



BASIC PROTOCOL

for the Nereis Park Experiment 07: Sediment reworking by Nereidae species

Sediment and organisms sampling

- Sample sediment and Nereis individuals at the same site.

Only one species has to be used (preferably *N. virens* or *N. diversicolor*). If you have both species in your area, you are free to build two separate experiments (ask for another set of tracers in this case).

Twelve individuals are requested for the experiment. They have to be weighted and measured (length, width). Chose them as close in size as possible. If possible do not use too small organisms.

Core preparation

- Sieve surface sediment (0-5 cm) with a 1 mm mesh (without adding water). Save some sieved sediment for TOC analysis (quantity depending of your own analysis need) and for grain-size distribution analysis (~20 ml; that will be shipped to LMGEM).
- Fill four core tubes with a 15-cm high column of sieved sediment. The core tubes should have a diameter of 8.5 -10 cm.
- Fill the remainder of the cores (~10 cm) with seawater (preferably from the sampling site) and leave the cores for a week with aerated seawater under in situ temperature (or at least as close as possible to in situ temperature).

Animal and luminophore introduction

- Introduce four individuals in each of three cores; the last core without introduced organisms will serve as a control.
- Let the cores acclimatized in the aerated seawater system for a week (12:12 dark:light cycle; *in situ* temperature).
- Make a suspension of each luminophore dose (i.e. each small plastic bag provided) with few ml of water (this will hydrate particles that will sink easily) and add one luminophore suspension to the overlying water of each core.
- Incubate the cores in aerated seawater (12:12 dark:light cycle; in situ temp.).

Sampling

- After 10 days of incubation, gently remove the overlying seawater (by siphoning) and save the first centimetre of water immediately above the sediment (it may contain luminophores).
 - Vertically section the sediment in 0.5-cm thick layers from the surface down to 2 cm depth, and then in 1-cm thick layers down to 15 cm for Nereis cores (only down to 5 cm for the control core).
 - Freeze-dry and then gently homogenise each sediment layer.
- Note: Freeze-dry the first cm of seawater and add it with the first sediment layer before homogeneization.



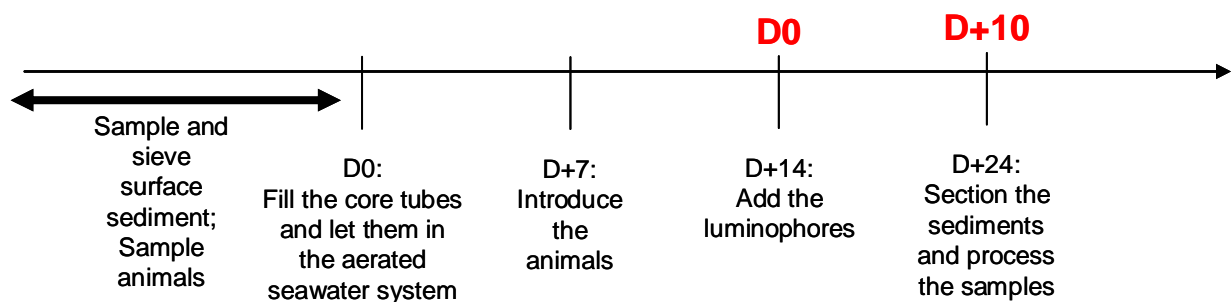
- Weigh each sample.
- Ship a ~10-g subsample of each layer to LMGEM for luminophore counting (you will keep then a spare set of samples).

Labelling of samples

Name of the partner (see below) – name of the core (N1 to N3 for each of the worm cores, and C for the control) – layer (1 to 17)

Ex: ISMER - N1 – 14

BASIC PROTOCOL



Data set to be provided by each participant:

- species used
- incubation temperature
- total worm wet weight in each core
- core tube diameter
- length and width of each worm (or total biovolume in each core)
- TOC of the starting (sieved and homogenized) sediment

Measurements by LMGEM (Marseille)

- grain-size distribution of the starting (sieved and homogenized) sediment
- luminophore profiles
- sediment reworking quantification



Participants		Organization	code
Philippe	ARCHAMBAULT	ISMER/IML, Québec, Canada	ISMER
Franck	GILBERT	UMarseille, France	UM
Georges	STORA	UMarseille, France	
Josephine	ALLER	MSRC, USA	MSRC
Robert C.	ALLER	MSRC, USA	
Suzanne	ERIKSSON	Göteborg University, Sweden	GOT
Stefan	HULTH	Göteborg University, Sweden	
Karl	NORLING	Göteborg University, Sweden	
Michael	TOWNSEND	NIWA, New Zealand	NIWA
Eric	KRISTENSEN	University of Southern Denmark, Denmark	USD
Stefan	FORSTER	Rostock University, Germany	UR
Robert A.	STEAD	Universidad de Los Lagos, Chile	ULL
Robert	KENNEDY	National University of Ireland Galway, Ireland	NUI
David	SHULL	Western Washington University, USA	WWU
Gary T.	BANTA	Roskilde University, Denmark	RUC
Filip	MEYSMAN	NIOO, The Netherlands	NIOO
Patrick	GILLET	Université Catholique de l'Ouest, France	UCO
Martin	SOLAN	Aberdeen University, UK	AU
Jonas	GUNNARSSON	Stockholm University, Sweden	SU
Nils	VOLKENBORN	AWI Bremerhaven, Germany	AWI
Stephen	VIDDICOMBE	Plymouth Marine Laboratory, UK	PLM
Lois	NICKELL	SAMS, UK	SAMS
Ming-Yi	SUN	University of Georgia, USA	UG
Xavier	DE MONTAUDOIN	Université de Bordeaux, France	UB
Jean-marie	JOUANNEAU	Université de Bordeaux, France	
Pascal	LECROART	Université de Bordeaux, France	
Sabine	SCHMIDT	Université de Bordeaux, France	
David	DUFOUR	Shanghai, China	DS
Frédérique	FRANCOIS-CARCAILLET	UMontpellier	USTL
Sam	BENTLEY	Memorial UNewfoundland	MUN
Duncan	MCILROY	Memorial UNewfoundland	
Sandor	MULSOW	Universidad Austral de Chile	UAC
Manuela L.	RUDLOFF	Universidad Austral de Chile	
Ronnie N.	GLUD	University of Copenhagen	UCOP
Mauro	FRIGNANI	Istituto di Scienze Marine Bologna, Italy	ISMB



OPTIONAL PROTOCOL

Sediment reworking and bioirrigation by Nereidae species

Sediment and organisms sampling

- Sample sediment and Nereis individuals at the same site.

