Project no. 512789

Project acronym: ALFA

Project title: Development of an automated innovative system for the continuous live feed production aquaculture hatchery units

Instrument: Co-operative Research Projects

Thematic Priority: Food quality and safety

Combined first and second activity reports for ALFA 512789

Period covered: 01 June 2005 to 30 November 2007
Date of preparation: 30th of November 2007

Start date of project: 1st of June 2005 Duration: 30 months

Project coordinator name: Patrick White
Project coordinator organisation name: Akvaplan-niva
Revision [draft]

1. Project execution

Project summary

Live food production in intensive marine fish hatcheries comprises of repetitive manual tasks that could be performed by specialised automated equipment. High use of manpower for repetitive work may result in human errors, differences in methodology, and poor efficiency of the unit. Such problems may affect the productivity of the hatchery with respect to both quantity and quality of live feed and fry. The project developed an innovative fully automated system for the continuous production of microalgae as live feed in aquaculture hatcheries. Two systems were developed, an large bore coil pipe system that used solar light energy and a large bore tube system using artificial light. This photobioreactors were integrated into the hatchery by a distribution supply of live algae to rotifer tanks, larvae tanks and a harvesting and temporary storage facility. The photobioreactors were automatically controlled to ensure optimal microclimatic and nutritional conditions for the stable growth of algae by using both natural and artificial illumination and controlling the temperature, the nutrient content, the pH and the CO2 concentration of the water.

Two systems were developed to be locally adapted for the culture of live feed in Northern Europe where there is low solar light using artificial light source and in Southern Europe where there is high solar light availability so that the photobioreactors are suitable for the range of cold, temperate and tropic waters. A novel optical algal density monitoring system based on colour image analysis techniques was developed for continuously assessing the algal density which allowed control of the growth rate and the quality of the culture. Since the algae production is partially used for feeding rotifers, a secondary objective of the proposed project was to link the new continuous algae production system to the existing rotifer production systems and to a continuous rotifer production system (CROPS). Another main objective of the proposed project was to develop an automatic harvesting, distribution system for efficiently managing the continuous algae production. In this way, the production is automatically harvested and transferred to other components of the hatchery according to demand, while any surpluses could be concentrated and stored. A Concentration and Storage Unit was developed for short to medium-term storage of the live algae. This allows excess production to be temporarily stored at the hatchery. A preservation and storage trial was undertaken to assess the algal viability and quality during storage.

Details about the project can be found at the project web site www.alfa.eu.com

2. Project objectives

The production system of phytoplankton (algae) is an important component of a hatchery. Algae are used for feeding both fry and other live food (rotifers and Artemia). The project aimed at developing an intensive, fully controlled continuous algae production system, to reduce the cost of algae production and improve its quality.

A second objective of this research was to integrate the algae bioreactor into other hatchery production modules by the developing an automated, reliable, and cost effective harvesting and transferring system, temporary storage and distribution system. This allowed automatic monitoring and control of the live feed production for intensive marine fish hatcheries in Mediterranean and Northern Europe. By automatically controlling density and increasing the volume of production, algae can be grown at the log phase of growth which provdes hatchery operators with live food of higher quality and at a lower cost in comparison to traditional techniques. (e.g. effective system for algae production and subsequently for the live feed production components depending on it).

Specifically, the objectives were the;

1 Development of photobioreactor for the continuous production of algae for aquaculture hatcheries taking into account locally adapted strains and local climatic conditions.
2 Integration of the algae reactor with continuous rotifer production systems in the hatcheries.
3 Development collection and storage unit for short to medium-term conservation of the live algae so that the excess production can be stored or sold to smaller hatcheries.
4 Development of a computerised transfer system for algae to the other components of the hatchery and/or the storage unit.

Main findings of the project

WP 1

In the first workpackage a baseline case model was developed for the current hatchery operation against which to compare any proposed technological modifications and a model was built for the best designed process using the new technology. The model was then used to evaluate the technology that was developed to allow optimization, assess the benefits based on the correct decision criteria.

Two full scale photobioreactors were designed, built and operated to determine the capital and operational cost and productivity. These results were then compared to the industry standard system the BioFence.

Boa bioreactor

The system is used to grow Nannochloropsis s.p.. The harvested capacity of the system at steady state is 85 liters per day (24 hours) at a concentration of 300 million Nannochloropsis cells per milliliter. This is a total of 25.5 trillion cells/day. The cost of the main unit constructed and installed by Hessy is 9,462 €. The control and automation cost 3500 €

Tubular bioreactor

The system is used to grow Nannochloropsis s.p.. The harvested capacity of the system at steady state is 83 liters per day (24 hours) at a concentration of 330 million Nannochloropsis cells per milliliter. This is a total of 27.39 trillion cells/day. The cost of the main unit constructed and installed by Hessy is 13,035 €. The control and automation cost 3500 €

The table that follows summarizes the economic evaluations of the BOA and Tubular commercial systems and compares them with the benchmark model (BioFence).

Systems under SUNLIGHT	**Production Cost of trillion cell of Nannochloropsis s.p**
BOA	2.00 €
BioFence	2.18 €

Systems under ARTIFICIAL LIGHT	**Production Cost of trillion cell of Nannochloropsis s.p**
Tubular	1.87 €
BioFence	3.63 €

The above show clearly that the two newly developed photobioreactors (BOA and Tubular) over-perform the benchmark current technology.

WP 2

In the second workpackage, four designs were developed for the Continuous Algae Production (CAP) with particular reference to materials and structure, energy saving, reliability and quality of the output. Each prototype had different characteristics and tested production efficiency in order to determine the optimal design to be chosen for development.

Design 1 – Large bore tube photobioreactor with internal paddles to ensure optimal capture of artificial light.
Design 2 – Small bore pipe photobioreactor with rapid light/dark phases angled optimally towards the sun.
Design 3 – Medium bore pipe photobioreactor in a spiral to capture sunlight using air as the carbon source.
Design 4 – Large sac photobioreactor in a triangle to capture sunlight and utilise artificial light efficiently.

The four systems were built in order to explore various aspects of a photo-bio-reactor, such as mixing rate in the reactor, mixing of air to the liquid, light-dark periodicity effect. Based on the productivity of the four prototypes 2 were chosen (Design 1 and 2) to be developed further and full scale systems built.

At the same time a novel algal density sensor was developed using light at the specific frequency that is absorbed by chlorophyll. This was tested and a prototype installed on the 2 full-scale prototype photobioreactors. This allowed a way of maintaining optimal algal density in the photobioreactor as a feedback mechanism for the control of quality and yield.

WP 3

In workpackage three, a review of continuous rotifer production systems was undertaken. Based on this, a continuous rotifer production system (CROPS) was developed and tested at one of the SME partner hatcheries initially using algae paste. The rotifer production system was linked to the photobioreactor and optimized by continuous supply of live algae to maintain optimal algae levels. In the second reporting period the project developed a Computer controlled Harvesting, Transferring and Packaging (CHTP) system for the algae production. New techniques were developed for concentration of excess production using a protein skimmer and a preservalion trial was undertaken using a number of preservatives, plastic bag type and temperature conditions to test the changes in quality and viability during short or medium-term preservation.

WP 4

The fourth workpackage aimed at optimising and evaluating the CAP and CHTP systems. Two full scale systems were constructed at different locations in Europe (Greece and Norway) corresponding to the extreme climatic zones and thefor cold and temporate aquaculture species. Evaluation of the operation of these systems were undertaken at full-scale and the new technology adapted to the local conditions and requirements. Thgis data was fed back into the stochastic model for analysis and comparison.

3. Contractor list

Partic. role	Partic. Type	Partic. No.	Participant name	Participant short name	Country	Date enter	Date exit
CR	SMEP	1	SagaFjord	SF	(NO)	Month 1	Month 30
CR	SMEP	2	Argosaronikos Fish Farms	SARONIC	(EL)	Month 1	Month 30
CR	SMEP	3	Viveireo Vila Nova	VILANOVA	(P)	Month 1	Month 24
CR	SMEP	4	Sagro Aquaculture	SAGRO	(CY)	Month 1	Month 30
CR	SMEP	5	HESY Bergambacht BV	HESY	(NL)	Month 1	Month 30
CO	RTD	6	Akvaplan-niva	APN	(NO)	Month 1	Month 30
CR	RTD	7	Agricultural University of Athens	AUA	(EL)	Month 1	Month 30
CR	RTD	8	Computer Technology Institute	CTI	(EL)	Month 1	Month 30
CR	RTD	9	University of Bergen	DFMB	(NO)	Month 1	Month 30

3. Project coordinator

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4. Summary of work performed and results achieved

In the first reporting peeriod, the live food production process in three of the SME hatcheries were analysed in depth for inputs, outputs, costs, quality, etc. The algae requirements were analysed for greening of tanks and feeding and enrichment of rotifers. From this data, the typical algae and rotifer requirement was analysed for a hatchery producing 10 million fry per year for seabass, seabream and cod.

A review of the biosecurity issues was undertaken to identify potential sources of contamination and find possible solutions to minimize potential contamination. An in depth review of all the present technologies used for continuous algal production (and technologies from other industries) was undertaken. In this way, the problems related with the current production methods and any bottlenecks in production were identified. A baseline stochastic simulation was made of present production methods so that impact of the new photobioreactor technology that were introduced through the other three workpackages could be assessed and measured.

In the first workpackage a baseline case model was developed for the current hatchery operation against which to compare any proposed technological modifications and a model was built for the best designed process using the new technology. This was undertaken during the first reporting period. This model has been developed, which yields operating data for a realistic but efficient hatchery operating with existing technology. This baseline case was continuously improved with ongoing laboratory and large-scale experiments and served as a benchmark model to:

- Compared the model against the performance of the two new designs.
- Conducted parametric investigation on the factors affecting the performance of the process
- Provided a tool for improving the design.

The model was then used to evaluate the technology that was developed to allow optimization, assess the benefits based on the correct decision criteria.

In the second workpackage, four designs were developed for the Continuous Algae Production (CAP) with particular reference to materials and structure, energy saving, reliability and quality of the output. Each prototype had different characteristics and tested production efficiency in order to determine the optimal design to be chosen for development.

Design 1 – Large bore tube photobioreactor with internal paddles to ensure optimal capture of artificial light.

The first system was a tubular bioreactor 30cm in diameter and 1.5m high. A variable speed motor controls the agitation speed. A series of curved blades force the liquid to move radially from the center of the tube to the wall and back. The reactor is lighted from the outside through a series of fluorescent lamps. The radial movement of the liquid caused by the curved agitation blades forces the liquid to reach the lighted wall, absorb the photons needed and return toward the center of the tube (dark phase) to complete the photosynthesis. Air is introduced at the bottom of the reactor through two submersed distributors. This bioreactor design gears to maximize the efficiency of capturing the available light and of maximizing the release/adsorption of the CO_2/O_2 of the liquid. The desin of the bioreactor was intended to improve algae productivity by improving the amount of photons captured and enhancing the breathing rate of the liquid.
Design 2 – Small bore pipe photobioreactor with rapid light/dark phases angled optimally towards the sun.

The second system, a 30 liter photo-bioreactor was built for use under outdoor (solar light) or indoor conditions. It consists of 40 tubes of 14mm inner diameter made of transparent PVC. The length of the tubes is 1 meter. Their total volume is 5.5 liters. The orientation of the solar collector was selected South and its inclination was set to 52^o, which is optimal for mid-winter conditions in Athens, Greece. The optimization of the light interception was achieved by creating a solar collector like structure made of thin tubes, which could be oriented and shaped optimally.

Design 3 – Medium bore pipe photobioreactor in a spiral to capture sunlight using air as the carbon source.
The third system was a two-phase coil type system (210 l) suitable for the continuous production of algae under a combination of natural light and artificial light (or even under controlled conditions). This design was based on the following basic principles: (i) Achieve full exploitation of solar radiation for maximum algae production, (ii) Develop a cheap and simple system with optimised design parameters, (iii) Allow for an optimal use of space through the geometric characteristics of the design and (iv) Design a modular and versatile system that may be adapted to include artificial light (supplementary or main). The two-phase system consists of two parallel flows: a) the culture media flow forced by a pump through a large diameter pipe (100mm PVC) and a tank and b) the air flow, released through an internal smaller perforated pipe (12mm PVC) continuously to the media flow along the whole length of the coil.

Design 4 – Large sac photobioreactor in a triangle to capture sunlight and utilise artificial light efficiently.
The fourth option is a large volume low density system. It is based on the conventional plastic 200l bag batch production system, which is modified to become continuous. Moreover illumination efficiency is improved by modifying the shape of the bioreactor from cylindrical into prismatic by using a steel frame supporting a wire grid. In order to eliminate stagnant regions and stratification at the bottom, a water pump is used to pump the culture from the bottom to the top. An air pump is providing the necessary CO2 to the culture, while the air flow also contributes in better mixing. This design was based on the following basic principles: (i)

Optimise room surface exploitation, (ii) increase productivity by allowing large volume cultures, (iii) improve productivity of conventional bag based systems.

The four systems were built in order to explore various aspects of a photo-bio-reactor, such as mixing rate in the reactor, mixing of air to the liquid, light-dark periodicity effect.

At the same time a novel algal density sensor was developed using light at the specific frequency that is absorbed by chlorophyll. This was tested and a prototype installed on the prototype photobioreactor. This allowed a way of maintaining optimal algal density in the photobioreactor as a feedback mechanism for the control of quality and yield.

In workpackage three, a review of continuous rotifer production systems was undertaken during the first reporting period. Based on this a continuous rotifer production system (CROPS) was developed and tested at one of the SME partner hatcheries initially using algae paste. The rotifer production system was linked to the photobioreactor and optimized by continuous supply of live algae to maintain optimal algae levels. In the second reporting period the project developed a Computer controlled Harvesting, Transferring and Packaging (CHTP) system for the algae production. New techniques were developed for concentration of excess production using a protein skimmer and a presenvalion trial was undertaken using a number of preservatives, plastic bag type and temperature conditions to test the changes in quality and viability during short or medium-term preservation.

In the second reporting period, the fourth workpackage aimed at optimising and evaluating the CAP and CHTP systems. Two full scale systems were constructed at different locations in Europe (Greece and Norway) corresponding to the extreme climatic zones and thefor cold and temporate aquaculture species. Evaluation of the operation of these systems were undertaken at full-scale and the new technology adapted to the local conditions and requirements.

The workpackage five concerns the management of the consortium, the dissemination, exploitation, and innovation activities, and the overall assessment of the new technology with respect to its exploitation potential and was undertaken during both reporting periods.

5. Intension for use and impact on industry sector

The development of a reliable continuous algae production system for marine fish hatcheries will boost both the fast growing aquaculture industry which depends on the production of high quality fry production.

The automation of the live feed production process will allow the following improvements;

- **Reduction in repetitive work and staff required**. Many of the problems during live food production are caused by human error. Rotifers and Artemia require to be fed in regular feeds though out the day and night requiring three shifts of workers to undertake this (day, afternoon/evening and night shifts). The proposed CHTP system will contribute to a more efficient distribution of the produced algae.
- **Reduce cost of production**. By reducing the labour required for producing live feed, operational costs will be reduced.
- **Improvement in hygiene**. Present production techniques utilise open, manually managed cultures, which can easily be contaminated. The equipment that will be developed will be housed in closed systems that will reduce this risk of contamination.
- **Improved reliability**. By automating the algae production system and ensuring that the cultures are maintained in optimal growing conditions, and allow the algae to be cropped at the best growth phase for optimal quality. Moreover, the automatic harvesting and transferring system will improve the reliability of the other modules of live food production in the hatchery.

The development of a modular continuous production system adapted to differing species of algae and different geographical areas of Europe will be able to be installed into most fish fry production hatcheries. The modular design will also allow it to be installed in different sizes of hatchery.

Patentable applications

The two photobioreactors differ from other systems due to the large volume of production and integration to the other live food and fry production processes within the hatchery. Although the photobioreactor design, algae density sensor and integrated feedback control systems were potentially patentable, it was decided not to undertake the patenting process as they could be easly be modigfied and copied. It was decided that HESY Bergambacht BV (one of the participating SMEs) would construct and sell the equipment modules commercially at an affordable price and that profits would be shared between the SME consortium (all SME partners) jointly..

The proposed project involved two main technical innovations.

1 A computer controlled Continuous Algae Production (CAP) system that was developed for improving the quality and the cost efficiency of the phytoplankton production and that could be. operated under different climatic conditions (arctic to tropic). Four pilot-scale models with different concepts and design were evaluated and the 2 most promising designs were upscaled to full-scale and tested in coldwater (Norway) and temperate (Greece) conditions.

2 New sensors for monitoring the growth of algae populations were developed.

3 A third innovative aspect of the proposed project was the development of a Computerised control system for ensuring optimal production from the CAP and controlling the delivery of live algae from the CAP with the other components of the hatchery. The automated algae production control system,is based on the standard technology of industrial control systems. However, new software and electronic hardware were developed taking into consideration the corresponding biological aspects.

6. Plan for using and dissemination knowledge

Exploitation of the results

The SME committee was responsible for developing an exploitation plan, for protecting the intellectual property generated by the project and for the commercialisation of the developed system. The SME committee decided not to patent the designs but allowed the designers to patent the designs if they so wished. The SME committee’s decision was to produce the 2 full scale photobioreactor designs commercially and sell them at an affordable price. The reactors would be continuously upgraded in the light of feedback so that new improved versions could be continually produced.

Two of the SMEs are already operating full scale reactors which are fully integrated into the hatchery systems. The other participating SMEs are considering the installation of improved versions.

HESY Bergambacht BV will construct and sell the equipment modules with a royalty made available for the benefit of the consortium and a consortium agreement to this effect was signed among the participating SMEs just before the starting of the project.

HESY Bergambacht BV is already manufacturing equipment for the aquaculture industry and is in the position of manufacturing the newly developed equipment and selling these in to the aquaculture market. They attend all the major aquaculture trade shows and would exhibit the newly developed continuous live food production systems.

A promotional sales brochure was prepared (see Appendix 1) and distributed at AquaNor, the largerst aquaculture exhibition, during August 2007. There was a lot of interest and HESY has already had enquiries and is preparing to start manufacture of the commercial versions.

Dissemination of results

The dissemination of the results will follow the consortium agreement limitation to ensure intellectual property protection and lucrative exploitation of the results by the SMEs. The Universities and the Research Institutes participating in the consortium have an intense disseminating policy and they plan to publish the obtained scientific results in agreement with their partners. Beyond the publication of the results in international scientific journals or international conferences, the participating research institutes will further disseminate these results through their consultant activities and extension services towards the fish farmers (end-users). All participants are willing to co-operate with the EU organisations and they can adjust their patent policies to the EU regulations with respect to this project.

Prototype installations will be used for training purposes. There, SME personnel will have the opportunity to interact with academic researchers, so that it is trained to the new technologies. Know how transfer from research institutions towards the SMEs is another benefit expected from the proposed project.

1. IMPORTANT FINDINGS AND THEIR IMPACT ON THE SME SECTOR

WORKPACKAGE 1

Economic and operational analysis of hatcheries

AUA were the participant with the main responsibility for this work package.

Work Undertaken and improrant findings

Reviews of the state of the Art

At the start of the projects various reveiws were undertaken on various aspects of algae biology and reactor design.

Review of Photobioreactors

Marine fish fry produced in hatcheries rely heavily on live feed. During its initial life stages, the fry feeds on phytoplankton (microalgae), zooplankton (rotifers and Artemia), while only at a later stage it consumes inert feed. These live feeds have to be produced locally in separate, specially equipped installations (tanks). Microalgae are also used as a culture feed and enrichment food for the rotifers as well being used directly in the fish culture tank for the green culture technique.

At present, the majority of live food in aquaculture hatcheries is produced in a batch culture, which is managed on a daily basis in order to satisfy the requirements of the fish larvae. This batch culture yields products of high uncertainty with respect to quantity and quality. For this reason, there is a recent tendency towards the replacement of such systems with continuous production systems. Continuous live food production has the advantage of a more stable production of consistent quality. This project aims at developing a computer-controlled, continuous production system for phytoplankton and integrating this system with the other production systems of the hatchery through a modular automation system.

There are presently three types of reactors for continuous production of algae. The most widely used consists of cylindrical plastic bags made of polyethylene with a diameter of approximately 50 cm, where phytoplankton (algae) is mixed in water enriched with nutrients. The bags are exposed to natural or artificial light under controlled temperature conditions in order for the algae to achieve fast photosynthetic growth. The main disadvantage of this system is the low algal density attained due to its poor photosynthetic performance. Photosynthetically active light can only penetrate approximately 3 cm in a dense culture of algae, therefore the illuminated area of culture is low and there are large volumes where no effective photosynthesis occurs.

For this reason two other systems recently appeared in the market, where the algae-water mix is forced to circulate through a fence-shaped or a coil-shaped tube in front of natural or artificial light. This tube has a diameter of 3-5 cm. In this way photosynthesis is intensified. These systems have been developed for temperate species of algae however there are no systems that have been developed for coldwater algae species cultured at lower temperatures. In addition, in southern Europe there are problems with under and over heating of the cultures and so new technology must be developed to allow systems to work under these low or high temperature conditions.

In most hatcheries, rotifers are grown in batch culture in cylindroconical tanks where only temperature and light are controlled using a thermostat and a timer. Recently continuous

rotifer production has been developed that allows higher celldensity and more consisten production.The project will build on the experience of the CRAFT project with the title "Development of a recirculation system for the high-density rotifer culture on commercial scale" to further develop a continuouse rotifer production system (CROPS) that will be fed with live algae from the photobioreactor. These two systems will be integrated with feedback monitoring and control of microalgae cell density to maintain optimal concentrations.

The condition of the algae and rotifer culture is monitored visually. The survival of this virtual "ecosystem", which consists of the mircoralgae, rotifers, and fish larvae grown in different tanks, depends on the timely transferring of correct quantities among the separate independent components. Transfer of live feed between the components of the system producing any of these live feeds is presently performed manually. Moreover, decision making for the selection of the correct timing fully depends on the judgement of the operator. Visual inspection and monitoring is in most cases the main feedback process used in the system. This approach results frequently in bottlenecks, uncertainty with respect to quality of the product, and even failure of production. For this reason, automatic transferring of biomass among the components of the system is necessary for the efficient operation of the hatchery.

Review of the use of short light-dark periods:

In photobioreactor (PBR) design light is considered to be one of the most important factors (e.g. Lee 1999). The effect of duration of light exposure on the cell is not well understood. Very little information is available as to how much time the cell should be in the light and dark. Many studies have used growth conditions that have a small dark period, others have used no dark period, and some have used dark periods up to18 hours (Park and Lee 2000, Kim et al. 2002). Merchuk and Wu (2003) suggest that an appropriate dark period is 6 seconds (s). Once a photon is absorbed, it needs 6s to reset itself (perform photosynthesis) so that it is ready to receive another photon (Merchuk and Wu 2003). Richmond (2004) on the other hand states that the rate of photosynthesis is governed by the turn over of electron transport that takes 1-15 milliseconds (ms). It is further asserted that an algal culture that has adapted to a high light intensity may require only 2 ms. This short dark period would make it difficult to move algae into and out of the lit region of a PBR, while the 6s dark period is more manageable. It has further been reported that flashing light enhances the biomass productivity and photosynthetic efficiency in PBR (e.g. Park and Lee 2000). In addition, high illumination of the algal antennas may also damage them necessitating longer dark periods to repair the damage (Wu and Merchuk 2001).

Review for algae biology and suitable algal strains for culture

Detailed literature review for algae biology and suitable algal strains for culture has been undertaken and completed. In addition two experiments; one investigating effects of light:dark periods on growth of live feed microalage and one investigating effects of LED light on growth of Nannochloropsis oculata has been undertaken and completed.

Review for current algae production systems, rotifer production systems, biosecurity issues in live food production.

Literature reviews have been completed on current algal production systems, rotifer production systems and biosecurity issues in live food production. The review on algal production systems examined the algal species cultured for different types of hatcheries and the methods of production from heterotrophic to phototropic, from batch to continuous and extensive to semi intensive and intensive systems. Commercially available systems were reviewed and the economic implications of these different methods of production were also considered. All aspects of the commercially available rotifer production systems were reviewed in order to finalise the design of the CROPS systems to be used in conjunction with the CAP yield and the CHTP system so that full integration of these systems within the hatchery operation can be carried out.

The review of the biosecurity issues in live food production systems highlighted the importance of sealed systems and sterile inputs so that the possibility of contamination is reduced. Algal starters should only be purchased from reputable algae collections that guarantee strain type and sterility. Pasteurisation of the water inputs is one of the only techniques that offer the best advantages for the continual operation of live feed systems.

Task 1.1 Build a baseline case model for the current hatchery operation against which to compare any proposed technological modifications.

A process model was built to simulate the algae production and utilization within a hatchery and the statistical variability of these operations by using stochastic simulation techniques. There are no adequate data for the detailed validation of the model and the validation work was based on observations of engineers experienced with running the algae production units. The experience of the equipment and consulting SMEs combined with a detailed literature search allowed us to build a model that simulates the operation of the most efficient hatchery with current technology (Benchmark).

The resulting model yields operating data for a realistic but efficient hatchery operating with existing technology. This baseline case will be continuously improved with ongoing laboratory and large scale experiments and will serve as a benchmark model to:

- Compare against the model the performance of any new design.
- Conduct parametric investigation on the factors affecting the performance of the process and target the design work
- Provide a tool for improving the design.

Benchmarking hatchery requirements for algae

An analysis was made of the current operation of the SME partners of the ALPHA project is summarised in the following Table:

Benchmaring hatcheries - live food production

Questions	Sagro Aquaculture Ltd	Viveiro Vilanova SA	Sagafjord Sea Farm AS
Juvenile production per year (mill)	**10 million**	**10 million**	**10 million**
Algal production season	October - End June	November to April	Whole year
Av daily algal req. - larvae	5000 - 6000 L	1000L av (min 900 max 1500L)	75 - 150 L (not every day)# ?
Av daily algal req. - rotifer culture	3000 - 5000 L		
Algal species cultured	Chlorella sp.	Nannochloropsis sp.	Tetraselmis suecica
Av Cell density cells /ml	Estimated @ 15x 10^6	35 x 10^6	11x10^6
Production scheme	Tanks only outside	Bag culture Indoor	BioFence indoor
Masters	No	Yes	Yes
Scale up	Yes	Yes - Petri dish to 200L bags	Yes – 250 ml to 20 L
Production volume	800L to 2m3 to 9m3	200 L bags	900 l continuos culture
Algae Vessel number and size	18 x 0.8 m3		1 x 300 lit
	8 x 2.0 m3		1 x 600 lit
	3 x 9 m3		
Algal culture temperature	Outside cultures	Indoor cultures	Indoor cultures
Winter	14-22 C	20C	23 – 25C
Summer	22-28 C	20C	23 – 25C
pH	8,2	7 ?	7.6
Particular problems	Ciliate contamination	Contamination - not usually found checked regularly for TBC and ciliates	Vorticella sp
Algal nutrients used	Agric fertiliser	Commercial nutrients	Commercial nutrients
	19 NH4/ 19P / 19K + trace elements + urea	Cell-Hi from Varicon Used as recommended	Cell-Hi F/2 and WP from Cellpharm
Fish species cultured	Sparus aurata	Sparus aurata	Gadus morhua
	D. labrax	D. labrax	
	P. puntazzo		
Larval rearing tank volumes m3	3m3 and 9m3	6m3	7m3
Larval tank number and size	3m3	18 x 6 m3	Bacth cycle of 4 x LR tanks
	9m3		75 – 150 larvae/litre
Type of LR stocking	Continuous as larvae available	Batch cycle of 9 x LR tanks	Batch cycle
	Continuous stocking	Stock for 1 week then stop	Stock for one week then stop
LR stocking density	200 larvae / litre	200 larvae / litre	
Algae added to LR tanks	day 4 to 25-28	day 3 to 22	Day 2 to 23
Freq of addition	3 x 40 litres in 9m3	120 litres / day/tank reducing last days	5 - 10 litres/day/tank #
	3 x 20 litres in 3m3		
Rotifer conc. used in LR	5-10 rotifers/ml	350 x 10^6 max no of rots/LR tank/day	4 – 6 rotifers/ml
			1 – 3 times/day
Rotifer culture general method	9m3 tank cultures	Scale up	Bacth cultures 3m3
	no small volumes		One small culture 1m3
Rotifer vessels	cylindroconical tanks		
Rotifer vessel number and size			
Rotifer culture feeding regimes	6 feeds per day	6 feeds	Every half an hour
	1 of algae	Culture Selco 3000 only	Fresh yeast
	5 artificial feed		Fish oil + vitamin mix
Quantity of algae fed to rotifers	For 2 x 10^9 rots 1m3 of algae		Ony small quant for cleaning
Rotifer enrichement		Enriched with Rich advanced	Phospholipids and Oils
Comments		If going for rotifer recirculation systems would like to use Nanno ?for culturing For enrichment would like to evaluate	Tetraselmis suecica Fed 12h before enrichment?
			# Depends of the daily concentration of algae in the LR tanks
		Nanno + Iso v's enrichment product	

The operations of the SME partners were analyzed in detail to estimate the algae requirements under current operating conditions and future hypothetical operating scenarios (i.e. the case the SMEs would like to grow rotifers on live algae or on a combination of yeast/live algae). The operating and capital cost is also presented where available. This analysis will benchmark the developmental effort and allow us to size and cost the developed units to satisfy the requirements of the Hatcheries and will allow us to build a stochastic model to simulate the performance of a benchmark process (best available process for algae production under current technology).

The information of the SME partners was supplemented with literature data and information from internal sources (Akvaplan-niva) to fill the gaps.

