

ECASA Study Site Report

Site name: Piran

Country: Slovenia

ECASA Partners: NIB

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1. Introduction to the aquaculture operation

1.1. Introductory background statement

The Company

The aquaculture company farming in this area is Fonda Ltd. which has been established in the area since 2003, (the first farm location was 400 m South of the current site) several companies operated farms in the area from 1992. The company operating before Fonda Ltd took over the site was Mr. Bojan Troha (Ribic Ltd.). This farm became bankrupt in 2006.

The existing Fonda Ltd farm produces mussels (*Mytilus galloprovincialis*) and sea bass (*Dicentrarchus labrax*) and employs 15 people. The company has one marine site with an output of 100 tons of fish per annum.

Field Campaigns

This survey was carried out by researchers from the Marine Biology Station of the National Institute of Biology. There were three field campaigns carried out, the first one on the Troha RibicLtd farm (details below).

Date: October 2005

The following samplings were carried out:

- Sediment collection: nutrients, sulphate, pH, A_{tot} , TCO_2 in pore waters and solid phase analyses, meio-fauna, micro-phyto-benthos
- Water column sampling: dissolved oxygen, salinity, pigments, phytoplankton groups, bacterial production, number of bacteria, nutrients, TSS, C_{org} , N_{org} .
- Sediment traps: TSS, C_{org} , N_{org} , sedimentation ratio

Water column sampling was taken twice- before feeding the fish and three hours after feeding.

The samples were taken at the depth of 5m.

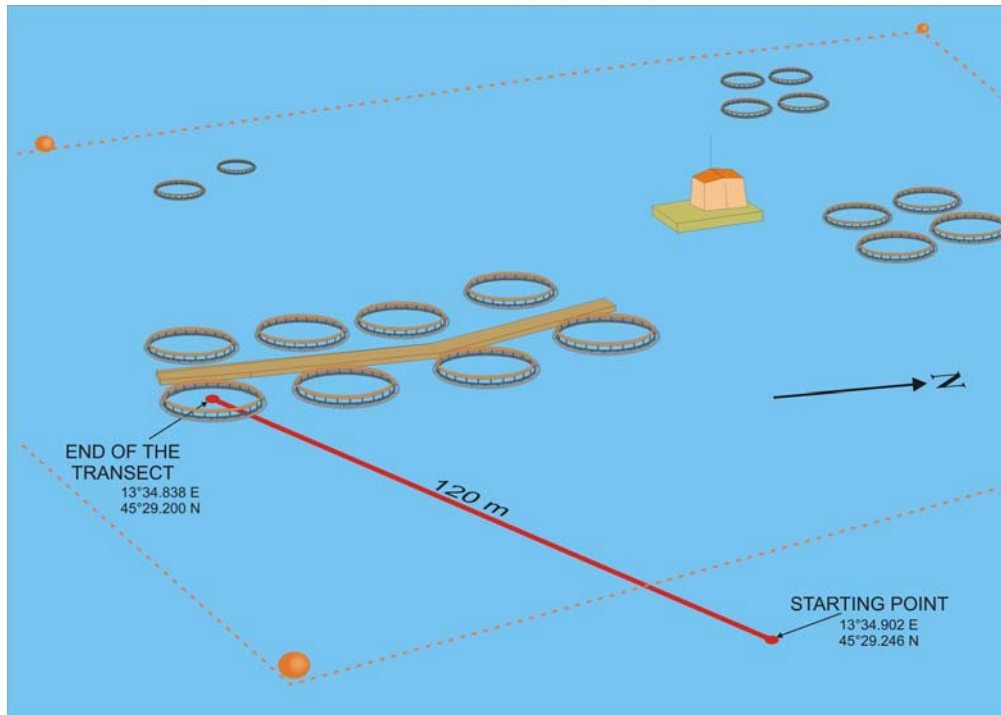
The following two field campaigns were carried out at the Fonda Ltd farm and its manager Irena Fonda has been the major source of information.

Date: April 2006

The parameters measured were the same as that from October 2005. As the position of the farm changed, we also changed the sampling strategy introducing some additional positions in order to get more information about the spread of effluents from the fish cages.

Date: July 2006

This sampling was taken to get information about seasonal differences. In addition to parameters surveyed in April 2006 redox potential was measured and macro-fauna samples were collected. A video transect of 120 m was taken. This is illustrated in Figure 1 below.



*The length of the transect is not related to the diameter of the cage.

Fig. 1. Schematic presentation of the fish farm and video transects

Methods used in the surveys can be found in the ECASA book of protocols; specific/new methods are described in Appendix 1.

1.2. Summary statement of key site specific environmental issues

Piran Bay is a semi enclosed bay, located in the Northern Adriatic. Its inner part has been declared as fisheries reserve since 1972. It is characterized by large annual temperature and salinity fluctuations (see Table 1). It is sporadically influenced by the Dragonja river, which has a torrential character and occasionally brings large amounts of water from the mainly agricultural surrounding land.

1.3. Information of farmer's environmental strategy

Fonda Ltd. follows codes of best environmental practice. The current policy is based on sustainable practice: the use of sustainable fishmeal sources and the cages are not protected with antifouling compounds, they are cleaned manually. In addition to this, the medical treatment for treating small fish is Floran, which is applied under the veterinary control.

Current regulatory status

The part of Piran bay was designated as the area of fish farming on the basis of the Water Act and Environmental Protection Act, details of which are given in Figures 2, 3 and 4.

2. Site specific regulatory and management background

2.1. The regulatory status of proposed location with respect to fish farming developments

The concession act (contract) between the farm Fonda Ltd. and State (Environmental Agency on behalf of Slovenian Government) was signed in 2004 on the base of the current legislation, described below.

Regulations for fish farming:

The Slovenian government accepted the “Decree on concessions of water use for the purpose of autochthonous *Dicentrarchus labrax* aquaculture at the territory of the Bay of Piran”. This decree is based on the “Water Act” and the “Environmental Protection Act”, which links to many EU directives. The Decree determines the area of fish farming, defines the fee to pay for the resulting environmental burden/impact. The concession is given for 20 years. The concession fee is divided between the state (50%) and the community (50%). The Concession is set on the basis of this decree: “Decisions are set depending on the average purchase price of the concession allowing the use of water for breeding autochthonous seawater fish for 2007”. The Concession changes on a yearly base and defines the price of a kg of fish.

All the acts/decrees/decisions mentioned are provided and followed by the Ministry of the Environment and Spatial Planning.

Separately, for the fish quality, rules are proposed by the government by the Ministry of Agriculture, Forestry and Food, such as: “The Rules on conditions to be met by aquaculture animals and products for the trade in the territory of EU” and are controlled by the Veterinary Administration of Slovenia. The Rule is linked directly to EU directives, and is based on the “Veterinary Practice Act”, which is also linked to many EU directives.

Regulations for shellfish farming:

The regulations for shell-fish farming are similar to those set for fin-fish farming, with additional laws regarding locating shellfish farms. These three extra regulations are: “Decree on the quality required of water supporting marine bivalves and gastropods” (EU directive 2006/113/ES), “Rules on the monitoring of the quality of waters supporting marine bivalves and gastropods” (EU directive 2000/60/ES + 2006/113/ES), and “Rules on determining marine areas where the quality of water is suitable to support marine bivalves and gastropods” (EU directive 2000/60/ES + 2006/113/ES). All the three regulations are linked to EU directives described in brackets, and are controlled directly by the Ministry of Environment and Spatial Planning. (See Figure 2.)

A separate set of regulation acts is established from the Ministry of Agriculture, Forestry and Food. The Veterinary Administration of Slovenia regulates: the “Instructions on the control of certain diseases affecting bivalve molluscs” (EU directive 95/70/ES), and the “Rules on conditions to be met by aquaculture animals and products for the trade in the territory of EU”, (5 different EU directives). Both the Instructions and the Rule are based on the “Veterinary Practice Act”. (See Figures 3 and 4)

Link to: <http://zakonodaja.gov.si/>

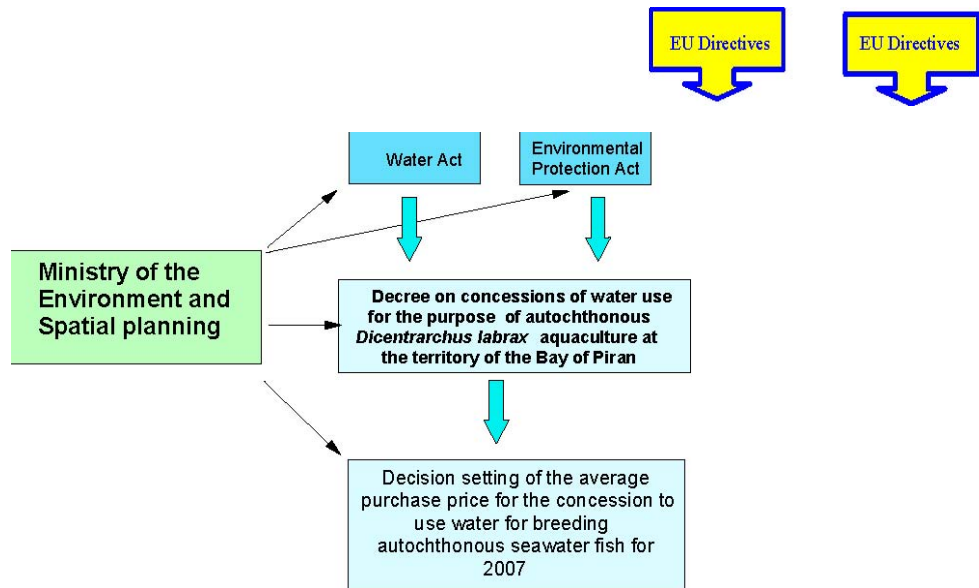


Fig. 2. Finfish farm regulations

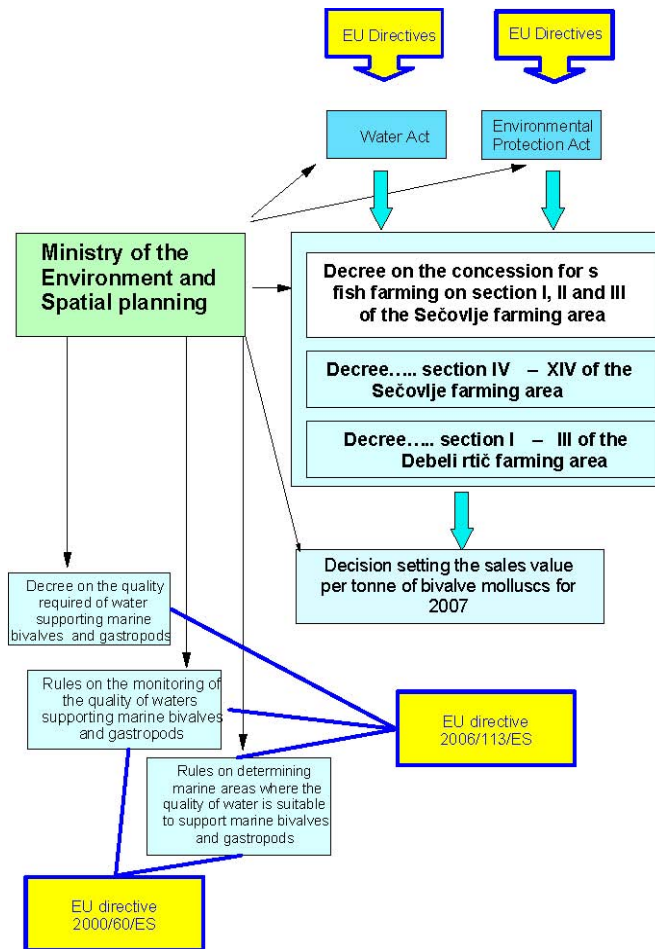


Fig. 3. Shellfish farming regulations

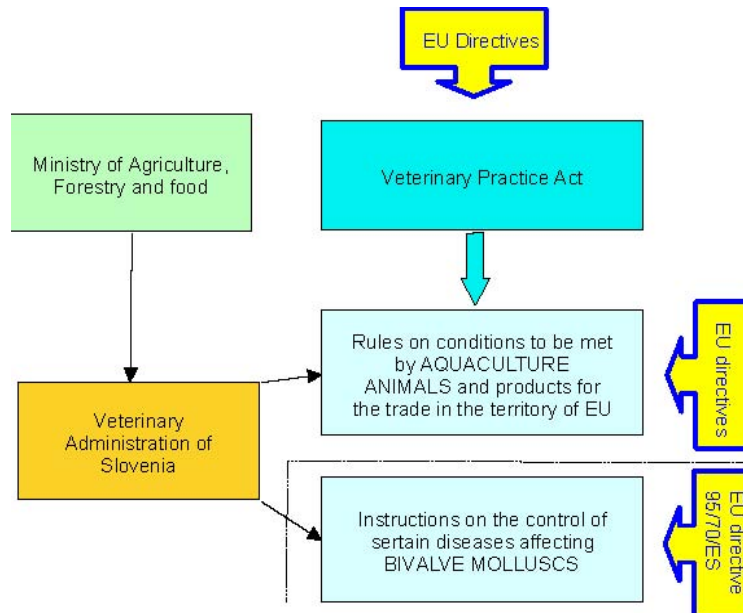


Fig. 4. Crop health control mechanism

2.2. Site description

Fish farm Fonda Ltd. is located inside Piran Bay (See Figure 5)

