Integrated fish monitoring in Sweden

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Foreword

The purpose of this report is to present a review and an evaluation of the strategy of integrated fish monitoring included in the Swedish national marine monitoring programme. As a background to the review an assessment is made of some of the long-term changes observed in monitoring.

The project is financed by the Environment Protection Agency (Contract no. 212 0326) and the Faculty of Science, Göteborg University. The project leader is Prof. Åke Larsson. Dr Olof Sandström, Skärgårdsutveckling SKUTAB AB, is contracted as external reviewer of the programme. Subproject leaders and participants in the programme have contributed with results and comments on the paper.

Introduction

The use of fish in environmental monitoring has a comparatively long history in Sweden. Systematic studies of coastal fish community structure, and the abundance, age distribution and growth of selected species, started in the 1960's as a result of the development of nuclear power. Impacts of the large cooling-water discharges had to be assessed, and monitoring programmes were established at the sites selected for the plants.

The development of this monitoring system continued and tests were made also at sites polluted by effluent from pulp mills and petrochemial industries. A consequence of the work on local pollution problems was a need of background data for comparisons, and search for suitable reference areas was initiated. Research on candidate species for sentinel purposes started. The studies were concentrated to perch (*Perca fluviatlis*) and viviparous blenny (*Zoarces viviparus*) and they could be recommended for regular monitoring (Jacobsson *et al.* 1993)

During the 1960's it became increasingly evident that the Baltic Sea was severely polluted by PCB, DDT and mercury (Jensen *et al.* 1969, 1972). This was also an incentive for monitoring, and the first standardized sampling programme started in the late 1960's, resulting in some of the longest time series available today on contaminants concentrations in biota (Olsson & Bignert 1997). The first step was to identify suitable sentinel species and sampling areas. Based on their migratory behaviour guillemots (*Uria aalge*) were found to represent conditions in the open Baltic proper, and egg samplings started in 1969. Herring (*Clupea harengus*) and cod (*Gadus morhua*) were included in the programme 1972 representing regional conditions within the Baltic basins. When the monitoring was expanded 1980 to cover also more localized coastal areas, the selection of perch as a sentinel species could be accepted also for contaminant monitoring purposes. In 1995 the coastal programme was expanded to include also viviparous blenny and blue mussel (*Mytilus edulis*).

Biomarker monitoring has been suggested for assessing the risk and impact of contaminants in the aquatic environment. The development started during the 1970's in Sweden, when ecotoxicologists began adapting diagnostic laboratory methods for use on field populations of fish (Larsson *et al.* 1985). The techniques were applied to fish populations exposed to metal pollution in the 1970's (Larsson *et al.* 1985) and pulp and paper mill effluent in the 1980's (Andersson *et al.* 1988; Södergren *et al.* 1989). It was soon realized among scientists that better background data on long-term trends were needed as well as more information about the natural variability in studied variables, and a first attempt to introduce biochemical/physiological methods for regular monitoring of fish health was made in 1988 in a coastal reference area of the Baltic Sea previously selected for fish community monitoring.

The monitoring activities started as separate series of measurements covering different levels of organisation aiming to describe contamination, physiological/pathological status and the status of populations and communities. There was only minor collaboration during sampling and data interpretations. Awareness was, however, growing that assessments based on chemical or biochemical data series alone could not be used for accurate extrapolations to describe changes on the level of population or community. It was also evident that analyses of whether observed population changes were associated with toxic exposure, eutrophication or natural alterations in habitats could not be performed without supporting information. It was felt that a higher degree of co-ordination was needed, and that the possibility to join activities within a common framework should be explored. Monitoring should also be developed towards more standardised long-term programmes. The weaknesses of non-integrated monitoring became increasingly obvious and could be summarized as:

- Contaminant concentrations can not alone indicate biological effects on the individual or population levels
- Biomarkers can indicate toxic exposure and biochemical/physiological effects, but they can rarely disclose responsible contaminants.
- The couplings between a certain change at a low level of biological organisation and an effect on growth, reproduction and survival are weak
- Changes on the individual organism level can indicate risk for population effects, but the causes are often unknown and relations between a specific change and an impact on recruitment, mortality and abundance are weak
- Changes on the population level may show ecologically relevant effects, but the causes are often unknown
- Support for analysing the importance of natural variations is usually lacking

The first example of a more integrated approach came during the pulp and paper mill effluent research in the 1980's. After a few years´ studies it was realized that the analysis of observed deviations and their biological significance could benefit from a higher degree of co-ordination between ecology, environmental toxicology and chemistry. A co-operation started, and sampling programmes as well as analyses of results could be integrated, which increased the analytical power of the investigations considerably (Larsson *et al.* 2003).

Development of integrated coastal monitoring was also discussed among the Nordic countries. A report on recommended communities and species for monitoring and criteria for selecting reference areas was prepared in the beginning of the 1990's (Nordisk Ministerråd 1992) by an expert group appointed by the Nordic Council of Ministers.

An opportunity to further strengthen project co-operation came when the Swedish National Marine Monitoring Programme (NMMP), run by the Environment Protection Agency (SEPA), was revised in 1992. During this process scientists within the fields of fish ecology, environmental toxicology and chemistry evaluated the existing monitoring and suggested a new strategy of integrated monitoring of coastal fish, including contaminants, biomarkers, and population and community indicators of ecosystem health in a common programme. The programme was accepted for national monitoring, and integrated samplings started in selected coastal areas.

The main purpose of the integrated fish monitoring is to provide a framework for assessments of ecosystem health by analysing observations from sub-cellular to population and community levels. Integrated effects of all stressors should be possible to detect. Although it is not the responsibility of regular monitoring to provide a full understanding of all observations, the integrated monitoring should allow a primary analysis of observed changes to verify whether or not changes are relevant and related to environmental stressors. When changes are detected but their relevance is unclear, this should lead to *a priori* designed analytical follow-up studies addressing the cause of the change and its further ecological significance. The objectives of the integrated monitoring of coastal fish were set to:

- monitor long term time trends in biological variables on different levels of biological organisation, i.e., from sub-cellular to population and community levels
- monitor long term time trends in contaminant concentrations
- provide data for comprehensive/integrated interpretations
- provide data on natural variations in biological variables
- estimate the response of measures taken to reduce the discharges of contaminants and nutrients
- act as watchdog to detect a renewed usage of banned contaminants and direct follow-up studies to possible new risk substances
- provide time series for contaminants of relevance for human and wildlife risk assessments
- provide reference data for local monitoring

The Swedish marine monitoring strategy stands on three legs: national, regional and local monitoring. National monitoring is performed in areas with no or very low local environmental impacts and is focusing on the large-scale development within basins. The regional programmes provide more detailed information and concentrate to the complicated pollution often seen in near-coast waters. Local monitoring is performed in areas exposed to industrial effluent. The national and regional programmes provide reference data for effluent area monitoring. All these monitoring activities are to a large extent performed according to common guidelines. It is important to note that data access is generally good through national, open, databases. It should also be emphasized that a clear connection between the three types of programmes was desired when the monitoring structure was established.

Integration starts with the selection of common monitoring sites and common sentinel species, and much effort was devoted to preparatory studies in potential monitoring areas (Ådjers *et al.* 1995, 1996) and to increase the knowledge about the biology of potential sentinel species. The national monitoring basically is a trend monitoring, which makes the statistical design important. Within the financial limits given, sampling was optimized and stratified with regard to species, season, gender, age and size of the fish. Considerable statistical research was made to improve assessments in contaminants monitoring, and the results did show the importance of location of sampling area, sample size and sampling frequency in studies of temporal and spatial variation in contaminant exposure (Bignert *et al.* 1993, 1994; Olsson 1994; Bignert 2003). As a consequence of these and other studies, monitoring of all variables included in the integrated programme is annually performed according to SEPA guidelines.

In this paper we present and evaluate the strategy of the integrated fish monitoring included in the Swedish NMMP, and the interpretation models developed to support assessments of observed deviations. The review is concentrated to the Baltic part of the programme. Observed trends indicating changes in the Baltic coastal environment are presented and discussed on this background, followed by an analysis of the integrative capacity of the monitoring. The Swedish Parliament has recently adopted new national environmental quality goals. The capacity of the present programme to meet monitoring criteria set up as a result of these objectives is evaluated.