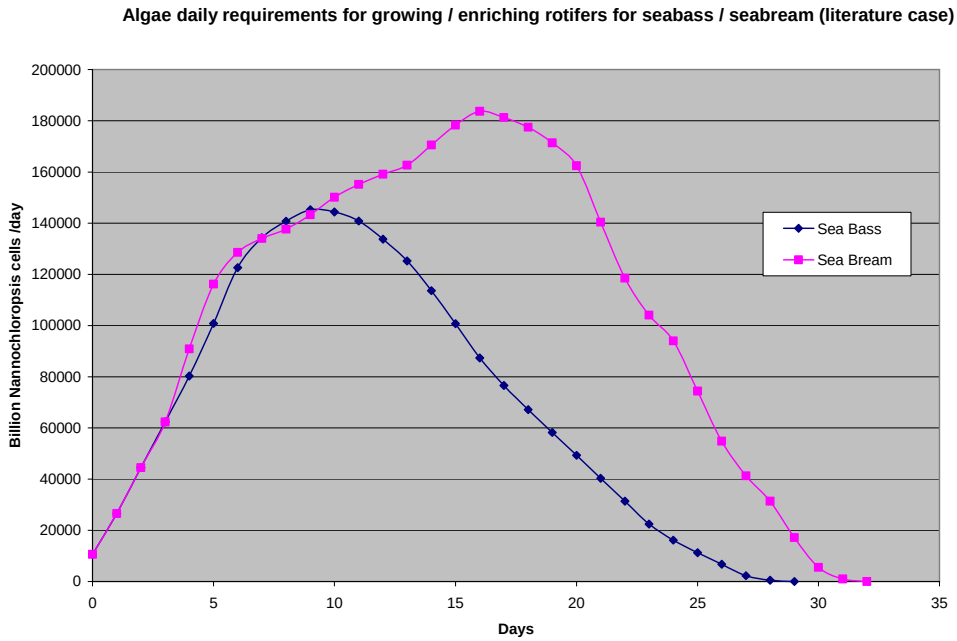
SEABREAM & SEABASS HATCHERY FROM PUBLISHED DATA

The hatchery data of the previous sections presented many gaps that were complemented with calculations based on the description of a Seabream, Seabass hatchery by a book by: **Moretti,**

Alessadro et al, "Manual on Hatchery Production of Seabass and Gilthead Seabream - Volume 1".

Table 2 and Figure 2 show the algae requirement for growing and enriching rotifers for Seabass and Seabream.

Figure 2



COMPARATIVE STUDY OF HATCHERY ALGAE REQUIREMENTS

The following table compares the SME peak Algae requirements for Larvae Rearing Tank greening and for growing rotifers on a combination algae/yeast* and for enriching* rotifers according to the following schedule:

*** GROWING ROTIFERS ON ALGEA / YEAST (low density cultivation)**

Algae requirements	Day 1	Day 2	Day 3	Enrichment	
Assuming fed with *Nanno* cells/rotifer/day	133000	66500	0	24000	TOTAL
Nannochloropsis cells consumed (billions)	29925	19950	0	6000	55875

The results can be used to calculate the capacity of the algae production unit that is required to satisfy the needs of the hatchery assuming that we cannot store algae in order to smooth the demand.

We can calculate the capacity of the algae producing unit by assuming that a Biofence unit is going to be used to produce algae and the productivity of a Biofence under steady state is:
Daily volume = 20% of the total volume of the unit
Concentration = 250 Mcells/ml for *Nannochloropsis* or chlorella and 11 Mcells/ml for *Tetraselmis*.

TABLE 2 Volume of Biofence unit producing Algae for the hatcheries in m3

	Greening	**Algae species**	**Rotifer Growing**	**Rotifer Enriching**	**Algae species**
SAGAFJORD	**0.43**	*Tetraselmis*	**6.3**	**1.89**	Nanno/Tetra
VIVEIRO VILANOVA	**0.75**	*Nannochloropsis*	**7.8**	**1.08**	*Nannochloropsis*
FAO	**0.27**	*Nannochloropsis*	**3.3**	**0.39**	*Nannochloropsis*
SAGRO	**1.50**	*Chlorella*	**3.6**	**1.30**	*Chlorella*

Table 2 shows that **a Biofence unit around 8 m3 can satisfy the peak algae requirement of a 10 million juveniles/ year hatchery.**

The above conclusions are made under the previously presented operating assumptions and under the assumption that algae cannot be stored to smooth the requirements. These calculations will be revised with the aid of the stochastic model.

TABLE 3 Comparative Study of Algae requirements by SMEs

	Capacity		Rearing Volume	Daily Peak Rotifer / day	Daily Peak Rotifer / day		Daily Peak Algae for greening	Daily Peak Algae for greening	Algae species
	million		m3 LR /cycle	millions	million /m3		billion	billion/ m3	
SAGAFJORD	10		105	1848	18		825	**8**	Tetraselmis
VIVEIRO VILANOVA	10		54	2250	42		37800	**700**	Nannochloropsis
FAO	3		28	832	30		13,440	**480**	Nannochloropsis
SAGRO	10		135	2700	20		75,000	**556**	Chlorela

	Daily Peak Algae Growing rotifer *	Daily Peak Algae for Growing /rotifer *	Algae species		Daily Peak Algae for Enrich rotifer	Daily Peak Algae for Enrich / rotifer	Algae species
	billion	thousand algae / rotifer			billion	thousand algae / rotifer	
SAGAFJORD	316,000	**171**	*Nannochloropsis*		4158	**2.25**	*Tetraselmis*
VIVEIRO VILANOVA	388000	**172**	*Nannochloropsis*		54000	**24**	*Nannochloropsis*
FAO	164,920	**198**	*Nannochloropsis*		19584	**24**	*Nannochloropsis*
SAGRO	182,250	**68**	*Chlorella*		64800	**24**	*Chlorella*

ECONOMICS OF ALGAE PRODUCTION

Introduction

The only continuous commercial systems in use for algae production in the hatcheries is apparently the Biofence (that is not described in this work). A template of costs for running a Biofence system producing *Nannochloropsis* was estimated for different parts of Europe (taking into account the real costs specific to this area). Finally the above calculated costs were compared with commercial algae paste.

Cost of Cephalonian Biofence Algal Unit

The cost of operating a biofence was prepared by Akvaplan-niva and represented the operational cost of a Biofence system at Cephalonia Fish Hatchery producing *Nannochloropsis* for seabass and seabream.

The daily cost including depreciation comes out to 52 Euros, which corresponds to 1.18 Euros per trillion *Nannochloropsis* cells.

Cost of Cephalonian Sack Culture Algal Unit

The cosy of operating algal sac culture was also prepared by Akvaplan-niva and represents the operational and capital cost of a Sac culture system at Cephalonia Fish Hatchery producing *Nannochloropsis* for seabass and seabream.

Because of the low productivity and the high labour, the cost of the sac-culture comes to 2.19 Euros/trillion *Nannochloropsis* cells that is higher than the cost of the Biofence (1.18 Euros/trillion *Nannochloropsis* cells).

Biofence Benchmark Costs

During the first annual meeting of the ALPHA project the group of the SMEs and RTDs reached a consensus on benchmarking the production cost of *Nannochloropsis* in a Biofence unit.

This benchmark work culminated in the following assumptions:
- *Nannochloropsis* is produced in a 1000lt unit (total volume), producing at steady state 200 lt/day of 250million *Nannochloropsis* cells / day.
- The unit is operating for 60 days at steady state.
- It takes 5 days for the unit to reach steady state.
- It takes 5 days to empty and clean reassemble and sterilize the unit each time the operation stops.
- There are 4 production cycles / year.

Using the above assumptions and the detailed costing for each country (due to the cost variabilities) Algal production costs were produced to benchmark the cost of a biofense producing *Nannochloropsis* unit at different countries. The costs are as follows:

Norway: 1.72 euro / trillion *Nannochloropsis* cells
UK: 1.44 euro / trillion *Nannochloropsis* cells
Portugal: 1.13 euro / trillion *Nannochloropsis* cells
Cyprus: 0.96 euro / trillion *Nannochloropsis* cells

Table 2.24 summarizes the cost of dead instant algae paste. As it can be seen from Table 2.24, the cost of *Nannochloropsis* algae paste varies from 1.01 to 1.7 euro per trillion *Nannochloropsis* cells depending on the size of the order.

TABLE 4

INSTANT ALGAE PURCHASE PRICE

Vilanova pays			**Cost in euros/trillion cells**
Chlorela from Japan	18.726 euro/lt		
Nannochloropsis from Reed	68.76 euro/lt	68 billion cells/ml	**1.011**
On Internet			
Nannochloropsis from Reed USA	$ 70/lt	68 billion cells/ml	**1.017**
transportation cost	$ 260 for 20lt		

PRICES FROM VARICON

Instant Algae® Products

Price (Euro) per trillion cells, 1 Case = 10 litres

	Million cells/ml	Wet weight	**1 Litre**	**1 Case**	**4 Cases**	**8 Cases**	**20 Cases**
Nannochloropsis 3600	68,000	18.4%	1.76	1.44	1.31	1.18	1.10
Tetraselmis 1800	750	18.9%	177.15	146.00	134.32	122.64	116.41
Isochrysis 1800	4,600	9.0%	15.87	13.01	11.90	10.79	10.00
Pavlova 1800	9,200	9.0%	7.93	6.51	5.95	5.40	5.00
Thal Weissflogii 1800	234	9.0%	311.97	255.81	233.97	212.14	196.54

a process model was built to simulate the algae production and utilization within a hatchery and the statistical variability of these operations by using stochastic simulation techniques.

There are no adequate data for the detailed validation of the model and the validation work was based on observations of engineers experienced with running the algae production units. The experience of the equipment and consulting SMEs combined with a detailed literature search allowed us to build a model that simulates the operation of the most efficient hatchery with current technology (Benchmark).

The resulting model yields operating data for a realistic but efficient hatchery operating with existing technology. This baseline case served as a benchmark model to:

- Compare against the model the performance of any new design.
- Conduct parametric investigation on the factors affecting the performance of the process and target the design work
- Provide a tool for improving the design.

In task 1.1, a stochastic model was produced to simulate and benchmark the existing photobioreactor designs for algae production.

Task 1.2 Build a model for the best designed process using the new technology.

During the 2nd reporting period the data from the laboratory and the pilot testing (see Deliverable 5) were used to build a stochastic model of the particular operation. This integrated model is used to optimise the design of the hatchery, eliminate inefficiencies and maximise synergies. This stochastic model is the best predictor for productivity, operating cost, resources utilisation, manpower, capital cost, yield, waste and every other parameter associated with the new process.

Some innovative prototype (BOA and Tubular) photobioreactors were built in this project (described in Deliverable 5). These photobioreactors were tested experimentally and the results of the experimental tests (described in Deliverable 5) are used here to adjust the parameters of the stochastic

model and predict the performance and economics of the scale-up models (cost and resource utilization) for the new designs, so as to quantify the benefits of the innovations.

Resources utilization and economics were analysed based on the experimental results of the prototypes, on the results of the stochastic model, and the scaled-up designs.

Task 1.3 Technology evaluation and decision criteria.

A quantitative evaluation of the new technology was conducted by comparing the optimised algae productivity within the hatchery using the new technologies (mega boa and mega tubular) and the baseline hatchery model using the current technology (Bio Fence).

The capital cost and start-up cost associated with the modification of the current hatchery were evaluated. Quantitative economic criteria for the adoption of the new technology have been developed.

In this Task the data from the testing in the hatcheries of the scaled-up units that (described in Deliverable D5) are used to build a stochastic model of the automated operation of algae production. This stochastic model allows us to conduct sensitivity analysis, to estimate operating cost, resources utilisation, manpower, yield, waste and every other parameter associated with the operation of the units. Comparing the model with the benchmark existing technology (described and modelled in Deliverable 1) will allow us to estimate the competitive advantages of the new technology and formulate the criteria for the adoption of the new technology.

As previously described, this project lead to the development of two reactor designs. The scaled-up versions that were built and installed one in Salamina (the BOA) and the other in Sagafjord (the Tubular).

Performance of the developed commercial photobioreactors.

This section describes the characteristics, performances and capital cost of the commercial units developed, built and installed in the hatcheries.

BOAS commercial unit at Salamina

The BOA commercial unit installed in Salamina and described in detail in Deliverable D5, has a capacity of 800 liters and it is operating with sunlight assisted by artificial light on a continuous fully automated mode. The concentration of the photobioreactor is monitored by a photosensor and an intricate computerized decision model that harvest automatically the algae produced and replenishes the harvested volume with fresh saline sterile water and F2 nutrient.

<u>Steady state operation:</u>
The system is used to grow Nannochloropsis s.p.. The harvested capacity of the system at steady state is 85 liters per day (24 hours) at a concentration of 300 million Nannochloropsis cells per milliliter. This is a total of 25.5 trillion cells/day.
The system requires the supervision of one person on an average of one hour per day.
The electricity consumption of the operating system is as follows:
For the recirculation motor: 1.87 KW
For the air blower: 1.5 KW
For the artificial lights: 800W
For the harvesting and control system: 600 W
The system consumes a bottle containing 37 Kg of CO_2 every week (7 days). This is equivalent to 5.28 Kg of CO_2 per day.

Shut down and maintenance:
The system needs to be cleaned after 60 days of production at the steady state
To dismantle, clean the pipes of the system, reassemble and disinfect the system, 2 persons are needed to work for four days.

Start-up
For the system to reach the harvesting steady state concentration are needed 7 days.
Capital cost:
The cost of the main unit constructed and installed by Hessy is 9462 €
The control and automation cost 3500 € and is composed by the following parts:

Parts	Price
Computer	700
Body of the sensor	340
Electronics parts of the sensor	10
Driving and collecting data circuits	140
Adaptor	20
Relays	40
PH –meter Transmitter	1200
PCI-bus Multifunction Card 100KS/s 12-bit	420
Wiring Terminal Board	70
68-Pin SCSI Cable 2m	50
PCI-bus Card 8-Channel Relay Actuator and 8-Channel Digital Input	140
PT-100 Temperature Sensor 0-45oC with Transducer	130
B13 pH electrode DIN	240
Total	3500

Tubular commercial unit at Sagafjord

The Tubular photobioreactor commercial unit installed at Sagafjord, is composed of two cylindrical reactors of 500mm diameter, 2.5m high made of transparent PVC and each reactor is having a 5KW motor with its proper frequency control in order to vary the rotation speed. Each reactor has a capacity of 300 liters that can be traduced to a useful liquid capacity of 230 liters. A total capacity of 460 liters.

The reactors are surrounded by 18 fluorescent lamps 2.5m each. Eight of these lamps are inserted to the tubes of the vertical baffles of the reactors (see Figure 1) and the rest 10 are set outside the reactor.
The control and harvesting systems are automated.

Steady state operation:
The system is used to grow Nannochloropsis s.p.. The harvested capacity of the system at steady state is 83 liters per day (24 hours) at a concentration of 330 million Nannochloropsis cells per milliliter. This is a total of 27.39 trillion cells/day.

The system requires the supervision of one person on an average of one hour per day.

The electricity consumption of the operating system is as follows:
For the agitation motors: 10 KW
For the air blower: 200W
For the artificial lights(18 X 58W): 1044W
For the harvesting and control system: 500 W
The system consumes 0.707 Kg of CO_2 per day.

Shut down and maintenance:
The system can operate for 60 days of production at the steady state
To clean the system and disinfect it, 1 persons is needed to work for one day.

Start-up
For the system to reach the harvesting steady state concentration are needed 7 days.

Capital cost:
The cost of the main unit constructed and installed by Hessy is 13035 €
The control and automation cost 3500 € and is composed by the following parts:

Parts	**Price**
Computer	700
Body of the sensor	340
Electronics parts of the sensor	10
Driving and collecting data circuits	140
Adaptor	20
Relays	40
PH –meter Transmitter	1200
PCI-bus Multifunction Card 100KS/s 12-bit	420
Wiring Terminal Board	70
68-Pin SCSI Cable 2m	50
PCI-bus Card 8-Channel Relay Actuator and 8-Channel Digital Input	140
PT-100 Temperature Sensor 0-45oC with Transducer	130
B13 pH electrode DIN	240
Total	3500

Algae concentration and storage system

The excess algae produced by the commercial photobioreactors is concentrated and stored. The maximum duration of the algae storage is of 3 weeks.

Economic evaluation of the commercial BOA and Tubular photobioreactors and comparison with the economics of the BioFence benchmark unit.

The economic evaluation of the commercial units described in section D3.1 is presented here as well as the economic evaluation of a BioFence unit with equivalent productivity. The BioFence is considered as the benchmark of the existing technology.

Economic evaluation of the BOA under sunlight

The table that follows summarizes the operating cost, the start-up and shut-down costs, the cost for repair and maintenance, the peripheral cost (required to prepare and maintain the inoculum) and the cost of amortization.

BOAS COSTING (in Euros 2007)					
Reactor total volume =	800	liters	**Annual production**		
Harvested daily volume =	85	liters	**6,120**	**(trillion cells /year)**	
Nannochloropsis concentration =	300	millcells/ml			
Daily Production at steady state (trilion nannocells/day) =	25.5	trilion cel /day			
Producing for aprox 60 days every two months	days/yr	240.00			
4 cycles /year					
Operational daily cost at steady state	**Units**	**Consumed**	**Unit Cost**	**Cost**	**Daily cost**
LABOUR					**16.57**
1 manhours/day	mandays/day	0.13	50	6.25	**Annual Cost**
CONSUMABLES					**3,976.31**
Nutrient F2 (9.26euro/Kg, 1Kg F2=>10m3 culture)	Kg/day	0.02	0.0085	0.00	
CO2	Kg/day	5.28	1.1	5.81	
ELECTRICITY					
Recirculating pump 1.87KW for 24 h	KWh/day	44.88	0.043	1.93	
Air blower 1.5 KW for 24 h	KWh/day	36.00	0.043	1.55	
LIGHTING					
800 W for 12 h	KWh/day	9.60	0.043	0.41	
Harvest and control 600W	KWh/day	14.40	0.043	0.62	
Startup Cost	**Units**	**Consumed**	**Unit Cost**	**Cost**	**Annual Cost**
Duration 7 days (4 times/yr)					**379.36**
LABOUR					
0.5 manhours/day	mandays	0.44	50	21.88	
CONSUMABLES					
Nutrient F2 (9.26euro/Kg, 1Kg F2=>10m3 culture)	Kg	0.08	9.26	0.74	
CO2 for 7 days	Kg	36.96	1.1	40.66	
ELECTRICITY					
Recirculating pump 1.87KW for 24 h	KWh	314.16	0.043	13.51	
Air blower 1.5 KW for 24 h	KWh	252.00	0.043	10.84	
LIGHTING					
800 W for 12 h	KWh	67.20	0.043	2.89	
Harvest and control 600W	KWh	100.80	0.043	4.33	
Shut Down Cost	**units**	**Quantities**	**Unit Cost**	**Cost**	**Annual Cost**
Every three months for 1 week (4 times/yr)					**1,700.00**
Labour for cleaning, dismantling	mandays	8.00	50	400.00	
Consumables (disenfectants)				25.00	
Maintenance Cost					**Annual Cost**
Maintenance, Repairs					**1,000.00**
Peripheral Cost				**Annual cost**	**Annual Cost**
Labour for master culture, autoclave, quality control	hrs/yr	120.00	12	1,440.00	**2,565.50**
Electricity for lighting master culture				200.00	
Master culture renewal twice /year				280.00	
Electricity for autoclave 45 KW , 120hrs/yr	KWh/yr	5,400.00	0.0825	445.50	
Others (glasware, chemicals etc)				200.00	
Amortization Cost					**Annual Cost**
Cost of boa including the control system	Euros	12,962.00			**2,592.40**
amortized in 5 years	years	5.00			
TOTAL ANNUAL COST (euros)		**Production cost**			
12,213.58		**2.00**	**(euros / trillion cells)**		

Economic evaluation of the BioFence system under sunlight

The table that follows summarizes the operating cost, the start-up and shut-down costs, the cost for repair and maintenance, the peripheral cost (required to prepare and maintain the inoculum) and the cost of amortization.

BioFence COSTING (in Euros 2007)						
Producing 12 trillion cells/day , 4 cycles/year (60 days/cycle)			**Annual production (trillion cells/year)**			
REACTOR SIZE = 400 lt reactor **			**2,880**			
Harvesting 20% of total volume per day **	80	liters/day				
Harvesting concentration Nannochloropsis **	150	milion cells/ml				
						Annual Cost
Operational daily cost at steady state	**units**	**Quantities**	**Unit Cost**	**Daily cost =**	**6.40**	**1,535.44**
LABOUR						
2 manhours/week	mandays/day	0.04	60.00	2.40		
CONSUMABLES						
Nutrient F2 (9.26 euro/Kg, 1 Kg F2 => 10m3 culture) **	m^3culture/day	0.08	0.926	0.07		
CO2 20 lt/hr *=0.48m3/day (22.4m^3=44Kg)	Kg/day	0.94	1.100	1.03		
ELECTRICITY						
Recirculation pump 600 W	KWh/day	14.40	0.043	0.62		
volume recirculated 340 lt/min*, pressure=2m H_2O, 600W						
Air flow pump, 100W, 20 lt/min	KWh/day	2.40	0.043	0.10		
LIGHTING (14 lamps at 150W to cover 10m long x 1.8m heigh)						
2.1 KW	KWh/day	50.40	0.043	2.17		
						Annual Cost
Startup Cost	**units**	**Quantities**	**Unit Cost**	**Cost =**	**32.92**	**131.68**
Duration 5 days every three months (4 startup per year)						
LABOUR						
2 manhours	mandays	0.25	60.000	15.00		
CONSUMABLES						
Nutrient F2 (9.26 euro/Kg, 1 Kg F2 => 10m^3 culture)	m^3 culture	0.40	0.926	0.37		
CO2 for 5 days (half)	Kg	2.35	1.100	2.59		
ELECTRICITY						
Recirculation pump 600 W	KWh	72.00	0.043	3.10		
Air flow pump, 200W, 100 lt/min	KWh	24.00	0.043	1.03		
LIGHTING (14 lamps at 150W to cover 10m long x 1.8m heigh)						
2.1 KW	KWh	252.00	0.043	10.84		
						Annual Cost
Shut Down Cost	**units**	**Quantities**	**Unit Cost**	**Cost =**	**600.00**	**2,400.00**
Every three months for 1 week (4 shut Down per year)						
Labour for cleaning, dismantling 40 hrs 2 persons	mandays	10	60.000	600.00		
						Annual Cost
Maintenance				**Annual cost**		**1,000.00**
Peripheral Cost				**Annual cost**		**Annual Cost**
Labour for master culture, autoclave, quality control	hrs/yr	120.00	12	1,440.00		**2,565.50**
Electricity for lighting master culture				200.00		
Master culture renewal twice /year				280.00		
Electricity for autoclave 45 KW , 120hrs/yr	KWh/yr	5,400.00	0.0825	445.50		
Others (glasware, chemicals etc)				200.00		
Amortization Cost				**Total invstm**		**Annual Cost**
Cost of biofense 9450 £**amortized in 5 years	Euros	14175		14175		**2,835.00**
TOTAL ANNUAL COST (euros)		**Production cost**				
10,467.62		**3.63**	**in Euros /trillion cells produced**			

* Sandnes et al, 2005 ** Cellpharm at http://home.bt-webworld.com/cellpharm/

Economic evaluation of the Tubular system under artificial light.

The table that follows summarizes the operating, start-up, shut-down, repair and maintenance, peripheral and the cost of amortization.

TUBULAR COSTING (in Euros 2007)					
Nominal volume of reactors	300	liters	**Annual production (trillion cells/year)**		
Actual volume of reactors	230	liters	**6,574**	**(trilion cells / year)**	
Daily harvested volume	83	liters			
Harvested Nannochloropsis concentration	330	millcells/ml			
Daily Production at steady state (trilion nannocells/day)	27.39				
Producing for aprox 60 days every three months	days/yr	240.00			
4 cycles /year					
Operational daily cost at steady state	**Units**	**Consumption**	**Unit Cost**	**Cost**	**Daily Cost**
					14.76
LABOUR					
2 manhours/week	mandays/day	0.04	50	1.79	**Annual Cost**
CONSUMABLES					**3,542.42**
Nutrient F2 (9.26euro/Kg, 1Kg F2=>10m3 culture)	m3 culture/day	0.08	0.926	0.08	
CO2 15 lt/hour = 0.36m3/day (22.4m3=44Kg)	Kg/day	0.71	1.1	0.78	
ELECTRICITY					
Motors (2 X 5KW = 10 KW for 24 hours/day)	KWh/day	240.00	0.043	10.32	
Air blower 200 W for 200lt/min at 3mH2O	KWh/day	4.80	0.043	0.21	
LIGHTING					
18 lamps X 58 W = 1.044 KWh for 24 hrs/day	KWh/day	25.06	0.043	1.08	
Harvesting and control system 500 W	KWh/day	12.00	0.043	0.52	
Startup Cost	**Units**	**Consumption**	**Unit Cost**	**Cost**	**Annual Cost**
Duration 7 days every three months					**561.98**
LABOUR					
1 day	mandays	1.00	50	50.00	
CONSUMABLES					
Nutrient F2	m3 culture	0.23	0.926	0.21	
CO2 for 7 days	Kg	4.95	1.1	5.44	
ELECTRICITY					
Motors (2 X 5KW = 10 KW for 24 hours/day)	KWh	1,680.00	0.043	72.24	
Air blower 200W for 200lt/min at 3mH2O	KWh	33.60	0.043	1.44	
LIGHTING					
18 lamps X 58 W = 1.044 KWh for 24 hrs/day	KWh	175.39	0.043	7.54	
Harvesting and control system 500 W	KWh	84.00	0.043	3.61	
Shut Down Cost	**Units**	**Consumption**	**Unit Cost**	**Cost**	**Annual Cost**
Every three months for 1 week					**300.00**
Labour for cleaning, dismantling 1 day	mandays	1.00	50	50.00	
Consumables (disenfectants)				25	
Maintenance Cost					**Annual Cost**
Maintenance, Repairs					**2,000.00**
Peripheral Cost				**Annual cost**	**Annual Cost**
Labour for master culture, autoclave, quality control	hrs/yr	120.00	12	1440	**2,565.50**
Electricity for lighting master culture				200	
Master culture renewal twice /year				280	
Electricity for autoclave 45 KW , 120hrs/yr	KWh/yr	5,400.00	0.0825	445.5	
Others (glasware, chemicals etc)				200	
Amortization Cost					
Cost of tubular and control system	Euros	16,535.00			**Annual Cost**
amortized in 5 years	years	5.00			**3,307.00**
TOTAL ANNUAL COST (euros)		**Production cost /trillion cells produced**			
12,276.90		**1.87**	**euros/trillion cells**		

Economic evaluation of the BioFence under artificial light.

The table that follows summarizes the operating, start-up, shut-down, repair and maintenance, peripheral and the cost of amortization.

BioFence COSTING (in Euros 2007)						
Producing 12 trillion cells/day , 4 cycles/year (60 days/cycle)			**Annual production (trillion cells/year)**			
REACTOR SIZE = 400 lt reactor **			**4,800**			
Harvesting 20% of total volume per day **	80	liters/day				
Harvesting concentration Nannochloropsis **	250	milion cells/ml				
						Annual Cost
Operational daily cost at steady state	**units**	**Quantities**	**Unit Cost**	**Daily cost =**	**6.40**	**1,535.44**
LABOUR						
2 manhours/week	mandays/day	0.04	60.00	2.40		
CONSUMABLES						
Nutrient F2 (9.26 euro/Kg, 1 Kg F2 => 10m3 culture) **	m^3culture/day	0.08	0.926	0.07		
CO2 20 lt/hr *=0.48m3/day (22.4m^3=44Kg)	Kg/day	0.94	1.100	1.03		
ELECTRICITY						
Recirculation pump 600 W	KWh/day	14.40	0.043	0.62		
volume recirculated 340 lt/min*, pressure=2m H_2O, 600W						
Air flow pump, 100W, 20 lt/min	KWh/day	2.40	0.043	0.10		
LIGHTING (14 lamps at 150W to cover 10m long x 1.8m heigh)						
2.1 KW	KWh/day	50.40	0.043	2.17		
						Annual Cost
Startup Cost	**units**	**Quantities**	**Unit Cost**	**Cost =**	**32.92**	**131.68**
Duration 5 days every three months (4 startup per year)						
LABOUR						
2 manhours	mandays	0.25	60.000	15.00		
CONSUMABLES						
Nutrient F2 (9.26 euro/Kg, 1 Kg F2 => 10m^3 culture)	m^3 culture	0.40	0.926	0.37		
CO2 for 5 days (half)	Kg	2.35	1.100	2.59		
ELECTRICITY						
Recirculation pump 600 W	KWh	72.00	0.043	3.10		
Air flow pump, 200W, 100 lt/min	KWh	24.00	0.043	1.03		
LIGHTING (14 lamps at 150W to cover 10m long x 1.8m heigh)						
2.1 KW	KWh	252.00	0.043	10.84		
						Annual Cost
Shut Down Cost	**units**	**Quantities**	**Unit Cost**	**Cost =**	**600.00**	**2,400.00**
Every three months for 1 week (4 shut Down per year)						
Labour for cleaning, dismantling 40 hrs 2 persons	mandays	10	60.000	600.00		
						Annual Cost
Maintenance Peripheral Cost				**Annual cost**		**1,000.00**
Peripheral Cost				**Annual cost**		**Annual Cost**
Labour for master culture, autoclave, quality control	hrs/yr	120.00	12	1,440.00		**2,565.50**
Electricity for lighting master culture				200.00		
Master culture renewal twice /year				280.00		
Electricity for autoclave 45 KW , 120hrs/yr	KWh/yr	5,400.00	0.0825	445.50		
Others (glasware, chemicals etc)				200.00		
Amortization Cost				**Total invstm**		**Annual Cost**
Cost of biofense 9450 £**amortized in 5 years	Euros	14175		14175		**2,835.00**
TOTAL ANNUAL COST (euros)		**Production cost**				
10,467.62		**2.18**	**in Euros /trillion cells produced**			

* Sandnes et al, 2005 ** Cellpharm at http://home.bt-webworld.com/cellpharm/

Summary of the Economic evaluations.
The table that follows summarizes the economic evaluations of the BOA and Tubular commercial systems and compares them with the benchmark model (BioFence).

