Sea-urchin bioassays as a tool for assessing toxicity of environmental samples

Concepción Martínez-Gómez, Instituto Español de Oceanografía (IEO)

Centro Oceanográfico de Murcia, Spain

<concepcion.martinez@mu.ieo.es>

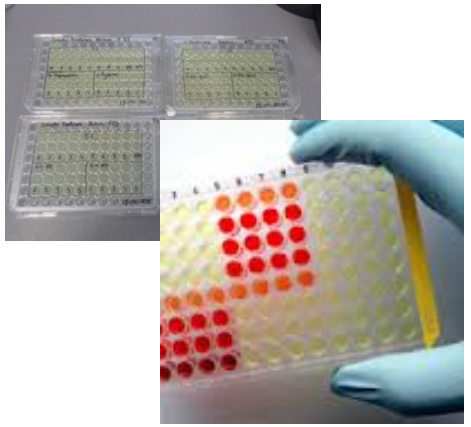
Content

- Bioassays and marine pollution environmental studies
- Sea urchin embryo test (SET)
- Sediment elutriates/water sediment interface/porewater/extracts
- SET Methodology
- Quality data and sources of error
- Data interpretation
- Discussion and questions

Bioassays

Bioassays are biological tests that measure the response of healthy organisms (*in vivo*) or of cell lines/cells (*in vitro*) after exposure to specific contaminants or environmental matrix under controlled laboratory conditions.

In vivo



In vitro

An acute toxicity test does not provide information concerning whether delayed effects will occur.

Common *in vivo* bioassays used in marine research

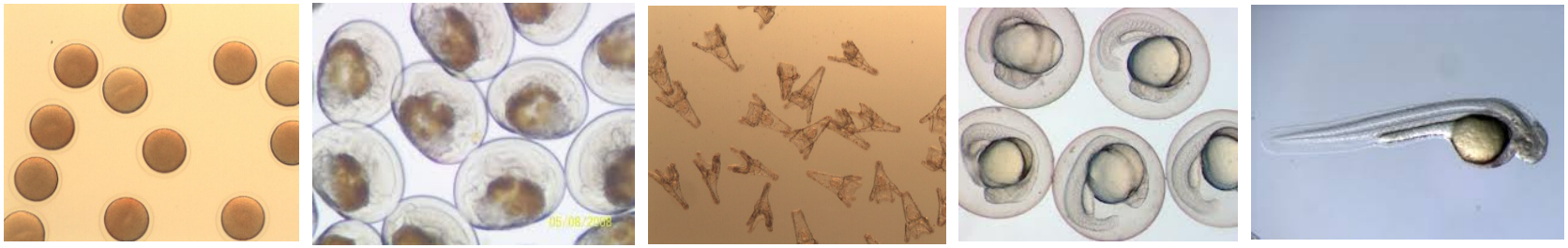
Organisms	Studied species	Experimental exposure
Fish	*Danio rerio, Oncorhynchus mykiss* *Gadus morhua, Sparus aurata* *Solea sp., etc*	Food seawater Whole sediment
Gastropods	*Hydrobia sp., Littorina sp,* *Murex sp.,*	Food seawater Whole-sediment
Bivalves	*Mytilus sp, Crassotrea sp.,* Cerastoderma sp., *Venerupis pullastra,* *Tapes decussates,* etc	Food Seawater Whole-sediment
Amphipods	*Corophium multisetosum* *Corophium volutator, Ampelisca brevicornis*	Whole-sediment
Polychaetes	*Arenicola marina*	Whole-sediment
Echinoderms	*Echinocardium sp.*	Whole-sediment
Copepods	*Tisbe sp., Acartia sp., Oithona davisae*	Seawater, sediment elutriates, pore water
Mysids	*Siriella sp* *Praunus sp*	Seawater, sediment elutriates, pore water
Microalgae	*Chorella vulgaris*, *Skeletonema costatum*	Seawater, sediment elutriates, pore water
Early developmental stages of fish and marine invertebrates	eggs, embryos and larvaes	Seawater, sediment elutriates, pore water, extracts

Common *in vivo* bioassays used in marine research

Organisms	Studied species	Experimental exposure
Fish	*Danio rerio, Oncorhynchus mykiss* *Gadus morhua, Sparus aurata* *Solea sp., etc*	Food seawater Whole sediment
Gastropods	*Hydrobia sp., Littorina sp,* *Murex sp., etc.*	Food seawater Whole-sediment
Bivalves	*Mytilus sp, Crassotrea sp.,* Cerastoderma sp., *Venerupis pullastra,* *Tapes decussates,* etc	Food Seawater Whole-sediment
Amphipods	*Corophium multisetosum* *Corophium volutator, Ampelisca* *brevicornis, etc*	Whole-sediment
Polychaetes	*Arenicola marina*	Whole-sediment
Echinoderms	*Echinocardium sp.*	Whole-sediment
Copepods	*Tisbe sp., Acartia sp., Oithona davisae,* *etc.*	Seawater, sediment elutriates, pore water
Mysids	*Siriella sp* *Praunus sp*	Seawater, sediment elutriates, pore water
Microalgae	*Chorella vulgaris*, *Skeletonema* *costatum*	Seawater, sediment elutriates, pore water
Early developmental stages of fish and marine invertebrates	**eggs, embryos and larvaes**	**Seawater, sediment elutriates, pore water, extracts**

Embryotoxicity bioassays

Early developmental stages of organisms are more sensitive to chemical stress than adults, being the weakest link in an organism's life cycle.

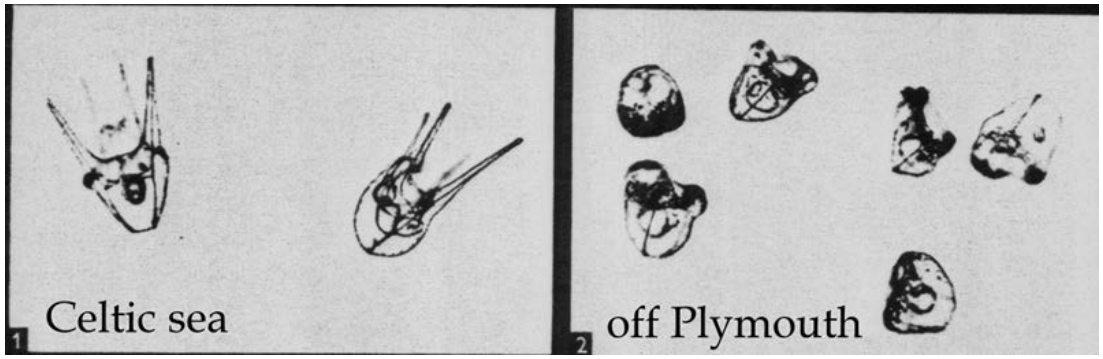


Deleterious effects observed to very low concentrations of contaminants (Kobayashi, 1995; His et al., 1999)

-1µg/L TBT and other antifoulings
-10 µg/L for Hg, Cu and Zn
-100 µg/L for Pb, Cd and other metals
- 0.1 mg/L organochlorine pesticides, detergents and refined oil
- 10 mg/L crude oil

Sea urchin embryotoxicity bioassay

Douglas P. Wilson (1951)
Plymouth Laboratory



Schinus suculentus

M. Bernhard (1955)
Stazione Zoologica de Napoli

Arbacia lixula

Sea urchin embryo test (SET)

Bioassay using embryos of the purple sea urchin *Paracentrotus lividus* (Lamarck, 1816), a species widely distributed in both Atlantic and European Mediterranean waters.

Method is directly applicable to other echinoid species such as

Strongylocentrotus droebachiensis
Echinus sculentus
Sphaerechinus granularis
Arbacia lixula

