

AGGREGATE METHODOLOGY WORKSHOP

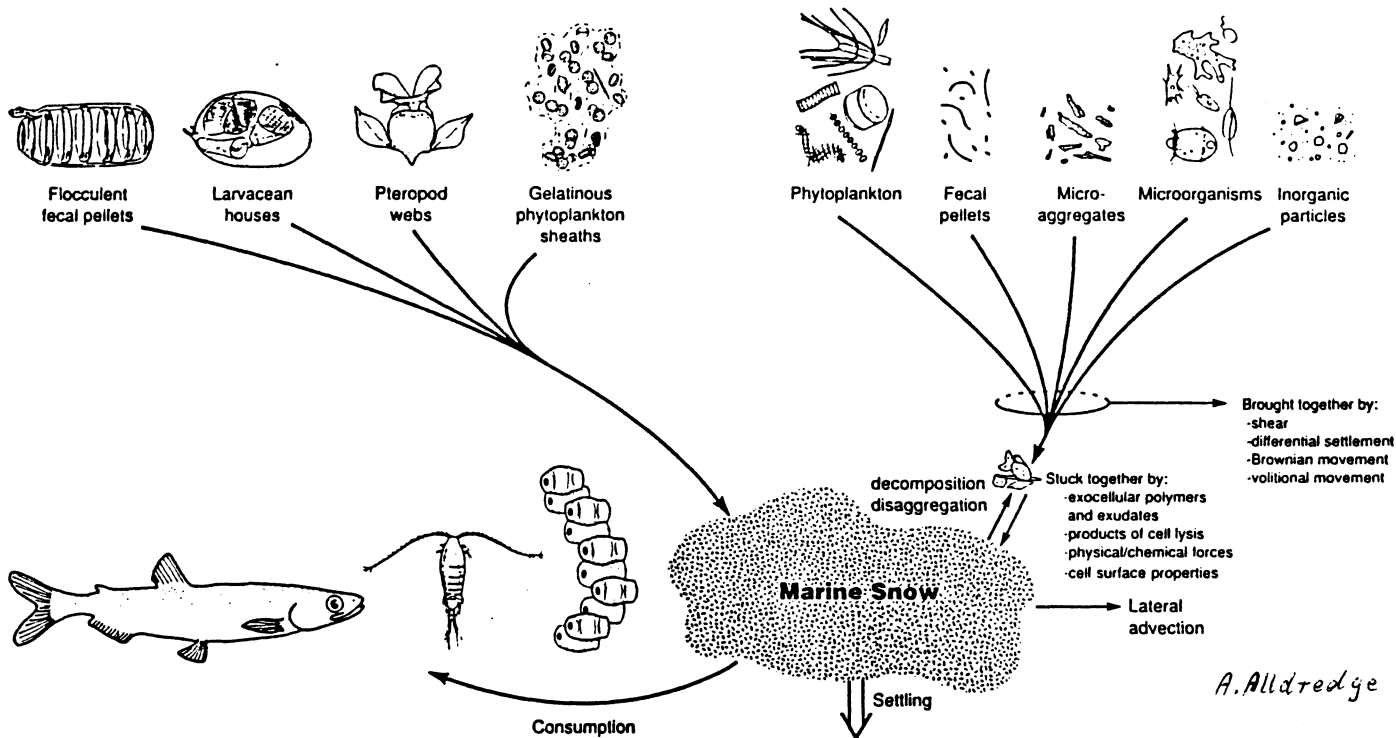
2 - 3 JUNE 1988 IN BREMERHAVEN

COLLECTION OF ABSTRACTS

Particle Dynamics of Marine Snow

de novo production
by marine organisms

Biologically-enhanced physical
aggregation of component particles



Alfred-Wegener-Institute for
Polar and Marine Research
- Biology II -
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AGGREGATE METHODOLOGY WORKSHOP

2 - 3 JUNE 1988 IN BREMERHAVEN

INTRODUCTION

A small workshop on marine particle aggregates was held this summer in the Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, F. R. Germany.

The importance of particle aggregation and disaggregation for biogeochemical processes in the sea need no longer be stressed thanks to intensive field studies carried out with divers and with moored instruments in the recent past. However, complementary in vitro studies are still in an early phase and as yet there is no consensus on how to carry out such experiments. We therefore felt it timely to call together a group of interested investigators to discuss methodological aspects of dealing with aggregates.

The main theme of this workshop as defined in the first circular was centred around laboratory work with aggregates:

- a) Methods for growing aggregates in the lab: e.g., choice of "standard detritus", bacterial-mediated aggregates, diatom-mediated aggregates, etc.
- b) Studying aggregate dynamics under simulated field conditions.
- c) Handling, measuring, analysing lab-grown aggregates with an aim to selecting parameters for characterizing aggregates: surface area, density, stickiness, consistency, fragility, chemical composition of matrix (mucopolysaccharide, glycoprotein, etc.).
- d) Protozoan and zooplankton activity related to formation and breakdown of aggregates.
- e) Rheological properties of aggregates (strength, elasticity) in relation to formation and breakup.
- f) Studying the influence of aggregates on water column properties (scavenging and adsorption etc.) by using tracers (isotopes, fluorescent dyes and beads, mineral particles etc.).

During preparation of the workshop, it became evident that laboratory work with aggregates is still in its infancy and that a regular methods workshop could not be expected at this stage. The shift in tenor of the workshop is reflected in the following paragraphs which are taken from a letter circulated to all participants of the workshop on May 10, 1988:

You will all agree that the study of aggregates differs from that of other fields of pelagic science in that it is much more visually based. The coining of the terms "marine snow" and "fluff" are proof of this fact. I would venture to say that the popularization of concepts during the last decade (such as energy flow models and their accompanying P vs. I or nutrient curves) have directed scientists inwardly towards abstract thought: visualizing graphs and getting a feel for numbers. The advent of aggregate science, in contrast, has had an immediate impact on the mode of our thinking, whether we are conscious of it or not. By directing our attention outwards, we are now attempting visualization of the environment itself; hence, we can assume that the

conceptual framework in which we classify and interpret our measurements will have changed.

The prescient work of Riley was not based on visualization so the community could not be fired by it. In my opinion it was the scuba divers who finally got the message across that we might have been missing something of importance. Of course, the observations were bolstered by the surge of new instruments and results - rapid sedimentation, microzones, anoxia in the mixed layer, to name a few. Now that we know there are aggregates out there, playing an important, perhaps crucial role, in shaping the biology and geochemistry of the sea, we must find new ways of coping with them. Sharing experience in this new venture is the goal of the workshop.

We do not intend running a hard-core methods workshop. We shall indeed share experience on handling aggregates. However, the accent is on the conceptual aspect of methodology. Obviously, there is tremendous scope for development of methods; what we should discuss are the directions which appear the most promising at the moment and what is still futuristic.

Before launching into the actual deliberations of the workshop, we feel it useful for the participants to know what the individual contributor is visualizing when making his or her comments. We, therefore, request you to bring along a few of your favourite aggregate and fluff slides to share with us during session 2. We will not be squinting at the parameters but will be treating ourselves to sea-scapes. Therefore, we should set an upper limit of 100 slides (about 3-10 per person) at an average of one slide per minute.

Victor Smetacek
(For the organizing committee)

The following is the report that has eventually come out of this meeting. The organizing committee consisted of (in alphabetical order): Biddanda, Bopaiah; Ehlken Sabine; Jenkinson, Ian; Nöthig, Eva-Maria; Riebesell, Ulf; Riemann, Franz; Rutgers v.d. Loeff, Michael; Scharek, Renate; Smetacek, Victor.

The "Organizing Committee" take this opportunity to extend their thanks to all participants of this workshop who shared their ideas and feelings while here. The mood was exciting and we all learned a lot!

This report was compiled and typed by Ingrid Lukait.

The following gives the abstracts of presentations.

ABSTRACTS

OPENING ADDRESS

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Macroscopic aggregates of detritus, living organisms and inorganic matter known as marine snow, have significance in the ocean both as unique, partially isolated microenvironments and as transport agents: much of surface-derived matter in the ocean fluxes to the ocean interior and the sea floor as marine snow. As microhabitats, marine snow aggregates contain enriched microbial communities and chemical gradients within which processes of photosynthesis, decomposition, and nutrient regeneration occur at highly elevated levels. Microbial communities associated with marine snow undergo complex successional changes on time scales of hours to days which significantly alter the chemical and biological properties of the particles. Marine snow can be produced either de novo by living plants and animals especially as mucus feeding webs of zooplankton, or by the biologically-enhanced physical aggregation of smaller particles. By the latter pathway, microaggregates, phytoplankton, fecal pellets, organic debris and clay-mineral particles collide by differential settlement or physical shear and adhere by the action of various, biologically-generated, organic compounds. Diatom flocculation is a poorly understood source of marine snow of potential global significance. Rates of snow production and breakdown are not known but are critical to predict flux and to understanding biological community structure and transformations of matter and energy in the water column. The greatest challenge to the study of marine snow at present is the development of appropriate technology to measure abundances and characteristics of aggregates in situ.