Systems under SUNLIGHT	**Production Cost of trillion cell of Nannochloropsis s.p**
BOA	2.00 €
BioFence	2.18 €

Systems under ARTIFICIAL LIGHT	**Production Cost of trillion cell of Nannochloropsis s.p**
Tubular	1.87 €
BioFence	3.63 €

The above Tables show clearly that the two newly developed photobioreactors (BOA and Tubular) over-perform the benchmark current technology.

Strategic and economic criteria for the adoption of the new technology.
As it is shown in the previous section the adoption of the new technology for the production of live algae under sunlight offers an economy of 9%. The adoption of the new technology on the production of live food under artificial light offers an economy of 48%. This means that in the case a new hatchery is built and wants to produce live food, it should adopt the new technology.

The case becomes more difficult if the existing hatchery is using a BioFence for the production of algae. In this case several factors come to play: the status of the existing equipment (how old it is and how well it is maintained), the size of the equipment and if it operates under sunlight or artificial light.

In the extreme case the BioFence used in the hatchery is new and produces on the order of 25 trillion Nannochloropsis cells/day under sunlight, it would be difficult to justify a 12000 € investment to replace the existing unit with a BOA that will save the hatchery 1080 € per year. On the other side, if the same unit operates under artificial light it might be worth investing 16500 € to replace it with a Tubular photobioreactor that will save the hatchery 10560 € each year. The older the existing photobioreactor, the easiest is the decision to invest for a replacement. The same holds true with the size of the photobioreactor. The biggest, the photobioreactor, the biggest is the improvement of the new technology.

Another big advantage of the new photobioreactors is that their footprint (as described in Deliverable D5) are considerably smaller than the equivalent BioFence. This makes it easier to replace an existing BioFence within the hatchery with one of the two systems and save space.

The above calculations are based on the production of Nannochloropsis s.p. for which the data of the newly developed photobioreactors were obtained. It is not clear how this economic evaluation will look with other species.

The argument might come also over live algae versus paste. The commercial paste (see Deliverable D1), comes cheaper than the production of the live algae. This is partly due to the scale at which the

algae is produced. The higher the production size, the cheaper the algae produced since size reduces the ratios of operating cost over production rate and capital cost over production rate. A key consideration in opting for live algae is the nutritional quality of the feed that impacts on the health of the fry. The economic impact of this is difficult to estimate and although it impacts the survival rate and the fattening rate of the healthy fish at a later stage of its development, it is not the only factor that impacts these two parameters.

WORKPACKAGE 2.
Design and development of a Continuous Algae Production (CAP) system

Work undertaken and important findings

Task 2.1 Biology of algal species suitable for aquaculture – Definition of biological requirements for high-density Continuous Algae Production

This work was undertaken in Reporting period 1.

Literature review – algal biology and nutritional qualities of algae

Marine micro-algae are important live food in hatcheries. They are widely used as food for many species of larval and juvenile fish, shellfish and shrimps, either directly or indirectly through zooplankton. Although, several alternatives for algae exist such as yeasts and microencapsulated feeds (Jones et al. 1987, Nell 1993, Heras et al. 1994, Nell et al. 1996), live algae are still the best and the preferred food source.

Mono-cultures are essential when a high quality feed source with known nutritional properties is required. Bacteria free cultures also reduce the risk of introducing unwanted pathogens into the animal cultures, which may result in mortalities in the cultured species. Live algae may even inhibit bacterial growth (Austin and Day 1990, Austin et al. 1992, Mezrioui et al. 1994) and this is an added advantage they have over artificial feeds such as microencapsulated feeds.
Many studies have been undertaken to determine the nutritional requirements of algal species, their biochemical composition and potential use as a food source (e.g. Volkman et al. 1981, 1989, 1991, 1993, Brown et al. 1989, 1993, Brown 1991, Brown and Jeffrey 1992, Brown and Miller 1992, Dunstan et al. 1992, 1994, de Roeck-Holtzhauer et al. 1993, Brown and Farmer 1994, Borowitzka 1997, Brown et al. 1997).

The biochemical composition, which differs among algal species, determines the nutritional value of the algae (e.g. Brown et al. 1999). The total concentrations of protein, lipid and carbohydrate can vary substantially between species and culture conditions. Accumulated data have shown that the quantity and fatty acid profile of algal lipids are crucial to the growth and development of the animals cultured (e.g. DePauw et al. 1984; Koven et al. 1989). The biochemical composition of algae is affected by the growth environment, which can be manipulated by changing the culture medium (Wikfors et al. 1984, Ben-Amotz et al. 1985), temperature (Redalje and Laws 1983, James et al. 1989), stage of harvest (Chu et al. 1982, Whyte 1987), photoperiod and stage of light cycle (Caron et al. 1988, Sicko-Goad et al. 1988), and irradiance (Cohen et al. 1988, Thompson et al. 1990). Other factors such as size, toxicity and digestibility of the micro-algae can also account for the differences in their food values (Webb and Chu 1983).

The algae production is an important component of hatcheries, however, it is costly and not always optimised with respect to the nutritional value of the species used. In order to identify optimal parameters for algal growth when designing a new algal bioreactor a literature review has been undertaken. The optimal growth conditions for algal species and their nutritional qualities will be reviewed and discussed. Particular emphasis has been made on the cold water species: *Phaeodactylum tricornutum*; temperate species: Isochrysis *galbana*, *Tetraselmis suecica*, *Nannochloropsis oculata* and semi-tropical species: *Isochrysis* sp. (T-iso, Tahitian strain), *Nannochloropsis* sp. (Kuwait strain). Potential new cold water species will also be considered.

Essential parameters important for growing algae in aquaculture

Size. Micro-algae within the size range 2-20 µm are widely used within rearing of zooplankton, shrimps, shellfish and larviculture of fish (e.g. De Pauw and Persoone 1988, Brown et al. 1989). The

size of the algal species used is important and the main reason for this is that the developing larvae are usually very small, fragile and not physiologically fully developed (Lavens and Sorgeloos 1996). The mouth size of the first-feeding larvae usually mechanically restricts the size of the food particles that can be ingested.

Growth rate. Fast growing species is preferred. The key to success of algal production is maintaining cultures in exponential phase of growth. During the exponential phase, cell density increases as a function of time t according to a logarithmic function: $Ct = C0 \cdot e^{\mu t}$
With Ct and C0 being the cell concentrations at time t and 0, respectively, and μ = specific growth rate. The specific growth rate can also be expressed as: $\mu = \ln(C1/C0)/t1-t0$
The specific growth rate is mainly dependent on algal species, light intensity and temperature. Cell division slows down when nutrients, light, pH, CO_2 or other factors becomes limiting.

Pigments *and absorption wavelengths*. Chlorophyll a (chl a) is the main photosynthetic pigment in all micro-algae. The accessory chl b is found together with chl a in the green algae (e.g. Prasinophyceae), whereas chl c is found with chl a in the chromophyte algal classes (e.g. Bacillariophyceae and Prymnesiophyceae). Chl a and β-carotene are common in the different classes. Chl a is located as a part of core and reaction center protein complexes and in the light-harvesting antenna (Richmond 2004). Other important pigments such as chl b and c, carotenes and xanthophylls act as supplementary pigments for light harvesting (Richmond 2004).

Light is often considered to be one of the most important factors in photobioreactors (Richmond 2004). Different types of algal cultures need different light and nutrient sources. The light requirement for algae is dependent upon the major pigments present in the algal cell. A detailed explanation of pigments and their importance is discussed in Kommareddy and Anderson (2003). Kommareddy and Anderson (2003) also discuss how different light wavelengths that are absorbed are converted to energy for the photosynthetic process.

Different pigments harvest different regions of visible light energy. Richmond (2004) showed the penetration depth of light spectra in *Nannochloropsis* sp. as a function of cell density. The important pigments of *Nannochloropsis* are chl a, and β-carotene. The light wavelengths corresponding to the absorption range of these pigments (approximately 400-500nm and 600-700nm) also corresponds to the light wavelengths with the least penetration depth because they are absorbed by algae. Another interesting aspect, which can be seen from Richmond (2004), is that when the concentration of algae in gL^{-1} is low, the absorption by the supplementary pigments is low. This suggests that supplementary pigments are not used to harvest light until there is a deficiency of light in the wavelengths absorbed by chl a. Richmond (2004) also showed that algal cultures with a density of 3 gL^{-1} effectively absorb all blue light (300-400nm). For blue light to have a greater penetration depth, the culture density must be less than 3 gL^{-1}.

Optimum culture conditions. The most important parameters regulating growth are temperature, salinity, nutrients, light, pH and mixing. These parameters may also be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for another. Optimum culture conditions, in terms of high growth rates, are, however, not necessarily the same as optimum conditions for nutritional quality (e.g. Sanchez et al. 2000). A compromise between the nutritional quality and growth kinetics will often have to be considered. For instance, it has been shown that the essential fatty acid, EPA, increase with decreasing light (Sukenik et al. 1989, Thompson et al. 1990).

Temperature. Optimum temperature for micro-algae used in aquaculture is generally between 18 and 24°C, although this may vary within strains and species. Many of the cultured species tolerate

temperatures between 16-27°C. Temperatures lower than 16°C will most likely result in slow growth, while temperatures above 35°C will lead to culture collaps (e.g. Acien Fernández et al. 2003). In order to keep stable temperatures, cultures can be cooled down by flow of cold water over the surface of the cultures or by controlling the air temperature with refrigerated air.

Optimum temperature reported for production is 19-21°C for *Nannochloropsis oculata* and *Tetraselmis suecica*, 20°C for *Nannochloropsis* sp. ("kuwaitian strain") and 24-26°C for *Isochrysis* sp. (T-iso) (James et al. 1989, Abu-Rezq et al. 1999). Optimal temperature reported for *Phaeodactylum tricornutum* is 18-22°C (e.g. Molina Grima et al. 1996) and 20°C for *I. galbana* (Molina Grima et al 1994).

Salinity. Marine micro-algae are in general tolerant to changes in salinity. In culture, most species grow best at a salinity that is a bit lower than found in their native habitat (diatoms at 20-25 ‰ and flagellates at 28-30‰). This can be obtained by diluting sea water with distilled water (Lavens and Sorgeloos 1996). Optimum salinity reported for production is 20-40‰ for *N.* sp. 20-35‰ for *T. suecica* and 25-35‰ for *I.* sp. (T-iso) (Abu-Rezq et al. 1999). Fabregas et al. (1985) and Renaud & Parry (1994) reported that the optimal growth rate for *I.* sp. (T-iso) was achieved when salinity was between 15 and 35‰, whereas *N.* sp. had a significant slower growth rate only at 35‰.

Nutrients. Cultures of micro-algae must be enriched with nutrients in order to sustain growth. Macronutrients include nitrate, phosphate and silicate. Silicate is mainly used by diatoms, which utilize this compound for production of an external cell covering. Micronutrients consist of various trace metals (Zn, Co, Cu, Mo, Mn, Fe) and vitamins (thiamine, cyanocobalamin and biotin). Two enrichment media are commonly used for growth of algae in aquaculture; Walne medium (Laing 1991) and f/2 medium (Guillard 1975). Various specific recipes for media are described by Vonshak (1986). The complexity and cost of media often excludes their use for large scale production. Alternative enrichment media for large scale production are often composed of agriculture-grade rather than laboratory-grade nutrients, and they often contain only the most essential nutrients (e.g. Palanisamy et al. 1991).

Light intensity and photoperiod. Micro-algae are photosynthetic organisms; they assimilate inorganic carbon and transform it to organic matter. Light is the energy source that drives this reaction. Light intensity, photoperiod and spectral quality need therefore to be carefully considered.
After identifying the type of algae culture to be grown, it is important to identify the right type of light source with appropriate wavelengths in order to achieve a high level of photosynthetic efficiency. Light may be natural or supplied artificial. Kommareddy and Anderson (2003) discussed energy produced by different light sources in the visible spectrum. The efficiency at converting electricity into light varies with different light sources and is further compounded when wavelength is considered. Light sources with descending order of efficiency are light emitting diodes (LEDs), grow flux/ fluorescent lights and incandescent/halogen lamps. Since LEDs are the most efficient light source for converting electricity into light with the desired wavelength, they should be given high priority for use. However, LEDs don't produce light in a broad white light spectrum which may make it necessary to use a combination of light sources or combination of LEDs.

Essentially any type of light sources which produces light between 400 nm–500 nm and 525nm to 680 nm should support growth of algae. The intensity of a light source gives the number of photons that are available for the photosynthetic process. The energy associated with photons with a wavelength of 680nm is the energy level required by chl a to initiate photosynthesis. Light with a wavelength of 680nm is near the longest wavelength of visible light. Therefore, most of the visible light has sufficient energy to support photosynthesis. If the wavelength is small the energy associated

with the wavelength is high. Light intensity plays an important role, but the requirements vary greatly with culture depth and cell density. At high depths and densities the light intensity must be increased, although too high intensity may result in photo-inhibition.

pH, CO_2, O_2 and mixing. The pH range for most cultured species is between 7 and 9, with the optimum range being 8.2-8.7 (Lavens and Sorgeloos 1996). Culture collapse can be the result when failing to keep an acceptable pH. This can be prevented be aerating the culture. At high cell densities, addition of CO_2 to the air allows to correct for increased pH due to high primary production. The CO_2 originating from air (~0.03%) is limiting growth when bubbled through a dense culture. Pure CO_2 may then be supplemented to the air supply usually at a rate of 1-2% of the volume of air. CO_2 addition furthermore buffers against pH changes. Mixing is necessary in order to prevent sedimentation, ensure that the cells are equally exposed to light and nutrients, avoid thermal stratification and to improve gas exchange between culture and air. Mixing can be achieved by stirring by hand (small volume flasks), aerating (bags, tanks), paddle wheels or jetpumps (ponds). Not all species can, however, tolerate vigorous mixing. In closed bioreactors oversaturation of O_2 due to respiration can limit the growth of algae. This can be prevented by ventilation of the cultures.

Nutritional qualities of algae

Gross composition. Protein, carbohydrate, lipids and minerals make up 90-95% of the dry weight of an algal cell. The remainder is accounted for by nucleic acids (5-10%) (Becker 1986, Fabregas et al. 1986, Fabregas et al. 1986). Protein is always the major organic constituent, followed by lipid and carbohydrate. Expressed as percentage of dry weight, the range of protein, lipid and carbohydrate are 12-35%, 7.2-23% and 4.6-23%, respectively. Variables such as photoperiod, light intensity, wavelength, temperature, nutrients and stage of growth at harvest can influence the gross composition (e.g. Myklestad 1974, Goldman 1977, 1979, Savidge 1980, Walsh and Legendre 1983, Fabregas et al. 1985, Hitchcock et al. 1986).

Protein and amino acids. The nutritional value of protein is determined by the content and availability of its constituent amino acids. Of the total amino acids in algae, 90-98% occurs in protein (Dortch et al. 1984). A number of studies have been conducted on total amino acid composition in micro-algae (e.g. Parsons et al. 1961, Chau et al. 1967, Enright et al. 1986a, Hayashi et al. 1986, Brown 1991). Brown (1991) showed that micro-algae have a well balanced amino acid composition. The proportions of individual amino acids do not vary greatly between different algal species. Differences in the nutritional quality of algae are therefore, mostly, unrelated to amino acid composition (Webb and Chu 1983, Brown 1991).The amino acid composition of micro-algae is quite similar to chicken egg protein (high nutritional value for humans), although the latter is richer in methionine and lower in arginine (Teshima et al. 1986).Some amino acids are unavailable for animal digestion and absorption if sections of the molecule are bound to other molecules (e.g. the free amino group of lysine can sometimes be bound to carbohydrate, particularly, during processing of harvested algae (like drying)).

Carbohydrate. Micro-algae provide a rich source of carbohydrate (Brown 1991). Few studies have been made on carbohydrate composition of micro-algae (Parsons et al. 1961, Chu et al. 1982, Whyte 1987, Brown 1991). The total carbohydrate fraction is composed of the polysaccharide fraction (45-97% of total carbohydrate fraction, Whyte 1987) and mono-and oligo-saccarides. Carbohydrate profiles of micro-algae can vary greatly. The principal sugars are glucose, galactose, mannose and ribose, with others in varying proportions.Differences in polysaccharides have been shown between the major groups of micro-algae. Diatom polysaccharides contain mainly chrysolaminarin (ß1-3 glucan) and mannans (Myklestad 1974, Whyte 1987), while flagellates mainly contain glucans like glucose and galactose (Whyte 1987).

Lipids. The lipids are grouped as polar and neutral lipids depending on their polarity. The polar lipids include the phospholipids and glycolipids. The neutral lipids include the triacylglycerides, diacylglycerides, hydrocarbons, alkenones, sterols and pigments.

Fatty acids (FA). The FAs constitute a major proportion of the lipid fraction in micro-algae (20-40% of total lipid on a weight basis, Cohen 1986). Fatty acids occur mainly in an esterified form with glycerol, and are found in tri- and di-acylglycerides, phospholipids and glucolipids. Most studies report only the total FAs, although some report the FA profiles (e.g. Fried et al. 1982, Sheffer et al. 1986). The different classes of algae show quite distinct distribution patterens (Chuecas and Riley 1969, Beach et al. 1970, Waldock and Nascimento 1979, Webb and Chu 1983, Volkman et al. 1989). Saturated FA constitute about 15-30% of the total FAs in green algae, while the range is 30-40% in diatoms and prymnesiophytes. Green algae are low in monosaturates (5-20%), but high in polyunsaturates (50-80%), whereas prymnesiophytes and diatoms have similar levels of both monosaturates (20-40%) and polyunsaturates (20-50%). The polyunsaturated fraction of the green algae is, however, dominated by 16 and 18 carbon chain length FAs, whereas higher carbon FAs (like 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA)) are typical for the other groups of algae. Despite these trends, the levels of specific FA may vary widely in closely related species within the same class (e.g. *I. galbana* and *I.* sp. (T-iso)). Most species contain moderate to high concentrations of EPA. Particularly *Pavlova lutheri*, *P. tricornumtum* and *N.* spp. are species rich in EPA, while *I.* sp. (T-iso) contains very low levels of this FA. Species that are rich in DHA are *I. galbana*, *I.* sp. (T-iso) and *P. lutheri*. Only *P. lutheri* is rich in both DHA and EPA. Green algae like *T. suecica* are usually rich in C16 and C18 PUFAs, in addition to ARA (arachidonic acid, 20:4n-6), but low in higher carbon fatty acids like EPA and DHA, which may contribute to low nutritional value.

Pigments. The major pigments of most algae are the green chlorophylls and the yellow, orange and red carotenoids, which contribute 0.5-5% of the dry weight of the cell (Parsons et al. 1961, Ben-Amotz et al. 1985). Blue-green algae, red algae and the cryptophytes also contain the red, protein-bound water-soluble, phycoerythrins and/or the blue phycocyanins. Chlorophylls and carotenoids are contained in the extracted lipid fraction of the cell. Carotenoids are made up of a number of isoprene units, functioning both as photoprotectants and light-harvesting pigments in photosyntesis (Cohen 1986). Each algal species may contain between 5 and 10 different carotenoids, and more than 60 different carotenoids are known from algae (Cohen 1986). ß-carotene (provitamin A) is a common constituent of the carotenoid fraction of micro-algae. It is found in highest concentration in the green algae. Although it generally constitutes less than 1% dw, it may accumulate levels up to 10% dw in halotolerant algae (Fried et al. 1982, Ben-Amotz et al. 1985). Algae of interest is prasinophytes (*Tetraselmis* spp), eustigmatophytes (*Nannochloropsis* spp), prymnesiophytes (*Isochrysis* spp), and bacillariophytes (*Phaeodactylum* spp). Some other species like *Euglena*, *Haematococcus* and *Chlorella* can produce significant quantities of other carotenoids such as astaxanthin. Astaxanthin and luthein may serve as a vitamin A precursor in fish that are not able to absorb ß-carotene (Torrissen and Christiansen 1995, Rønnestad et al. 1998). Green algae, like for instance *T. suecica*, have also shown to contain adequate amounts of luthein (Rønnestad et al. 1998).

Minerals. The mineral fraction of the algal cell can constitute a major proportion of the dry weight, ranging from 6-39%, but there are few detailed analyses. Algae can be a major source of a number of minerals. They can also accumulate trace- and heavy metals, which can be a disadvantage if the metals are toxic (Sakaguchi et al. 1981, Fisher 1985, Fabregas and Herrero 1986). Major ions that are of biologically importance are phosphorous, silica, calcium, sodium, potassium, chlorine, iron, magnesium, and zinc; manganese, copper and cobalt occur in trace amounts.

Vitamins. Algae are a significant source of nearly all the vitamins. However, few studies have been conducted (Kanazawa 1969, Aaronson et al. 1971, Dubinsky et al. 1978, Brown et al. 1999). The major vitamins identified are thiamine (vitamin B1), riboflavin (B2), pyridoxine (B6), cyanocobalamin (B12), biotin, ascorbic acid (vitamin C), nicotinic acid, pantothenic acid, choline, inositol, tocopherol (E) and ß-carotene (provitamin A). In addition, vitamin K has been detected in trace amounts in *Porphyridium cruentum* when grown heterotrophically (Antia et al. 1970) and vitamin D precursors have also been isolated from algae (Hollick 1984). Many algae also have specific vitamin requirements, particularly thiamin, cyanocobalamin and biotin have shown to be of importance (Provasoli and Carlucci 1974).

Quality variation. Algal species vary significantly in their nutritional value under different growth conditions (Brown et al. 1997). A carefully selected mixture of algae can offer an excellent nutrition for larval fish, either directly or indirectly through enrichment of zooplankton. Algae that have been found to have good nutritional qualities include species like *Chaetoceros calcitrans, C. muelleri, P. lutheri, I.* sp. (T-iso), *T. suecica, Skeletonema costatum* and *Thalassiosira pseudonana* (Enright et al. 1986a, Thompson et al. 1993, Brown et al. 1997) amongst others. The gross composition of micro-algae can influence the nutritional value (e.g. Enright et al. 1986b), however, it may seem that it is the balance of other key nutrients that are of more importance.

Micro-algae have an important role in enriching zooplankton fed to fish and other larvae. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential PUFAs, pigments and sterols, which are transferred through the food chain.
Rotifers fed microalgae become rapidly enriched with ascorbic acid (AsA). After 24 h, rotifers fed on I. sp. (T-iso) and *N. oculata* contained 2.5 and 1.7 mg g^{-1} DW, respectively, whereas rotifers fed on baker's yeast (itself deficient in AsA) contained only 0.6 mg g–1 DW (Brown et al. 1998). After an ensuing 16 h of non-feeding, rotifers lost <10% of their AsA, retaining ≈ 50% of total ingested AsA. Similarly, concentration of AsA in *Artemia* may be enriched by feeding with microalgae (Merchie et al. 1995). Little information is available on the transfer of other vitamins from microalgae through the food chain to fish larvae, but a similar mechanism would be suspected. Rønnestad et al. (1998) showed that micro-algal pigments transferred through to zooplankton may contribute to nutritional value. They found that the dominant pigments in the copepod *Temora* sp. were lutein and astaxanthin, whereas in *Artemia* it was canthaxanthin. When these prey items were fed to halibut larvae, adequate amounts of vitamin A were found in halibut fed on copepods, but not with halibut fed on *Artemia*. The authors ascribed this to the ability of the larvae to convert lutein and/or astaxanthin, but not canthaxanthin, into vitamin A. They recommended that *Artemia* should routinely be enriched with astaxanthin and lutein (the latter pigment common in 'green' microalgae, e.g. *Tetraselmis* spp.) to improve their nutritional value.

Manipulation of biochemical composition. Under nutrient sufficient conditions, cells synthesise mainly proteins to support growth and division (Myers 1980). However, when a culture is deprived of an essential nutrient, cell division stop and the fraction of carbon allocated to lipids and carbohydrates can be greatly increased at the expense of protein synthesis. In most algae, enhancement of lipid accumulation is imposed by nitrogen deficient conditions (Spoehr and Miller 1949, Shifrin and

Chisholm 1981, Piorreck et al. 1984, Cohen et al. 1988). Herrero et al. (1991) showed that the protein content per cell was more susceptible to medium induced variation than the other cellular constituents.

Other properties. A common procedure during the culture of both larval fish and prawns is to add micro-algae (i.e. 'green-water') to intensive culture systems together with the zooplankton prey (Tamaru et al. 1994). Addition of the micro-algae can improve the production of larvae in such systems, though the exact mechanism of action is unclear. Theories advanced include (a) light attenuation (i.e. shading effects) have a beneficial effect on larvae, (b) maintenance of the nutritional quality of the zooplankton, (c) an excretion of vitamins or other growth-promoting substances by algae, (d) a probiotic effect of the algae and e) stabilizing the water quality in static rearing systems (remove metabolic by-products, produce oxygen). Most likely, the mechanism may be a combination of several of these possibilities. The most popular algal species used for green-water applications are *N. oculata* and *T. suecica*. More research is needed on the application of other micro-algae, especially, species rich in DHA to green-water systems. Contaminants with bacteria, protozoa or other micro-algae can be a major problem in algal production. Contamination often comes from the culture medium, the air, culture vessel and/or the starter culture. Different methods to avoid contamination are listed in Lavens and Sorgeloos (1996).

Nutrient value of micro-algae concentrates. High costs and risks posed by on-site production of live micro-algae have prompted a search for alternatives, such as preserved micro-algal concentrates (Coutteau and Sorgeloos 1992, Knauer and Southgate 1999). Different techniques have been used for concentrating and preserving micro-algae, including centrifugation, flocculation, spray- and freeze-drying, refrigerating and freezing (Knauer and Southgate 1999). A lower rate of biochemical degradation has been demonstrated by cold storage than by air- or freeze- drying (Cordero Esquivel and Voltolina Lobina 1996). By using centrifuged algae it has also been shown that the chemical and microbial loads associated with direct feeding may be reduced. O`Connor and Nell (1992) found that centrifuging algae before feeding reduced the bacterial numbers introduced to larvicultures. Centrifugation of algae into paste (micro-algae concentrates) and subsequent refrigeration until required is widely applied in North America by oyster hatcheries. The limited shelf-life and the high prizes of the commercially available algal pastes (>US$ 200/kg DW) have, however, kept many from using them. Also, a major constraining factor to wider commercial use has been the inability to create pastes that retain high nutritional value through storage. The resuspended cells from pastes need to rival that of the original live cells; retain palatability, be able to be ingested and digested by the animals being cultured. The process of concentrating algae may also damage the cells or leak their contents during concentration, in addition to autolysis and microbial degradation which can occur during long storage. Not all species are equally hardy for this process.

Companies like Reed Mariculture grows their algae in closed photobioreactors, using natural light and a growing medium modified from standard f/2. Their products contain no chemical preservatives and some have up to 3-month refrigerated shelf life. These concentrates contain, however, mostly cells that are not live and can therefore not be used as starter cultures. Reed has advocated some advantages using their non-viable algae in hatcheries others than mentioned earlier, such as stable nutritional profile, super high concentrations, easier to capture, faster feeding, excellent suspension, no fouling or clumping (http://microalgae.reed-mariculture.com).

Loss of nutrients during storage of concentrates may affect their efficacy as diets for aquacultured animals (Brown 1995). Brown (1995) found that centrifuged *C. calcitrans* lost ascorbic acid with storage age. Molina Grima et al. (1994) and Montaini et al. (1995) found that PUFAs remained constant in centrifuged *I. galbana* and *T. suecica*. Other losses of nutrients such as total protein,

carbohydrate and lipid can also affect the development of larvae. However, few experiments have been conducted and it would be very useful to measure effect of storage age on a broader range of nutritional components. In addition, the storage conditions of the concentrates such as light, temperature, oxygen and preservatives would also have to be evaluated. Heasman et al. (2000) found that high-speed centrifugation was the most appropriate method for harvesting micro-algae for developing extended shelf-life concentrates. Bioassay evaluation of stored micro-algae concentrates revealed, however, major discrepancies between closely related species with regard to the impact of harvesting method on both short-term nutritional quality and shelf-life of stored concentrates. They found very good retention of nutritional quality of *Tetraselmis* and *C. calcitrans* beyond 8 weeks storage. In contrast, *P. lutheri, I.* sp. (T-iso) and *C. muelleri* exhibited rapid and profound losses in nutritional quality as a consequence of high-speed centrifugation.

Potential new algal species for cold waters

Temperate and sub-tropical species like *I.* sp. (T-iso), *I. galbana, T. suecica* and *N. oculata* are widely used feed organisms, in addition to greening and enrichment in cold water hatcheries. Today, algal species isolated from cold waters are hardly in use. Therefore, new and more suitable species isolated from local areas for use in cold water hatcheries are highly demanded. Some attempts have been made on isolating new species from cold waters, but they have so far not been implemented in intensive hatchery production. Based on biochemical composition, particularly the FAs, some promising candidates have emerged from the algal classes; cryptophyceae, chlorophyceae, dinophyceae, bacillariophyceae, prasinophyceae and dictyochophyceae (Leirvoll et al. 2001, Solbakken and Johnsen 2004). These species have yet to be tested for their suitability as feed organisms in high density cultures.

