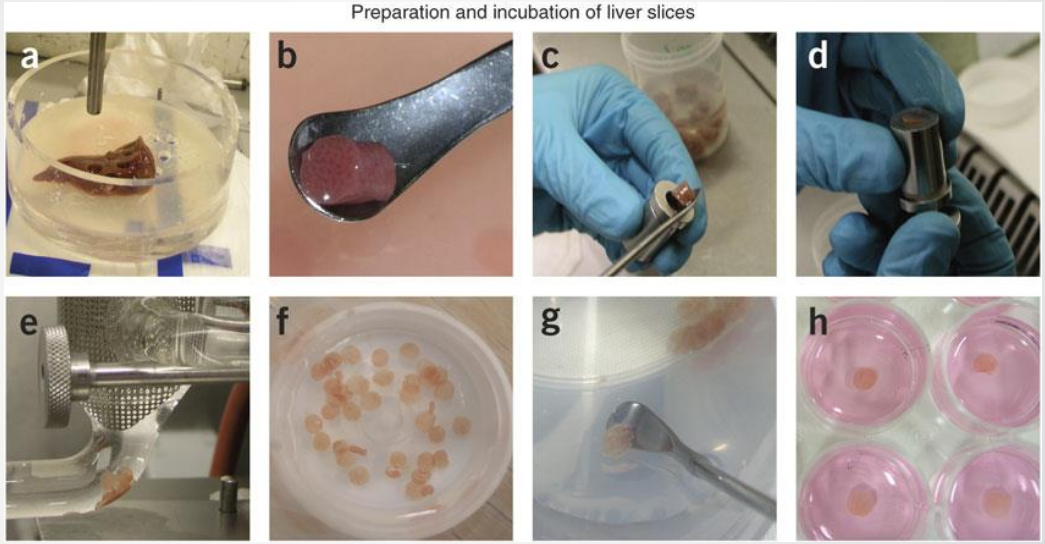
New models, old models and new approaches

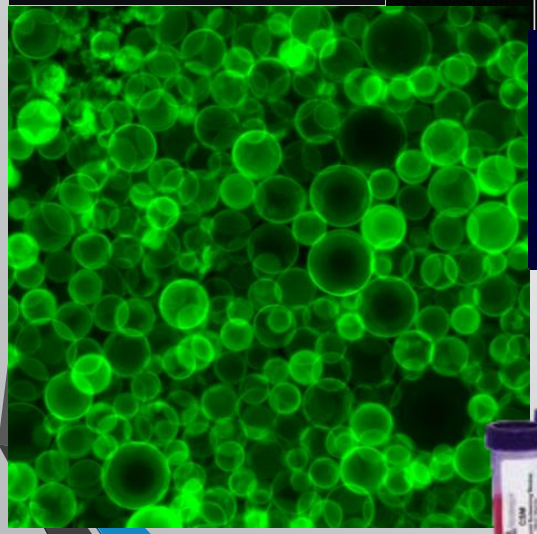


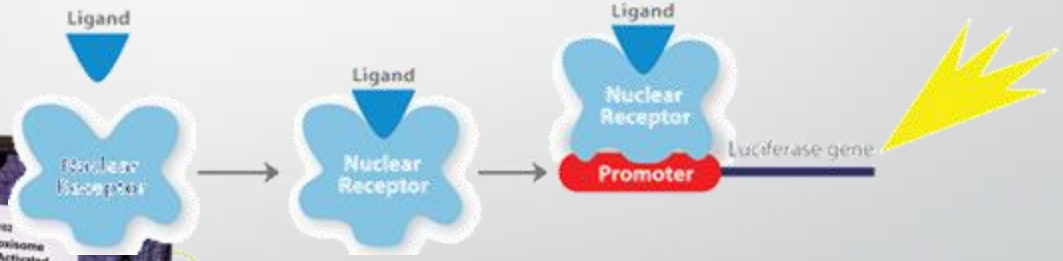
Not only in vivo…
also new in vitro models

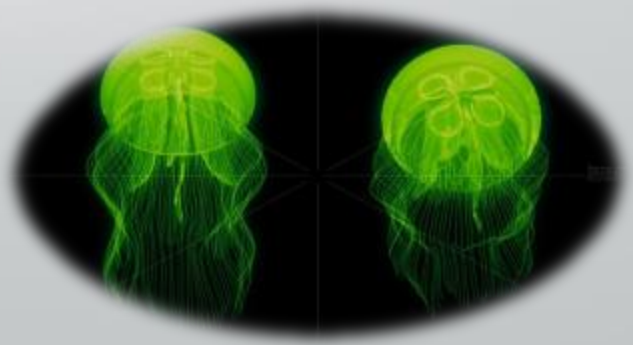






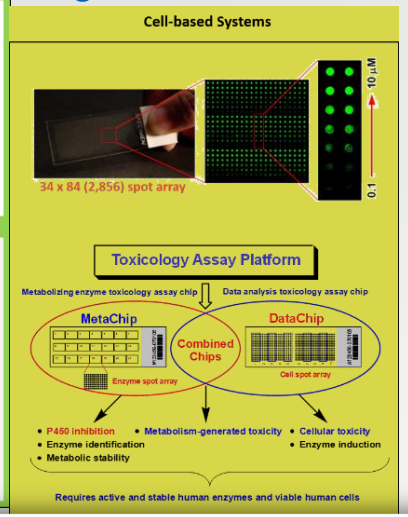




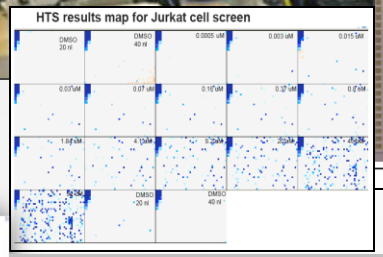


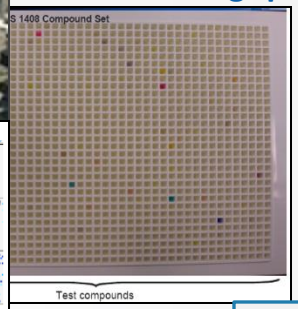
New analytical technologies

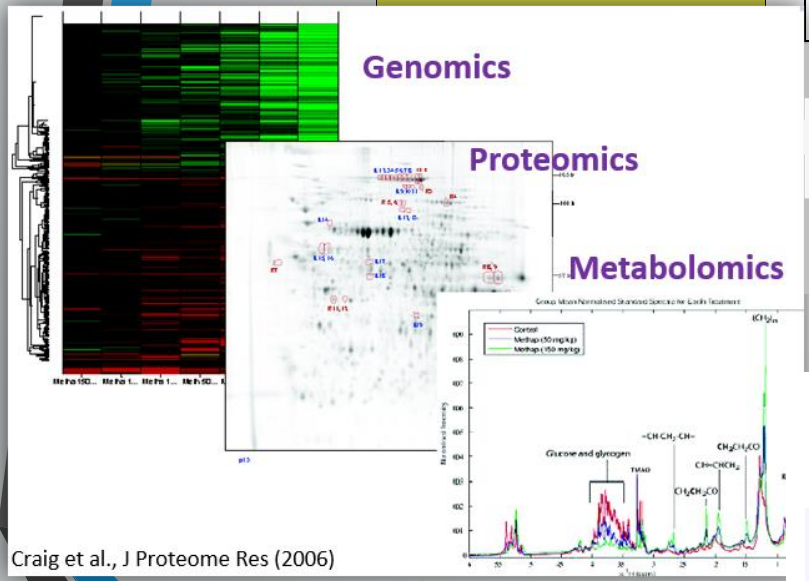
Single Molecule-Based Screening

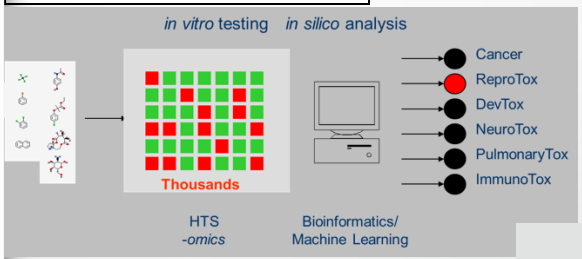


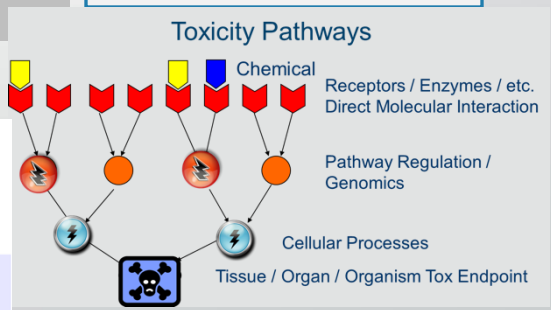
Cell Culture-Based Quantitative High Throughput Analyses

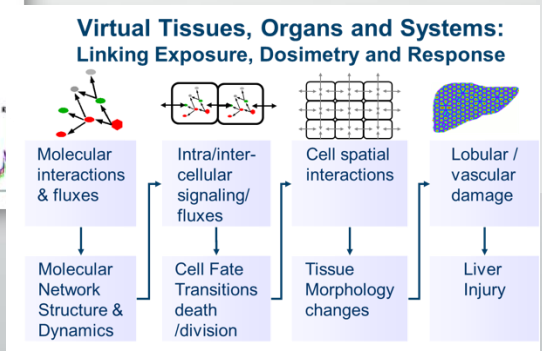


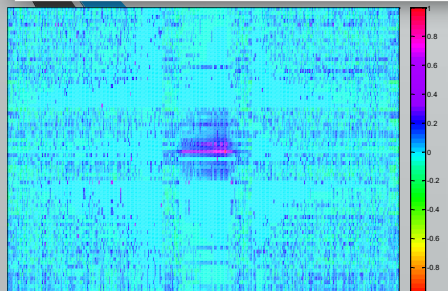






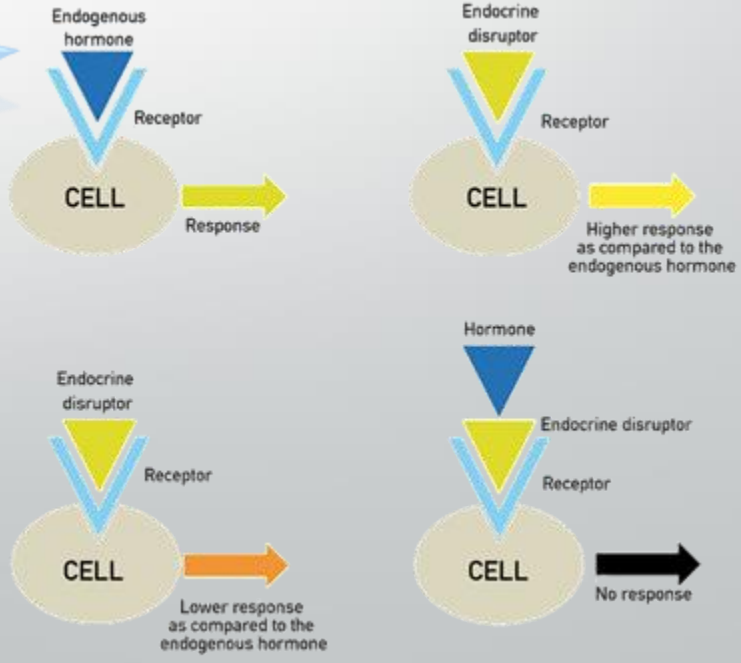




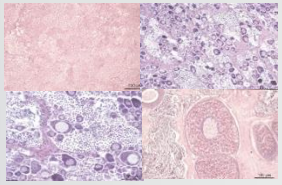


Engineered Tissue-Based Screening

ENDOCRINE DISRUPTORS



EFFECTS OF ENDOCRINE DISRUPTORS



FISH
Testicular tissue with infiltration of

GASTROPODS
Imposex, infertility, masculinization

ALLIGATORS (FLORIDA)
High embryo mortality rate, low testosterone concentration

SEA LIONS (SAN FRANCISCO)
Reduction of reproductive success

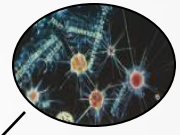
POLAR BEAR
4 % hermaphroditism [Pbde]

BELUGA WHALE (St Lawrence estuary) alterations of the reproductive system,

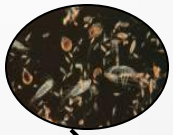
Seagull124 ppm

X 50.000

Phytoplankton 0.0025 ppm



Zooplankton 0.123 ppm



Seagull eggs 124 ppm

Juveniles 1.04 ppm

Trout 4.83 ppm

SEA BIRDS
Hormonal imbalances and sex ratio alteration, fragile shell eggs

AMPHIBIANS
Malformations and neonatal death.

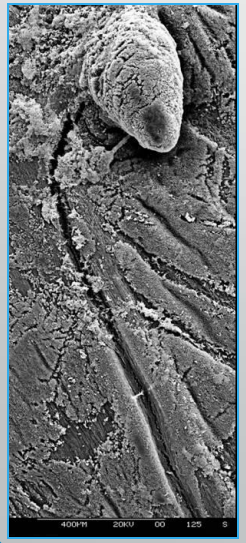
Labroides dimidiatus

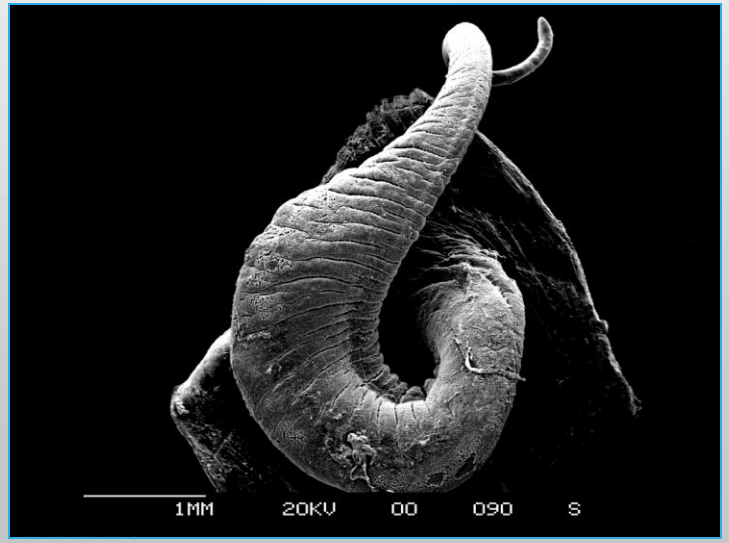
- Tropical cleaner fish
- In addition to cleaning, it also provides tactile stimulation to the customer
- Exposure to oestrogens such as oestradiol or its agonist alters behaviour, compromising survival:
  - More time for tactile stimulation
  - Less time for feeding and reproduction

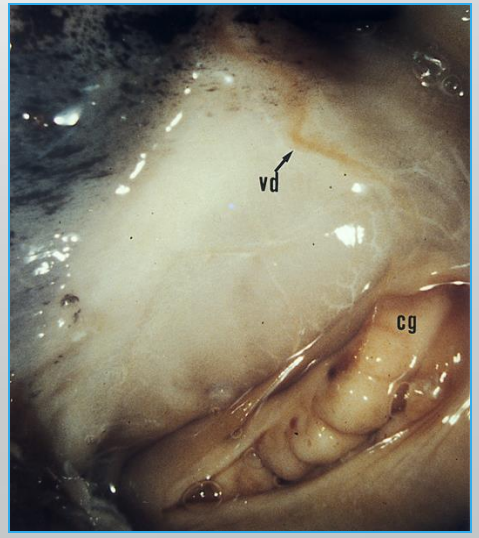
MPAs and contamination by chemicals: the TBT case

Imposex is an expression of environmentally controlled pseudohermaphroditism in gonochorist gastropods and consists in a hormonal imbalance superimposing male characters, namely penis and vas deferens, on females' genitalia or, also, abnormal development of sexual traits in males.