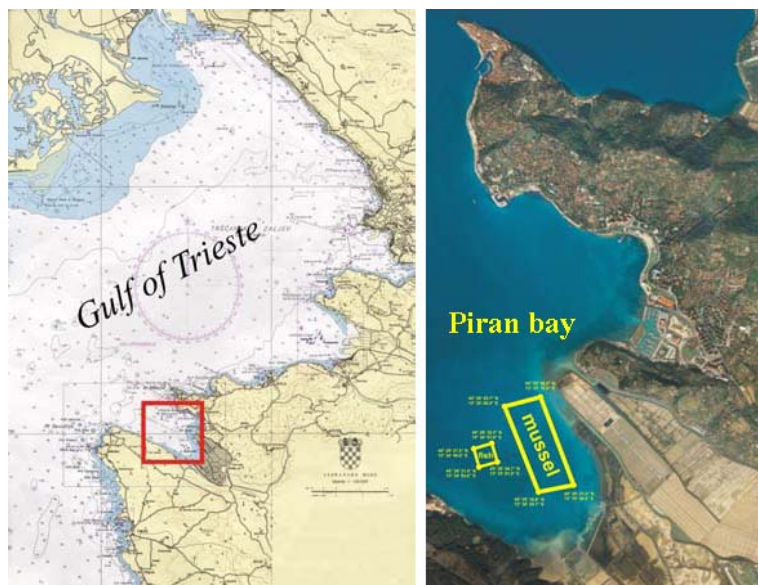


Fig. 5. Location of the Fonda farm in Piran Bay

The area is occasionally influenced by the small torrential Dragonja river. On the south-eastern part of the bay there are the Secovlje salt pans, where salt is produced according to traditional methods. The entire area of the salt pans is protected as a Regional park (Decree on the Sečoveljske soline Landscape Park, 2001). Close but separated by the Seca peninsula is the large Portoroz Marina, with more than 1.000 mooring berths.

The main external sources of nutrients for the Bay of Piran are the Dragonja and Drnica rivers, with a cumulative annual input of about 60 tons of nitrogen and 1.2 tons of phosphorous (Turk & Potočnik 2001). Their input is markedly seasonal being the lowest during summer. In contrast, input of dissolved and particulate nutrients from fish farms is the highest during summer due to the temperature-dependent increase of the metabolism of fish and more intensive feeding. It has been estimated that the release of nitrogen from the fish farm during summer may be roughly 1/4 to 1/3 of that introduced by the Dragonja river (Červek, 2002).

In winter the water column is vertically homogeneous, while thermal stratification generally develops from late April through September. Records of monthly data of temperature and salinity obtained at two locations in the inner part of the Bay of Piran (Malačič & Forte, 2003) are presented in Table 1.

Table 1. Simple statistics of 1362 temperature and salinity data obtained during 1974/2002 in the inner part of the Bay of Piran. The average value is denoted with <> and deviation with SD. Sampling was performed at four depths (0.5, 5, 10 m and about 0.5 m above bottom)

| | T (°C) | S (PSU) | ΣT (kg/m ³) |
|-----|--------|---------|-------------------------|
| <> | 15.67 | 36.88 | 27.16 |
| SD | 5.42 | 1.02 | 1.73 |
| Min | 5.85 | 28.77 | 20.69 |
| Max | 27.63 | 39.83 | 30.89 |

The phytoplankton standing crop and primary production are modest during late autumn/winter but increase in late winter/spring due to a diatom bloom. A shift to a micro-flagellate and cyanobacteria dominated phytoplankton community during summer is generally accompanied by lower chlorophyll *a* concentrations with values below 1 µg/dm³ (Malej *et al.* 1995). Such seasonal phytoplankton dynamics is mainly linked to freshwater inputs of nutrients (Mozetič *et al.* 1998)

2.3. Detailed description of the farm:

Fonda Ltd. is a sea bass farm, which have been operating at alternate site locations for more than 15 years. Close to the fish farm (about 200m) there are long line mussel farms, which are managed by different owners. Table 2 lists a detailed description of the farm.

Table 2. Detailed description of the farm infrastructure and activities.

| Farm Description | Detailed Information |
|--------------------------------------------|---------------------------------------------------------------------|
| Farm layout | |
| Cage shape | Cages are circular, with diameter 12 m (20 cages) and 8 m (8 cages) |
| Depth of cages | 6-9m |
| No. of cages | 20 cages – 12m diameter 8 cages – 8m diameter |
| No. of rows, | 3 rows |
| Distance between cages | 4-8m |
| Distance between rows | 50m |
| The total area of farm. | 40.000m |
| The total area of cages. | 2.019m ² |
| The total Volume of cages | 11.040m ³ |
| Reduction factor for trough-flow (0-1) | unknown |
| Fish species | Dicentrarchus labrax |
| Fish data | |
| Start weight | 5-8 g |
| End weight | 800-1000 g (in 4 years) |
| Content of protein | unknown |
| Content fat | unknown |
| Sinking speed of faeces | 0.6 cm/s (measured in lab) |
| Food data | |
| Content of fat | 18-22% |
| Content of protein | 43-47% |
| Content of carbohydrate | 16-17% |
| Content of ash | 7,3-7,5% |
| Sinking speed of feed | 5-15cm/s – farmers data, 6-8 cm (measured in lab) |
| Food factor | |
| Feed/growth- based on the farming practice | 2-2,4 |
| Critical values | |

| | |
|---------------------------------------------------------------------|------------------------------------------------------------------|
| Lowest acceptable oxygen in the water in the cage | unknown |
| Highest acceptable ammonium concentration in the water in the cage | Unknown, LC ₅₀ = 40mg/l of total ammonia (literature) |
| Lowest acceptable oxygen concentration at the bottom below the farm | 2 ml/L |

2.4. Proposed management strategy: biomass, medicines, chemicals, cycle, feed inputs, growth measurements:

The production cycle of sea bass is based on imported fingerlings from the hatchery in Italy. The life cycle for sea bass is 48 months, this relatively long life cycle results in a high quality fish product. Certified organic feeds (Ecolife 68) are used. The total production is approximately 100 tons and is planned to increase to 240 tons/year. Floran is used by prescription and a veterinarian controls the use of medicines.

A farmer continuously measures the growth of the fish and temperature of the water.

2.5. Physical farm logistic, including type of gear used (Cages, long lines, rafts....), moorings, access, lighting and anti – predator measures (use maps, diagrams)

The net depth is 6-9m below the surface. There are 8 circular cages, diameter of 8m and 20 cages, diameter 12 m organized in three rows. Distance between cages is 4-8m and distance between rows is 50m. The total area of the farm is 40.000m²; the total area of cages is 2.019m². Total volume of cages is 11.040m³. Anti-predator nets are used to prevent birds from attacking the fish. There is no protection against dolphins. During the night the farm is not illuminated.

2.6. Production and Processing

Fish are removed by herding and netting and are immediately put in -8 °C cold water (the ice shock method used to killing the fish). The fish are then transported to the shore where they are sorted, tagged and packed in ice. Majority of the production goes to the individual customers. The rest is sold at a local fish market.

3. Description of the site and quantification of effects on the environment – existing information only, not collected by ECASA

3.1. Land use, landscape and visual quality



Fig. 6. The surrounding land class is characterized by agriculture



Fig. 7. One of the founders of the Fonda Ltd., M.Sc. Ugo Fonda

The surrounding land use is mainly agricultural. Other land use is related to the tourist industry, e.g. the marina, airport and campsite. Large part of the closest land is salt pans, which are protected on the basis of the Ramsar convention. Settlements are approximately 1.5 km distant and include Sečovlje with 1760 inhabitants and Lucija with approx. 6700 inhabitants.

A study carried out after a decade of open cage fish farming indicated increased sedimentation of total particulate matter and higher concentration of hydrogen sulphide in pore waters along with changes in the meio-fauna community beneath the cages (Kovač et al. 2001) however clear impacts on the pelagic

environment were not detected.

In addition to the data collected during the ECASA surveys several RTD projects and other studies have collected data on the area studied. These are:

-Frumen, Aleksandra: Fouling organisms on artificial substrate near fish farm cages in the bay of Piran (Graduation thesis, 2002)

-Červek, Urban: Use of floating artificial substrate to reduce environmental impacts of fish farming (Graduation thesis, 2002)

-Forte, Janez: Environmental impact of fish farming in the bay of Piran (Graduation thesis, 2001)

-Kovač, Nives: Assessment of the oligotrophic environment of the Slovenian coastal sea (Final report, IAEA Research Contract No. 11103/UN, 2002)

-J. Faganeli, R. Planinc, J. Pezdič, B. Smodiš, P. Stegnar and B. Ogorelec: Marine geology of the Gulf of Trieste (northern Adriatic): Geochemical aspects: *Marine Geology*, 99 (1991) 93–108

-B. Ogorelec, M. Mišič and J. Faganeli: Marine geology of the Gulf of Trieste (northern Adriatic): Sedimentological aspects: *Marine Geology*, 99 (1991) 79–92

-Pokleka, Danijel: Biology of grown mussel (*Mytilus galloprovincialis*) in Piran bay (Graduation thesis, 1978) – only in Slovene language

-Peter, Tušnik – Biological and ecophysiological characteristics of *Mytilus galloprovincialis* from polluted and unpolluted environment (Thesis, 1985) – only in Slovene language -

3.2. Hydrography and water quality:

There are large historical databases from different monitoring programs which include data on: BOD5, density, nitrate, nitrate to orthophosphate ratio, orthophosphate, oxygen saturation, pH, temperature, total alkalinity, total ammonium, total CO₂, total oxidized nitrogen, total phosphorus, urea, nitrite, salinity.

Marine pollution

Sanitary quality at public beaches along the Bay of Piran meets the interim quality criteria for the use of the sea for recreational purposes (Turk *et al.*, early reports 2000-2005). Households are connected to the sewage system with a central wastewater treatment plant in Piran, and preliminary treated sewage is discharged through two underwater pipes, 3600 m from the shore toward the middle of the Gulf of Trieste. However, high faecal coliform concentrations have been detected in the estuaries of the Drnica and Dragonja rivers. Both rivers receive untreated urban wastewater from the inland households. Faecal contaminated water can spread in different directions toward the middle of the bay, depending on the meteorological and hydrographic conditions. Lower values are usually observed during periods of high river flow due to greater dilution; the increased flow of water also spreads the polluted water further into the Bay. The opposite situation is observed during the summer months, when brackish water with concentrated effluents is retained in the estuary, and high bacterial activity and consumption of oxygen may lead to hypoxic/anoxic conditions. The concentration of faecal pollution rapidly declines with the distance from the pollution source but contaminated water may occasionally influence the quality of the sea water around the aquaculture area. High concentrations for some chemical parameters were also measured at the estuaries of the Drnica and Dragonja rivers. In the period from 2001 to 2006 the yearly average input of total suspended solids was 430 t/y, 740 t/y of nitrogen, and 19 t/y of phosphorous.

According to chlorophyll, oxygen saturation, mineral and total nitrogen and phosphorus the ecological status of the coastal bay was tested by using the trophic index TRIX (Vollenweider *et al.* 1998). Along the transect from the inner part of the Bay of Piran to the south-eastern Gulf of Trieste the values of TRIX index varied between 6 and 4.8. The average value of TRIX units was 6.9 in the estuary of the Dragonja river. The average value was below 4 in the Bay of Piran, which is typical for mesotrophic/oligotrophic seas. The sediment in the inner part of the Bay of Piran at the entrance of the Portoroz Marina is contaminated by aliphatic and polyaromatic hydrocarbons. Lower concentrations were measured in the middle of the Bay of Piran. Hydrocarbons in the surficial sediments are mostly of pyrogenic origin, with a rather small contribution of petrogenic origin of the aromatic fraction.

Harmful algal blooms

Increased abundance of some dino-flagellate and diatom species may result in shell-fish becoming contaminated and being unsuitable for human consumption. Two main types of contamination occur in the Gulf of Trieste: diarrhetic shellfish poisoning (DSP) which is caused mainly by various dinoflagellate species of the genus *Dinophysis*, and paralytic shellfish poisoning (PSP) which is caused by some dinoflagellate species of the genus *Alexandrium*. At shellfish farms on the Slovenian coast DSP occurs almost every year, resulting a ban on selling shell-fish... PSP as well as ASP (amnesic shellfish poisoning) however, has not been observed in this area. Temporary bans of shellfish from farms have been recorded since 1989/90 (Sedmak *et al.* 2003). The abundance of the harmful micro algae is high at the edges of mussel (*Mytilus galloprovincialis*) growing areas in the inner part of Piran Bay. The genus *Alexandrium* prevails in colder months (early spring, autumn), while the number of species of the genus *Dinophysis* increases with increasing sea temperature in May and reached the peak during the warmest months (Francé and Mozetič, 2006).