Conclusion

- Optimum culture condition varies between species and strains. Essential parameters regulating growth are temperature, salinity, nutrients, light, pH, CO2 and mixing. Optimum culture conditions for the selected species are as follows:
    - *N. oculata* and *N.* sp. (“kuwaitian strain”); temperature: 19-21°C, salinity: 20-40‰, light intensity: 100-200 µmol m^{-2} s^{-1}, pH: 7-8.7 and CO_2: 1-2%.
    - *I. galbana*; temperature: 20°C, salinity: 25-35‰, light intensity: 100 µmol m^{-2} s^{-1}, pH: 8.2-8.7 and CO_2: 1-2%.
    - *I.* sp. (T-iso); temperature: 24-26°C, salinity: 25-35‰, light intensity: 100 µmol m^{-2} s^{-1}, pH: 6.8-8.2 and CO_2: 1-2%.
    - *T. suecica*; temperature: 19-21°C, salinity: 20-35‰, light intensity: 100 µmol m^{-2} s^{-1}, pH: 6.8-8.2 and CO_2: 1-2%.
    - *P. tricornutum*; temperature: 18-22°C, salinity: 20-30‰, light intensity: 125-250 µmol m^{-2} s^{-1}, pH: 7.7-8.9 and CO_2: 1-2%.
- There are no consensus in the literature on what is an appropriate photoperiod.
- Gross composition differs among micro-algal species. Protein is always the major organic constituent, followed by lipid and carbohydrate (12-35%, 7.2-23% and 4.6-23%, respectively).
- The protein content and quality of all micro-algae is high. Micro-algae have a well balanced amino acid composition and the proportions of individual amino acids do not vary greatly between different species.
- Micro-algae are a rich source of carbohydrate and the carbohydrate profiles can vary greatly between species. The sugar composition is variable; particularly the polysaccharides glucose and mannose, and can in some instances affect the nutritional value.
- Fatty acids constitute a major proportion of the lipid fraction (20-40% of total lipid). The essential PUFAs EPA and DHA are key nutrients in animal nutrition, and most algae are rich in one or both of these acids.

  - Most species contain moderate to high concentrations of EPA. *Pavlova lutheri, P. tricornumtum* and *N*. spp. are rich in EPA, while *I.* sp. (T-iso) contains low levels of this fatty acid.
  - Species that are rich in DHA are *I. galbana, I.* sp. (T-iso) and *P. lutheri*. Only P. lutheri is rich in both DHA and EPA.
  - Green algae like *T. suecica* are usually rich in C16 and C18 PUFAs, in addition to ARA, but low in EPA and DHA. This may contribute to low nutritional value.
- Micro-algae are rich sources of pigments like chlorophylls and carotenoids. ß-carotene is a present in most micro-algae. *Chlorella* spp. and *T. suecica* can also produce significant amounts of astaxanthin and luthein, respectively.
- Micro-algae are rich sources of almost all vitamins, particularly the vitamins C (ascorbic acid), however, some species lack specific vitamins. Because of this, mixed algal diets are necessary to provide high concentrations of all vitamins.
- Biochemical composition of micro-algae can be manipulated by changing the growth conditions, but varies between species.
- The conditions that increase the biomass productivity and the PUFA contents are often in opposition..
- Micro-algae can effectively transfer nutrients to other feed organisms.
- A balanced diet of two or several algal species is recommended in order to ensure the best optimal nutritional value for the feed organisms.
- Micro-algae concentrates are promising products for feeding, greening and enrichment of marine larvae. However, the nutritional quality of the pastes may change during storage and better storage conditions have to be developed.
- Promising new algal species for cold waters are a cold water strain of *P. tricornutum*, the dinoflagellate *Amphidinium* sp. and species from the genuses Pyramimonas, Cryptomonas, Hemiselmis, Mantoniella and Pseudoscourfielda.

Task 2.2 Design optimisation of a CAP

Evaluation of articial light sources

This was undertaken during the first reporting period.

Objective – motivation

Under several climatic conditions (low solar irradiance, long nights, very low temperature) algae production should remain indoors. In this case, artificial light replaces solar radiation. Moreover, prolonging growth during night time is also important under climatic conditions with high solar irradiation. For this reason, the photosynthetic performance of various existing artificial light sources has been investigated as a part of the task 2.2 of the project.

In the present experiment only the algae nannochloropsis occulata was considered. This phytoplankton contains only chlorophyll-a as photosynthetic molecule [1]. Its main absorption lines are at 400, 435, 480, 490, 669, 681 and 690 nm. The absorption in the blue part of the spectrum is more intense than in the red.

Based on this existing information we investigated the performance of four different categories of lamps:

1. Fluorescent
2. High pressure sodium
3. Metal halide
4. LED

Materials and methods

Five light sources were constructed made of

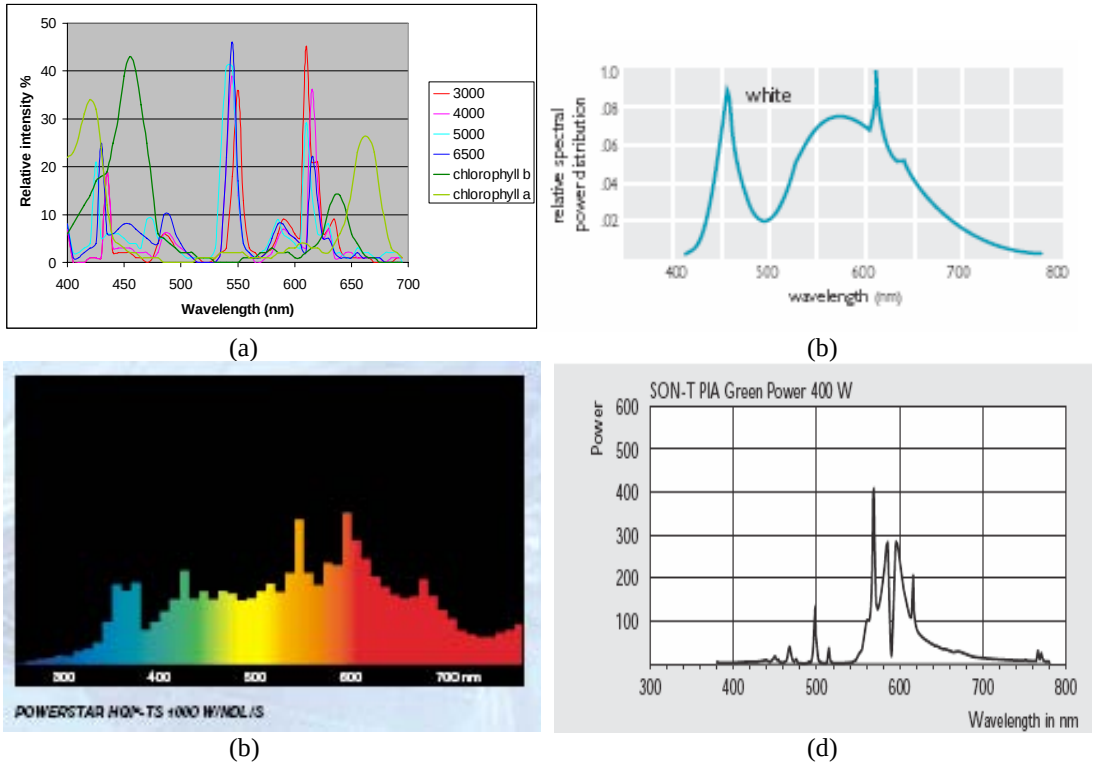
1. Two (2) fluorescent white (colour: 4000oK) 11W
2. Two (2) fluorescent cool daylight (colour: 6500oK) 15W
3. Nine (9) White 12LED spot lamps 1.8W
4. One (1) Metal Halide warm daylight (OSRAM HQI-TS-150W/WDL)
5. One (1) Sodium High Pressure 150W

These light sources were built to provide almost uniform luminance of 6000 lux on a vertical flat surface of dimensions 25x25 cm. Luminance was regulated for all types of lamps by adjusting their distance from the illuminated surface.

The emission spectrum of these light sources differs. Figure 3 presents the spectra of the lamps used in the current experiment.

Two experiments have been performed. In the first, four sets of three PET bottles were used. Each bottle contained a 1l nannochloropsis occulata culture made of the same inoculum. The salinity was 3.5%. Nutrient F2 (100mg/l) was added. Air was added through air pumps at 1l/min. Each set of three bottles was illuminated with a different light source. Luminance was regulated for all types of lamps equal to 6000 lux by adjusting their distance from the illuminated bottles. A fluorescent lamp of white colour (4000oK) was used in this test.

In the second experiment, only four bottles were used, each illuminated by a different light source. A fluorescent lamp of cool daylight colour (6500oK) was used in this test.



(a) (b)

(b) (d)

Fig 3. Emission spectrum of lamps: (a) fluorescent with colour in the range of 3000-6500^{o}K, [3] (b) white LED, [4] (c) metal halide with normal daylight colour (NDL), [5] (d) high pressure sodium [6].

Results

A fast growth was observed for all four light sources (fig. 4). The high pressure sodium (NAV) and the metal halide (HQI) lamps provide a slightly stronger growth.

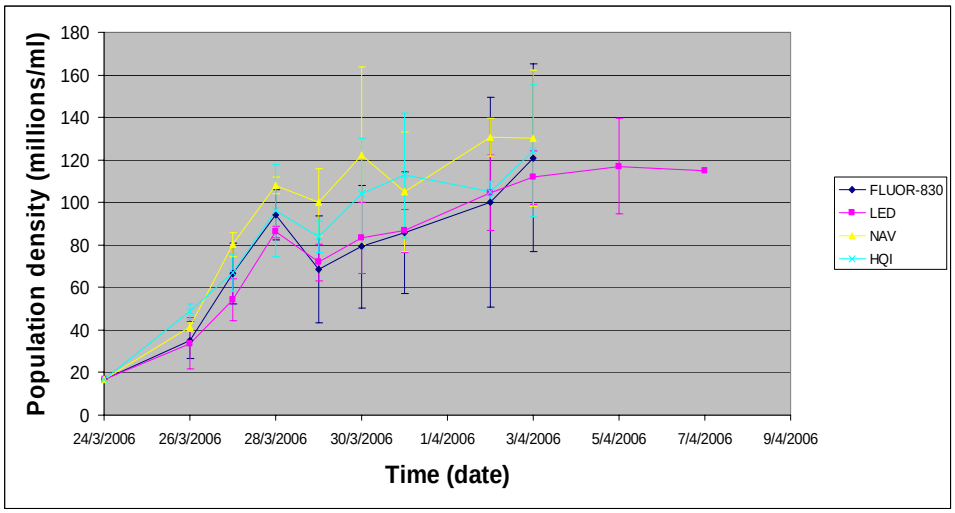


Fig 4. Growth of *nannochloropsis occulata* under four different light sources: FLUOR-830= fluorescent warm white, LED= white LED, NAV= High pressure sodium, HQI= metal halide

However, the quality of the cultures was poor after ten (10) days of batch growth with the exception of the LED. A possible explanation of the brownish colour of the cultures could be the exhaustion of nutrients. The possible lack of important spectral regions in the corresponding light sources could also influence the final quality of the cultures.

Very large standard deviations were recorded among the three cultures illuminated by each light source. The poor quality of these results does not allow reaching a safe result with respect to the photosynthetic performance of the studied lamps.

Energy and cost efficiency of the studied lamps

The photosynthetic efficiency of a lamp can not be easily determined, since different chlorophyll pigments have different absorption spectra. A simplified method for defining the photosynthetic efficiency of a lamp is to characterise it using its efficacy in lm/W. Although this efficacy expresses the performance of the lamp with respect to human vision, it may also be used for the characterisation of photosynthetic efficiency, because PAR roughly coincides with visible light (wavelength 400-700 nm). The lm/W efficacy of lamps available in the market can be obtained for datasheets provided by the lamp producers and are shown in the following table 2.

Table 2. Comparison of lamps with respect to efficacy in visible light emission

LAMP	Efficacy (lm / W)
Fluorescent [3]	89-104
Metal Halide [5]	80-100
Sodium High Pressure [6]	130-147
LED [7]	32-80

The possibility of using LEDs as a replacement of incandescent light bulbs or fluorescent lamps in algae production systems has been discussed widely lately. LEDs used for illumination applications are known as solid-state lighting (SSL) and are packaged as a cluster of white LEDs grouped together to form a light source (pictured). LEDs are moderately efficient: the average commercial SSL

currently outputs 32 lumens per watt (lm/W), and new technologies promise to deliver up to 80 lm/W. The long lifetime of LEDs make SSL very attractive. They are also more mechanically robust than incandescent light bulbs and fluorescent tubes. [7]

SSL (LEDs) are more efficient than incandescent bulbs, which generate 16 lm/W (domestic tungsten bulb) to 22 lm/W (halogen bulb) [7]. Fluorescent tubes are more efficient, providing 80 to 100 lm/W, but are bulky and fragile.

High pressure sodium lamps provide very high efficacy in visible light emission. However, this does not correspond to high photosynthetic efficiency, because they emit mostly in the orange-yellow part of the spectrum. Thus the photosynthetically important blue and red parts of the spectrum are weaker. In particular, the blue component of the spectrum is very weak in these lamps, while it is very important for algae growth.

Metal halide lamps provide similar efficacy and spectrum type with fluorescent lamps. Their main difference is that they can generate high intensities (400-600 W per lamp), while the power of a typical fluorescent tube is 58W.

The cost efficiency of lamps can be expressed in power consumption per price (W/euro) and some values concerning market prices in Greece are shown in the following table 3.

Table 3. Cost efficiency of lamps

LAMP	**Cost (W / euro)**
Fluorescent	6.5
Metal Halide	5.0
Sodium High Pressure	5.0
LED	0.2

The above cost analysis is based on the cost of the lamps purchased for the purpose of the experiments described in this report.

Conclusions

Although the experimental data obtained through the above described experiments could not lead to safe and detailed evaluation of the various tested lamps, a few rough conclusions can be reached.

- The Sodium High Pressure lamps are the most efficient with respect to visible light emission. However, their photosynthetic efficiency is not higher than the other lamps, because the blue part of their spectrum is weak.
- Sodium and metal halide lamps can provide high intensities. For this reason, they are used for the illumination of roads and other public places. High light intensities are not required in algae production.
- SSL lamps made of LEDs provide sufficient light intensity for photosynthesis but they are very expensive compared to other lamps.
- Nannochloropsis, contrary to land vegetation, needs more blue than red light. This is probably an acclimatisation effect in the aquatic environment, where water absorbs stronger the red than the blue light. Therefore cool daylight fluorescent lamps give better results than warm-white lamps.
- The best choice for an artificial light source to be used for algae production is the fluorescent cool daylight lamp (colour: 6500oK) or the FLUORA (OSRAM) lamp [8], depending on availability and prices.

Design of prototype CAPs

Four small-scale CAP prototypes were built and tested during the first reporting period. In this way various important characteristics of the photo-bio-reactor have been explored. Moreover, the effect of existing artificial light sources to algae growth has been investigated by a lab experiment.

Pilot 'Boas' CAP system coil type photobioreactor system

Design concept

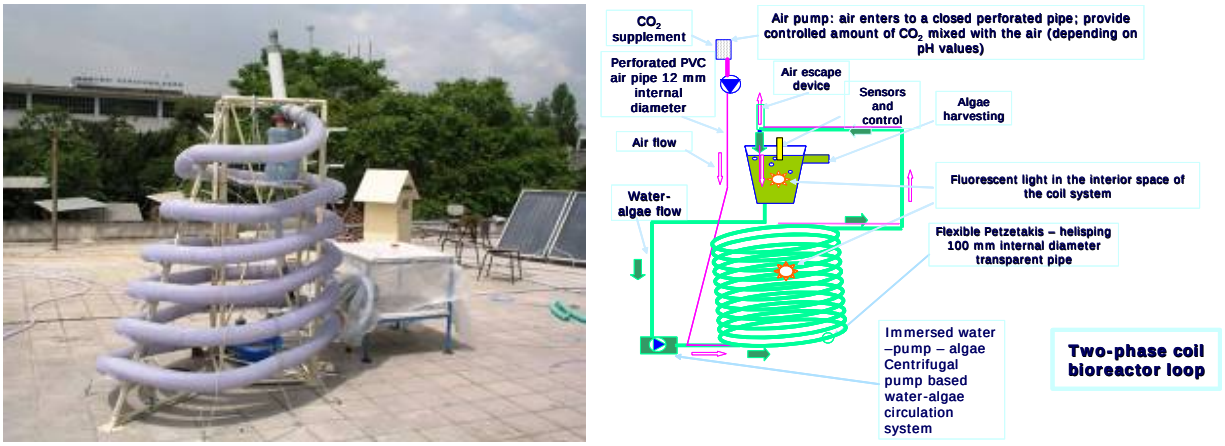
An innovative two-phase design of a coil type system suitable for the continuous production of algae under a combination of natural light and artificial light (or even under controlled conditions) has been designed in the framework of the Alfa project. This new design of a coil type system, named 'Boas' CAP system is based on the following basic principles:

- Achieve full exploitation of solar radiation for maximum algae production
- Develop a cheap and simple system with optimised design parameters
- Allow for an optimal use of space through the geometric characteristics of the design
- Design a modular and versatile system that may be adapted to include artificial light (supplementary or main)

The design allowed further investigation and possibly modification or optimization of some of the design parameters of the system. The main optimisation parameters of the system included:

- Flow characteristics of the two-phase system
- Cooling system design alternatives
- Complimentary artificial light system
- Possible supply of CO_2 through the air pipe.

The two-phase system consisted of two parallel flows: a) the culture –media- flow forced by a pump through a large diameter pipe and a tank and b) the air flow, released through an internal smaller perforated pipe continuously to the media flow along the whole length of the coil. Full details of the system are given in D5 (Section II).



Design and construction of the pilot-scale boa photobioreactor

Pilot photobioreactor using thin transparent pipes

A 30 l photo-bioreactor was built for use under outdoor conditions (solar light). It consisted of 40 tubes of 14 mm inner diameter made of transparent PVC. The length of the tubes was 1m. Their total volume is 5.5 l. The thin tubes were connected to four manifold tubes (32mm diameter) made of black LDPE. A tank representing the dark phase was connected to the manifolds and the pump by

flexible tubes of 50mm diameter. The flow was controlled by a pump with flexible (plastic) rotating impeller. It provided a flux of 115 l/min at 900 rpm.

A similar prototype was designed and constructed for indoor operation using artificial light. Four tubular fluorescent lamps (58W each) were used for illuminating the thin tube light collector, while the tank was illuminated by two 20 W fluorescent spot lamps.

Design and testing of the thin tube bioreactor outdoors and indoors with artifical lights

This system failed to produce high algae densities and the culture collapsed a few days after inoculation. The conclusions are described below:

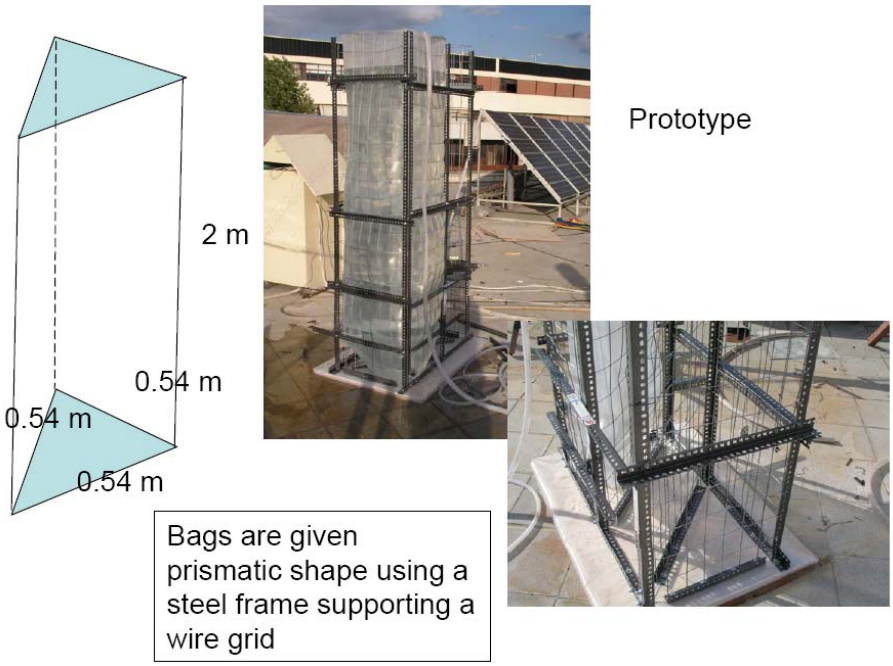
- The indoor system performed better than the outdoor one. A possible reason for this is photo-inhibition due to the high intensity of the solar radiation during the initial growth stage when the culture is thin. Therefore optimization of the orientation and inclination of the thin-tube solar collector to enhance light interception is **not** necessary.
- A 15 s light-dark cycle can not prevent photo-inhibition and does not improve enough photosynthesis in order to compensate for the stress due to fast circulation. On the contrary, frequent passage of the cells through the pump resulted into severely damaging the algae. This is in agreement with similar observations reported in two other marine microalgae species (Skeletonema costatum and Haslea ostrearia).
- Low Reynolds number in the pipes is not sufficient for reducing stress due to flow.
- The inclination of pipes combined with the upward flow of the liquid prevents the deposition of algae on the illuminated surface of the tubes.

Pilot Photobioreactor triangular sacs

Using large volume low-density cultures is another option for achieving the algae production, which is necessary for covering rotifer and larvae needs. A system was developed based on the conventional plastic bag batch production system, which is modified to become continuous. Moreover illumination efficiency is improved by modifying the shape of the bioreactor from cylindrical into prismatic by using a steel frame supporting a wire grid. In order to eliminate stagnant regions and stratification at the bottom, a water pump is used to pump the culture from the bottom to the top. An air pump is providing the necessary CO_2 to the culture, while the air flow also contributes in better mixing.

A single 200 liter prismatic photo-bioreactor was built and tested for the production of nannochloropsis occ. in artificial light conditions. It reached a maximum density of 50-60 Mcells/ml, which was almost half of the expected density. The reason for this poor performance is probably due

to the inefficient mixing and air supply. Therefore further optimization is required but this is not possible in the framework of the current project due to lack of time and funds. For these reasons, it has been decided that the proposed upscaled system will not be built through the ALFA project.



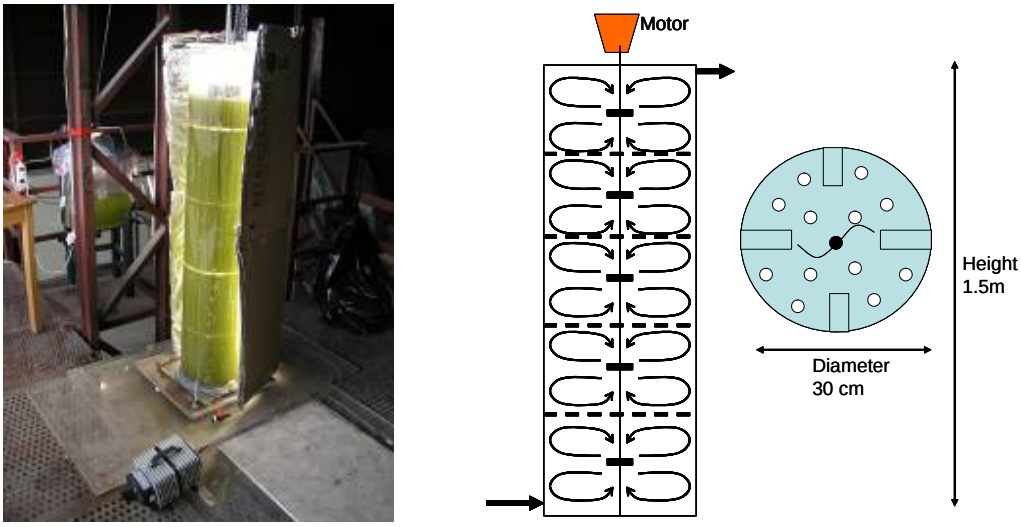
Design and testing of the prismatic photobioreactor

Pilot Photobioreactor Cylindar type

A novel cylindrical photobioreactor with axial agitation was designed, constructed and tested for growing Nanochloropsis sp. This photobioreactor is basically a transparent cylinder lighted from the outside, with an agitator containing several curved propellers. Upon rotation, the propellers project the algae solution radialy from the centre to the wall of the cylinder where the light source is located. It is claimed that this radial movement increases the productivity of the photobioreactor by moving the algae from an area where the light cannot penetrate to the walls of the cylinder that are lighted. The intense agitation, finely disperses the CO_2 enriched air increasing drastically the gas-liquid interface and consequently reducing the CO_2 consumption. An economic comparison of this photobioreactor with a widely commercial photobioreactor (BioFence) is attempted and shows that the higher productivity and lower CO_2 and air consumption of the new photobioreactor produces algae 20% more economically.The compact geometry of the cylindrical photobioreactor provides reduced footprint.

The prototype photobioreactor was built from plexiglass. The plexiglass cylinder measures 30 cm in diameter and 1.5m high. It has four horizontal plexiglass diaphragms with holes that separate the cylinder into five communicating compartments. The cylinder has in its centre an axle made of stainless steel and has five curved agitator blades placed in mid distance between the horizontal diaphragms. The blades are curved to move the liquid radialy from the axle to the cylinder surface and back. A motor of variable speed controls the agitation speed between 0 and 11 rounds per second. Four plexiglass vertical baffles running symmetrically along the cylinder prevent the circular movement of the liquid during the rotation of the agitator. Air enriched with CO_2 is introduced at the bottom of the reactor through two submersed porous stone distributors. The photobioreactor is surrounded by eight 58 W fluorescent lamps 1.5 meter long. The intensity of the light at the surface of the cylinder was measured with a luxometer. It varies from 5,500 to 19,000 lux (depending on the

position of the wall from the lamps). The average is close to 15,700 lux. Harvesting is done from the top of the reactor while the fresh nutrients are introduced from the bottom.



Design and testing of the large bore tubular photobioreactor

The experimental results showed that an increase in the agitation speed results in faster growth and in higher stationary phase algae concentration. This can be explained since at low algae concentrations the light penetrates through the entire cylindrical reactor and the Nannochloropsis sp growth is unimpeded. As the concentration increases, the light penetration drops and the growth takes place only in the annular zone where the light is able to penetrate. By forcing the algae to penetrate the lighted zone through radial movement, it is possible to maintain its growth. The higher the agitation speed the more frequently the algae cells penetrate the lighted zone leading to higher growth rates and higher final algae concentrations. The agitation has equivalent effect to increasing the light penetration in the reactor. The photobioreactor without agitation reaches concentrations bellow 100 million cells per millilitre, while at high agitation (11 rounds per second) the concentration exceeds 350 million cells per millilitre.

During the second reporting period the pilot-scale boa was used to test the culture of a second algae species Isochrysis. Full details are given in D5 (Section II)

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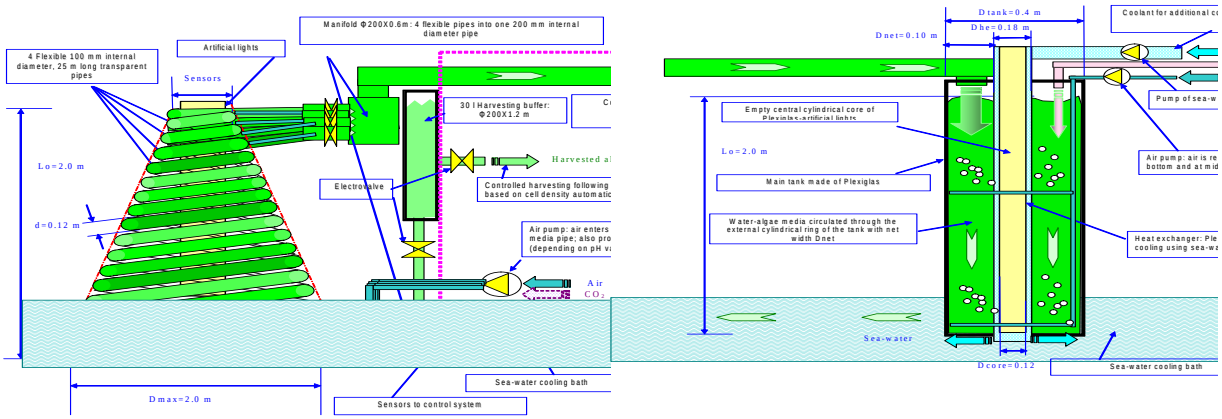
Up-scaling photobioreactors from pilot-scale to full-scale

During the second reporting period the 2 most promising design (Boa and tubular) concepts were up-scaled to close to full commercial size.

Upscale 'Boas' CAP system

The up-scale 'Boas' CAP system has been redesigned, based on the pilot design findings (see D5-Section II), so as not to change its main principle of operation and on ArgoSaronikos needs as these were determined by the consortium (see D5-Appendix A). The basic design adaptations concern the total volume of the system and its optimization with respect to its exposure to natural and artificial light and the continuous along its whole length air supply, in order to obtain as much possible quantity of algae, using the same area of installation. Full details of the system are given in D5 (Section III).