Monitoring approach

An effects-based approach (Figure 1), rather than a strictly stressor-based approach, was chosen for the design of the monitoring strategy although many potential stressors were known already when the programme started. In the past many monitoring activities aimed to study known pollutants and their specific biological impact. Since the environment receives a mixture of numerous different contaminants it is hardly possible to monitor concentrations

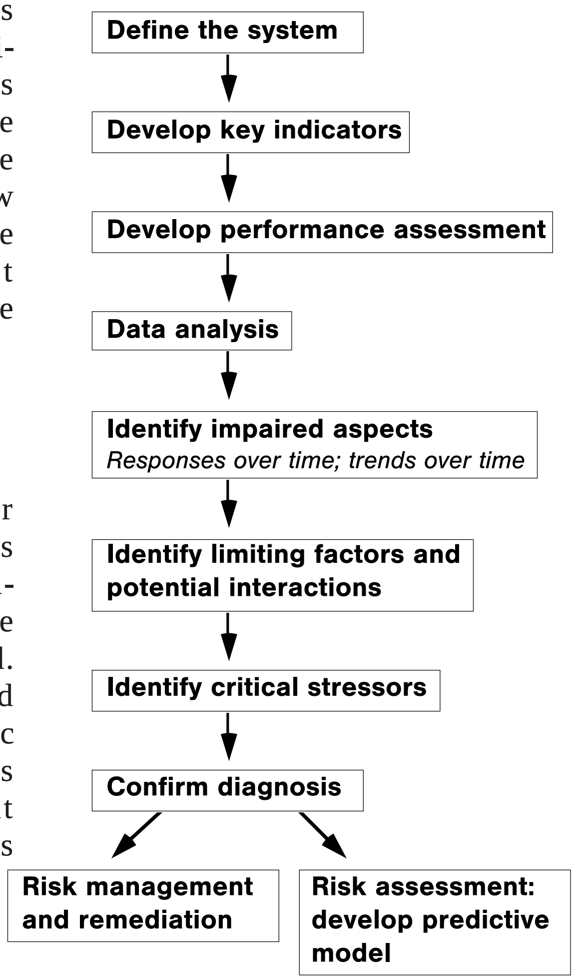

Figure 1. *Basic steps conducted during effects-driven assessments (from Munkittrick et al. 2000).*

of them all. The picture may be even more complicated as toxic contamination often is accompanied with high plant nutrient concentrations and other environmental stressors.

An effects-based assessment serves as an indicator of effects of known, none foreseen, unknown or known but ignored stressors. Documentation of stressor identities is not required, and initial analysis can be made without knowing the identity of stressors (Dubé & Munkittrick 2001). An effects-based approach can be useful to evaluate observed changes for their ecological relevance. However, effects-based assessments require substantial field collections, which can be time consuming and expensive unless the monitoring variables are carefully selected and directed to key areas and critical stages in the life history of the fish.

The biological monitoring variables were selected to indicate population impacts through either recruitment or adult mortality (Figure 2). Once changes are observed, a primary analysis should be made to evaluate whether impacts are related to toxicity, productivity or temperature. Steps for further follow-up studies should be taken when the cause of the change still is unclear or when critical stages have to be identified for a deeper understanding of the impact. E.g., if a negative trend in relative gonad size (gonadosomatic index, GSI) is observed,

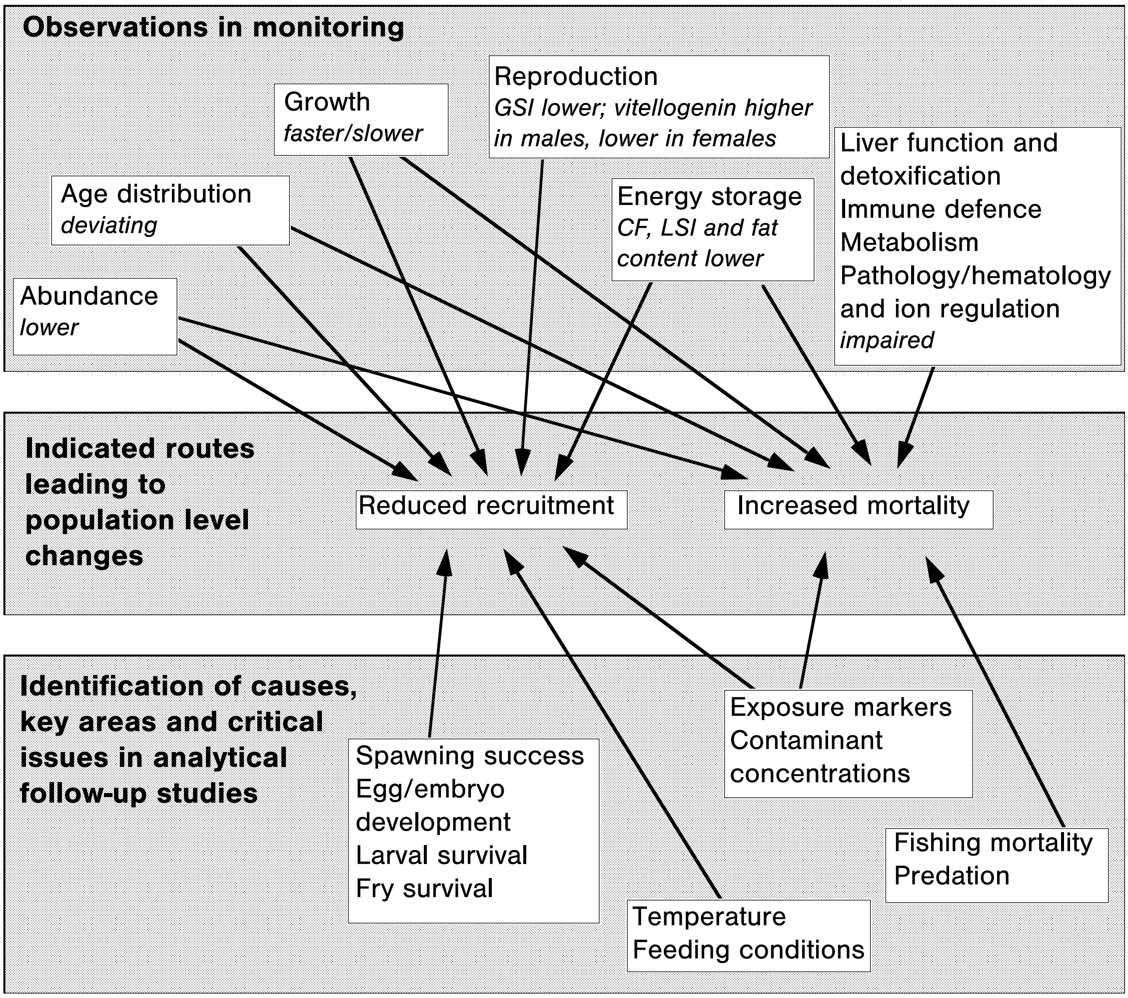

Figure 2. *A conceptual model explaining the strategy of effects-driven fish monitoring. Modified after Neuman & Sandström (1996).*

this indicates a reduced quality of the spawn. To understand the *cause* of the change follow-up studies may be directed to biochemical variables indicating toxic/endocrine disturbance as well as to contaminants not included in the programme although judged to be of potential importance. The *relevance* of the change in GSI can be addressed in follow-up studies directed to spawning success, embryo development and larval survival to identify critical life-stages and to assess the ecological significance of the effect (Karås *et al.* 1991).

Follow-up studies are also possible to do in the laboratory, allowing verifications of the field observations and closer analyses of responsible substances once effects have been documented. Support will be available from, e.g., human and veterinary toxicology where similar biochemical, physiological or pathological variables are used for indicating toxic responses and health impairment in higher vertebrates.

The Swedish fish monitoring can be compared with the Canadian EEM for pulp and paper industries (Lowell *et al.* 2003). An Adult Fish Survey is included in this programme. The survey has many similarities with the Swedish integrated fish monitoring, but there are also differences. It is strongly concentrated to whole organism variables. Biochemical tests are not included, as it is felt that rather than deciding *a priori* which tests to be run, it is more practical to have *a priori* analytical tests ready and decide which ones to run according to whole organism indications (Munkittrick 1992). The same argument is applied to the monitoring at the population level. The ecological relevance of effects may be addressed in follow-up studies. The interpretation of changes is based on a predictive response model (Gibbons & Munkittrick 1994).

This approach is cost-effective in pollution site monitoring, but it is not equally optimal when studying trends on a basin-wide level. The possibilities to make retrospective studies to elucidate cause of effects once changes are detected and evaluate their significance on higher levels are generally small. Banking of materials for retrospective chemical analyses of persistent compounds is a practice included in the contaminants monitoring programme (Olsson & Bignert 1997). Similar preservations of samples are, however, difficult for non-persistent contaminants as well as for biochemical and physiological purposes although some materials are kept for future use. In many cases it is also technically impossible to store tissue samples for future trend analyses. The lack of information about changes in populations and communities, which can never be reproduced, would be the most serious disadvantage. This explains why the integrated fish monitoring can not be concentrated to only one level of organisation.

The selection of monitoring areas

The selection of adequate sampling areas is a critical step in the assessment of ecosystem health (Nordisk Ministerråd 1992; Munkittrick 1992). The Swedish national coastal monitoring has to provide reference data for local programmes at industrial sites, besides following the large-scale environmental effects on the coastal ecosystem. Selected sites thus should be as free from local environmental impacts as possible. Coastal monitoring has a special relevance in Swedish waters, due to the large and unique archipelagos. Such ecosystems are important parts of Swedish nature and, according to the environmental objectives defined by the Parliament, deserve particular attention in national monitoring.