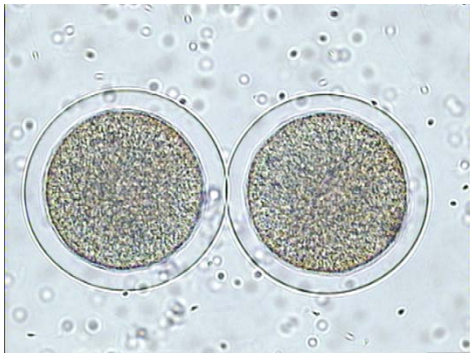
Paracentrotus lividus

Seawater quality

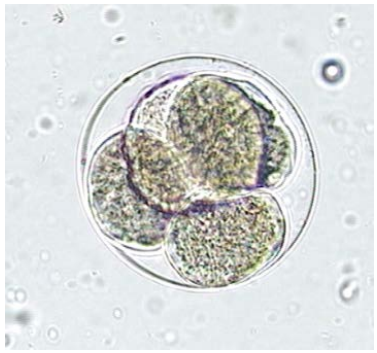
Sediment quality

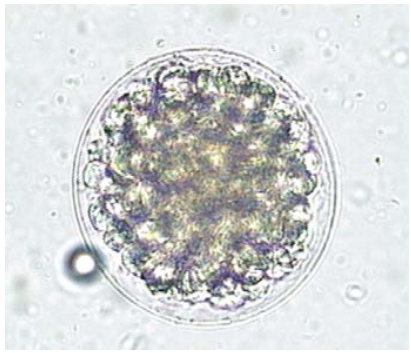
Toxicants

Echinoderm embryology

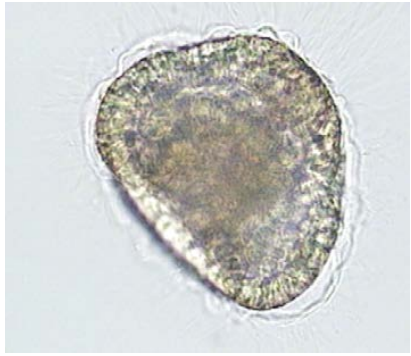


Fertilize eggs = 92 ± 3 µm





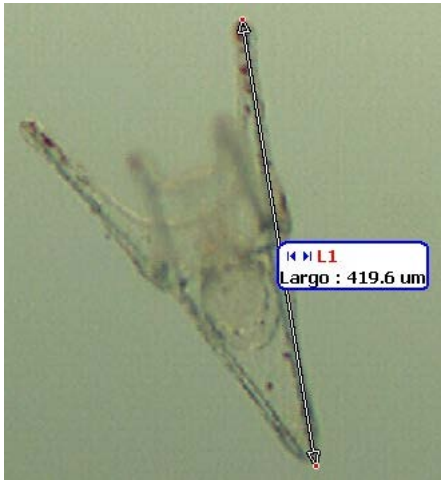
Morula



Blastula = 110-130 µm

Gastrula-Prisma = 110-130 µm

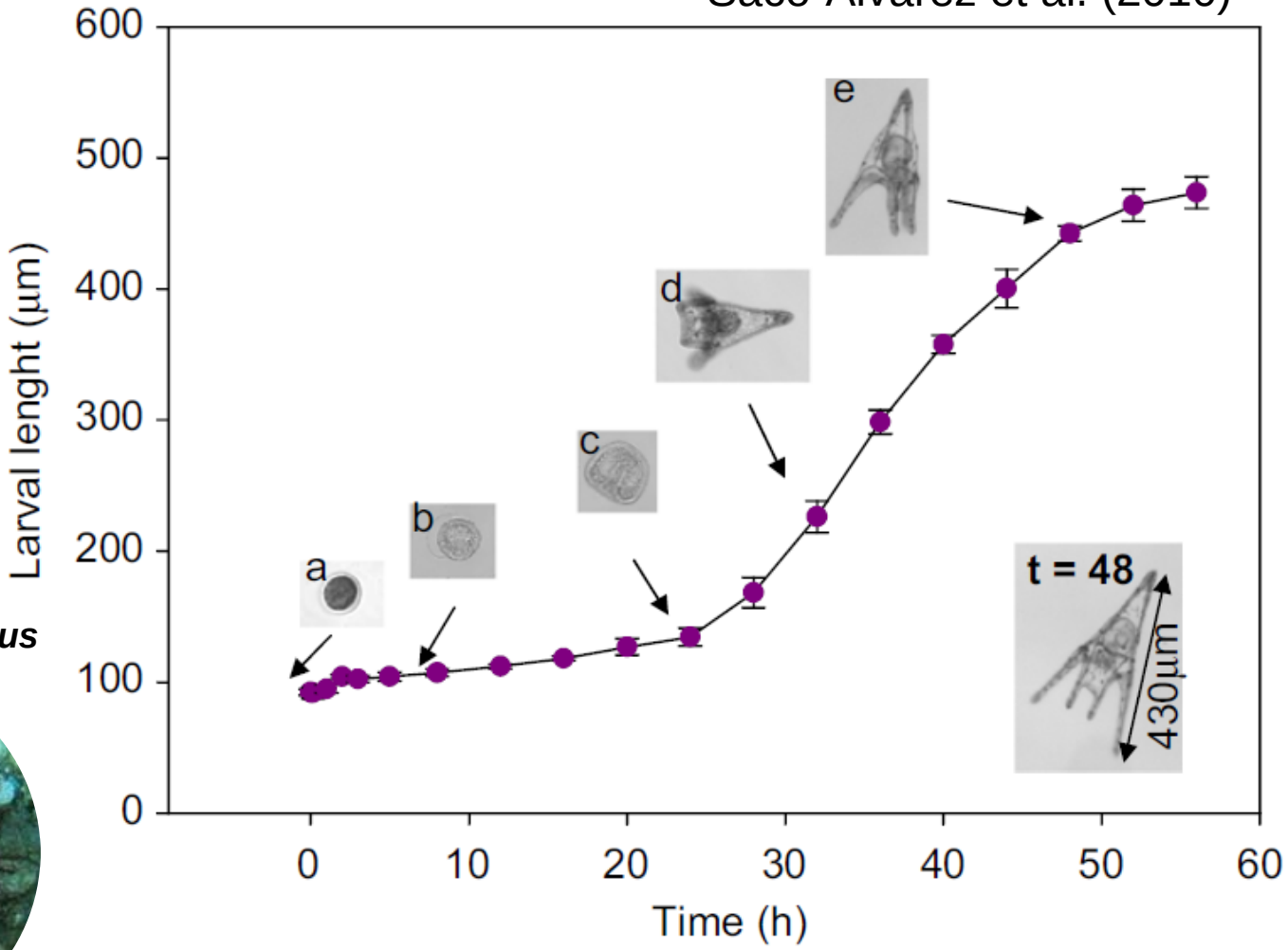
Prepluteus = 150-290 µm



Pluteus = 300-450 µm

Echinoderm embryology

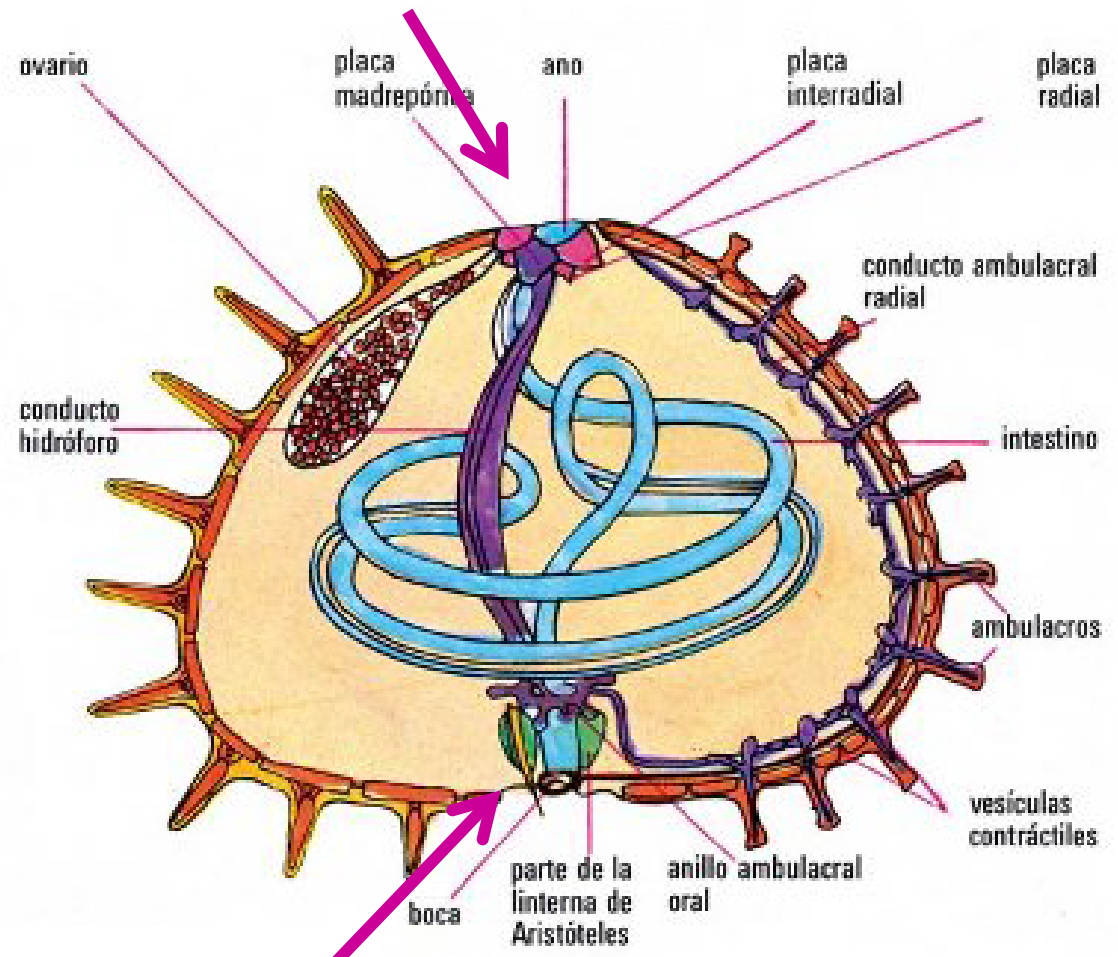
Saco-Álvarez et al. (2010)



Paracentrotus lividus

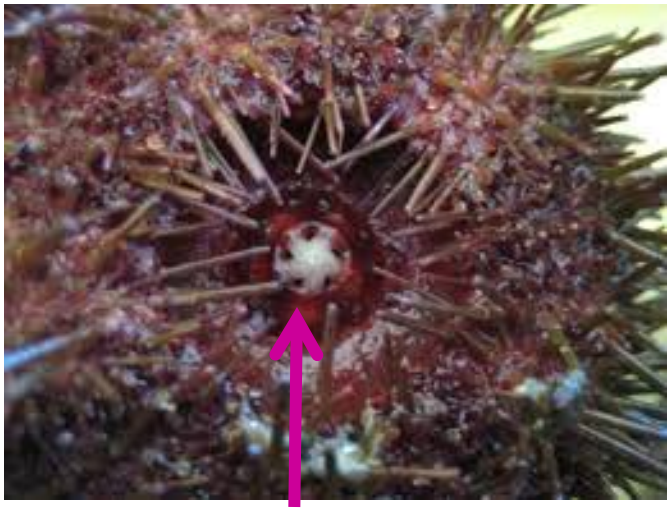
Spawning and fertilization

Gonopore



Peristomal membrane

Spawning and fertilization



Peristomal membrane

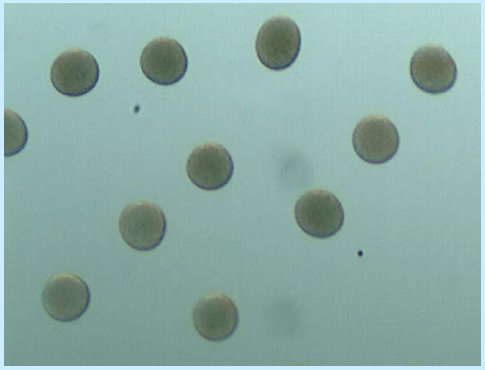
- direct stripping of the gonad

- mild electric shock (35 V)

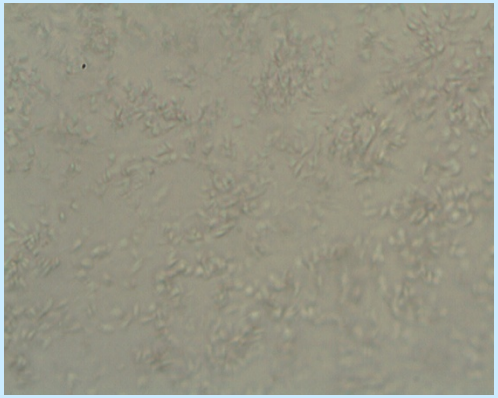
- osmotic-shock-induced spawning:
1 ml KCl 0.5 M

Spawning and fertilization

Gamete viability

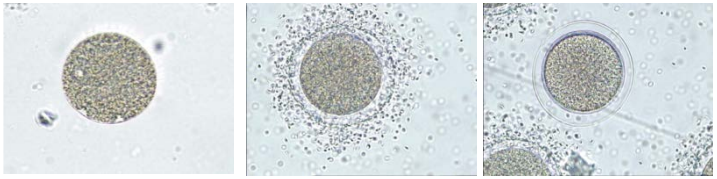


Roundness, 100 µm
free of germinal vesicles

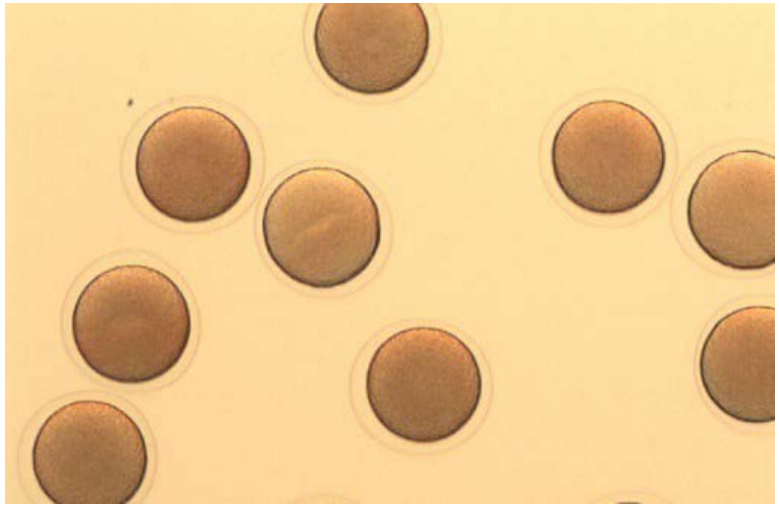


High sperm motility

Spawning and fertilization



Espermatocytes/oocytes 2000-20000
After 7 minutes fertilized eggs



20 µL (x4)