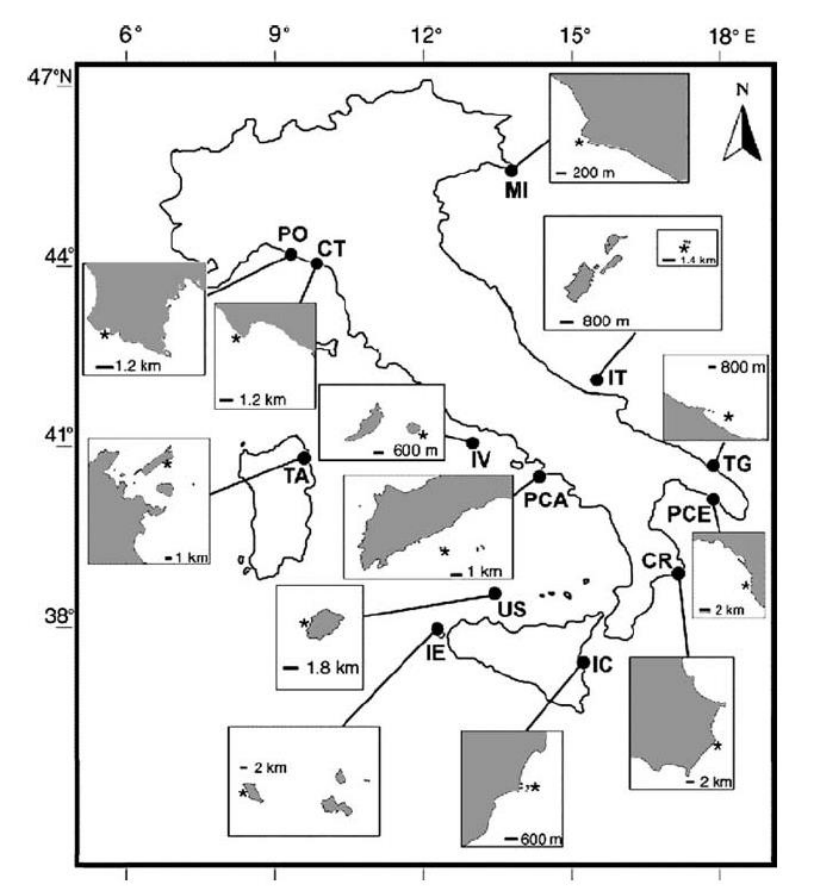
It is caused by tri-butyltin (TBT), a toxic contained in massively-used self-polishing antifouling paints



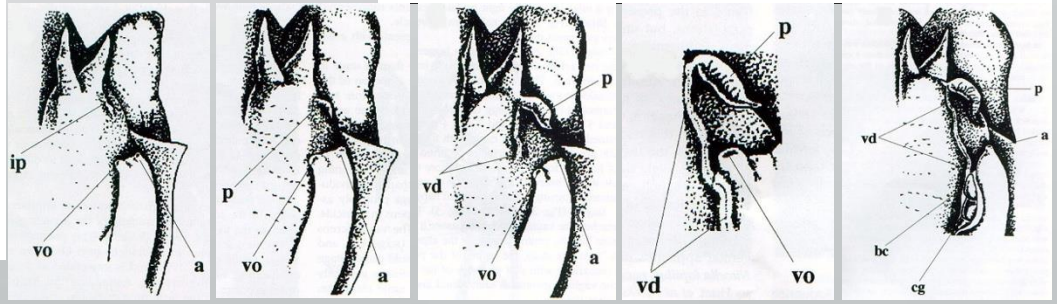




The spread of contaminants: TBT and morphological alterations of sexual apparatus in mollusc populations within MPAs



Marine protected area	Sex	No. of individuals	% Imposex	VDSI
CAPO RIZZUTO = CR	M	20		
	F	7	100	3.94
CINQUE TERRE = CT	M	8		
	F	12	100	3.82
ISOLE CICLOPI = IC	M	18		
	F	26	100	4.05
ISOLE DI VENTOTENE E S. STEFANO = IV	M	11		
	F	21	100	2.68
ISOLE PELAGIE = IP	M	20		
	F	13	30.8	0.62
ISOLE TREMITI = IT	M	13		
	F	19	10.5	0.16
MIRAMARE = MI	M	25		
	F	7	100	4.13
PORTO CESAREO = PCE	M	16		
	F	24	54.2	0.11
PORTOFINO = PO	M	20		
	F	21	100	3.86
PUNTA CAMPANELLA = PCA	M	12		
	F	14	100	3.33
TAVOLARA = TA	M	23		
	F	14	100	4.04
TORRE GUACETO = TG	M	12		
	F	24	25	0.66
USTICA = US	M	27		
	F	18	38.9	0.77



Terlizzi et al., 2004 Mar Poll Bull

IMPOSEX IN PRE-POLLUTION TIMES: IS TBT TO BLAME?

42 European institutions contacted

Replied positively: 6

- Muséum National D'Histoire Naturelle – Paris - Prof. P. Bouchet
- Zoologisches Museum der Universitaet Hamburg – Dr. Bernard Hausdorf
- Institute of Systematic Zoology, Museum fur Naturkunde, Humboldt University, Berlin - Dr. Frank Koehler
- Swedish Museum of Natural History - Dep. Of Invertebrate Zoology, Stockolm - Dr. Anders Warén
- Universiteit van Amsterdam, Zoologisch Museum, Dept. Of Malacology - Dr. Robert G. Moolenbeek
- Zoological Museum University of Copenhagen - Dr. Ole Secher Tendal

Total number of specimens: about 70 (55 analyzed) (38 females and 17 males)

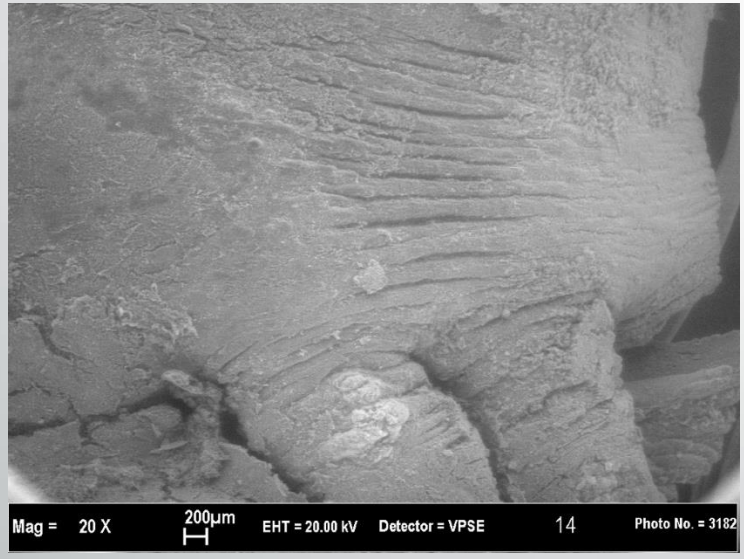
Period: 1890-1957

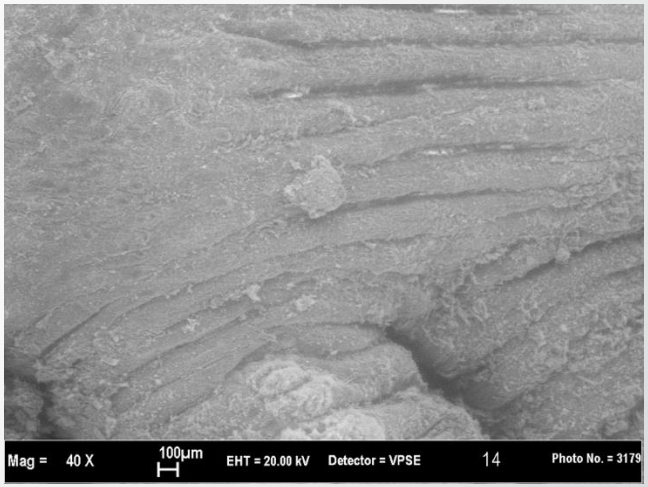
Garaventa et al., 2006 Mar Poll Bull

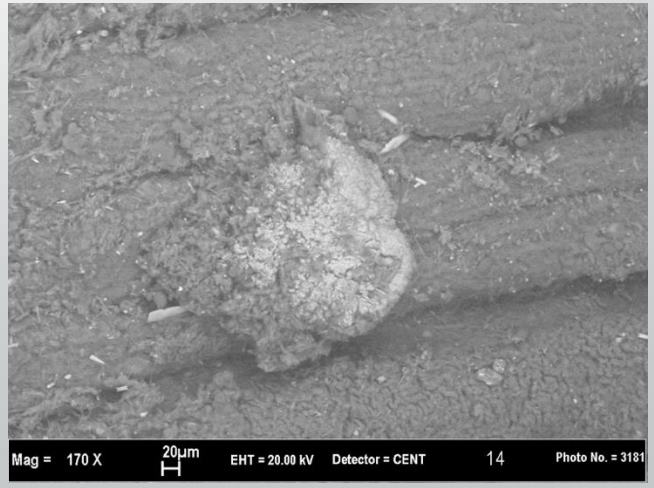
Results

3 females with imposex – 8% incidence

SAMPLE 14: Napoli 1875-1940

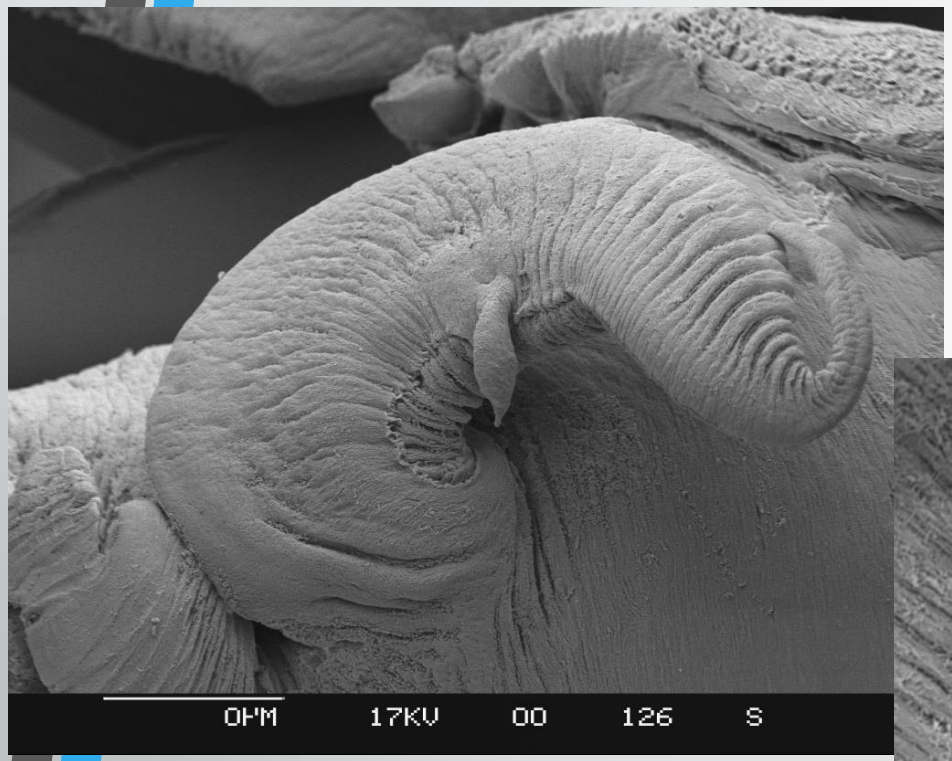




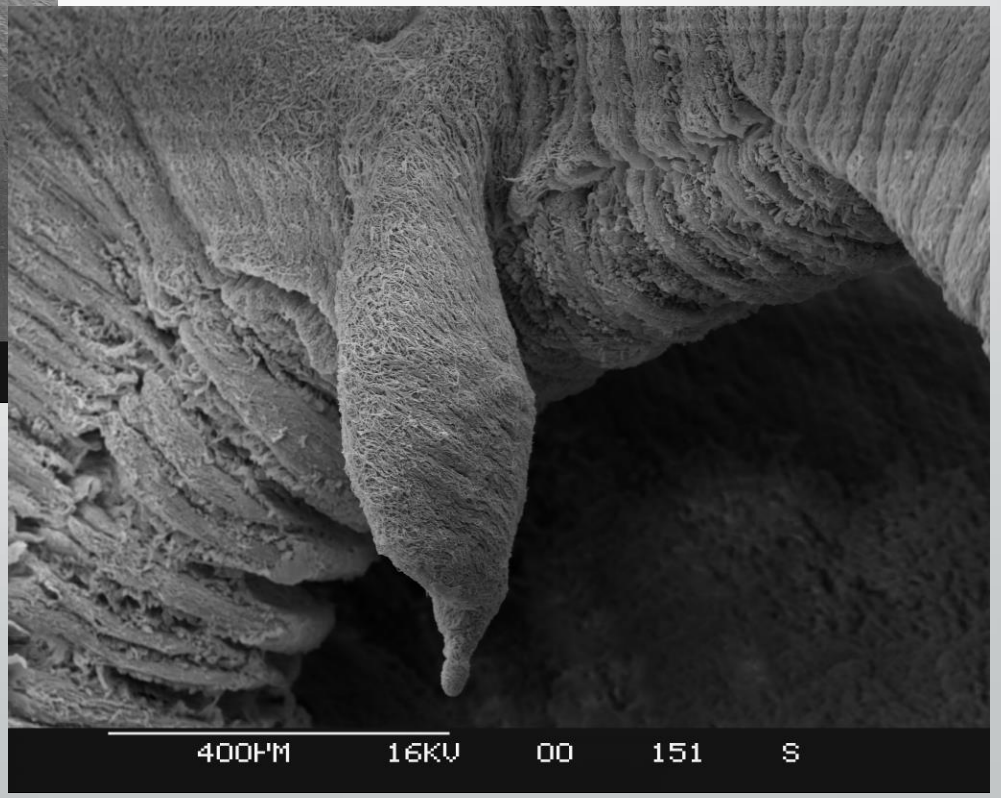


Results

Sample 39 Adriatic Sea 1875-1940



1 biphallic male– 6% incidence



Induced IMPOSEX

For each specimens, 5 µl in EtOH of:

TBTCl	PCB - Aroclor 1260
8 mg/l (40 ng/injection/organism)	25 mg/l (125 ng/injection/organism)
16 mg/l (80 ng/injection/organism)	50 mg/l (250 ng/injection/organism)

Experimental design

Treatment: 7 levels

Ctr nt – Ctr $MgCl_2$ – Ctr EtOH – TBT 8 – TBT 16 – PCB 25 – PCB 50

Tanks: 2 levels

V 1 V 1

n=40 → Specimens sampled in Porto Cesareo MPA (VDSI = 1.3)