Design of the full-scale boa phytobioreactor

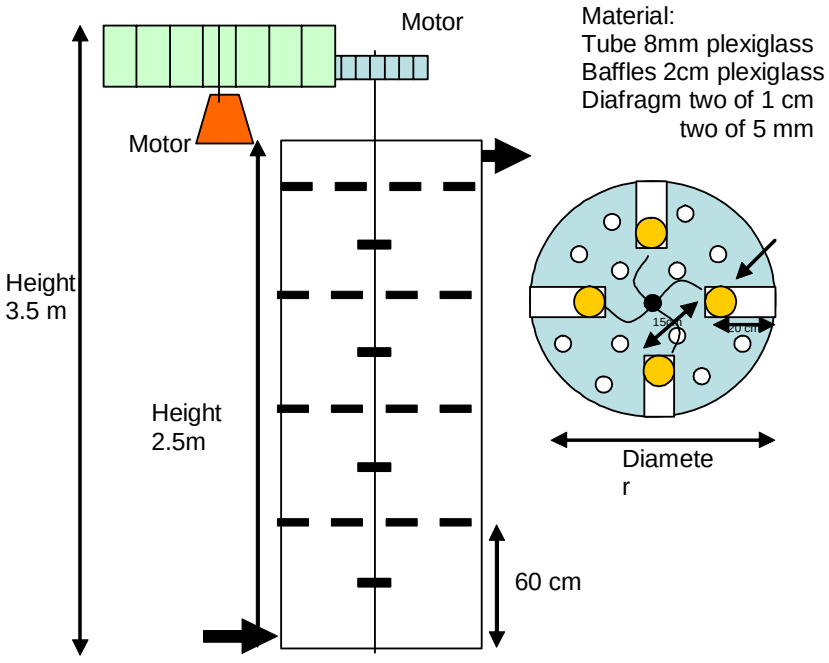
The full scale Boa was constructed at HESY, Holland and transported and erected at Argosaronikos, Greece.

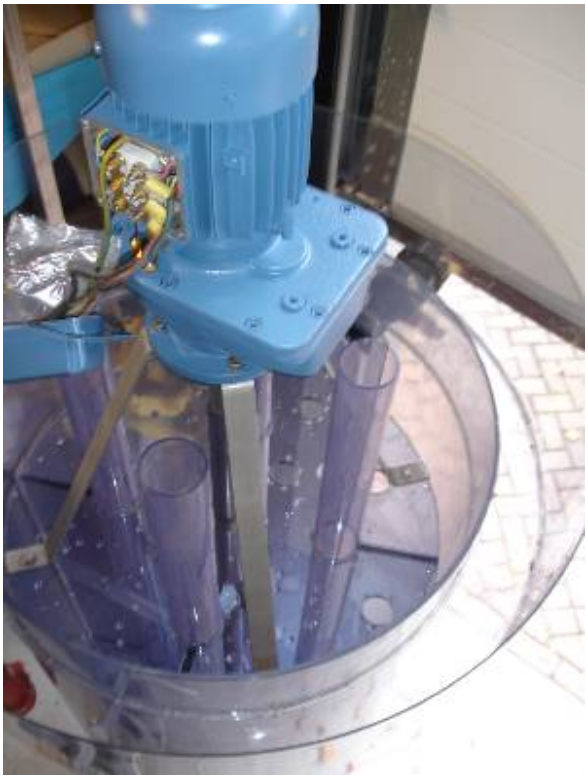
Boa operational durin daylight

Boa operational at night.

Upscale 'Tubular' CAP system

Based on the experimental performance of the indoor cylindrical photobioreactor with axial agitation and by adopting very conservative estimates, we proposed two scale-up designs; one for an indoor unit and one for one outdoor unit. The two designs differ on the artificial light and cooling requirements as well as on the spatial arrangement of the units.





Details of the full scale tubular photobioreactor

Double phtotbioreactor with and with out internal lights on

Full design details of the system are given in D5.

The full-scale tubular photobioreactor was constructed at HESY, Holland and transported and erected at SagaFjord, Norway.

Task 2.3 Monitoring and Control of the CAP system

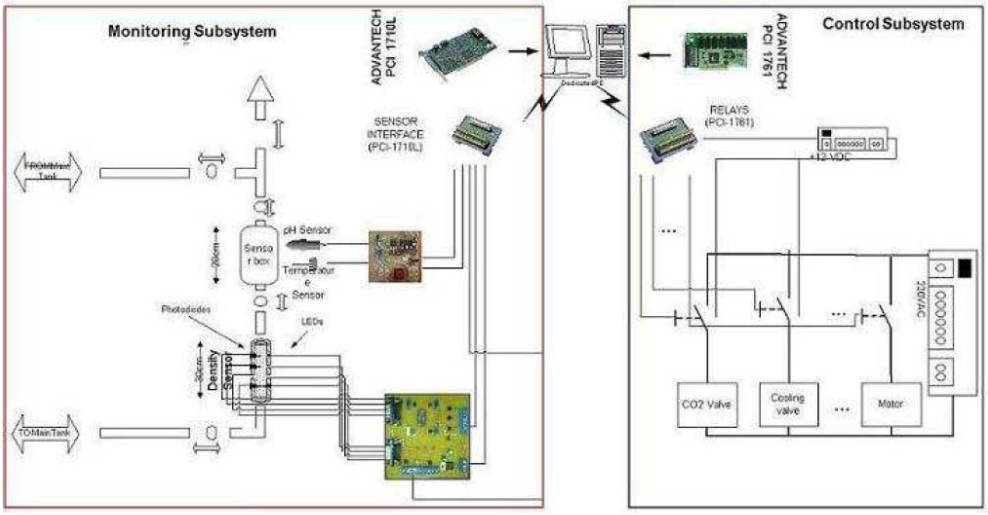


Figure1:Schematic diagram of the monitoring and control subsystem

The automation required for the operation of the CAP subsystem is provided by a PC based control system. The activation and driving of the control functions will be done with the use of some industrial control cards installed in a PC backplane. Several sources of data have been studied, in order to acquire sufficient information that has been used as a basis for our design and development of the computerized monitoring and control system for managing the CAP unit. These include research of the current trends in modern industrial setups, review of the control systems already in use at the sites of ALFA partners as well as discussion of the draft design and interaction with the SMEs in an attempt to continuously improve the design and address many nontrivial issues.

Detailed designs of the hardware and software parts of the automation systems have been initially developed. Based on these designs, prototypes have been constructed and installed. Special purpose controlling software has been also developed. The designs are flexible and the computer software has been designed and implemented as modular as possible, to allow easy modifications, in order to address issues that arise during infield usage in the SMEs hatcheries.

This report contains technical information on the monitoring and control subsystems of the CAP unit. The information herein describes and enumerates the components which are required for one monitoring subsystem and one control subsystem. It is important to mention that both subsystems require one dedicated PC with two available PCI slots. This PC will run the software developed by the CTI, which performs all the monitoring, data logging and control functions.

Monitoring subsystem

The monitoring subsystem consists of:

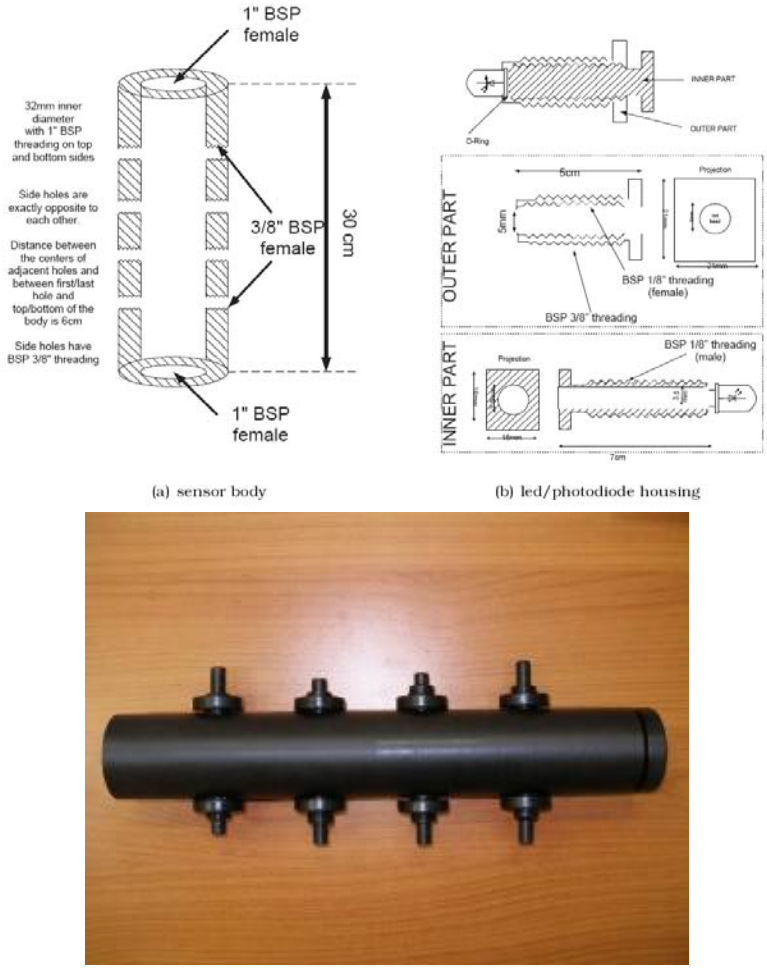
- One custom made sensor, appropriate for measuring algae density.
- Two commercially available sensors, one for monitoring pH and one for temperature.
- Two custom made electronic boards and wiring, one for driving the algae density sensor and one for the temperature and pH sensor.
- One commercially available electronic PCI card that is installed on a computer, which provides analog input channels for reading the output of the sensors.
- One bypass structure, which attaches on the main tank of the algae production unit and hosts the sensors

Design of a new density sensor

In deliverable 6 we provide details for two CTMS sensors that were developed and tested. Their principle of operation was based on the observation that the algae concentration can be estimated by measuring turbidity. Experiments with two different types of algae indicated that both sensors had satisfactory performance in estimating the density of the culture. The two sensors differed in the way that could be mounted on a continuous algae production system, and in the dynamic range of their output values. The first sensor, mentioned as the non flow-through sensor, required an extra control unit, that will be responsible for the refilling and the evacuation of the receptacle with the sample. The other one, mentioned as the flow-trough sensor in contradiction to the aforementioned was suitable for on-line measurements. It could be mounted on a tube for continuously monitoring the algae concentration, as it passes through the tube. Furthermore, the wide output range of the non flow-through sensor made the interconnection of the sensor with other electronic devices possibly more complex. The above facts indicate that the flow-through sensor is a more appropriate device for continuously monitoring the algae growth.

A new flow through design that incorporates 4 pairs of sensors in one device has been designed. It aim at the exploitation of diverse measurements that arise from the use of different light sources. This prototype was constructed by a mechanic and some sketches along with pictures are shown in Fig. 2. The sensor's colour has been selected to be black, to reduce the reflections that may occur in the internal side of the sensor body
In these design all the photoresistors have been replaced by photodiodes

- Photodiodes give robust & repeatable readings regardless of manufacturing imperfections (distance & angle perturbations).
- The use of photodiodes simplifies the sensor's construction, since they have exactly the same physical structure with Leds.



(a) sensor body (b) led/photodiode housing

Figure2: Sketches and photo of the sensor body and led/photodiode housing

Design & construction of custom made electronic boards for the interconnection of the sensor to the PC card

In order to make accurate and repeatable measurements, a sensor needs to be driven by a special circuit that drives the excitation and/or the sensing components, in order to make the readings both repeatable and comparable. The algae density meter is a custom made sensor and thus we needed to design and implement a special circuit for driving and controlling this sensor. This circuit needs to provide the following facilities to the sensor:

- Ensure that the luminance of the LEDs is stable. The datasheets of the LEDs (see deliverable 6) that have been used in our design suggest that the luminance of the LEDs is controlled by the electrical current that flows through them. Thus, the circuit has to keep this current as stable as possible during operation, and also provide the sensor with the same current value every time that it is powered on.
- Provide the operator with a way to fine-tune the luminance of the LEDs. This is an important facility for any calibration process, because it allows the operator to control the output of the sensor and adjust it as required for a known concentration.

- Ensure that the leds are turned on sequentially, and four different interference free, output values are obtained. This means that each output value is affected only from the light that is emitted from the turned on LED. As it was mentioned above and is analyzed in deliverable 12, one can take more accurate measurements by exploiting the diversity that is introduced from the different LED-photodiode pairs.

The prototype that was designed and constructed for driving the CTMS has the following key characteristics:

- It can be powered with a 12V DC voltage provided by the Data Acquisition Card (PCI – 1710L)
- 4 calibration trimmers let the user adjust the current that supplies 4 Leds (the Leds must be provided with constant current of 20 mA)
- Supplies power to the photodiodes & converts the output current of the photodiodes to voltages.
- Provides a control unit that allows the leds to be turned on or off by the software

In Figures 3, 4, a prototype of such driving circuit is shown.

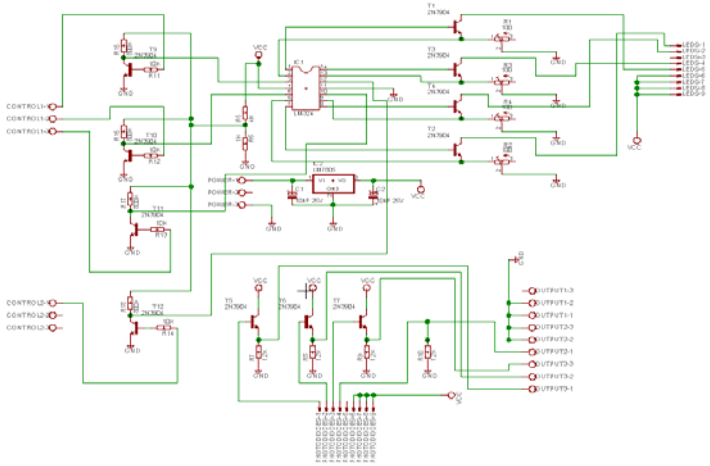


Figure3: Schematic diagram of the pH transmitter and the pH probe

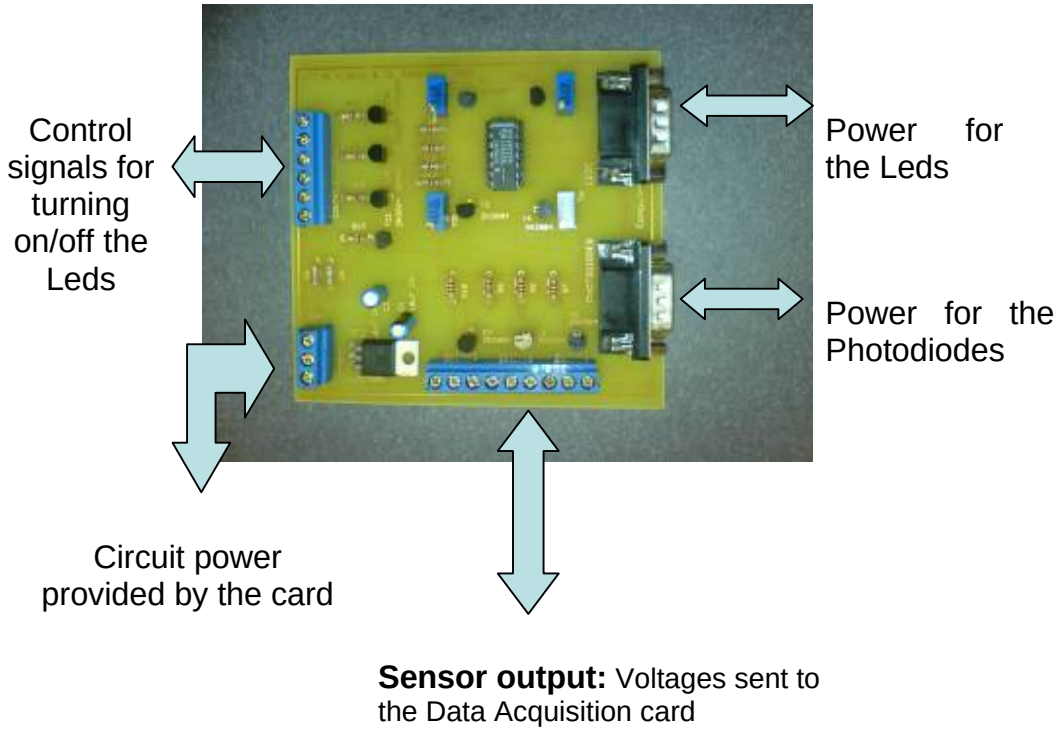


Figure 4: CTMS Driving circuit

Exploitation of the diverse measurements

The CTMS unit consists of four cascaded Led/Photodiode pairs. The output of each pair and the turbidity (or concentration) of the phytoplankton are related through exponential processes (see deliverable 6). Initially we calculated the exponential curves that fit the measurements that we took in the Lab with Nannochloropsis. These curves are presented in figure 5. We repeated the above experiments with different samples and observed that the infrared led photodiode pair gives robust & repeatable readings regardless of manufacturing imperfection (distance & angle perturbations), by paying the price of a less accurate exponential fit. Furthermore, the Red, Blue LED photodiode pairs offer higher resolution by achieving better exponential fit by paying the price of high sensitivity to perturbations. The use of data fusion techniques, may lead to more accurate results by combining the outputs of the different sensors.

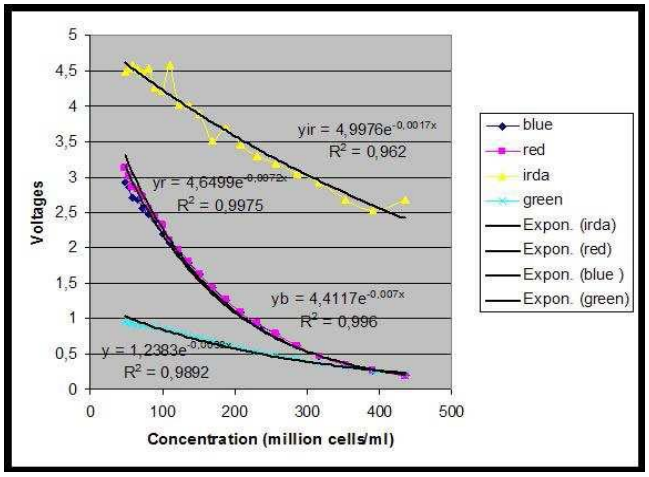


Figure 5: Exponential curves that fit the measurements for the different Led/photodiode pairs.

For each sample we get four different measurements and we form a 4 × 1 vector:

$$S(c) = \begin{bmatrix} B(c) \\ G(c) \\ R(c) \\ IR(c) \end{bmatrix},$$

where B(C), G(C), R(C), IR(C) are the output voltages that correspond to the photodiodes responses opposite the blue, green, red and infrared led respectively and C is the concentration of the sample inside the CTMS sensor. The objective is to obtain the estimation of C by using the 4 above measurements. This seems to be a pattern recognition problem that can be easily solved with use of a neural network.

Figure 6: Reduce estimation problem into a pattern recognition problem

In order to solve the problem of estimating the concentration we employed a multilayer feed-forward neural network with four inputs (B(C),G(C),R(C), IR(C)), one hidden layer and one output layer as the one presented in Fig. 7.

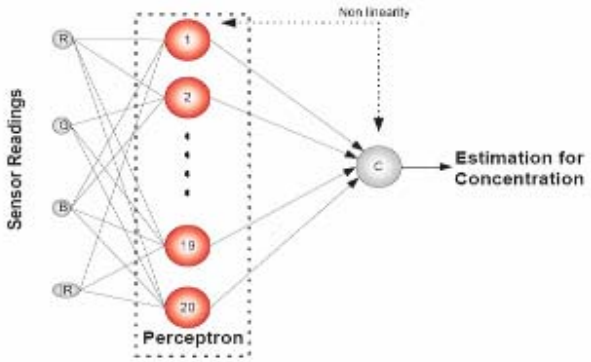


Figure 7: Architecture of the fully connected feed-forward Neural Network with one hidden layer and one output layer.

Sensors for measuring pH and Temperature

The pH meter has been purchased from Oxyguard. The pH probe is connected to the Oxyguard Alpha transmitter. The Oxyguard pH Alpha is a single channel pH measurement transmitter with manual or automatic temperature compensation. The temperature can be shown on the display. It has a 420mA analog output. The unit is a simple, accurate and very rugged pH measurement unit intended for function as a primary measurement device in industrial or other control and monitoring systems. It is built into a solid, waterproof metal cabinet with a clear plastic lid covering the front panel. In Fig. 8 we present some photos of the pH transmitter and the probe that have been purchased by Oxyguard.

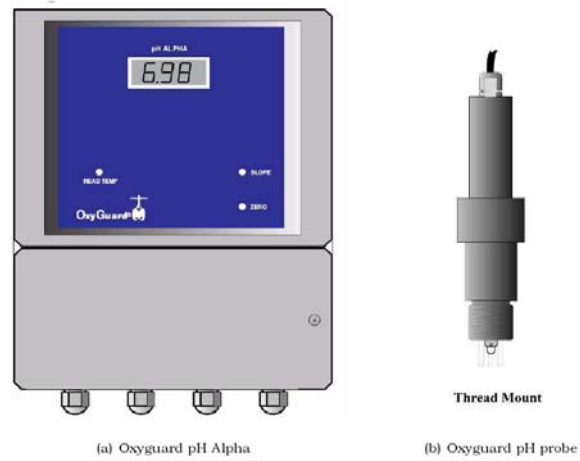


Figure 8: Schematic diagram of the pH transmitter and the pH probe

The temperature meter is a PT100 temperature probe. It is a stainless sensor that operates as electrical resistance, which varies its value according to its temperature. The probe comes together with a 4-20 mA transducer, for connecting it to the analog input of the data acquisition card. The transducer has been programmed to function in the range $0 - 45^0$C and the probe must be thread mounted on the bypass system with an R 1/2" BSP thread. Thus, we have used a stainless steel case for the thermoresistor and the transducer with threading, which offers reliable sealing against both negative and positive pressures. The schematic diagram of the case with the PT100 sensor is presented in Fig. 9

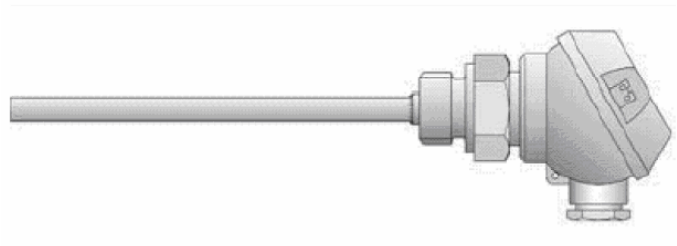
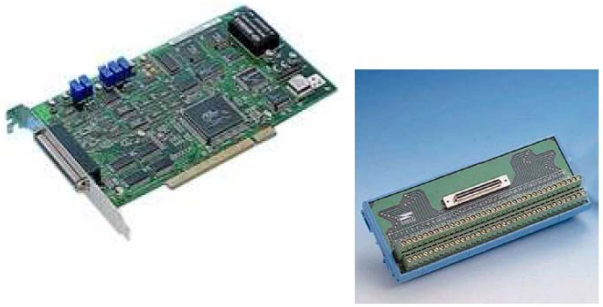


Figure 9: Schematic diagram of the temperature sensor.

For interconnecting the pH and temperature probes to the data acquisition card we constructed a small circuit for transforming the output currents to output voltages.

Data acquisition Card



(a) PCI 1710L Card (b) ADAM-3968 wiring terminal

Figure 10: Data acquisition card and wiring terminal

The data acquisition card is an Advantech PCI1710L card. It is a multifunction card for the PCI bus, appropriate for reading the outputs of the sensors. Its advanced circuit design provides higher quality and more functions, including the five most desired measurement and control functions: 12bit A/D conversion, D/A conversion, digital input, and counter/timer. Its sampling rate covers the needs of the project and includes shortcircuit protection.
In the four double ended channels, the four outputs of the CTMS driving circuit that corresponds to the response of the four photodiodes, are connected. Two extra single ended channels are used for reading the output of the temperature and pH driving circuit. The connections between the driving circuits and the cards are made trough a wiring terminal that is connected on the PCI 1710L card. In Fig. 10 we provide some photos of the data acquisition card that is installed in a PCI slot of a PC and the wiring terminal that is connected through an industrial cable to the PCI1710L card.

Attaching the sensors to the CAP

In order to attach the three sensors to the continuous algae production system we constructed a bypass system that consists of a sensor box, the CTMS measurement system and some plastic pipes. Two valves have been used for isolating this system from the main tank, for cleaning purposes. The temperature and the pH sensors are threaded on the sensor box and the CTMS system is pipelined to the box. The sensor box has been made transparent, so that one can easily verify that the algae inside the bypass system is recirculated and its cover can be easily removed for cleaning purposes. This system was also constructed by a mechanic and has been installed in Salamina. A detailed sketch and some images of the actual construction installed in Salamina is shown in Fig. 2.8.

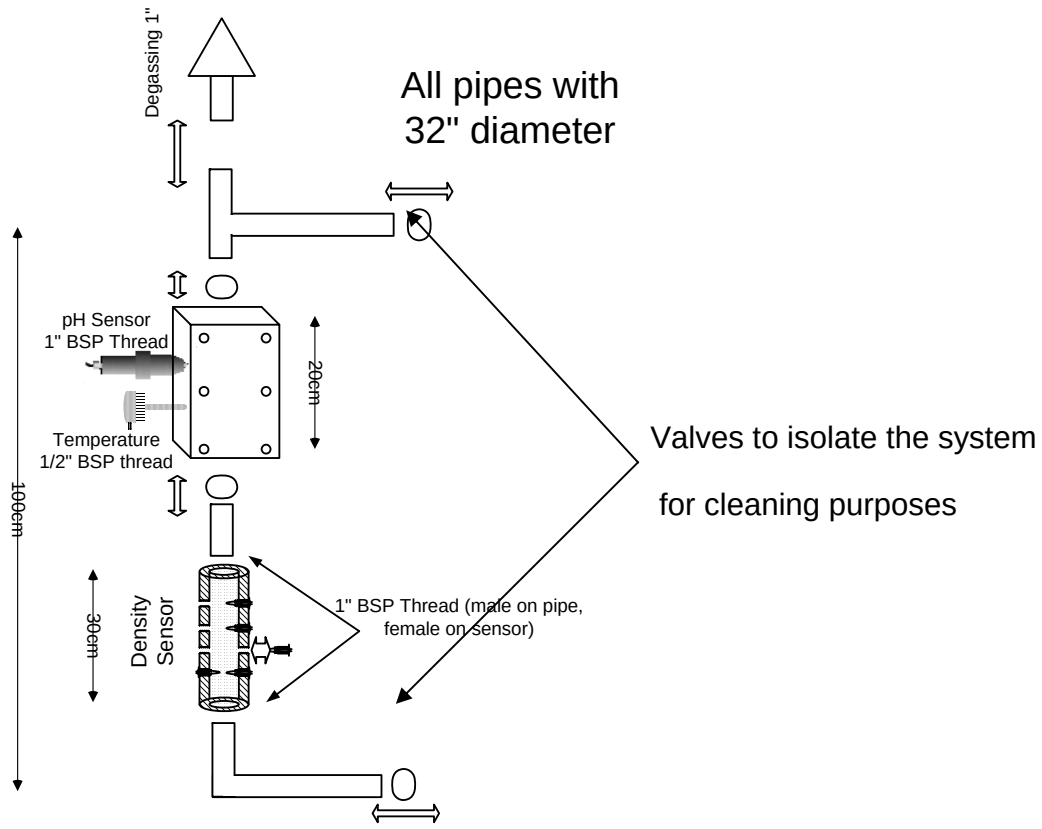


Figure 11: Sketch of the Bypass system that was constructed and installed in ARGOSARONIKOS

Control Subsystem

This part of the report is dedicated on the appropriate hardware parts for maintaining the appropriate environmental and cultivation parameters. These hardware parts that comprise the control subsystem are listed below:

- One commercially available electronic PCI card that is installed on a computer (relay output card) .
- Eight electrical relays .
- A 12Volt power source capable of supplying DC current of at least 1 Ampere.
- Cabling

The functionality of both the monitoring and control subsystems is performed by the central computer system. The computer, through software, gives commands to the two subsystems and reads their feedback. The tasks performed on the control system include:

- Controlling on/off devices such as electric pumps and electrovalves. .
- Determining actions that need to be taken in order to regulate the pH within the acceptable limits set by the operator, based on the feedback of the monitoring subsystem and a predefined algorithm.
- Determining actions that need to be taken in order to regulate the temperature within the preset limits, based on the feedback from the monitoring process.

Electric pumps and electric valves are turned on/off by electrical relays. More specifically, we need one electrical relay dedicated for the regulation of pH, one for the regulation of temperature and some relays for performing the harvesting according to the measurements that were taken with the use of the CTMS unit. Further details on the algorithms that specify the actions that need to be taken to perform harvesting and maintain the environmental parameters within predefined limits are given in deliverable 13. Thus, we need eight relays in total, to take the actions mentioned above.

At this point it is important to note that the the functionality of both the monitoring and control subsystems is performed by the central computer system. The computer, through software, gives commands to the two subsystems and reads their feedback. Thus, we purchased a relay output card from ADVANTECH (PCI1761) with eight relays that can be controlled by the dedicated PC. It is an add on card for the PCI bus and it can control up to 8 electrical devices. We also purchased the following peripherals: .the wiring terminal ADAM3937 and a cable (PCL101373) for the interconnection of the PCI card and the terminal

The relay card and the wiring terminal are shown in Fig. 12.

Figure 12: Pictures of the PCI Relay card and the wiring terminal for this card

We have used a second layer of electrical relays to switch on/off the different devices. The card relays control the next layer of relays that are able to switch on/off 250V/30A AC currents, when powered with 12V DC. Thus we have also purchased a power source, to power the relays through the relays of the card, in order to switch the current. Any power source able to deliver 12VDC and at least 1 ampere should be adequate.