The following criteria were set up for the selection of sampling sites and the delimitation of coastal monitoring areas in the NMMP:

- The area must represent an important Swedish coastal environment
- There should be no local environmental impact
- The probability of future impact must be low
- The area must be large enough to assure that the probability of irregular impact from the surroundings is low
- Strong populations of stationary fish must occur, allowing long-term sampling of sentinel species for monitoring
- Habitats suitable for all life stages of sentinel species must be available within the selected area

It is also important that reference areas provide habitats suitable for other organisms than fish for expanded integration purposes, e.g. benthic flora and fauna for eutrophication studies, and bird and mammalian top predators, i.e., species with a comparatively advanced metabolic capacity, for monitoring effects of contaminants.

Scanning the coasts, we found that local impact was significant in most regions. Pulp and paper mills, metal and petrochemical industries, nuclear power plants, large cities, shipping, and agriculture influenced large parts of the Swedish coast. However, representative areas with little or no local impacts could be identified, and basic studies were made to see if they could meet the criteria set up for national monitoring. The first monitoring of fish community and population variables started in 1962 in Kvädöfjärden, a bay at the SE coast of the Baltic proper. Monitoring of contaminants started in the area in 1984, and physiological variables were included in 1988. The monitoring in Kvädöfjärden has been expanded to cover also other ecosystem compartments besides fish.

Four reference areas have so far been accepted for the NMMP (Figure 3): one in the Northern Quark separating the Bothnian Bay from the Bothnian Sea (*Holmöarna*), two at the coast of the Baltic proper (*Kvädöfjärden* and *Torhamn/Gåsöfjärden*) and a fourth (*Väderöarna/Fjällbacka*) at the Skagerrak coast of the North Sea. Additional reference data are available from regional programmes and from other countries bordering the Baltic (Figure 3). A network has been established within the HELCOM monitoring (COBRA; Ådjers *et al.* 1995). These programmes, however, are focused on fish communities and populations, and only occasionally comprise contaminants and biological effects monitoring. Monitoring of contaminants is not only restricted to coastal fish within the NMMP. Supporting information is available from time-series representing also open sea biota (Figure 3).

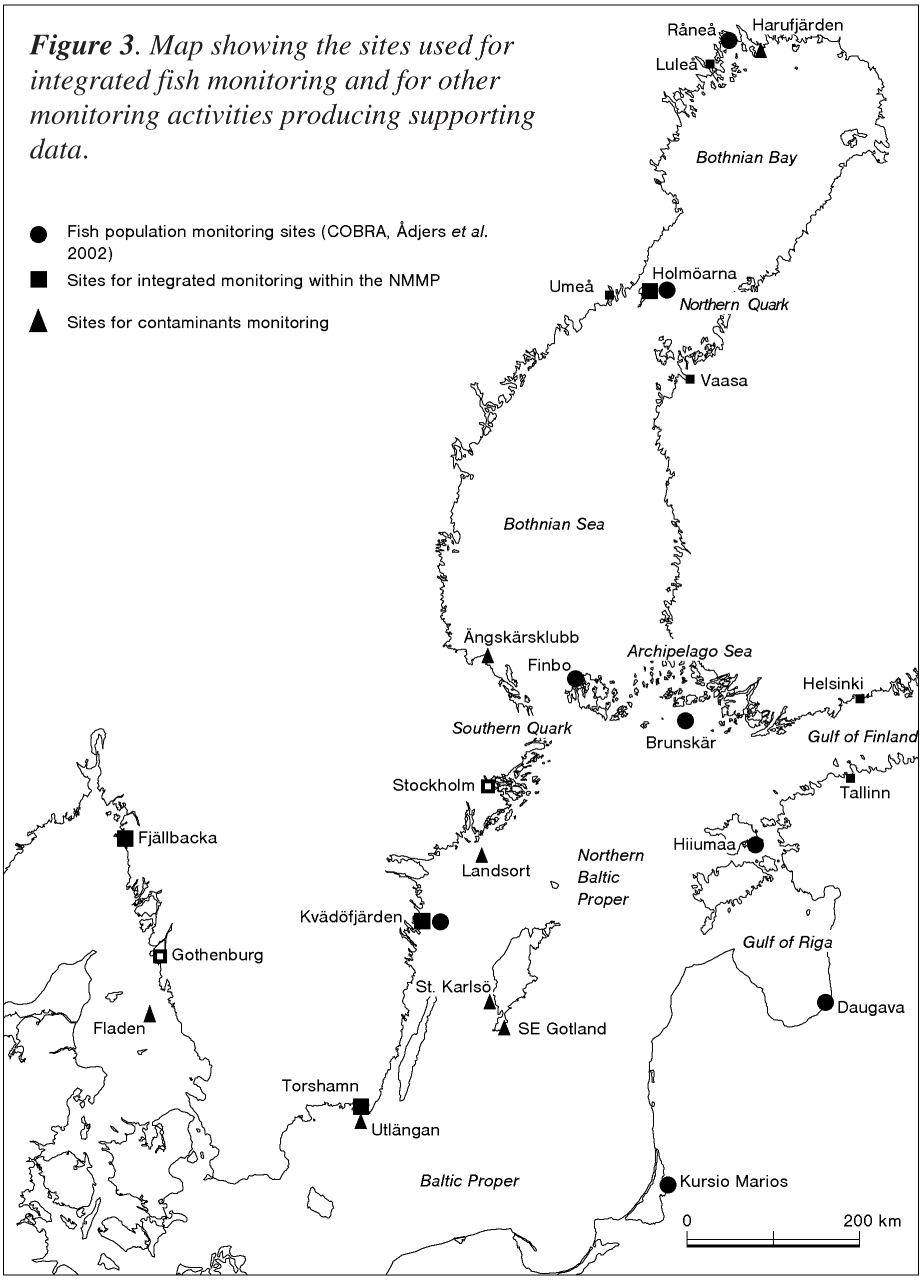

***Figure 3**. Map showing the sites used for integrated fish monitoring and for other monitoring activities producing supporting data.*

Monitoring variables

The monitoring variables included in the programme are selected to indicate:

- Changes in fish community structure relative to eutrophication or climate change.
- Changes on the population and organism levels due to metabolic disturbance or deviating feeding conditions.
- Changes on cellular or sub-cellular levels following exposure to toxic/endocrine disrupting substances.
- Trends in contaminant concentrations in specified matrices to indicate changes in exposure.

The contaminant variables are also selected to serve in human and wildlife risk assessments and to assess the results of regulatory measures.

Data from several coastal sub-programmes within the NMMP and from different monitoring areas can be compared for comprehensive interpretations. Coastal data can moreover be compared with open sea monitoring, showing the general development within the different basins.

A support from other programmes is often needed to:

- Distinguish eutrophication effects from toxic reactions.
- Indicate the general development in contaminant concentrations and nutrient levels within basins.
- Analyse the importance of changes in natural ambient conditions.
- Analyse the importance of fishing and predation.

Information on, e.g., contaminants in different matrices, nutrient concentrations, phyto-benthos and benthic macrofauna is accessible from other sub-programmes in the NMMP. Although these programmes are not always running in the same areas as were selected for integrated fish monitoring, the data show general patterns and trends within the different basins.

Coastal fish community monitoring is directed towards the stationary fish assemblage. The significance of observed community changes in the Baltic coastal fish monitoring is briefly presented in Table 1. Secchi disk depths and water temperatures are measured in connection with test fishing. Temperature is also recorded all through the growth season to provide data for, e.g., recruitment analyses and growth comparisons.

***Table 1**. Variables in Baltic coastal fish community monitoring and an initial tentative explanation to observed changes from values regarded as normal.*

Variable	*Interpretation/significance*
Species distribution	Shift towards cyprinids indicates eutrophication
Abundance/biomass	Increase indicates eutrophication or higher temperature, decrease indicates exploitation, predation or lower temperature
Disease prevalence	Interpretation unclear; increased occurrence could indicate an impact on, e.g., immune defence induced by pollutants. Disease outbreaks may affect survival and eventually community structure

Influence from fisheries and predation on the monitored populations may obscure assessments of other environmental impacts. However, data collected by the programme can be used for evaluating changes in size-distributions and adult fish mortality. Fishery statistics although poor in Swedish coastal waters, and bird censuses etc., can provide additional information for the assessments.

The two species selected for sentinel monitoring, perch and viviparous blenny, have a stationary behaviour and a life history which makes them representative of the local area and also in other respect suitable for monitoring (Jacobsson *et al.* 1986). Perch belongs to the dominating species in the Baltic archipelagos, while viviparous blenny is common at the open coasts of the Baltic up to the Northern Quark and in the Kattegatt and Skagerrak coastal waters.

