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Giménez Casalduero F.

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Biondicators. Tools for the impact assessment of aquaculture activities on the marine communities

F. Giménez Casalduero
Unidad de Biología Marina, Dpto. Ciencias Ambientales y Recursos Naturales,
Universidad de Alicante, Campus de S. Vicente del Raspeig, Apdo 99, 03080 Alicante, Spain

SUMMARY – The use biotic indicators for the assessment of the impact of organic pollution is a very common monitoring technique. The present paper presents detailed information about the different types of biological indicators (physiology and immunology bio-indicators, indicators species and biological pollution indices), which can be based on indicator species, on trophic strategies, on the structural heterogeneity, or those incorporating biomass. All these tools can help us to interpret the state of a community and the possible changes due to certain environmental pressure. The combined use of different environmental descriptors makes it possible to identify various parameters that are capable of determining the area of impact.

Key words: Aquaculture, biotic indicators, impact, marine communities.

RESUME – "*Bioindicateurs. Des outils pour l'évaluation de l'impact des activités aquacoles sur les communautés marines". L'utilisation des indicateurs biotiques dans l'évaluation de l'impact de la pollution organique est une technique très courante de surveillance. Cet article présente des informations détaillées sur les différents types d'indicateurs biologiques (bioindicateurs physiologiques et immunologiques, espèces indicatrices, et indices de pollution biologique) qui peuvent être basés sur des espèces indicatrices, des stratégies trophiques, l'hétérogénéité structurelle ou ceux incorporant la biomasse. Tous ces outils peuvent nous aider à interpréter l'état de la communauté et les changements possibles provoqués par une certaine pression de l'environnement. L'utilisation conjointe de différents descripteurs de l'environnement permet l'identification de divers paramètres capables de déterminer la zone de l'impact.*

Mots-clés : *Aquaculture, indicateurs biotiques, impact, communautés marines.*

Introduction

At the same time that concerns for the environment have significantly increased during last decades, all food producing sectors, including fisheries and aquaculture, are facing problems of environmental degradation and increasing land and water scarcity. In this context, it has become necessary to explore for the variables which can help us to detect and quantify easily the perturbations caused by mariculture. These variables should give us immediate and effective answers.

Approaches may include chemical testing or biological studies. As regards chemical testing this is based in the investigation of changes in chemical components, such as oxygen, dissolved inorganic nutrients, or organic compounds in benthic fauna that are accumulated (e.g. antibiotics). Another way is to study changes of community structure because the modifications of biota abundance is very sensitive to environmental changes (Satsmadjis, 1985).

The biological assessment of water quality is now largely developing because the inclusion of biological indicators in water quality guidelines and in the assessment of environmental impact. To this respect, it is noted that some regulations and management procedures for coastal aquaculture in northern Europe already contemplate the assessment of impacts from nutrient wastes and chemicals used in finfish aquaculture by modelling and survey techniques, which study the sediment fauna and compare it with general standards (Telfer and Bevereidge, this volume).

Indicators

Blandin (1986) defined the term bioindicator as an organism or group of organisms allow to characterise the state of an ecosystem based on biochemical, cytological, physiological, ethological or ecological variables. The term "pollution indicator" was defined originally as any measures of corporal fluids, cells, tissues or other biological variables estimations that indicate the presence and magnitude of stress caused by environmental variations. This definition includes different characteristics of organisms, populations or communities influenced by the environmental impact.

We can distinguish different types of biological indicators according to composition and the variables used (Fig.1).