Finally, we mention that for the interconnection between the wiring terminal relays, and relays devices we used 3conductor cables for electricity, with sufficient lengths.

WORKPACKAGE 3.

Design and development of a Computerised Harvesting, Transferring and Packaging (CHTP) system for algae.

Work undertaken and important findings

Task 3.1 Design and development of a harvesting, temporal storage, and transferring system linking the new CAP system with the other modules of the hatchery

In the first reporting priod a literature review was undertaken for algae harvesting methods and algae preservation methods. In addition, the most advanced transferring system of the SME hatcheries (Partner SF) was analysed and was a bench mark for the design of the new system.

One of the main objectives of the ALFA project was to develop a computerised harvesting, transferring, and packaging system for algae. To achieve this, the project has developed a monitoring system for the continuous algae photobioreactor and a control system for managing the algae production, harvesting, and transferring process.

The hardware and software control mechanisms are described in the following Deliverables;

- Deliverable No. 10 Data Logging Unit based on computer controlled feedback systems.
- Deliverable No. 11 Special purpose hardware solution including the density sensor, interconnection printed circuit boards.
- Deliverable No. 12 Signal Processing Unit which was implemented as computer software
- Deliverable No. 13 User Interface Software a computer application for the control of harvesting.

These innovative electronic hardware and software modules allow continuous and automatic transfer of the algae production to the other components of the hatchery and/or the Concentration and Storage Unit.

A comprehensive review of the equipment, methods available for the harvesting, transfer and packaging of live algae was undertaken and recommended the most appropriate system so that the computerised harvesting, transferring, and packaging system could be designed and installed in the hatchery facility of the SME partner Argosaronikos.

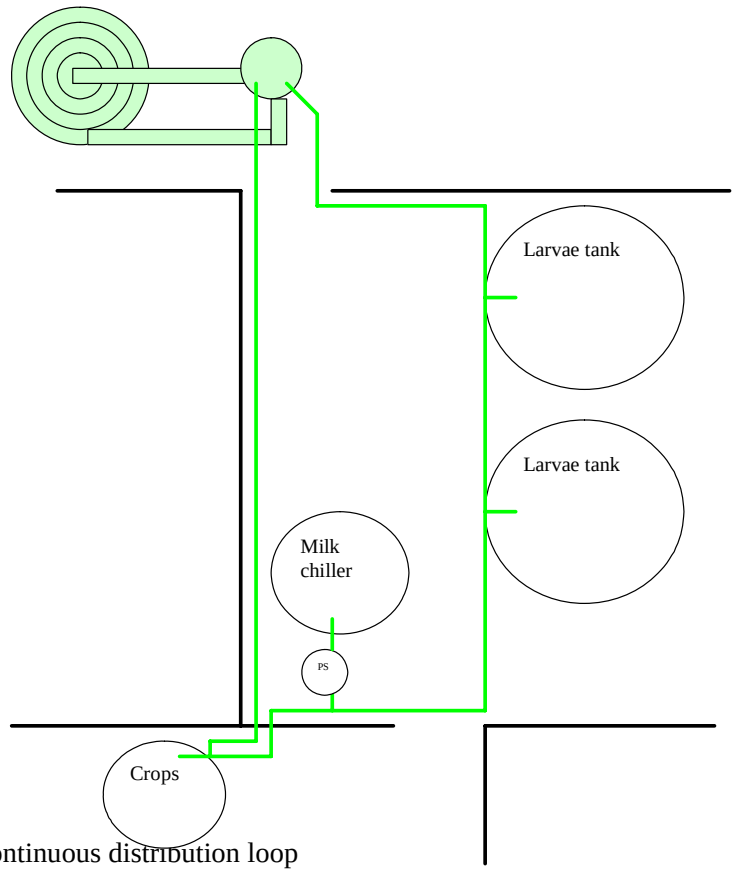
The protocol relied heavily on a comprehensive study done by Heasman et al. who evaluated the harvesting, preservation and storage of various species of live algae. This allowed us to select the most appropriate methods to test without duplication or repeating previous research. In addition, the systems used in the partner SMEs were analysed and the best were adapted for the test facility.

The production of algae from the photobioreactor is pumped through insulated pipes to the rotifer tanks, larval atnks and protein skimmer and back into the bioreactor. The density of the algae in the reactor is controlled by the algae density sensor which monitors the debnsity of algae and if the density reaches above a set point an electrovalve is opended to the protein skimmer and the temporary clilled storage tank (milk chiller). This tank has a capacity of 300

liters and will be able to store a full day's production from a 1 m3 bioreactor. This tank stores the excess concentrated live algae.

Electrovalves can be used to control the dosing of the CROPS and larval tanks (possibly controlled by an algae density sensor) with the remaining unused algae returning to bioreactor.

Integration of subsystems with the photobioreactor
The up-scaled 'Boas' CAP system was connected with a 300 l cooling storage tank (milk chiller) as well as with the CROPS and the larvae tanks (for the greening effect) through a continuous distribution loop from the CAP system and back to it.



Continuous distribution loop

Temporary storage
Harvested algae will be pumped to a chilled buffer tank comprising of an adapted fresh milk chiller tank.

300 liter milk chiller installed at Argosaronikos with concentrated algae.

Circulation pipe

The pipe is small bore 32 mm PE pipe to reduce the quantity of algae circulating, isolated with 15mm isolation material to reduce heating or cooling during the circulation through the hatcery.

Electrovalve

Initially the control system to the larval tanks and rotifers was manual but it would be possible to use an electronically controlled solenoid to dose algae to CROPS and the larvae tanks using solenoid valves such as those manufactured by Burkert: 24V, 0,124 A, PN0-3 Bar.

Example of an electronically controlled solenoid valve at SagFjord

Larvae tanks with live algae distribution system.

The distribution system is still under development and the manual valves will be replaced by electrically controlled solenoid valves controlled by the computer system.

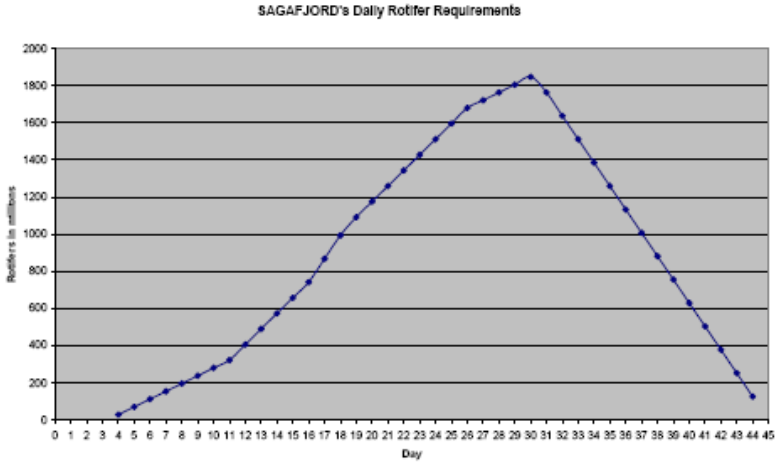
Task 3.2 Linking CAP with existing production systems for rotifers adapted to cold, temperate and tropical conditions

Several systems are available for the continuous production of rotifers using recirculation technology. Two are produced commercially in the USA, while an Australian design is available for private commissioning and one system developed by the University of Gent is not yet commercially available. Therefore it was decided by the partners of the ALFA project to manufacture our own system based on these well-publicised systems so that we would be able to test it in line with our algal production system. The sizing of the CROPS would reflect the rotifer requirements as calculated from the stochastic model presented in deliverable 1.

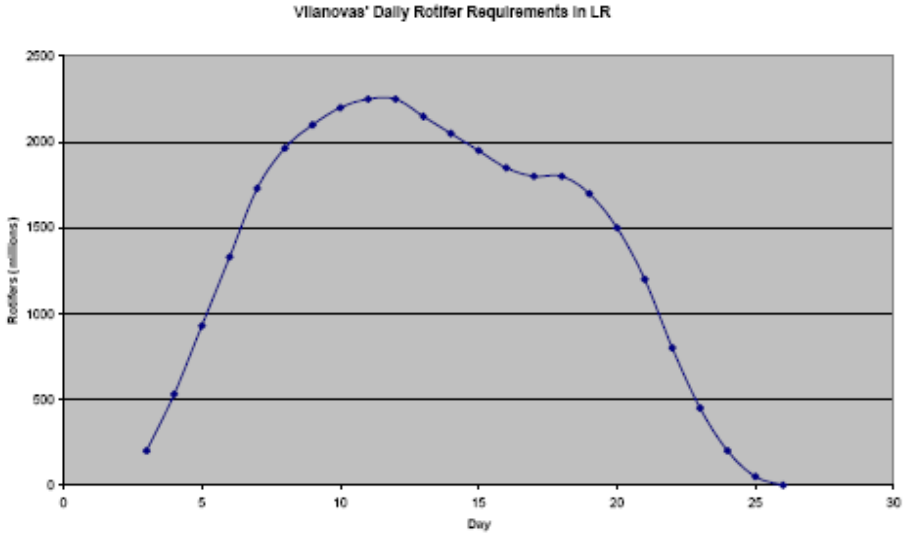
DESIGN OF A CONTINUOUS ROTIFER PRODUCTION SYSTEM (CROPS) FOR THE ALFA PROJECT.

Hatchery requirement.

A typical hatchery, designed to produce 10 million fry per year, requires approximately 2.4 billion rotifers per day. The daily rotifer requirement at Saga Fjord varies through the production cycle with a maximum requirement of 1.84 billion rotifers/day.



The way the rotifer production is organized at Saga Fjord, 2.5 billion rotifers are harvested daily from which 2 billion are enriched.



Viveiro Vilanova's daily rotifer requirements peaks at 2.25 billion rotifers/day while Sagro's daily rotifer requirement during the production cycle peaks at 2.7 billion rotifers/day.

Hatchery (10 million fry per year)	Peak production requirements (billion)
SagaFjord	1848
Viveiro Vilanova	2250
Sagro	2700
FAO (Theoretical)	2787
Average	2396

Commercial production systems

A typical continuous rotifer production system has a rotifer density of 3,175 rotifers/ml

Production system	Average density (rotifers/ml)
Depart of Business, Industry and Resource Development, Australia (DBIRD)	1,250
Reed Mariculture	2,250
University of Gent	2,700
Aquatic Ecosystems	6,500 (3,000 to 10,000)
Average	3,175

A typical harvest rate from the rotifer production systems is 46.9%/day

Production system	Daily harvest Rate (% of volume)
Depart of Business, Industry and Resource Development, Australia	50% (40 to 60%)
Reed Mariculture	50% (40 to 60%)
University of Gent	40%
Aquatic Ecosystems	47.5% (25 to 70%)
Average	46.9%

These harvest rates are those reported by the manufacturers but only limited data is given on the feeding regimes used and the egg percentages obtained to know if these harvesting rates are sustainable in an industrial unit during a production run. From the experience of several of the

partners with continuous flow through systems using both fresh concentrated algae and chlorella algal paste harvesting percentages of 25-30% could be maintained so it was decided to factor this into the dimensioning of the CROPS.

Assumptions used for specifying the design CROPS

Two CROPS will supply the peak daily rotifer demand of 2.4 billion rotifers per day for a 10 million fry per year hatchery. Therefore each CROPS will produce 1.2 billion rotifers/day.

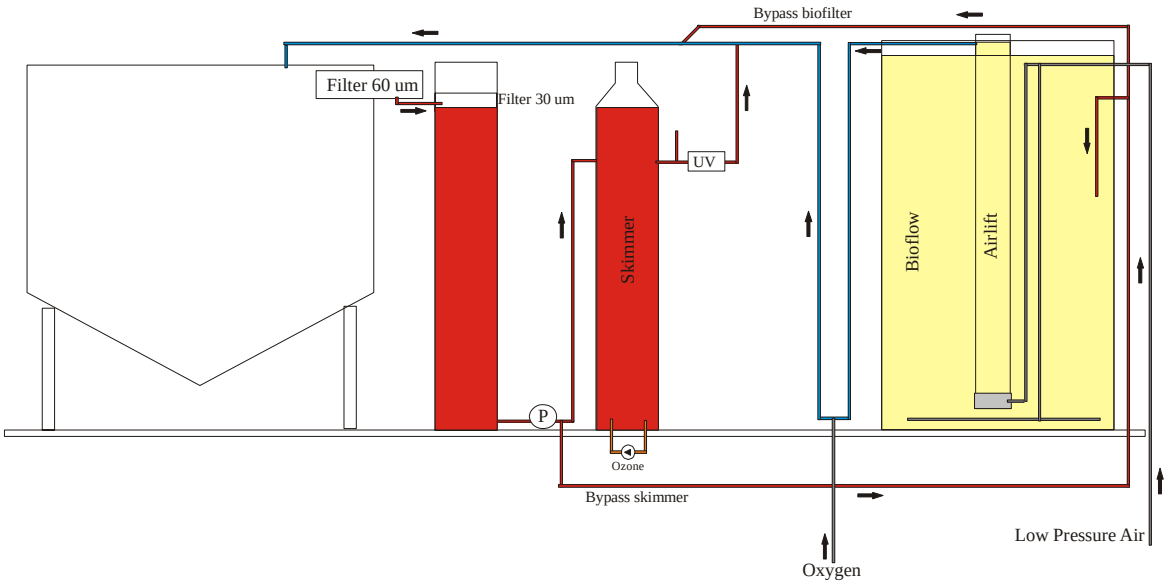
Standing stock required to produce 1.2 billion rotifers/day is
1.2 billion X 46.9 % = 2.558 billion rotifers
1.2 billion x 30% = 4billion stock
If this stock is held at an average density of 3,175 rotifers/ml requires a volume of 2.558 billion rotifers @ 3175 r/ml = 805 liters of culture space.
4.0 billion rotifers @ 3175 r/ml = 1260 litres of culture space
In addition to this their will be the volume of the filter ~ 50 litres and a freeboard of ~ 10% required.

Design discussions by the partners

One Festoon meeting and 3 Skype meetings were held with all the interested partners to discuss the details of the CROPS design. During these discussions several drawings were considered and the advantages and disadvantages of various combinations of equipment were considered and the decisions of these meetings are outlined in the next section.

CROPS design specifications / parameters

The basic design components are shown in the figure below:-



Components of the system

- **Culture tank** – this is envisaged as Ø 1.50 diameter x 1.200 height with a conical floor (effective volume 1.5m^3 water). This should be placed on a stand so that the water level is a maximum of 1.40 m above the floor. A bottom drain should be placed at the bottom of the cone to discharge waste material and an outlet should be placed in the middle part of the tank to drain rotifers to a holding tank of 400 litres.
  In addition an air and oxygen ring should be attached 20 cm above the cone.

- **Filter** – a filter with a screen size of 60 microns should be placed in the outlet of the tank to the settling tank
- **Flock traps** should be designed, constructed and be located in the tanks but with ease of access for cleaning. Alternative cleaning the production tank with a magnetic wiper or by using Bioflow or beads in the system water should also be investigated.
- **Settling tank** – Ø 315 x 1.400 envisaged, this could be a square box.
- **Temporary collection tank** (Settling tank or for continuous harvest collection)
- **Pump**. Single puming is envisaged with a pump of 200 litres/hour connected to pump from the settling tank. 20% or 40 l/h of the water should be pumped over the protein skimmer with ozone injection and 80% or 160 l/hr pumped over the biological filter and oxygenated to 120% over saturation. The water should return to the system by gravity.
- **Protein skimmer** - this is envisaged to be Ø 500 x 1.400 and will be injected with ozone supplied by an aquarium ozonator. The water leaving the skimmer should enter the bio-filter after UV treatment.
- **Biofilter** – the biofilter volume should be 1.5m^3 filled with 500 litres of Bioflow 800 m^2/m^3. An aeration ring and airlift will keep the material moving. The dimensions should be Ø 1.250 x 2.000 with 0.5 m^3 with 0.5m^3 bioflow 9 (Kalnes).
  The water leaving the bio-filter will flow back into the production tank by gravity.
  Flushing of the bio-filter should take about 30 minutes and during this period the water must be by-passed.
- **Oxygen injection** – After the bio-filter there should be a U-tube with oxygen injection to bring the water to over-saturation with oxygen of 120%.
- The temperature and salinity of the production system will be determined by the inlet water. However a temperature of 26- 28°C and a salinity of 26ppt are to be preferred.
- **Temperature, salinity and oxygen measurements** should by made using hatchery hand held instrumentation.
- **Continuous feed** to be provided by means of a small dosing pump adding a controlled quantity of feed to the culture tank.
- **Harvesting** of the rotifers can be batch or continuous. Either by siphoning a fixed volume out of the tank through the harvesting port and refilling the tank or continuous by the gradual addition of new fresh water. Consequently an identical quantity of rotifer culture flows out of the harvesting port into a receiving vessel.

SYSTEM SPECIFICATIONS

A test system was constructed by Hesy Bergambacht B.V. based on the design parameters and assumptions decided in sections 2 and this is summarised in the table below and described in detail following this:-

Parameter	**Design**	**Experimental system**
Culture tank	Ø 1.50 diam x 1.200 with a conical floor (0.22 depth)	Ø 1.80 diam x 1.00 with a conical floor(0.22 depth)
Total volume	2.3 m3	2.8 m3
Effective volume	1.8 m3	2.06 m3 (70 cm depth)
Settling tank L	Ø 315 x 1.4 - 108 litres	Ø 370 ID x 1.1 - 118 litres
Bio-filter L	Ø 1.25 x 2.0 - 1.5m3 filled with 500 litres Bioflow 800 m2/m3 fitted with aeration ring and airlift	Ø 1.30 x 2.00 Supplied with 200Litres of Kaldnes Bioflow 9 media
Total volume	2.45 m3	2.65 m3
Working volume	1.5m3	2.12m3

Water flow	200 l/hour	257 l/hour (Pump max – can be reduced)
Tank vol: biofilter vol	1.8: 1.5 = 1.2:1	2.57: 2.12 = 1.21:1
Tank vol: settling tank vol	1.8 : 0.108 = 16.7:1	2.57: 0.118 = 21.8:1

INTEGRATION WITH THE PHOTOBIOREACTOR

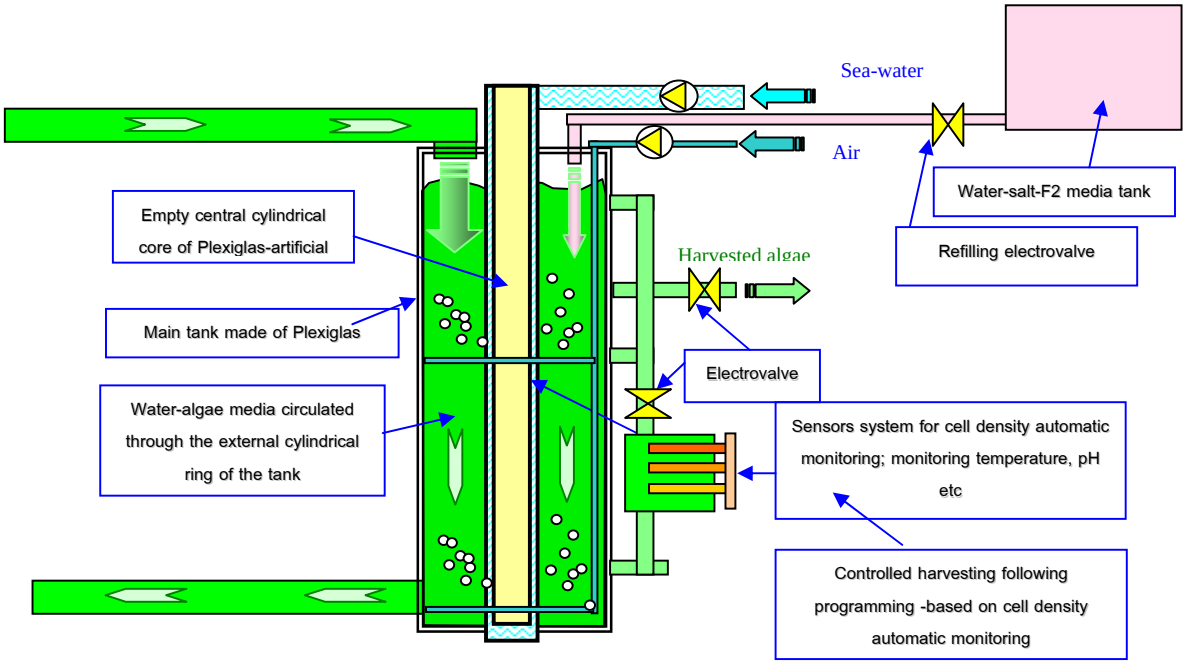
Following the testing phase the unit in Viveiro Vilanova in Portugal it was transported to SME Partner Saronikos, Greece and integrated with the photobioreactor.

CTI has concluded to an experimental setup in order to test the Colour & Turbidity Measurement System, which is under development, in the environment of a rotifer tank. The main goal of this setup is to determine whether the sensor is able to provide algae concentration estimations inside the rotifer tanks, given the different environmental properties, compared to the Continuous Algae Production system. In particular, inside the rotifer tank not only the algae concentration is significantly lower than in the CAP system, but it also contains extra turbidity due to the rotifers. It is thought that in practice, the rotifers are going to be filtered out of the sensor, and the sensor will be only supplied with the algae water from the rotifer tank. As a first step, experiments in the laboratory will be conducted. CTI will obtain samples of algae water from a rotifer tank without rotifers (rotifers will be filtered out). Measurements will be received by the sensors, using various sensor setups. Slight increases of the algae concentration in each measurement step will be achieved by adding algae paste. The research team will study the results in order to conclude whether the sensor has the potential of giving reliable estimates under these different conditions. Attempts to slightly modify the sensor and/or its calibration methods will be made.

The up-scaled 'Boas' CAP system was connected with a 300 l cooling storage tank as well as with the CROPS and the larvae tanks (for the greening effect) through a continous distribution loop from the CAP system and back to it (full details are given in D5; Section III). The harvesting system was operated through the control system designed and installed under task 3.4. The 'Boas' CAP production and continuous harvesting system was integrated to the CROPS system through the automatic monitoring sensors system that was connected to the concentration and storage unit (CSU) developed under Task 3.3 and the CROPS system, as well as to the fry tanks, while special system were designed to fill-up the 'Boas' CAP system with media replacement for the material harvested, feeding, CO2 etc, according to the needs and the thresholds set at the central control system of Task 3.4 and read by the system of special sensors installed in the CAP system.

Harvesting from the bioreactor

The algae is harvested from the tank of the photobioreactor and is pumped to the hatchery facilities including the rotifer tanks.

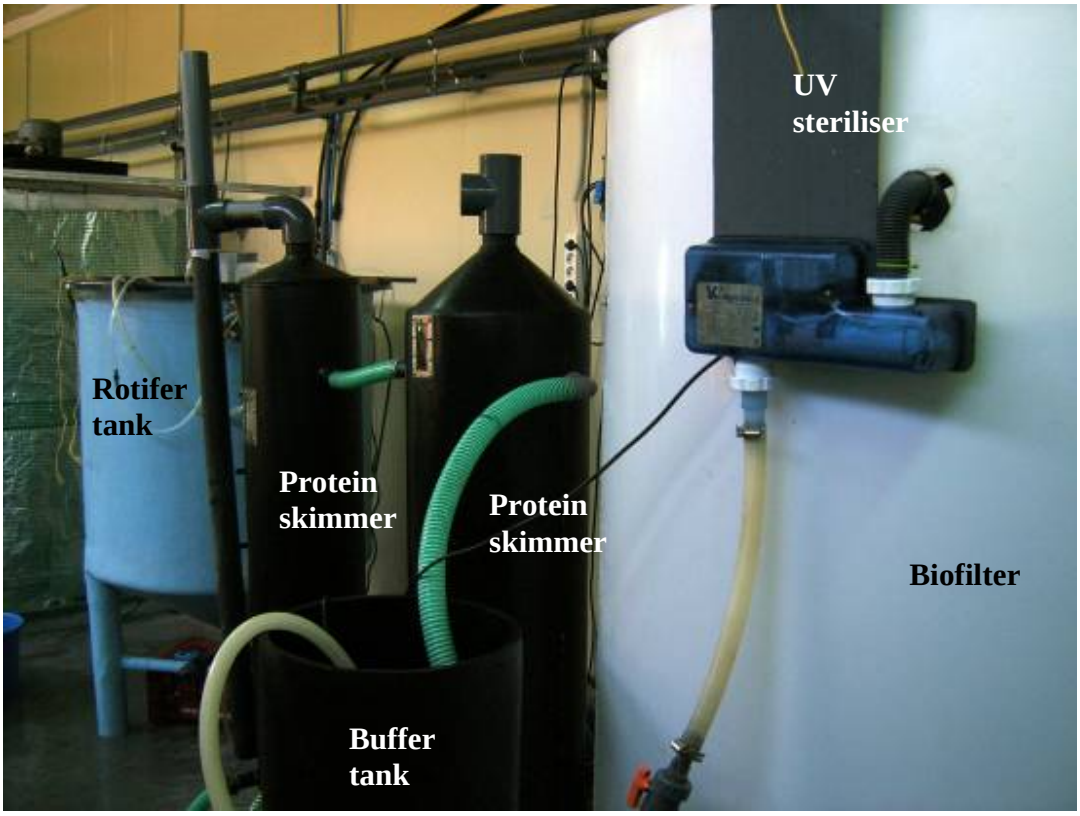


Schematic of the harvesting system from the boa photobioreactor.

Design and production optimization of the continuous rotifer production system (CROPS).
During the first reporting peiod, the continuous rotifer production system was designed then constructed by HESY, Holland and shipped to Viveiro Vilanova, Portugal for initial testing before being shipped to Argosaronikos, Greece to be integrated with the photobioreactor.

During the second reporting period, a number of trial production runs were made in ArgoSaronikos hatchery with the CROPS using live algae produced by the MegaBoa photobioreactor. Full details can be found in Deliverable 8.

CROPS layout
The CROPS was adapted for the ArgaSaronikos hatchery using a cylindroconical rotifer tank which allowed easier cleaning.



CROPS cylindroconical tan. 2 protein skimmers, buffer tank with submersible pump, UV disinfection and biofilter.

Rotifer tank with flock trap and internal filter

Three trials were undertaken

Crops trial 01 conducted between 16/06/2007 – 10/07/2007. Rotifer density increased from 100 rotifers/ml to over 700 rots/ml.
Egg production was variable between 10 to 55%.
The trial was stopped when NO2 in the CROPS biofilter was not able to cope with the nutrient out put and the level in the CROPS outlet reached over 80 mg/lit. The rotifers were washed and the culture restarted.

CROPS trial 02 was conducted between 09/07/2007 – 06/08/2007. Rotifer density reached 900 rotifers/ml and egg percentage ranging between 10 and 45%.
The biofilter was still relatively unstable with NO2 again reaching levels of 80 mg/lit.

CROPS trial 03 was undertaken between 07/08/2007 – 02/09/2007.
A density of over 1,000 rotifers/ml was achieved.
The biofilter was more stable with the levels of NO2 kept below 20 mg/lit.
The biofilter required over 2 months to be able to cope with the nutrient output by the rotifers.

CROPS Feeding regime spreadsheet

A feeding regime was devised for the continuous feeding of live algae from the photobioreactor during the day and a single feed of concentrated algae paste at night. This was undertaken as a saty measure so that the rotifer filter could not be blocked at night and so prevented overflow of rotifers from the crops.

	Rotifer appetite		
1	Rotifers eat	100,000	cells nano/day
1,000,00			
0	Rotifers eat	100,000,000,000	cells nano/day
1,000,00			
0	Rotifers eat	100,000	million cells nano/day
1	million Rotifers eat	100,000	million cells nano/day
600	million rotifers eat	60,000,000	million cells nano/day
		60,000	billion cells nano/day
		60	trillion cells nanno/day
4	Feeds per day	15.00	trillion cells nanno/feed
	Rofiter feeding		
	Nanno paste	68	billion cells/ml
		68	trillion cells/lit
	feed of nanno		
1	paste	0.221	lits paste/feed
		221	**ml paste per feed**
	Boa density		

500	Million cells/ml	500	billion cells/lit
		0.5	trillion cells/lit
1	feed of Boa	**30**	**lits Boa/feed**
3	feeds Boa	90	lits Boa/ day
11	Hours of feeding/day	8.2	lits/h
	Boa flow rate	**136**	**ml/minute**

Task 3.3 Development of a Concentration and Storage Unit (CSU) for phytoplankton

In the first reporting period a literature review was undertaken on concentration systems for algae and storage regimes for live algae and some preliminary work was undertaken on the use of breathable bags.

The 6 main techniques for harvesting Algae were evaluated in Deliverable 7 during reporting period 1.

- Sedimentation by centrifuge
- Micro filtration
- Chemical flocculation
- Electro-flocculation
- Concentration by ultrasound
- Flocculation by micro bubble

Two techniques were chosen to be evaluated further

- Concentration by ultrasound
- Flocculation by micro bubble

Ultrasound harvest testing

In order to harvest microalgae conventional processes such as centrifugation, membrane filtration and flocculation are widely used, but they all have some constraints (Ryll et al. 2000). Recently, a new separation technique based on gentle acoustically induced aggregation has been reported (Bosma et al. 2003). Bosma et al. (2003) showed that when algae are exposed to an ultrasonic standing wave, they experience a force that almost instantaneously drives them into the planes of the pressure nodes. Subsequently, agglomeration of the cells occurs into the knots of the ultrasonic field and when the field is switched off, the aggregated cells sediment rapidly from the fluid due to gravity forces.
In this study we wanted to test the cell viability after harvesting microalgae by ultrasound.