In this paper we present the variables selected for monitoring of perch. The selection of variables aims to reflect population characteristics and to illustrate vital physiological functions: growth and energy storage, reproduction, liver function and metabolism, and immune defence. Biomarkers are used to indicate toxic exposure, and concentrations of selected contaminants in specified tissues are analysed. Furthermore, annually collected tissue samples are stored in a Specimen Bank (Olsson & Bignert 1997) to allow future retrospective studies of contaminants.

The interpretation and significance of changes in population characteristics and in single morphometric, biochemical/physiological or chemical variables is based on common knowledge about their respective diagnostic capacity (Table 2). The variables have been grouped to illustrate a) important population characteristics, and b) vital physiological functions, thereby increasing the analytical capacity when assessing risks for population impacts (Larsson *et al.* 2000). It should be stressed that the interpretations given in the table is an initial tentative attempt to explain the meaning of an altered value in measured variables. When changes have been detected, this primary interpretation has to be followed by a careful scrutinising of alternative explanations.

A key question has been when an observed deviation should be considered to be an unacceptable disturbance, relative to the environmental objectives, and become an incentive for technical amendments. It has been suggested when reviewing pulp and paper mill data (Larsson *et al.* 2000) that if three or more variables in the same functional group are significantly affected, this should be interpreted as an unacceptable disturbance of the function. An unacceptable disturbance of two or more physiological functions should be interpreted as an unacceptable disturbance of fish health and an evident risk of population effects through increased mortality. If one or two variables in a functional group deviate, further investigations are needed to confirm the responses and to analyse their wider significance.

Table 2*. Population characteristics, physiological functions and environmental contaminants studied in perch monitoring. Priority variables, interpretation guidance and limits of unacceptable impact are presented (modified from Larsson et al. 2000; Sandström et al. 2003).*

Population characteristics, Physiological functions and Contaminants	*Variables*	*Interpretation/significance*	*Limit of unacceptable impact*
Population structure	Abundance (Catch-per-unit-of-effort)	Natural ambient conditions may influence the abundance. Lowered values may indicate a reduced recruitment or increased adult mortality due to toxic influence.	Negative impact on abundance is unacceptable if interpreted as a toxic response.
	Age distribution	Age distributions indicate mortality as well as recruitment; data should be supported by observations of reproduction disorders.	Deviating age distributions are unacceptable if interpreted as toxic responses (indicated by health indicators and contaminant analyses).
Reproduction	Gonad size (GSI)	Lowered GSI indicates low fecundity, reduced oocyte growth or decreased energy allocation to reproduction. High GSI indicates increased energy allocation to reproduction or disturbed hormonal regulation of gonad growth.	Impact on more than one reproduction variable is unacceptable.

***Table 2**. continued*

Population characteristics, Physiological functions and Contaminants	*Variables*	*Interpretation/significance*	*Limit of unacceptable impact*
	Plasma vitellogenin	The vitellogenin level in male fish and juvenile fish is normally zero and therefore occurrence of vitellogenin indicates disturbed reproduction due exposure to estrogenic substances. In female fish a low level indicates disturbed reproduction due to hormonal imbalance.	See above
Growth	Annual length increment	Temperature, feeding condititons and metabolic disturbances may influence the growth; sex and sex stage must be considered.	Strongly reduced growth is unacceptable when coupled with supporting data from other health indicators.
Energy storage	Condition factor (CF)	Temperature, feeding conditions and metabolic disturbances may influence the condition.	Strongly reduced condition is unacceptable when coupled with supporting data from other health indicators.
	Liver size (LSI)	Reflects nutritional and metabolic status of the liver	Strongly reduced LSI is unacceptable when coupled with supporting data from other health indicators.
	Fat content in tissue analysed for contaminants	Temperature, feeding conditions and metabolic disturbances may influence the fat metabolism.	Strongly changed fat content is unacceptable when coupled with other indicators of metabolic disturbances.
Liver function and detoxification	Liver histology (necrosis, degenerated cells)	Structural changes indicate damages caused by exposure to pollutants or due to infections or parasites.	Strong structural changes are unacceptable if interpreted as toxic responses.
	Liver size (LSI)	Enlarged livers indicate high metabolic activity and/or induced detoxification system; reduced liver size indicates nutritional imbalance, metabolic disturbance or necrosis.	If one or two liver function variables deviate: continue investigations; if three or more variables deviate: unacceptable impact on the liver function.
	EROD activity	Reflects detoxification/metabolism of chemical substances (phase I). Induction of EROD indicates exposure to certain toxic organic molecules.	See above
	Glutation reductase (GR) activity	Reflects detoxification and protection against oxygen radicals and organoradicals. Increased GR activity may indicate oxidative stress.	See above
	Catalase activity	Catalase is involved in the protection against oxygen radicals.	See above
	Metallothionein (MT)	Induction of MT indicates presence of heavy metals; MT binds metals thereby reducing the metal toxicity.	See above
	DNA adducts	An increased formation of DNA adducts indicates exposure to genotoxic contaminants.	See above
Metabolism	Condition factor (CF)	See above	If one or two metabolic variables deviate: continue investigations; if three or more variables deviate: unacceptable impact on the metabolic functions.
	Liver size (LSI)	See above	See above
	Blood glucose	High blood glucose levels may indicate sampling stress or stress response due to toxic exposure. Changes coupled with supporting data for other health indicators may indicate metabolic/hormonal disturbances.	See above

***Table 2**. continued*

Population characteristics, Physiological functions and Contaminants	*Variables*	*Interpretation/significance*	*Limit of unacceptable impact*
	Blood plasma lactate	High lactate levels indicate a stress response due to sampling or ambient factors (e.g. toxic exposure). Changes coupled with supporting data for other health indicators may indicate altered metabolism or hormonal regulation.	See above
Immune defence	Lymphocytes	Low values may indicate suppressed immune defence; high values may indicate stimulated immune defence due to cell/tissue damage, acute bleedings, or bacterial infections. Sampling stress may influence the results.	If one immune defence variable deviates: continue investigations; if two or more variables deviate: unacceptable impact on the immune defence.
	Neutrophilic granulocytes	These white cells are involved in immune defence, phagocytosis and inflammatory responses. Low values indicate suppressed immune defence; high values indicate cell/tissue damage, inflammations or bacterial infections; also acute stress results in high levels.	See above
	Thrombocytes	Thrombocytes are involved both in immune defence mechanisms and blood clot formation. Low values may be due to chronic stress or certain infections; high values may reflect acute anemic or inflammatory conditions.	See above
Pathology, hematology and ion regulation	Internal and external pathological changes (fin erosions, skin damages, wounds, malformations)	Pathological changes may be caused by natural factors but more often by exposure to pollutants. The observations are often supported by data for other health indicators (e.g. variables reflecting immune defence, liver function or metabolism) and contaminant analyses.	Increased frequency of serious pathological changes are regarded as an unacceptable environmental impact.
	Hematocrit	Hematocrit reflects the capacity of oxygen transport in the blood; low values indicate anemia or hemodilution due to gill damage or impaired osmoregulation; high values may reflect increased oxygen demand but also a polycythemia due to acute stress or gill damage/ impaired osmoregulation.	If one or two hematological or plasma ion variables deviate: continue investigations; if three or more variables deviate: unacceptable impact on blood functions and ion regulation.
	Hemoglobin	See above	See above
	Plasma chloride	Plasma chloride reflects osmo- and ion regulation; low values indicate hemodilution due to disturbed osmotic regulation or impaired active uptake due to gill damage; high values indicate disturbed water balance, and, in marine fish, impaired excretion by the gills.	See above
	Plasma sodium (new variable 2003)	Plasma sodium reflects osmo- and ion regulation; interpretation of changes: see above for chloride.	See above

***Table 2**. continued*

Population characteristics, Physiological functions and Contaminants	*Variables*	*Interpretation/significance*	*Limit of unacceptable impact*
	Plasma potassium (new variable 2003)	Plasma potassium is involved in nerve and muscle cell functions and is a good indicator for the ion regulation; high potassium levels indicate ion leakage due to cell and tissue damages; low values indicate impaired active uptake in gills and/or intestine, or reduced kidney retention.	See above
	Plasma calcium (new variable 2003)	Plasma calcium reflects the calcium balance; low values may indicate kidney damages (e.g. induced by metals such as cadmium); high levels may indicate disturbed calcium regulation.	See above
Contaminants	Cd, Cu, Cr, Ni, Zn, Pb in liver; Hg in muscle; PCBs, DDTs, HCHs and HCB in muscle	The measured contaminant concentrations reflect the current exposure situation and the risk for biological effects. Decreased levels indicate positive effects of regulatory measures; elevated levels indicates continuing inputs to the environment and increased risk for biological disturbances.	Steadily increased levels of metals and organic pollutants are unacceptable according to the national environmental quality goals.