→% fecundation
→oocyte density

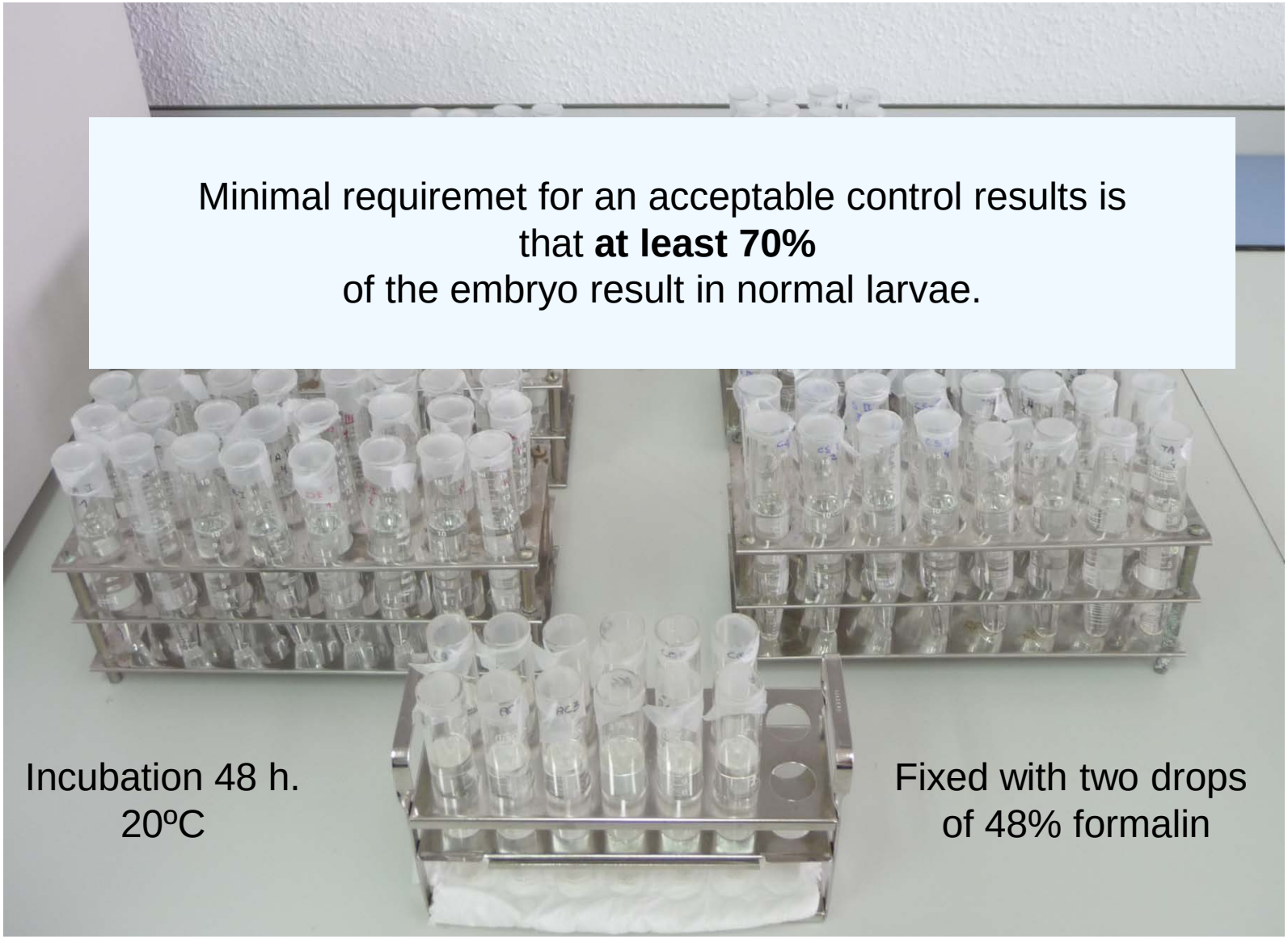
20-40
Fert. eggs / mL

< 30 min starting incubation

Egg fertilization should be > 90%

- FERTILIZED EEGS FIXED AFTER DELIVERY (T=0)
- CONTROL OF FSW
- UNDILUTED SAMPLES
- ½, ¼, AND 1/10 DILUTIONS IN FSW

Incubations



Minimal requiremet for an acceptable control results is that **at least 70%** of the embryo result in normal larvae.

Incubation 48 h.
20ºC

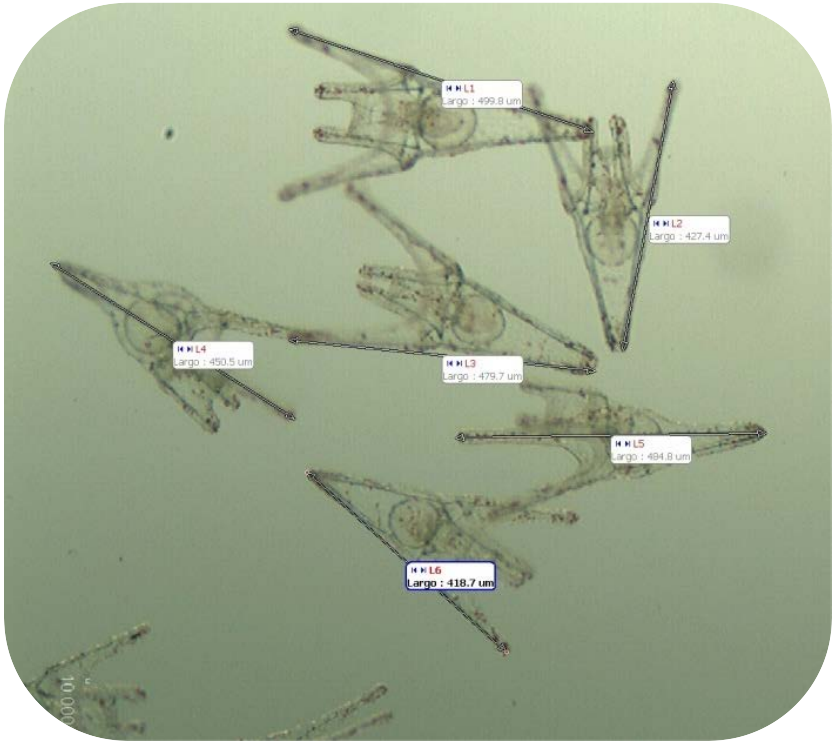
Fixed with two drops of 48% formalin

Readings

i) Morphological normality of the larvae
(qualitative) (N=35)

ii) Size increase
(quantitative) (N=35)
Maximum length of individuals

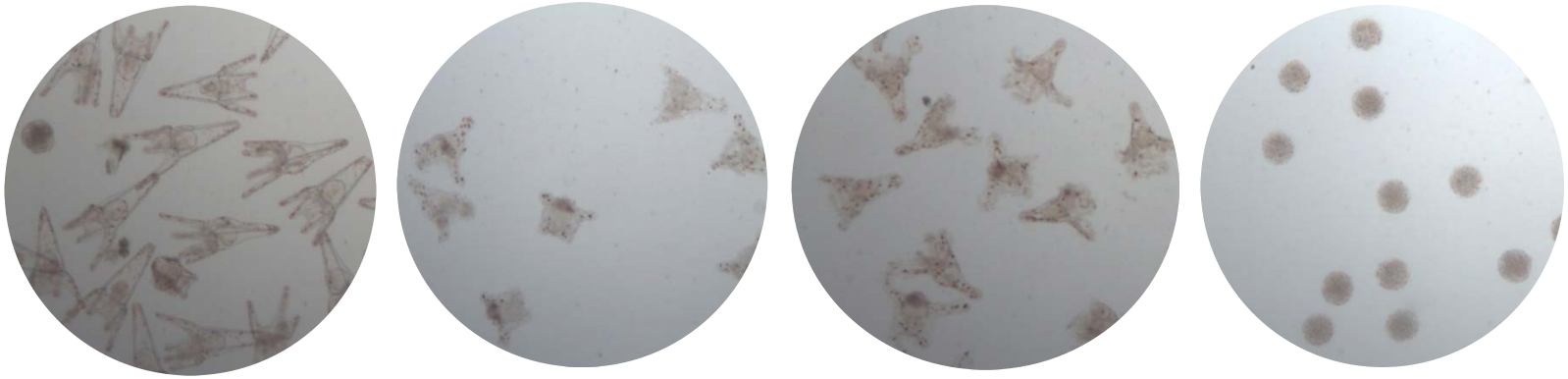
Normal pluteus: four arms well developed (aprox. 300-450 µm)



SET results

i) Morphological normality of the larvae (qualitative) (N=35)

Pc



X 4 X 4 X 4 X 4

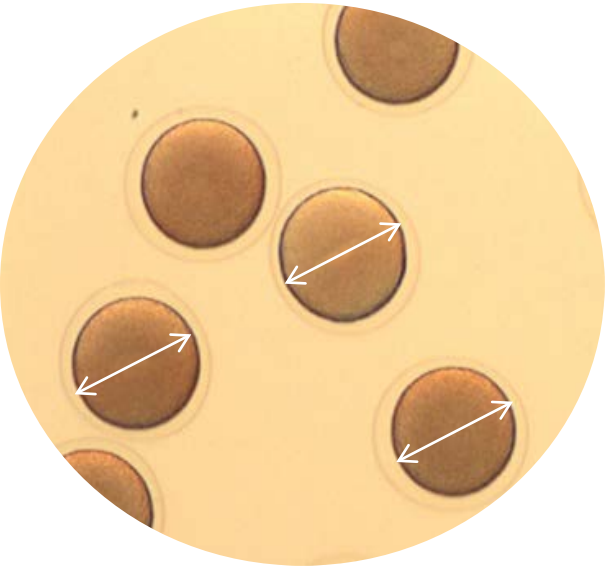
Abbot formula (Emmens, 1948)

$$P' = (P-P_c/100-P_c) \times 100$$

SET results

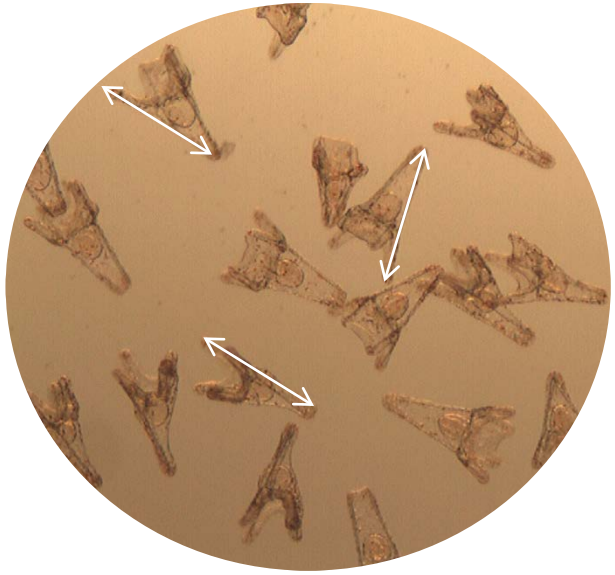
i) Size increase (quantitative) (N=35)