SLIDE SHOW: Snow and fluff kaleidoscope

About one hundred favourite slides contributed by the participants were shown here.

I. FIELD METHODS AND OBSERVATIONS

Methods of Investigating Marine Snow in Situ

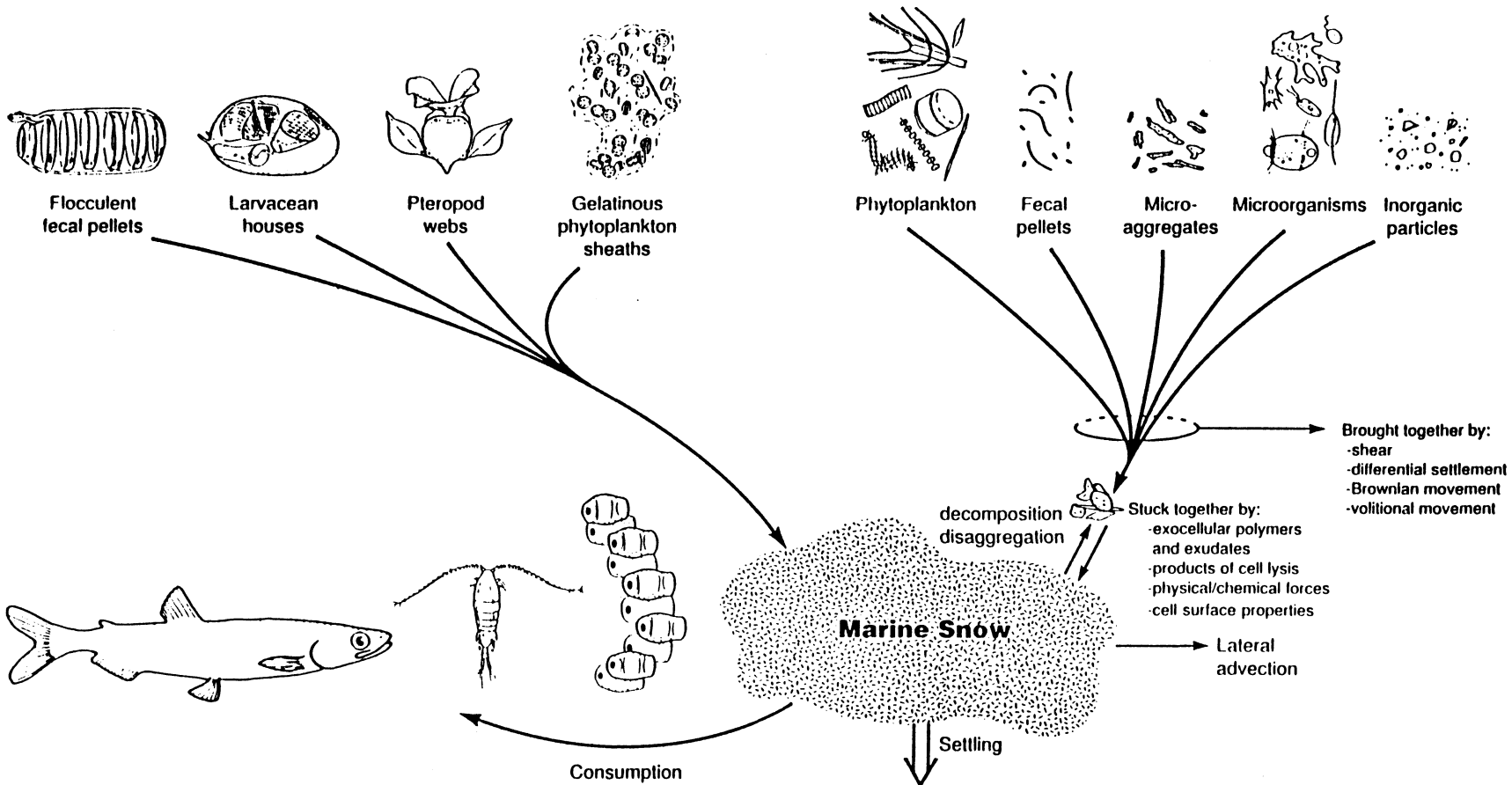
Alice L. Alldredge

Marine snow has been investigated and collected in situ using two approaches: 1) scuba diving in the ocean surface layer and 2) submersibles in the ocean interior. Using scuba, divers can collect aggregates of marine snow individually in plastic syringe barrels stoppered at both ends with syringe plungers. Aggregates may be collected in large quantities by sucking them into large volume syringes with a minimum volume of surrounding seawater. Aggregate densities are determined visually by counting the aggregates passing through a hoop attached to a hand-held flow meter. Additional sampling tools include underwater tape recorders, dye-rifles for delivery of neutrally buoyant dye spots, and syringe samplers for collection of μl sized samples within and around aggregates. Studies of marine snow with submersibles are limited by expense, by the small numbers of aggregates which can be collected per dive, and by the difficulties of performing manipulative experiments in situ.

Particle Dynamics of Marine Snow

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In Situ Observation of Flocs

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Particle characteristics such as size, shape, density and settling rate are important factors determining suspended sediment transport. Usually these characteristics are determined after sampling in one way or another. Natural flocs, however, although stable under natural conditions in the water, are easily destroyed by increased turbulence or shock waves, such as occur during sampling and analysis. Because of this fragility, almost all determinations have been done on fragments of natural flocs. This does not mean that such data cannot be used in a meaningful way, but in situ observations are required when size, shape and structure of the flocs, and their association with organisms are studied. Large flocs, stringers, and large structures can be seen with an unaided eye and photographed with a normal underwater camera or from a submersible. Careful sampling by divers may bring flocs intact to the laboratory. To observe small particles in situ several optical techniques have been used: in situ camera systems with oblique or parallel-transmitted light, laser diffraction, holography. Some new developments in Holland to observe flocs in situ down to less than 10 micron size are discussed.

Algae in Marine Snow

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Over the last 7 years, the UCSC group has been studying algal associations with marine snow using sediment trap collections in the VERTEX program. Such collections provide comparatively large samples and represent averages for particles obtained over periods of a few months. In this presentation, I review advantages and disadvantages of sediment trap collections for obtaining snow associated algae and then briefly review some of the highlights of our findings in VERTEX.

Sediment trap contents in our North Pacific VERTEX sites are dominated by marine snow, thus making traps excellent, time-averaged collectors of sinking snow. Traps also contain abundant living and decomposing organisms, including algae, and many of these - particularly small cells with low sinking rates - likely enter on marine snow. Sediment traps also provide samples of snow at all depths, thus overcoming the limitations of hand collections, which are restricted to surface waters, or of submersibles at depth, which are very expensive. However, it is never clear from traps which algae entered independently and which entered on marine snow, since mixing of trap contents occurs during the long field deployments. Furthermore, the timing of particle entry (e.g. whether most entered in a few, pulsed events), is unknown. Algal identification in the complex trap mixtures is also a problem. Live algae in traps are best recognized by autofluorescence, but pigment fading appears to be significant in traps in the upper few 100 m over the week to month deployments. Thus algae may be underestimated at these shallower depths. Furthermore, it is not yet clear how trap design affects efficiency of snow capture: cylindrical traps appear to differ in their snow collecting efficiency as compared with cone-shaped traps. Lastly, "swimmers" such as larvaceans may introduce mucus feeding structures with

their associated algal populations, a phenomenon difficult to reconstruct from trap collections.

Results of our VERTEX deployments indicate large numbers of algae, predominantly very small cells ($>5 \mu\text{m}$), settle through the water column, presumably in association with marine snow: typically 10^6 cells $/\text{m}^2$ each day. However, these large numbers generally represent only a few percent or less of the standing crops of surface populations, thus suggesting the losses do not deplete surface populations. Exceptions occur sporadically with large centric diatoms, which may be settling independently of marine snow. These results suggest that algal association with snow involves only a minor fraction of the surface crop, or, alternatively, that the types of marine snow that sink are comparatively algal poor. The numbers of cells decrease by several orders of magnitude in the lower euphotic zone and upper mesopelagic zone, mirroring the disappearance of marine snow and other particles in the flux. Furthermore, VERTEX findings and those of other recent studies on particle flux suggest that the average life expectancies of settling particles may be limited. Thus associations between algae and marine snow should be considered temporary ones, and models of such systems should reflect the likelihood that these communities are transitory, at least the communities on sinking marine snow.