3.3. Bathymetry, geology and habitats

Beneath the fish farm the sea bed is uniformly flat; depth varies between 13 and 14m. More recent grain size and mineral composition analysis of the sediments in the Bay were done by Ranke (1976) and later by Ogorelec *et al.* (1991). The general pattern of sediment distribution is strongly affected by the geological composition of the coastal area (Flysh on the northern and northeast hinterland and limestone at the south) and the fresh water sediment input due to the Dragonja river inflow. According to Ogorelec *et al.* (1991) the majority of the sediment in the Piran bay belongs to a dark grey clayey silt containing up to 40% of clay and less than 5% of sand. The median ranges between 3-10 μm , while the carbonate content ranges between 30-40%. The biogenic component is composed of ostracods, molluscs and foraminifera. The comparison between grain-size and carbonate content show that with the increasing carbonate content the grain size increases as well. The second most abundant mineral in all fractions is quartz. Its amount varies between 16-35%. It originates from the flysh hinterland. Feldspar is less frequent amounting to about 2-10% in the silt and clay fraction. The clay minerals are composed of illite, chlorite and mixed structure illite/montmorillonite (Ogorelec *et al.*, 1991) amounting in total to about 30-50%. Beside aragonite and high-Mg calcite, authigenic minerals found include pyrite and gypsum. Pyrite was present in all analyzed samples and is formed during diagenesis of organic matter bellow the sediment water interface (Ogorelec *et al.*, 1987; Ranke, 1976). It indicates reducing environment in the sediment and is normally observed few millimeters below the sediment surface. During the ECASA project special attention was paid to the uppermost 10 cm of the sediment close to the study area (Cermelj, pers. comm.). Sediment cores sliced in 2 cm thick intervals were analyzed for grain size distribution. The grain size distribution does not change with depth in this upper 10 cm, what changes is its biogenic content. The sediment belongs to dark grey clayey silt with practically no sand present. Silt prevails over clay that varies from 1020%. The biogenic component composed of shells and molluscs varies greatly and is more abundant in the vicinity of the shell and fish farm. Elemental C and N analyses showed varying concentration of organic carbon and

total nitrogen content in the sediment. The natural background values, about 1% of organic carbon and 0.1% of total nitrogen content, are substantially enriched in the vicinity of the fish farms rising up to 2.5 and 0.25 respectively. In most of the cases the content of both analites exponentially decreases to a depth of 10 cm, which corresponds to the beginning of the farming activities about 15 years ago.

Habitat types

The bay of Piran is a wide submerged valley of the small Dragonja river. The bay has high sedimentation rates (Ogorelec *et al.*, 1991). The rocky bottom is limited to short segments of coast that in many cases are formed by allochthonous material, which have been strewn in this area about thirty years ago. These rocky strips of the upper infralittoral belt are covered mostly by algal turf, which is composed by masses of small, scrubby, tiny, filamentous algae with canopy heights of less than 10 mm. In the summer months it is composed of species from the family Ciampiaceae, from the genera *Ceramium*, *Laurencia*, *Dictyota* and *Polysiphonia*, *Wrangelia penicillata*, *Cystoseira compressa* var. *rosetta* and by the mucus produced by Cyanophyceae and Diatomeae (Orlando Bonaca, 2003). On rocks and boulders are growing also some species of benthic macroalgae, mostly *Padina pavonica* and *Alsidium corallinum*.

The sandy-muddy bottom starts at a depth of about 2-3 m, and it is mostly covered by a *Cymodocea nodosa* meadow, which is the most common and abundant sea grass species in Slovenian coastal waters. Near Bernardin, large mats of the green alga *Cladophora prolifera* have been found (Orlando Bonaca, 2003). In this site, *C. prolifera* forms extensive, unattached, drift algal mats 10-70 cm thick and half a kilometer long on inshore waters, which overgrows the *C. nodosa* meadow. The mats are composed of individual plants which fragment easily. The spread of this alga has been related to cultural eutrophication and its productivity increases significantly with nutrient enrichment (Lapointe & O'Connell, 1989). Decomposition of mats results in hypoxia, which causes a reduction in infaunal and epifaunal benthic species diversity (Lapointe & O'Connell, 1989).

3.4. Benthos and sediments:

Main information on benthic environment comes from extensive sampling, analyses and assessment carried out in for the application the European Water Framework Directive in Slovenia (Lipej *et al.*, 2006). Macrophytes survey from the upper infralittoral zone along the rocky coasts revealed that in the upper infralittoral belt of the bay of Piran there are mostly growing settlements constituted by the brown alga *Padina pavonica* and the red alga *Alsidium corallinum*. Less abundant are *Dictyota dichotoma*, *Dictyota linearis*, *Flabellia petiolata* and *Cladophora* spp. In some sites also some typically nitrophilic algae (*Dictyopteris membranacea* and *Ulva rigida*) have been found. Within WFD project sampling and analyses of soft bottom macrozoobenthos was also carried including two locations (VT5-P4 and VT5-P6) in close vicinity of our study site (Fig. 8). The dominant benthic taxa within the water body 5 (VT5) including our study site were polychaeta followed by mollusca and echinodermata (Table 3).

Table 3. Dominant taxonomic groups of benthic invertebrates in different water bodies

| Proportion (%) | VT2 | VT3 | VT4 | VT5 |
|----------------|------|------|------|------|
| Polychaeta | 81,9 | 67,9 | 67,7 | 67,1 |
| Mollusca | 11,9 | 21 | 25 | 19,2 |
| Crustacea | 4,1 | 5,3 | 4,3 | 2,2 |
| Echinodermata | 1,1 | 4,9 | 2,3 | 10,1 |
| Other groups | 1 | 0,9 | 0,7 | 1,4 |

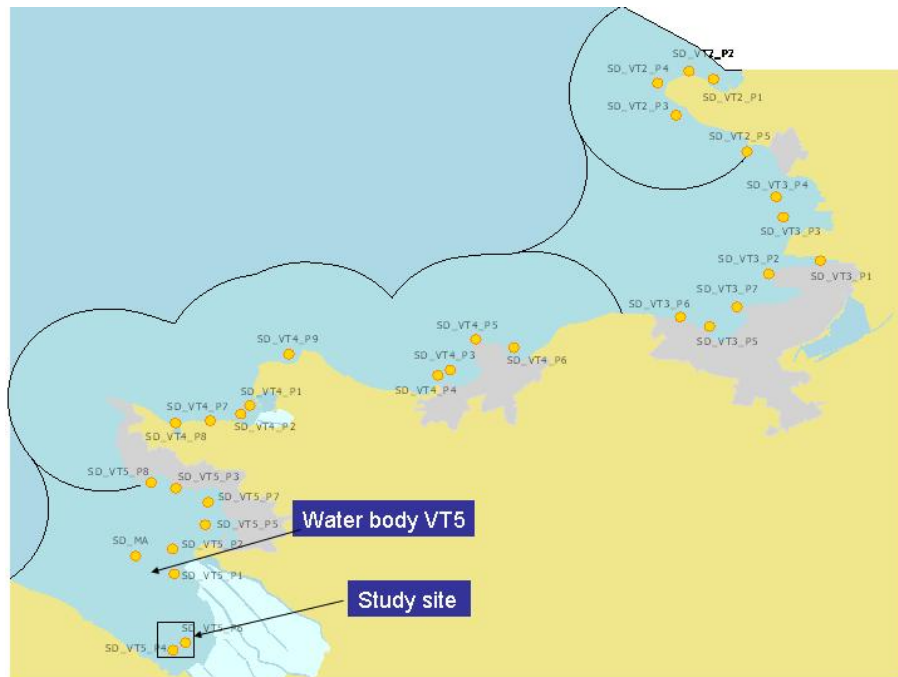


Fig. 8. Water bodies with sampling locations as used for the WFD assessment, study site is located within VT5 including two sampling location near study site

Soft bottom communities were dominated by only 5 macrozoobenthic taxa within the whole water body VT5 with the highest proportion of polychaeta *Lumbrinereis gracilis* at two locations close to the study site (Table 4).

Table 4. Dominant macrozoobenthic taxa in the water body VT5 including two locations near study site

| Dominant species (%) | SD_VT5_P1 | SD_VT5_P2 | SD_VT5_P3 | SD_VT5_P4 | SD_VT5_P5 | SD_VT5_P6 | VT5 |
|--------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-------|
| <i>Lumbrinereis gracilis</i> | 9,62 | 9,26 | 11,32 | 19,16 | 11,64 | 21,77 | 13,79 |
| <i>Heteromastus filiformis</i> | 19,24 | 15,58 | 14,78 | 7,62 | 4,0 | 14,74 | 12,66 |
| <i>Corbula gibba</i> | 3,80 | 2,93 | 15,47 | 9,34 | 6,18 | 1,59 | 6,55 |
| <i>Amphiura chiajei</i> | 6,84 | 7,00 | 4,39 | 5,90 | 4,73 | 6,58 | 5,90 |
| <i>Lumbrinereis latreilli</i> | 3,98 | 8,35 | 2,54 | 2,46 | 4,36 | 3,85 | 4,23 |

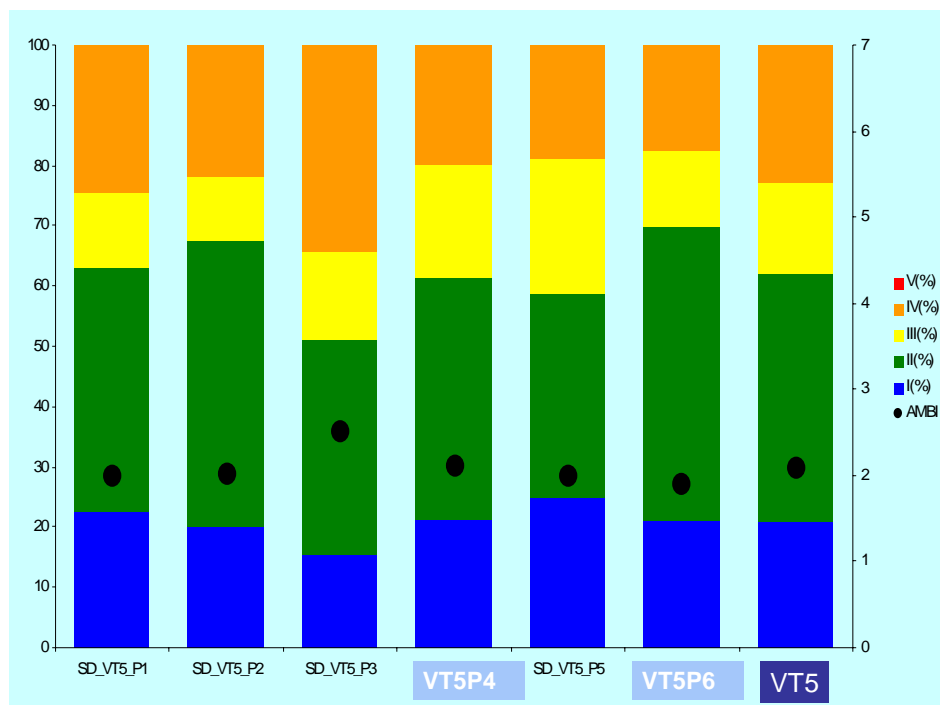


Fig. 9. AMBI for the water body VT5 and two locations near the study site

3.5. Marine mammals

There is one species permanently living in North Adriatic, the bottlenose dolphin, *Tursiops truncatus*. The population is estimated to be 40 animals. There were no interactions noted between the fish farm, the dolphin population or any other cetaceans. The dolphin population is regularly surveyed by the marine mammal research and conservation society, Morigenos (see Table 3).

Table 5. Bottle nose dolphin population in Piran Bay from 2002 - 2005

| Date | Estimated number of animals |
|------------------|-----------------------------|
| 25 February 2003 | 16 |
| 28 February 2003 | 15 |
| 1 March 2003 | 4 |
| 6 July 2003 | 5 |
| 21 March 2004 | 2 |
| 12 April 2004 | 5 |
| 27 April 2004 | 8 |

3.6. Birds

There were 248 species of birds (Table 6) found on the shore where the Sečovlje salt pans are located. At least 91 species regularly nest there. The fish farm is located about 1 km from the salt pans and there are 36 species of birds found regularly or occasionally on the sea surrounding the farm trying to feed from the cages or just resting on the buoys or the structures of the farm. are 36 species of birds found regularly or occasionally on the sea.

