A novel approach for the detection and quantification of phthalates in marine environments

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Plastic and Micro plastic in the ocean

Huge amount of plastic material is carried away into the ocean every year

It severely effects marine organisms

Phthalates are plastic additives known to be as Endocrine disruptors

Phthalates are easily leach into the environment

The ocean has a very high dilution factor

Bio-Accumulators as *in-situ* concentrators

Who are Ascidians and why using them as bio-indicators?

- ✓ Phylum: Chordata, subphylum: Tunicata, class: Ascidiacea
- ✓ Sessile filter-feeding invertebrates
- ✓ **Filter high volumes of water – approx. 100L/day**
- ✓ **Retain micro particulate matter**
- ✓ **Very successful invaders with wide distribution – present globally**

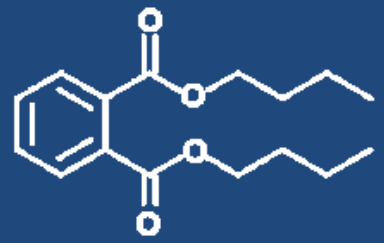
Microcosmus exasperatus

Photo: Rossana Rocha Animation: Gal Vered

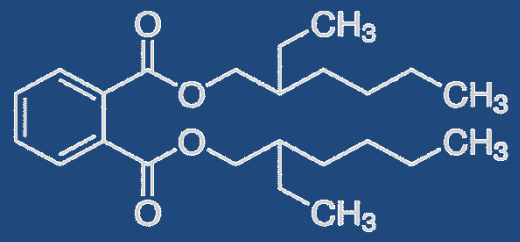
Research Goals

1. Developing a method for detection and quantitation of phthalates di-esthers from ascidians body
2. Implementing the method to examine ascidians, sampled at different sites, along the Israeli coastline

Target compounds: Dibutyl Phathalate, Bis(2-ethylhexyl) Phthalate, Di-n-octyl Phthalate



DBP



DEHP

DnOP

Recent studies on phthalates detection in aquatic organisms

Animal and Environment	Di-Esther Phthalates [ng/g]	Extraction Method	Detection Method	Reference
Atlantic blue fin tune, muscle, Mediterranean sea	DEHP: 9.14 ± 3.27	Ultra-Sonic Bath	HPLC-MS	Guerranti, et al. 2016
superficial neustonic/planktonic, Mediterranean sea	DEHP: 18.38 ± 44.39	Ultra-Sonic Bath	HPLC-MS	Fossi et al. 2012
superficial neustonic/planktonic, Mediterranean sea	DEHP: 23.42 ± 32.46	Ultra-Sonic Bath	HPLC-MS	Fossi et al. 2012
Rutilus rutilus (a fish), liver, Orge river, France	DEHP:3,052 ± 3,854 DnOP: 653 ± 1,285	Ultra-Sonic Bath	GC-MS	Valton et al., 2014
Rutilus rutilus (fish), muscle, Orge river, France	DEHP:523 ± 309 DnOP: 245 ± 239	Ultra-Sonic Bath	GC-MS	Valton et al., 2014

Agilent GCMS 6890/5973 parameters

Injection volume: 2µL

Inlet mode: Pulse splitless 20psi for 30sec

Column head pressure: 8psi

Column: Restek RXI-1ms. 15mX250µmX0.25µm

Oven:

Stage	°C/min	Temp. °C	Hold[min]
init	-	70	1
Ramp	15	320	0
Post run	-	320	9

Agilent GCMS 6890/5973 parameters (cont.)