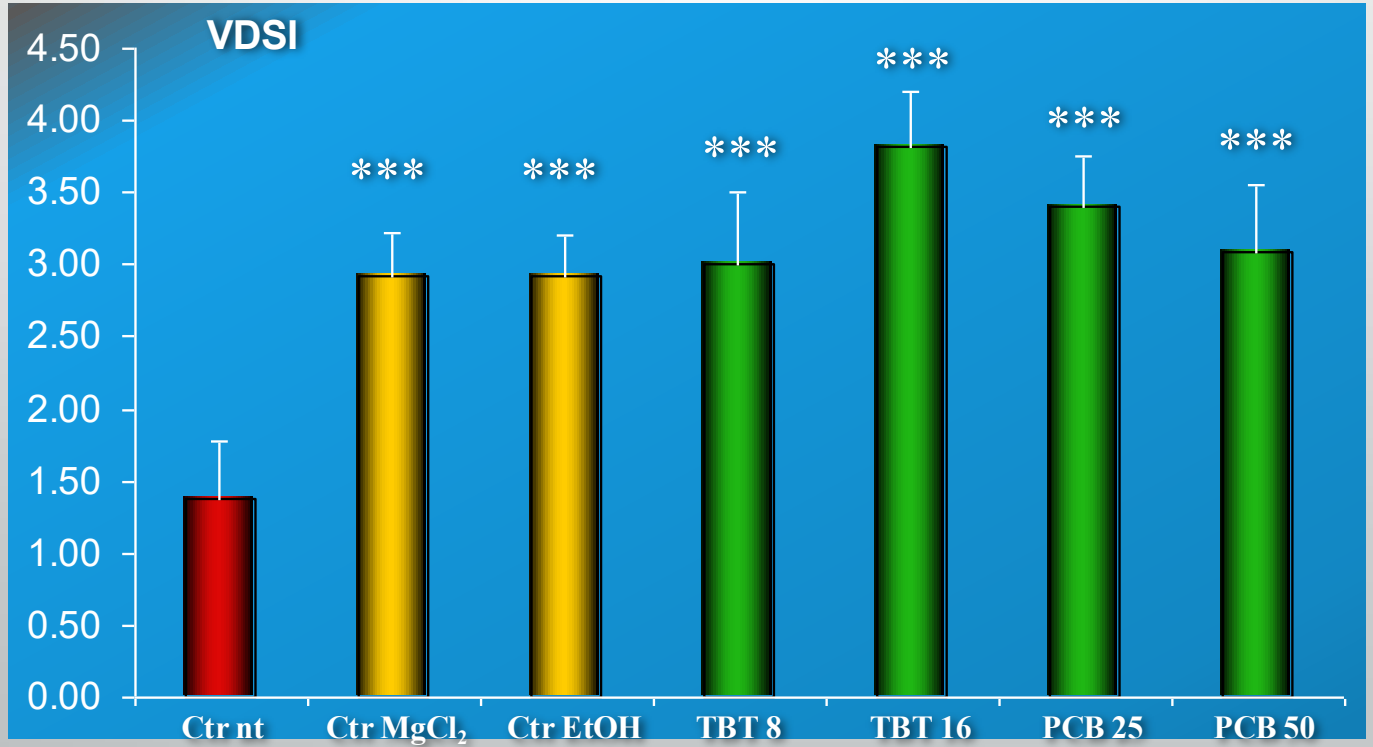
Time of exposure: 2 weeks – 4 injections

Garaventa et al., 2008 Cell Bio, Toxicol

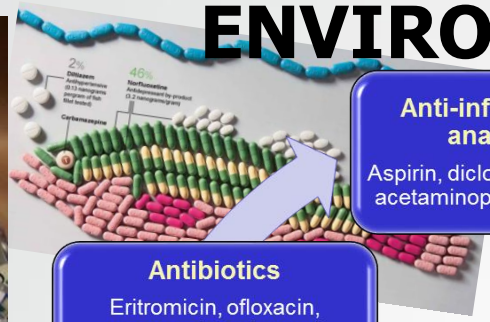
Results

Source	df	SS	MS	F	p	F denominator	
Treat	6	82.85	13.808	10.581	0.0033	Ta(Tr)	***
Controls vs Toxic	1	35.071	35.071	26.874	0.0013	Ta(Tr)	***
[a]Among Controls	2	38.339	19.169	14.689	0.0031	Ta(Tr)	***
Among Toxic	3	9.4403	3.1468	2.4113	0.1522	Ta(Tr)	NS
Tank(Treat)=Ta(T	7	9.135	1.305	0.7178	0.6571	Residual	NS
Residual	154	280	1.8181				

[a]SNK TEST : CtrlNT<Ctrl$MgCl_2$=Ctrls EtOH

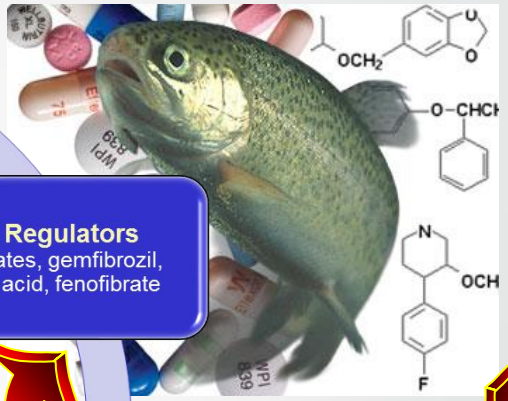


PHARMACEUTICALS IN AQUATIC ENVIRONMENTS



Anti-inflammatory, analgesics
Aspirin, diclofenac, ibuprofen, acetaminophen, naproxen…

Antibiotics
Eritromicin, ofloxacin, streptomycin, spiramycin, pennicillin, oxytetracycline, ciprofloxacin…



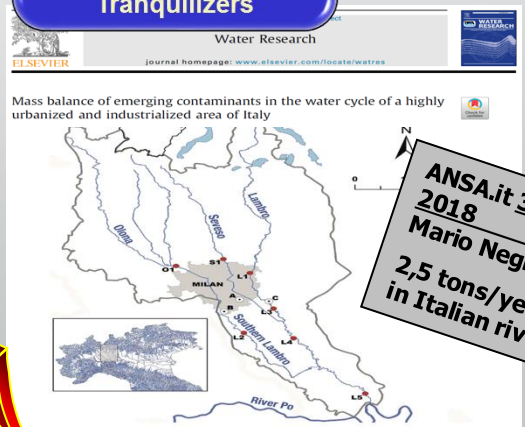
Lipid Regulators
Bezafibrates, gemfibrozil, clolfibric acid, fenofibrate

Steroids and hormones
17-B-estradiol, 17-a-ethinyl estradiol, diethylstilbestrol, diethylstilbestrol acetate

Beta-blockers
Metoprolol, Propranolol, Nadolol, Atenolol. Sotalol, Betaxolol

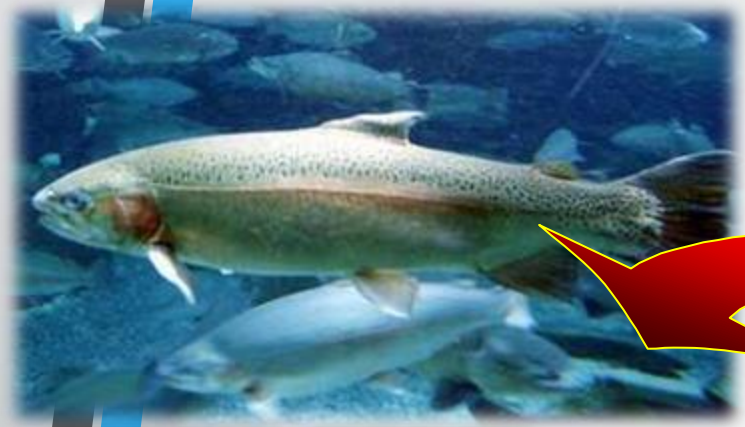
Anti cancer agents
Diuretics
Antiepileptic
Antidepressants
Tranquilizers

La Repubblica, 4 august 2005: «Cocain in the Po river, a constant flow of 4 kilos per day"

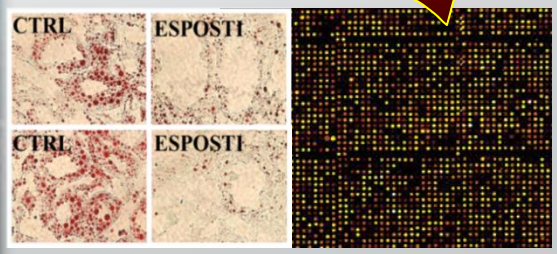


ANSA.it 30 January 2018
Research of Mario Negri Institute
2,5 tons/year of drugs in Italian rivers

EFFECTS OF DRUGS IN AQUATIC BIOTA



17α- etinilestradiolo
Diclofenac
Gemfibrozil
Anti-depressivi (Prozac)
Anti-infiammatori (NSAID)



DRUGS IN MUSSELS FROM ADRIATIC AND TIRRENIAN SEA

All the analyzed mussels accumulated, at least, one of the investigated pharmaceuticals

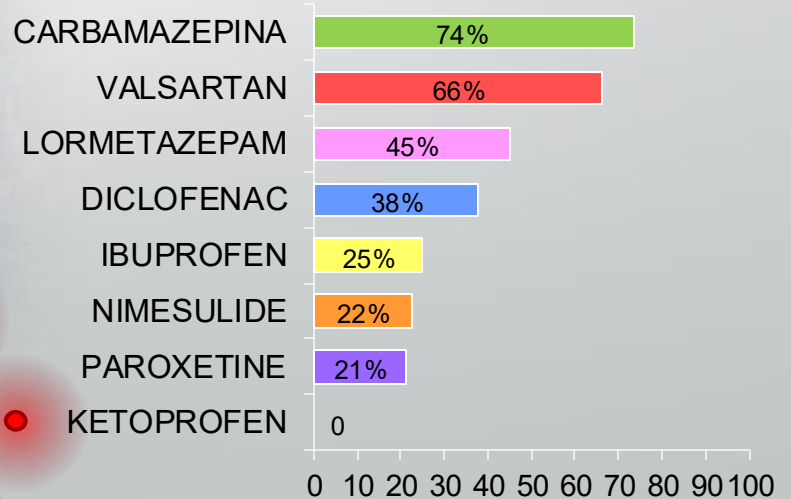
Carbamazepine was the most common molecule found

100%

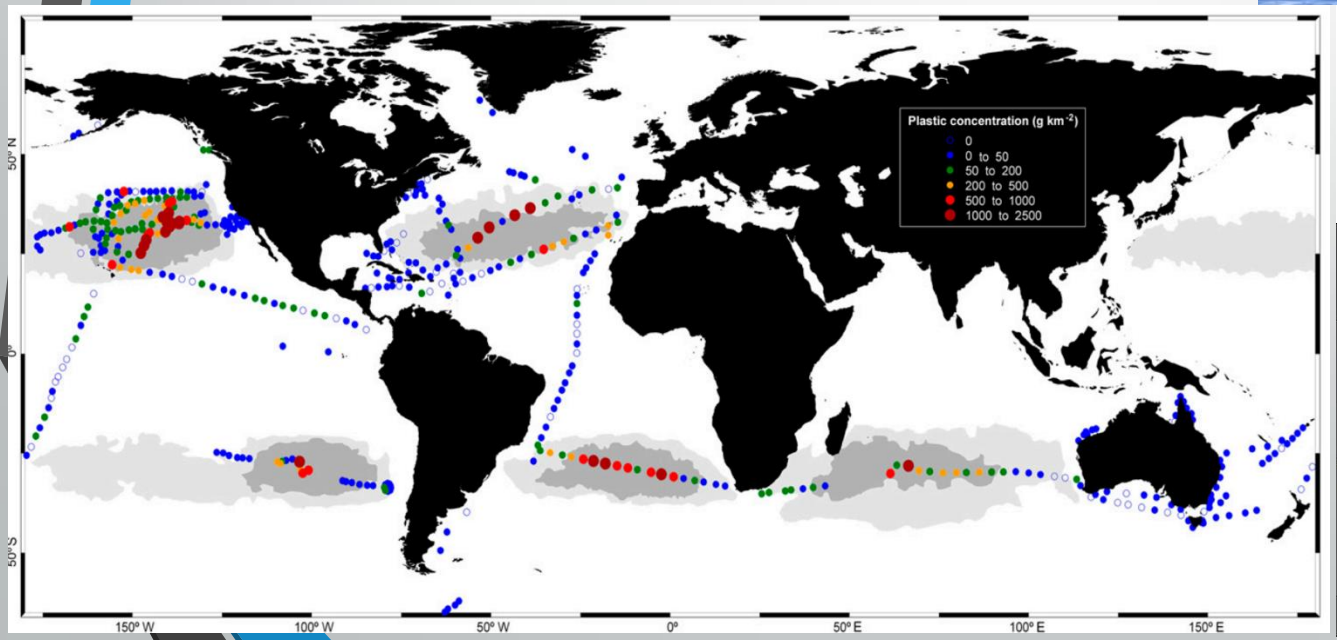
92%

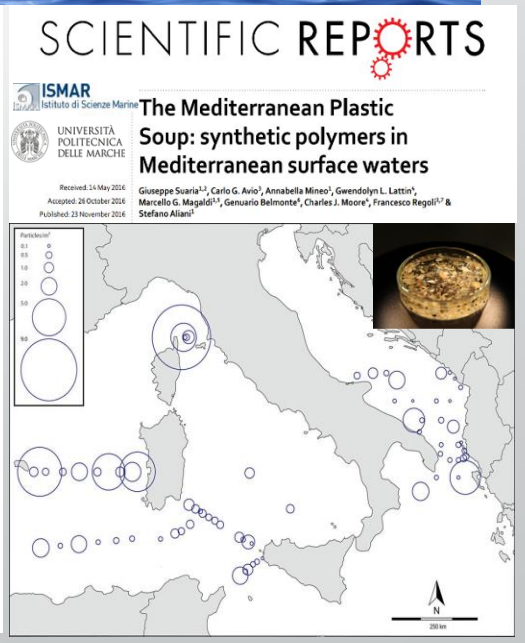
65%

29%



PLASTICS IN MARINE ENVIRONMENT



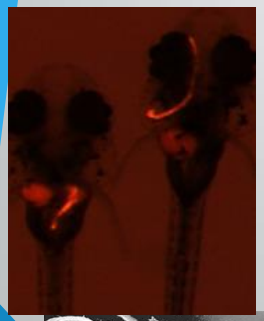


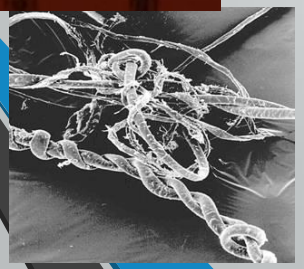
… THE TIP OF THE ICEBERG

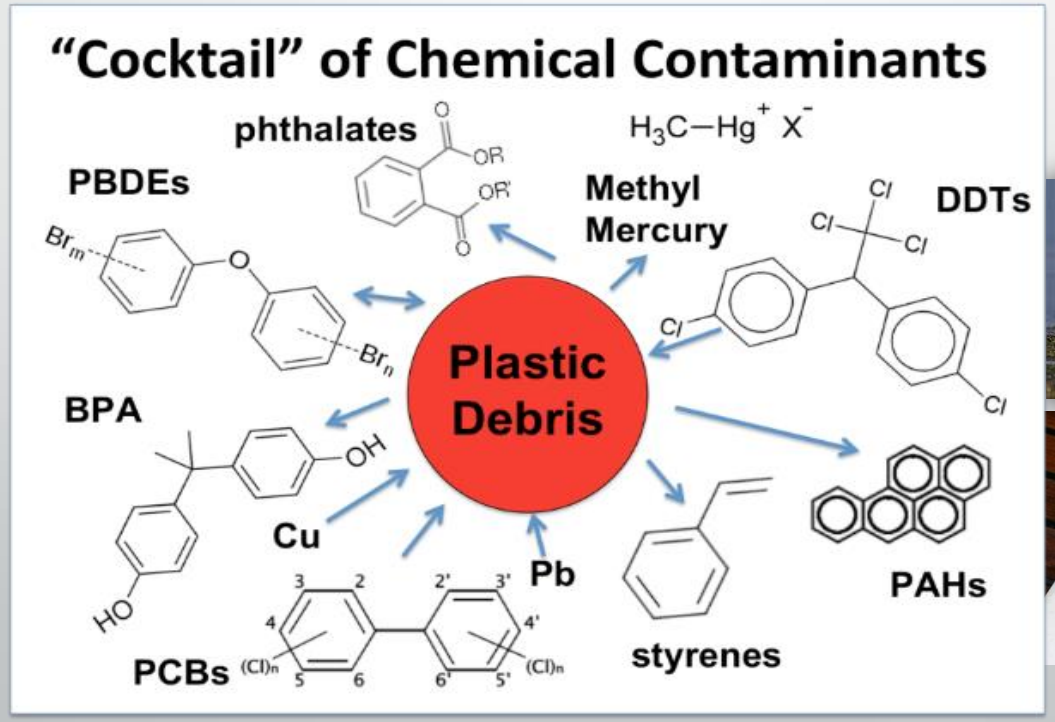
Plastic: source and sinks

NEW ECOTOXICOLOGICAL RISKS: MICROPLASTICS

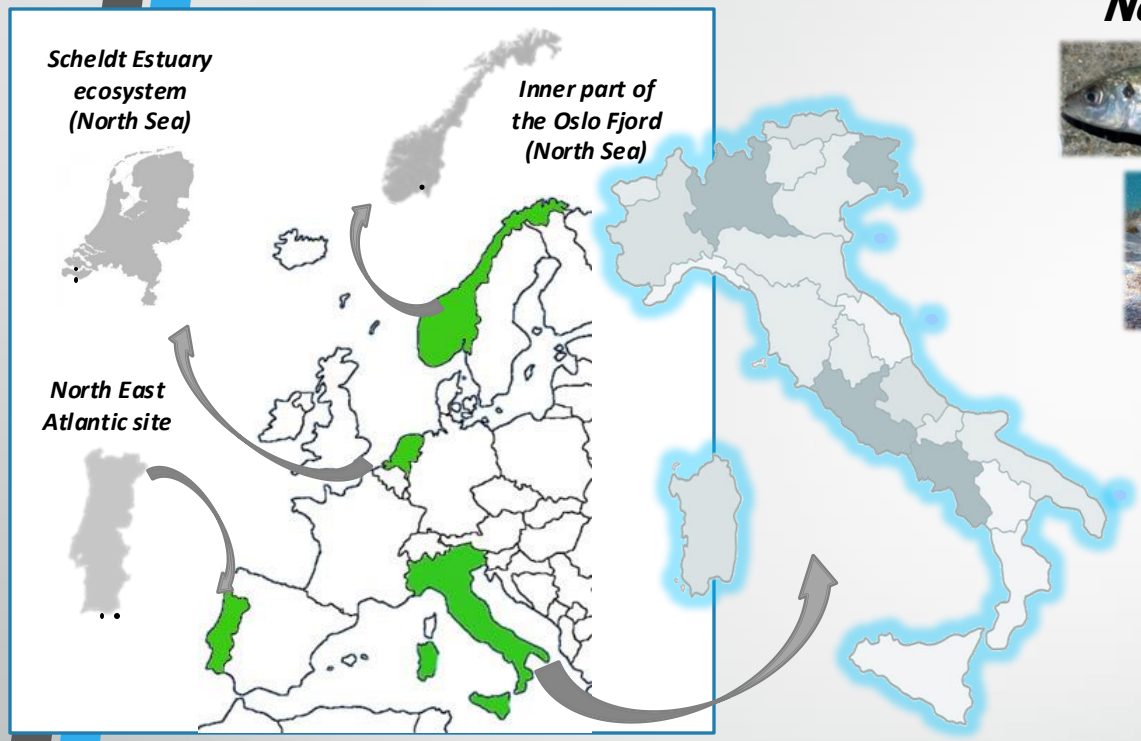
Pseudo-satiety, physical and mechanical damage,
release of additives, accumulation of pollutants, trophic transfer



