LABORATORY ANALOGUES OF ESTUARINE ECOSYSTEMS,

Ramón Margalef (*)

INTRODUCTION

Estuarine ecosystems exhibit regulatory feedback at different levels. An increase of the inflow of freshwater in the head of an estuary may augment the flow of denser sea water over the bottom in the opposite direction. Any strong exchange, by way of tides or otherwise, means a heavy loss of suspended organisms, but in certain situations introduces new nutrients in the system; selection is then active in favour of either fixed organisms or of planktonic species with a high rate of increase, capable of support important loses. On the contrary, when flow decreases, floating organisms with a relatively low rate of increase can maintain themselves, mobility is an asset and, in such conditions, dinoflagellate blooms may be apparent. An estuary rarely can be studied as a nearly closed system; most of the time it receives materials from freshwater and exports production to neighbor systems, contributing to their maintenance with algae, detritus and, in general, food. Such contribution is more important when flow of energy (primary production) per unit of biomass is high, a condition obviously fulfilled if flow and water exchange remain at a high level. In populations organized under such constraints, there is usually a dominance of one or a few species and a high ratio primary producers/animals. Several authors remarked that estuarine plankton po-

(*) Instituto de Investigaciones Pesqueras, Barcelona, Spain.
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pulations have a low biotic diversity when compared with coastal and oceanic populations and concrete evidence has been furnished (Hulburt, 1963). In this case, low diversity is related to high turnover of populations, and may be accompanied, but is not necessarily accompanied, by a high density of populations. At any event, it is an indicator of poor organization under the influence of changing conditions.

The purpose of laboratory models is not to duplicate or imitate natural situations, but to have simple situations that facilitate the identification of operating mechanisms, first in the experiment and later in the nature.

The experimental approach is dependent from the focus of interest. Useful data on the ecology of estuaries can be gathered through the customary flask culture, in which selected organisms are growth under different conditions of water quality, water agitation and temperature, and the experimenter records the rates of growth, or if they growth at all, and tries to find correlations with the different factors, nutritional or others.

The design of the present experiments started from the consideration of estuaries as self regulatory systems, and the immediate purpose was to gain understanding of the particular quantitative values found in estuaries -or in analogous situations- of certain characters dealing with the structure and function of ecosystems. A promising approach was the use of flow cultures, where stationary populations are the expression of equilibrium in dynamic processes that are made more or less independent of time, independence that cannot be obtained in simple and closed culture flasks. It has been thought more constructive to present the necessary information to perform certain relatively simple experiments, than to discuss at length the results of the perfor-

med experiments, that are of a preliminary nature and did not exploit fully the possibilities of the method.

An important point is to decide about the most important parameters in the study of mixed populations. Its selection is dependent of the purpose of the worker: assesment of resources calls for estimates of biomass, projects of exploitation need also an estimate of production. In any consideration of the ecosystem as a cybernetic system one has to overvalue certain synthetic and dynamic properties that include some reference to time, as time rates (primary production per unit of total biomass) or as structures result of historical processes (biotic diversity of populations, or plant pigment diversity expressed in the practice by ratio D_{430}/D_{665} of the absorbancies, at the stated wavelengths, of aceton extracts).

THE CHEMOSTAT

The principle and theory of the chemostat have been discussed by several authors, that often present useful hints for the construction. The following are a few selected, basic or specially useful references: Monod, 1950; Novick & Szilard, 1950; Novick, 1959; Bulder, 1960; Herbert, 1961; Pfennig & Jannasch, 1962; Watts & Harvey, 1963. Here it is only necessary to remind the basic principles of the device and describe the disposition that seems more appropiate as an analogue of estuaries or of any running water system.

In a chemostat we have a population, which density is controlled by a density dependent factor, suspended in a constant environment that can be selected or changed at will. The culture vessel receives a constant input of fresh medium of definite composition and the same amount of the well mixed and uniformized content of the vessel is taken away.