Interpretation models

A multiple stressor response model is essential for the selection of monitoring variables and the primary interpretation of observed changes. Theoretically, fish respond in a predictable manner to different stressors (Colby 1984). The interpretation model developed for the integrated fish monitoring is based upon general life-history theory and results from more directed research on the selected sentinel species. It has many similarities with the model originally proposed by Colby (1984) and further elaborated by Munkittrick & Dixon (1989) and Gibbons & Munkittrick (1994). The model presented here, however, also includes predicted responses on the sub-cellular and cellular levels due to contaminant exposures, and changes in the fish community following climate shifts or ecosystem productivity change. The response model is still general in many respects, and it should be used primarily for a first tentative assessment and to direct further analytical steps towards critical aspects and key areas. It can be especially difficult to analyse cause and effect when several stressors simultaneously act on the population, and environmental changes influencing fish populations may move in different directions obscuring the analysis of long-term monitoring data.

Only changes in single biochemical variables can seldom indicate population effects as extrapolations generally are difficult. However, they can be regarded as early signals of a toxic influence and should lead to further analytical steps. When signals at sub-cellular or cellular levels really should be considered to be relevant indicators of population effects has been a matter of debate when, e.g., results from pulp and paper mill effluent studies have been presented. One proposed solution was to analyse functional groups of variables and decide whether changes are of a magnitude indicating that the specific physiological function is impacted (Table 2). If there is an impact on reproduction, this may act on the population through a reduced recruitment (Figure 2). Other impairments of physiological functions may influence the population through an increase of mortality (Figure 2).

Identification of contaminant impact depends on the ability of the monitoring to distinguish changes in survival and energy allocation from changes associated with alterations in habitat and natural variability (Munkittrick 1992). Since fish represent high trophic levels, populations reflect the flow of energy through the ecosystem. Eutrophication changes the energy flows, generally increasing fish production, but also other ecosystem changes can affect fish populations. Stationary fish populations are not only governed by energy flow, but often to a higher degree by over-growth of spawning grounds, changes in turbidity, and other physical changes in recruitment habitats (Sandström & Karås 2002). The result can be a shift in species composition. The Baltic coastal fish community is comparatively poor in species. Two species generally dominate the stationary shallow-water community: perch and roach. Ecosystem changes related to eutrophication are known to influence the relation between species (Neuman & Sandström 1996). Abundance and biomass of common cyprinids like roach and silver-bream (*Blicca bjoerkna*) generally increase during moderate eutrophication while perch and many other species do not respond. Changes on the community level, reflected as shifts in species distributions and the abundance and biomass of fish in test-fishing, thus should be interpreted according to this predictive response model. Also other indicators of community stability have been suggested, e.g. species diversity and phylogenetic width to illustrate biodiversity and changes in trophic levels (Pauly *et al*. 1998). How to include these indicators into a response model for the Baltic coastal fish community is, however, still unclear but deserves attention in a future development of the assessment strategy. The protection and if necessary restoration of biodiversity are important parts of the national environmental quality objectives.

The relations between energy expenditure, energy storage and reproduction are essential in the life history of fish. There is a considerable literature on, e.g., how sexual development is influenced by growth rate and fat content (Policansky 1983; Stearns & Crandall 1984; Roff 1984; Rowe *et al*. 1991; Thorpe 1994; Svedäng *et al*. 1996). More specific data on the life history of perch and reactions to different stressors were obtained during research on populations exposed to cooling water and pulp mill effluents. Interactions between growth, storage and reproduction were studied in a perch population exposed to cooling-water in an enclosed research facility, the Biotest basin at the Forsmark nuclear power plant (SW Bothnian Sea; Sandström *et al*. 1995). The high temperature allowed very fast juvenile growth, which enabled an early sexual maturation at a small size. However, spent fish had depleted their energy stores to such low levels that repeated spawning was inhibited in many fish. The reactions of this perch population could be explained in terms of life-history strategy (Stearns & Crandall 1984).

When population structure, recruitment and life history variables were studied in a perch population exposed to effluents from a kraft pulp mill, the results were conflicting. Growth was faster and the condition factor higher in exposed fish, but reproduction was inhibited (Sandström *et al.* 1988). Recruitment was impaired, and adult fish appeared in low abundance in the effluent area (Neuman & Karås 1988; Karås *et al.* 1991). The response pattern indicated a metabolic disturbance stimulating growth and inhibiting reproduction, which differed from the response seen in cooling-water exposed fish. Exposure to toxic substances was indicated by biomarker studies, and some changes in vital physiological functions were of a character that was serious enough to indicate increased adult mortality (Andersson *et al.* 1988), which also could be verified when age distributions were analysed (Sandström & Thoresson 1988). It was concluded that the response pattern indicated metabolic disruption caused by toxic substances in the effluent with effects on all levels of organisation.

Parallel to the Swedish studies a Canadian group of scientists investigated fish in a pulp mill effluent area in Lake Superior, and found a very similar response pattern (McMaster *et al.* 1991). Results of a larger survey confirmed the results (Munkittrick *et al.* 1994) and the finding of such responses also at modernized pulp mills contributed to the development of fish monitoring (Adult Fish Survey) to be included in the Environmental Effects Monitoring requirements for pulp mills (Lowell *et al.* 2003). Evaluations in 2002 disclosed a national pattern of a decrease in gonad weight and increases in liver weight, condition and weight at age. This was believed to indicate metabolic disturbance in combination with nutrient enrichment (Lowell *et al.* 2003).

The predicted response patterns in coastal perch populations can be summarized as follows:

- Nutrient enrichment (when food is limiting) as well as increased temperatures (within reasonable limits in relation to the optima of the fish) should lead to faster growth, higher condition, larger livers, earlier maturation, and increased gonad size. Biomarkers for exposure to toxic/endocrine disrupting substances do not react. This response is in agreement with basic life-history theory.
- Exposure to toxic or endocrine disrupting substances can lead to faster growth, higher condition, larger livers, later maturation and smaller gonad size. Impairments of physiological functions can be expected and biomarkers for exposure can react, depending upon active substances. This response is not in agreement with basic life-history theory, as increased growth should allow earlier maturation and larger gonads. Higher energy use for growth and storage and lower commitment to reproduction thus should be interpreted as a metabolic disruption.
- In a situation where toxicity becomes very severe, a change towards slower growth, lower condition, smaller livers, later maturity and smaller gonad size will occur, even if feeding conditions and temperature stay constant. Impairments of physiological functions are expected and biomarkers for exposure will react. Adult mortality may increase. This is a strong signal that the ecosystem really is at risk.
- Exploitation by fisheries or predation by birds and mammals can lead to increased adult mortality, lower adult abundances and changes in size distributions. Biochemical and physiological markers do not react. If there are food limitations, and if the reduced abundances will lead to lower competition

for resources, growth rate will increase as well as condition and liver weight. Maturation will be earlier and gonad sizes larger.

In perch populations which are not regulated by density dependent factors, exploitation or predation will mainly influence abundances and size distributions with no effects on individual growth, maturation and GSI. This can be expected in Baltic archipelagos where populations are considered to be primarily recruitment regulated.

It should also be noted that severe eutrophication with increased turbidity and dense over-growth may cause negative effects on perch recruitment.

Observed time trends in monitoring

Data from perch monitoring in the Kvädöfjärden bay were used to test the interpretation tools and the integrative properties of the system, partly because we have the longest time series from this area, partly as trends have been documented which need a comprehensive analysis. The various time trends recovered from the Kvädöfjärden monitoring area are reported elsewhere, together with descriptions of sampling methods, materials and analytical procedures (Ådjers *et al.* 2001 for fish community and perch population monitoring; Hansson *et al.* 2003 for perch biochemistry/physiology; Bignert & Asplund 2003 for contaminants). In this paper we have selected some of these series for an integrated analysis of observed changes.

The data presented by Ådjers *et al.* (2001) together with unpublished data from 2002 were further elaborated to show possible effects of eutrophication or climate change on fish community structure. The relation between cyprinids (nine species, dominated by roach, silver bream and rudd) and perch in catches was calculated and related to the Secchi disk depth (Figure 4). There have been rather small variations in Secchi disk depth between years with no significant trend. Cyprinids dominated over perch at the beginning of the study period, but there was a change towards more equal shares with a ratio close to 1 in 2001 and 2002. The shift in species dominance was a result of both decreased catches of cyprinids and increased catches of perch. Total biomass, however, did not change significantly over time (Figure 5). The catches of 15–20 cm perch, which is the dominating size-class, increased significantly during the study period (Figure 6).