SO



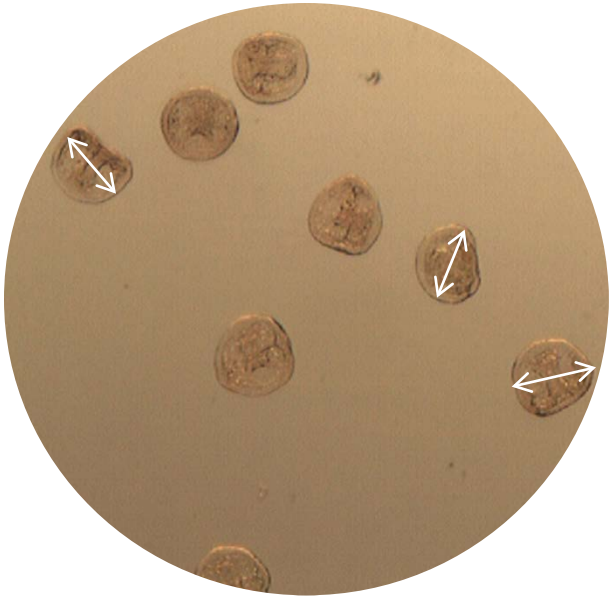
X 4

SC



X 4

S1

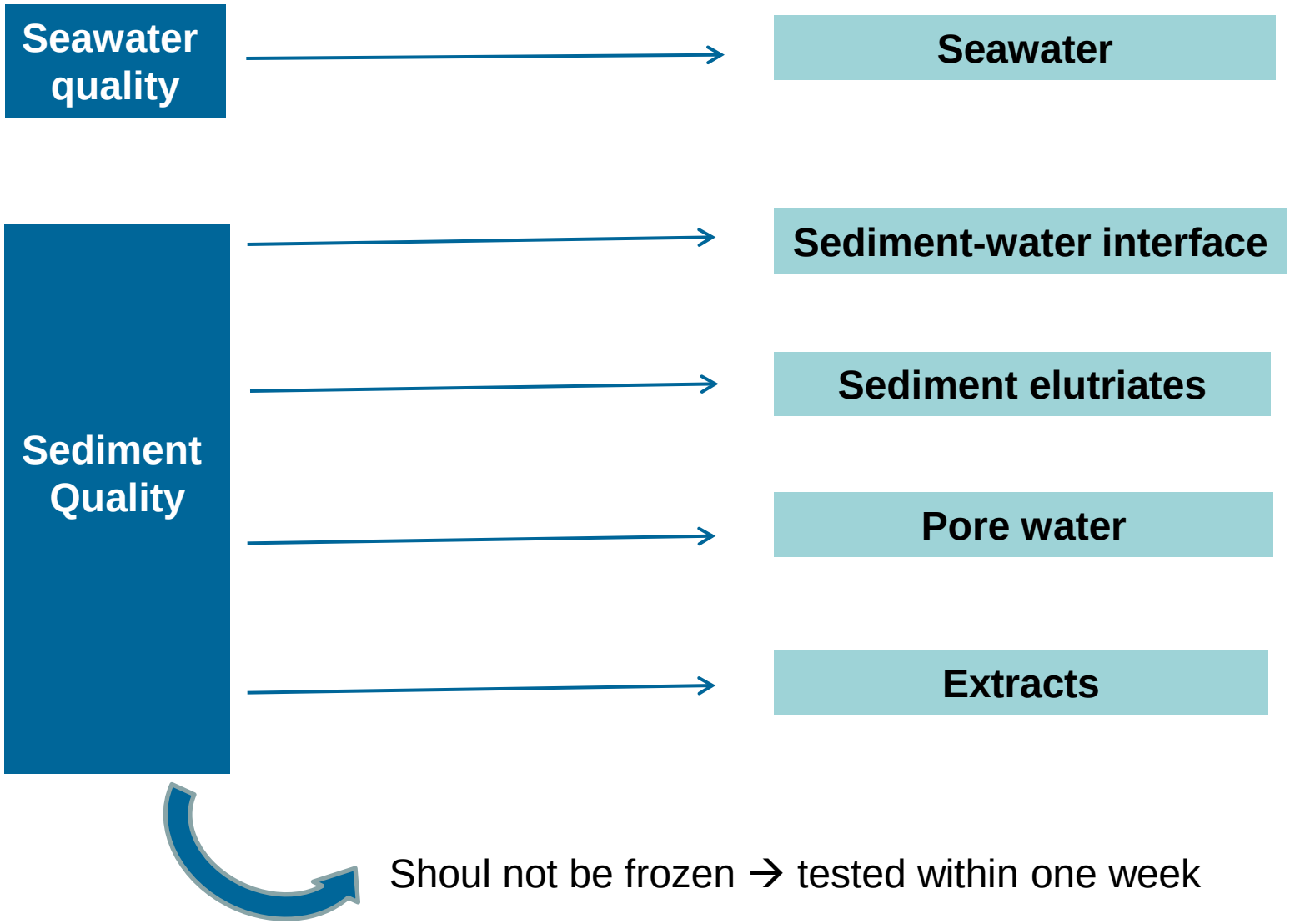


X 4

PNR (Percentage Net Response)

PNR = (S1-S0) / (SC-SO)

Sea urchin embryo test (SET)



Sea urchin embryo test (SET)

Seawater quality

Sediment quality

Reference Toxicants

- *In vitro* fertilization
- Preparation of the sediments elutriates/sediment-water interface
- Serial dilutions of the testing samples
- Control incubations

✓0.22 µm filtered seawater of oceanic characteristics (FSW)

✓Artificial seawater (ASW) (Lorenzo et al., 2002).

Caution for trace metals impurity content!

Reference toxicants: EC_{50}

Reference toxicants are used to assess the organism sensitivity

→ To obtaining information of the organism conditions
→ To validate protocols
→ To compare sensitivity of biological material used in different experiments

NH_4Cl Amonium Chloride
SDS Sodium-dodecyl-sulphate
$CdCl_2$ Cadmium chloride

www.epa.gov/enviro/html/emci/chemref/complete_index.html

- To prepare dilutions of the reference toxicants to estimate **EC_{50}**

(i.e. 0, 1, 2, 4, 8, 16 and 32 mg/L)

- Stock solution of the toxicant **0.1 -1.0 g/L** (Using **FSW**!!!)

Fernández, 2002; Bellas et al., 2005

Reference toxicants: EC_{50}

1L Stock solution 0.4 g/L $CdCl_2$

Molecular weight Cd^{2+} = 112.4
Molecular weight $CdCl_2$ ($CdCl_2 \bullet 2.5\ H_2O$)= 228.34
1L x 0.4 g/L x (228.34 g de $CdCl_2$ / 112.4 g Cd^{+2}) = 0.813 g

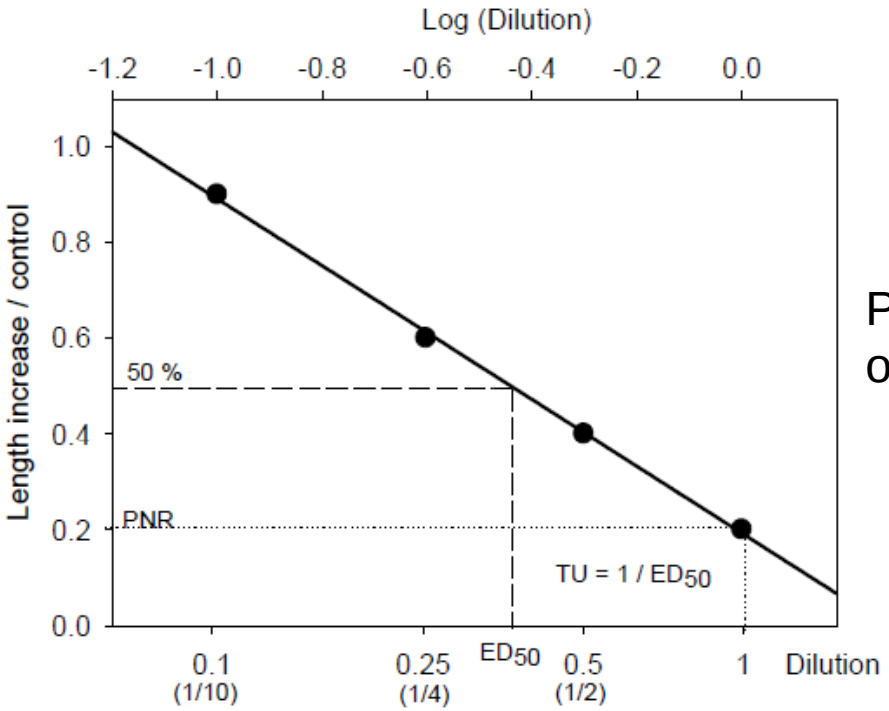
mg / L	mL FSW	mL Stock solution	mL tube	Replicates
0	20.00	0.00	20	5
1	19.95	0.05	20	5
2	19.90	0.10	20	5
4	19.80	0.20	20	5
8	19.60	0.40	20	5
16	19.20	0.80	20	5
32	18.40	1.60	20	5

Reference toxicants: EC_{50}; LC_{50}

Median: Lower variance than other percentiles

Calculo EC50:

Softwares →combination of Moving average, Probit, Logit and Binomial (Rodríguez and Esclapes, 1995)



Probit

P = @ (A+B) (1)

P= Dead probability of the organisms exposed to concentration X

EC_{50} found in *Paracentrotus lividus*

Area	Reference toxicant	EC_{50} (± 95%)	Source
Adriatic (Italy)	NH4+ total (µ/L)	5700 (5300-6100) pH 7.7 4200 (3900-4600) pH 8.0 3100 (2900-3300) pH 8.3	Arizzi Novelli et al., 2003
B. Country (Spain)	NH4+ total (µ/L)	4980 (4760-5300)	AZTI, 2009
Galician (Spain)	Cd (µg/L)	9240	Fernández and Beiras, 2001
Galician (Spain)	Cd (µg/L)	8628 (8456-9135)	Fernández, 2002
Venice (Italy)	Cd (µg/L)	2300 (1900-2700)	Arizzi Novelli et al., 2003
B. Country (Spain)	Cd (µg/L)	7520 (7310-7740)	AZTI 2009
Aveiro (Portugal)	SDS (µg/L)	4150-4170	Rolland et al., 1999
Galicia (Spain)	SDS (µg/L)	4100 (3750-4580)	Fernández, 2002
Mar Menor (Spain)	SDS (µg/L)	1710 (1430-1990)	Marín Guirao et al., 2005
Galicia (Spain)	SDS (µg/L)	4277	Bellas et al., 2005
B. Country (Spain)	SDS (µg/L)	4235(4094-4378)	AZTI, 2009