Losses of the population are proportional to the flow and to the density of population, thus density dependent and effective in the regulation. Let be N the density of population, r the rate of growth, and F the flow, that is, the fraction of the total volume of the culture that is exchanged in unit time. In stationary state we should have

$$N = N e^{r} - F N ,$$

$$r = \log_{e} (1 + F)$$

The regulation of the population density is very noticeable in a simple chemostat with a unialgal culture. When flow (= exploitation, = exchange) is increased, the ratio production/biomass has to be higher, since production/biomass = $e^{r}-1 = F$. This is a relation of fundamental interest in general ecology and , specially, in the ecology of estuaries.

In conditions of decreased light, decreased nutrient input or increased flow, rate of increase , r, of a unispecific culture , or of any of the species in a mixed culture, can become insuficient to cope with exploitation. If such situation arises, biomass will decrease steadily until a new stationary level is reached, or perhaps indefinitely until the population vanishes, the ratio production/biomass always going up until the late moment. This means that the turnover of such population is too low to allow the passage of a certain energy flow, imposed by the conditions of the experiment.

Since conditions of competition in a chemostat vessel may be practically reduced to differences in the rates of increase in definite conditions, and it is highly improbable to have two organisms with exactly the same rates of increase, a chemostat inoculated with a mixed culture leads generally to a unispecific population, demonstrating Gause's law. But the situation turns more complex if the issue of competition is not only decided by prolificity.

The merits of the chemostat for the simulation of situations that are found in estuaries, increases when several chemostats are connected together in a row, in the way that the outflow of the first chemostat is the inflow of the second, and so on. Malek (1952) and then other authors describe such disposition and the writer has been using compound chemostats for several years, with very stimulating results, of which only a short account of a particular experiment has been published (Margalef and Ryther, 1960).

Like a single chemostat vessel, a system of many vessels approaches a stationary state, under constant environmental conditions. The basic expression for equilibrium resembles the one for the simple chemostat, exceptuating that the loss of cells due to the flow is not only dependent of the density attained in the particular vessel under consideration (N_i), but of the difference between this density, and the density in the precedent container (N_{i-1}),

$$N_i = N_i\, e^r - F\,(N_i - N_{i-1})$$

The composition of medium remains constant in time, and changes in a progressive or directional way in space. Going from one flask to the next, concentration of nutrients decrease, and metabolites increase.

A simple row of flasks, the linear compound chemostat, is an excellent analogue of any planktonic system in running waters, or in a single layered estuary, or in a layer of a stratified one.

The theoretical limit of the construction of a linear compound chemostat would be a simple tube, as an analogue of a river. The writer has experimented with plastic tubes, up to 40 m long, but such device presents certain diadvantages respective to the multichambered chemostat. First: A flow system divided into chambers separately stirred, and interconnected by rather narrow tubes, is more appropiate for sampling

the volumes, rather large, necessary for performing the diverse analysis. Second: Under conditions of laminar flow, any suspended population is fatally washed away. In the need of accept a certain turbulence, the model made up by a row of chambers separately stirred and with exchange in one direction is more easily understood and managed by the biologist unfamiliar with hydrodynamics.

In a compound chemostat made up of a simple row of culture vessels, with unidirectional flow, and populated by planktonic algae, we have, going in the direction of the flow: An increase of biomass, a decrease of the ratio primary production/biomass, an increase of the ratio D_{430}/D_{665} of plant pigments, and, if the experiment is not designed to prevent it by limitation of the assortment of species, an increase of the biotic diversity of populations. In such experiments there is a steady state that is maintained during long periods and they attain a certain independence from time. Externally controlled rate of flow, if increased, moves the entire pattern downstream; if it decreases, the pattern is displaced upstream.

This confirms the interpretation of patterns ocurring in nature. Such experimental disposition is to recommend for instructional purposes and also for exploring quantitative relations between rates of increase, biomass, production, biotic diversity and pigments, under different conditions of flow, nutrients, light and temperature. But the approach based in the application of the expressions already given is limited to systems containing only free, non attachable species, and requires to discard the experiments when some organisms begin to settle on the walls.