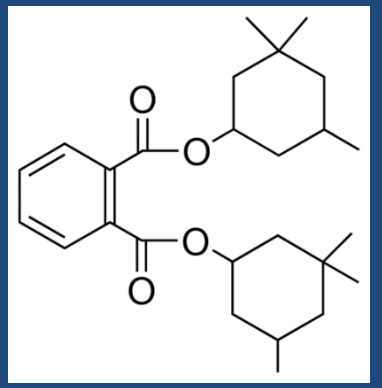
Acquisition mode: SIM

Solvent delay: 5.0min

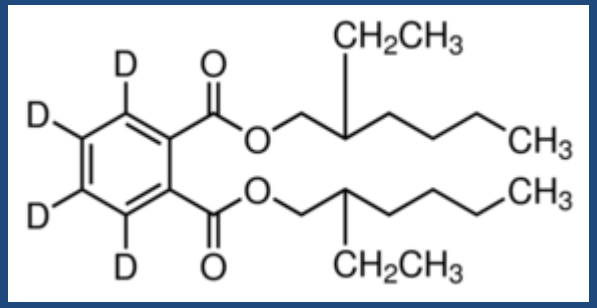
Compound	m/z (for quantitation)	m/z (qualifier)	RT [min]
DBP	149.05	205.10 223.10	8.701
DEHP	149.05	167.05 279.10	12.060
DnOP	149.05	279.10	12.942
TMCHP (internal standard)	149.05	109.00	12.602
DEHP-d4 (surrogate)	153.05	171.00 283.20	12.054

Internal standard and surrogate

- ✓ Internal standard: Trimethylcyclohexyl phthalate (TMCHP) Not expected to be present in samples (rare use in industry)

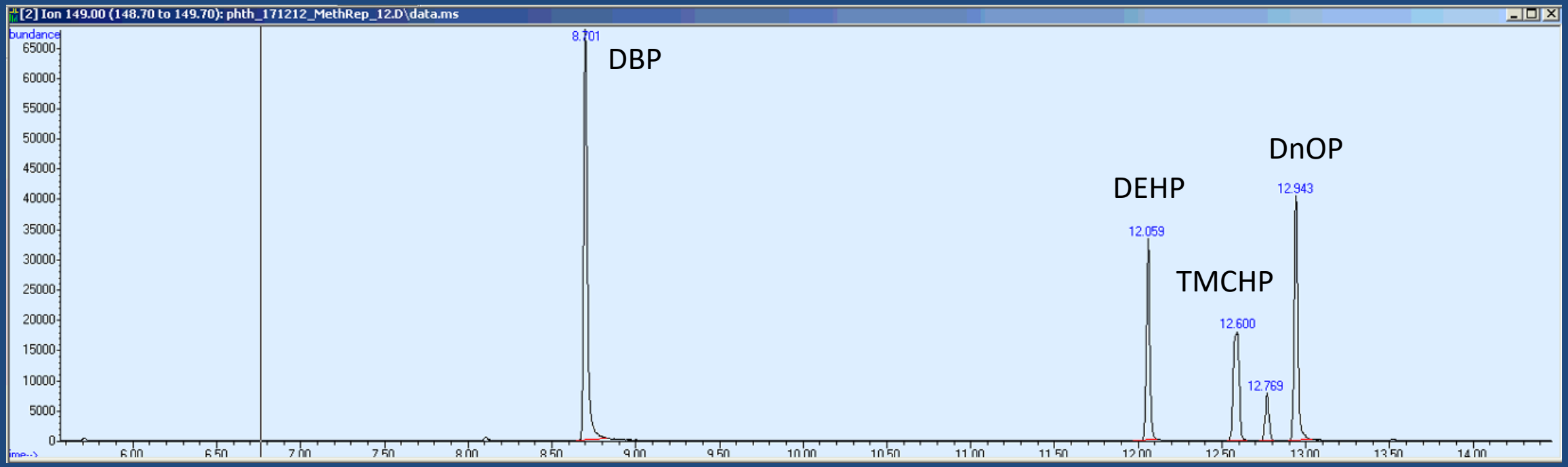


- ✓ Surrogate: DEHP-3,4,5,6-d_4 Labeled isotopic standard for the extraction efficiency