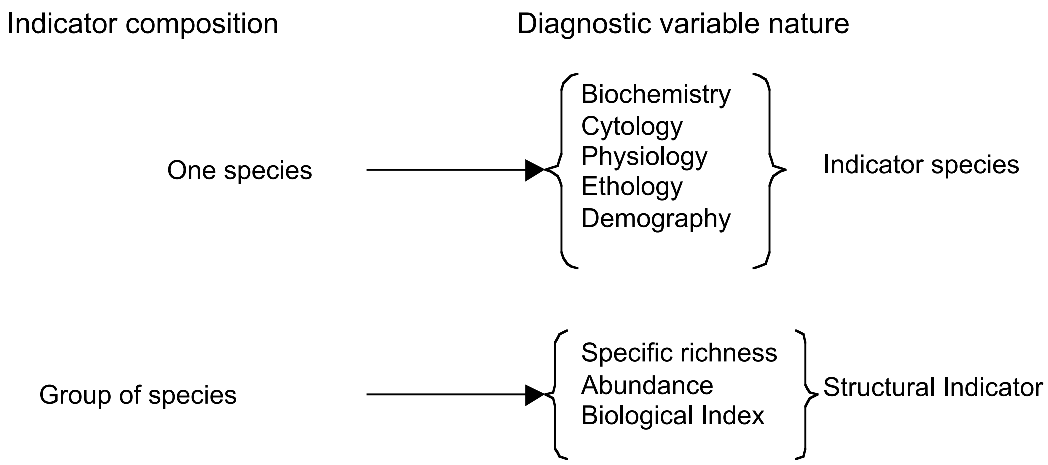


Fig. 1. Different types of biological indicators according to their composition and the variables used in nature (based on Birje and Gravez, 1991).

An efficient bioindicator should hold the following characteristic (Salas *et al.,* 1999):

(i) It should no need very specialised works or complicated calculations (simplicity and easy handling)

(ii) It is basic to be able to detect states of slight contamination, when the situation is even repairable (sensibility to low variations of contamination levels).

(iii) It is necessary to recognise and specify different types of contamination (it is important to identify the causes and evaluate the consequences).

(iv) It should be autonomous of reference states. It must be independent of previous studies from affected area neither control areas.

(v) It should be an efficient administration and planning tool in a widest possible scope (useful in extensive geographical areas).

(vi) It should hold wide ecological range to be applicable in any time of the year, ambient, community or ecosystem. It should be independent of the natural demographic fluctuations.

The election of the bioindicator depends on the pursued objectives. The objective can be to assure the health of our culture, or on the other hand, to control the possible environmental alterations caused by the culture wastes, in each case the chosen bioindicator will be clearly different. Such it is exposed subsequently.

Environmental impact of aquaculture activities on the marine communities

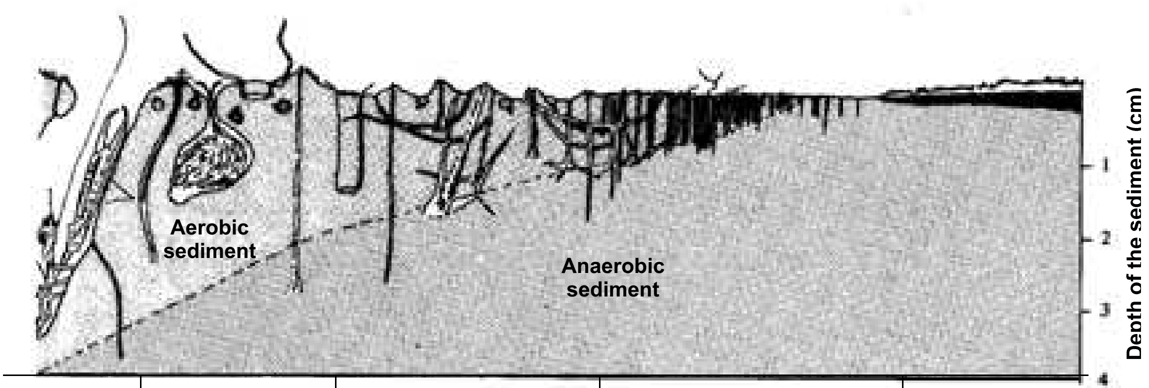
An environmental impact is essentially a perturbation causing alteration of the population's density, size, frequency, or behaviour of some members of assemblage of plants and/or animals (Kingsford and Battershill, 1998). Negative impact of mariculture derives mainly of particulate and dissolved nutrients from animal excretion and uneaten food (Krom and Neori, 1989). Soluble waste is incorporated in the water column. The non-soluble components are capable of incorporate to the sediment. These dissolved nutrients cause organic enrichment and, in the worst situation, can produce an eutrophication.