Preparation of elutriates

(4 FSW: 1 SED)

Preparation of elutriates

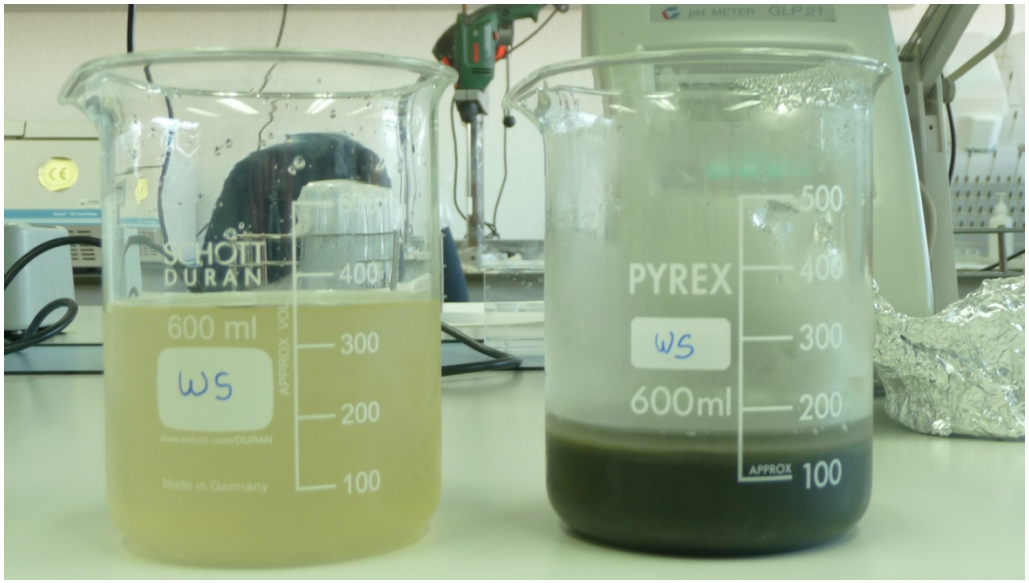
Rotatory stirring 60 rpm polypropylene flasks

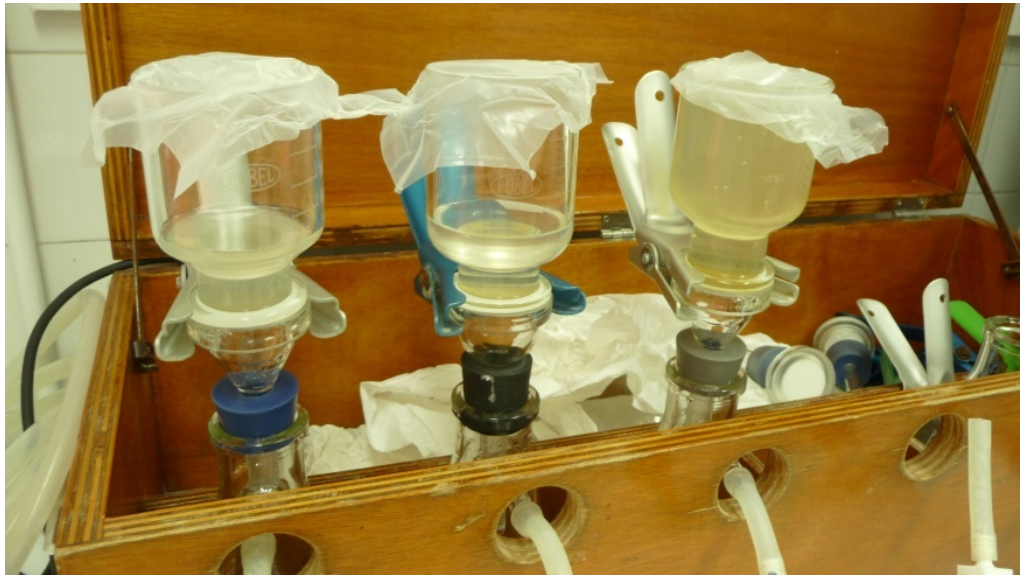
Magnetic stirring 45 min

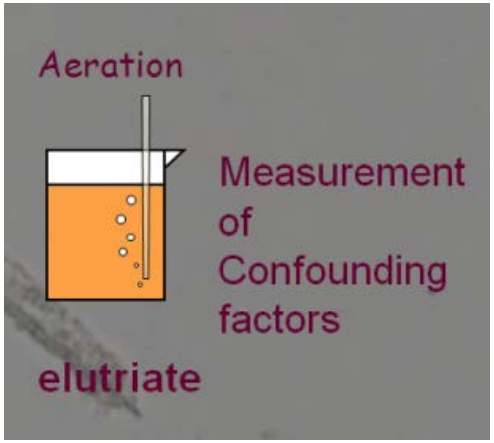
→ **Decantation 24 hours (4ºC in dark)**

→ **Decantation 12 hours (20ºC in dark)**

Preparation of elutriates

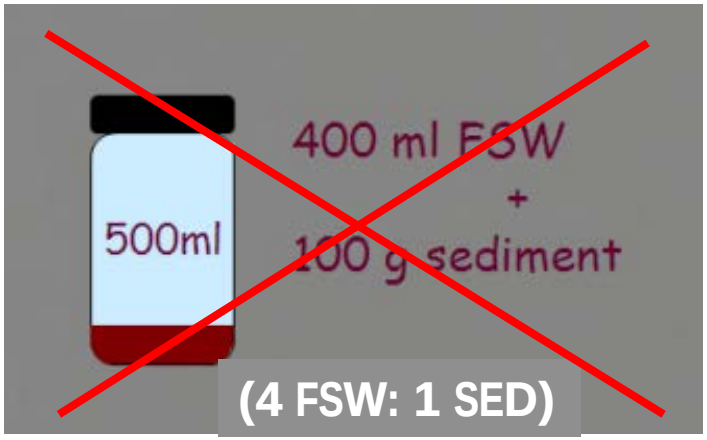






Obtention of porewater

2000 rpm
10000 xg



Enhanced sensitivity

Carr and Chapman, (1995)

Obtention of sediment-water interface

Dilution FSW stabilize 24 hours with whole sediment (4 FSW: 1SED)

César et al. (2004)

Working with extracts

→ Solvent should be kept to a minimum, **not exceeding 0.5 mL/L**

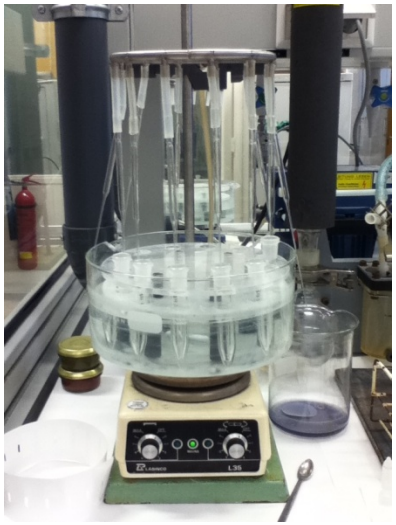
→ Include a solvent control containing highest concentrations in treatments

→70% normal larvae in solvent control

- **Triethylene glycol** –Low toxicity and low volatility

- Water miscible organic solvents (**methanol, ethanol, acetone**): Stimulate undesirable growths of microorganisms, volatile

- Organic solvent (**DMSO**) → **Reagent grade**



ASTM 1563 - 98(2012)

Experimental exposure conditions

- 300 embryos/10 ml → 4-5 replicates
- Control samples
- Incubation at 20ºC
- Stopping the development with formalin after 48h
- Measuring endpoint.

i) Morphological normality of the larvae
(qualitative)

ii) Size increase
(quantitative)

Sources of Error

- Quality assurance of the biological material

- Confounding factors

Sources of Error

❑ Quality assurance of the biological material

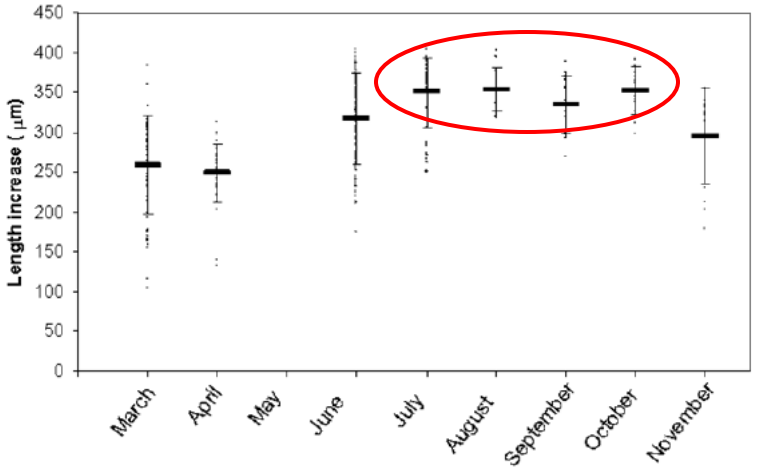
1. CONTROL TREATMENT

Mean response in Control exceeds a size increase of

218 µm for FSW
253 µm for ASW

Beiras et al., 2012

	SIZE INCREASE (µM)		
water	FSW	ASW	FSW and ASW
Mean (95% CI)	287.9 (272.8; 291.0)	345.1 (335.5; 354.6)	312.3 (306.0; 318.7)
n	167	139	226
5th percentile	218	253	245



Beiras et al, 2012. *TICES TIMES. Nº 51.*

Intralaboratory control charts with reference toxicants (Cu or Zn)

→CV 12-20% normal larvae

Phillips et al., 1998; Volpi Ghirardini et al., 2005.

Sources of Error

❑ Quality assurance of the biological material

1. CONTROL TREATMENT

Mean response in Control exceeds a size increase of

218 µm for FSW
253 µm for ASW

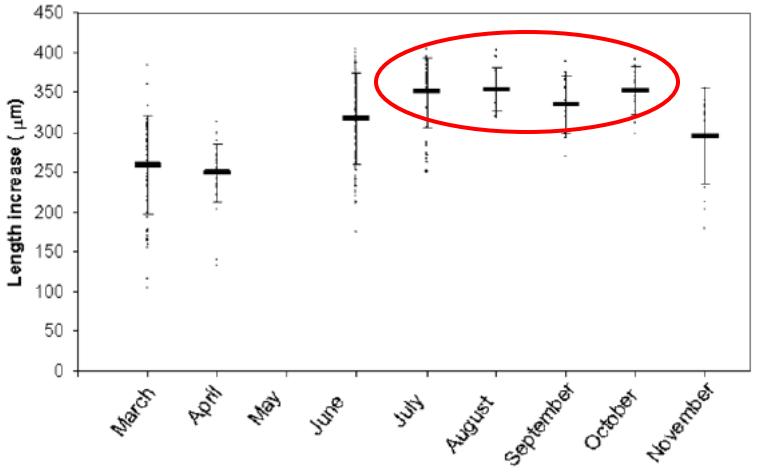
Beiras et al., 2012

	SIZE INCREASE (µM)		
water	FSW	ASW	FSW and ASW
Mean (95% CI)	287.9 (272.8; 291.0)	345.1 (335.5; 354.6)	312.3 (306.0; 318.7)
n	167	139	226
5th percentile	218	253	245

Intralaboratory control charts with reference toxicants (Cu or Zn)

→CV 12-20% normal larvae

Phillips et al., 1998; Volpi Ghirardini et al., 2005.