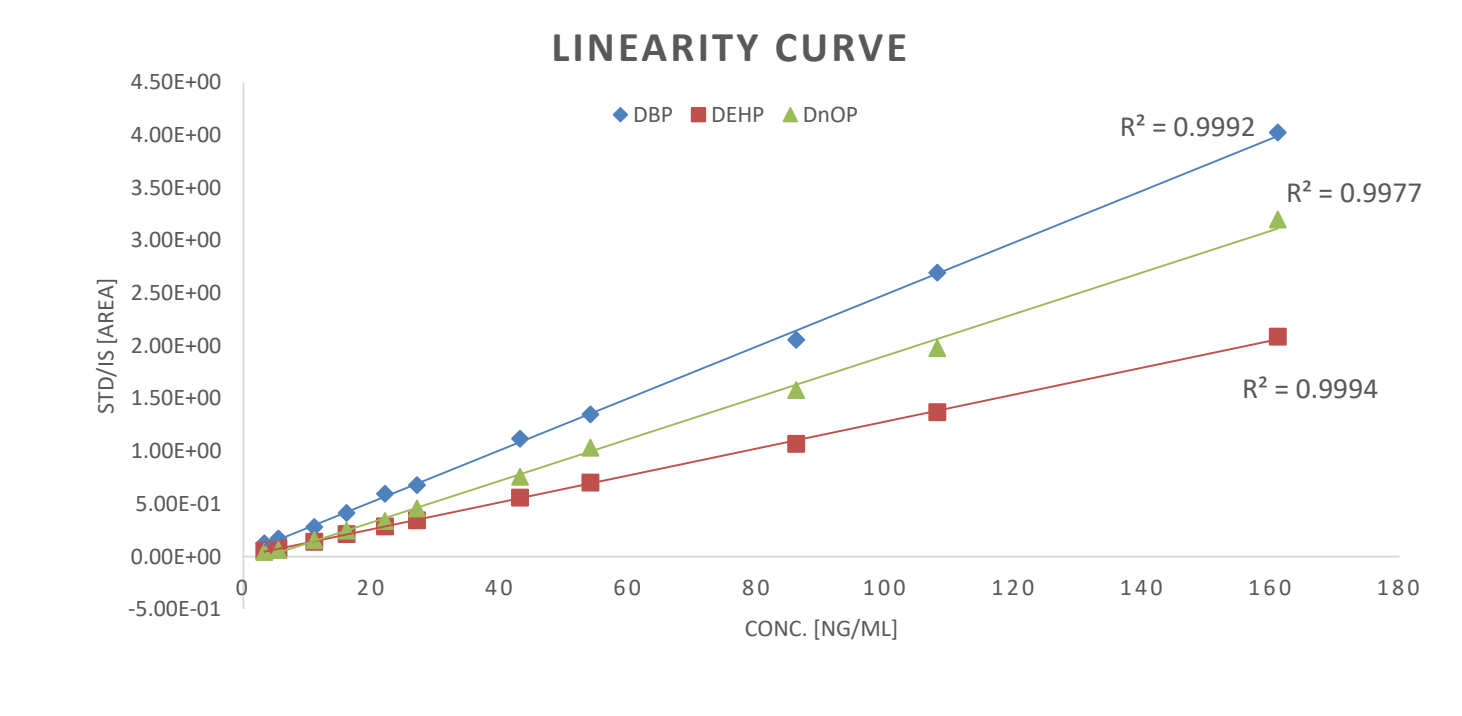
Typical GCMS standard chromatogram

m/z=149



Linearity, LOQ & Range

Linearity:



LOQ: 3.2ng/mL

Method range: 3.2-269ng/mL (***eq. to 80-6725ng/g dry weight sample***)

Sample Preparation

Pressurized Liquid Extraction (PLE):

- ✓ Accelerated Solvent Extraction (Thermo ASE™ 350)
- ✓ Stainless steel cell filled with dry sample and a filler
- ✓ High temperature (up to 160°C)
- ✓ High pressure (~1500psi)
- ✓ Solvent induces in cell under extreme conditions

Sample Preparation (cont.)

ASE™ Optimized Parameters:

Cell:	10mL with glass-fiber filter
Filler:	Diatomaceous earth
Solvent:	Hexane
Temperature:	120°C
Static time:	10min
Cycles:	2
Volume obtained:	~20mL