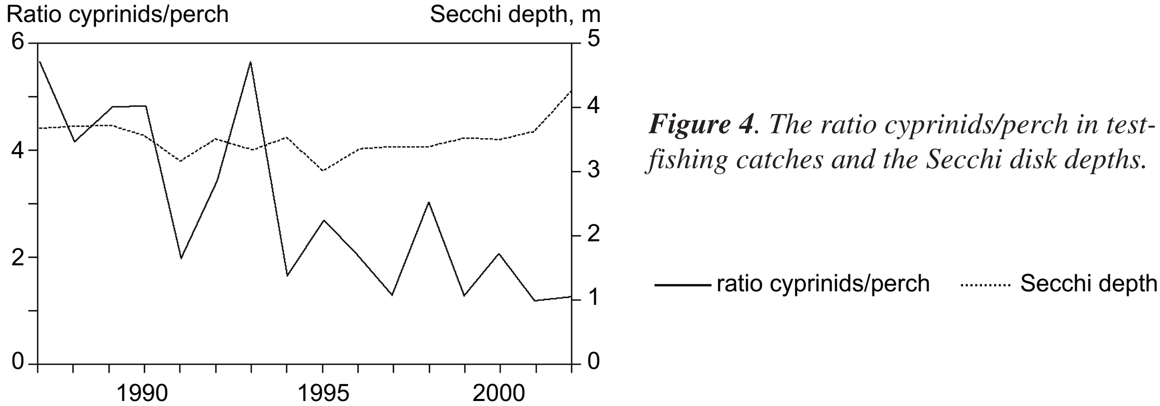

***Figure 4**. The ratio cyprinids/perch in test-fishing catches and the Secchi disk depths.*

Age and growth estimations were made on the materials collected during test fishing. About 400 females, distributed over the size range from 12 cm to ca. 30 cm, were sampled annually. Operculum bones were used for the age and growth analysis. Back-calculated growth was estimated for different ages.

Individual length growth has increased in all ages analysed, from the 1st to the 6th growth season (Figure 7). Significant trends were detected for ages up to three. As growth rate is influenced by temperature, the relation between summer water temperatures (May–September), recorded manually every week, in the monitoring area and the annual length increase was analysed.

The mean annual length increase over the period 1985–2000 was calculated for each age group each year of catch. A normalized growth value was calculated as the ratio between the annual mean and the grand mean for all years. These normalized growth estimates were used to analyse the correlation between temperature and growth. There was a strong statistically significant dependence between annual length growth and temperature variations (Figure 8; r^2= 0.78).

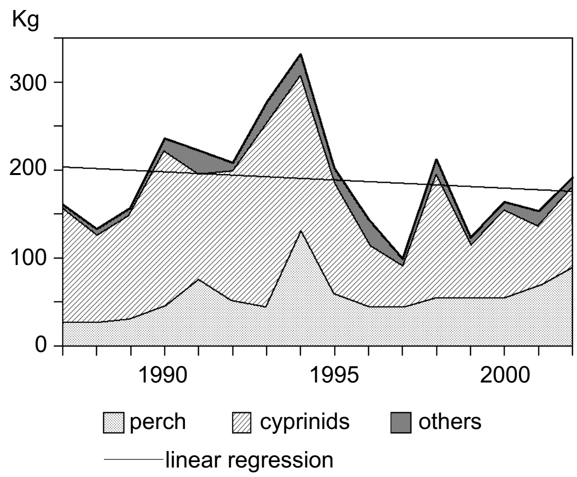

***Figure 5**. Total biomass (kg) of perch, cyprinids and other species in test-fishing catches.*

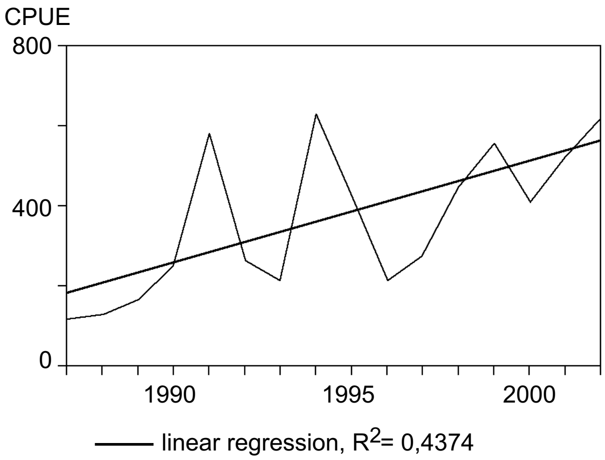

***Figure 6**. Abundance (CPUE) of perch (15-20 cm) in test-fishings.*

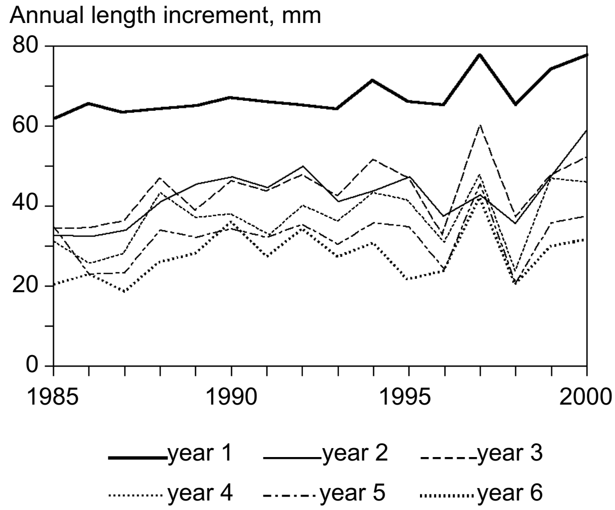

***Figure 7**. Annual length increment in perch of ages 1 to 6 during different calendar years.*

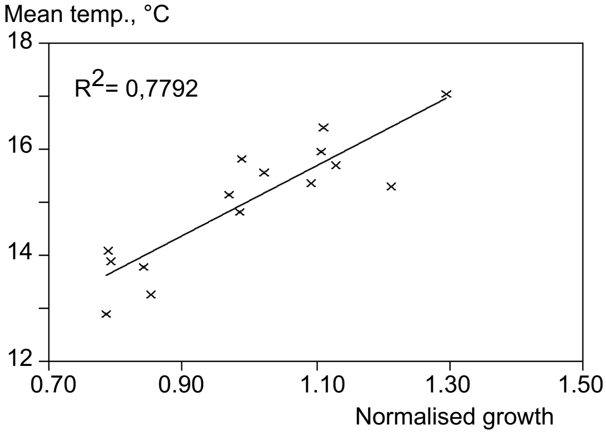

Figure 8. *The relation between normalised growth and summer temperatures.*

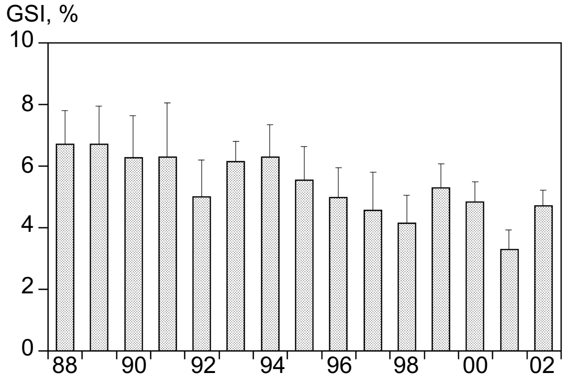

Figure 9. *Relative gonad size (GSI) in female perch.*

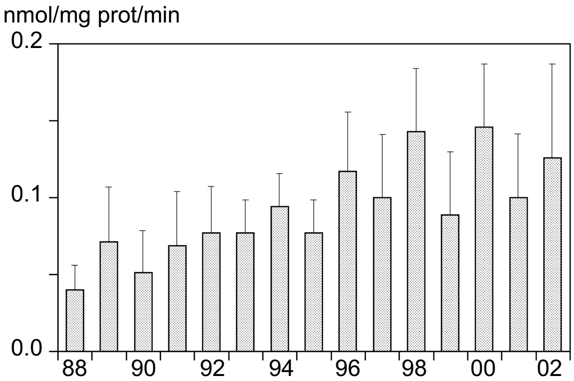

Figure 10. *EROD-activities in female perch.*

Gonadosomatic index (GSI), vitellogenin in plasma, condition factor (Cf) and liversomatic index (LSI) were measured on samples collected for biochemical/ physiological analyses (25 mature female perch within the size range 20–30 cm. An additional sample of 10 males was collected for vitellogenin analyses). These four variables reflect reproductive capacity and the energy storage of the fish. Cf and LSI (measured since 1988) differed little between years with no significant trends (Hansson *et al.* 2003). A significant reduction of GSI was, however, evident (Figure 9). The yearly decrease was about 2.5% of the mean annual value for 1994 (selected as reference since it is in the middle of the sampling period). Vitellogenin in male plasma, indicating exposure to estrogenic substances, was included in the programme in 1998. It is thus too early to expect a trend in plasma vitellogenin concentrations. The concentrations have, however, been low.