Phytodetritus or "Fluff" from the Sea Bed in the N. E. Atlantic at 4.500 m

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Layers and pockets of phytodetrital aggregates were found on the sediment surface at 4.500 m in the northeastern Atlantic ($47^{\circ}20'N$, $20^{\circ}W$) during the BIOTRANS (Biological Vertical Transport in the Deep Sea) programme during July and August 1986. The "whitish, older" component of these fluffy aggregates contained cells characteristic of the spring bloom while the "greenish, fresher" aggregates contained intact coccolithospheres and dividing cyanobacteria characteristic of summer production in these waters. The abundance of intact, dividing cyanobacteria indicates that these photosynthetic picoplankton are transported rapidly from the euphotic zone to the deep sea by attachment to larger aggregates.

Rapid growth by deep sea adapted bacterial and flagellate populations, under shipboard pressure incubations, resulted in fast decomposition and transformation rates of the phytodetritus.

Calculations based on the distribution and concentration of the phytodetritus on the sea bed and the microbial decomposition rates indicate that the seasonal sedimentation of aggregates from the surface waters to the deep-sea may make a significant contribution to the ocean flux of carbon.

For further details see:

Lochte, K., & Turley, C.M. (1988). Bacteria and cyanobacteria associated with phytodetritus in the deep sea. *Nature* **333**, 67-69

Suess, E. (1988). Effects of microbe activity. *Nature, News and Views*, **333**, 17-18.

Turley, C.M.; Lochte, K. & Patterson, P.J. (1988). A barophilic flagellate isolated from 4.500 m in the N. E. Atlantic. *Deep-Sea Res.*, (in press)

Gelatinous Phytoplankton Detritus Aggregates on the Atlantic Deep-Sea Bed: Structure and Mode of Formation

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A rapid deposition of phytoplankton detritus to the deep-sea bottom and a close coupling of this phenomenon to spring and summer blooms of surface phytoplankton has been described earlier from the Porcupine Seabight, North Atlantic. However, the causes of this large amount of phytodetrital sedimentation to the deep-sea bed was unknown.

During a cruise with the German RV "Meteor" (BIOTRANS IV) to an adjacent but more remote site (47°N/20°W) with depths of about 4500 m, the occasion arose to collect some of the relevant detritus material in samples from the bottom and the water column. The specific deposits most often encountered at the bottom were greenish gelatinous aggregates (ca. 1 cm in diameter) consisting of an amorphous mucous substance enclosing single, dispersed algal cells, skeletal elements of protists, and small faecal pellets. Coccolithophorids (sometimes with preserved cytoplasm and nuclei) were abundant. The most prominent parts in terms of numbers and volumes within the gelatinous aggregates were round faecal pellets having a diameter of 5 to about 80 µm, and consisting of unidentifiable detrital material, folded membranes, and masses of green chlorophyta cells, 3 µm in diameter.

The faecal pellets were identical to those found in Phaeodaria (Radiolaria) which were collected at the same time in the water column. It is proposed that the loading of drifting phytoplankton-containing mucus flakes with phaeodarian faecal pellets and the entangling of this mucus with spiny phaeodarian cells, are important factors in the formation of fast-sinking gelatinous aggregates.

II. LAB METHODS AND OBSERVATIONS

Laboratory Methods and Observations on Algal Binding

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Visual and microscopic observations show that most aggregates do contain phytoplankton cells. Species like *Chaetoceros*, *Phaeocystis*, *Navicula* were frequently reported. Even the large quantities of foam deposited on Dutch and German beaches as a result of *Phaeocystis* bloom include high number of cells or cellular fragments and can therefore be considered as massive aggregates. The role of phytoplankton and more especially the type of phytoplankton community in the induction of marine aggregates is therefore questionable.

In this direction, it seems that colonial forms should have a key role. Indeed, microscopic observations of natural colonies of *Chaetoceros socialis* or *Phaeocystis* show a clear morphological evolution with the age of the colonies: healthy colonies are always spherical with well-distributed cells in it although old colonies are irregular and characterized by a higher cells density. Some of them are illustrated by Fig. 1. Moreover, contrary to healthy

colonies, old forms are always colonized by bacteria and protozoa even by other phytoplankton species like *Navicula*.

Do these morphological changes constitute the different steps up to the colony death or better do they correspond to an adaptative behaviour of these colonies in response to environmental stress? And then can we consider that healthy colonies should be regarded as an extreme form of aggregates?

The link between environmental factors, morphological changes and biological function or behaviour is far from being understood:

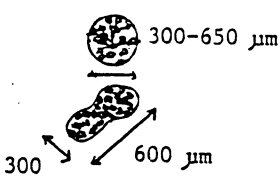
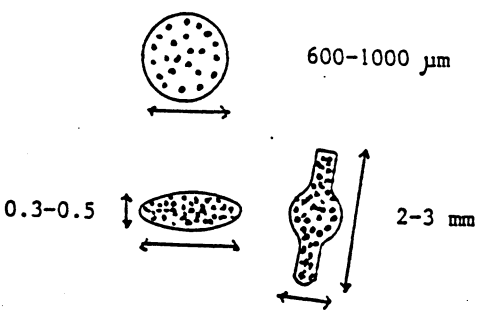
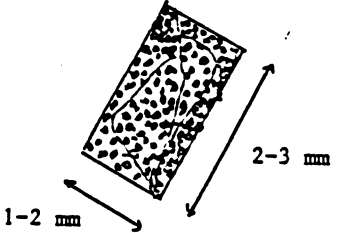
<u>Induction</u>	<u>Form</u>	<u>Function</u>
Triggering factors?	spherical	floating versus sinking?
Disruption factors?	irregular	increase or decrease nutrient? uptake capacity
(Physical, chemical, hydrodynamic, chemiotaxis)		survival strategy? induction of micro-environment?

The idealized experiment should combine simultaneously visual and microscopic morphological observations, physiological studies, behaviour observations like sedimentation or rising velocity and hydrodynamical and physico-chemical corresponding data. Physiological experiments should be conducted on basis of a conceptual model of phytoplankton metabolism expressing the main metabolic activities bound not only to phytoplankton growth like protein synthesis, but to important processes in colonies or aggregate formation like mucilaginous substances secretion. As an example of this concept, Fig. 2 shows the main metabolic activities associated with a mixed phytoplankton community composed of diatoms and *Phaeocystis* colonies. In this diagram secretion of polysaccharides by *Phaeocystis* is explicitly considered. Such an idealized conceptual model constitutes a framework for developing new experiments, the results of which would in turn add some complexity to the existing model and improve it. In such an approach, interaction between the model and laboratory and field experiments is constant in such a way that each feeds the other. Complementary to these experiments, measurements of bacterial and protozoan activities should be conducted with the same philosophy.

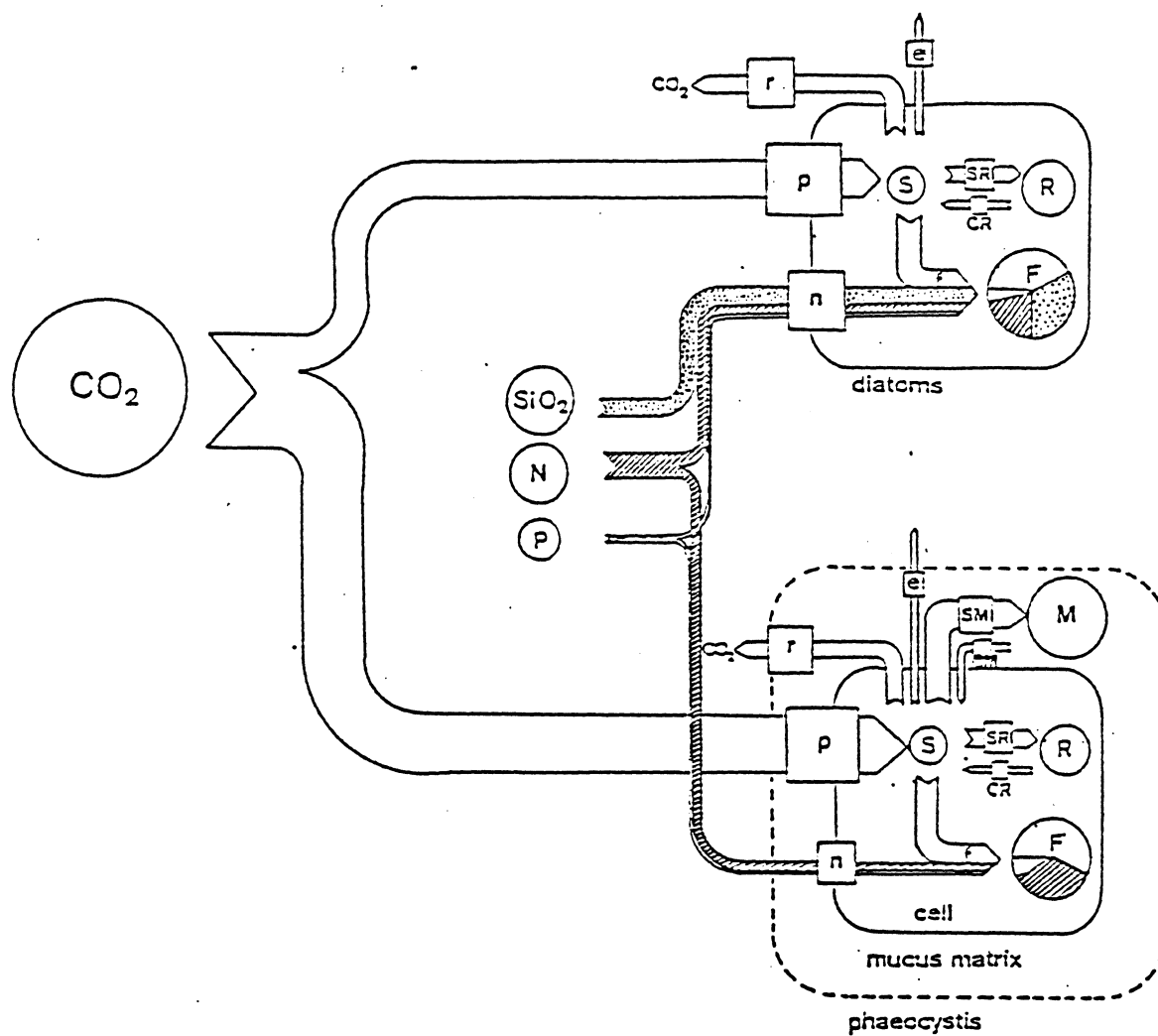
In our opinion, this approach constitutes the only way to understand the fundamental link between morphology and behaviour and to predict accurately aggregate formations on basis of the knowledge of phytoplankton physiology and of its control by environmental factors. This would constitute then an important way to success in an accurate estimate of the aggregates' importance in the biological functioning of the whole pelagic and benthic system.