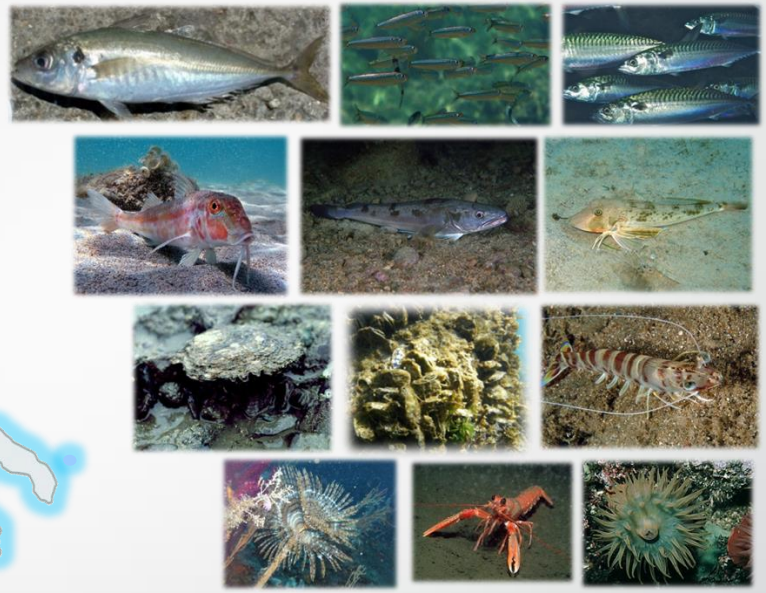




SURVEYS ON THE PRESENCE OF MICROPLASTICS IN MARINE FOOD WEBS

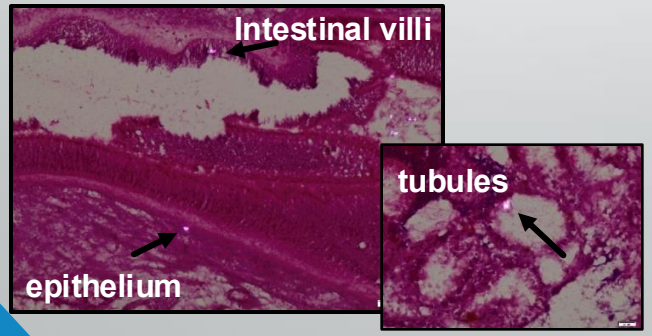


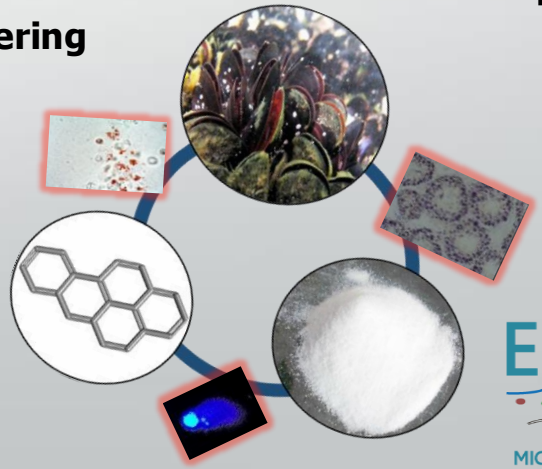
North-Central-South Adriatic Sea



25% of analyzed individuals ingest microplastic

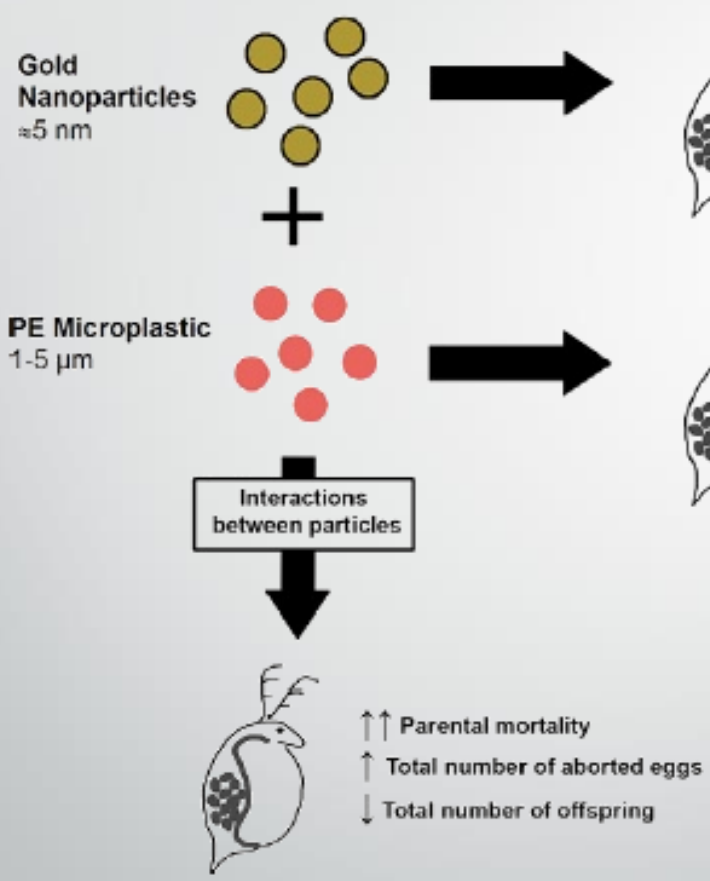
Microplastics adsorb organic pollutants transfering them to the wild fauna





EPHEMARE
ECOTOXICOLOGICAL EFFECTS OF MICROPLASTICS IN MARINE ECOSYSTEMS

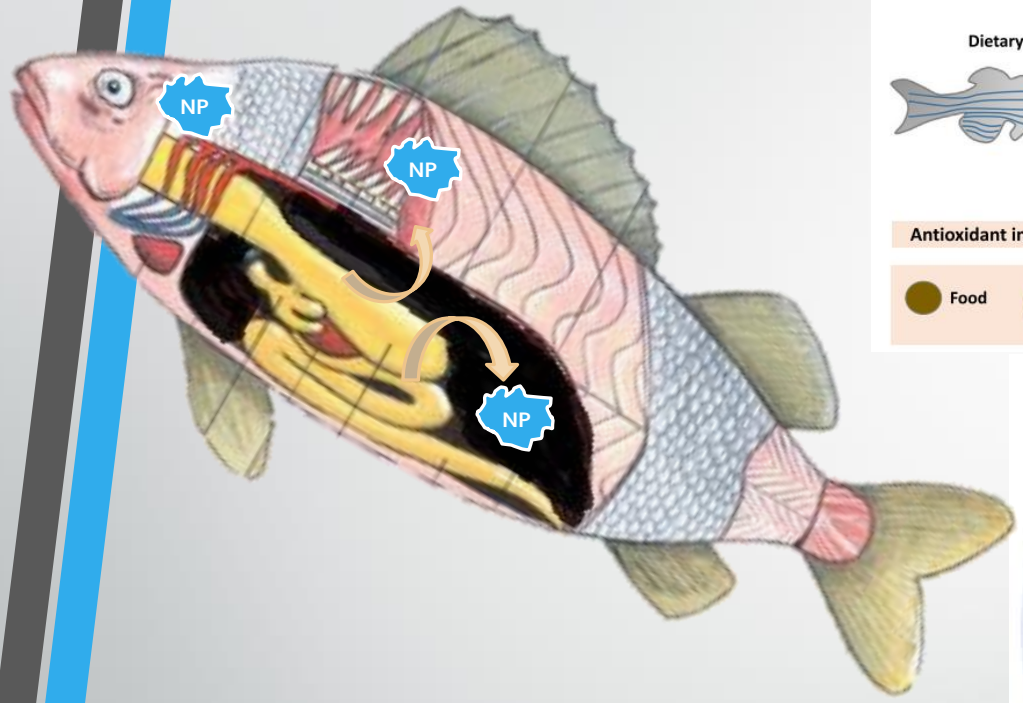
Toxicological interactions induced by chronic exposure to gold nanoparticles and microplastics mixtures in *Daphnia magna*

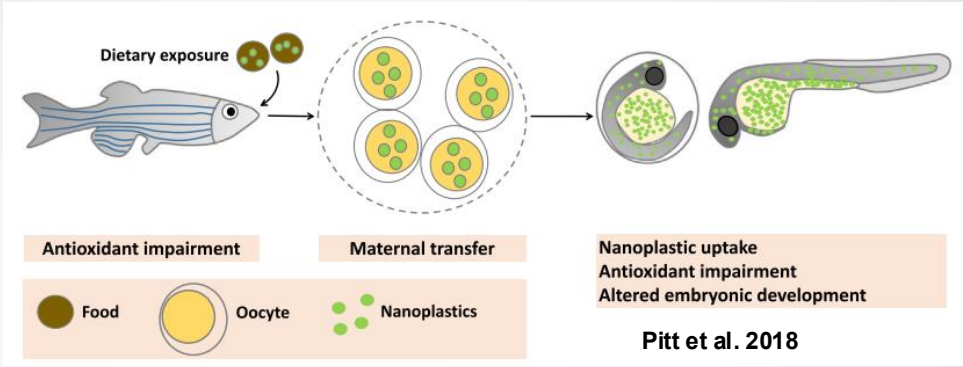


- 21-day *D. magna* exposure to 5 nm AuNP caused mortality and reproduction impairment.
- 21-day *D. magna* exposure to 1–5 µm MP caused mortality and reproduction impairment.
- Mixtures of AuNP and MP caused higher effects than the components individually.
- Based on mortality, synergism at high concentrations of mixture components was found.

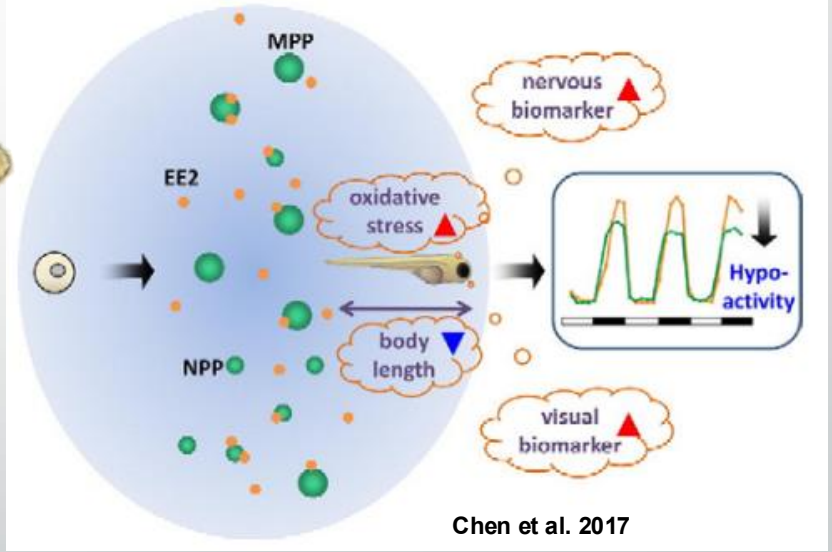
Pacheco et al. (2018) Science of the Total Environment 628–629: 474–483

Biological effects





Pitt et al. 2018



Chen et al. 2017

Documented effects on aquatic organisms:

- Reduction of predatory behaviour
- Induction of oxydative stress
- Genotoxic and neutotoxic activities
- Growth retard
- Reducrion of reproducion acctivity
- Death

Marine Pollution Bulletin 157 (2020) 111317

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Review

Nanoplastics in the oceans: Theory, experimental evidence and real world

Manuela Piccardo[a,b], Monia Renzi[a,*], Antonio Terlizzi[a,b]

[a] Department of Life Sciences, University of Trieste, 34127 Trieste, Italy
[b] Stazione Zoologica Anton Dohrn, 80121 Napoli, Italy

«PAZIENTE N° 0»

22-05-2018

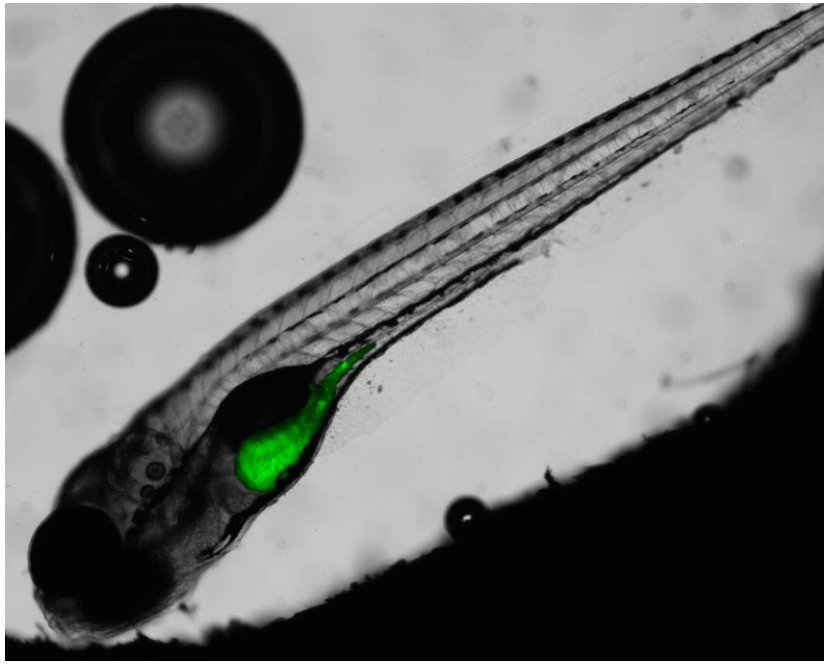
Unica somministrazione, 10 mg/L

Larve di 4 dpf; tempo d’esposizione: 29h

3 larve in 3 ml di working solution (boccettine di vetro con tappo non avvitato)

Ingrandimento 5x (ZEISS IMAGER.Z2, camera AXIOCAM 506 mono, software Zen pro)