Any system of chemostats affords an ideal way for mapping time series into space, since going down the row of flasks is equivalent to progres-

sion along an ecological succession. The chemostats become important when we are interested in a pattern as the expression of processes. We may be more interested in clouds as such, than in the behavior of definite molecules of water or of the atmospheric gases; we may be more interested in plankton patches than in the destiny of definite algal cells. In any case it is necessary to state clearly our wishes. An aspect of this problem is often a subject of discussion among planktologists, but never, to my knowledge, has been properly and formally presented in print: Are planktonic populations better studied with reference to a geographically fixed coordinate system, or with reference to a deformable coordinate system displaced with the water or with the organisms ? The vessels of a chemostat are like a frame of geographical coordinates, a rigid reference for the study of populations. The populations themselves are transient or stationary states, expression of processes of cell multiplication, of diffusion, of flow, going on.

It is possible to speculate about the requirements of a good chemostat, for the study of planktonic populations. It may consist in any high number of culture vessels, to be interconnected in topologically proper relations, in order to form a tridimensional lattice. An appropiate control of the flow of medium among them would be attained through the use of connecting tubes of elastic material, actuating as elements of peristaltic pumps, according to a program. The pumps could forward the fluid in either direction and by this device the effects of turbulent mixing could be simulated, pumping fluid from one vessel to another, stirring, and pumping back again the same volume to the first, and so on. One can realize the interest of a system of, say, 10 x 10 x 10 vessels, every one subjected to appropiate conditions duplicating distributions of temperature, light, etc. in

natural situations, ~~xxxxxxxx~~ It would be possible to lead into a given vessel, in a point of the tridimensional lattice, a continuous flow representing a local upwelling or a spot source of nutrients, program the exchange between diffefent vessels, according to selected conditions of flow and turbulence, and wait for a steady state. We could gain a dynamic insight on how spatial patterns develop. It is seriously contemplated to investigate the possibilities of such expanded approach, but enthousiasm has been somewhat damped for reasons better explained latter. The presence of walls becomes soon a nuisance.

THE ADOPTED CHEMOSTAT UNIT.

The disposition now in use, after considerable trial and error, is satisfactory for many purposes. The essentials are given in figure 1 and in the following lines.

The adopted flasks are of conical shape and 500 to 600 ml of capacity, with fluted sides to increase turbulence when the contents is stirred. At a level that confines beneath a volume of 300 to 400 ml, there are one or two simple inlets, with receiving ground glass joints, and an outlet that starts close above the bottom and ends with a tapering male glass joint. Another opening, placed high above, serves to equilibrate atmospheric pressure. The contents of every vessel can be stirred magnetically, using iron bars covered with teflon.

Two types of ground glass stoppers have been used: simple or with conduction. The stoppers of the last type are hollow, have an inflow, an inferior vertical outlet made of small bore tube (about o.8 - 1.0 mm diameter) and an emergency outflow, that serves to equilibrate atmospheric pressure and to allow an escape of the accumulated liquid in the case of eventual stopping of the small bore tube. The purpose of the chamber in the hollow stopper is to provide a better equilibration of the temperature of

the medium, if necessary. The small bore tube secures a slow incorporation of the fresh medium to the contents of the culture vessel. The discontinuous feeding mechanism fills the stopper, but the passage of the liquid ffom stopper to the culture vessel approaches or attains continuity. The end of the small bore tube lies high over the culture medium, preventing any ascendent contamination.

The culture vessels can be separately sterilized, closing the lateral openings with appropiate stoppers, or using connecting segments with taps. The units can be assembled in rows or, as there is provision of flasks with two inlets and one outlet, in more complex patterns, that include branching as T or Y. The special hollow stoppers used as inlets of fresh medium are inserted only in the head of the rows, or, sometimes, also in intermediate position if the effect of a dilution or of a special adition in certain stage of the development of a population is to be investigated. The other flasks receive simple stoppers.