Beiras et al, 2012. *TICES TIMES. Nº 51.*

Sources of Error

❑ Confounding factors

Natural conditions of the samples are not suitable for the target specie

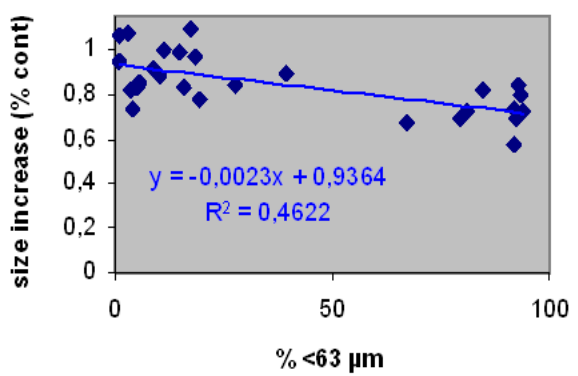
→ testing elutriates from highly reduced sediments

Paracentrotus lividus (Saco Alvarez et al., 2010)

- ✓ Salinity 31 $^{0}/_{00}$ -35 $^{0}/_{00}$
- ✓ pH 7.0-8.5
- ✓ Dissolved oxygen > 2 mg/L
- ✓ H_2S < 0.1 mg/L
- ✓ NH_3 < 40 microg/L)

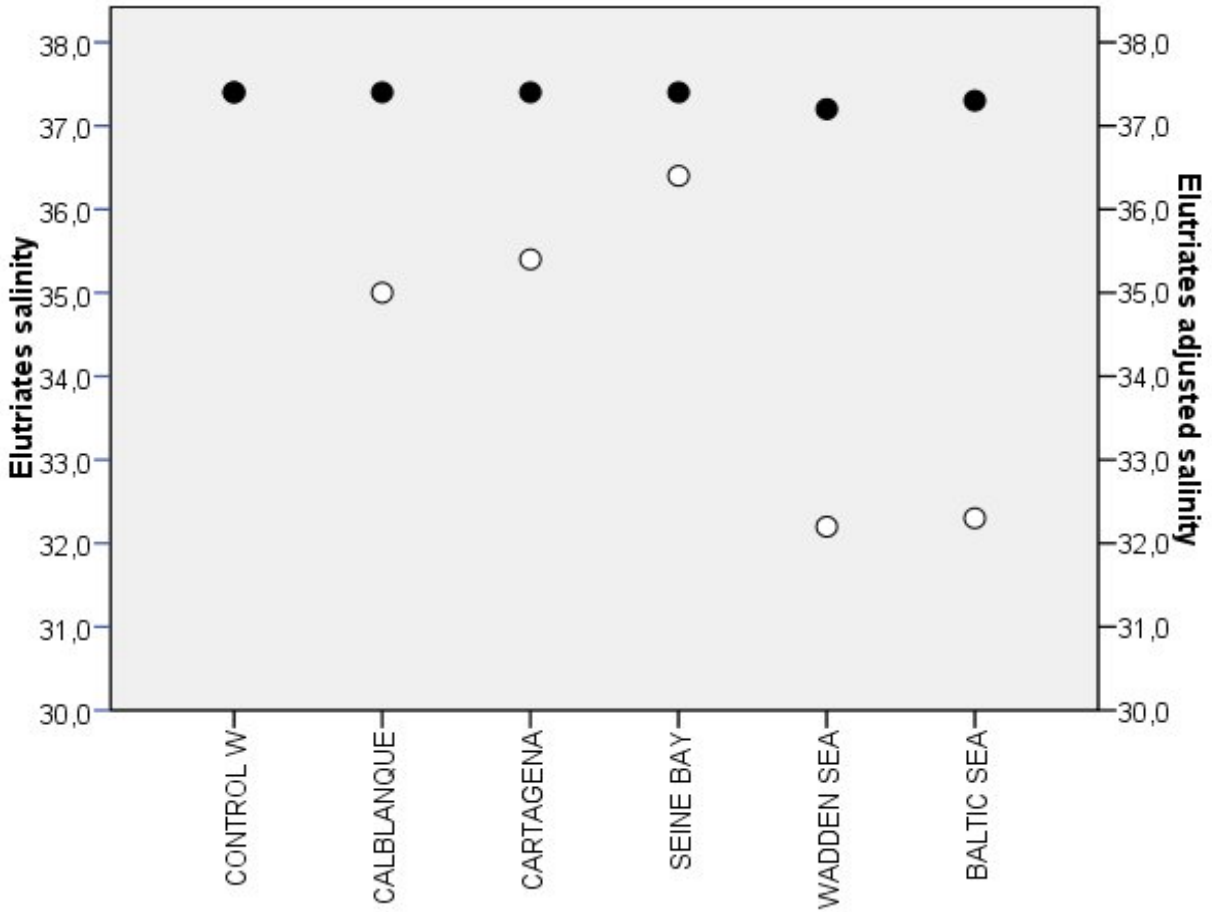
Measurements at the beggining and at the end of the incubation (1 replicate without formaline)

Particle size effect



Beiras et al, 2012. *TICES TIMES. Nº 51.*

Sources of Error



Assessment of toxicity

Assessment Criteria SET	Background response	Elevated Response	High and cause for concern response
% abnormality	0-10	>10-50	>50

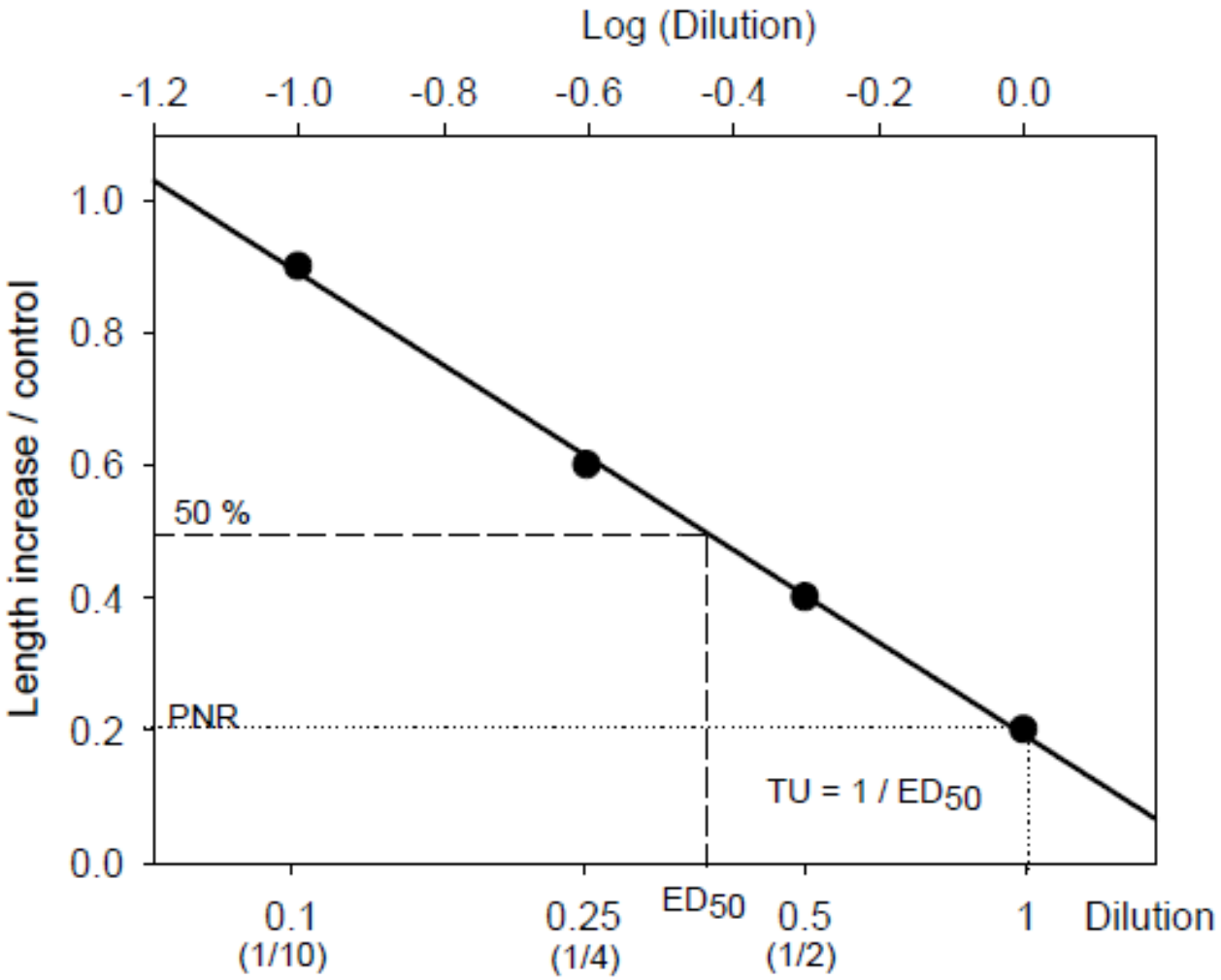
Davies et al., 2012. *Technical Annex 30. Assessment Criteria fro biological effects measurements. ICES. Cooperative Research Report. No 315.*

	High or good	Moderate	Poor or bad
PNR	> 0.7	0.7-0.5	<0.5
% Inhibiton growth	< 30	30-50	> 50

Davies et al., 2012. *Technical Annex 30. Assessment Criteria fro biological effects measurements. ICES. Cooperative Research Report. No 315.*

Arc sen $\sqrt{}$ abnormality → Parametric analisis ANOVA

Calculation of Toxic Units: Sediment elutriates



1. Liner regression PNR vs lg Dilution
2. DE50 = dilution causing 50% decrease
3. Obtention TU

TU (Toxic Units)

TU = 1 / DE50

Assessment of toxicity

Assessment Criteria SET	Background response	Elevated Response	High and cause for concern response
% abnormality	0-10	>10-50	>50

Davies et al., 2012. *Technical Annex 30. Assessment Criteria fro biological effects measurements. ICES. Cooperative Research Report. No 315.*

	High or good	Moderate	Poor or bad
PNR	> 0.7	0.7-0.5	<0.5
% Inhibiton growth	< 30	30-50	> 50

Davies et al., 2012. *Technical Annex 30. Assessment Criteria fro biological effects measurements. ICES. Cooperative Research Report. No 315.*

Sediment quality status	High or good	Moderate	Poor or bad
TU	< 0.27	0.27-0.86	> 0.86

Beiras et al, 2012. *TICES TIMES. Nº 51.*

Advantadges of SET

•In vivo bioassay with a high ecological relevance

•Acute and sub-lethal toxicity

•Sampling, maintenance in the laboratory conditions, easy to get gametes and embryos, short embryological development period

•The Percentage of Net Response (PNR) is a quantitative, observer-independent, automatically readable response.