The Mediterranean Sea is an oligotrophic (poor in nutrients) system. In extreme situations, column water enrichment favours the emergence of opportunistic algae which grown very fast, in detriment of mature species of Mediterranean benthic system (such as *Cystoseira* spp.; Leskinen *et al.*, 1986; Rosenthal *et al.*, 1988). The main effects of non-soluble waste when is incorporated to the sediment, are anoxia, decrease in abundance and biomass, and alteration in benthic community structure.

The composition of community richness change under pollution. The most sensitive species disappear. When pollution becomes more severe the species number is progressively reduced, simplifying the community structure. Finally, only a few species survive.

A known situation exist during pollution of sediment process. The communities become qualitative and structurally simpler, with a reduction of the habitat complexity in some cases. Firstly, biomass decrease and the production/biomass (P/B) rate increase. The opportunistic k-strategic species are replaced by r-strategic species. In an extreme situation macrobenthos community disappear, even the opportunistic species cannot survive and the biota is limited to heterotrophic fungus, bacteria and some cyanophiceas (Gray, 1981).

We can observe different phases of a degradation process due to organic pollution on macrobenthic communities living in soft sediment affected by mariculture (see Fig. 2):



Zone:	Normal	Transition zone (stressed)	Polluted zone		Zone of pollution extreme
Dominant macrofaona	Syllids Great wealth of species Wide diversity	*Hyalonoecia, Eunice vittata* Abudant biomass Wide diversity	*Hyalonoecia* *Neanthes caudata* *Capitella* *Capitomastus*	*Capitella*	No macrofauna

Fig. 2. Diagram of changes in fauna and sediment structure along a gradient of organic enrichment in Mediterranean sediment (based on Grey, 1981).

(i) Non impacted area. Number of species and diversity are high.

(ii) Stress area. Medium pollution level shows a high diversity value, abundance and species richness. We find a high number of indicator species of organic pollution (e.g. the polychaeta *Notomastus latericerus, Nicolea venustula, Nematonereis unicornis, Lumbrineris latreilli*). Species such as *Hyalonoecia bilineata* could be very dominant in this situation.

(iii) Very polluted area of 2nd order. Number of species decrease, community is dominated by high organic pollution indicator species like *Capitella capitata*, or *Capitomatus minimus* link to another species with a low abundance.

(iv) Very polluted area of 1st order: richness of species and also diversity has minimum values. Only indicators of strong pollution species survive (*Capitella capitata, Capitomatus minimus* or *Cirratulus cirratus*).

(v) Area of extreme pollution. Whole macrofauna disappears. Even opportunistic species do not be able to survive in this area.

Physiological and immunological bio-indicators

Although it is well know that the physiology and immune systems of wild and farmed fish and shellfish are greatly influenced by different environmental parameters (temperature, oxygen, etc.), the influence caused by pollutants are complex and poorly understood. The effects on health and physiological conditions vary considerably with species, size, age and previous history of exposure to each dissolved constituent in question (Wedemeyer, 1997).

As for the physiological responses to contaminants there are studies aiming to develop different biomarkers based on different parameters. As an example, it is here mentioned the study of Fernandez *et al*. (1996) who have reported the application of several biomarkers (acetyl-cholinesterase inhibition, induction of metallothionein, and cytochrome P450) in sea bream, and the study of Heath *et al*. (1997) about the physiological responses of fathead minnow larvae to rice pesticides.