Bright field e fluorescenza overlapped



Environmental Pollution 367 (2025) 125566

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

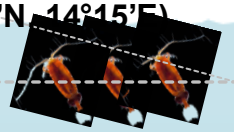
Epipelagic community as prominent biosensor for sub-micron and nanoparticles uptake: Insights from field and laboratory experiments*

Carola Murano [a,b,1], Tecla Bentivoglio [c,1], Serena Anselmi [c], Leonilde Roselli [d], Iole Di Capua [d], Monia Renzi [a,**], Antonio Terlizzi [a,b,e,*]

[a] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy
[b] NBFC, National Biodiversity Future Center, Piazza Marina 61, 9013, Palermo, Italy
[c] Bioscience Research Center, Via Aurelia Vecchia, 32, 58015, Orbetello, Italy
[d] Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Naples, Italy
[e] Department of Life Sciences, University of Trieste, Via Licio Giorgieri, 34127, Trieste, Italy

Field

LTER MareChiara – Italy
Gulf of Naples (40°49'N, 14°15'E)



Phytoplankton

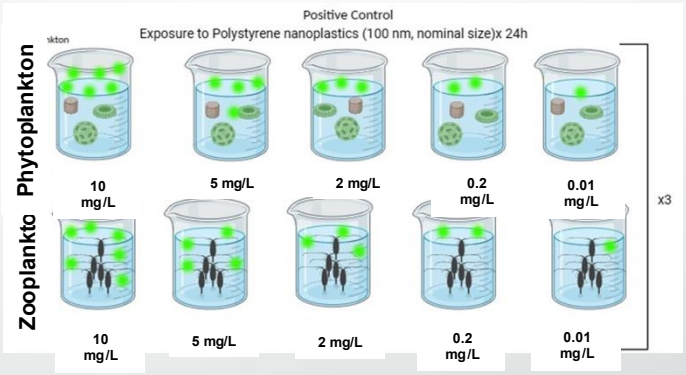
Zooplankton

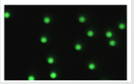
Engraulis encrasicolus

Scomber colias

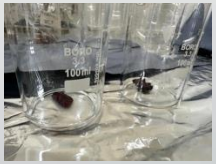
Laboratory

Exposure to Polystyrene nanoplastics (100 nm, nominal size) for 24h





PS-NPs excitation maxima of 441nm and emission maxima at 485nm (FITC)



Particles extraction from organisms with 5% KOH and Na_2ClO

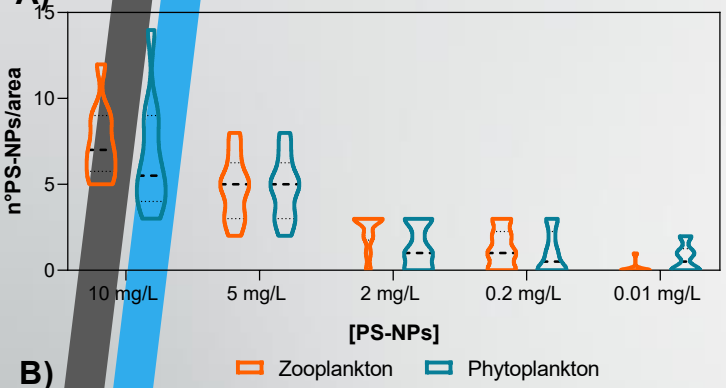
Filtration through anodisk 0.22 µm

Particle identification

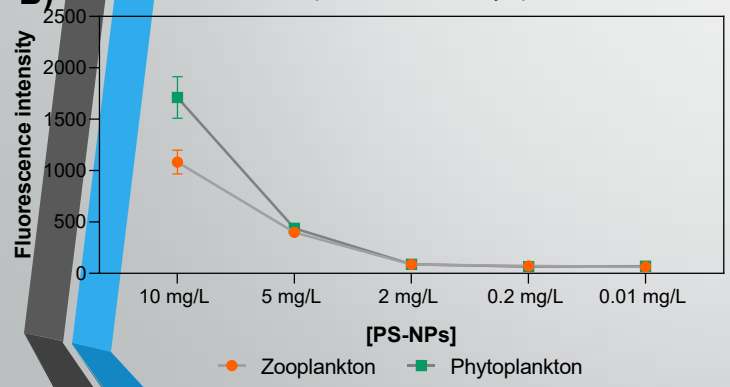
All these steps followed quality assurance and quality check procedures

Concentration-dependent Uptake

A)



B)



Quantification of PS-NPs uptake by zooplankton and phytoplankton communities after digestion process. (A) Results represents the number of particle within an optical surface area of 250 µm. (B) Fluorescent intensity of digested samples of zooplankton and phytoplankton.

C)

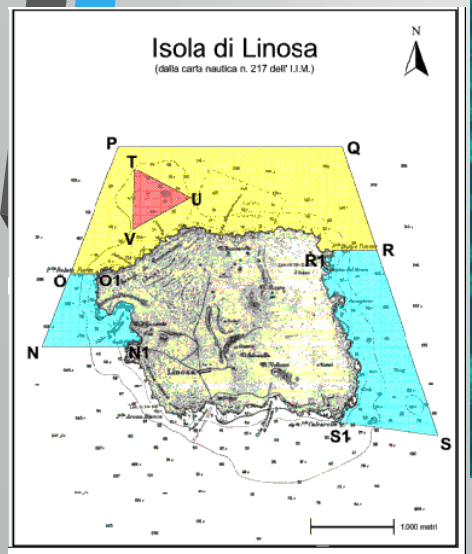
Phytoplankton				
[PS-NPs]	P/A	Area (µm²)	N° PS-NPs	PS-NPs (µm)
10mg/L	P	2500	66	0.6 ± 0.2
5mg/L	P	2500	48	0.57±0.14
2mg/L	P	2550	14	0.46±0.14
0.2mg/L	P	2420	10	0.46±0.13
0.01mg/L	P	2520	7	0.73±0.14

Zooplankton				
[PS-NPs]	P/A	Area (µm²)	N° PS-NPs	PS-NPs (µm)
10mg/L	P	2520	74	0.54±0.1
5mg/L	P	2500	48	0.57±0.1
2mg/L	P	2540	23	0.43±0.1
0.2mg/L	P	2490	13	0.46±0.05
0.01mg/L	P	2500	1	0.51±0

(C) Total number of PS-NPs internalized by 10 specimens of zooplankton and phytoplankton for each concentration tested.

Viral Nervous Necrosis*: from acquaculture to protected wild populations*

Betanodaviruses are causative agents of viral nervous necrosis (VNN), a devastating disease of cultured marine fish worldwide



Vendramin *et al. BMC Veterinary Research* 2013, **9**:20
http://www.biomedcentral.com/1746-6148/9/20

BMC Veterinary Research

CASE REPORT Open Access

Viral Encephalopathy and Retinopathy in groupers (*Epinephelus* spp.) in southern Italy: a threat for wild endangered species?

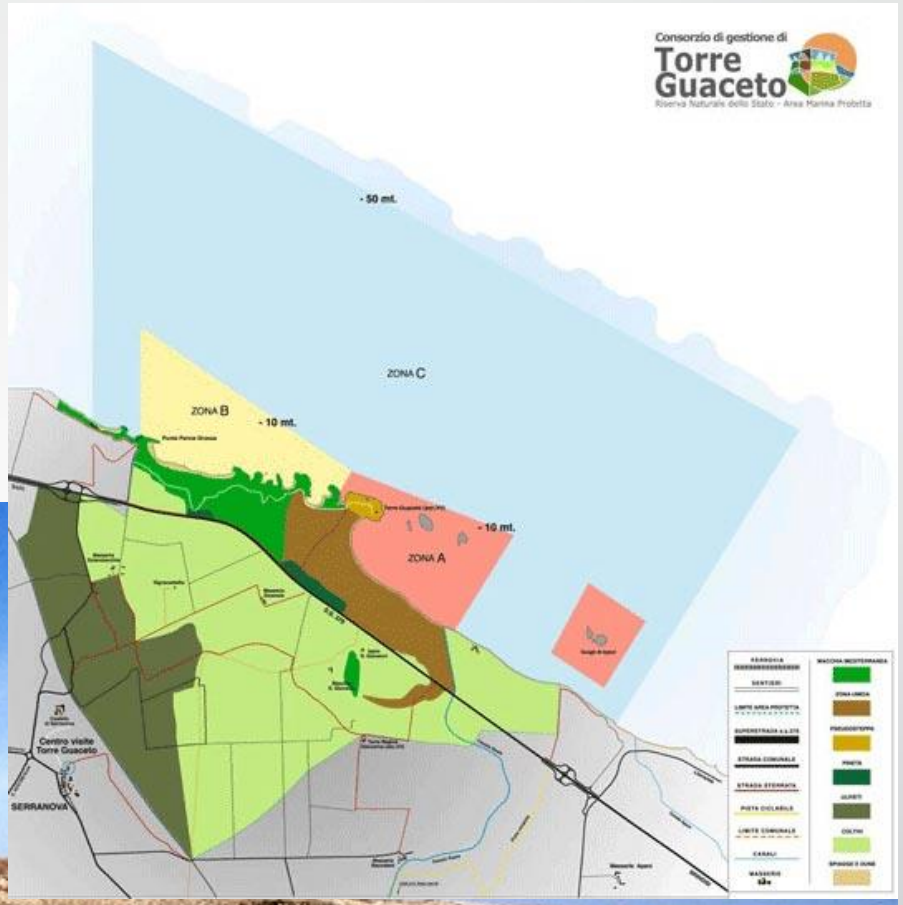
Niccolò Vendramin[1,2], Pierpaolo Patarnello[4], Anna Toffan[1*], Valentina Panzarin[1], Elisabetta Cappellozza[1], Perla Tedesco[3], Antonio Terlizzi[3], Calogero Terregino[1] and Giovanni Cattoli[1]

Munday et al., 2002

Picture: Dr Pierpaolo Patarnello

Kara et al., 2014

* Sampling Area #1: Torre Guaceto AMP



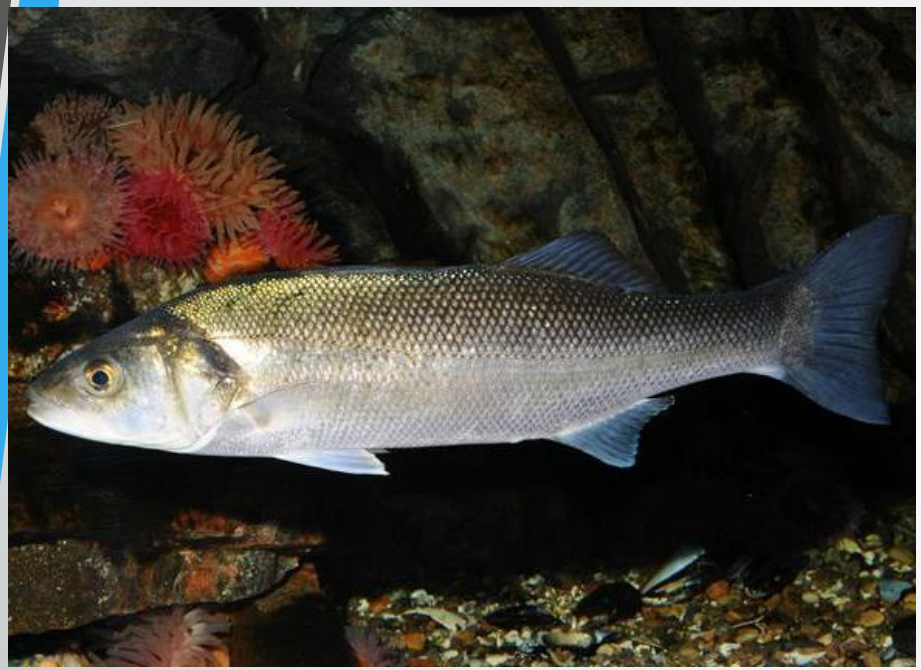
* Sampling Area #2: Porto Cesareo AMP

The detection of Betanodavirus in wild specimens from two no take-no access MPAs in the Southern Italy

Samples analysed by real-time TaqMan PCR (Panzarin et al., 2010).

Family	Species	examined/positive
Labridae	*Coris julis*	3/0
	Labrus viridis	1/1
	Symphodus mediterraneus	2/0
	Symphodus ocellatus	1/0
	Symphodus tinca	26/8
	Xyrichtys novacula	27/18
Serranidae	*Epinephelus costae*	3/1
	Serranus cabrilla	2/0
	Serranus scriba	5/0
Moronidae	*Dicentrarchus labrax*	20/3
Mugilidae	*Mugil cephalus*	1/0
Mullidae	*Mullus surmuletus*	9/2
Sparidae	*Sarpa salpa*	1/0
	Diplodus sargus	2/0
Scombridae	*Scomber scombrus*	2/0
Centracanthidae	*Spicara maena*	1/0
Scorpaenidae	*Scorpaena porcus*	2/0
Carangidae	*Trachurus trachurus*	1/0
Gobiidae		1/0

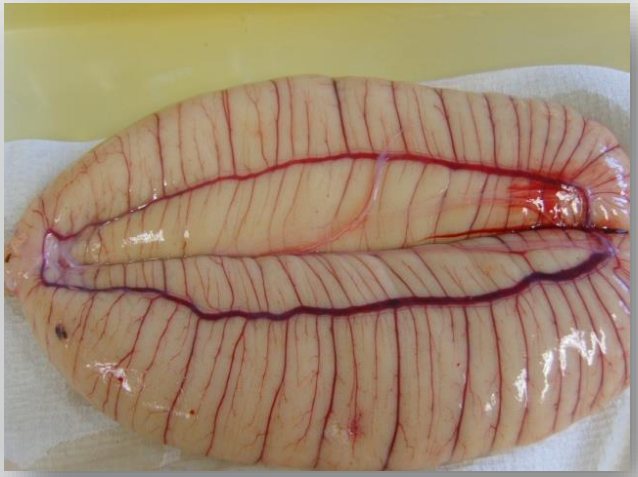
Terlizzi et al., 2011 Aquaculture

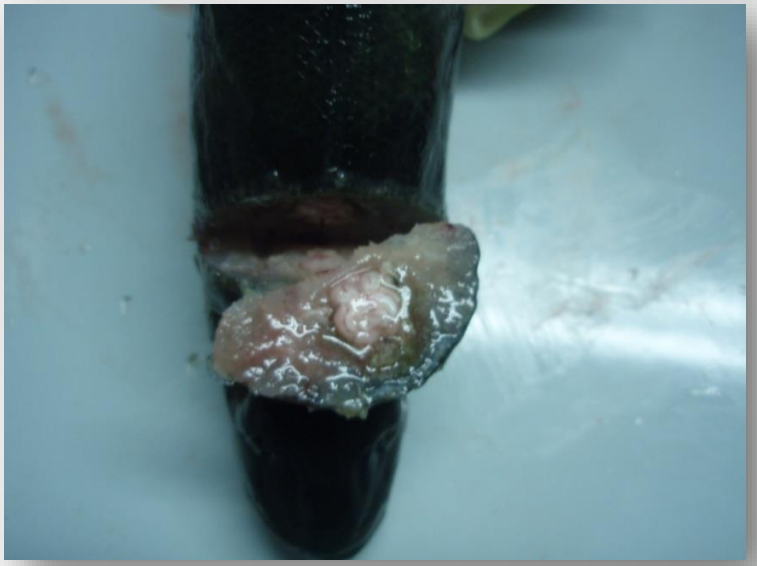


* The highlighted positivity for *D. labrax* and *E. costae* confirms the susceptibility of these species against the isolated genotype that is the same responsible for the deaths that occurred in the past against individuals belonging to these two species and other species of the genus *Epinephelus*.