Christiane Lancelot

COLONIAL PHYTOPLANKTON IN COASTAL NORTH SEA DURING THE SPRING BLOOM

SPECIES	SHAPE & DIMENSIONS
<u>DIATOMS</u>	
CHAETOCEROS SOCIALIS	 300-650 μm 300 μm 600 μm
<u>PHAEOCYSTIS</u>	
	 600-1000 μm 0.3-0.5 μm 2-3 mm 2-3 mm
	 1-2 mm 2-3 mm "folded sheet": contains bacteria & protozoa

Christiane Lancelot



S : small metabolites (monomeric precursor for macromolecular synthesis)
 R : storage macromolecules (polysaccharides & lipids)
 F : functional & structural macromolecules (proteins, DNA...)
 M : mucilaginous matrix

p : photosynthesis
 S_R : synthesis of storage products
 C_R : catabolism of storage products
 n : nutrient uptake
 r : respiration

Figure 2

Bacterial Attachment

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Two types of bacterial adhesion are common in natural environments. These are adhesion to particles or other solid surfaces and adhesion to other bacteria or microbes. Both appear to play a role in aggregate formation.

Adhesion is the result of attractive physicochemical interactions between the two surfaces. These interactions include van der Waals forces, electrostatic and polar interactions, hydrogen bonding, and hydrophobic interactions. The types of interactions involved in any particular adhesive event will depend on the compositions of the two surfaces and of absorbed or constituent polymers.

When attempting to answer the question "how do bacteria attach?", it is useful to ask the reverse question, what is that can keep them dispersed? Bacteria, which are of colloidal size, will tend to stay dispersed in suspension by (i) electrostatic repulsion, because both bacteria and almost all natural surfaces bear a net negative charge, and (ii) steric effects, due to the hydration of surface polymers and their "resistance" to conformation changes which impose more order or less freedom of movement. Thus, for aggregation to occur, attractive interactions must overcome steric or electrostatic repulsion. Also, daughter cells may remain associated after replication.

Many different surface polymers may be involved in the adhesion and aggregation of bacteria. These include extracellular or O-antigen (lipopolysaccharide, LPS) polysaccharides, proteins, the lipid moiety of LPS, and organized structures such as fimbriae and flagella. Aggregation may be enhanced by the addition of flocculents (e.g., metal ions, polyelectrolytes, lectins, proteins) or changes in environmental factors, (e.g., ionic strength, pH, cation concentration, temperature, mixing characteristics).

Key questions relating to aggregate formation which are now ripe for investigation are:

- (i) To what extent are polymers involved in cell-cell and cell-particles aggregation?
- (ii) Which cells produce these polymers? Are they derived from bacteria, from algae, or from both? To what extent are they degradation products?
- (iii) What is the time-sequence of adhesive polymer production?
- (iv) To what extent and in what way is adhesive polymer production, hence aggregation, controlled by environmental conditions?

*In Smetacek, V. (convenor), Aggregate Methodology
Workshop, 2-3 June, 1988, AWI, Bremerhaven.*

Microbial aggregation, degradation, and disaggregation of phytodetritus in sea water.

Bopaiah A. Biddanda and *Lawrence R. Pomeroy

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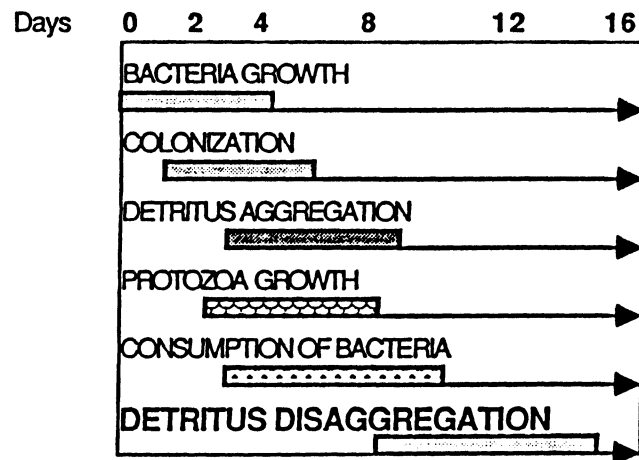
Some rather serendipitous in situ observations of degrading blooms of the planktonic blue green alga, Trichodesmium sp. were made at sea. It was observed that post bloom bundles of trichomes were rapidly transformed by microbial activity (heterotrophic bacteria and bacterivorous protozoa) into an irre recognizable status in a matter of just four days in the surface waters of the Sargasso Sea during the summer period.

These observations in nature, led to an additional study of detritus behaviour in the laboratory. Detritus was generated from three different phytoplankton sources (Cylindrotheca fusiformis - a diatom; Dunaliella sp. - a phytoflagellate; Synechococcus sp. - a cyanobacteria) and kept in suspension in natural unfiltered sea water. Initial aggregation of particulate matter into macroaggregates was mediated by microbial colonization. In time, disaggregation resulted following the microbial activities of assimilation and breakdown of particulate matter. Bacterivorous protozoa seemed to accelerate this process of degradation and disaggregation in a manner analogous to the activity of invertebrates in soil and in forest litter.

A remarkably similar microbial succession involving heterotrophic bacteria and bacterivorous protozoa was observed on detritus derived from all the three sources (Fig. 1). This similarity in microbial processing of all detritus may be the reason why most particles in the sea appear similar. Further, there was a regular process of aggregation and disaggregation of particulate organic matter associated with the microbial utilization of detritus. It appears that detritus aggregation, degradation, and disaggregation are a continuous processes in the water column. A sample from the coastal water, therefore, contains all stages in this process.

Figure 2, describes a schematic model of detritus behaviour or life history in the water column. The model is driven by inputs from primary and secondary production. It is, on the whole, more or less internally consistent, since loss of aggregates by degradation and sinking are compensated through new inputs from primary and secondary production as well as from resuspension from the deeper layers and the benthos. In a general sense, we see the fate of detritus particles in the water column as aggregation- disaggregation sequences in time and space.

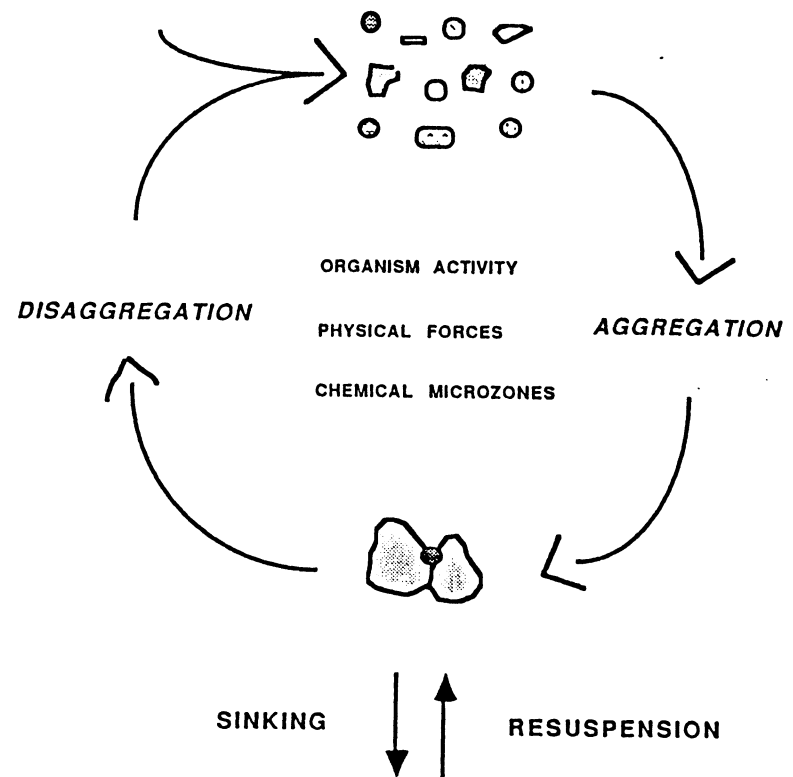
1 MICROBIAL SUCCESSION DURING DETRITUS DECOMPOSITION



2

AGGREGATES IN THE SEA

Inputs from Primary and Secondary Production, etc.