Table 6. Bird species found in the Sečovlje salt pans (about 1 km distance from fish farm)

| | Scientific name | Common name | Status | On the sea | Also on net pens | Also on buoys |
|----|----------------------------------|------------------------|--------|------------|------------------|---------------|
| 1 | <i>Gavia arctica</i> | BLACK-THROATED DIVER | Wg | + | | |
| 2 | <i>Gavia stellata</i> | RED-THROATED DIVER | Wg | + | | |
| 3 | <i>Podiceps cristatus</i> | GREAT CRESTED GREBE | Wg | + | | |
| 4 | <i>Podiceps grisegena</i> | RED-NECKED GREBE | Wg | + | | |
| 5 | <i>Podiceps nigricollis</i> | BLACK-NECKED GREBE | Wg | + | | |
| 6 | <i>Tachybaptus ruficollis</i> | LITTLE GREBE | NePe | + | | |
| 7 | <i>Phalacrocorax carbo</i> | CORMORANT | Wg | + | + | + |
| 8 | <i>Phalacrocorax aristotelis</i> | SHAG | Wg | + | + | + |
| 9 | <i>Phalacrocorax pygmeus</i> | PYGMY CORMORANT | Wg | + | | + |
| 10 | <i>Egretta garzetta</i> | LITTLE EGRET | Wg | + | + | |
| 11 | <i>Egretta alba</i> | GREAT WHITE ALBA | Wg | + | + | |
| 12 | <i>Ardea cinerea</i> | GREY HERON | Wg | + | + | |
| 13 | <i>Cygnus olor</i> | MUTE SWAM | Wg | + | | |
| 14 | <i>Anas penelope</i> | WIGEON | Wg | + | | |
| 15 | <i>Anas crecca</i> | TEAL | Wg | + | | |
| 16 | <i>Anas platyrhynchos</i> | MALLARD | NePe | + | | |
| 17 | <i>Anas acuta</i> | PINTAIL | Wg | + | | |
| 18 | <i>Anas clypeata</i> | SHOVELER | Wg | + | | |
| 19 | <i>Aythya ferina</i> | POCHARD | Wg | + | | |
| 20 | <i>Aythya fuligula</i> | TUFTED DUCK | Wg | + | | |
| 21 | <i>Somateria mollissima</i> | EIDER | Wg | + | | |
| 22 | <i>Clangula hyemalis</i> | LONG-TAILED DUCK | Wg | + | | |
| 23 | <i>Melanitta nigra</i> | COMMON SCOTER | Wg | + | | |
| 24 | <i>Melanitta fusca</i> | VELVET SCOTER | Wg | + | | |
| 25 | <i>Bucephala clangula</i> | GOLDENEYE | Wg | + | | |
| 26 | <i>Mergus albellus</i> | SMEW | Wg | + | | |
| 27 | <i>Mergus serrator</i> | RED-BREASTED MERGANSER | Wg | + | | |
| 28 | <i>Fulica atra</i> | COOT | NePe | + | | |

| | | | | | | |
|----|-----------------------------|--------------------|------|---|---|---|
| 29 | <i>Larus melanocephalus</i> | MEDITERRANEAN GULL | Wg | + | + | + |
| 30 | <i>Larus ridibundus</i> | BLACK-HEADED GULL | Wg | + | + | + |
| 31 | <i>Larus canus</i> | COMMON GULL | Wg | + | + | + |
| 32 | <i>Larus cahinnans</i> | ? | NePe | + | + | + |
| 33 | <i>Sterna sandvicensis</i> | SANDWICH TERN | Wg | + | + | + |
| 34 | <i>Sterna hirundo</i> | COMMON TERN | Ne | + | + | + |
| 35 | <i>Sterna albifrons</i> | LITTLE TERN | Ne | + | + | + |
| 36 | <i>Alcedo atthis</i> | KINGFISHER | Wg | + | + | |

Legend:

Ne: only nesting

Wg: winter guest

NePe: nesting and permanent presence

3.7. Fisheries and wild fish populations:

The farm is located inside the fisheries reserve where special permission from the Ministry of agriculture, forestry and food must be acquired. This permission allows the fishermen to catch mullets (*Mugil auratus* and *Mugil cephalus*) once per year during the wintertime when they occur in large shoals.

There are many species of wild fish which can be found around the fish farm. Beside the species listed in Table 5 below, a hybrid fish resulting from inter-breeding between *Dentex dentex* and *Sparus auratus* (*orantice*) occurs. This fish has probably escaped from other fish farms in Italy.

Table 7. List of wild fish species found in Piran Bay

1. *Mugil cephalus*
2. *Liza aurata*
3. *Liza saliens*
4. *Boops boops*
5. *Sarpa salpa*
6. *Belone belone*
7. *Dentex dentex*
8. *Diplodus puntazzo*
9. *Diplodus vulgaris*
10. *Diplodus annularis*
11. *Dicentrarchus labrax*
12. *Sparus aurata*
13. *Pagellus erythrinus*
14. *Lithognathus mormyrus*
15. *Lichia amia*
16. *Spondylisoma cantharus*
17. *Conger conger*

18. *Symphodus tinca*
19. *Lipophrys sp.*
20. *Trachurus trachurus*
21. *Balistes carolinensis*
22. *Scorpaena porcus*
23. *Solea sp.*
24. *Umbrina cirrosa*
25. *Mustellus mustellus*

3.8. Noise

There is a small airport about 3 km from the fish farm. The aircraft take off in the direction of the farm. The airport is busy only during the high season, and planes are small, able to carry a maximum of 48 passengers.

In 2006 an off-shore boat race was organized, some 3.5km from the farm. During the race the noise under water was measured and was found to exceed the allowed level, but further information is not available.

Inside the farm area there is a speed limit which applies to all craft, which will also decrease the noise impact. There are no other sources of noise.

3.9. Transport

The site is accessed by boat on a daily basis.

3.10. Socio – economic impact

The company employs 15 people but not all of them work as farmers, some staff is highly skilled and carries out specialized tasks.

4. Results of ECASA field studies: Indicators and Models applied and evaluated

4.1. Background to field program: dates, staff, boats, stations sampled, etc

Three surveys were performed with an aim to find the differences between the area affected by fish cage and an unaffected area. For this purpose a system of anchored sampling bottles was built (Figure 8).

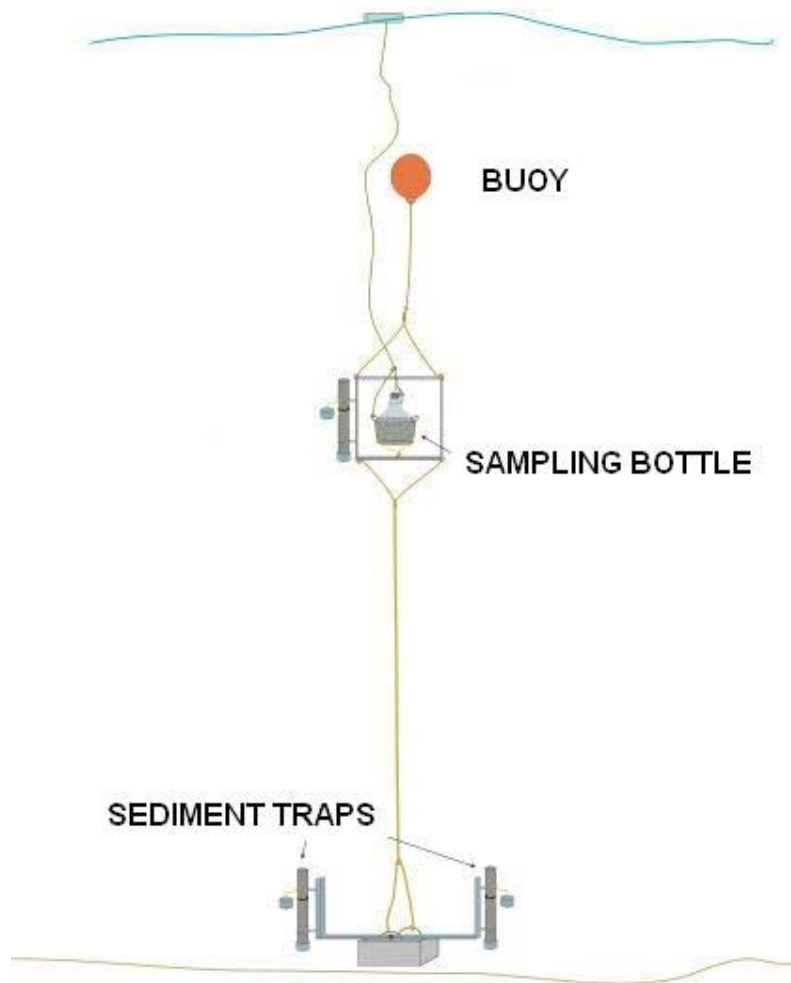


Fig. 10. One of 13 anchored sampling systems, arranged around the fish cages

The bottles were deployed at 5 m. When sampling, all the bottles were opened simultaneously (some of them from small boats, the others were opened by divers). On each of the field surveys two samples for each sampling location were taken: one before the fish were fed and the second 3 hours after feeding. The system was tested in October 2005 and deployed during April and July 2006 (Figures 9 – 12).

Table 8. List of sampling locations for ECASA

| Field campaign | Sampling point | Longitude | Latitude |
|----------------------------------------------------|----------------|-------------|-------------|
| First farm - Ribic; October 2005 | 00CC | 13° 34,724' | 45° 29,417' |
| | 00N1 | 13° 34,724' | 45° 29,420' |
| | 00N2 | 13° 34,724' | 45° 29,428' |
| | 0EN1 | 13° 34,728' | 45° 29,418' |
| | 0EN2 | 13° 34,737' | 45° 29,422' |
| | 0ES1 | 13° 34,728' | 45° 29,415' |
| | 0ES2 | 13° 34,737' | 45° 29,412' |
| | 00S1 | 13° 34,724' | 45° 29,414' |
| | 00S2 | 13° 34,724' | 45° 29,406' |
| | 0WS1 | 13° 34,720' | 45° 29,415' |
| | 0WS2 | 13° 34,711' | 45° 29,411' |
| | 0WN1 | 13° 34,720' | 45° 29,418' |
| | 0WN2 | 13° 34,710' | 45° 29,422' |
| | ZBMA | 13° 34,200' | 45° 30,200' |
| | ZCOB | 13° 33,032' | 45° 32,928' |
| | ZBWC | 13° 34,150' | 45° 29,225' |
| | ZBSC | 13° 35,050' | 45° 28,967' |
| ZBEC | 13° 34,900' | 45° 29,633' | |
| Second farm - Fonda; April, 2006; July, 2006 | CESY | 13° 34,835' | 45° 29,209' |
| | E200 | 13° 34,973' | 45° 29,263' |
| | E400 | 13° 35,105' | 45° 29,317' |
| | N200 | 13° 34,745' | 45° 29,317' |
| | N400 | 13° 34,673' | 45° 29,407' |
| | N600 | 13° 34,595' | 45° 29,503' |
| | S200 | 13° 34,919' | 45° 29,101' |
| | S400 | 13° 34,991' | 45° 29,011' |
| | S600 | 13° 35,069' | 45° 28,915' |
| | W200 | 13° 34,691' | 45° 29,155' |
| | W400 | 13° 34,559' | 45° 29,101' |
| | F0CC | 13° 34,838' | 45° 29,200' |
| | F0N1 | 13° 34,838' | 45° 29,203' |
| | F0N2 | 13° 34,838' | 45° 29,211' |
| | FEN1 | 13° 34,842' | 45° 29,202' |
| | FEN2 | 13° 34,851' | 45° 29,206' |
| | FEN3 | 13° 34,964' | 45° 29,287' |

| | | | |
|----------|------|-------------|-------------|
| | FES1 | 13° 34,842' | 45° 29,198' |
| | FES2 | 13° 34,851' | 45° 29,195' |
| | F0S1 | 13° 34,838' | 45° 29,197' |
| | F0S2 | 13° 34,838' | 45° 29,189' |
| | F0S3 | 13° 34,950' | 45° 29,161' |
| | FWS1 | 13° 34,834' | 45° 29,198' |
| | FWS2 | 13° 34,825' | 45° 29,194' |
| | FWN1 | 13° 34,834' | 45° 29,202' |
| | FWN2 | 13° 34,825' | 45° 29,205' |
| | FWN3 | 13° 34,664' | 45° 29,178' |
| | | | |
| Bioassay | S50 | 13° 34,873' | 45° 29,227' |
| Bioassay | S100 | 13° 34,947' | 45° 29,300' |
| Bioassay | REF | 13° 34,286' | 45° 29,508' |
| Bioassay | CESY | 13° 34,835' | 45° 29,209' |

Besides the usual analyses in the water column, filters were photographed and analyzed using Corel Photo-paint program in order to see whether it is possible to distinguish filters taken close to the cage from the filters taken away from the cage, and those taken before feeding from those taken after feeding the fish, (see Figure 10).



Fig. 11. Digital photographs of filters. BF – before feeding, AF – after feeding.



Fig. 12. Digital photos of filters along the transect from the zone C towards farm

Figures 13, 14, 15, 16 give details on sampling locations during the three surveys Sediment sampling: on all locations scuba divers took samples for analyses of interstitial waters. The same method was used to sample meio-fauna, micro-phyto-benthos and during the last field survey for macro-fauna.