- The genus *Epinephelus* is highly sensitive to betanodavirus
- *Epinephelus* sp. strongly declined in the past decades
- Many species are now considered at risk (IUCN Red List)

* **Traditionally, overfishing and illegal fishery are considered the main drivers of the decline**
* **Mass mortalities due to diseases are likely to be underestimated**





* Sampling and analyses

* Brain
* Optical nerve
* Gonads

* Real Time RT-PCR
* Histology
* IHC
* virus isolation cell culture and genotypization

Istituto Zooprofilattico Sperimentale delle Venezie

Pinna nobilis mass mortality outbreak

Mass mortality events of the endemic bivalve *Pinna nobilis* (pen shell) have been recorded in the Western Mediterranean populations over the last year and a half

They first occurred in the south-east of the Iberian Peninsula and Balearic Islands in late 2016 and have been rapidly spreading ever since, causing the mortality of around 99% of individuals in all infected populations. To date, the mortality has been confirmed along the entire Spanish coast and in several locations in Corsica and Italy

L'EMERGENZA

La Pinna nobilis muore, a Taranto è a rischio estinzione

I ricercatori del Cnr ipotizzano la moria diffusa a causa di un agente patogeno che colpisce il mollusco bivalve

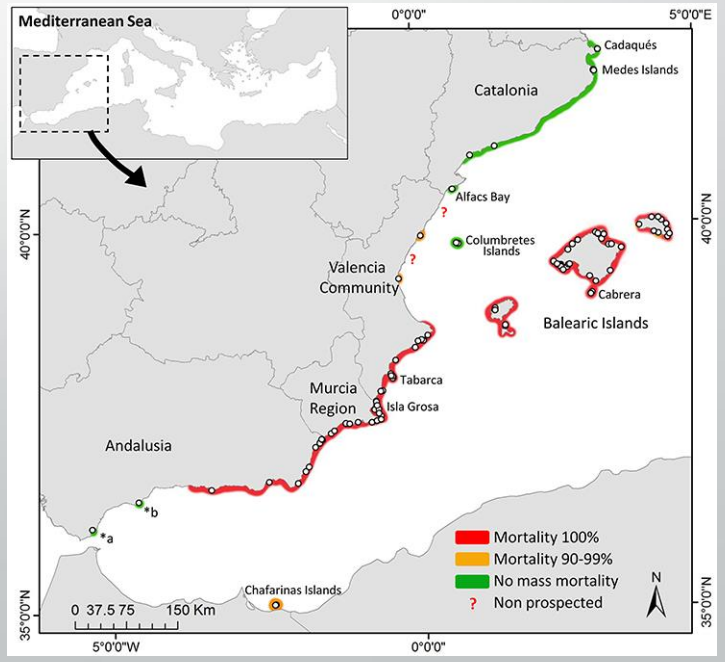
di **FABIO DI TODARO**

25 Giugno 2018

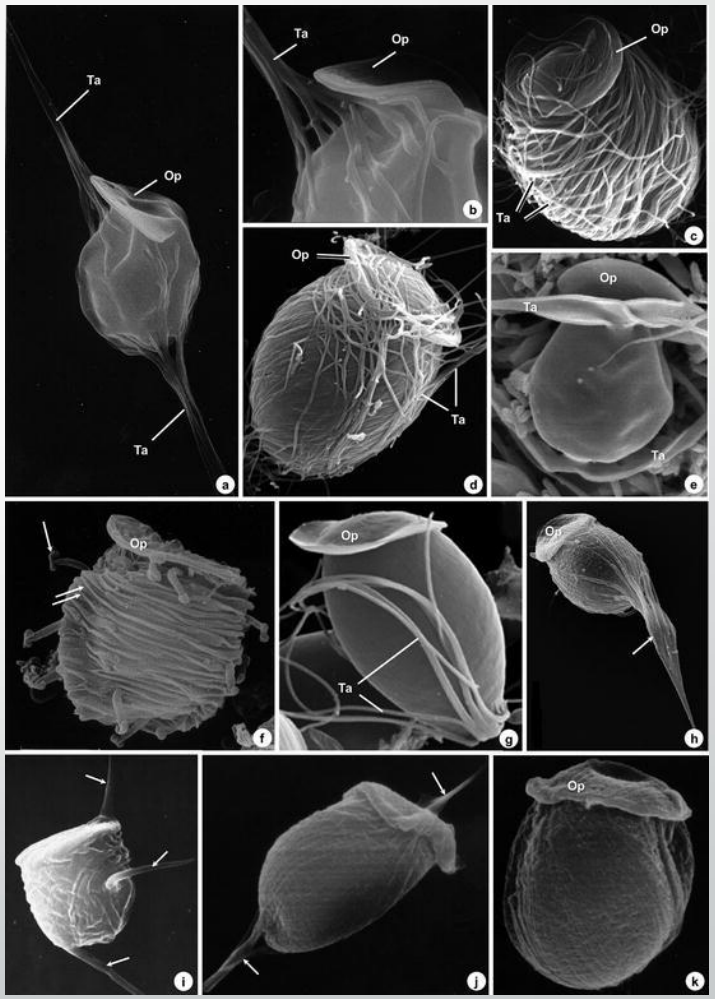
Porto Cesareo, morti migliaia di molluschi Pinna nobilis

La causa potrebbe essere la presenza di un batterio. È il più grande mollusco bivalve presente nel Mediterraneo. Il fenomeno sarebbe avvenuto negli ultimi sei mesi

di Antonio Della Rocca

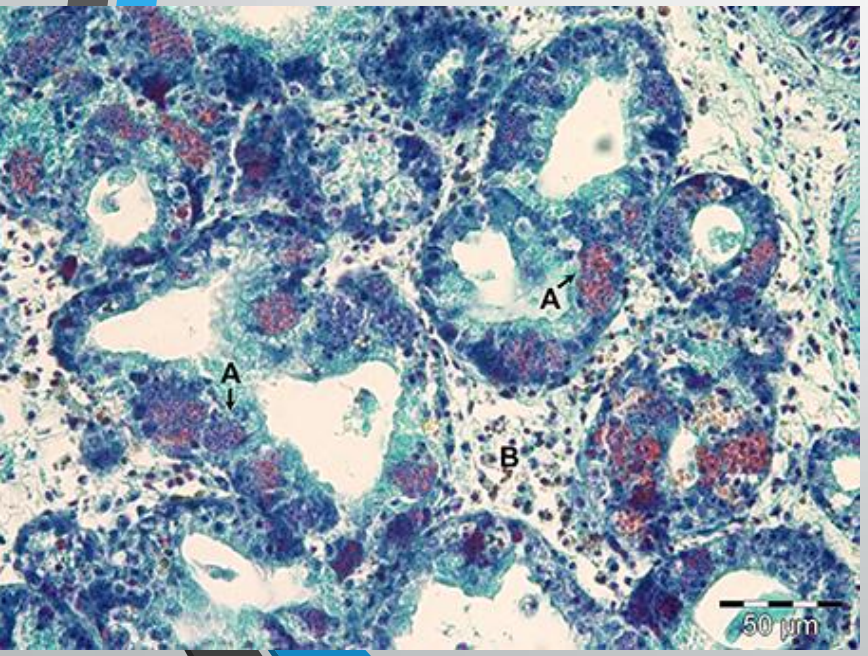


Pinna nobilis



Pinna nobilis mass mortality outbreak

Outbreak is caused by a new species of haplosporidian parasite (unknown species, under study) which is found in digestive glands of infected individuals. When infected in their natural environment, pen shells display mantle retraction, no reaction to stimuli and open valves, as they are no longer able to close their shells. The death is attributed to direct blockage of the digestive gland by the parasite and subsequent starvation of the bivalve. Once the populations are infected, the likelihood of survival of the individuals is very low, with no possibility of creating buffer zones.



Digestive gland of *P. nobilis*. Mature ellipsoidal uninucleate spores were found in the epithelia of digestive tubules **(A)**. A heavy inflammatory host response, with infiltration of the connective tissue by hyaline hemocytes, was associated with infection **(B)**. Note that the epithelium height of the digestive tubules was reduced, resulting in a wider lumen.

Recommendations from the IUCN Centre for Mediterranean Cooperation

1. INCREASE MONITORING FREQUENCY in Western and Central Mediterranean. The monitoring should be conducted particularly every month during summer time, including:

- Monitor (by visual means) the status of the populations of *Pinna nobilis* and *Pinna rudis* on a monthly basis.
- Carry out tissue biopsies (histological and molecular analysis) of the populations in each area to find out the presence or absence of the parasite. Even if individuals look healthy, is possible that the parasite just arrive.
- Report any unusual changes in the populations. If a mass mortality of the population is observed, a rescue programme to the populations that have not being affected (and with the confirmation that the parasite is not present) should be put in place. The report information of these mortality events would also allow us to alert and prepare other areas.

2. PREPARE A RESCUE PROGRAMME

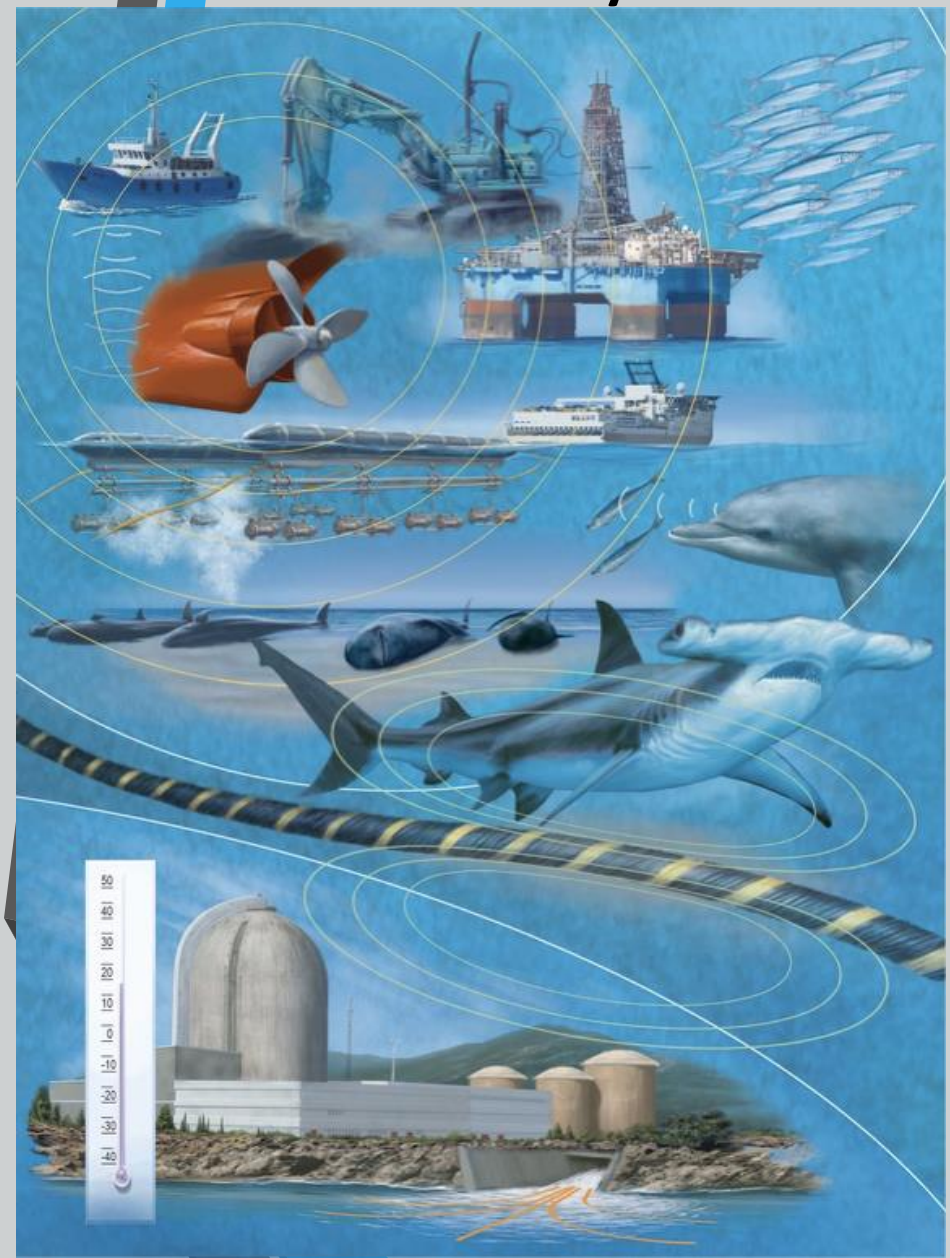
- The development of a rescue programme closed to the affected areas is paramount and it should be developed as soon as possible in areas where there are important density of *Pinna nobilis* and the parasite has confirmed not arrived. These measures should be conceived respecting the different local realities and in relation to the evolution of this highly dynamic phenomenon. Rescue programme should be targeted to adults and juveniles.
- To enhance the resilience of natural *Pinna nobilis* populations, individuals could be protected by exclusion cages to decrease predation. Reducing anthropogenic impacts and declaring protected areas or microreserves around *Pinna nobilis* populations in these areas are key to the survival of the species if the spread of the parasite continues.

  Captivity breeding programme for future restocking of resistant juveniles could be a possibility. For this, is important to complete the hatchery culture of the pen shell.
- Monitoring the natural recruitment with larvae collectors as well as using/testing the ropes of aquaculture farms

3. RAISE THE ISSUE AT NATIONAL LEVEL AND ADVOCATE FOR THE DEVELOPMENT OF A RESCUE PROGRAMME that would target both adult and juvenile specimens. First rescue programmes are already underway in Spain and France, with the aim of studying the pathogen under different conditions, starting a captivity breeding programme and exploring the possibility of transplantation of the juveniles.

4. COLLABORATE IN THE IDENTIFICATION OF PINNA NOBILIS HOTSPOTS in the entire region. Populations with high densities in areas that still haven't been affected by the pathogen, should be priority sites for the early implementation of management measures

Introduction of energy (including underwater noise) does not adversely alter the ecosystem



SOURCES OF NOISE IN MARINE ENVIRONMENT



100 Hz - 100 KHz

Natural

- Earthquakes
- **Volcanic explosions**
- Wave
- Thunder
- Wind

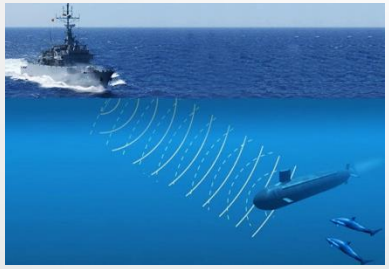
Bigclaw snapping shrimp *(Alpheus haterochaelis)*

Biological

Noise created by several marine species to feed, communicate, orient and protect themselves