Lab-Made Marine Snow: It's the Real Thing

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This research reports on a simple method for manipulating and studying intact marine snow in the lab. Cylindrical tanks filled with unfiltered seawater were rotated on a roller table. Within 0.5 to 1.5 hours particulates in the seawater flocculated forming what appeared to be marine snow. Marine snow sampled in the field on 7 dates and at two sites was compared to that formed in the lab from water collected concurrently. The size (projected area), concentration (#/l), and total amount (total projected area) of field caught and lab made marine snow were not significantly different. There was a significant difference in the shape of the two types of marine snow; lab made marine snow had longer perimeters and was more globular than marine snow in the field. Under the microscope the subjective and quantitative impression was that the composition of the two types of marine snow was very similar. Both types of marine snow were composed of detritus and living phytoplankton surrounded by a matrix of mucus. There was no difference in the percentage of the total biomass of phytoplankton bound up in the two types of marine snow, but a significantly smaller percentage of the total mass of detrital particles were contained in the lab made marine snow. In both types of marine snow diatoms and dinoflagellates were on average only slightly enriched relative to the water surrounding the marine snow while detrital particulates were highly enriched. There was no significant difference in the concentration of phytoplankton or detrital particulates within the two types of marine snow. Over all, the lab formed marine snow appears nearly identical to natural marine snow suggesting that this mechanism of forming and handling marine snow allows researchers to easily experiment in the lab on intact marine snow.

LABORATORY AGGREGATES - SOME EXAMPLES, THEIR DESCRIPTION AND NON-DESTRUCTIVE HANDLING DURING LABORATORY CONTROLLED EXPERIMENTS.

(Carol Turley, Bopi Biddanda, Sabine Ehlken, Paulo Abreu, Ulf Riebesell, Ian Jenkinson)

On the premise that there is a vast range of aggregate types in the sea and not one "model aggregate" we looked to produce a variety of different aggregates in the laboratory with as diverse features as possible (different sizes and shapes, degrees of biological complexity, mineral content, fragility, strength and stickiness etc).

A. Description

We describe three very different aggregates, in terms of origin, content and rheometric qualities, using autofluorescence under different excitation wavelengths, fluorescent DAPI staining of DNA and SEM's.

1.) AGED SEA WATER AGGREGATES. These were formed in North Sea water enclosed in polyethylene tanks which were left to stand unmixed for over a year in subdued lighting at 20°C. These aggregates were the most dense of all examined with no visible "water spaces" between the components of the aggregate. They also were the most surface active, and were hardest to handle because of their stickiness and attraction to surfaces. The sticky matrix of this aggregate seems to be comprised of mucus produced by bacteria as they seemed to be the main biological component of the aggregate. Intermixed with this sticky bacterial matrix are inorganic mineral particles.

2.) SKELETONEMA AGGREGATES. Produced from a 2 month old, aerated, declining batch culture of *Skeletonema costatum* where bacteria, flagellates and ciliates are an important active component of the aggregates. Despite the decline of the culture the autofluorescence from the chlorophyll *a* in the *Skeletonema* is still strong indicating that regeneration processes by the microbial loop may be playing some role in their continued activity despite their aggregation. In this aggregate *Skeletonema* cells seem to comprise the main matrix. However, it looks like bacteria and cyanobacteria may play some role in aggregation as chains of both types of cells were a feature of all these aggregates and protruded from the edges of the aggregate. Do they play a role in increasing aggregate size and the ability of the aggregate to scavenge other aggregates and particles? Indeed, this type of aggregate was the only one that changed in size during an experiment we ran - it increased in size, was this growth!?

3.) PLANKTON-TOW AGGREGATES. These aggregates were formed in the laboratory from natural planktonic components sampled, using a 150 µm mesh sized plankton net, during March at the onset of the spring bloom in the North Sea just South of Helgoland. The algal components were nearly totally comprised on *Coscinodiscus wailesii*, known to be an important mucus producer, and a filamentous cyanobacteria. The zooplankton were a mixture of different larval stages, copepods, sagitta, shrimps, zoea etc. The sample was gently shaken under low light until aggregates >0.5 mm approximate diameter were formed approx. 2 weeks later. These are composed of fragile, loose aggregates incorporating decomposing/decomposed bodies of zooplankton, algal cells and chains often still autofluorescing and bacterial cells embedded sometimes in a thin layer of mucus. However, most striking were the chains of small autofluorescing cells (presumably cyanobacteria) and bacteria which seem to connect up the larger aggregate components and may again

play an important role in increasing aggregate size. These could be seen by the naked eye as a fuzzy halo around the aggregates.

B. Handling

We felt that it was essential to be able to handle the aggregates without affecting their shape and size. Furthermore, we wanted to be able to characterize them using non-destructive methods so that we could use the same aggregates in an experiment to investigate the sinking rates of the various aggregate types and sizes. We were even greedier, as we wanted to examine the effects of different temperature gradients and structures on aggregate sinking rates. Therefore, we wanted to be able to recover each aggregate and run it through different laboratory thermocosms with controlled temperature structures. After this, we wanted to check for any change in size of the aggregates and then measure some of the rheometric characteristics of the aggregate to see if these were in anyway related to aggregate origin, size, composition or sinking characteristics.

THE PARTICLE PEEPER (see fig. 1). Simple, cheap and effective. You can sample individual aggregates gently, as the glass reduces the shear likely to occur using a syringe and needle alone. The aggregate, during sampling, does not make contact with air, which is highly destructive! The peepers can be used to transfer the aggregate through a series of washes, store individual aggregates prior to experimentation, and of course where it gets its name from, to characterize the aggregate by microscopic examination through the glass wall of the peeper. We used a drawing arm extension to the microscope to draw the shape of each aggregate, measured the area using a planometer and with an estimate of the third dimension we were able to have a non destructive method of determining aggregate volume. Release of the aggregate from the peeper into the experimental thermocosms was simply achieved by upending the peeper and allowing the aggregate to settle out under its own sinking speed. Some patience is required! Retrieval of the aggregate was simple and could be achieved, on arrival on the bottom or mid water, by attaching the same peeper to the end of an AGGREGATE RETREIVER. This is based on the same simple principle as the peeper but with a connection at one end to receive the peeper (see fig. 1). In this manner, we were able to keep tabs on each aggregate as it had its own individual peeper. Our success rate of bringing 30 aggregates of each type through 3 thermocosms and onto the rheometer for the more fragile skeletonema and plankton tow aggregates was around 90% and for the more troublesome sticky aged seawater aggregates around 75%. This was far better than we had hoped for. An hour of practice and steady hands results in reasonable handling ability of the laboratory produced aggregates.