Fish are protected of pathogenic agents by an immune system related to other vertebrates. The stress can commit the effectiveness of the immune system, altering the physiological routes of unspecific response either the specific one. Basic mechanisms can be affected to obtain an effective immune defence, those most affected are: (i) inflammatory initial answer; (ii) recognition of the antigen; (iii) antigen transport to the central system organs; and (iv) cells and molecules ejectors activation. These facts are manifested in an indirect way such as the increase of sensitivity to micro-organisms. Also the responds to the environmental stress, the reduction of the immune system, can be interpreted as a fish disease produce for bacteria or virus (Anderson, 1990).

Both cellular and humoral immune responses have been shown to be affected by exposure to contaminants. Thus, recently, Viola *et al*. (1996) have reported the effect of in vitro Cadmium exposure on Natural Killer (NK) cells of catfish, *Ictalurus melas*; and Dixon *et al*. (1996) have showed how the production of antibodies to lymphocytes disease virus in flounder, *Platichthys flesus* L., may be affected by the exposition to contaminated harbour sludge.

The immune response due to environmental stress induces the next physiological changes (Romano, 1999): (i) increase in the corticoid concentration in blood, this compound hinders the antigens; (ii) increase the catecolamine concentration, this hormone accelerate the immune-suppression mechanisms, activating molecules and cells inhibitors of the immune answer; and (iii) increase in the encephalins syntheses that restrain the immune response.

All these variables (corticoid, catecolamine or encephalins levels) can be used like a pollution indicators.

Experience gained from farm operations and pollution monitoring programs in natural waters has shown that the occurrence of stress-mediated fish diseases can serve as useful early warning indicators that unfavourable conditions are developing in the aquatic environment (Wedemeyer, 1996). This diseases can provide useful information on the indirect effects of supposedly sublethal water quality conditions.

Although considerably information is available on the acute and chronic toxicity of dissolved heavy metals, very little is known on the trace metal concentration required to alter or even promote physiological health and disease resistance. For example, fluoride and selenium are potentially toxic but may improve resistance to Bacterial Kidney Disease (BKD) (Lall *et al.,* 1985).

Indicators species

Characteristic species susceptible to be used to define the structure and the space-temporal dynamic of biocenoses will be able to be used like bioindicators. They are the species or groups of species that put in evidence a specific environmental factor.

Many different species have been used such as pollution indicators: benthic algae (Bouderesque, 1970), annelids (Nicolaidou and Papadopoulou, 1989), crustaceans amphipods (Bellan-Santini, 1980), and another taxa but, the more used organisms are polychaeta and gastropods species (Gray and Pearson, 1982).

It is apparent that a "indicator" taxa should be identified on the bases of their numerical dominance in the sample.

Some polychaeta species are considered as representative of polluted areas (*Capitella capitata, Scolelepis fuliginosa, Nereis caudata;* Bellan, 1985). Pearson and Rosemberg (1978) concluded that species of the first stages of ecological succession are abundant in the most polluted areas. However such species are not universal, but they change in different geographic areas. The identification of such pool of species in the different marine environments is largely based in local ecological studies (Gray and Pearson, 1982).

The species considered as pollution indicators can be dominant due to: (i) they are directly favoured for the increase of organic composes because they exploit the nutrients as a resource; and (ii) some species can manifest a higher resistance to the pollutant effects than other competitive species or potential predators.

Several researchers have proposed the opportunistic polychaeta *Capitella capitata* such as an universal indicator of organic pollution. Abundant populations of *Capitella* appear in deteriorated areas. After a very quick colonisation of high densities, a fall of the number of individuals takes place. This density slope is due to the food exhaustion and the accumulation of toxic substances (Valiela, 1984).

It may not be possible to use the same species of organisms as indicators of polluted waters throughout the world because many species have either limited geographical or ecological distribution. However, the most widely used marine indicator, *Capitella capitata*, is cosmopolitan in distribution. The fact that the same assemblage of species would not necessarily be a universal indicator of polluted or semi-polluted conditions should not be a deterrent to the indicator concept being used (Reish, 1972).