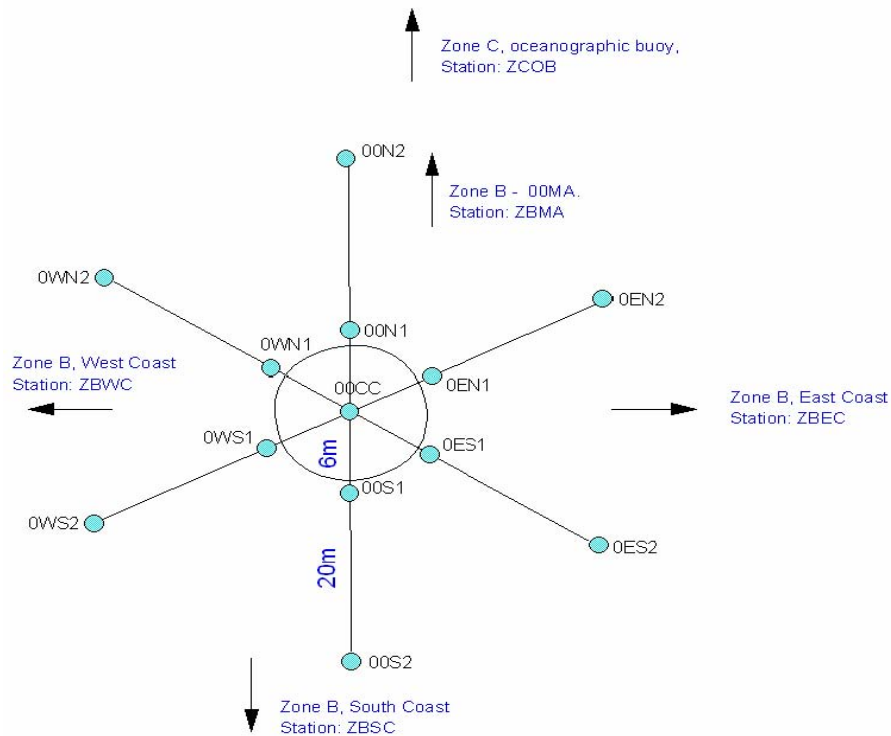


Fig. 13. Locations of the water column sampling from the center of fish cage (00CC), October 2005



Fig. 14. Locations of the water column sampling from the center of fish cage (00CC), April and July 2006

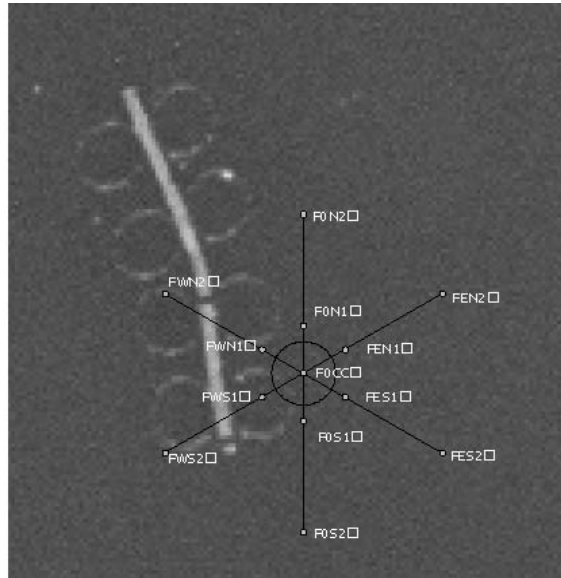


Fig. 15. Sampling locations for sediment and benthos parameters (April and July 2006); in addition we surveyed 3 locations 100 m from the fish cage (directions: NE, NW and S); these are not shown on the figure (FEN3, FWN3, F0S3)

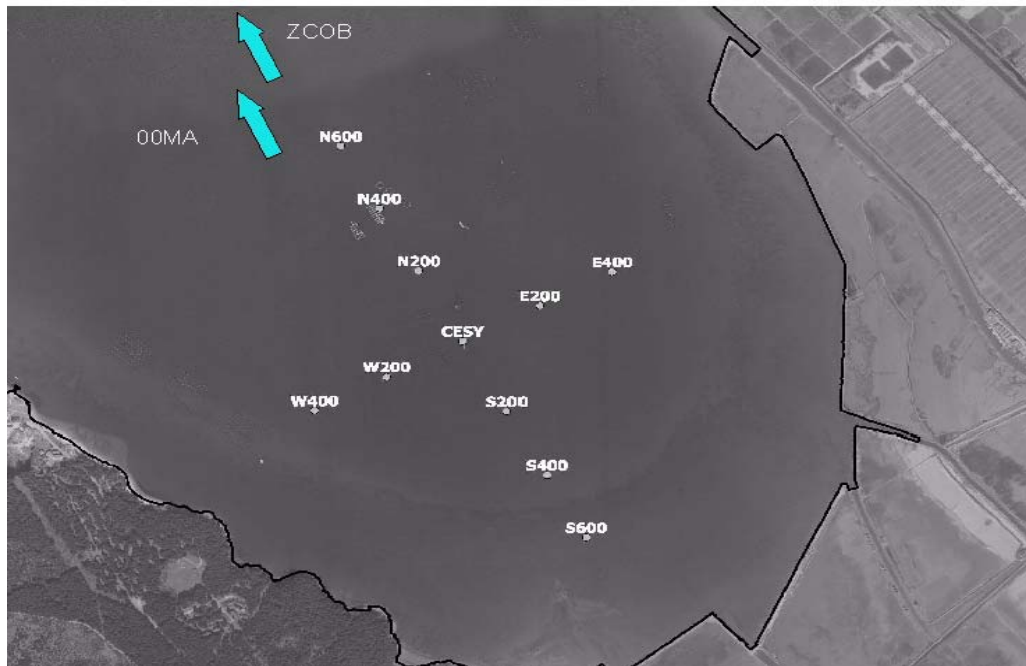


Fig. 16 Sampling locations for water column and sediment traps in zones B and C

4.2. Sampling methods and materials, analytical methods. (Refer to the book of protocols for detailed methods and appendix 1)

Benthos and sediment

Three benthic surveys were carried out in Piran bay: first in October 2006, second in April 2006 and the third in July 2006. The locations are presented in Figures 15 and 16. All the sampling was done by scuba divers. For meio-fauna, microphytobenthos (chlorophyll a and phaeopigments), pore water and bacteria cores of 10 cm² were employed; for each of parameters 3 replicates were collected. (Figure17). Macro-fauna was sampled with cores with the diameter of 10 cm, 4 replicates per location. The same size of cores was used during the third field campaign for redox measurements.

4.3. Description of methodology employed



Fig. 17. Samples for microphytobenthos and meiofauna

Water column

During the field campaign in October 2005 there were two sets of samples were collected at 5 m below the surface (Figure 8): the first one before feeding and the second 3 hours later, after feeding the fish. Orthophosphate, total phosphorus, nitrite, nitrate, ammonia, silicate, total nitrogen, total suspended solids, organic C and N were measured. From the same samples subsamples were taken for bacterial production and bacteria counts. Pigments were measured with HPLC methodology. At the same day 24 hours measurements of currents were done with ADCP.

Sediment traps

During the field campaign in October 2005 sediment traps were deployed for 24 hours on two different depths: 5m and 0.80 above the bottom (Figure 8). The dimensions of the trap were 7 cm (diameter) x 50 cm (height). From the content total suspended matter, organic C and N were measured. During the field campaigns in April and July 2006 the sampling locations were different (Figure 14).

4.4. Models used and their parameterization

We were not involved in WP but sent our results to partners.

4.5. Results. (Give tables of bulk/data model outputs electronically and/or in appendices. Here only give highlights and summary information)

Sediment:

Redox:

Redox potential was measured only during July 2006. Results show large deviation of redox values just below the center of the fish cage (F0CC) compared to the other locations that had similar values (Figure 18).

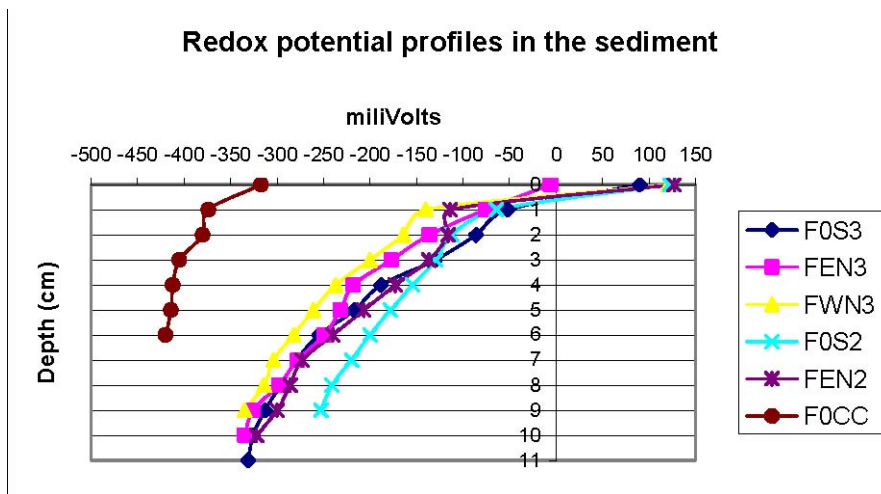


Fig. 18. Redox potential below the fish cage and at locations 20 m and 100 m from farm

Sedimentation rates, TOC and TN in sediments and historical organic carbon content

Sedimentation rates (expressed as N, P and C) at study site and reference locations in the zone B and C for spring and summer periods are presented in Fig 19 and there was no any clear relation to fish farm.

On the contrary, there was a very clear trend of enrichment in the organic carbon content in the sediment below fish cage (Fig. 20): from rather low contents in the layer 10-8 cm below surface to a significantly higher content in the surficial (2-0 cm) layer. Estimated sedimentation rate in the Bay of Piran is about 2.5-3 mm/year (Ogorelec et al. 1991). First fish farms were established at the study site in early 1980-ties and it may be assumed that organic carbon increase in recent sediment could be related to this activity.

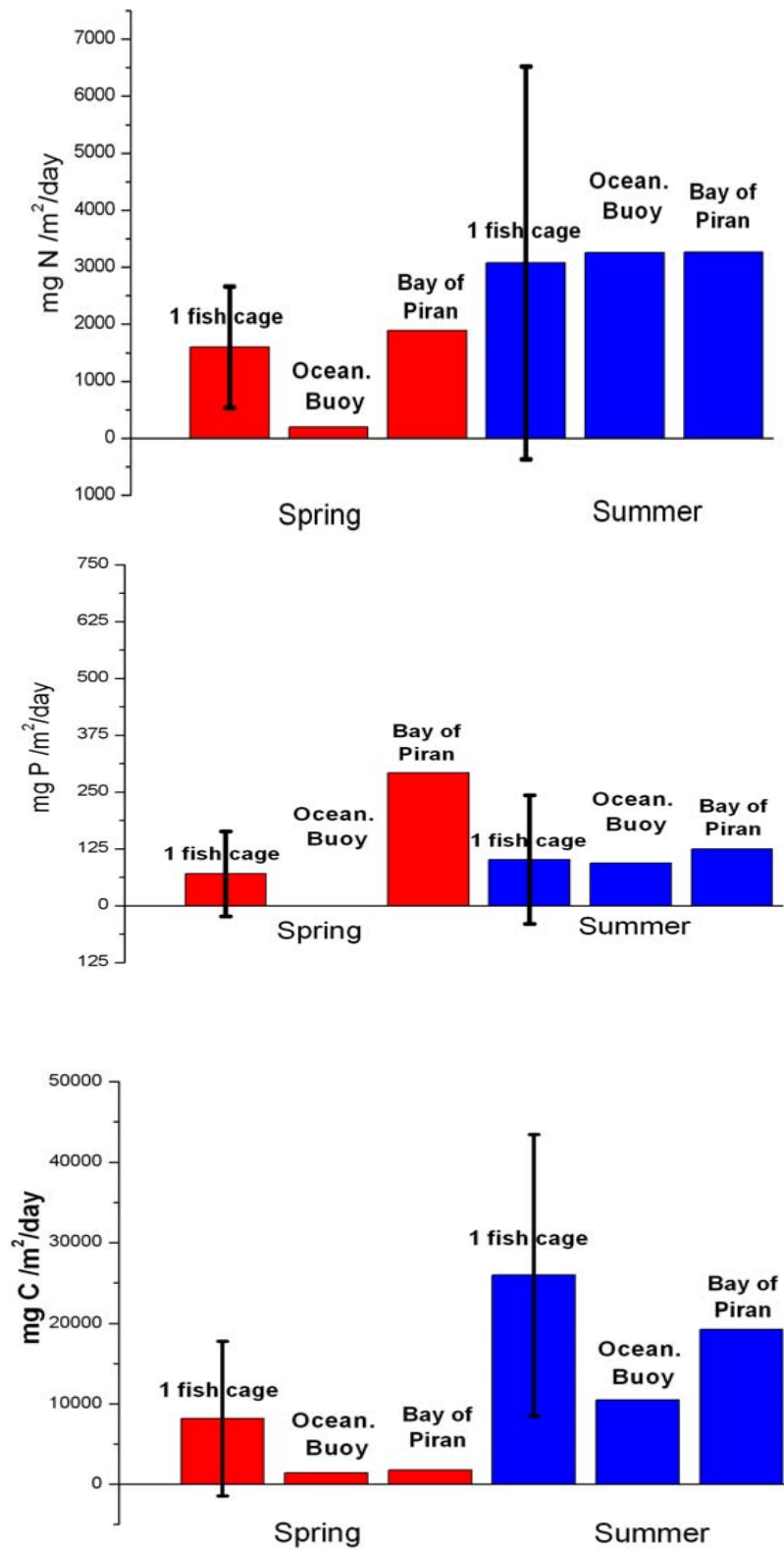


Fig. 19. Sedimentation rates expressed as N (top), P (middle) and C (bottom) at fish cage site (zone A), in zone B (Bay of Piran) and zone C (Ocean. Buoy) in spring and summer

The total organic carbon in the superficial sediment bellow the fish cages vary from 1.079 % to 2.437 % with an average of 1.473 % (Table 9). These values are far above the background values of the bay of Piran with an average of 0.988 %. The same is true for total nitrogen in these sediments where the average concentration of the total nitrogen in the superficial sediments bellow the fish cages varies from 0.067 to 0.245 % with an average of 0.116 %.