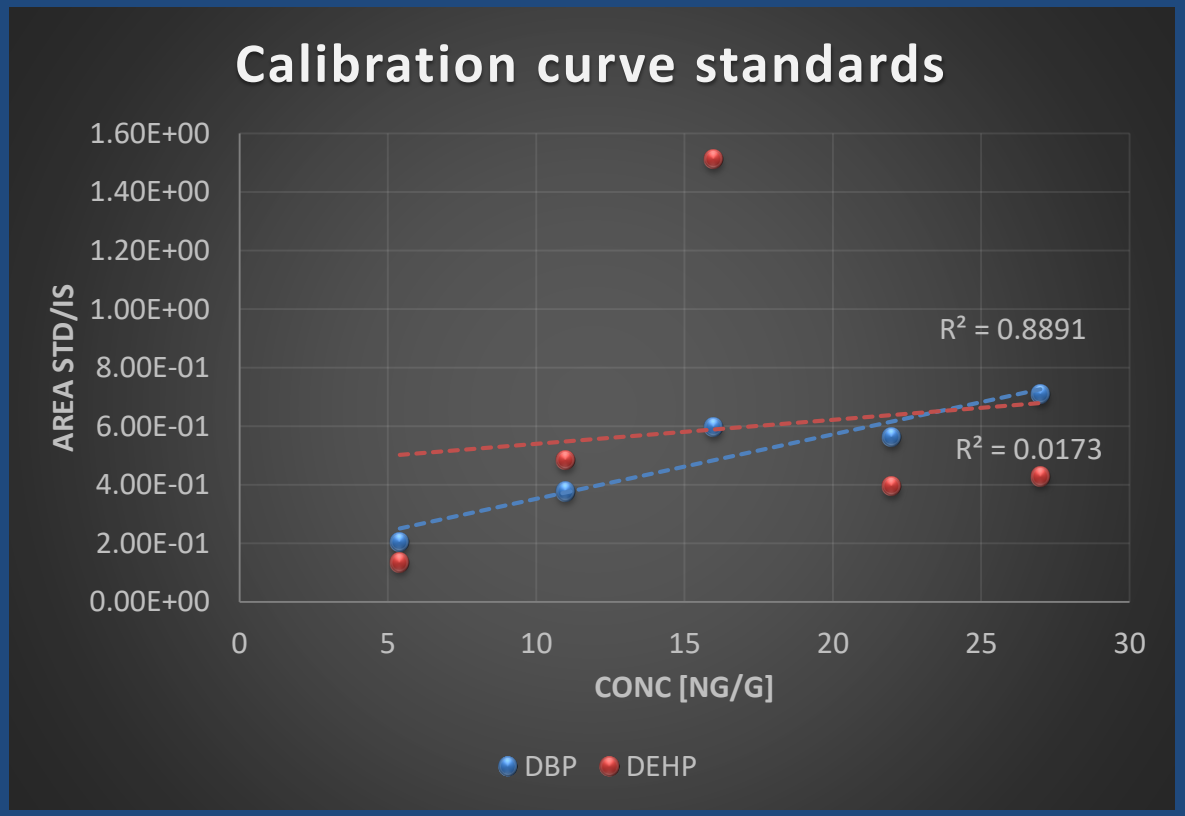
Sample Preparation (cont.)

Final Procedure:

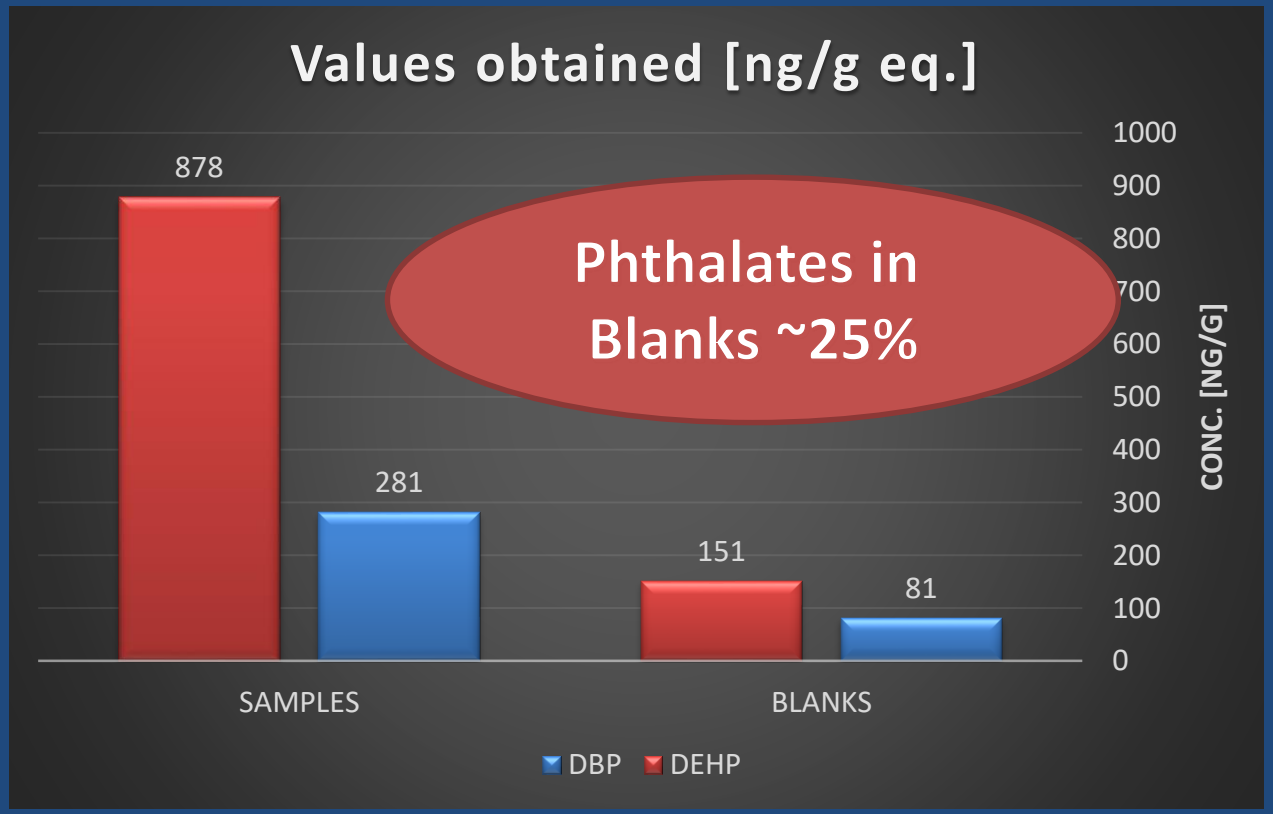
1. Sample ascidians and separate body from tunic
2. Freeze dry Ascidians samples
3. Mill sample to powder
4. Weight ~1.0g of dried sample
5. Fill the ASE™ cell with sample mixed with diatomaceous earth
6. Spike surrogate to the cell (~6µg of DEHP-d4)
7. Extraction by ASE™
8. Transfer extracted solvent to 25mL volumetric flask
9. Add 1.0mL internal standard (TMCH at ~5 µg/mL) and make up to volume with hexane

Phthalates contamination – glassware and samples

Phthalates in calibration curve:
(Origin: residue on glass?)



Phthalates in BLANK samples:
(Origin: Seals? Filler?)



Phthalates contamination – glassware and samples (cont.)

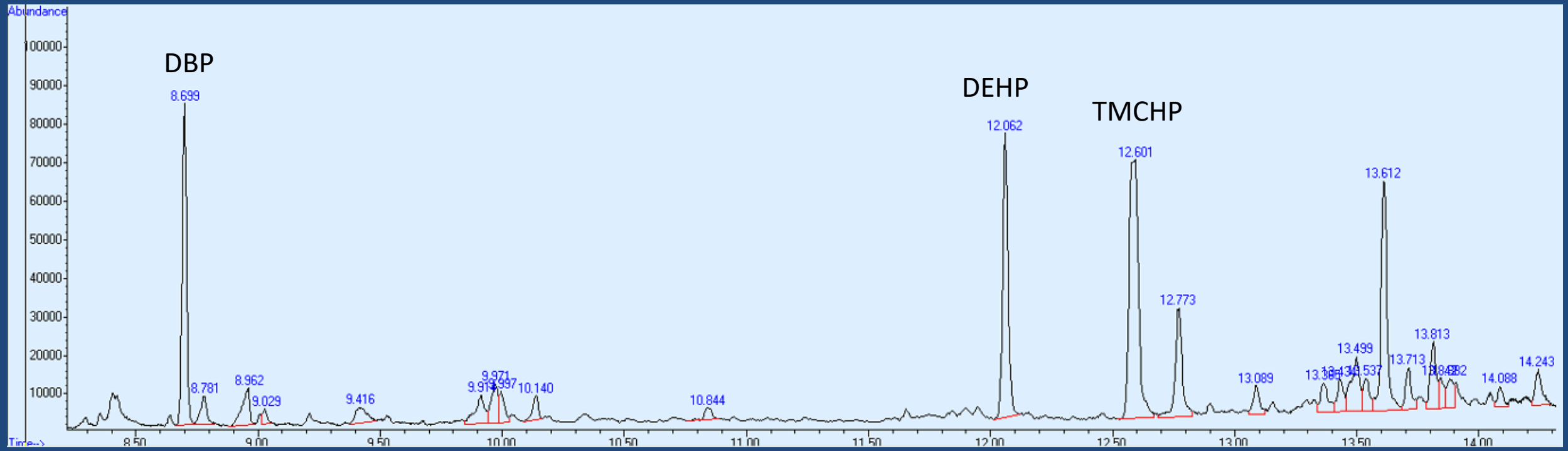
- ✓ **NO PLASTIC USED!!**
- ✓ Full extraction of blank cell (only filler) prior to sample load
- ✓ Use of the same equipment (including seals)