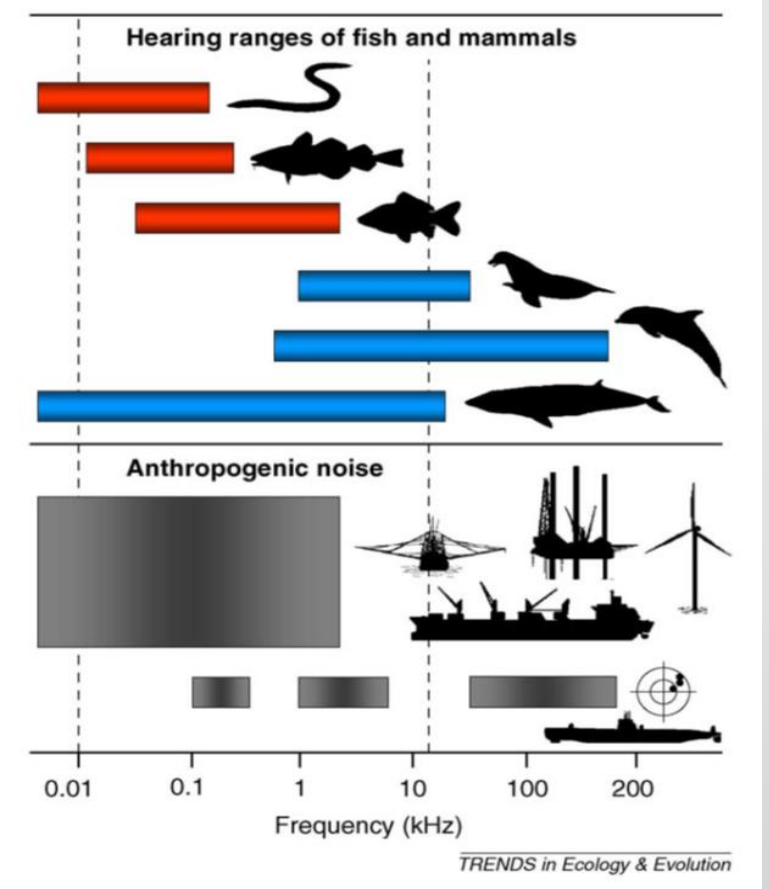
UNDERWATER SOURCE OF NOISE

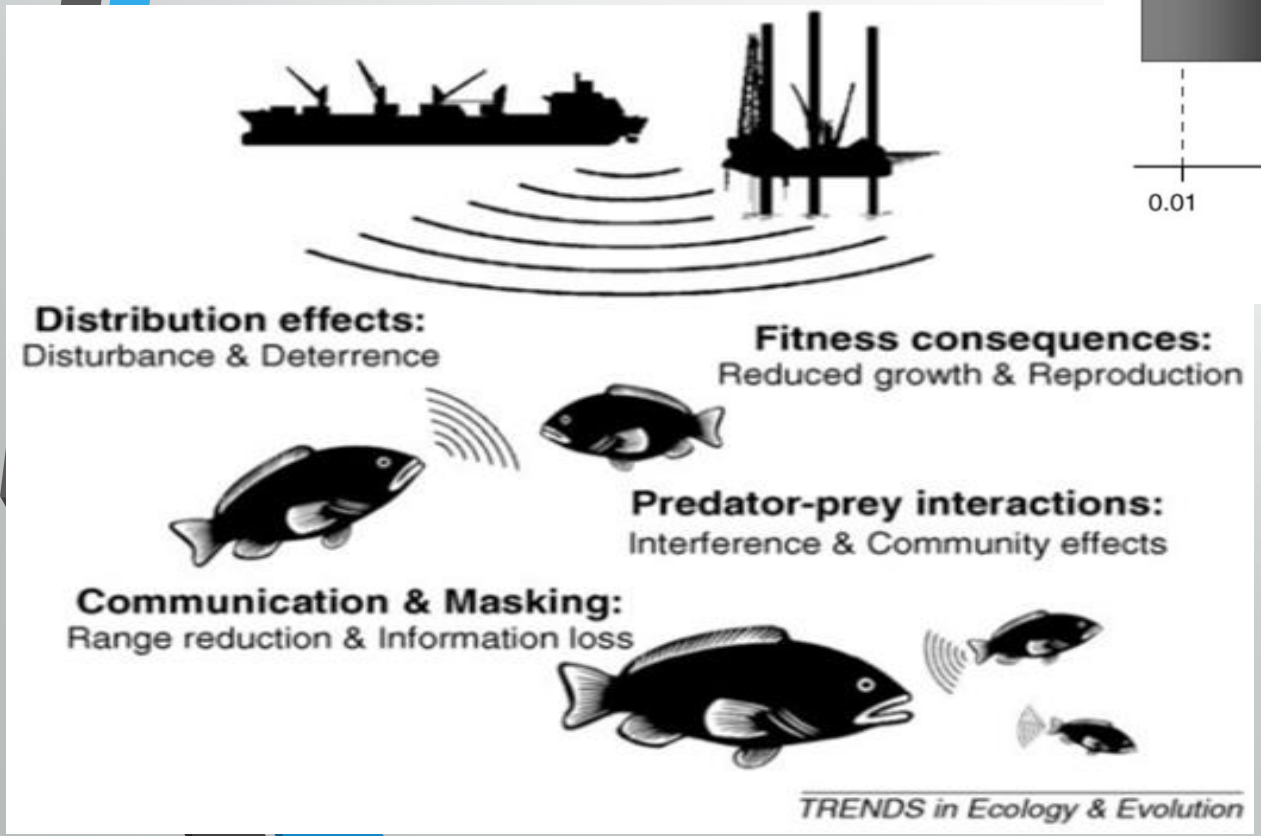


ANTHROPOGENIC

- Nuclear explosion
- Seismic survey
- Maritime traffic and fisheries
- Drilling
- Military sonar
- Wind farms
- Scientific explorations
- Coastal building development


Hearing ranges of fish and mammals
Anthropogenic noise
0.01
0.1
1
10
100
200
Frequency (kHz)
TRENDS in Ecology & Evolution


Distribution effects:
Disturbance & Deterrence
Fitness consequences:
Reduced growth & Reproduction
Predator-prey interactions:
Interference & Community effects
Communication & Masking:
Range reduction & Information loss
TRENDS in Ecology & Evolution

IMPACT ON MARINE ANIMALS

PHYSIOLOGICAL

BEHAVIOURAL

- BEACHING
- ALTERATION OF BEHAVIOUR
- DIFFICULTY IN MATING AND FEEDING
- ANTAGONISM
- ESCAPE FROM NATIVE AREA

PERCEPTUAL

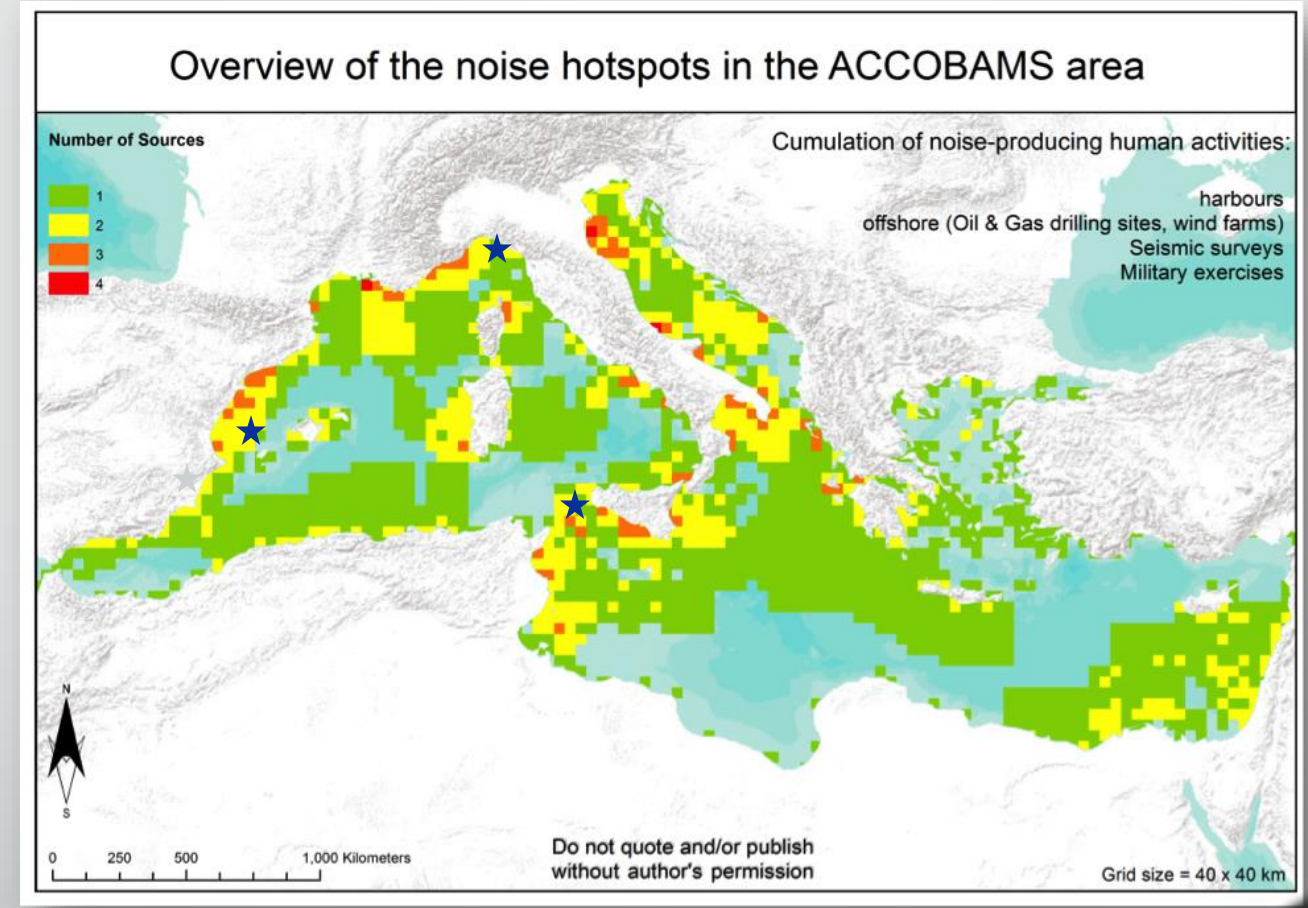
- MASKING OF ACOUSTIC SIGNALS NEEDED FOR INTRASPECIFIC COMMUNICATION
- MASKING OF OTHER IMPORTANT SOUNDS SUCH AS THOSE EMITTED FROM PREDATORS
- INTERFERENCE WITH ECOLOCALIZATION CAPACITY

CHRONIC

- CUMULATIVE AND SYNERGIC IMPACTS
- IPERSENSITIVE TO NOISE
- INUREMENT TO NOISE

Jasny et al., 2005

MAP OF UNDERWATER NOISE IN THE MEDITERRANEAN SEA



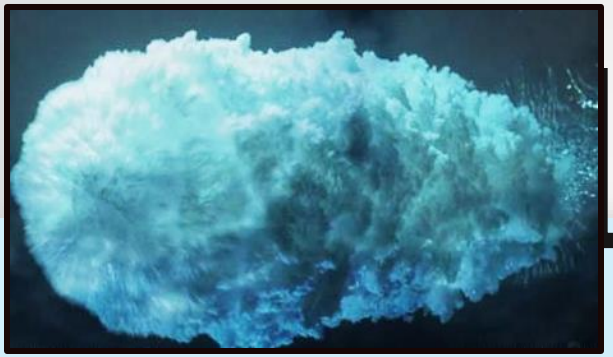
CRITICAL AREAS:

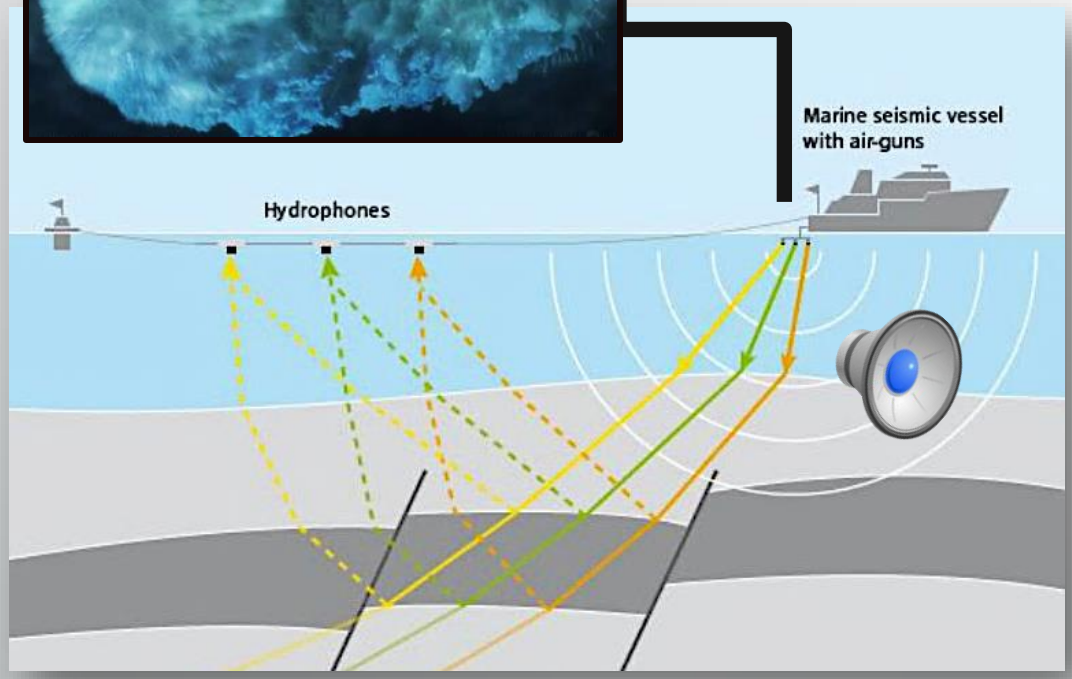
- Pelagos Sanctuary;
- Ligurian Sea;
- Sicily channel and parts of Hellenic Trench;
- Water body between Balearic Island and continental Spain

ACCOBAMS

Agreement on the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and Contiguous Atlantic Area

SEISMIC SURVEY (AIRGUNS)





Frequency: 20 Hz - 150 Hz
Source level: 260 dB

every 9 -10 seconds
Time interval: months

To detect:

- Ship
- Airguns – Source
- Streamer } Reception system
- hydrophones

EFFECTS ON CETACEANS

Most common effect → **Mass beaching**

"Gas and fat embolic syndrome: rapid emersion cause auditory disorders and death from embolism"

- ✓ 10/12/2009: beaching of 9 sperm whales on the Gargano coast (2 saved – 7 dead).
- ✓ 12/09/2014: beaching of 7 sperm whales in Vasto (4 saved – 3 dead).

EFFECTS ON CETACEANS



- Goold, 1996: Escape of *Delphinus delphis* individuals during seismic prospecting in the Ireland Sea
- Engel et al., 2004: Beaching of *beaked whales* in California and *humpback whale* along the Brazilian coast in 2002, recorded after geophysical prospecting
- Bowels et al., 1994: Reduction of sperm whale's clicking, with the interruption of feeding due to the seismic impulses coming from a ship distant more than 300 km away; perceived sounds were of 115dB
- Parente et al., 2007: Significant reduction of cetacean species diversity with increased number of geophysical prospecting in Brazil (1999-2004)

MASS BEACHING IN 2015

TOTAL BEACHING
4703

TOTAL INDIVIDUALS
4821

(1986-2015)

LEGENDA

- Balaenoptera
- Physeter macrocephalus
- Grampus griseus
- Stenella coeruleoalba
- Tursiops truncatus
- Ziphius cavirostris
- Indeterminato
- Altre Specie

ACCOBAMS

MINISTERO DELL'AMBIENTE

Fonte: Banca Dati Spiaggiamenti 2015

BIOLOGICAL ROLE OF SOUNDS FOR OTHER MARINE SPECIES (NO CETACEANS)

Bony fish (Teleostei)
Phylum: Chordata
Class: Actinopterygii

- Sensitive to sounds between 100 Hz and 2 kHz
- Bladder and otoliths
- For predation, defense of territory, intra-specific relationship, direction, swimming and escape from predators

Cartilaginous fish (Elasmobranchii)
Phylum: Chordata
Class: Chondrichthyes

- Perception of sounds between 200 Hz and 600 Hz
- Use of low-frequency sounds to localize preys

Crustacea
Phylum: Arthropoda

- Response to acoustic signals
- Internal mechanoreceptors for the reception of acoustic vibrations / Snapping Shrimp - Alpheidi

Phylum: Mollusca
Classes: Bivalvia, Caphalopoda, Gastropoda, Scaphopoda, Monoplacophora

- Reception of acoustic stimuli - Cephalopods

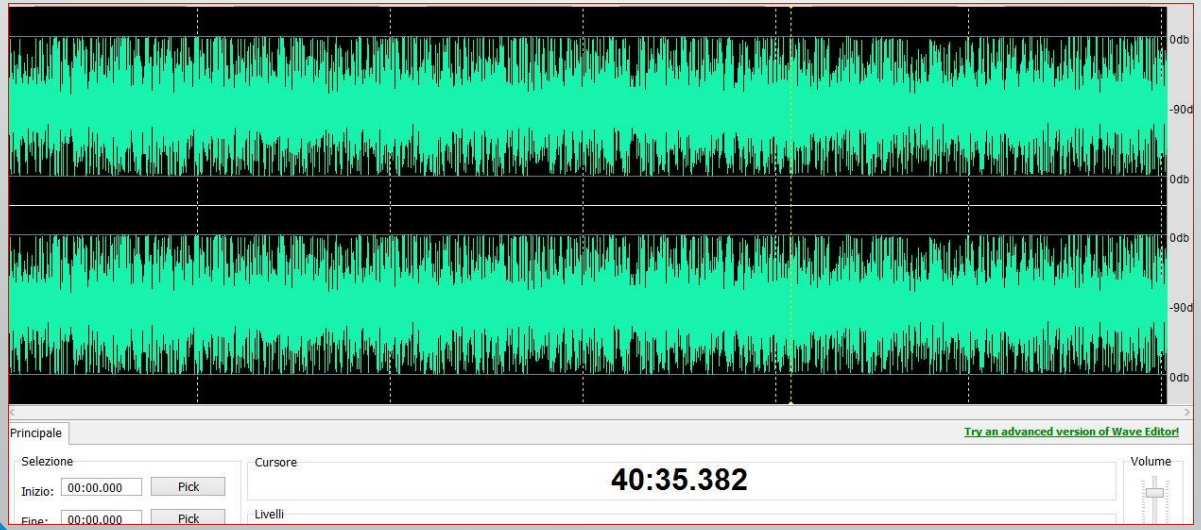
Phylum: Cnidaria
Classes: Anthozoa, Cubozoa, Hydrozoa, Scyphozoa