The feeding mechanism gave considerable trouble. A discontinuous mechanism is much simpler to construct and more reliable than the use of a continuous feeding system. The system must resist sterilization. Solenoid operated valves have been in operation for a time and rejected. Long segments of capillary tubing have been tested also as regulators of flow, without giving satisfaction. Slow peristaltic pumps seemed to promise a good solution, but at a rather high cost. Finally, a very sturdy, sure and cheap device has been adopted. Two glass valves, each one consisting of a ground tapered plug in its casing, are connected by a piece of elastic tube. The whole is easily made sterile, together with the medium reservoirs. An electromagnetically activated pusher compresses the elastic tube and the whole acts as a pump. Selecting

the diameter and length of the compressible segment it is possible to fix approximatively the amount of fluid displaced at each stroke, but it is rather difficult to make it exactly coincide with a prefixed amount. The best thing to do is to measure a posteriori the amount of liquid actually flowing, and introduce in the computations this value. Fortunately the device is very stable, in the sense that the delivered fluid, in ml/day, remains constant weeks over. An electrical timer controls the electromagnetic pusher and affords a further possibility of regulation.

In the course of experiments to be described in part later, it has been found convenient to have a flow of about 100 ml/day. The electromagnetic pusher was activated every 20 minutes, and the amount of fluid introduced by every stroke was around 1.5 ml.

It was found appropiate to make stirring discontinuous, about half time, and to link the operation of the stirrer to the same timer that controls input of fresh medium. Stirring starts before the injection of fresh medium and stops after the last drop has fallen from the hollow stopper

The final outflow of the whole system, at the outlet of the last culture flask, is led to a measuring vessel. This reservoir contains a few drops of a fixative (saturated solution of iodine in saturated solution of potassium iodide in water, plus 20 % of sodium acetate). Every day, or more frequently, if convenient, the amount of fluid collected in this reservoir is measured and the cell concentration counted. Here is a good check of the regularity of flow, general behavior of the system and a test for stability. In a few days, after the initial changes, density of organisms in the discharge approaches a stationary state. Only then it is possible to accept that the system has reached equilibrium, disconnect the vessels and analyze the partial populations enclosed in the different flasks.

No elaborate equipment is required for making the exchange between succesive flasks rigorously unidirectional. It suffices to use special connecting pieces and have the flasks placed at different levels. In the experiments performed, notwithstanding, the flasks have been placed always at the same level and connected by the simple glass joints. A certain amount of diffusion and contamination "upstream" has been possible, ressembling more to the conditions present in natural systems.

OPERATION OF COMPOUND CHEMOSTATS

In the second section have been summarily described the results of the operation of a single row of culture vessels with a single inlet in the first vessel (Compound linear chemostat), using unialgal cultures of planktonic, that is, non sticking algae. If we have still a compound linear chemostat, but several planktonic species of algae in it, experimentation demonstrates the simplification of structure (= drop of diversity) of the mixed population, when flow (= exploitation) is sustained or increased. In most runs, in the first vessel all but one of the species are soon eliminated. Competition has an issue decided by differences in the rate of increase.

If in a compound linear chemostat inflow of fresh medium is not limited to the first vessel, but every flask receives a certain input of culture medium, flow and diffusion accelerate along the system, like in an estuary with a wide mouth, and/the population reacts keeping a high ratio production/biomass. Expansion of population in a "fresh" environment is accompanied by a rejuvenation. A possible variant of this sort of experiment would be to lead water of increasing

salinities into the successive flasks. This model would duplicate situations often found in estuaries, but so far has not been tested.

Other possible modifications include to make conditions (light, temperature) less constant in the first vessel or in other vessels; changes in the intensity of stirring have often a pronounced effect.