- ✓ Cleaning procedure for all glassware:
  - ➢ DCM
  - ➢ Acetone
  - ➢ Oven at 200°C overnight (>8hr)
  - ➢ Hexane (just before use)

Phthalates in Blanks Reduced to <5%

Typical sample chromatogram

m/z=149



Method Repeatability (homogenous sample)

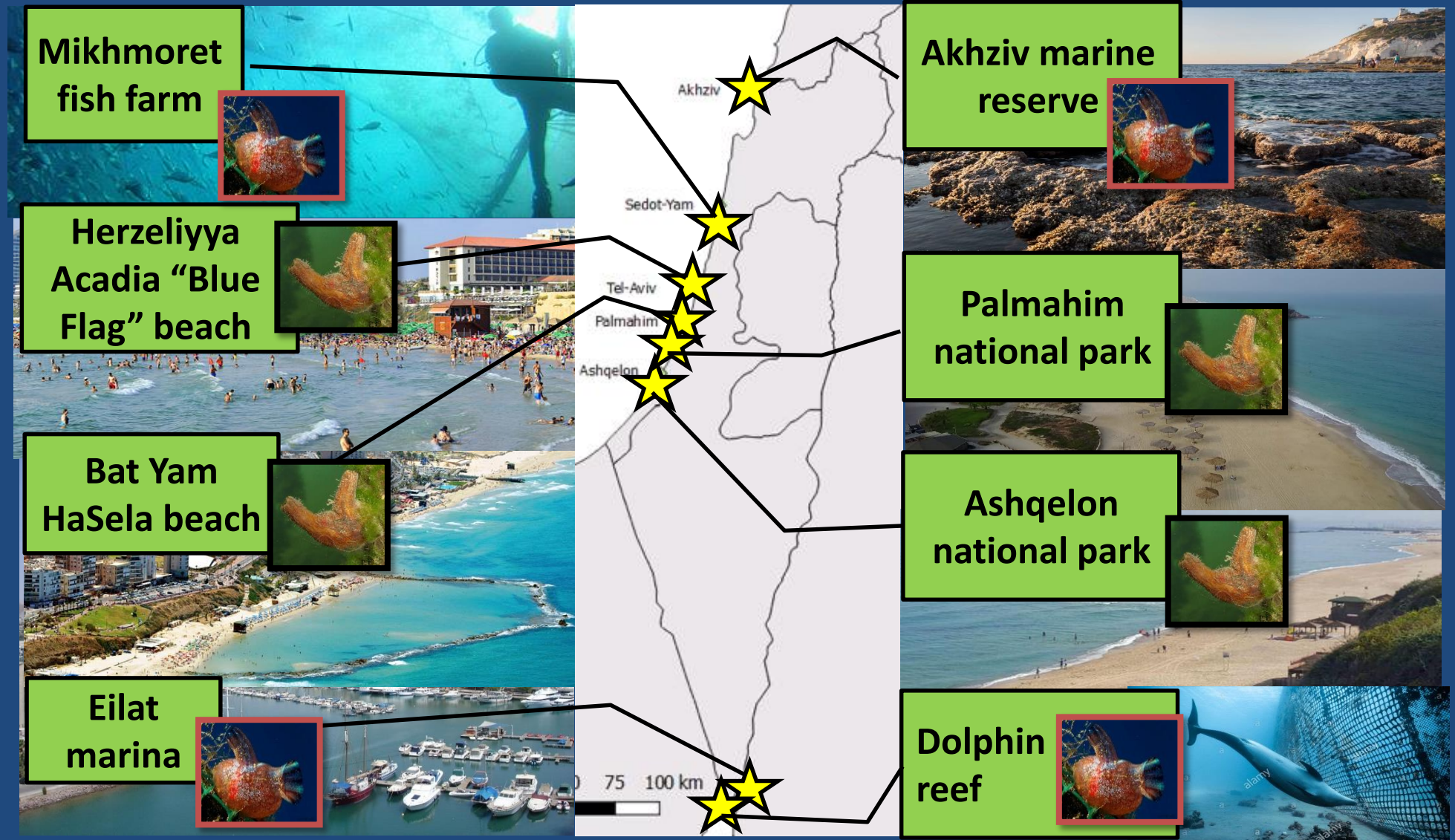
	Final conc. ng/g			
Sample	**DBP**	**DEHP**	**DnOP**	**Dry weight (g)**
1	585.6	1220.8	ND	1.006
2	612.7	1115.2	ND	1.0375
3	649.2	1084.7	ND	1.0739
4	669.8	1185.1	ND	1.0151
5	643.1	1152.4	ND	1.0685
6	647.8	1178.5	ND	1.0455
AVG.	**634.7**	**1156.1**		
%RSD	**4.8%**	**4.3%**		