Particle Peepers

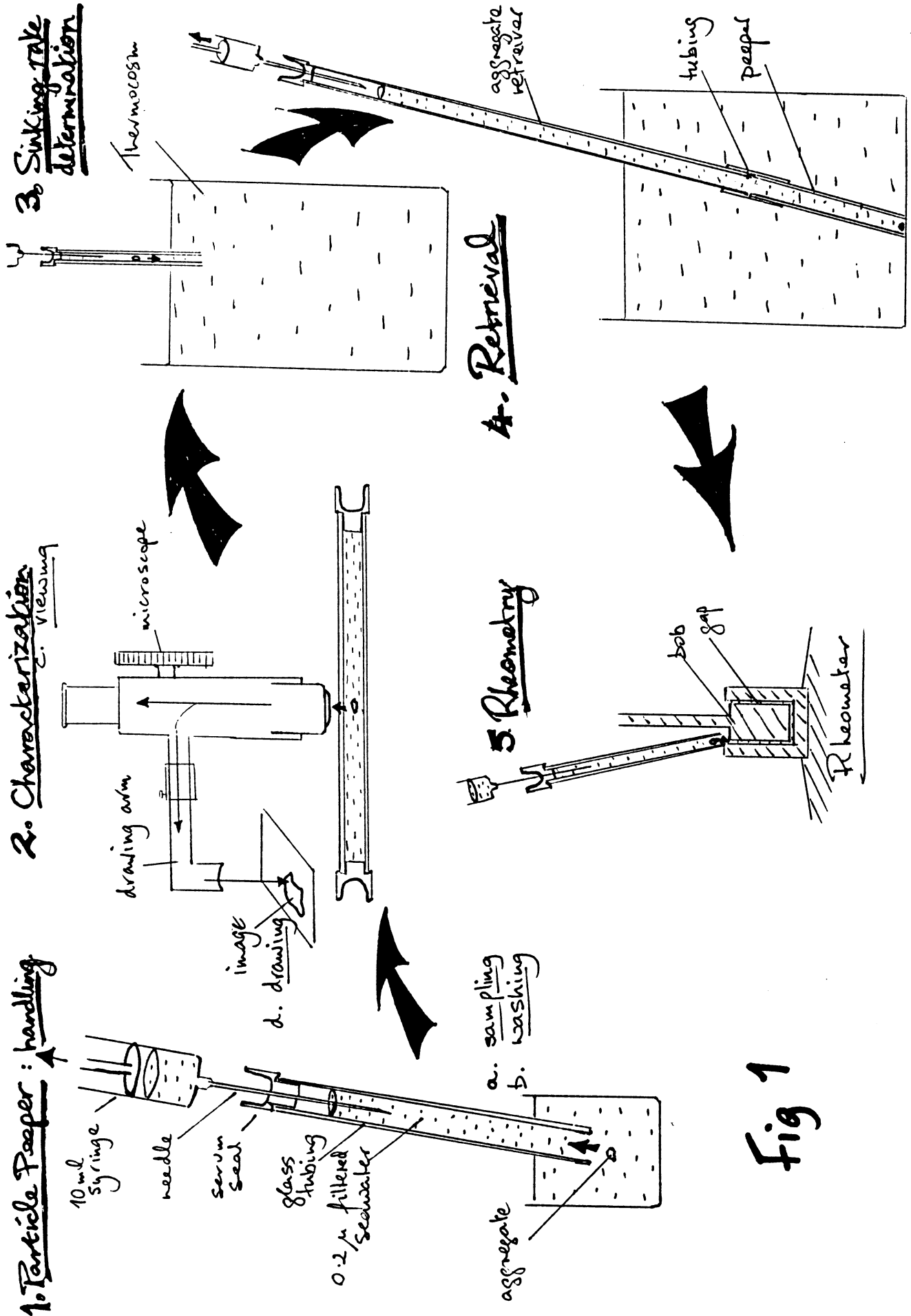


Fig 1

Microprobes

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At present there are microelectrodes for the measurement of oxygen, pH, and sulfide that have successfully been used in the marine environment.

Purchasable micro- and minielectrodes do not meet the size requirements for work on aggregates. Thus sensors have to be self-constructed, demanding much skill and training.

Amongst the oxygen microelectrodes those operating with a separate reference will in both spacial resolution (1-4 μm tip diameter) and response time ($T_{90} \approx 0.2$ s) be the ultimate choice. This makes them most useful in answering questions on small scale heterogeneity in photosynthesis studies and during investigations on small objects in general.

A more stable type of oxygen-sensor including both cathode and anode within a glass casing can be constructed with a tip diameter of 4-6 μm and response time well below one second. Electrical stability and less interference from other ions are the advantage of this more complex construction. This might be of interest under conditions of high Ca^{2+} - ion concentrations and if physical stress on the electrode is for some reason anticipated. Sulfide- and pH-electrodes have dimensions of 100 μm and 20 μm and less, respectively.

For a number of ions miniature (1-4 μm) ion-sensitive electrodes have been constructed. However, these have not been applied in a saltwater medium and interference is substantial.

Near-future use of microelectrodes on marine aggregates seems to be most promising under lab conditions in order to study processes taking place in short time scales. In situ use will pose severe technical problems.

III. TRANSFORMATION PROCESSES

Phytoplankton-bacteria interactions: Possible Role in the Genesis of Marine Aggregates

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We hypothesize that phytoplankton-bacteria mutualism results in the nucleation of marine aggregates in the following scenario: Nutrient-limited phytoplankton exude cell-associated exopolysaccharides which cause the accumulation of bacteria and their microbial predators within the microenvironment of the phytoplankton. The physical nature of the mucoid microzone, and the enhanced production-decomposition activities and elevated nutrient levels within it, impart such microzones a marine-snow-like character. Conceivably, the coalescence of the ephemeral "micro-snow" realms and sticking of micro-snow to other marine particles results in the formation of larger, visible, marine aggregates. Elegant experiments by Biddanda and Pomeroy have shown that bacteria can cement phytoplankton cells to generate marine snow. However, an additional role of bacteria in our hypothetical scenario is to physiologically elicit the production of

phytoplankton exopolysaccharides which then act to produce aggregates. We suggest that phytoplankton-bacteria mutualism may provide a framework for developing a mechanistic understanding of the genesis of certain types of marine aggregates.

Interaction Aggregate/Sea Floor

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Aggregates function as vehicles of transport for various materials from the surface waters to the sea floor. Once they reach the sea-floor the particles change profoundly. Firstly, their very patchy distribution on the sediment is largely determined by the often complex near bottom currents. Secondly, any current-induced movement across the sediment leads to re-aggregation and to incorporation of benthic components. Analysis of a number of different "fluff" samples revealed a strong variation in dry weights, content of organic carbon and bacterial numbers, which were uncorrelated. This indicates the simultaneous presence of different types of "fluff" probably of different origin, age and degree of decomposition. Studies on microbial metabolic activity under simulated deep-sea conditions showed barophilic characteristics in the community of the benthic boundary layer and these micro-organisms were capable of a breakdown of particulate detrital organic matter of 1.4% to 1.8% per day. Thus, once aggregates reach the sea-floor they obviously become "contaminated" by benthic bacteria able to degrade the detrital material. Indications of seasonal variations in the sediment biomass further substantiate that microbial breakdown of organic matter is indeed a major pathway of energy flow in the seep-sea.

The Role of Zooplankton in Transforming Marine Aggregates

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Aggregates will be modified both physically and chemically during their residence time in the water column. Nearly all of the published studies on the dynamics of aggregates have considered only the fauna intimately associated with them. The larger organisms which swim between aggregates and may be considered as transient associates have largely been neglected. One type of aggregate which has been of particular interest for many years is the faecal pellet. Although within the context of this workshop they may not be considered as "typical", the processes affecting their disintegration and remineralization may be very similar to those affecting more typical aggregates. Furthermore, they are readily produced and handled in the laboratory, a feature not shared with aggregates of looser construction.

There are a number of parameters of particular interest when considering the fate of a particle. These are the rates of degradation, sinking, and production. For faecal pellets empirically derived estimates of these parameters are incompatible with field observations on their abundance distribution and flux. It seems that the lifetime of a pellet may be far less than expected. The implication from this is that the microbiota associated with the pellets may not be the principle destructive agents. Experiments have been carried out to determine if other faunal groups could be responsible for a greater rate of destruction than the microbiota. It was found that copepods are capable of destroying large numbers of their own pellets without ingesting significant

quantities, a process termed coprorhexy. This behaviour provides a ready explanation for many of the "difficult" field data.

Studies on aggregate modification should not ignore the potential role of the zooplankton and nekton. They may play a crucial role in converting aggregates with high sinking velocities into small particles which sink very slowly.

IV. MODELS AND PHYSICS

Micro Turbulences in Relation to Particle Sizes

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Turbulence in the water plays a very important role in the flocculation process.

The basic aggregates of flocculation are the so-called microflocs. These are mainly quite stable, bound by sticky material and can resist rather strong forces. The package is also compact. When these microflocs are involved in the flocculation process, there will be formed the so-called macroflocs. Macroflocs are, on the other hand, very fragile and break quickly if the forces are too high. They are also very porous.

Turbulence has great influence on the existing of macroflocs, which can be divided into two main mechanisms.

The first main mechanism is bringing the particles together. Because of the greater fluctuations in the velocity (increase of turbulence), the probability that two particles collide gets bigger. This is the reason that the flocculation process is going faster.