Only one species has to be used (preferably *N. virens* or *N. diversicolor*). If you have both species in your area, you are free to build two separate experiments (ask for another set of tracers in this case).

Twelve individuals are requested for the experiment. They have to be weighted and measured (length, width). Chose them as close in size as possible. If possible do not use too small organisms.

Core preparation

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- Fill four core tubes with a 15-cm high column of sieved sediment. The core tubes should have a diameter of 8.5 -10 cm.
- Fill the remainder of the cores (~10 cm) with seawater (preferably from the sampling site) and leave the cores for a week with aerated seawater under in situ temperature (or at least as close as possible to in situ temperature).

Animal and luminophore introduction

- Introduce four individuals in each of three cores; the last core without introduced organisms will serve as a control.
- Let the cores acclimatized in the aerated seawater system for a week (12:12 dark:light cycle; *in situ* temperature).
- Make a suspension of each luminophore dose (i.e. each small plastic bag provided) with few ml of water (this will hydrate particles that will sink easily) and add one luminophore suspension to the overlying water of each core.
- Incubate the cores in aerated seawater (12:12 dark:light cycle; in situ temp.).

Bromide introduction

Add Br⁻ (e.g., as NaBr) at a final concentration of approximately 10 mM to the overlying seawater of one core with worms, approximately 2 (core 1), 1 (core 2) and 0.5 (core 3) days before the end of the incubation (10 days), respectively. Two days before the end of incubation, also add Br⁻ at a concentration of 10 mM to the overlying seawater of the control core. Note the time of Br⁻ addition to each core exactly. After approximately 15 min. of incubation, take a water sample of the overlying water (a few ml) and store until later determination of the initial Br⁻ concentration (see below).



Sampling

- After 10 days of incubation, remove most of the overlying seawater and store a subsample (several ml) until later Br⁻ analysis (see below).

Note: carefully remove the water close to the sediment (~1cm) in order not to avoid sediment (and luminophore resuspension) and save this water sample for luminophore analysis (it may contain luminophores).

- Vertically section the sediment in 0.5-cm thick layers from the surface down to 2 cm depth, and then in 1-cm thick layers down to 15 cm. Note the time of core sectioning exactly.

- Extract porewater from each sediment slice by centrifugation in double centrifuge tubes at 1500 rpm for 10 min.

- Store the porewater at 4°C in closed containers to prevent evaporation until later Br⁻ analysis. We assume that your laboratory can perform this analysis, usually by ion chromatography or spectrophotometrically (e.g., after Presley 1971 as cited in Aller & Aller 1992), but if that is not the case analysis at another laboratory will be arranged.

- Freeze-dry and then gently homogenise each sediment layer.

- Weigh each sample.

- Ship a ~10-g subsample of each layer to the LMGEM for luminophore counting (you will keep then a spare set of samples.).

Labelling of samples

Name of the partner (see below) – name of the core (N1 to N3 for each of the triplicate, C for the control) – layer (1 to 17)

Ex: ISMER - N1 – 14

Data set to be provided by each participant:

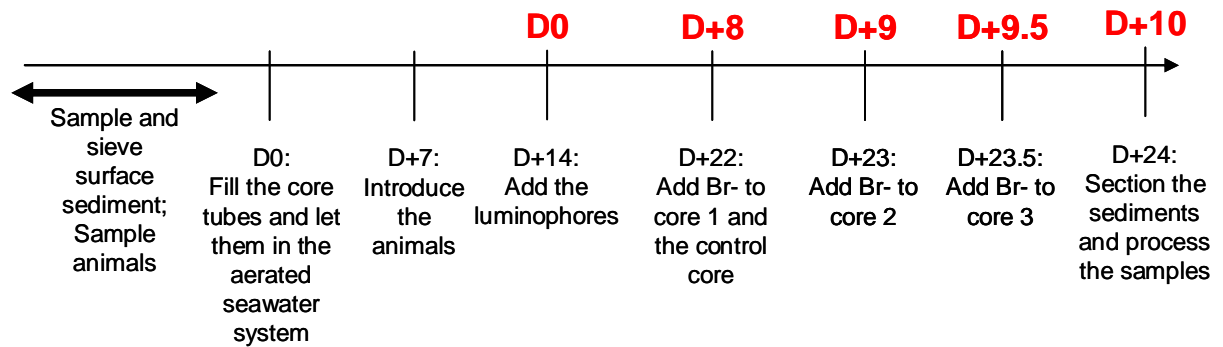
- species used
- incubation temperature
- total worm wet weight in each core
- core tube diameter
- length and width of each worm (or total biovolume in each core)
- TOC of the starting (sieved and homogenized) sediment
- bromide profiles (unless agreed to be measured elsewhere)
- incubation time for Br⁻ tracer

Measurements by LMGEM (Marseille)

- grain-size distribution of the starting (sieved and homogenized) sediment
- luminophore profiles
- sediment reworking quantification



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