Table 9. Total organic carbon (TOC) and total nitrogen (TN) below the fish cages, zone B (MA) and zone C (OB)

| Sampling location | TOC (weight %) | | TN (weight %) | |
|--------------------------|-----------------------|---------|----------------------|---------|
| FEN1 | 1.216 | ± 0.036 | 0.077 | ± 0.002 |
| FEN2 | 1.162 | ± 0.035 | 0.086 | ± 0.003 |
| FEN3 | 1.215 | ± 0.036 | 0.067 | ± 0.002 |
| FES1 | 1.923 | ± 0.058 | 0.185 | ± 0.006 |
| FES2 | 1.079 | ± 0.032 | 0.073 | ± 0.002 |
| FOCC | 1.487 | ± 0.045 | 0.121 | ± 0.004 |
| FON1 | 1.354 | ± 0.041 | 0.107 | ± 0.003 |
| FON2 | 1.626 | ± 0.049 | 0.125 | ± 0.004 |
| FOS1 | 1.386 | ± 0.042 | 0.124 | ± 0.004 |
| FOS2 | 1.341 | ± 0.04 | 0.103 | ± 0.003 |
| FOS3 | 1.269 | ± 0.038 | 0.087 | ± 0.003 |
| FWN1 | 1.219 | ± 0.037 | 0.091 | ± 0.003 |
| FWN2 | 1.854 | ± 0.056 | 0.193 | ± 0.006 |
| FWN3 | 1.132 | ± 0.034 | 0.075 | ± 0.002 |
| FWS1 | 2.437 | ± 0.073 | 0.245 | ± 0.007 |
| FWS2 | 1.301 | ± 0.039 | 0.096 | ± 0.003 |
| Zone B MA | 1.323 | ± 0.04 | 0.106 | ± 0.003 |
| Zone C OB | 0.652 | ± 0.02 | 0.04 | ± 0.001 |

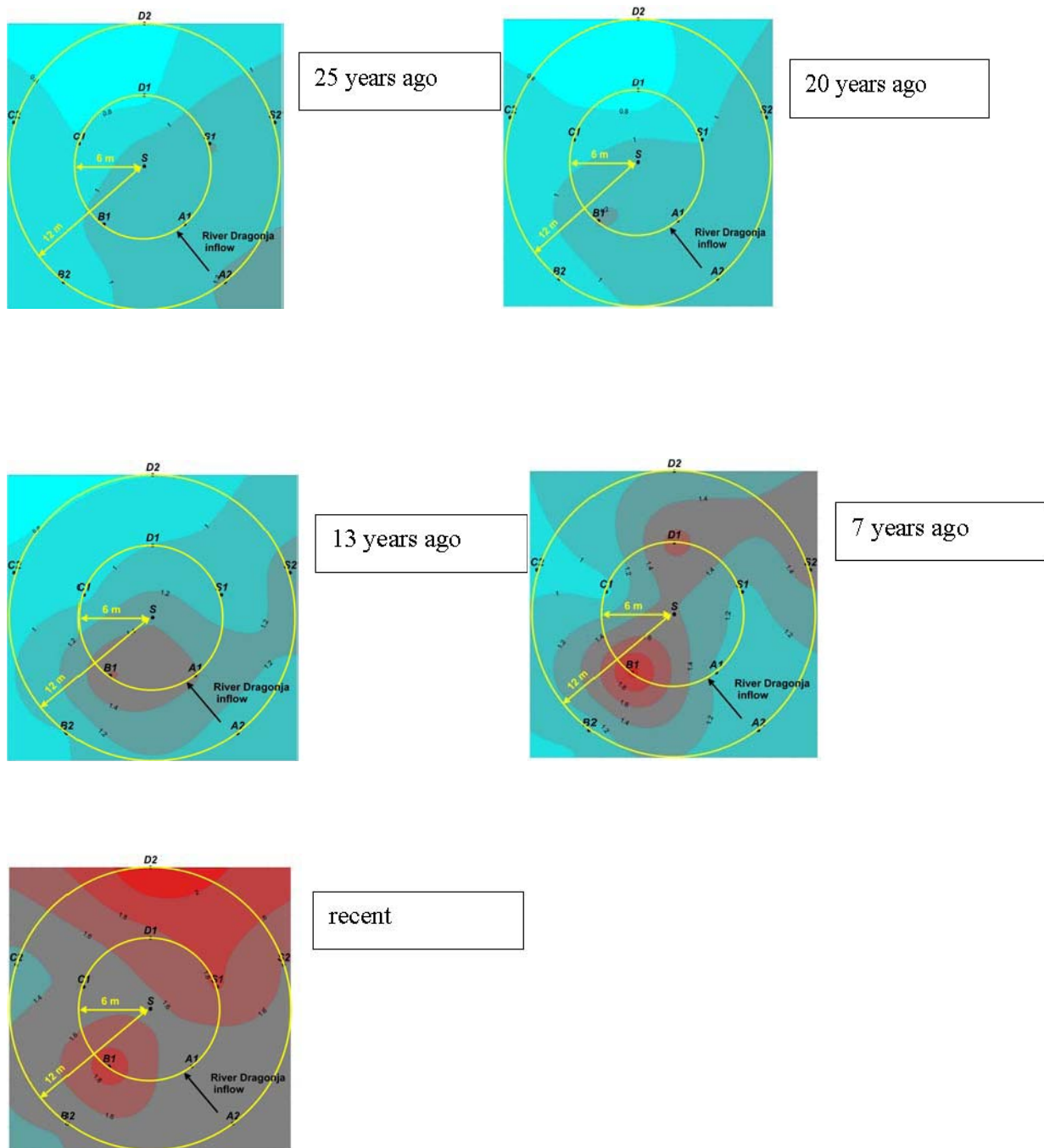


Fig. 20. Concentrations of organic carbon in the sediment cores at location below fish cage: 10-8 cm, top left (≈ 25 years ago), 8-6 cm, top right (≈ 20 years ago), 6-4 cm, middle left (≈ 13 years ago), 4-2 cm, middle right (≈ 7 years ago), and 2-0 cm, bottom (recent)

Microphytobenthos

Concentration of chlorophyll *a* and phaeopigments of the microphytobenthos followed the same spatial trend but differed in two seasons (Fig. 21) being higher in the inner circle (6 m from the center of fish cage) in April compared to July (Fig. 22).

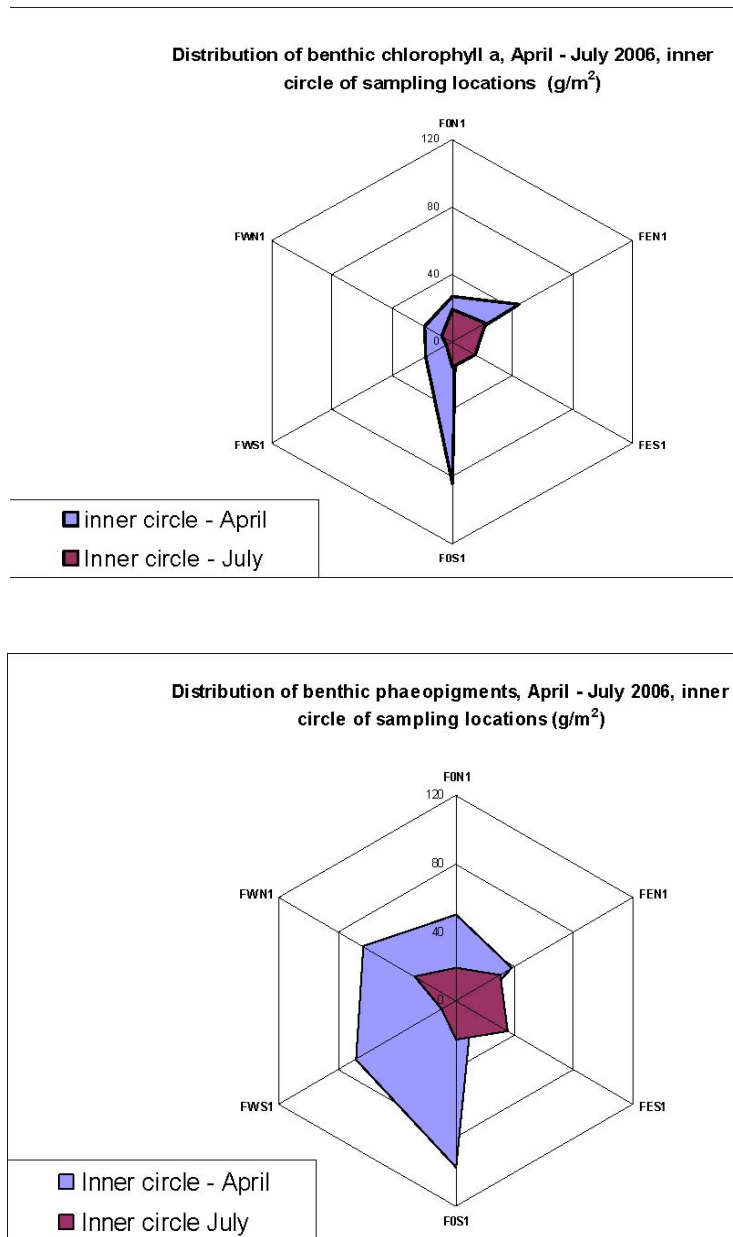


Fig. 21. Spatial distribution of microphytobenthic Chl *a* and phaeopigments

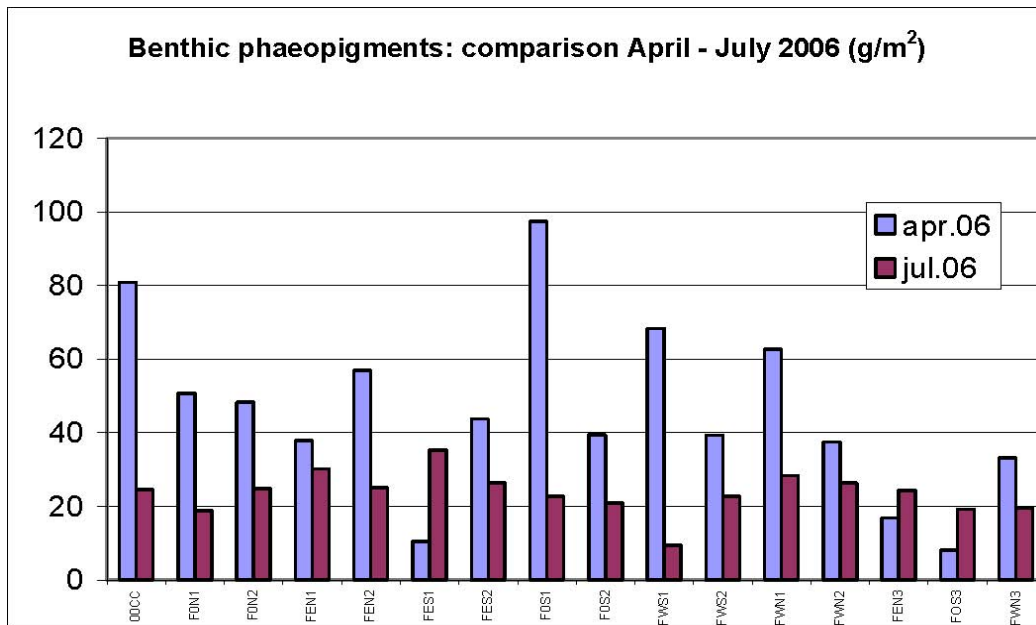
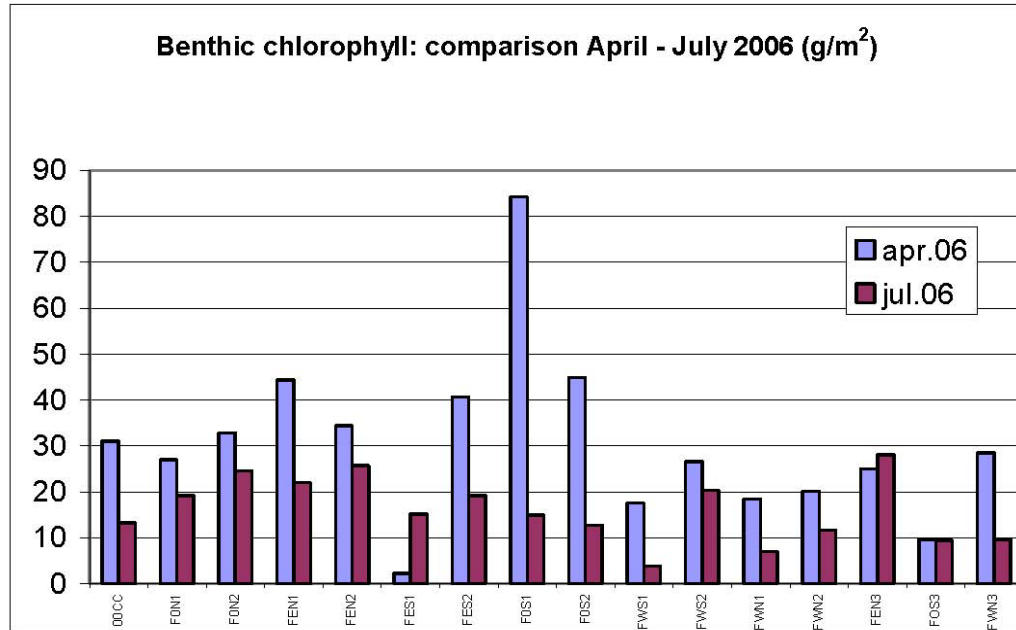


Fig. 22. Seasonal difference in concentrations microphytobenthic Chl *a* and phaeopigments

Meiofauna

Meiofauna composition was sampled along three transects (directions NE, NW and S) from the center of the fish cage (0 m, 6 m, 20 m, to 100 m distance from the cages in each transect). Triplicate samples were taken. This sampling strategy was applied in April 2006 and July 2006 to analyse possible seasonal differences.