EROD activities increased about 3-fold during the study period with rather small between-years variations (Figure 10). The trend was significant. As there may be expected a relationship between gonad size and EROD-activity in female fish through, e..g., a stimulative influence of estradiol on gonad growth and a possibly suppressive effect on EROD activities, the correlation between EROD activities and GSI was analysed. There was a statistically significant correlation between the EROD increase and the GSI decrease. By statistical means the EROD activities were adjusted for the effect of lower GSI. The adjusted values gave a slightly lower increase rate, from about 7.4 to about 5% per year, but the adjusted EROD trend still was significant.

Apart from the change in EROD activities only one significant trend, an increase of about 1% per year in chloride concentrations in plasma, was detected among the biochemical and physiological variables monitored.

Concentrations of most contaminants analysed have decreased since the samplings started (Bignert & Asplund 2003) except for cadmium. Concentrations of cadmium in perch liver have increased rapidly during the last years (Figure 11a). The pattern of generally lower concentrations of PCB, although not significant (Figure 11b), is confirmed by monitoring in other areas of the Baltic and using other matrices like guillemot eggs (Bignert 2003).

The statistical power was calculated for all variables where this was relevant (Table 3).

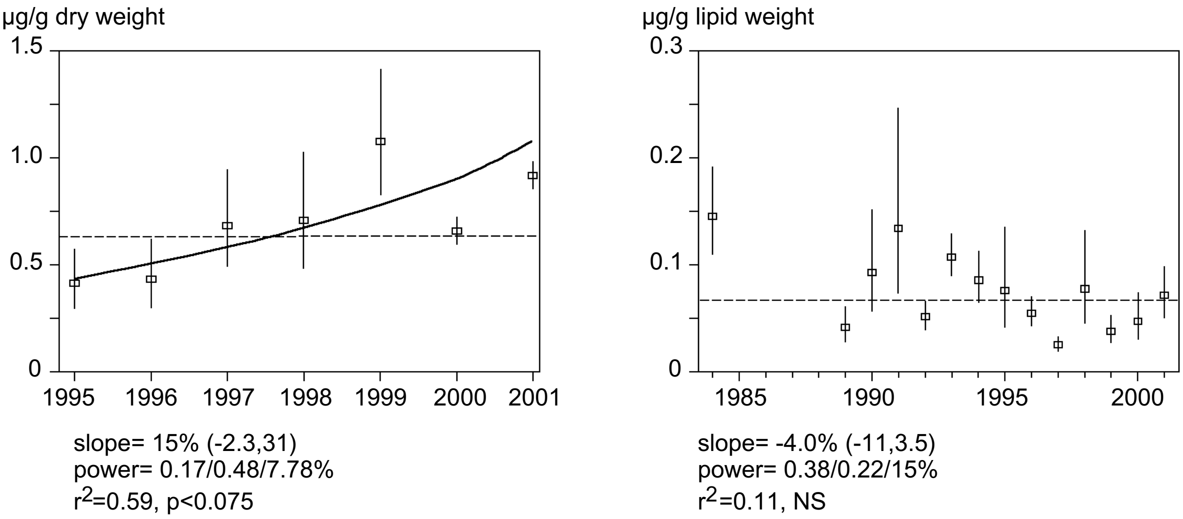

Figure 11. *Cadmium concentrations (µg/g dry weight) in perch liver (11a) and PCB-153 lipid concentrations in perch muscle (µg/g lipid weight) (11b). The trend is presented by a regression line (plotted if p < 0.10, two-sided regression analysis).*
slope= slope, expressed as the yearly percentual change together with its 95% confidence interval.
r^2= the coefficient of determination together with a p-value for a two-sided test (H_0: slope = 0), i.e., a significant value is interpreted as a true change, provided that the assumptions of the regression analysis is fulfilled.

Interpretations of the monitoring results

Community structure, total fish biomass and Secchi disk depths in the Kvädöfjärden monitoring area did not change in directions reflecting progressing eutrophication. The shift from a dominance of cyprinids to a community with shares close to 50% on the contrary indicates a commenced recovery towards normal conditions for Baltic archipelagos (Neuman & Sandström 1996). Supporting information from nutrient monitoring confirms that the increasing trend in concentrations was broken at the end of the 1980's and that there has been a tendency for decreasing concentrations during later years (Figure 12). The community reaction thus can be seen as an anticipated reaction to reduced eutrophication.

Table 3. *Results of analyses to estimate the power to detect log-linear trends in the time series. (Nicholson & Fryer, 1991). The minimum slope for indicating a relevant change for individual effect variables is estimated based upon general knowledge about their respective natural variability and when a deviation indicates a risk that significant biological or ecological effects may occur. When the minimum slope possible to detect within 10 years is considerably higher than the slope to indicate relevant change, significant impacts may remain undetected by the monitoring.*

Variable	*n of years available*	*Power to detect a slope of 5% for a period of 10 years*	*n of years required to detect a slope of 5% at a power of 80%*	*minimum slope possible to detect in a period of 10 years at a power of 80%*	*Minimum slope to indicate relevant change in a period of 10 years*
Abundance, CPUE	16	73	17	12	10
Juvenile (1+) growth	16	~100	6	1.5	5
Adult (3+) growth	16	~100	10	4.7	3
Condition factor (Cf)	15	~100	4	0.8	1.5
Gonad size (GSI)	15	96	9	3.7	2.5
Liver size (LSI)	15	~100	6	2.0	2.5
Hematocrit (Ht)	15	100	6	1.9	3.0
Lymphocytes	4	98	9	3.5	4.0
Neutrophilic granulocytes	4	~100	7	2.5	3.0
Trombocytes	4	64	12	6.2	5.0
White blood cell count (WBC)	4	99	8	3.3	4.0
Blood glukos	8	85	10	4.7	5.0
Hemoglobin (Hb)	8	97	9	3.5	3.0
Plasma lactate	14	27	18	12	10.0
EROD, liver	15	65	12	6.1	7.5
GR, liver	9	34	16	10	10.0
Plasma chloride	14	~100	4	0.7	1.5
GST, liver	9	47	14	8.0	10.0
Catalas, liver	8	38	15	9.3	10.0

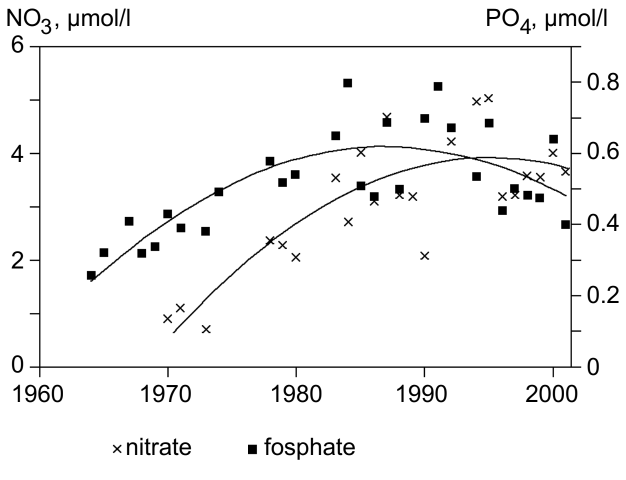

Figure 12*. Trends in nutrient (nitrate and phosphate, Jan–Feb) concentrations at station BY 32, NW Baltic proper. Data from the National Marine Monitoring Programme.*

Perch abundances increased significantly during the studied period. This is not an anticipated response to lower productivity, unless eutrophication has been severe. However, perch recruitment is strongly influenced by temperature and the generally warmer springs and summers during later years may explain the increase in catches due to increased growth and hence survival during the first year of life (Karås 1986).

During the last years several exposure and/or biochemical and physiological effect indicators have been included in the programme, but so far significant trends are only seen in EROD activities (Figure 10) and plasma chloride concentrations (Hansson *et al.* 2003). The EROD response indicates the presence of toxic and/or endocrine active substances. Generally EROD induction in fish is interpreted as a reaction to exposure to Ah-receptor ligands including planar compounds, e.g., halogenated dioxins, furans and biphenyls as well as certain polyaromatic hydrocarbons (Stegeman *et al.* 1992; Goksøyr & Förlin 1992; Andersson & Förlin 1992). Experience from pulp mills, oil refineries, petrochemical plants and other industrial or municipal activities producing complex effluent has shown that a common reaction to exposure is an increase of EROD activity (Andersson *et al.* 1988; Goksøyr & Förlin 1992; Förlin *et al.* 1994; Vetemaa *et al.* 1997; Stephensen *et al.* 2000; Lindesjöö *et al.* 2002). In a review, Sandström (1996) found an indication of metabolic disturbance (faster growth and inhibited reproduction) in 7 cases where fish had been exposed to pulp and paper effluent. In all these cases there was also a significant increase in EROD activity.