- Organs for receiving noises produced by nearby fish in order to capture them with stinging tentacles – Sea Anemones

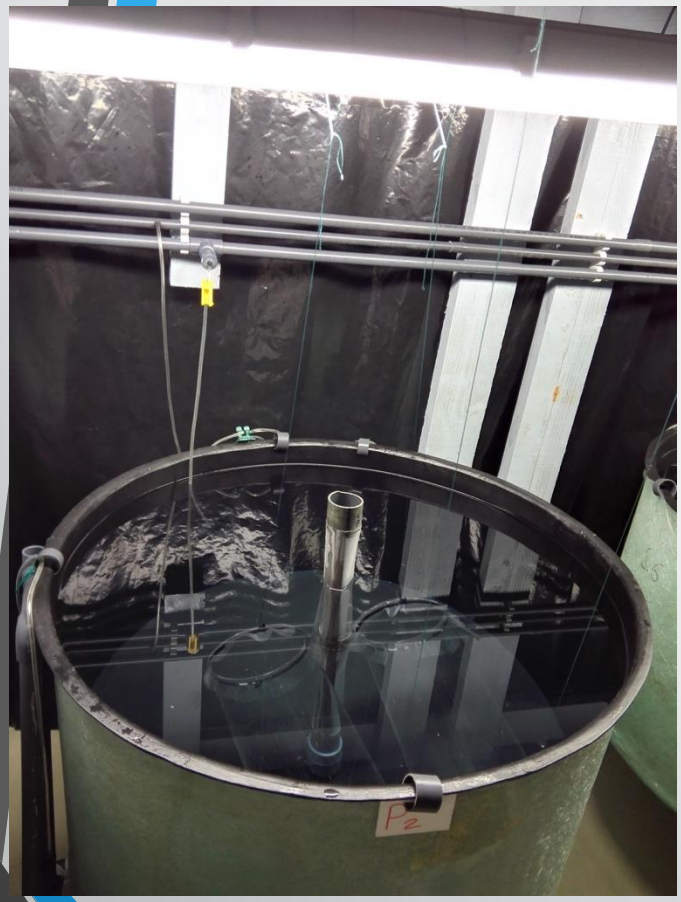
Effects on other species (no cetaceans)

- McCauley et al., 2003: Irreversible damage to the inner ear in several fish species → compromised survival
- Engas et al. 1996; Hirst et al., 2000; Wardle et al., 2001: Reduction of fish catches that remains for many days after the end of the activities; reduction of eggs probably caused from the prolonged exposure to low-frequency sounds
- Pearson et al., 1992: Alarm reactions (change of direction and speed of swimming) in some species of scorpionfish *(Sebastes sp.)* to the noise from airguns remaining about 60 min after switching off the source.
- McCauley et al., 2000: *Marine turtles*, alarming behavior and escape responses as reaction to reaction to sound impulses of airguns and increased number of sightings during non-activity periods
- MacKenzie, 2004: mass beaching of *giant squids* along the Spanish coast; damage in inner organs were observed in analysed individuals (seismic survey, 2001 and 2003)

The Habitat Soundscape



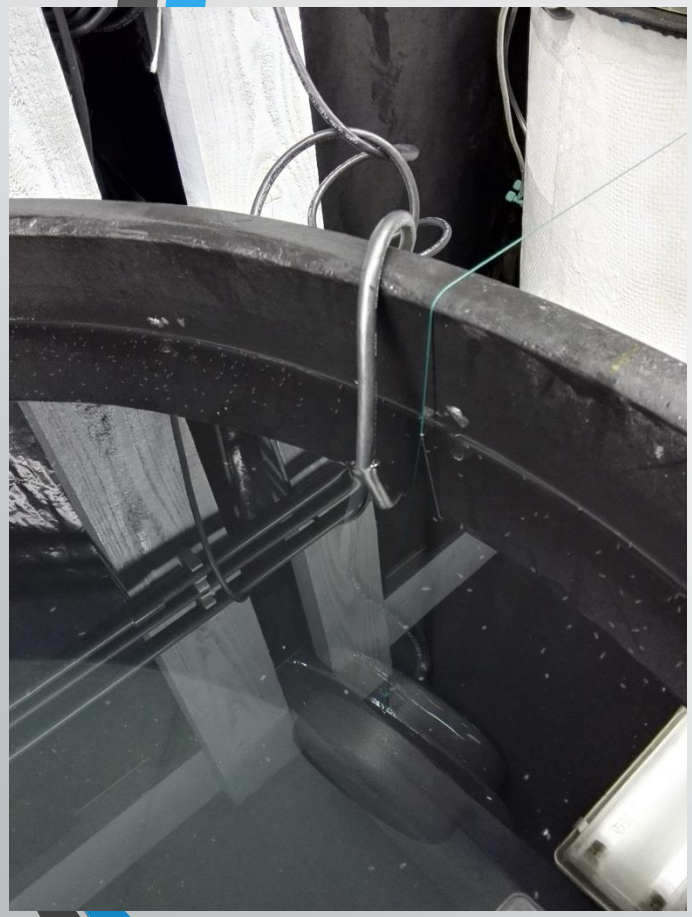
Experimental setup

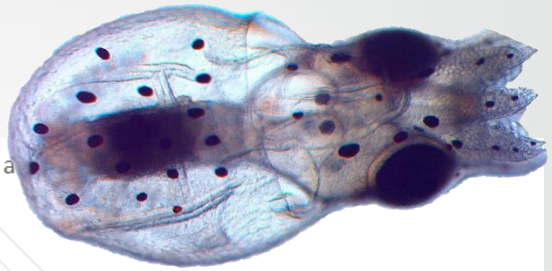


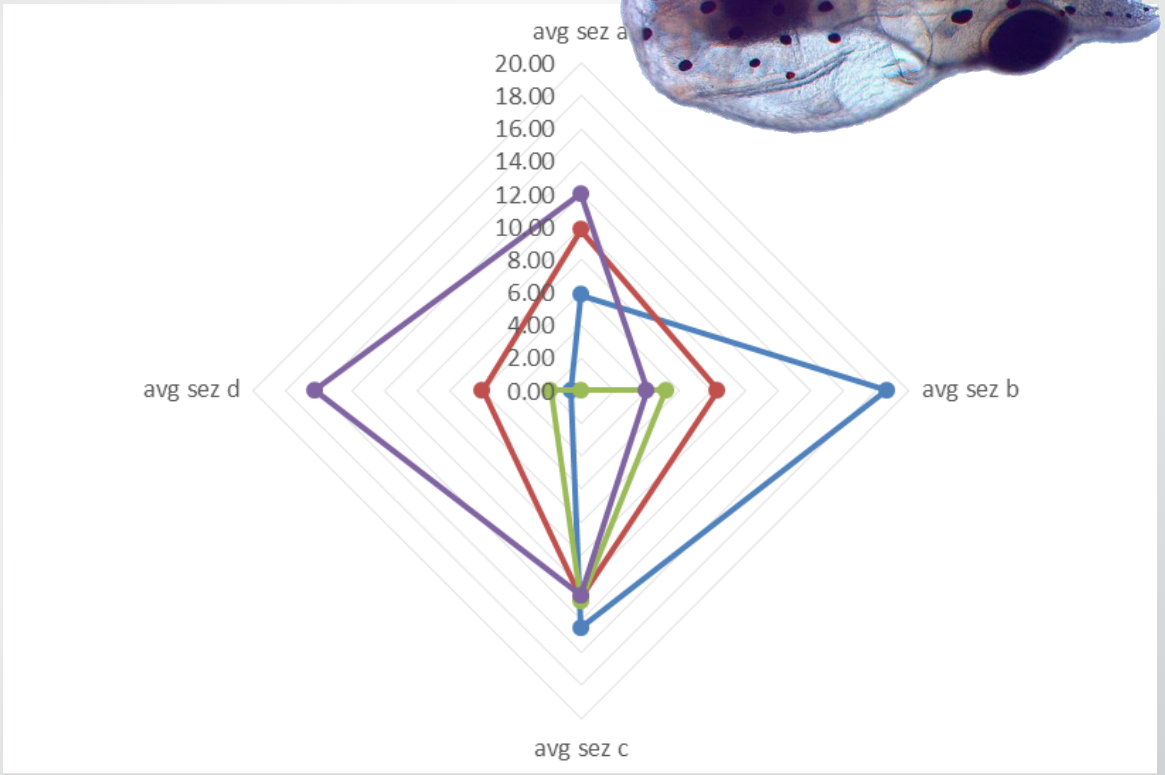
Octopus hatchlings (mean body length: 1.45 ± 0,1 mm) were reared in 500 L fibreglass cylinder conical tanks of a flow-through seawater system with a 60 mL min-1 water flow, at a density of 2 paralarvae L-1 (1000 individuals per tank). Paralarvae were fed twice a day with enriched *Artemia* (final concentration in tank: 0.3 art/ml).

A light intensity of 200 lx and a photoperiod of 12L : 12D (light from 8:00 a.m. to 8:00 p.m.) were maintained throughout the experiment. Green water technique was applied to all rearing tanks, daily adding 200,000 cells mL-1 of *Chlorella* spp. to each tank, prior lights were turned on. The mean water temperature of the rearing tanks was 22.42 ± 0.26 ° C, salinity was 36.8 ± 0.14 g L-1 and dissolved oxygen was near saturation.

Results







Blue : control, day 0;
Green : experimental, day 0;
Redlines: control, day 3;
Purple lines: experimental, day 3.

Results

Gene	Protein description
sod	superoxide dismutase
gst	glutathione S-transferase
gsh-s	glutathione synthetase-like isoform X3
gpx	glutathione peroxidase
NFkB	NFkB protein
tnf	lipopolysaccharide-induced TNF-alpha factor
piezo2	piezo-type mechanosensitive ion channel component 2
vglut1	vesicular glutamate transporter 1

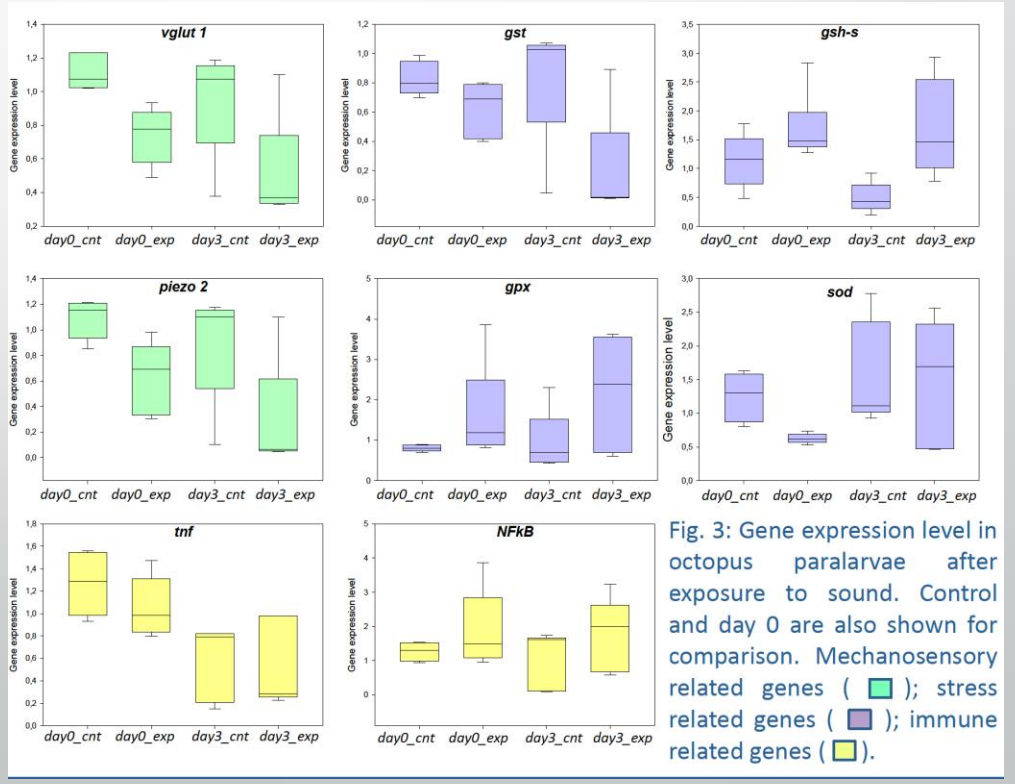
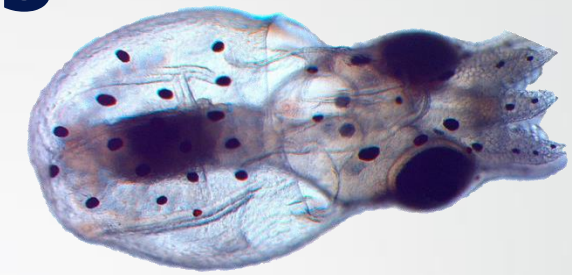


Fig. 3: Gene expression level in octopus paralarvae after exposure to sound. Control and day 0 are also shown for comparison. Mechanosensory related genes ( ); stress related genes ( ); immune related genes ( ).

Conclusions



Paralarvae significantly distributed in proximities of the origin of the sound compared to “silent” conditions (G: 18,760; $p < 0.0001$).

Gene expression analysis of mRNA from paralarvae at different time points and different conditions showed that genes related to mechanosensation (*piezo 2* and *vglut1*) are down-regulated after exposing to sound

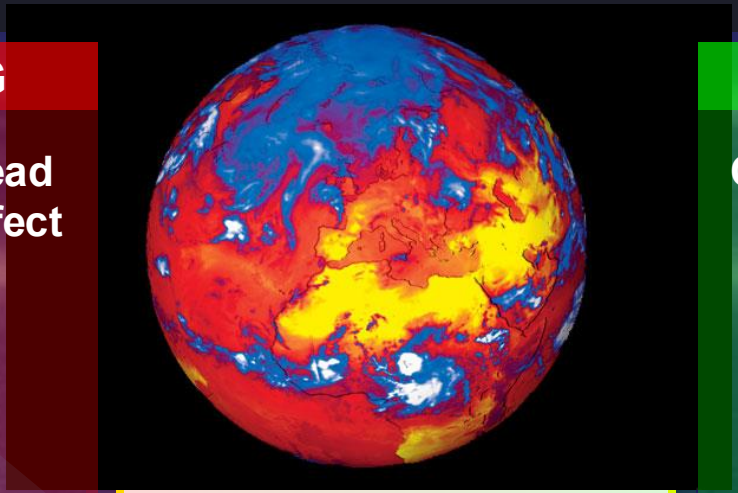
No-changes in the stress related genes, i.e. *sod, gpx, gst* and *gshs*.

Immune related genes (*tnf* and *nfkb*) show no difference between the two conditions.

EMERGING ENVIRONMENTAL ISSUES: CLIMATE CHANGE

GLOBAL WARMING

Higher CO_2 emissions lead to higher greenhouse effect



OCEAN ACIDIFICATION

Ocean took up about 30% of anthropogenic CO_2

DIRECT EFFECTS ON MARINE ORGANISMS HEALTH STATUS

MULTIPLE STRESSORS AND MULTIPLE LEVELS