Material and methods

Microalgae cultures

Cultures of *Nannochloropsis oculata*, *Isochrysis* sp (Tiso) and *Tetraselmis suecica* were obtained from the culture collection at the University of Bergen. They were grown in triplicate (250 ml) under continuous white fluorescent light at 100 µmol m^{-2} s^{-1}, at 20°C and in F/2 medium (Guillard 1975).

Cell numbers were recorded daily using a haematocytometer (Fuchs Rosenthal, 0.2 mm deep). The cells were counted at 400 x magnification in a Leitz Dialux-20 light microscope with phase contrast. At least 400 cells were counted in each sample giving a counting error of ± 10 % (Andersen and Throndsen 2003).

Experimental design

Based on the findings of Bosma et al. (2003) dense cultures of microalgae were exposed to ultrasound waves by using an ultrasound water bath (Bransonic 3510). In contrast to Bosma et al. (2003) where they harvested by using a continuous flow of culture going through a resonance chamber, culture flasks were put directly into the ultrasound bath. The power input of the ultrasonic field was 130W, the acoustic field was switched on and off for 60, 120 and 300 s, respectively, at an operating resonance frequency of 42 kHz.

Results

Dense cultures of Nanno, Tiso and Tetra (Fig 1, day 15) were exposed to ultrasound waves for 60, 120 and 300 s. However, none of the cultures did sediment. Even when the exposure time was increased up to 480 s the cultures did not sediment. Therefore, testing cell viability by ultrasound harvesting was not possible.

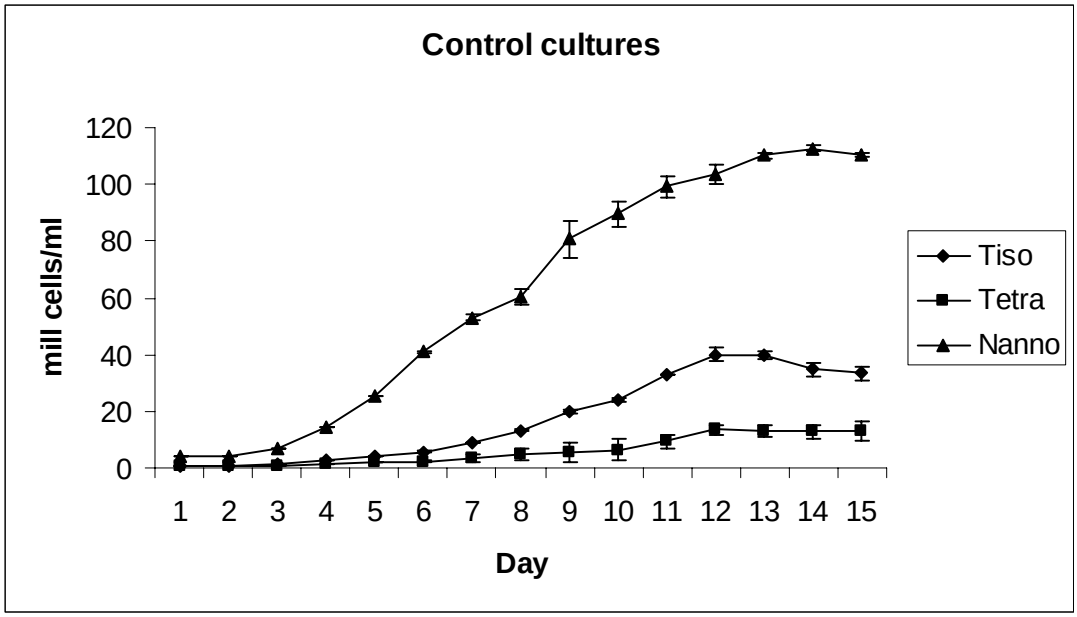


Fig 1. Growth of *Nannochloropsis oculata* (Nanno), *Isochrysis* sp (Tiso) and *Tetraselmis suecica* (Tetra).

Discussion
The results may have been different if the exact same power input and resonance frequency as Bosma et al (2003) was used, but this was not achievable with the instrument that was used in this experiment. The resonance frequency used in this experiment was probably too low.
In order to test cell viability from ultrasound harvesting an ultrasound machine generating 2.1 MHz should be tested. We were, however, not able to get hold of such a machine.

Concentration by microbubble - Protein skimmers
Protein skimmers or foam fractionators are a device used mostly in saltwater aquaria to remove organic compounds from the water before they break down into nitrogenous waste.

All skimmers have key features in common: water flows through a chamber and is brought into contact with a column of fine bubbles. The bubbles collect algae and other substances and carry them to the top of the device where the foam, but not the water, overflows from a funnel. Here the foam condenses to a slurry, which can them be easily collected from the system.

The two primary factors for the efficiency of the protein skimmer are
(1) the total air/water surface area determined by the number and size of bubbles
(2) the dwell time, or the amount of time these bubbles have to react with the water.

The Protein skimmer is a reactor to maximize these two by operating in a counter-current flow. In a counter-current system, air is forced into the system under pressure and moves against the flow of the water for a while before it rises up towards the collection funnel. Because the air bubbles are in contact with the water for a longer period in a counter-current flow system, protein skimmers of this type are more effective at removing organic wastes.

The protein skimmer consists of
- aeration/contact zone,
- venturi
- froth forming zone
- Froth collector

The bubbles (medium size) formed in the protein skimmer will be 100–600 micrometer in diameter.

The main advantages are
- high throughput,
- high efficiency
- moderate equipment cost
- No moving parts,
- low power consumption and low maintenance costs.

The protein skimmers that were used for the concentration process.

Bubble size can be reduced by adding low quantities of ozone to the air feed of the venturi. Ozone can be added at 0.05 mg ozone per litre of flow which is is sufficient to achieve a redox of 300–400 mV which will in addition disinfect the water. However, care must be taken not to overdose with ozone as a much higher dose rate of 0.2–0.5 mg of ozone per litre of flow will achieve redox levels of 700–800 mV at which the water can be considered sterilised but unfit for livestock and will kill the algal cells.

Preservation trial protocols

Algae (Nannochloropsis,) from the boa was collected at log phase growth and concentrated using an adapted protein skimmer to a density of 600 million/ml.

Algae slurry was mixed with the following preservatives

- Cryo-protectant, glycerol (SIGMA G-7893) at 10% (w/w). Glycerol has been widely demonstrated as an effective cryo-protectant for marine micro-algae
- Anti-oxidant, ascorbic acid (=Vitamin C) (SIGMA A-7506) at 1% (w/w). Ascorbic acid is an anti-oxidant because widely used in the food industry due to it's low toxicity, dual action as a food acid and potential benefit as a dietary vitamin supplement.

Aliquots of 500 g of concentrated algae were stored in sealed PE or breathable bags (with small air bubble) on shelves in an adapted refrigerator (Argsaronikos) and commercial refrigerator (University of Bergen) with a digital temperature thermostat set at ±0.5^{o}C.

These samples remaind refrigerated for a period of 6 weeks and be sampled regularly every 2nd week to assess the quality of stored micro-algae concentrates.

The criteria that were evaluated were:

- gross appearance and odour of concentrates prior to resuspension;
- microscopic examination of resuspended cells for evidence of physical damage to or degradation of cell walls and of intracellular structures and materials;
- use of specialist stain (Evan's Blue) to establish the integrity or otherwise of cell membranes.

Preservation and storage trials

In order to have a centralised production and supply of microalgal concentrates for use in aquaculture hatcheries extended shelf-life of stored concentrates are the key to success.

A major constraining factor has been the inability to create concentrates that retain high nutritional value and viability through storage. The concentrates need to rival that of the original live cells; retain palatability, be able to be ingested and digested by the animals being cultured. The concentrating process often damages the cells causing leakage of cell contents, in addition to autolysis and microbial degradation which can occur during long storage. Not all species are equally hardy for this process.

Stored microalgal concentrates are susceptible to rapid loss of nutritional value if not stored under appropriate conditions. In order to increase the shelf-life the integrity of the cell wall or membranes, and the cell contents and chemical integrity must be maintained. A minimum shelf-life of 4 to 6 weeks should be obtained. Because such a period spans the duration of hatchery and preliminary nursery rearing cycles for most fish and other marine species used in aquaculture.

The extension of shelf-life can be undertaken by using three promising techniques.
1) Use of non-toxic preservatives such as anti-oxidants, food acids or cryo-protectants.
2) Low temperature storage and
3) Low density slurries.

The three additives that are generally used for enhancing shelf-life of microalgal concentrates are glycerol, ascorbic acid (vitamin C) and citric acid. Results have shown that a combination of these three additives gave the best results in terms of extended shelf-life (Heasman et al. 2001). Heasman et al. (2001) also showed that the best results were obtained when microalgal concentrates were stored in low density slurries (10 g/l) and at low temperatures (2°C). A lower rate of biochemical degradation has been demonstrated by cold storage than by air- or freeze- drying (Cordero Esquivel and Voltolina Lobina 1996).

Loss of nutrients during storage of concentrates may affect their efficacy as diets for aquacultured animals (Brown 1995). Brown (1995) found that centrifuged *Chaetoceros calcitrans* lost ascorbic acid with storage age. Molina Grima et al. (1994) and Montaini et al. (1995) found that polyunsaturated fatty acids remained constant in centrifuged *Isochrysis galbana* and *Tetraselmis suecica*. Other losses of nutrients such as total protein, carbohydrate and lipid can also affect the development of larvae. However, few experiments have been conducted measuring effects of storage age on a broader range of nutritional components, in addition to storage conditions such as light, temperature, density and preservatives.

Based on the literature findings the ALFA-project wanted to test the recommended preservation and storage conditions, as well as the nutritional quality, of the production of *Nannochloropsis* sp. from the newly developed automated continuous live feed production system.

Material and methods

Algae (*Nannochloropsis* sp.) from the boa was collected at log phase growth and concentrated using an adapted protein skimmer to a density of 600 million/ml.
Algae slurry was mixed with the following preservatives

- Cryo-protectant, glycerol (SIGMA G-7893) at 10% (w/w).
- Anti-oxidant, ascorbic acid (=Vitamin C) (SIGMA A-7506) at 1% (w/w).

Aliquots of 500 g of concentrated algae were stored in sealed polyethylene (poly) or breathable bags (Kordon), with small air bubble, on shelves in an adapted refrigerator (Argosaronikos) and commercial refrigerator (University of Bergen) with a digital temperature thermostat set at 2 ± 0.5^{o}C. 5 poly bags and 5 Kordon bags remained at Argosaronikos, while 5 poly bags and 5 Kordon bags were sent to the University of Bergen.
These samples remained refrigerated for a period of 6 weeks and were sampled regularly every 2nd week to assess the quality of stored micro-algae concentrates.
The criteria used to assess retained quality of stored concentrates were:

- gross appearance and odour of concentrates prior to resuspension;
- microscopic examination of resuspended cells for evidence of physical damage to or degradation of cell walls and of intracellular structures and materials;
- use of specialist stain (Evan's Blue) to establish the integrity or otherwise of cell membranes;
- microbial contamination;
- nutritional quality in terms of fatty acids, vitamins B1, C and E.

Cell damage

In order to evaluate cell damage 1 % (w/v) Evans's Blue was used to stain the microalgae (e.g. Molina Grima et al. 1994, Heasman et al. 2001). A 20 mL sample was treated with 1 mL of Evan's Blue and left at room temperature for at least 30 min before stained and un-stained cells were counted in a light microscope using a Fuchs Rosenthal haematocytometer with a counting error of $\pm$ 10 % (Andersen and Throndsen 2003).

Cell viability
A small sample of stored microalgae was resuspended in F/2 medium (1 l), at 18°C and in continuous white fluorescent light at 100 µmol m^{-2} s^{-1} at an approximately cell density of 3 x 10^6 cells ml^{-1} – 30 x 10^6 cells ml^{-1}, in order to detect cell viability. Cell counts were made daily in a haematocytometer.

Odour, colour and pH
Colour change and offensive odours were assessed. Colour change was made by visual inspection. Odour was measured as 0=normal fresh, 1=different, but not offensive and 2=offensive. Ph was measured by using a pH-meter (Beckman).

Microbiological contamination
Sanoguard® BDS-G (Inve Aquaculture Health) bacterial dip slides were used to monitor bacterial growth. Both, growth of heterotrophic bacteria on marine agar and *Vibrio* spp selective growth on TCBS agar were registered. The slides were incubated for 12 to 18 hours at a temperature of 25 to 35 ‘C. The colonies were counted on the MA and TCBS sides. The slide were then incubated further and recounted the following day.

Ease of resuspension
Ease of resuspension was evaluated according to the time required for the concentrated algae to be fully resuspended: 1=2 seconds, 2=3-5 seconds, 3=5-10 seconds and 4=10 seconds.

Nutritional quality
Immediately upon arrival at UoB, the bags were place in a temperature controlled room at 2±0.5°C and at a continuously white fluorescent light intensity of 80 µmol m^{-2} s^{-1}.
Analyses for fatty acids, vitamin C, B1 and E were conducted at storage week 0, 2, 4 and 6. The samples were analysed at National Institute of Nutrition and Seafood Research (Bergen, Norway with Norwegian accreditation test 050). The fatty acids were analysed with extraction- methyl-polar-phase GLC-FID detection (method 041). Determination of Thiamine (vitamin B1) was performed by using HPLC equipped with fluorescence detector (NIFES method 239). Vitamin C (ascorbic acid) was determined by using inverted phase HPLC equipped with amperometric electrochemical detetector (NIFES method 221). Vitamin E (tokoferol-isomers *α, ß, γ, δ*) was determined with extraction and normal phase HPLC with fluorescence detection (NIFES methode 042).

Results

Preservation and storage trials conducted at ArgoSaronikos (Greece) – Domestic refrigerator
The results from the assessment of the stored microalgae from ArgoSaronikos at storage week 0, 2 and 4 are summarized in Table 1. The temperature recorded in the domestic refrigerator was between 7-10.3°C (Table 1).

Table 1. Assessment of stored *Nannochloropsis* sp. at storage week 0, 2 and 4 at ArgoSaronikos. Kordon = breathable bags, Poly = bags made of polyethylene.

	Kordon			**Poly**		
Storage week	**0**	**2**	**4**	**0**	**2**	**4**
Temperature (°C)	7	10.3	9.6	7	10.3	9.6

Odour	0	1	2	0	1	1
Colour	greenish	green/brown	brown	greenish	green/brown	green/brown
pH	3.7	3.2	2.7	3.7	3.5	3.2
Bacteria on marine agar (CFU/ml)	1660	700	60	1160	120	60
Bacteria on TCBS (CFU/ml)	240	0	40	240	0	40
cell damage (% dead)	6.4	35	31	6.4	28.6	16.1

In both types of bags the odour was normal and fresh (0) at the start of the experiment (Table 1). The odour turned into really offensive (2) at storage week 4 in the breathable bags, but was different and not offensive (1) in the poly bags. The colour was green for both types of storage bags in the beginning of the experiment, but turned into brown and green/brown for the kordon and poly bags, respectively. pH varied between 3.7 and 2.7 for all of the bags.

There was a trend of decreasing pH in all of the bags during the storage period. In the breathable bags the pH fell from 3.7 to 2.7, compared to the poly bags were pH fell from 3.7 to 3.2. There were some bacteria present in both types of storage bags at the start of the experiment (Table 1). More bacteria were present in the Kordon bags compared to the Poly bags during storage week 0 and 2. High numbers of *Vibrio* spp bacteria were recorded at the start of the experiment in both types of bags. The *Vibrio* bacteria were not recorded on storage week 2, but reoccurred on storage week 4. Cell damage, measured as % dead cells varied between 6.4% to 35% in the Kordon bags and 6.4% to 28.6% in the Poly bags. After 4 weeks of storage 69% of the cells in the breathable bags were live, compared to 83.9% live cells in the poly bags. Cell viability was tested as re-inoculating the stored concentrates into fresh medium (Fig 1). Growth was fairly good at storage week 0 for both types of bags until day 7 after reinoculation. At storage week 4 declining growth were seen after only 3 days for both types of bags, indicating poor cell viability. The concentrated microalgae were equally resuspended immediately when dissolved in new medium.

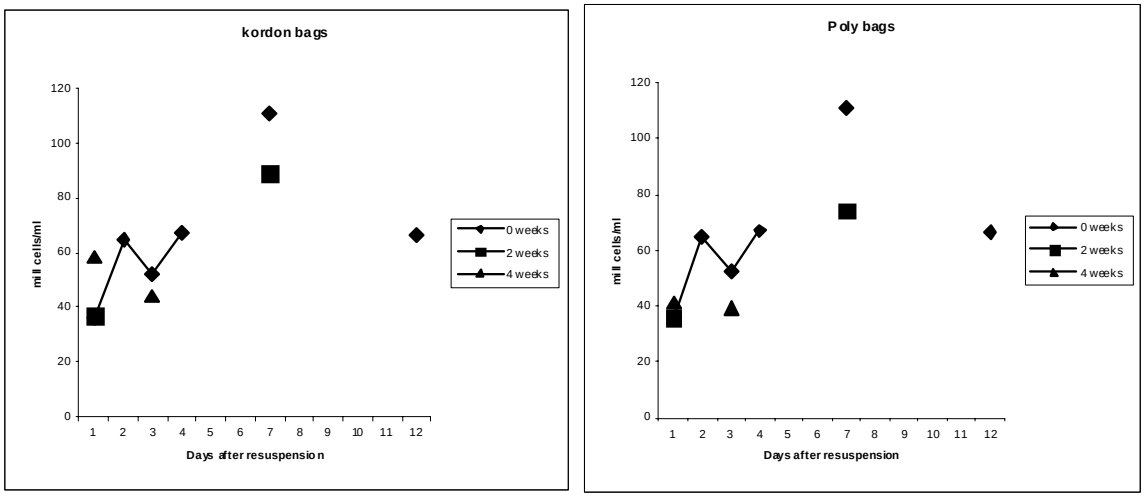


Figure 1. Cell viability after 0, 2 and 4 weeks of storage in Kordon (breathable bags) and polyethylene (Poly) bags.

Preservation and storage trials conducted at University of Bergen

The initial evaluation of cell damage, cell viability, pH, odour, microbial contamination and ease of resuspension was undertaken at ArgoSaronikos one day after storage and was then repeated at week 4 and 6 of storage at the University of Bergen.

The results from the assessment of the stored microalgae received from ArgoSaronikos at storage week 4 and 6 are summarized in Table 2. The temperature was stable at 2.0-2.2 °C during the period (Table 2). The odour was normal and fresh (0), except at week 6 for the Poly bag where the odour was different, but not offensive (1). The colour was brownish for the Kordon bags during the whole period. The colour was dark greenish for the Poly bags at week 4, but turned into brownish at week 6. pH was stable for all the bags at pH 4.8-4.9. There were some bacteria present in both types of storage bags. There was a trend to more bacteria present in the Kordon bags compared to the Poly bags (Table 2). *Vibrio* spp bacteria were recorded in all of the bags. Cell damage, measured as % dead cells was considerable higher in the Kordon bags than in the Poly bags. The percentage of dead cells was 45.3 at storage week 4 and as high as 85.1 at storage week 6. The poly bags had a percentage of dead cells of 40.4 at storage week 4 and 58 at storage week 6. No growth was recorded when cell viability was tested in the Kordon bags at storage week 4 and 6, while some, but slow growth were measured in the Poly bags when the concentrated microalgae were reinoculated into fresh F/2 medium (Fig. 2). The concentrated microalgae were equally resuspended immediately when dissolved in new medium.

Table 2. Assessment of stored *Nannochloropsis* sp. at storage week 4 and 6 at University of Bergen.

	Kordon		**Poly**	
Storage week	**4**	**6**	**4**	**6**
Temperature (°C)	2	2.2	2	2.2
Odour	0	0	0	1
Colour	brownish	brownish	dark greenish	brownish
pH	4.8	4.9	4.8	4.9
Bacteria on marine agar (CFU/ml)	1020	560	520	440
Bacteria on TCBS (CFU/ml)	40	40	40	20
cell damage (% dead)	45.3	85.1	40.4	58
Cell viability (see fig 2)	No growth	No growth	Some, but slow	Some, but slow

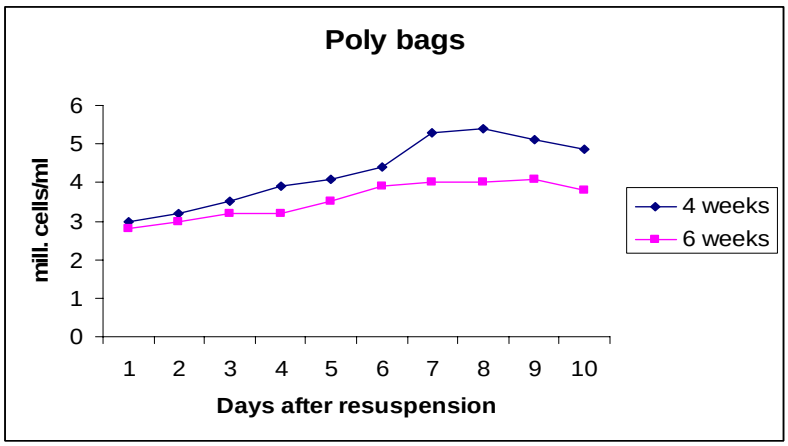


Figure 2. Cell viability after 4 and 6 weeks of storage in sealed polyethylene (Poly) bags.

Nutritional quality in terms of fatty acids, vitamins B1, C and E.

The concentration of vitamin B1, C and E are summarized in Table 3. Vitamin B1 was low during the experimental period. A rapid loss of vitamin C was observed in the Kordon bags compared to the Poly bags. Vitamin C was better preserved in the Poly bags (44.6% left of initial concentration) compared to the Kordon bags (17.2% left of initial concentration).

Table 3. Vitamin B1, C and E profile (mg/kg) of *Nannochloropsis* sp concentrates at storage week 0, 2, 4 and 6. Kordon = breathable bags, Poly = polyethylene bags.

	0	**0**	**2**	**2**	**4**	**4**	**6**	**6**
	Kordon	**Poly**	**Kordon**	**Poly**	**Kordon**	**Poly**	**Kordon**	**Poly**
Thiamine (B1)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Vitamin C	9280	9580	5930	8400	3480	6370	1600	4270
Vitamin E:								
α-tokeferol	0.47	0.50	0.18	0.30	0.14	0.32	0.05	0.25
β-tokeferol	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
γ-tokeferol	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
δ-tokeferol	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
α-toketrienol	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
β-toketrienol	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
γ-toketrienol	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
δ-toketrienol	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Vitamin E also dropped rapidly in the Kordon bags compared to the Poly bags. Vitamin E was better preserved in the Poly bags (50% left) at storage week 6, compared to only 0.1% left in the Kordon bags (Table 3).

The fatty acid composition (%) of *Nannochloropsis* sp. is summarised in Table 4. No big difference could be observed between the different storage bags during the experimental period, except the Kordon bag at storage week 6. There was a relatively high and stable level of 16:0, 18:2n-6, 18:3n-3 fatty acids present during the experiment. Arachidonic acid (ARA, 20:4n-6) and docosahexanoic acid (DHA, 22:6n-3) were not detected. Eicosapentanoic acid (EPA, 20:5n-3) was detected at a low (0.2-0.3%) and stable level during the experimental period (Table 4).

Table 4. Fatty acid composition (%) of *Nannochloropsis* sp. at storage week 0, 2, 4 and 6. The concentrates were stored in breathable bags (Kordon) or bags made of polyethylene (Poly).

	Kordon	**Poly**	**Kordon**	**Poly**	**Kordon**	**Poly**	**Kordon**	**Poly**
Storage week	**0**	**0**	**2**	**2**	**4**	**4**	**6**	**6**
14:0	0.7	0.7	0.8	0.7	0.7	0.6	1.4	0.8
14:1n-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15:0	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2
16:0	23.3	22.0	23.4	21.8	27.2	22.7	42.2	23.9
16:1n-9	2.1	2.3	2.3	2.2	2.4	2.2	2.9	2.4
16:1n-7	1.0	1.0	1.0	1.0	1.0	0.5	1.2	1.0
17:0	0.1	0.0	0.0	0.0	0.1	0.0	0.5	0.2
16:2n-4	0.5	0.2	0.5	0.4	0.6	0.5	0.0	0.3
18:0	2.2	1.8	1.7	1.7	2.3	2.0	4.0	1.8
16:3n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:1n-11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:1n-9	9.5	8.4	8.6	8.2	9.9	8.8	14.0	8.2
18:1n-7	0.8	0.8	0.7	0.7	0.7	0.6	0.6	0.6
16:4n-3	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.2
18:2n-6	23.6	23.8	23.9	23.7	21.9	23.6	13.5	23.3
20:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
18:3n-3	15.7	17.1	16.2	17.0	13.1	16.3	4.1	15.8
20:1n-11	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
20:1n-9	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0
20:1n-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:4n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:2n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:3n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
22:0	0.0	0.0	0.0	0.0	0.1	0.0	0.2	0.0
20:3n-3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
20:4n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:1n-11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:1n-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:4n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:5n-3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3
24:0	0.2	0.2	0.1	0.1	0.2	0.2	0.3	0.3
24:1n-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:5n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:6n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum diverse	19.4	20.8	20.0	21.6	18.8	20.9	14.0	20.6
Sum identified	80.6	79.2	80.0	78.4	81.2	79.1	86.0	79.4
Sum total	100	100	100	100	100	100	100	100
Sum saturated	26.9	25.2	26.4	24.8	31.1	26.1	49.1	27.5
14:1n-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum 16:1	3.1	3.3	3.3	3.2	3.4	2.7	4.1	3.3
Sum 18:1	10.3	9.2	9.3	8.9	10.6	9.5	14.6	8.8
Sum 20:1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0
Sum 22:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

24:1n-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum monoens	13.5	12.6	12.5	12.1	14.2	12.2	18.8	12.2
Sum n-3	16.0	17.5	16.6	17.4	13.5	16.7	4.4	16.3
Sum n-6	23.6	23.8	23.9	23.7	21.9	23.6	13.7	23.3
Sum polyenes	40.2	41.5	41.0	41.5	36.0	40.9	18.1	39.8
n-3/n-6	0.68	0.73	0.69	0.74	0.62	0.71	0.32	0.70

Discussion

There were some differences when comparing the storage results from ArgoSaronikos and University of Bergen (UoB) (Table 1 and 2). The major difference was the temperature. The storage temperature at ArgoSaronikos was higher and variable compared to UoB. The pH was also measured lower at ArgoSaronikos than at UoB. This could be due to different pH-meters or the fact that citric acid was not initially added as a preservative. Heasman et al. (2001) showed that the best results were obtained when all three of the preservatives (Vitamin C, citric acid and glycerol) were added. The fact that no citric acid was added in this experiment could therefore partially explain why the stored microalgal concentrates performed relatively bad.

There was also a trend that the breathable (Kordon) bags performed worse than the bags made of polyethylene (poly). The microbial activity was higher in the Kordon bags compared to the poly bags (Table 1 and 2). The *Vibrio* spp. bacteria should not be present and could be a sign that the water treatment was not efficient enough. The breathable bags are designed in a way that CO_2 gets out and O_2 gets into the bags. The optimal for storage of microalgae is the reverse. A high content of O_2 could be toxic to the microalgae and may explain why this type of storage bags did not perform as good as expected.
The vitamins (B1, C and E) were better preserved when stored in Poly bags compared to Kordon bags. The loss of vitamins was quite rapid after only two weeks of storage. Vitamin B1 was relatively low during the whole experiment. This could be due to naturally low content of this vitamin in this strain of *Nannochloropsis* sp. or that the growth medium (F/2) contained low concentration of this vitamin initially. The nutrient concentration in the medium was, however, not measured.

The fatty acid profile (FA %) showed little variation between the different storage bags, except at storage week 6 (Kordon bag), indicating that the FAs were relatively good preserved during 6 weeks of storage. However, a relatively high and stable level of saturated FAs could be observed (31.1-49.1%) in the samples. Sukenik and Wahnon (1991) showed that nitrogen limitation increased abundance of saturated (16:0) and monounsaturated (18:1) FAs and decreased the percentage of DHA. This is in accordance with the observed results from this experiment, indicating that the growth system probably was initially nutrient limited. In this experiment the PUFA fraction was within the range of 18.1-41.5%. Reported levels of *Nannochloropsis* strains are often within the range of 50-80% (e.g. Volkman et al 1989). The PUFA fraction is usually dominated by C16 and C18 chain length FAs, which was also the case in this experiment. EPA was present in rather low and stable levels (0.2-0.3%), while DHA and ARA were not detected. Usually, *Nannochloropsis* sp. is considered as a species rich in EPA. Levels of EPA commonly reported are within the rage of 20-30%

(e.g. Hu and Gao 2003). DHA has been reported in low levels (0.4-1.4%) in other *Nannochloropsis* strains, while ARA may either be absent or present in relatively high levels (Brown et al. 1989, James et al. 1989, Hu and Gao 2003). Another reason that might explain the low levels of PUFAs in the samples is that the light intensity might have been too high either when the microalgae were produced in the Mega Boa or when they were stored under light conditions. Thompson et al. (1990) investigated the influence of irradiance on the FA composition and found that for most species there was a trend that EPA increased and DHA decreased with decreasing intensities. Sukenik et al (1989) showed that increasing intensity (35-550 µmol $m^{-2}s^{-1}$) imposed an exponential reduction in the proportion of ARA and EPA, and an increase in 16:0, 16:1. Cells grown under limiting conditions (35 µmol $m^{-2}s^{-1}$) contained high levels of EPA.