There are several problems associated with the use of indicator species if their abundance is to be used in some absolute sense as a measure of the intensity of perturbation. In certain situations many of them may occur naturally in relatively high densities. Usually, less subjective aspects of community structure indicate stress effects before indicator species unequivocally suggest a pollution situation, so these indicators are probably best used as confirmatory evidence or as part of a suite of other pollution assessment. Also, their appearance is not universal, and while their dominance may be used as an indicator of pollution, their absence certainly cannot be taken to indicate the absence of pollution (Warwick, 1993)

Biological pollution indices

The main advantages of biotic indices is that they are based on what is known about the responses of a range of different taxa to habitat changes or pollution. There are many different kind of indices which can be classified depending of the variables used (Boudouresque, 1970; Margalef, 1975; Bellan, 1980; Bellan-Santini, 1980; Belsher, 1982; Gray and Pearson, 1982; Ros, *et al.* 1990):

Indices based on indicator species

Bellan (1980) established an index based in polychaeta species for rocky bottom: *"key species of pollution"* usually appear in populations of polluted environments.

IP = Σdominants key species / Σdominants indicators of clean waters

Dominance of a species is expressed by individuals % of this species presents on the total of polychaeta species collected. This index is used in a comparative study among sites. IP will be > 1 only in polluted areas.

Ros *et al.* (1990) modified this index for applying to soft sediments. Bellan-Santini (1980) used the same index for the amphipod assemblages.

Belsher (1982) proposed an index using the algae:

Qualitative dominance = (% species of a taxonomic group / Σ population species) x 100

and

Quantitative dominance = (Σ cover area by a group / total cover area)

The ratio between qualitative and quantitative dominance is called tension Ψ and Boudouresque (1970) has used it as polluted index for rocky bottom.

Indices based on trophic strategies

Rafaelli and Mason (1981) described *nematode-copepods ratio* based on trophic strategies. This index is based on the rate of two taxonomic groups abundance (Margalef, 1975). Both groups have different life strategies. Along a gradient of organic enrichment, nematode number tend to increase in fine sediment whereas copepod number show the reverse trend. The organic matter input favour nematodes abundance because they are sedimentivorous; oppositely copepods are disfavour. The nematode-copepod ratio does illustrate trends where there are clear organic enrichment gradients and it may well be useful as an additional tool such as diversity indices and methods based on the distribution of individuals among species (Amjad and Gray, 1983).

Ratio of polychaeta with different feeding strategies is used such as a structure indicator of environmental quality (Bianchi and Morri, 1985; Tena, 1992). Dominance of sedimentivorous over filter-species is related with high sedimentation discharges rate, fine particles sediment dominance and also organic matter enriched sediments.

Infaunal Trophic Index (ITI) (Word, 1978; 1990) was originally developed as an aid to identifying changed and degraded environmental conditions as a result of organic pollution in the coastal waters of southern California. The approach is based on the allocation of species to one of four groups based on the type of food consumed by the animal and where the food was obtained from. As such it is a strictly a trophic index. However, Word (1990) demonstrated a relationship between the total abundance of animals in each of the four groups and sediment BOD values such that the ITI could be used to indicate pollution.

The ITI is calculated by determining the total abundance of the taxa belonging to each of the four groups and combining them in the following formula:

$$ITI = 100 – \{[33.33x\ [(0n_1 + 1n_2 + 2n_3 + 3n_4) / (0n_1 + 1n_2 + 2n_3 + 3n_4)]\}$$

Where n_{1-4} is the number of individuals in groups 1-4 and the coefficients in the numerator are simply scaling factors. Values of the index vary from 0-100 with low values indicating degraded conditions. ITI values were used to classify areas of seabed into either "normal" (ITI values 100-60), "changed" (60-30) or "degraded" (30-0) (Bascom *et al.*, 1978).