A first clear result indicated a reduced number of taxa in the vicinity of the cages (0m and 6m), illustrating a negative impact on meiofauna biodiversity (Figure 23).

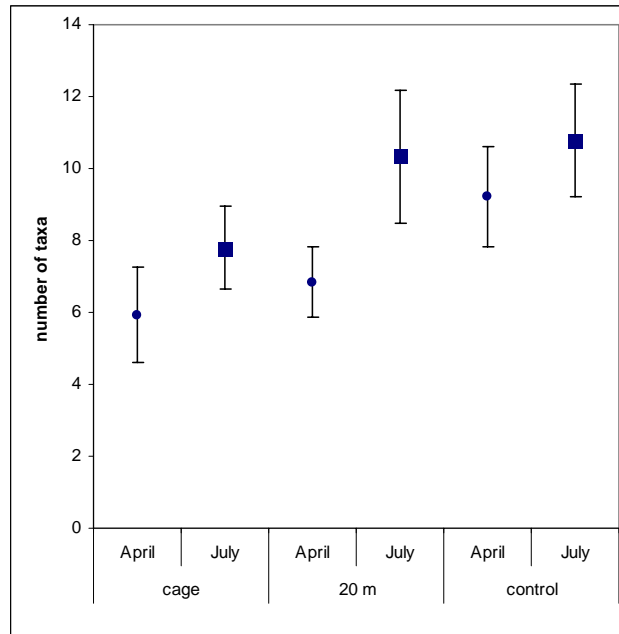


Fig. 23. The trendline of the number of taxa in relation to the distance from the fish farm

In terms of meiofauna main taxa densities, a nMDS analysis showed three groups (Fig. 24): group 1 consisting of samples 20 to 100 m away of cages (samples from both April and July - lower left part of the Fig. 24); group 2 comprised samples under the fish farm (0-6 m) in April, and group 3 comprising samples under the fish farm (0-6 m) in July. The differences in samples under the fish farm between April and July can be explained by a significantly higher feeding frequency in July. The 100 m samples, representing the 'natural environment' (background values) showed no difference between seasons.

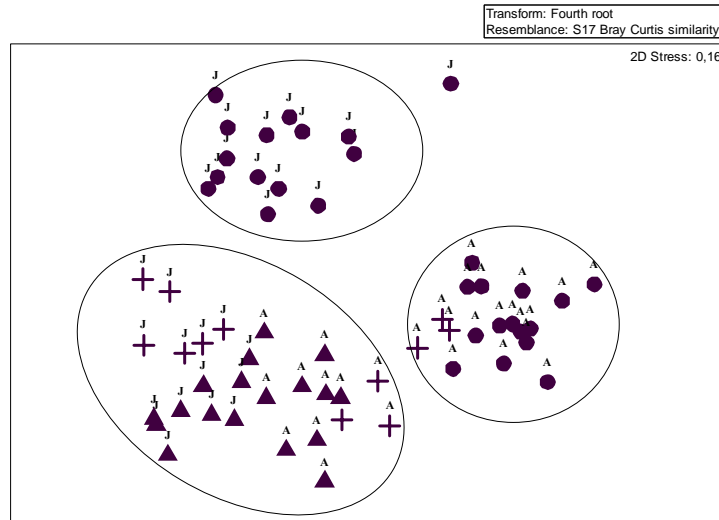


Fig. 24. The non-metric Multi Dimensional Scaling (nMDS). A= April, J= July

The meiofauna taxa which contribute most to the difference between the samples are the kinorhynchs and the harpacticoid copepods (SIMPER analysis) and their abundance is increasing in the direction away from cages. A two-way ANOVA showed that harpacticoid copepods are the group where the distance from the cage has a statistically significant impact ($p=0,00004$) whereas the season has not ($p=0,32946$). Therefore, harpacticoid copepods can be set forward as suitable indicators for possible effects of fish farming, regardless of the season. In contrast there was clear seasonality in abundance of kinorhynchs that show a general density increase with increasing distance away from the cage.

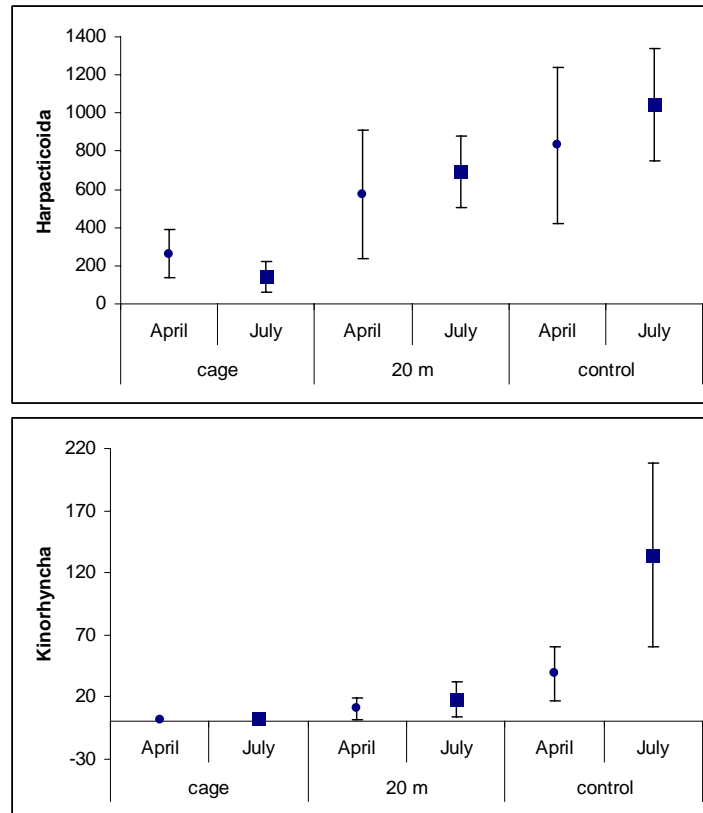


Fig. 25. Abundance of Harpacticoid copepods and Kinorhynchs in relation to the distance from the fish farm

Water column

Bioassay study

The results of bioassay experiment for phytoplankton biomass expressed as Chlorophyll *a* (Chl *a*) and TSS are presented in Table 9. Phytoplankton biomass was significant higher (Table 10) in the filtered sea water in dialysis bags incubated in the vicinity of fish cages. The results from other stations were for 50% lower but comparable along transect. The chlorophyll *a* concentrations incubated in filtered sea water showed similar significant decrease (Table 11) with distance from the fish cage towards the reference station. At all locations the chlorophyll *a* and total suspended solids were lower in dialyses bags with pre-filtered sea water, probably because of the dominance of smaller cells and insignificant influence of grazers.

Table 10. Bioassay experiment: chlorophyll *a*, phaeopigments and total suspended solids (TSS) means and standard deviations obtained for filtered (FSW) and non filtered (SW) seawater at the sampling stations located near (Cages), 50 meters (50mt), 100 meters (50mt), and 500meters (REF) from the fish cages.

| Parameter | | Cages | 50 m | 100 m | REF |
|-------------------------------------------|-----|------------------|------------------|------------------|------------------|
| | | mean \pm sd | mean \pm sd | mean \pm sd | mean \pm sd |
| Chl <i>a</i> (μg/l) | FSW | 1.11 \pm 0.55 | 0.51 \pm 0.22 | 0.60 \pm 0.11 | 0.42 \pm 0.14 |
| | SW | 1.61 \pm 0.57 | 0.62 \pm 0.23 | 0.74 \pm 0.10 | 0.46 \pm 0.19 |
| Phaeopigment (μg/l) | FSW | 0.55 \pm 0.13 | 0.34 \pm 0.17 | 0.38 \pm 0.07 | 0.29 \pm 0.09 |
| | SW | 0.77 \pm 0.27 | 0.44 \pm 0.17 | 0.36 \pm 0.02 | 0.25 \pm 0.07 |
| TSS (mg/l) | FSW | 34.48 \pm 5.00 | 30.25 \pm 0.51 | 30.09 \pm 1.70 | 31.54 \pm 0.64 |
| | SW | 33.25 \pm 4.76 | 30.33 \pm 0.35 | 33.24 \pm 3.14 | 30.19 \pm 1.13 |

Table 11. The results of two way ANOVA applied to the Chlorophyll *a*, Phaeopigments and Total suspended solids (TSS) transformed data in relation to distance and type of incubated seawaters in bioassay study.

| | Df | Sum Sq | Mean Sq | F value | p |
|-----------------------------|----|---------|---------|---------|----------|
| Chlorophyll <i>a</i> | | | | | |
| Distance | 3 | 1.0191 | 0.3397 | 14.737 | 0.000012 |
| Type_of_water | 1 | 0.0809 | 0.0809 | 3.511 | 0.073180 |
| Distance:Type_of_water | 3 | 0.0216 | 0.0072 | 0.313 | 0.815450 |
| Residuals | 24 | 0.5531 | 0.0230 | | |
| Phaeopigments | | | | | |
| Distance | 3 | 0.6128 | 0.2042 | 10.098 | 0.000172 |
| Type_of_water | 1 | 0.0211 | 0.0211 | 1.046 | 0.316525 |
| Distance:Type_of_water | 3 | 0.0563 | 0.0187 | 0.928 | 0.442356 |
| Residuals | 24 | 0.4854 | 0.0202 | | |
| TSS | | | | | |
| Distance | 3 | 54.988 | 18.329 | 2.155 | 0.122200 |
| Type_of_water | 1 | 0.209 | 0.209 | 0.024 | 0.876800 |
| Distance:Type_of_water | 3 | 26.283 | 8.761 | 1.030 | 0.398600 |
| Residuals | 22 | 187.114 | 8.505 | | |

Nutrients, phytoplankton pigments and heterotrophic bacteria

Summary results of total suspended matter, nutrients, chlorophyll *a*, bacterial biomass and production along the gradient from center of fish cage to zone B (200 m) are given in Table 12.