When experiments are performed with a wide assemblage of species, taken from natural populations, the systems develop a flaw, from the point of view of the planktologist. In a certain way it is a fortunate flaw, because it throws light into the dynamics of populations in estuaries and in other natural environments. Species able to attach themselves to the walls of the culture vessels become much successful in competition. The same fact was observed by bacteriologists working with flow cultures. Graziosi (1959) states that in continuous cultures there is a positive selection of mutants that grow on the wall of the vessel. The selective advantage has to be measured quantitatively, as a greater probability of remaining in the vessel.

The development of organisms with a propensity to attach to the glass is the most serious drawback in the use of chemostats as analogues of plankton systems. Species that are often used as models of planktonic algae, as Nitzschia closterium, and even some small species of Chaetoceros, are found attached in some way. Propensity to attachment seems to be different according to the conditions of nutrition, to accompanying bacterial flora and to the time elapsed since cleaning of the flasks and start of the experiment. The multiplication of particular mutants cannot be excluded, neither. Stirring does not check attachment of algae on the walls. The design of a chemostat proper for experimentation with complex planktonic

populations awaits the improbable discovery of the bottle without walls. Ice walls do not help.

Several runs have been made in compound linear chemostats with Platymonas (attaching easily), associated ordinarily with Nitzschia and/or Skeletonema. Now the results are almost opposite to the expected ones in totally free species. Biomass accumulates more in the firsts vessels, and the usual regularity in the distribution of the ratio production/biomass is distorted. The ratio D_{430}/D_{665} of plant pigments behaves in an interesting way. It is between 2 and 4 in healthy and normally growing cultures of Platymonas, and between 3 and 6 in analogous cultures of Skeletonema. In a run including a mixed culture of both species, Platymonas dominates in the firsts vessels, not by a higher rate of increase -it has not-, but by a higher propensity to remain attached to the glass. On the contrary, Skeletonema makes a higher proportion of the population in the flasks placed downwards in the flow. Thus, we have seen the customary pattern of increasing values of the ratio D_{430}/D_{665} going down with the flow. But later -one to two weeks- things change: Populations of Platymonas accumulated in the vessels at the head of the series profit of the flow of the medium (the so called "eutrophic effect of running water"), but their rate of increase decline, and the ratio D_{430}/D_{665} increases as happens in old and senescent populations. On the contrary, the populations of Skeletonema are relatively more abundant towards the vessels in the tail of the system; as unattached, they are subjected to a strong exploitation that maintains a high ratio production/biomass, and a relatively low ratio D_{430}/D_{665}. Thus, at a certain moment, the distribution of this ratio along the vessels is inversed; in fact it decreases from first to the last flask. For

instance, in an experiment with Platymonas, Skeletonema (and bacteria), after 15 days of a flow of 130 ml/day, the ratios D_{430}/D_{665} in the mixed pupulations of the four consecutive vessels, were respectively, 17.5, 13.9, 7.2 and 5.5. But here, decrease of the value of the ratio is not achieved by exaggerate dilution downwards, but by exaggerate condentration upwards. As time goes on, the attached species invade progressively all the flasks and the image becomes much blurred. The elegant simplicity of the experiments with free floating algae is lost. The brutal competition for dominance based on rates of increase has given way to more subtle prowesses and, in fact, the chemostat is prevented to attain a stationary state. The situation is interesting as an example of development of organization; more organization than was desired by the experimenter.

Fig. 2

An attempt has been made to measure the degree of attachement of the cells, counting cells in suspension; then detaching and suspending also the cells settled on the walls and counting again, and checking the obtained data with measurements of production, flow and final output of the system. The results cannot be very accurate, and are expressed as the average probability for a cell to move with the water going through the flask. P = 1 , in the true planktonic and pasive algae, non attached and flowing with the medium. P = 0, if all the cells are solidly attached. Mobile organisms pose special problems, but so far have not been used in the experiments discussed here.

The introduction of P in an expression describing a steady state situation would be

$$N_i = N_i\, e^r - F\,(N_i P_i - N_{i-1} P_{i-1})$$

but such expression is used with reluctance, since no true

stationary state is reached. Although the output of the final outlet of the system may remain approximatively constant for a few days, there are rearrangements in the populations of the separate flasks.