Indices based on the structural heterogeneity

From the considerable assortment of indices designed by ecologist we chose *Margalef Index* (I), to compute species richness (Clifford and Stephenson, 1975):

$I = (n - 1) / (\log_e N)$

Where n is the number of species found and N is the total number of individuals.

Shannon-Weinner's index (H') based on the information theory to compute heterogeneity (Pielou, 1975):

$H' = -\Sigma\, p \log p_i$

Where n is the number of species, and p_i is the proportion of the biomass of species i in a community where the species proportions are p_1, p_2, p_3..... p_i, p_n.

The *log-Normal distribution:* the majority of natural communities has a log normal distribution of the numbers of individuals among species (Taylor, 1978; Sugihara, 1980). Several authors (Gray and Mirza, 1979; Gray 1981; Gray and Pearson, 1982) suggested that unpolluted benthic communities have the log normal pattern of species abundance on a probability scale plotted against geometric abundance classes which produces a linear plot. Deviation from this in the form of a break or breaks in the line was considered to characterise pollution. This method has been critiqued subsequently on the grounds that undisturbed benthic communities were not always observed to be log normal (Sanders, 1968; Hughes, 1984). Even Gray and Pearson (1982) pointed out the utility of the log-normal distribution methods in assessing moderate or early-stage organic pollution impact.

Indices incorporating biomass

As a measure of the relative functional importance of species, biomass is better than abundance, and production in turn is better than biomass.

The *epiphytes biomass* can be a good indicator of eutrophication affecting on *Posidonia oceanica*. The leaves of *P. oceanica* are covered with a dense layer of epiphytes in the meadows situated in the proximity of facilities farm (per. obs.). The epiphyte development is directly correlated with the amount of nutrients and light available in the environment (Sand-Jensen and Borum, 1983; Buia *et al.,* 1992). Nutrient input into oligotrophic waters is particularly disturbing for *P. oceanica* meadow because it tempt an increase in the epiphytic cover of the plant leaves. Epiphytes may reduce the photosynthetic rate of the plant acting as a barrier to carbon uptake and reducing the light intensity reaching the plant. However, the epiphytic cover represents an important source of food for the herbivorous species, especially the fish *Sarpa salpa* (Linnaeus, 1758) or sea urchin *Paracentrotus lividus*, which leave clearly visible traces on the *P. oceanica* leaves, causing important damage. Seagrasses are key ecosystems, and are very sensitive to anthropic disturbance (Delgado *et al.*, 1999; Pergent *et al.*, 1999).

Abundance-biomass comparison plots (ABC) method, (Warwick, 1986) is a method based of determining level of disturbance (pollution-induced or otherwise) on benthic macrofauna communities. Under stable conditions of infrequent disturbance the competitive dominants in macrobenthic communities are *k*-selected or conservative species with the attributes of large body size and long life-span: these are rarely dominant numerically, but are dominant in terms of biomass. Also present in these communities are smaller *r*-selected or opportunistic species with a short life-span which are usually numerically dominant but do not represent a large proportion of the community biomass. When pollution perturbs a community, conservative species are less favoured and opportunistic species often become the biomass dominants as well as the numerical dominants. Thus, under pollution stress, the distribution of numbers of individuals among species behaves differently from distribution of biomass among species.

The ABC method involves the plotting of separate *k*-dominance curves for species abundance and species biomass on the same graph and making a comparison of the forms of these curves. The species are ranked in order of importance in terms of abundance or biomass on the *x* axis (logarithmic scale) with percentage dominance on the *y* axis (cumulative scale). In undisturbed communities (A) the biomass is dominated by one or a few large species, each represented by rather few individuals, while the numerical dominants are small species with a strong stochastic element in the determination of their abundance. Thus, the *k*-dominance curve for biomass lies above the curve for abundance for its entire length (Fig. 3).