Method Accuracy

The Accuracy by %recovery of spiked surrogate (DEHP-3,4,5,6-d_4) into extraction cells with sample:

Avg. %Recovery (n=52)	**89.2%**
S.D.	0.09
RSD%	10.3%

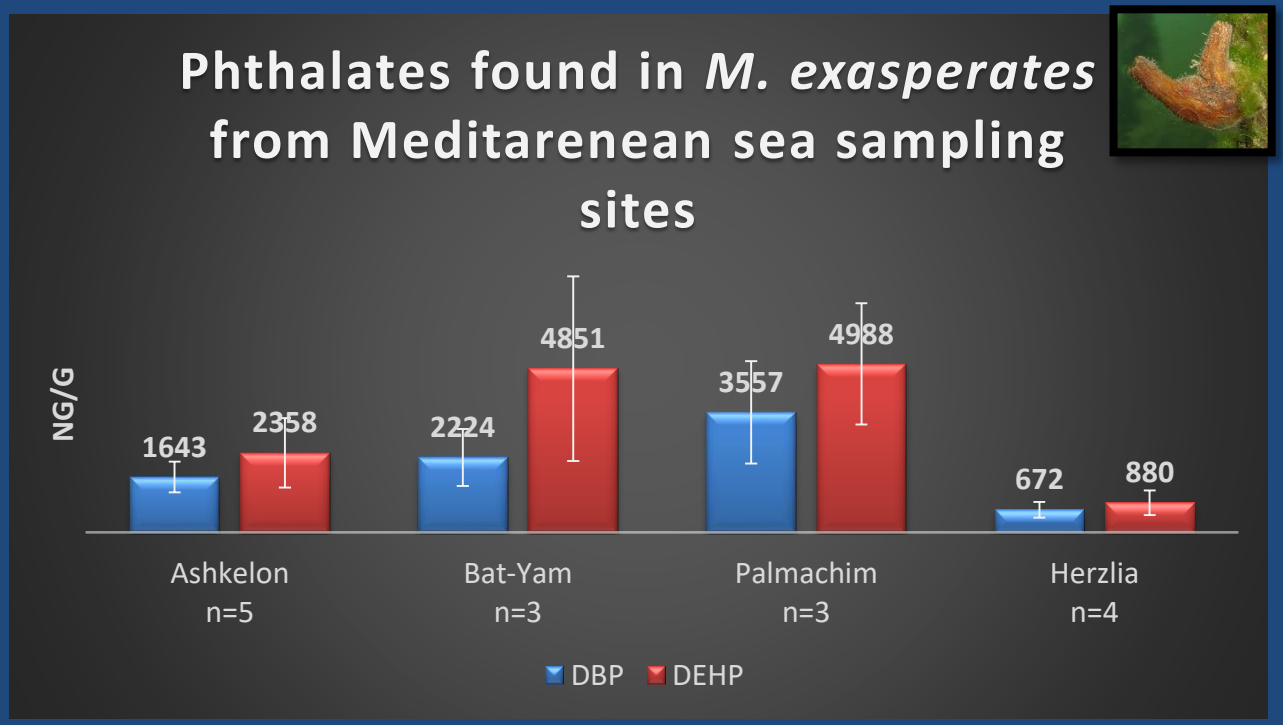
Sampling sites and organisms



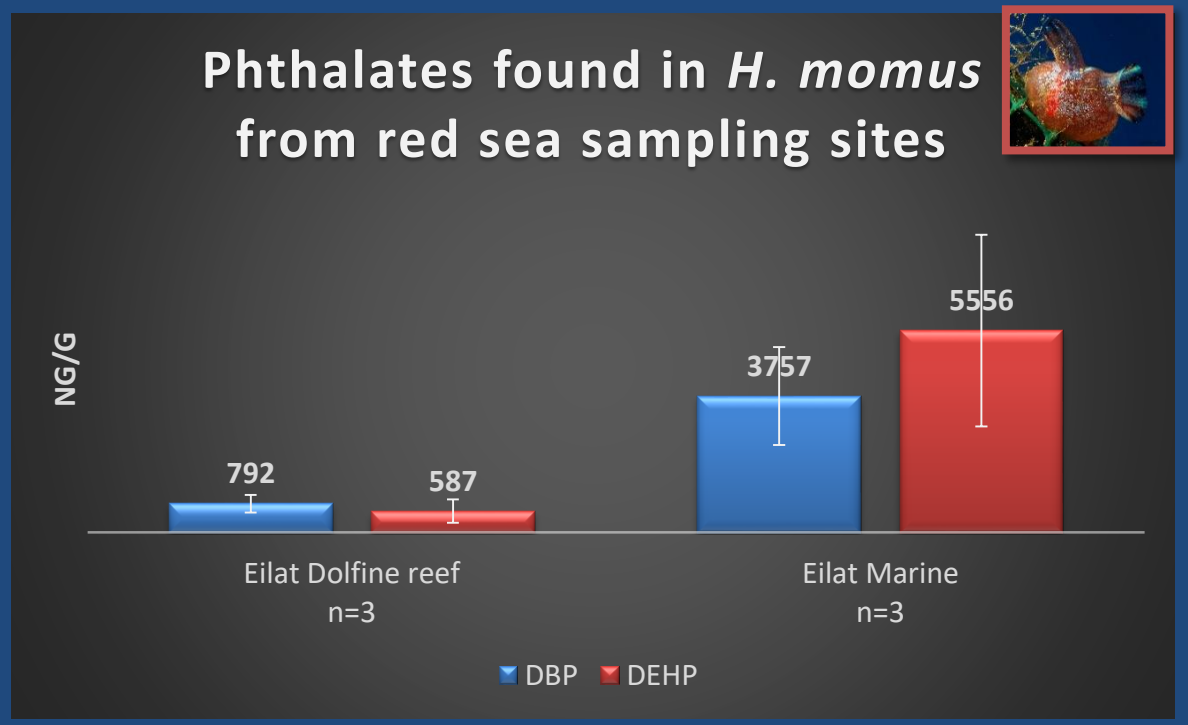
H. Momus

M. exasperates

Results – Eco-sampling (non-homogenous)



	DBP[ng/g]	%RSD	DEHP[ng/g]	%RSD
Ashkelon	1643	28%	2358	44%
Bat-Yam	2224	38%	4851	56%
Palmachim	3557	43%	4988	36%
Herzlia	672	35%	880	42%



	DBP[ng/g]	%RSD	DEHP[ng/g]	%RSD
Eilat Dolfine reef	792	30%	587	54%
Eilat Marine	3757	36%	5556	47%

Summary

- ✓ **Novel approach for phthalates quantitation in marine environments:**
  - ➢ Use ascidians as bio indicators and as bio filters for *in-situ* compounds concentration
  - ➢ Accelerated Solvent Extraction (ASE™) for sample preparation
- ✓ Simple and repeatable method
- ✓ Could be applied anywhere around the world, due to the wide spreading ascidians
- ✓ Good indications for plastic contamination in marine environment, both in the water column and in animals body

Thank You!