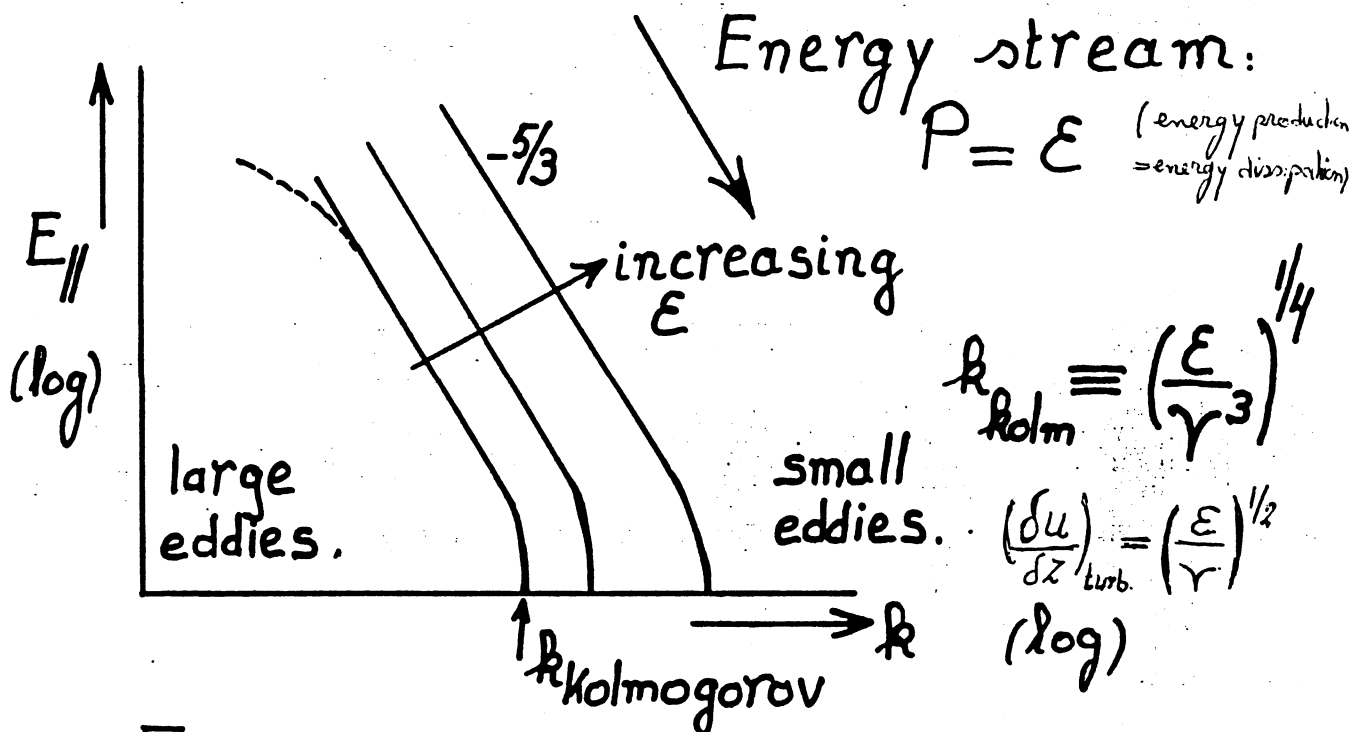
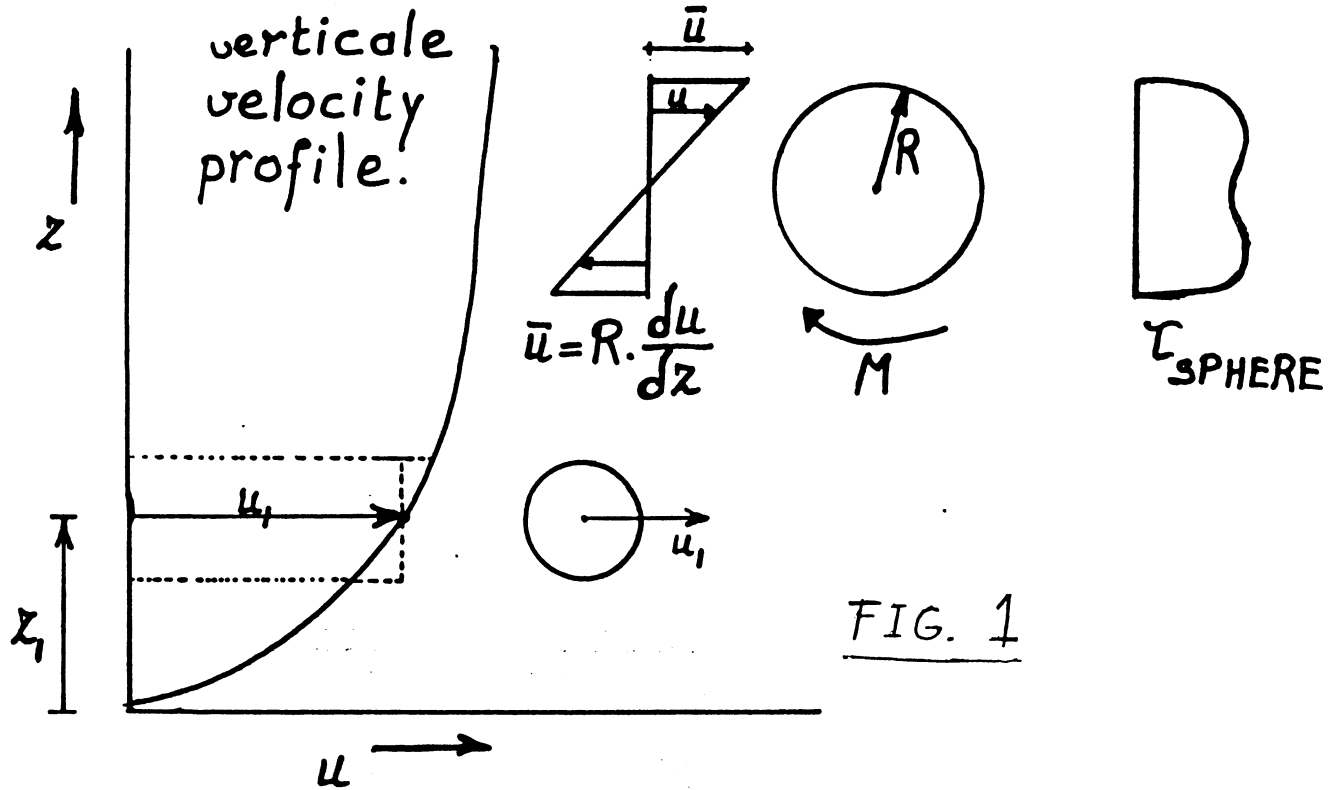
The second main mechanism is the break up of the macroflocs. By increasing turbulence the stress on a particle due to this turbulence is also increasing. When the force on the particle is bigger than the internal force in the particle, the particle will break up.

The force on a particle is dependent on the velocity gradient (shear stress) over the particle, as is shown in Fig. 1.

The same reasoning as for a vertical velocity profile can be set up for the case of turbulence. Therefore there must be a relation between the measure of turbulence and the shear stress the turbulence is causing.

An often used measurement of turbulence is the energy dissipation. The energy in the system will be transferred from the mean flow to the large turbulent scales and from here further to the small turbulence scales. From these small scales the turbulent energy will be dissipated by the viscosity of the water. In this process there is always a smallest scale of turbulence present, depending on the amount of turbulent energy available in the flow. This can be seen in Fig. 2. The smallest scale that can exist is defined by Kolmogorov. Kolmogorov defined a length scale; a time scale and a velocity scale on dimensional reasons.

Thys Schuhmacher



Energy spectrum of Turbulence.

FIG. 2

Out of these relations there can be defined a measure for the shear stress over a particle in the water that is existing over the distance defined by Kolmogorov.

By larger distances this shear stress is not stable any more and forces to other directions can be expected. Therefore, the largest forces on the particles appear at those length scales where the particles are in the order of the smallest scale of turbulence (defined by Kolmogorov).

Out of measurements we have indications that the largest particles are somewhat smaller than the smallest scale of turbulence. On these particles the shear stress is so large that the particle will break up.

We expect that there will be a dynamic equilibrium of the particle size distribution, belonging to a constant rate of energy dissipation of the turbulence.

Modelling Bacterial Attraction to Large Particles

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The ability of chemosensory organisms, such as bacteria, to find and attach to aggregates depends on the interaction of such diverse phenomena as fluid flow around the particle, chemical substrate concentrations, and organism behaviour. By combining models of bacterial sensory response with models of water flow and chemical concentration, one is able to examine the interaction of the different aspects of bacterial attraction to falling particles. Results of model simulations show that the effectiveness of chemokinesis is highly dependent on the size of the particle to which the bacteria are attracted, the sensitivity of the bacterial chemosensor, and the bacterial swimming speed. Fast swimming bacteria of acute sensitivity should be able to stay near a falling particle despite the fact that the particle falls faster than the bacterium can swim. Bacterial chemokinesis also increases the rate of bacterial adhesion to particle surfaces. This enhancement is greater when the surface is less sticky. Models can focus experimental studies to determine the nature of organism responses to micro-environments in the water column.

V. NEW METHODS AND OUTLOOK

MEASUREMENTS OF AGGREGATE STRENGTH

Ian Jenkinson, Paulo Abreu, Bopiah Biddanda, Sabine Ehlken, Ulf Riebesell and Carol Turley

It is generally recognized that the size of aggregates of solid material suspended in a liquid is determined by a balance between aggregative processes and breaking processes.