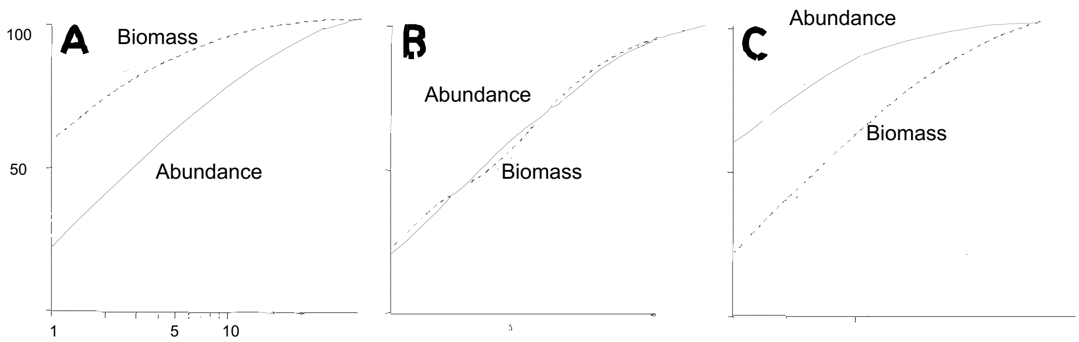


Fig. 3. Hypothetical curve species of k-dominance for abundance and biomass show an unpolluted (A), polluted (B) and extremely polluted (C) situation (based in Warwick, 1986).

Under moderate pollution (B), the large competitive dominants are eliminated and the inequality in size between the numerical and biomass dominants is reduced so that the biomass and abundance curves are closely coincident and may cross each other one or more times.

As pollution becomes more severe (C), benthic communities become increasingly dominated by one or a few very small species and the abundance curve lies above the biomass curve throughout its length.

These three conditions (undisturbed, moderately disturbed and grossly disturbed) should be recognisable in a community without reference to control samples in time or space, the two curves acting an "internal control" against each other.

Discussion

Species indicators and indices of pollution can help us to interpret the state of a community and the possible changes due to certain environmental pressures. However, few indicators complete all the requirements to be considered such as an effective tools. One of the typical problem is the taxonomic difficulty. Polychaeta are one of those more used as pollution indicators, but it need a high technical specialisation. The same difficulty appears using the indexes based on indicators species in spite of indicator species. Also, the pollution indicators species groups defined in a geographical zone may not apply to other habitat types or zoogeographical regions (Rygg, 1985).

The structural indices show another kind of problems. The lack of knowledge of species feeding behaviour (trophic index), or the scarce interpretation of community changes along natural gradients (copepods presence diminishes in depth) could produce misunderstanding. We can find a dominant species and its abundance can be attributed to pollution when the population abundance is caused by a natural stress.

In the determinant of pollution effects, Warwick (1993) suggests that any information is lost working at the level of families. Useful information is still present when working at the phylum level. More case studies need to be undertaken to establish the validity of these more cost-effective approaches.

The combined use of different environmental descriptors makes it possible to identify various parameters that are capable of determining the area of impact of the fish farm. Species abundance and composition react to changes in environmental conditions. However, knowledge of undisturbed community structure, including natural long-term successions changes, is pre-requisite for detection of these effects. Early stages of environmental change or sublethal effects are especially difficult to detect, although major changes in community structure have been readily demonstrated after the fact (Hargrave and Thiel, 1983).

Gray's (1981) suggestion that the most species adapt to pollution by varying their life history and not by gaining tolerance to stress implies that observations of population structure sensitive to change may be effective monitoring tools.

Monitoring studies are designed to detect any changes from the present state. It is crucial that sampling be designed to detect effects of predetermined importance (e.g. 30% change in abundance). By definition, monitoring studies involve repeated sampling in time and the study should also be replicated in space (Kingsford and Battershill, 1998).

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