•SET can be used to compare sensitivities of different species and different test materials

•Statistical methods and assessment criteria to classify water and sediment samples according to their biological quality status are developed

Drawbacks

- Toxicity of hydrophobic contaminants might be underestimated
- Availability of mature seaurchin can be difficult in some areas/times

SET in marine environmental management

Ciencias Marinas (2006), 32(1B): 139–147

Nota de Investigación/Research Note

Ejercicio interlaboratorio con bioensayos marinos para la evaluación de la calidad ambiental de sedimentos costeros. III. Bioensayo con embriones del erizo de mar *Paracentrotus lividus*

Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. III. Bioassay using embryos of the sea urchin *Paracentrotus lividus*

MC Casado-Martínez[1]*, N Fernández[1], J Lloret[2], A Marín[3], C Martínez-Gómez[4], I Riba[5], R Beiras[6], L Saco-Álvarez[6], TA DelValls[1]

[1] Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Polígono Río San Pedro s/n, 11510 Puerto Real, Cádiz, España. * E-mail: mcarmen.casado@uca.es

[2] Servicio de Cultivo Celulares, SACE, Universidad de Murcia, 30100 Murcia, España

[3] Departamento de Ecología e Hidrología, Facultad de Biología, Universidad de Murcia, 30100 Murcia, España

[4] Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, Varadero, I. Lo Pagán, 30740 San Pedro del Pinatar (Murcia), España

[5] Consejo Superior de Investigaciones Científicas, Instituto de Ciencias Marinas de Andalucía, Polígono Río San Pedro s/n, 11510 Puerto Real, Cádiz, España

[6] Laboratorio de Ecoloxía Mariña, Universidade de Vigo, Campus Lagoas-Marcosende, E-36200, Vigo, España

SET in marine environmental management

GOBIERNO DE ESPAÑA · MINISTERIO DE FOMENTO · MINISTERIO DE AGRICULTURA, ALIMENTACIÓN Y MEDIO AMBIENTE · CEDEX CENTRO DE ESTUDIOS Y EXPERIMENTACIÓN DE OBRAS PÚBLICAS

INFORME TÉCNICO

para

SECRETARIA DE ESTADO DE MEDIO AMBIENTE.
DIRECCIÓN GENERAL DE SOSTENIBILIDAD
DE LA COSTA Y EL MAR

BORRADOR DEL REAL DECRETO POR EL QUE SE REGULAN LAS CONDICIONES PARA LAS OPERACIONES DE DRAGADO Y LA REUBICACIÓN DE LOS MATERIALES DRAGADOS EN AGUAS DEL DOMINIO PÚBLICO MARÍTIMO TERRESTRE.

INFORME FINAL

TOMO ÚNICO

Clave: 23-411-5-006 Madrid, diciembre de 2012

Centro de Estudios de Puertos y Costas

CEDEX

BIOENSAYO EN FASE LÍQUIDA BASADO EN LA EMBRIOGÉNESIS DEL ERIZO DE MAR *PARACENTROTUS LIVIDUS*

(Protocolo Operacional Estándar)

1. Introducción

Distintas especies de equinodermos se han empleado tradicionalmente en la evaluación ecotoxicológica de sedimentos (Geffard et al., 2000; Marina et al., 2001; Brils et al., 2002; Volpi Ghirardini et al., 2005; Giuliani et al., 2007) y han sido expuestas a distintos tipos de contaminantes tales como surfactantes (Volpi Ghirardini et al., 2001), pesticidas (Dinnel et al. 1989) y metales (Fernández & Beiras, 2001).

La aplicación ecotoxicológica de equinodermos está ampliamente estandarizada, se inchrye en diversas reglamentaciones nacionales para la gestión del material de dragado (Environment Canada, 1992; RIKZ, 2000; ASTM, 2004; ICRAM-APAT, 2006) y ha sido sometida de forma satisfactoria a ensayos de intercalibración (Arizzi Novelli et al. 2007).

Los bioensayos con estadios embrionarios y larvarios de invertebrados marinos son considerados un método rápido y sensible para la caracterización de la ecotoxicidad de los sedimentos marinos.

La obtención de gametos y su fecundación in Vitro son simples y debido a la rapidez con que se completa el desarrollo embrionario pueden obtenerse resultados en un corto periodo de tiempo (Casado-Martínez, et al. 2006).

Existen abundantes y relevantes investigaciones relativas a distintos efectos de la contaminación sobre la especie *Paracentrotus lividus*, tales como la bioacumulación (Radenac et al., 2000), el crecimiento larvario temprano (Fernández & Beiras, 2001; Fernández Méijome et al., 2006), la embriogénesis (Fernández & Beiras, 2001; Volpi Ghirardini et al., 2003; Arizzi Novelli et al., 2004) y el éxito en la fecundación (Volpi Ghirardini et al., 2003; Arizzi Novelli et al., 2004; Lera et al., 2006; Lera & Pellegrini, 2006) que fundamentan el presente protocolo. La posibilidad de estudiar efectos ecotoxicológicos subletales resulta especialmente interesante teniendo en cuenta la tendencia internacional existente (Convenio de Londres, 2008) en esa dirección para la caracterización ecotoxicológica del material a dragar.

La selección de la especie *Paracentrotus lividus* para el desarrollo de este protocolo se fundamenta además en su amplia distribución en las costas españolas y en la posibilidad de ser cultivado en laboratorio (Catoira Gómez et al., 1995; Spirlet et al., 2001; Kelly, 2005; Schlosser et al., 2005; Plan Nacional del Cultivo del Erizo 2006-2009).

El presente protocolo se fundamenta en las referencias bibliográficas expuestas al término del documento.

2. Equipo y material necesarios

Material

- Erizos *Paracentrotus lividus*, maduros (30-60 mm de diámetro de testa)
- Sedimento problema

SET in marine environmental management

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Highlighted Article

Methodological basis for the optimization of a marine sea-urchin embryo test (SET) for the ecological assessment of coastal water quality

Liliana Saco-Álvarez [a], Iria Durán [a], J. Ignacio Lorenzo [a], Ricardo Beiras [a,b,*]

[a] Laboratorio de Ecoloxía Mariña (LEM), Facultade de Ciencias do Mar, Universidade de Vigo, E-36200 Galicia, Spain
[b] ECIMAT, Universidade de Vigo, Illa de Toralla, E-3631 Galicia, Spain

ARTICLE INFO

Article history:
Received 10 February 2009
Received in revised form
21 January 2010
Accepted 22 January 2010
Available online 18 February 2010

Keywords:
Larval growth
Sea-urchin
Embryo
Larva
Bioassay
Method

ABSTRACT

The sea-urchin embryo test (SET) has been frequently used as a rapid, sensitive, and cost-effective biological tool for marine monitoring worldwide, but the selection of a sensitive, objective, and automatically readable endpoint, a stricter quality control to guarantee optimum handling and biological material, and the identification of confounding factors that interfere with the response have hampered its widespread routine use. Size increase in a minimum of $n=30$ individuals per replicate, either normal larvae or earlier developmental stages, was preferred to observer-dependent, discontinuous responses as test endpoint. Control size increase after 48 h incubation at 20 °C must meet an acceptability criterion of 218 µm. In order to avoid false positives minimums of 32‰ salinity, 7 pH and 2 mg/L oxygen, and a maximum of 40 µg/L NH_3 (NOEC) are required in the incubation media. For *in situ* testing size increase rates must be corrected on a degree-day basis using 12 °C as the developmental threshold.

1. Introduction

A central issue in standardization of a bioassay is the choice as test endpoint of a sensitive but observer-independent biological

SET in marine environmental management

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 29, No. 5, pp. 1192–1198, 2010

Printed in the USA
DOI: 10.1002/etc.136

ASSESSMENT CRITERIA FOR USING THE SEA-URCHIN EMBRYO TEST WITH SEDIMENT ELUTRIATES AS A TOOL TO CLASSIFY THE ECOTOXICOLOGICAL STATUS OF MARINE WATER BODIES

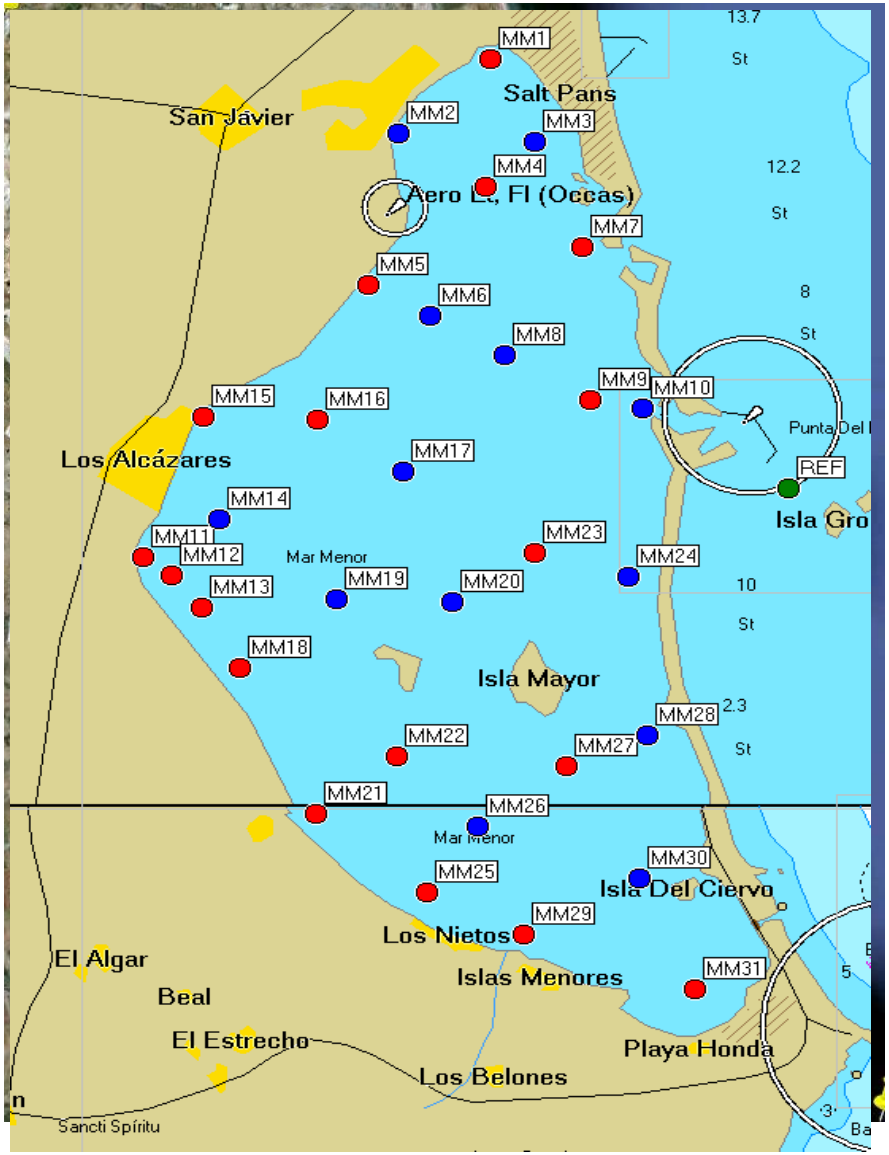
IRIA DURÁN†‡ and RICARDO BEIRAS*‡
†Laboratorio de Ecoloxía Mariña, Departamento de Química Analítica e Alimentaria, Facultade de Ciencias do Mar, Universidade de Vigo, Estrada Colexio Universitario s/n. E-36310, Vigo, Galicia, Spain
‡Toralla Marina Science Station, Universidade de Vigo, Illa de Toralla, E-36331, Coruxo, Galicia, Spain