Breaking is produced by shear forces acting through the surface of the aggregate. Such shear forces are produced, firstly by turbulent and laminar shear in the liquid, and secondly by shear around the aggregate due to viscous resistance to its sinking. If the sum of the shear stress acting on an aggregate is greater than its yield stress (or "strength"), the aggregate will be pulled apart.

For homogeneous aggregates, the material will be "smeared out", irrespective of aggregate size, if stress due to shear in the liquid exceeds the yield stress of the material. In the domain where shear stress is dominated by viscous resistance to sinking, however, stress is related to aggregate size and to excess

density. So material strength and excess density together place an upper bound on possible aggregate size, and hence also on sinking rate.

When aggregates are internally heterogeneous, however, breaking will follow lines of weakness, and breaking will then increase strength at the same time as decreasing size.

The aggregates that we used for this study were of three types: aged seawater, *Skeletonema* culture, and plankton tow (Turley et al., abstract in this volume). They were handled and introduced into a *Contraves Low Shear 30* rheometer using "peepers", which were also developed by the Bremerhaven Aggregate Group (Turley et al., loc. cit.). The aggregates were compressed between the bob and cup of the rheometer to about 0.5 of their original size. Slow rotation of the cup was started to give a shear rate, $\dot{\gamma}$, of between ≈ 0.01 and 0.05 s^{-1} . Typically one of two types of curve was obtained. The first type was plastic-type behaviour (like trying to break well-chewed chewing gum) (Fig. 1a), shown principally by the aged seawater and *Skeletonema* aggregates, and the second type was brittle behaviour with some plastic behaviour superposed (perhaps like trying to break a müsli or granola bar) (Fig. 1b), shown mainly by the plankton tow aggregates. Behaviour intermediate between these two types also occurred.

Preliminary treatment of the data indicates that shear strength, τ_y , showed a lot of variation between aggregates, being typically between 0.1 and 1 Pa for all three types. Aged seawater and *Skeletonema* aggregates typically showed more plastic behaviour than the plankton tow aggregates. Aged seawater aggregates sheared more before yielding (yield shear, γ_y , about 1.5 to 6) than the *Skeletonema* and plankton tow aggregates (γ_y about 0.5 to 3). So more work was required to cause the aged seawater aggregates to yield than in the case of the *Skeletonema* and plankton tow aggregates.

It would be interesting to carry out similar measurements on aggregates taken from the field, as well as to compare "strength" measured by different techniques.

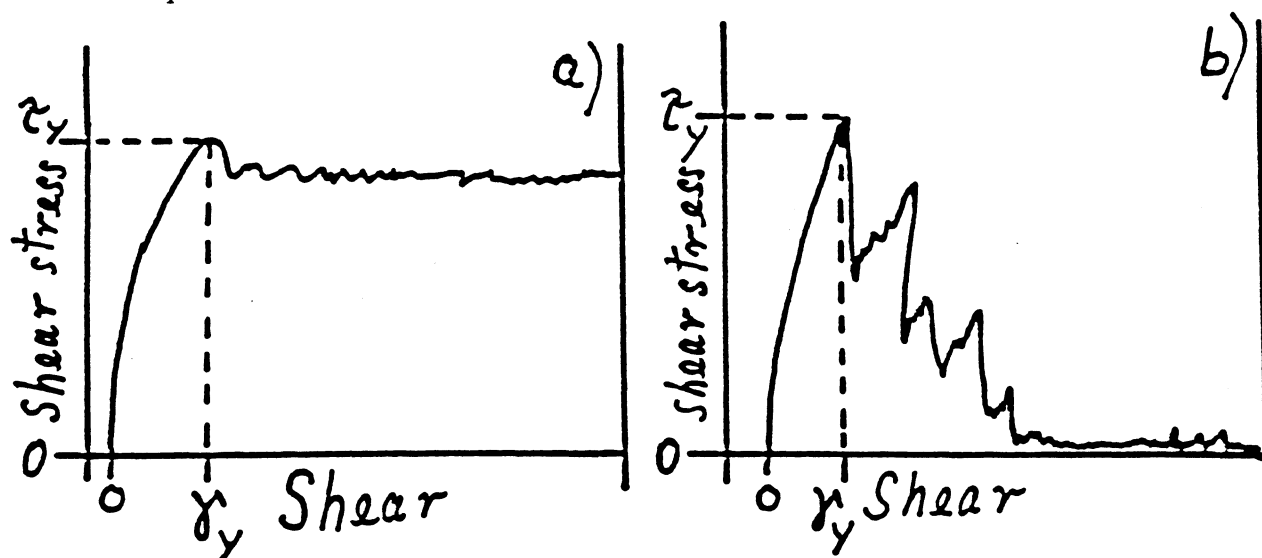


Fig.1 Schematic "typical" graphs of shear stress, τ , vs. shear, γ , where γ is increased uniformly from zero. γ_y is the shear at which yield occurs. τ_y is yield stress (or "strength"). a) almost plastic behaviour: only a small decrease in τ occurs after yield (typical of aged seawater and *Skeletonema* aggregates); b) Multiple brittle breaks with a very small amount of plastic behaviour superposed (typical of plankton tow aggregates).

Natural Radionuclides as Tracers for Aggregate Formation

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Literature data:

The natural Uranium decay series produce radioactive tracers in the water column that are very useful for the study of aggregate formation. Most suitable is the combination of a well soluble mother nuclide (U, Ra) and a highly particle-reactive daughter nuclide (Th, Pb, Po). Such mother-daughter pairs exist for a range of time scales from ^{238}U - ^{234}Th (24 days halflife) to ^{234}U - ^{230}Th (75000 y halflife). The scavenging of the water column was originally described by a simple model of adsorption on particles and subsequent sedimentation. However, this model failed to explain the experimental data (Bacon and Anderson, 1982; Bacon et al., 1985):

- 1) There exists a reversible exchange of ^{230}Th between the dissolved and particulate phase.
- 2) The distribution of dissolved and particulate ^{230}Th in the ocean indicates that the average sinking rate of particulate ^{230}Th is 1-3 m/day, (corresponding to an average residence time in the water column of the deep ocean of a few years). Since the main vertical flux is thought to be in the larger particles that settle much faster (in the order of 100 m/day), this suggested a turnover of these larger particles by disaggregation and aggregation, resulting in the net slow sinking rate.
- 3) The seasonal changes in particle mass flux (measured with sediment traps) strongly affect the fluxes to the sea-floor of isotopes as ^{230}Th that are produced in the entire water column. This means that aggregation of small to large particles in the deep sea is influenced by changes in the flux of large particles from the upper ocean.

Recent data from the Bransfield Strait

In Oct/Dec 1987 the distribution of ^{234}Th in the water column was measured before and during the spring bloom. In the beginning of November, the winter situation prevailed: Few particles, nearly all ^{234}Th in dissolved form, no depletion of ^{234}Th or nitrate. Mid-November we met a *Phaeocystis* bloom. Over 80% of ^{234}Th was taken up by particles, but there was no depletion of total ^{234}Th , which means that the particles had not started yet to settle out. In the beginning of December, 2/3 of the ^{234}Th in the surface water had been taken up by particles, and the surface water was depleted by 20% relative to ^{238}U , indicating that the sedimentation of aggregates had set on.

Our understanding of aggregate behaviour can be improved by studies combining detailed analyses of the aggregates with data from sediment traps and radionuclide distributions in the water column.

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VI. SOME AFTERTHOUGHTS

MARINE AGGREGATES - AN INVESTIGATION IN THE PERFECT WORLD!

Carol Turley

Sometimes I find that if I make a list (A WHAT IF I COULD LIST?!), of all the things I would like to measure IF the methods, technology, time, people, funds, etc. were available, it concentrates the thoughts, adds realism to what we can do, what perhaps our future aims should be and what we just have to put on standby for a few more years. So, in a perfect world, these are what I would like to measure and model. How about you, I am sure your perfect world is different to mine? How many do you think are presently achievable?

A. THEIR CHARACTERIZATION:

1. Aggregate density)
2. Aggregate water content) perhaps a ratio of 1-3
3. Aggregate volume)
4. Aggregate shape factor.
5. Aggregate biological composition (numbers and biomass of organisms).

B. THEIR RATES AND PROCESSES:

1. Aggregate sinking rates.
2. Aggregate decomposition (eg. through microbial respiration or disassociation) or loss rate (through shear during sedimentation, dissolution, ingestion and repackaging).
3. Aggregate growth rates (eg. through carbon dioxide fixation, nitrogen and DOC uptake, particle scavenging or ingestion and repackaging).

C. MODELLING:

1. In an ideal world, from all the above information we should be able to plug this data into various models, e.g.
 - a) Models of the microbial loop on and off a sinking aggregate.
 - b) Models of the gain to (growth) and loss from (shrinkage) aggregates of different types and sizes as they pass through the whole water column.
 - c) Models of the relative importance of aggregates in the flux of material (carbon, nitrogen, inorganic minerals etc.) from the euphotic zone to the deep sea.

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2 - 3 June 1988

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