Nevertheless, discouragement has not prevented further experiments with complex populations in compound chemostats, either with a single row of flasks, or with two rows converging in a single final row. Such model was assembled in order to have an analogue of two water masses that meet and mix in a boundary. Flow in the vessel that receives a double input ([e] in table I) is higher and requires a sharp increase in the ratio production/biomass, if attachement of organisms is not excessive. In fact, if the two converging flows consist of water of different composition, the success of attached species in front of suspended ones seems to be not so important as in other circumstances.

As illustration, table I reports the principal results of one of the runs.

CORRELATIONS IN CHEMOSTAT POPULATIONS

It is instructive to find certain correlations between selected parameters of populations grown in chemostats. They are maintained under different conditions of flow, of temperature and of species composition(Table II).

The positive correlations between number of cells counted in a sedimentation chamber, number of particles counted with an electronic dimensional particle counter, and chlorophyll content were expected.

There is a significant positive correlation between the ratio production/biomass and the average probability of being carried away by the flow. Production was measured by 14-C fixation at the end of

experiment, after interrupting the flow and disconnecting the flasks. Both values are negatively correlated with the ratio D_{430}/D_{665} in plant pigments. There were hopes to find, also here, a positive correlation between ratio D_{430}/D_{665} and biotic diversity. But species diversity behaved rather disappointing, since not significant differences were found between different vessels; it is true that the assortment of species was artificially limited.

The probability of being washed away is unrelated to the intensity of flow.

Between the quantitative, instantaneous, measures of the biomass, in number of cells or chlorophyll, and the second group of characteristics correlated between them, correlation is lacking or poor. The characteristics of this second group (ratio production/biomass, ratio D_{430}/D_{665}, perhaps also, after all, species diversity) are specially relevant when the ecosystem is considered as a cybernetic system.

INTERPRETATION OF ESTUARIES.

It was pretended to have in the compound chemostat an analogue of estuaries, but we have been led somewhat astray by the operation of factors that, although active also in estuaries, made difficult the interpretation of our models.

Compound chemostats illustrate the drop of diversity in planktonic estuarine populations when exchange is strong. Under such conditions, the populations not only show dominance of one or few species, as Chaetoceros socialis, Skeletonema costatum, and others, but these are species with a high rate of increase, and ratio production/biomass is high. Rate of exchange controls the planktonic population. When it drops, higher organization of the pelagic community is apparent, ratio

production/biomass drops and, sometimes, red tides follow.

This is to be applied to suspended and drifting populations, but population dynamics is controlled also by the probability of being washed by the flow, or by its converse, the capacity of keeping a position. In natural estuarine ecosystems, benthic mode of life reduces the probability of losing the place. Fixed plants can be prevalent in some situations, if the time necessary for development is not interrupted by drastic changes in the environment.

Another resource leading to a decrease of the probability of being carried away is a special form of behavior, as in euryhaline animals that migrate vertically and are found alternatively in layers flowing in opposite directions (Cronin, Daibert & Hulbert, 1962). Such populations compete with advantage or exploit with success the less stable, truly planktonic or drifting populations.

The more or less fixed communities gather and preserve organization (information) better than passively vagant communities. Their higher organization is linked with higher homeostasis and with lower energy flow (primary production per unit total biomass). An increased flow or another form of heavy exploitation leads to a restructuration and simplification of the system, and the new structures are more resistent to further changes or further exploitation.

The compound chemostat can visualize many situations concerning the general ecology of estuaries. This would be a purpose rather didactic, but probably the usefulness of the approach does not stop here. Compound chemostats facilitate the quantitative approach to the problem of correlate metabolism and structure in ecosystems, and identify responsible regulatory feedback circuits. Carefully conducted experiments can answer questions regarding nutrition, competition and

importance, for the survival in place, of different adaptations concerning probabilities of being grazed or displaced by the flow of water.

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