(*Submitted* 23 *September* 2009; *Returned for Revision* 4 *December* 2009; *Accepted* 14 *December* 2009)

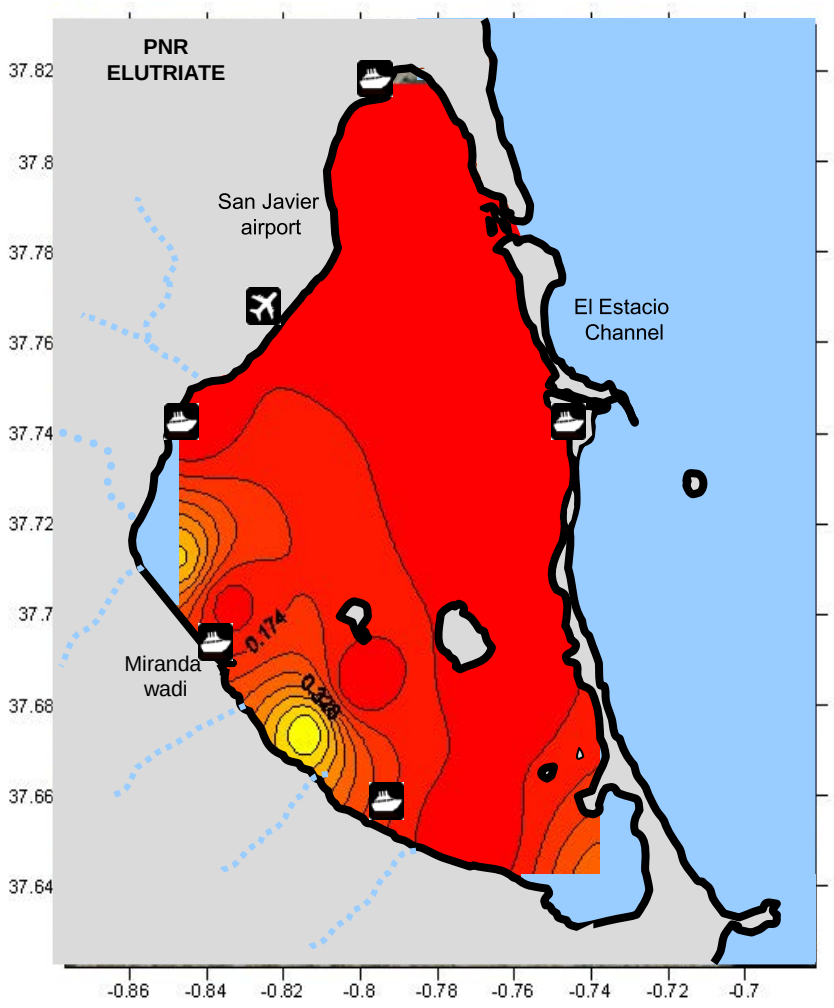
Abstract—A large, multiyear data set was generated by pooling the sediment elutriate (SET) results collected during previous studies conducted in the Galician Rias (northwest Iberian Peninsula) that met the acceptability criteria in the controls, to ensure optimum quality of data ($n = 162$). Two subsets of equal to reference and lower than reference sites were identified by comparing the Percentage Net Response (PNR) value from each sampling site with nontoxic, cruise-specific, reference sites by using the *t* test with the unequal variance assumption. Ecotoxicological Assessment Criteria (EAC_0, EAC_1, EAC_2, and EAC_3) were then derived from those two subsets to classify the SET results into five categories of ecotoxicological status: high, good, moderate, poor, and bad, in line with the European legislation. The 50th and 5th percentiles of the PNR distribution of the equal to reference sites subset were $EAC_0 = 0.879$ and $EAC_1 = 0.694$. An $EAC_2 = 0.508$ was obtained from the 50th percentile of the lower than reference sites subset. Because the PNR values of the entire database showed a distribution that can be adjusted to two normal populations, the $EAC_3 = 0.240$ PNR was calculated as the intersection between the first and second normal distributions identified. Power analysis proved that the limit between acceptable and unacceptable status (EAC_1) corresponded to a detectable PNR difference to control with a confidence level >99% and a power of 95%. Environ. Toxicol. Chem. 2010;29:1192–1198. © 2010 SETAC

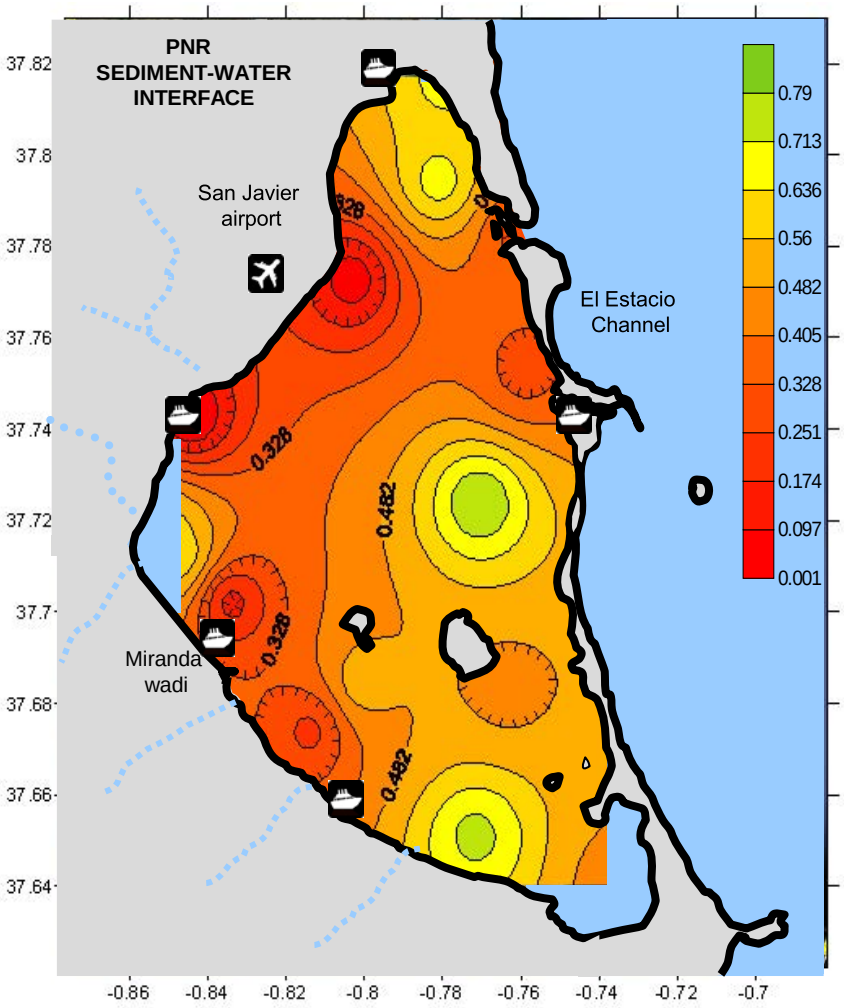
Keywords—Sediment toxicity Water quality Ecotoxicological status *Paracentrotus lividus*

SET in local environmental monitoring



SET in local environmental monitoring





SET in human health research

Research | Article

The Sea Urchin Embryo as a Model for Mammalian Developmental Neurotoxicity: Ontogenesis of the High-Affinity Choline Transporter and Its Role in Cholinergic Trophic Activity

Dan Qiao,[1] Lyudmila A. Nikitina,[2] Gennady A. Buznikov,[1,2,3] Jean M. Lauder,[2] Frederic J. Seidler,[1] and Theodore A. Slotkin[1]

[1]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina, USA; [2]Department of Cell and Developmental Biology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA; [3]N.K. Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia

Embryonic development in the sea urchin requires trophic actions of the same neurotransmitters that participate in mammalian brain assembly. We evaluated the development of the high-affinity choline transporter, which controls acetylcholine synthesis. A variety of developmental neurotoxicants affect this transporter in mammalian brain. [^{3}H]Hemicholinium-3 binding to the transporter was found in the cell membrane fraction at stages from the unfertilized egg to pluteus, with a binding affinity comparable with that seen in mammalian brain. Over the course of development, the concentration of transporter sites rose more than 3-fold, achieving concentrations comparable with those of cholinergically enriched mammalian brain regions. Dimethylaminoethanol (DMAE), a competitive inhibitor of choline transport, elicited dysmorphology beginning at the mid-blastula stage, with anomalies beginning progressively later as the concentration of DMAE was lowered. Pretreatment, cotreatment, or delayed treatment with acetylcholine or choline prevented the adverse effects of DMAE. Because acetylcholine was protective at a lower threshold, the DMAE-induced defects were most likely mediated by its effects on acetylcholine synthesis. Transient removal of the hyaline layer enabled a charged transport inhibitor, hemicholinium-3, to penetrate sufficiently to elicit similar anomalies, which were again prevented by acetylcholine or choline. These results indicate that the developing sea urchin possesses a high-affinity choline transporter analogous to that found in the mammalian brain, and, as in mammals, the functioning of this transporter plays a key role in the developmental, trophic activity of acetylcholine. The sea urchin model may thus be useful in high-throughput screening of suspected developmental neurotoxicants.
Key words: cholinergic phenotype, choline transporter, dimethylaminoethanol, hemicholinium-3, sea urchin embryo. *Environ Health Perspect* 111:1730–1735 (2003). doi:10.1289/ehp.6429 available via *http://dx.doi.org/* [Online 30 July 2003]

"pre-nervous" developmental stages. The concentration of ACh exhibits distinct peaks during early cleavage divisions, but the major, sustained increases occur after the beginning of gastrulation, in tandem with transcription of zygotic genes and attendant rises in choline acetyltransferase, the enzyme that synthesizes ACh (Buznikov et al. 1968; Buznikov and Podmarev 1990; Falugi et al. 2002). In accord with the trophic role of ACh, both ACh antagonists and agonists that are known to exert developmental neurotoxicant actions in mammals perturb sea urchin development, with periods of sensitivity corresponding to the surges in ACh levels (Buznikov 1990; Buznikov et al. 1968, 1996, 1997, 2001a, 2001b; Buznikov and Podmarev 1990; Buznikov and Rakic 2000; Gustafson and Toneby 1970; Pesando et al. 2003).

To our knowledge, no studies have appeared on the ontogeny and trophic role of the high-affinity choline transporter in the sea urchin. In the mammalian brain, choline transport, and not the activity of choline acetyltrans-

Questions and discussion