A well known reason for an increased EROD-activity in fish is the exposure to dioxins and co-planar PCBs. However, PCB concentrations have decreased in the studied perch population. We lack data for dioxins in perch, but the guillemot egg monitoring has shown a decrease in concentrations from the 1970´s to the 1980´s. Since then concentrations have stabilized (Bignert & Asplund 2003). A similar development has been found also in Baltic herring (Bignert & Asplund 2003), indicating that the general environmental contamination by these substances has decreased in the western part of the Baltic proper. The only positive contaminant trend documented in monitoring is cadmium in perch. However, we have so far no evidence of causality between EROD activity and cadmium effects. Among contaminants not covered by the monitoring the PAH´s are known to induce EROD. Data about PAH´s are, however, so far lacking.

Although the trend in EROD activity indicates increasing exposure to toxic Ah-ligands, the response may also be related to the successively decreasing relative gonad size suggesting an altered sex hormone metabolism and/or gonad development in female perch. Sex hormones like estradiol were never measured but it is known that EROD activities are generally higher in juvenile female perch than in adult females with maturing gonads and high plasma levels of estradiol. Another possible explanation could be an effect of the nutritional state of the fish. Mattsson *et al.* (2001) studied the reactions of rainbow trout to restricted diet in a laboratory experiment, and found that starvation resulted in reduced growth and a significant increase of EROD activity. It is thus not likely that the fast growth observed in perch explains the EROD response. The trend in EROD activity in Kvädöfjärden perch should after the first interpretation step be seen as an indication of increased toxic exposure.

The weak but statistically significant increase of the plasma chloride level suggests an altered ion regulation. The reason for such an alteration is at present difficult to explain. Cadmium concentrations have increased during the studied period, but cadmium should not be a likely candidate for the observed responses. The unchanged hematological variables and blood glucose levels speak against a role of cadmium at the present concentration levels (Larsson *et al.* 1985).

In conclusion, the monitored biochemical and physiological variables did not indicate any apparent disturbance of vital functions indicating a risk for adult mortality, which also was verified by the significant increase in perch abundance. However, the increased EROD activity and the trend in plasma chloride concentrations might be early signals of toxic effects, which should be subject for further studies.

The increased growth rate and reduced gonad size noted in Kvädöfjärden perch is not an anticipated normal response pattern. Fast growth should indicate nutrient enrichment (in populations where food is restricted) and/or increased temperature. This positive impact on growth should result in higher commitment to reproduction. A lack of this coupling between growth and reproduction should after a first evaluation step be considered as an indication of metabolic disruption or a possible toxic effect on the reproduction process.

According to the interpretation model, these changes are not in agreement with life-history theory. However, predictive response models are general in character, and results should be tested for natural variability and confounding factors. The integrated fish monitoring allows a second analytical step incorporating some possible factors. Growth is considered to be a driving force in fish reproduction, and the understanding of why growth rate has changed is important. In pulp mill research, which has produced much of the empirical data behind the theoretic development of response patterns (Sandström *et al.* 1988; McMaster *et al.* 1991; Gibbons & Munkittrick 1994), growth stimulation in concert with inhibited reproduction is seen as a change in energy allocation. In this respect, growth stimulation is not a natural response.

Many different stressors can influence perch growth, among them temperature which is the perhaps most forceful environmental factor in Baltic coastal waters (Neuman 1976; Karås & Neuman 1981). The analysis of temperature versus annual length increase did reveal that the growth response in Kvädöfjärden perch to a considerable extent could be explained by changes in temperature. Improved feeding conditions could contribute to the growth effect. However, neither this nor other monitoring has provided any evidence that productivity has increased during the last 15 years in these parts of the Baltic Sea. Enhanced growth consequently was a natural response to a temperature change, not a sign of metabolic disruption. Indicators of energy storage – Cf and LSI – did not react significantly during the study period.

Although the faster growth could be explained as a natural response, this should still have resulted in a positive effect on reproduction, not a negative. There may be natural explanations also to the observed reduction in GSI, but these solutions tend to come in conflict with life-history theory or be difficult to analyse on available data. One possible explanation to the trend in GSI may be the lower age of the fish in later years (Hansson *et al.* 2003). However, there was no change in the mean length of the sampled fish. It is known that the relative gonad size depends upon the size of the fish (Sandström, unpublished data), at least in the smaller length classes (15–20 cm). If this is an effect of size in itself or if it is related to age or whether the fish are first-time or repeated spawners is not known. A follow-up study of the relations between size, age, growth and GSI has to be made for a final assessment.

After this initial tentative analysis of the data we can conclude that there is a signal indicating metabolic disturbance still remaining to be explained, and that a toxic exposure can not be excluded. The observed changes may be related to natural events, but follow-up studies are needed for a full understanding.

Integrative capacity

Integration starts with the practical arrangements during sampling. The experiences have shown that much has been gained from using a common infrastructure during fishing and sample treatment. The same local assistants have been engaged in different activities, which has improved sampling performance. Laboratory facilities, e.g. for age reading, could be used for different subprogrammes. This has been an important contribution to quality assurance.

Data accessibility is another important aspect of integration. There have been no problems to have free access to each other's data, and during assessments data have been processed according to common routines, often in single comprehensive analyses.

The next step to consider is the possibility to use all or most variables in integrated interpretations. The basis for this is the interpretation model. Most studied variables fit into the predictive response model used, but it is realized that more effort should be directed towards improving the model and revise the variable list accordingly, if needed. The analysis presented in this paper, however, has shown that when trends are observed the system has an integrative capacity to provide the initial, tentative, analysis. Lacking information could also be identified and at least some of this can be available after a further treatment of stored materials and data and through directed follow-up studies.

Finally, it should be possible to produce integrated assessments. For this purpose it is important that scientists and institutes are willing to co-operate and that all participants share a common opinion about the monitoring needs and the basic strategy. A good indicator of co-operative functions should be the turnover rate of institutes and project leaders. All institutes involved today in the Swedish integrated fish monitoring took part already from the start of the programme. Although some of the initial project leaders have been replaced, most are still active in the programme. Besides contributing to the stability of the system, this long-term co-operation has certain advantages during assessments and has facilitated the production of comprehensive reports.

A well-designed and focused monitoring programme must be able to identify and analyse impacts on all levels in a cost-effective way. There are, however, several demands set up for the fish monitoring which are difficult to fit into an integrative framework. Contaminants monitoring must show changes relevant for human risk assessments even if levels are too low to harm the fish. Biodiversity is another concept, which is not easily included in the basic integrative theory. There is no model yet available for biodiversity which can be adapted to our predictive response model, but we should still monitor changes indicating that biodiversity is affected. The national environmental quality objectives are clearly directed to the preservation of biodiversity. Other parts of the programme fulfil the need of references for local pollution studies. Basic research can also be justified even if the results do not provide support for comprehensive interpretations. For example, new biomarkers must somewhere be developed and tested, and why not in an integrated programme where practical arrangements can be facilitated and where comparisons with other data will be possible.

Conclusions

As a conclusion, this review has demonstrated strong as well as weak properties of the programme. The positive aspects are:

- It was possible to co-operate in the same areas, at the same time of year, on the same species and for some variables on the same specimens
- It was possible to show convincing trends in contaminant concentrations
- It was possible to show shifts in community structure which could be interpreted in terms of ecosystem productivity
- It was possible to show a trend in population abundance
- It was possible to show a trend in individual growth rate
- The trends in abundance and growth could be explained by a temperature increase
- It was possible to show a trend in relative gonad size, GSI
- It was possible to show a trend in detoxification enzyme (EROD) activity
- A first, tentative analysis of these data was possible to perform
- The use of a predictive response model, based on life-history theory, was a constructive approach to interpretations
- The concept of physiological functions in monitoring was a constructive approach to interpretations
- Good data on natural variability were available for most variables supporting the interpretations of observed changes
- The data series were long enough for estimations of statistical power for most variables
- Only small adjustments of the programme should be needed to meet anticipated monitoring criteria set up as a result of the new environmental quality objectives.

Among negative aspects or weaknesses of the programme, the following can be mentioned:

- We have a lack of data on certain suggested risk substances like the PAHs, dioxins and other “modern” contaminants like the brominated compounds.
- We have a lack of data about the identity of suspected endocrine disrupters
- Biomarkers of exposure are included, but we do not know if they react to all relevant risk substances
- We have a possibility to analyse couplings between contaminants concentrations and biochemistry/physiology, but this has not yet been fully explored in assessments
- There is still a lack of good markers for reproduction; further development is needed
- We lack sufficient information for understanding the life-history of monitored species; further research is needed for development of response models
- We lack data on sexual maturation – a critical variable in reproduction studies
- Sex rate is not included in the programme as an indicator of endocrine disruption, but this can easily be made
- Population monitoring is probably enough well developed for the purpose, but the possibility to analyse mortality should be further explored
- Supporting information on exploitation and predation is mainly of a qualitative nature
- Supporting information on ambient conditions, e.g., temperature and water transparency is available for interpretations, but a closer integration with other sub-programmes in the NMMP would be desirable.
- We lack sufficient knowledge of how fish communities react to different stressors from a biodiversity perspective, although preservation of diversity is a priority environmental objective.

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