Table 12. Means \pm standard deviations of the results for nutrients, phytoplankton (chlorophyll *a*), bacterial biomass and bacterial carbon production rates (BCP) for each parameter according to distance from the fish cage.

| | Cages mean | 6 m mean \pm sd | 20 m mean \pm sd | 200 m mean \pm sd |
|------------------------------------|----------------------|-----------------------------|------------------------------|-------------------------------|
| TSS (mg/l) | 2.40 | 2.12 \pm 0.56 | 2.55 \pm 1.79 | 2.09 \pm 0.35 |
| Nitrate (μ mol/l) | 0.95 | 0.33 \pm 0.07 | 0.34 \pm 0.12 | 0.38 \pm 0.18 |
| Ammonium (μ mol/l) | 0.68 | 0.59 \pm 0.12 | 0.57 \pm 0.04 | 0.82 \pm 0.28 |
| TotN (μ mol/l) | 31.13 | 33.80 \pm 4.86 | 31.72 \pm 3.92 | 42.46 \pm 9.20 |
| Orthophosphate(μ mol/l) | 0.12 | 0.10 \pm 0.01 | 0.11 \pm 0.04 | 0.11 \pm 0.01 |
| TotP (μ mol/l) | 0.32 | 0.29 \pm 0.02 | 0.30 \pm 0.04 | 0.29 \pm 0.06 |
| Chlorophyll <i>a</i> (μ g /l) | 1.06 | 1.23 \pm 0.12 | 1.23 \pm 0.15 | 1.20 \pm 0.28 |
| Bacterial biomass (μ gC/l) | 43.95 | 53.21 \pm 12.33 | 44.15 \pm 5.62 | 37.38 \pm 10.34 |
| BCP (μ gC/l/day) | 10.31 | 9.27 \pm 2.72 | 10.89 \pm 2.26 | 1.65 \pm 1.08 |

Results of chemical and biological analyses of seawater collected simultaneously at all sampling locations (in the center of cage – 0 m, 6 m, 20 m and 200 m away in four different directions: S, N, E, W) showed significant difference in several parameters (concentrations of orthophosphate, total phosphate, ammonium and bacterial carbon production) before and after feeding period (Table 13) while distance from the cage (20 m) was significant only for Chlorophyll *a* values

Table 13: Statistical evaluation (three way ANOVA) of nutrients (orthophosphate **P-PO₄**, total phosphate **TotP**, ammonium - **N-NH₄**) chlorophyll *a* (**Chl *a***) and bacterial carbon production - **BCP**) before and after feeding), distance (0m, 6m, 20m) and different directions (South, North, East, West) from the cages

| Parameters/treatment | Df | Sum Sq | Mean Sq | F value | <i>P</i> |
|----------------------------|----|---------|---------|---------|----------|
| P-PO₄ | | | | | |
| Before/After feeding | 1 | 1.917 | 1.917 | 25.090 | 0.0001 |
| Distance (0-20m) | 2 | 0.066 | 0.033 | 0.435 | 0.655 |
| Direction | 1 | 0.000 | 0.000 | 0.004 | 0.950 |
| Feeding:Distance | 2 | 0.106 | 0.053 | 0.696 | 0.513 |
| Feeding:Direction | 1 | 0.050 | 0.050 | 0.649 | 0.432 |
| Distance:Direction | 1 | 0.108 | 0.108 | 1.413 | 0.252 |
| Feeding:Distance:Direction | 1 | 0.062 | 0.062 | 0.814 | 0.380 |
| Residuals | 16 | 1.223 | 0.076 | | |
| TotP | | | | | |
| Before/After feeding | 1 | 0.2545 | 0.2545 | 37.0805 | 0.0000 |
| Distance (0-20m) | 2 | 0.0002 | 0.0001 | 0.0134 | 0.9867 |
| Direction | 1 | 0.0000 | 0.0000 | 0.0034 | 0.9543 |
| Feeding:Distance | 2 | 0.0143 | 0.0071 | 1.0413 | 0.3757 |
| Feeding:Direction | 1 | 0.0145 | 0.0145 | 2.1172 | 0.1650 |
| Distance:Direction | 1 | 0.0001 | 0.0001 | 0.0103 | 0.9206 |
| Feeding:Distance:Direction | 1 | 0.0009 | 0.0009 | 0.1320 | 0.7211 |
| Residuals | 16 | 0.1098 | 0.0069 | | |
| N-NH₄ | | | | | |
| Before/After feeding | 1 | 0.2489 | 0.2489 | 8.6114 | 0.0097 |
| Distance (0-20m) | 2 | 0.0505 | 0.0252 | 0.8730 | 0.4367 |
| Direction | 1 | 0.0944 | 0.0944 | 3.2652 | 0.0896 |
| Feeding:Distance | 2 | 0.0329 | 0.0165 | 0.5695 | 0.5769 |
| Feeding:Direction | 1 | 0.0460 | 0.0460 | 1.5918 | 0.2252 |
| Distance:Direction | 1 | 0.0233 | 0.0233 | 0.8059 | 0.3827 |
| Feeding:Distance:Direction | 1 | 0.0061 | 0.0061 | 0.2093 | 0.6535 |
| Residuals | 16 | 0.4624 | 0.0289 | | |
| Chl <i>a</i> | | | | | |
| Before/After feeding | 1 | 0.0071 | 0.0071 | 0.8237 | 0.3776 |
| Distance (0-20m) | 2 | 0.2274 | 0.1137 | 13.2654 | 0.0004 |
| Direction | 1 | 0.0108 | 0.0108 | 1.2632 | 0.2776 |
| Feeding:Distance | 2 | 0.0712 | 0.0356 | 4.1548 | 0.0352 |
| Feeding:Direction | 1 | 0.0100 | 0.0100 | 1.1690 | 0.2956 |
| Distance:Direction | 1 | 0.0002 | 0.0002 | 0.0244 | 0.8779 |
| Feeding:Distance:Direction | 1 | 0.0266 | 0.0266 | 3.1004 | 0.0974 |
| Residuals | 16 | 0.1371 | 0.0086 | | |
| BCP | | | | | |
| Before/After feeding | 1 | 143.115 | 143.115 | 18.805 | 0.001 |
| Distance (0-20m) | 2 | 7.718 | 3.859 | 0.507 | 0.612 |
| Direction | 1 | 2.822 | 2.822 | 0.371 | 0.551 |
| Feeding:Distance | 2 | 18.768 | 9.384 | 1.233 | 0.318 |
| Feeding:Direction | 1 | 28.493 | 28.493 | 3.744 | 0.071 |
| Distance:Direction | 1 | 4.192 | 4.192 | 0.551 | 0.469 |
| Feeding:Distance:Direction | 1 | 0.128 | 0.128 | 0.017 | 0.899 |
| Residuals | 16 | 121.767 | 7.610 | | |

Heterotrophic bacteria

Bacterial abundance

In the vicinity of the fish cage the abundance of heterotrophic bacteria was 2-times higher ($2,4-3,1 \times 10^9$ cells L⁻¹) compared to the measured abundances at the sampling station, located about 1500 m (ZBMA) away from the center of the fish cage ($1,4 \times 10^9$ cells L⁻¹) and 2,5 times higher compared to numbers measured at the reference station ZCOB ($1,1 \times 10^9$ cells L⁻¹). Figure 33 shows that significantly elevated values of measured bacterial abundance in the vicinity of the fishery decrease with the distance from the origin of pollution.

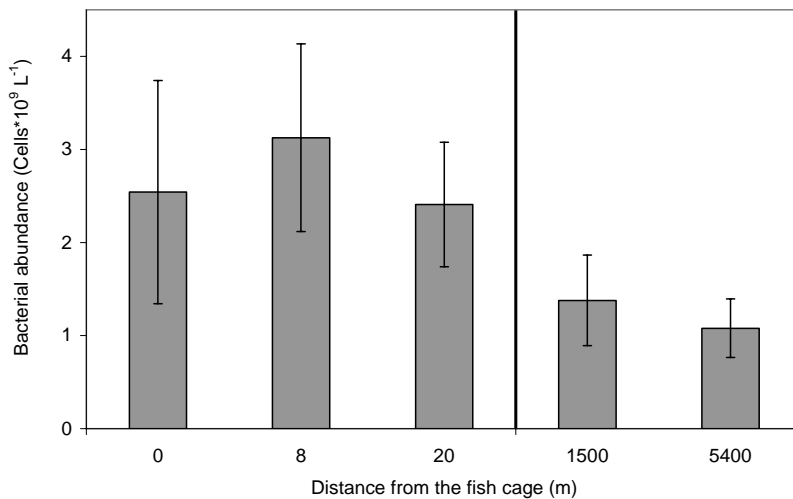


Fig. 26. Bacterial abundance along the transect from the center of fish cage in October 2005

Bacterial Carbon Production

In the vicinity of the fish cage bacterial production was on average up to 7times higher ($7-10,8 \mu\text{g C L}^{-1} \text{ day}^{-1}$) compared to values measured at the sampling station, located 1500 m away ($1,3 \mu\text{g C L}^{-1} \text{ day}^{-1}$), and 3times higher compared to bacterial production measured at reference station ($3,1 \mu\text{g C L}^{-1} \text{ day}^{-1}$) (Fig. 34). Bacterial biomass was calculated from bacterial abundance using conversion factor 19,8 fg carbon per bacterial cell (Lee & Fuhrman, 1987). In the vicinity of the fish cage calculated ratio between bacterial production and biomass (P/B) was on average up to 4,5times higher compared to calculated P/B ratio at 1500 m distant sampling station and 1,5times higher compared to calculated values at for the reference station. Higher P/B ratio reflects higher daily biomass production, which could be a consequence of the more intensive bacterial metabolic process around the fish cage.

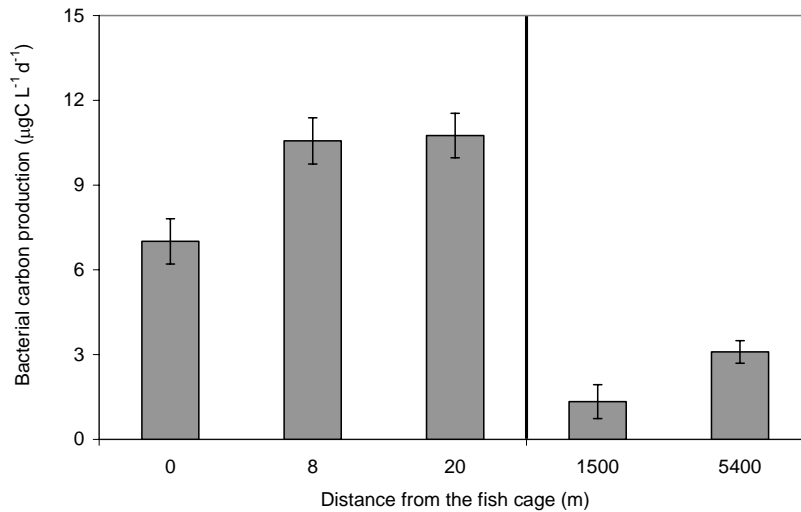


Fig. 27. Bacterial C production along the transect from the center of fish cage in October 2005

Differences of the bacterial abundance and production before and after feeding the fish

The bacterial abundance and carbon production were measured before and three hours after feeding the fish. The results for each parameter were expressed as a ratio ($K = C_{bf}/C_{af}$) between the values before and after feeding. The results in the (Figures 35, 36) are presented as the percentage of either an increase or decrease of the particular parameter. The size of the circle gives values, red colour represents the increase of the measured values and blue colour represents the decrease.

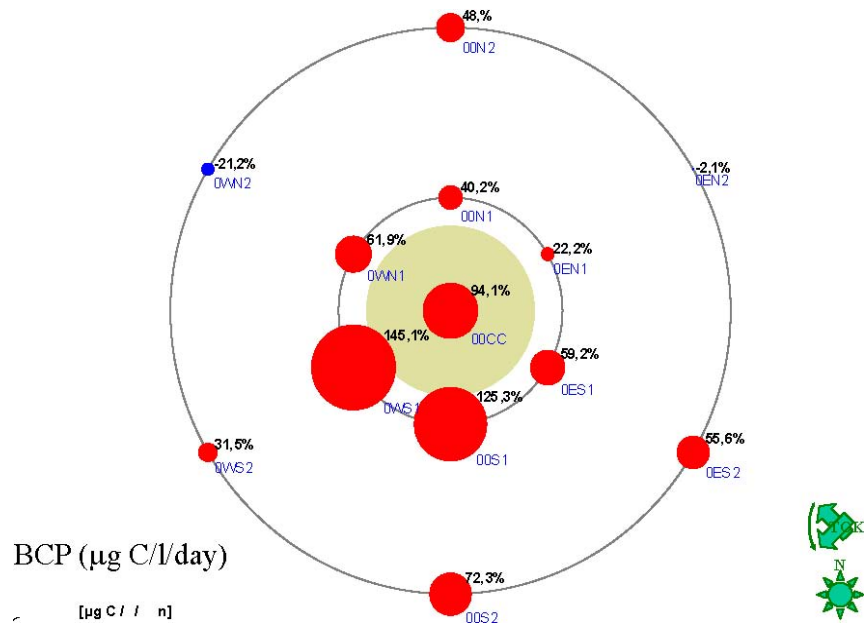


Fig. 28. Temporal differences in the bacterial carbon production (BCP) in the time period between the feeding of the fish and three hours after the feeding in the vicinity of the fish cage (size of the circle shows the increase/decrease of the value, red colour means increase/blue colour means decrease)

According to our evaluation (historical data, results of field study and pre-set criteria) the best indicators of impacts of aquaculture on environment are: redox, meiofauna, sediment organic carbon and nitrogen followed by pore water ammonium concentration, and macrofauna.

4.7. Evaluation of model performance

We were not involved in WP but sent our results to partners.

4.8. Site specific conclusions

The ECASA field study in 2005-2006 and the previous surveys in the area indicated an impact of fish farm on the water column parameters only in limited distance from fish cages (< 10 m), while at 20 m distance effects were generally not detectable. Similarly, enrichment effect was observed near fish cages in bioassay experiment but not at distance of 50 m. One reason for weak gradients in trophic state from fish cages to control locations could be proximity of river Dragonja discharge (at distance < 2 km from farm) which is an important local source of nutrients. We found, however, significant differences for most pelagic parameters (Table 13) before and after feeding the fish indicating short-term impacts on water column. More clear were the impacts in the sediment that were detectable also at 20 m distance from fish cages. Parameters that showed clear deviations from background values were redox, meiofauna structure and abundance, total organic carbon and total nitrogen.

4.9. Culture type and environment type conclusions

5. Acknowledgements

6. References

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Appendices

Appendix 1 Details of all methods used (indicators, models, etc)

Appendix 2 Environmental data