Conclusion

The bags made of polyethylene were better for storage of *Nannochloropsis* sp. than the breathable bags tested. The Poly bags showed lower cell damage, better cell viability, lesser microbial contamination and better preservation of the nutritional quality after 6 weeks of storage.

Based on the results from the preservation and storage trials a shelf-life of 3 weeks is recommended for the production of *Nannochloropsis* sp. from the Mega Boa.

Task 3.4 Development of a Control System for managing the algae production, harvesting, and transferring process

The automation required for the operation of the CAP subsystem as well as the control and management of the algae production and the harvesting and transferring process will be provided by a computer system. The choice of a computer system over a solution with PLCs not only allows faster implementation of the whole system, but also offers the potential of supporting a variety of additional auxiliary services such as extended data logging as well as organization and storage of large data, while maintaining the total cost within reasonable limits. Typically, a computer system that provides automation for an industrial process needs to perform the following general tasks:

I. Perform monitoring of the microenvironment parameters within the process.
II. Perform Data Logging, i.e. record the monitored parameters into a database.
III. Evaluate the current state of the process, based on the data obtained from monitoring and using known and predefined metrics to assess the current state and make decisions, based on predefined algorithms that evaluate the logged data and generate decisions for corrective actions. Speaking more specifically, this part of the software concerns the maintenance of PH, temperature and concentration between pre-defined desired values.

Several sources of data have been studied, in order to acquire sufficient information that will be used as a basis for our design and development of the computerized monitoring and control system for managing the CAP & CHTP units. These include research of the current trends in modern industrial setups, review of the control systems already in use at the sites of ALFA partners as well as discussion of the design and interaction with the SMEs in an attempt to continuously improve the design and address many non-trivial issues.

We have implemented a monitoring and control system for two different systems, the mega-boa and the two tubular. The design of the systems is based on the designs that AUA provided us. The two systems have some parts that are different, so we had to specifically design the parts for each system to meet the desired requirements. The system consists of a commercially available PC with the ADVANTECH cards and MS Windows XP installed. The software is implemented in .NET Visual Basic. The software is divided in several units that are concerned with different processes. More specifically the parts are:

- The Data logging unit which logs the measurements of PH, Temperature and Concentration in a database related with the software.
- The signal processing unit which calculates the concentration, the PH and the Temperature, according to the values of the sensors that are collected with the ADVANTECH card.
- The PH regulation unit which supplies the tank with CO_2 when the PH exceeds a predefined value.
- The Temperature regulation unit which cools the tank with water when the Temperature exceeds a predefined value.
- The harvesting unit which initiates the harvesting process of the tank if its concentration is above a predefined value and then initiates the washing procedure which cleans the tank, the sensors and the pipes that are attached to the system.
- The user interface, which depicts the current state of the bioreactor (PH, temperature and concentration values) and provides several options that, can be accessed by the user.

The following paragraphs describe these basic parts of the software implemented for the mega – boa bioreactor. In task 4.1, we will describe the parts of the software that are different in the two – tubular system.

Architecture of the automation system

First we give a brief description for the hardware and software architecture of the automation systems which had been developed and tested. It should be noted that the level of details given in this chapter is only as high as necessary in order to demonstrate the context into which the part of the software integrate and function. Further details can be found in the deliverables that concern the automation systems or in the corresponding paragraphs of this report.

Hardware components

From the hardware point of view, the automation system can be partitioned into two subsystems; the monitoring and control parts. Both subsystems communicate with a central computer system, which controls them and read their feedbacks. This architecture is shown in figure 1. The monitoring subsystem consists of a pH and a temperature sensor, one custom made sensor (algae density meter) (see deliverable 6), one commercially available electronic PCI card that is installed on a computer (data acquisition card ADVANTECH PCI1710L), two custom electronic boards for the interconnection of the sensors and the PC cards and wiring. This subsystem communicates with the central PC through the wiring terminal of the

ADVANTECH PCI1710L. The schematic of the sensors subsystem occupies the left part of figure 1.

The control subsystem consists of one commercially available electronic PCI card that is installed on a computer (relay output card ADVANTECH PCI1761), eight electrical relays capable of closing a 220V - 30A AC circuit when powered with 12VDC, a 12VDC power source capable of supplying DC current of at least 1 Ampere and the necessary cabling. The wiring terminal of the ADVANTECH PCI1761 is the point where this subsystem interfaces with the central PC. The schematic of this subsystem is visible on the right part of figure 1. In figure 2 we depict how the electrical relays are connected with the relay output card ADVANTECH PCI1761.

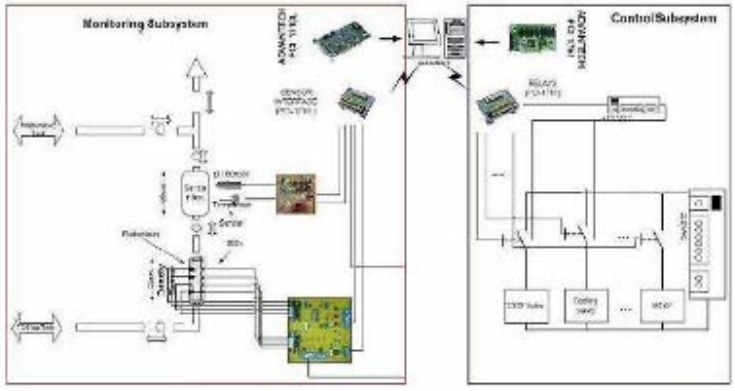


Figure 1: Hardware architecture for the monitoring of the CAP system.

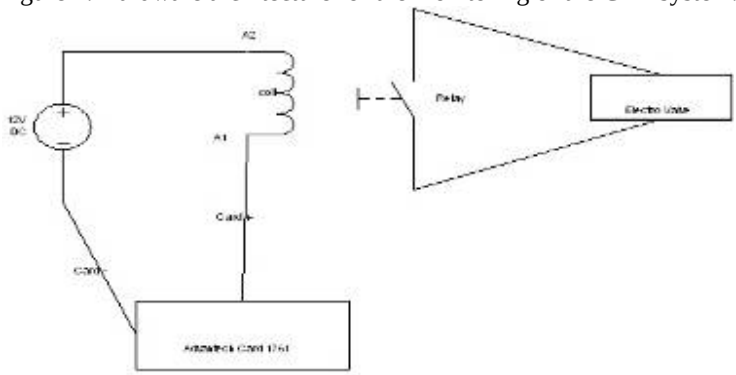


Figure 2: The complete diagram of the control system.

Both the monitoring and control subsystems can generate data that need to be logged. The monitoring process generates output such as temperature values, pH values, concentration estimations, or alarms indicating values that are out of range. Every such value, together with the relevant timestamp, is logged. The actions of the control automations are also logged. This allows the operator to recall and evaluate the actions that were performed automatically by the system.

The logs must be searchable and provide mechanisms of retrieving specific information fast and with minimal effort from the operator. In addition to that, the logging system must be

reliable, in order to ensure that no actions or data is lost during operation. The Data Logging Unit was designed and implemented in order to meet these requirements.

Software architecture

The central PC runs specially designed software which has been implemented by the Research Academic Computer Technology Institute for the project ALFA. This software includes all the algorithms that the central computer must execute in order to carry out all the automation tasks and coordinates the function and communication of the monitoring and control subsystems. This includes performing periodic monitoring of the environment inside the bioreactor and run algorithms in order to evaluate each parameter and produce decisions for correcting these values in the case that they are out of the desired range. With the aid of the specially designed software application, the central computer controls and reads the various sensors, processes the readings, performs logging, makes automatic decisions and executes the necessary actions through the control subsystem. The schematic diagram of the software architecture is displayed in figure 3. The main consideration in software design was to keep it as modular as possible, during all the phases of development. In software engineering, this principle can lead to higher reliability, allow faster development and easier maintenance.

Each module is depicted in figure 3 as a separate block. It can be easily seen that the basic functionalities and the coordination of the modules are provided by the *Main Control Thread*. This module is constantly executed and carries out all the periodic operations, decides about the actions that need to be taken and coordinates the other modules. The *Sensor Input* and *Relay Output* software layers drive the special purpose hardware of the monitoring and control subsystems, respectively, according to commands issued by the Main Control Thread. The signal processing unit processes the voltages received by the sensors and produces the respective values of real - world parameters. As depicted in figure 3, the Main Control Thread has two-way communication with the *Data Logging Layer*. This layer provides logging and data retrieval services to the main application.

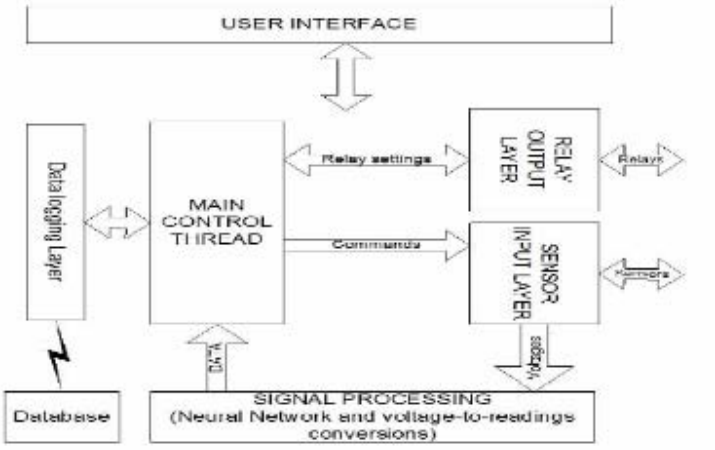


Figure 3: Software architecture for the monitoring and control automations.

The communication of the Main Control Thread with the operator is facilitated by the *User Interface* module, which presents the various data and receives configuration commands.

The data logging unit

The Data Logging Unit consists of two modules: the Data Logging Layer and the Database. This is illustrated in figure 3. The data is stored in tables in the Database module. The Data Logging Layer interfaces the Main Control Thread with the Database. The Data Logging Unit stores and manages the following information:

- Environmental parameters in the system, i.e. pH, Temperature, Concentration estimation and the voltage outputs of the density meter, for debugging.
- Actions that were taken automatically by the central computer or manually by the operator.
- Alarms of any kind of abnormality.
- Settings of the software application.
- Training data for the density sensor. This data is collected and inserted by the operator in the training phase for the density meter.

Signal Processing Layer

The sensors are electronic devices which vary an electrical parameter, such as current or voltage, according to the physical phenomenon that they are observing. However, in practice, we are interested for the values of the actual phenomena that these voltages correspond to. The role of the ‘‘Signal Processing Layer’’ is to receive the voltages read by the ‘‘Sensor Input Layer’’ and convert them to Temperature/pH/Concentration. Those values are then supplied to the ‘‘Main Control Thread’’ for further processing. In this chapter we describe the operation of this software module. In the following sections we describe the voltage to temperature converter, the data fusion technique applied for the estimation of the algae concentration and the voltage to ph converter.

Conversion of Voltage to Temperature

The temperature sensor that has been used for the monitoring system consists of a PT100 temperature probe and a transducer. The transducer drives the PT100 probe and converts its response to a current in the range of 4 – 20 mA. More details on the hardware of the temperature sensor can be found in the report accompanying the Deliverable 11. The output of the transducer is a linear function with respect to the temperature of the PT100 probe, according to its manufacturer. We have adjusted the transducer to read temperatures in the range of 0 - 45^{o} C. This means that we have the following information, which must be taken into account in the design of the voltage to temperature converter: For 0 ^{o}C, the response of the transducer is 4mA. For 45^{o} C, the response of the transducer is 20mA. The response of the transducer is linear with respect to the temperature of the PT100 probe. The output current of the transducer is converted to voltage over a resistor of 250. This means that the currents mentioned above are transformed in the voltages 1V and 5V respectively. From the above data, we can conclude that the function which connects the output voltage and the temperature is linear, and can be easily calculated as: $T = 11.25 \times (V - 1)$ where T is the temperature in ^{o}C and V is the voltage returned from the Sensor Input Layer module.

Neural Network architecture

The CTMS unit consists of four cascaded Led/Photodiode pairs. The output of each pair and the turbidity (or concentration) of the phytoplankton are related through exponential processes (see deliverable 6). Initially we calculated the exponential curves that fit the measurements that we took in the Lab with Nannochloropsis. These curves are presented in figure 6. We repeated the above experiments with different samples and observed that the infrared led photodiode pair gives robust & repeatable readings regardless of manufacturing imperfection (distance & angle perturbations), by paying the price of a less accurate exponential fit. Furthermore, the Red, Blue LED photodiode pairs offer higher resolution by achieving better exponential fit by paying the price of high sensitivity to perturbations. The use of data fusion techniques, may lead to more accurate results by combining the outputs of the different sensors.

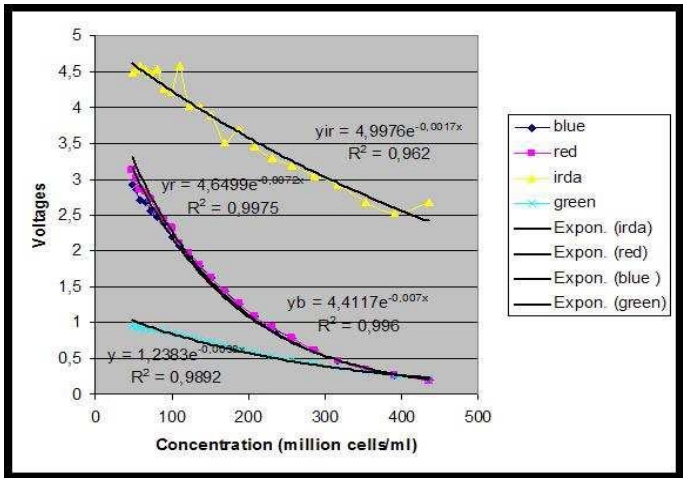


Figure 6: Exponential curves that fit the measurements for the different Led/photodiode pairs.

For each sample we get four different measurements and we form a 4 × 1 vector:

$$S(c) = \begin{bmatrix} B(c) \\ G(c) \\ R(c) \\ IR(c) \end{bmatrix},$$

where B(C), G(C), R(C), IR(C) are the output voltages that correspond to the photodiodes responses opposite the blue, green, red and infrared led respectively and C is the concentration of the sample inside the CTMS sensor. The objective is to obtain the estimation of C by using the 4 above measurements. This seems to be a pattern recognition problem that can be easily solved with use of a neural network.

Figure 7: Reduce estimation problem into a pattern recognition problem

A NN is a massively parallel distributed processor made up of simple processing units, which has a natural propensity of storing experiential knowledge and making it available of use. It resembles the brain in two aspects:

1. Knowledge is acquired by the network from its environment through a training process.
2. Interneuron connection strengths, known as synaptic weights, are used to store the acquired knowledge.

The procedure used to perform the training process is called a training algorithm, the function of which is to modify the synaptic weights of the network in an orderly fashion to attain a desired design objective. The modifications of synaptic weights provide the traditional way for design the neural networks. In general we may identify three fundamentally different classes of network architectures:

- Single – Layer Feed-forward Networks
- Multilayer feed-forward networks
- Recurrent networks

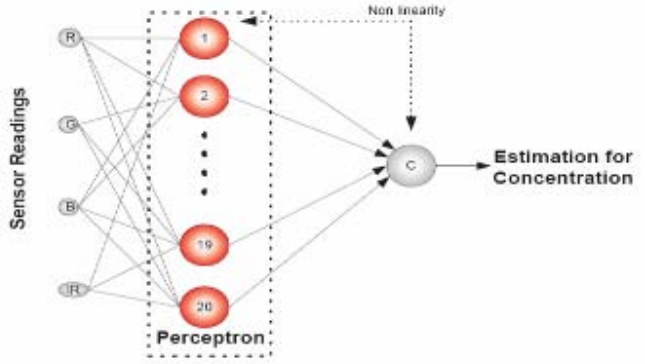


Figure 8: Architecture of the fully connected feed-forward Neural Network with one hidden layer and one output layer.

In order to solve the problem of estimating the concentration we employed a multilayer feed-forward neural network with four inputs (B(C),G(C),R(C), IR(C)), one hidden layer and one output layer as the one presented in Fig. 8.

Training and testing the NN

We have exhaustively tested many different combinations of number of layers and number of nodes per layer. We thoroughly studied and tested many algorithms for training the NN in the lab. The NN that we implemented consists of 1 hidden layer with 20 hidden nodes. The weight tuning algorithm that we finally adopted for training is the Levenberg-Marquadt algorithm. During the design phase, all the simulations and tests were carried out in an interactive system that allows you to solve many technical computing problems, as the one of designing, training and testing a NN. This system is called MATLAB and is a commercial product from the MathWorks, Inc. At the implementation phase the NN was implemented by using the programming language C++. For compatibility reasons, the NN model is saved on a text file with a specific format that is used both from the MATLAB and C++ functions.

Fine tuning of the NN

The computer software features a mechanism for collecting data that can be used for re-training the neural network structure. The training process requires pairs of sensor readings and the corresponding algae concentrations with which those readings were obtained. Additional calibration might be needed in order to improve the sensor's output or for training the sensor with other algae species.

Conversion of Voltage to pH

The pH sensor that has been used for the monitoring system consists of a pH probe and an ALPHA Transmitter manufactured by Oxyguard. The transmitter drives the pH probe and converts its response to a current in the range of 4 - 20 mA. More details on the hardware of the pH sensor can be found in the report accompanying the Deliverable 11. The output of the transmitter is a linear function with respect to the pH of the solution where the probe is sank in, according to its manufacturer. We have adjusted the transmitter to read pH values in the range of 2-12. This means that we have the following information, which must be taken into account in the design of the voltage to pH converter: For pH = 2, the response of the transmitter is 4mA. For pH = 12, the response of the transmitter is 20mA. The response of the transmitter is linear with respect to the pH. The output current of the transmitter is converted to voltage over a resistor of 250Ω. This means that the currents mentioned above are transformed in the voltages 1V and 5V respectively. From the above data, we can conclude that function connecting the output voltage and the pH is linear, and can be easily calculated as:

$$pH = 2.5 \times V - 0.5$$

where V is the voltage returned from the Sensor Input Layer module.

The main control thread

This is the core of the software application. This module is running constantly on the central PC. It starts from the moment the application is launched and keeps executing until the application terminates. The Main Control Thread was programmed taking into account that it might need to perform multiple tasks in parallel; for example it might need to take action in order to regulate the pH in the bioreactor and at the same moment monitor the temperature and take action for regulating this parameter too, simultaneously. The Main Control Thread executes in the background and achieves parallelism by using Multithreading programming techniques and the ability of the modern computers to perform multitasking. The ‘‘Main Control Thread’’ is responsible for:

- Executing periodic operations.
- Requesting measurements from the Sensor Input Layer.
- Checking if the measurements are within the preset bounds .Deciding about actions that should be taken on the bioreactor's components.
- Providing the connection and coordination between the system's modules and the user's interface.

Periodic operations that need to be executed include periodic monitoring and evaluation of the monitored parameters, data logging and harvesting. The Main Control Thread consists of four Control Sub-threads which run in parallel and each one executes very specific periodic

tasks. Apart from adding the ability to perform multiple tasks in parallel, this choice also improves the robustness of the controlling software. For example, if one sub-thread crashes, then only a part of the application's functionality will be lost, while the rest of the functions will continue smoothly. Each sub-thread checks the current value of a variable, and if that value exceeds the predefined desired limits, opens and closes specific relays to adjust the value of the variable that is under observation. The four sub-threads are:

- Monitoring thread
- PH regulator
- Temperature regulator
- Harvesting regulator

In the following paragraphs we will describe more analytically these modules.

The Monitoring sub-thread

The temperature, pH and algae concentration are monitored every 60 seconds. The values are logged in a database, through the Data Logging Layer. In order to perform this task, the Monitoring sub-thread executes the following algorithm:

```
1. Send command to the Sensor Input Layer, to request reading for pH.
2. Read the value of the pH through the Signal Processing Module.
3. Send command to the Sensor Input Layer, to request reading for
   temperature.
4. Read the value of the temperature through the Signal Processing Module.
5. Send command to the Sensor Input Layer, to request reading for algae
   concentration.
6. Read the value of the algae concentration through the Signal Processing
   Module.
7. Prepare the data record by including the read data and the current
   timestamp and pass it to the Data Logging Layer.
8. Wait 60 seconds and go to 1.
```

Relay Output Layer

The following three sub-threads use the relays to adjust the value of the PH, temperature and concentration. The ''Relay Output Layer'' is responsible for driving the relay outputs of the system, thus for controlling the Control Subsystem (right part of the figure 1). So, in this paragraph we present this layer which is of great importance to the sub-threads of the following paragraphs. This layer performs all the low level operations on the hardware that are necessary in order to switch on/off the requested relays on the PCI relay card. When the Relay Output Layer receives a command from the Main Control Thread to toggle a relay, it performs the following:

- Isolates the output channel on the relay card, which corresponds to the relay which we want to toggle.
- Requests from the card's driver to toggle the relay.

Turning on or off a switch, results in turning on or off a pump or an electric valve. The ‘‘Relay Output Layer’’ receives commands to toggle relays when one of the control sub-threads of the ‘‘Main Control Thread’’ make a decision that some specific action has to be taken on the automation system, i.e. pH/temperature regulation or harvesting. The high-level operations of the Relay Output Layer are implemented in the main application’s code. The low-level operations of the Relay Output Layer (i.e. the access and control of hardware resources) are implemented in the programming language C as a separate software library (DLL file) which provides the rest of the software with those low-level functions.

PH regulator

The main purpose of the PH regulator is to maintain the PH value of the culture below a pre-defined limit. If the PH value exceeds that limit, then the PH regulator opens a certain relay and as a consequence the bioreactor tank is supplied with CO_2. Then, the pH regulator measures again the PH and if the value of PH is not under the specified limit, it opens the relay again. This iterative procedure is repeated until PH value decreases under the specified value. The pH regulation sub-thread can be disabled by the operator, by clicking on the appropriate button on the user interface.

Temperature regulator

As in the case of the above paragraph, the main purpose of the temperature regulator is to maintain the temperature value of the culture below a pre-defined limit. If the temperature value exceeds that limit, then the temperature regulator opens a certain relay and as a consequence the bioreactor tank is exposed to fresh water of constant temperature value. Then, the temperature regulator measures again the temperature and if the value of temperature is not under the specified limit, it opens the water supply again. This iterative procedure is repeated until temperature value decreases under the pre-defined value. The temperature regulation sub-thread can be disabled by the operator, by clicking on the appropriate button on the user interface.

Description of Harvesting and Washing Software unit

The part of the software that concerns the harvesting and washing sub-system is based on the diagrams that AUA provided us. These diagrams are depicted in figure 11

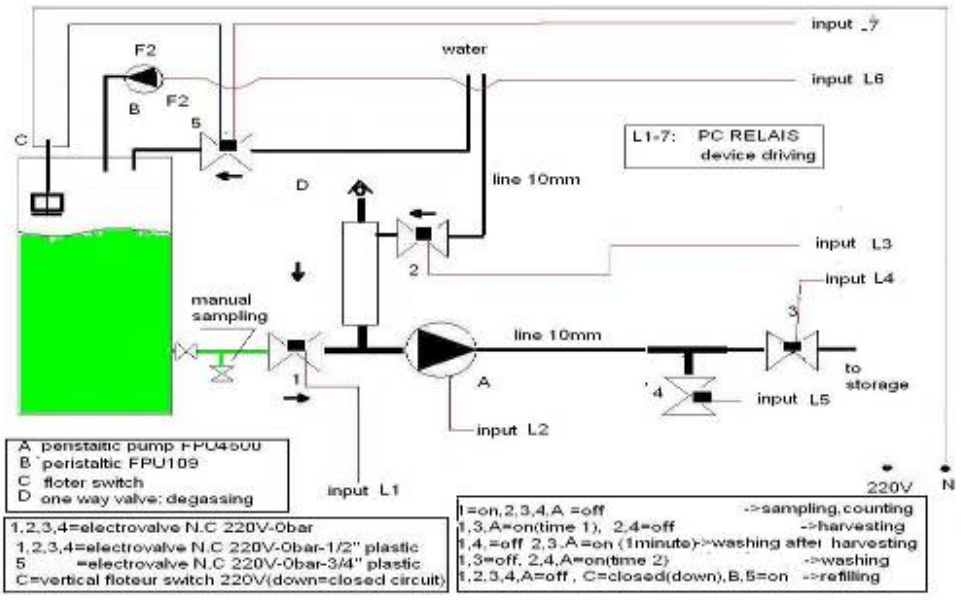


Figure 11: Harvesting and washing sub – system.

For the implementation of the procedures that described in the above diagram, we insert a new "Thread" in our software that its main purpose is to observe the concentration and take several decisions according to its value. More specifically, if the concentration exceeds a predefined value, the Harvesting Thread initiates the harvesting process and a predefined quantity of algae is transferred to the storage. When the harvesting process is completed, the harvesting thread initiates the washing process. This process cleans the sensors and the pipes with the use of water. Finally the Harvesting process, initiates the refilling process that refills the mega- boa tank with water and fertilizer. This procedure is done every time by opening and closing a specific set of relays (and as a consequence a specific set of electro - valves - pumps).

User Interface

The User Interface is the part of the Monitoring and Control Software application that is actually visible to the user. It appears automatically by launching the computer software, since the other modules of the software (depicted in figure 3) are running in the background. The user of the computer software is assumed to be a hatchery operator. The operator is not expected to have any extra knowledge or expertise on electronics or personal computers. For this reason, the user interface was designed as simple as possible. However, the operator is required to be familiar with the basics of using a simple computer program on a PC which runs Microsoft Windows XP. The user interface serves a twofold purpose:

- Receive commands and settings from the user
- Present information to the user

The interface consists of a full screen window which features a drop down menu. The area of the full screen window is used for displaying message boxes, control panels and information windows. Control panels include forms for defining the desired operation parameters (i.e. the Environmental Manager window) or forms that allow the operator to take immediate action

on the actuators of the automation system (i.e. the Control Manager window). Information windows include tables of logged data (such as the Collected Data Viewer window) or graphs. The drop down menu is used in order to allow the operator to launch control panels and to view information. The main options of the drop down menu include the Configuration, View and Tools menus. With the launch of the application, the main window automatically displays the current status of the bioreactor.

Installing and running the application

The installation of the software application on a computer is a relatively complex task and it should be performed by an expert. Up to the time of the writing of this document, all the installations have been performed by the CTI team.

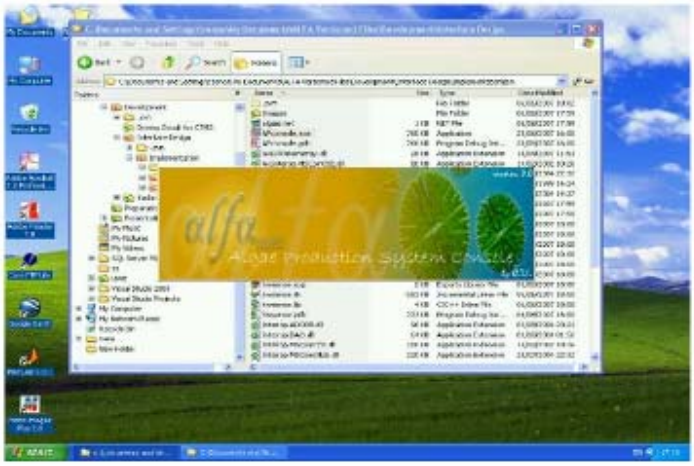


Figure 12: Splash screen shown during the initialization phase of the software application.

However, all the mentioned software modules and files can be made available to any interested partner or the reviewer, upon request. It should be noted that the custom application developed by the CTI will only run properly if the database engine is properly installed and configured and the specialized hardware parts are present, properly installed and configured. This is because it is automation software, which -in order to operate properly - needs to access the proper sensors and actuators. In order to launch the application, the program ‘‘APConsole.exe’’ must be executed. Normally, this program is configured to start automatically, as soon as the computer system completes its boot sequence. When the application starts, during its initialization phase, a splash screen is displayed as in figure 12. After this phase, the main window, the main menus and the Current Status window appear, as in figure 13.

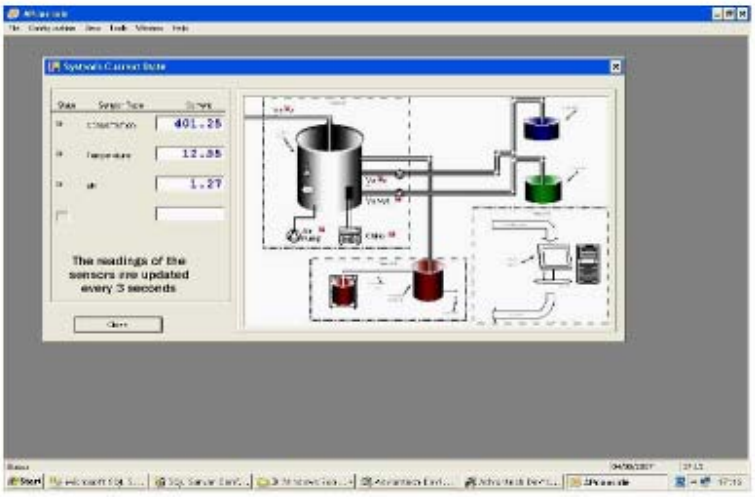


Figure 13: Monitoring the current state of the automation system (the values are for demonstration purposes only).

Monitoring of the current status

In order to monitor the current status of the automation system, the operator must open the ‘‘System’s Current State’’ window. This window is opened by default, when the application starts. Figure 13 displays an example screenshot of this window. As it can be seen, on the left part of the window the operator can see the current readings of the monitoring system. On the right part of the window, a sketch of the system is displayed. At the points where actuators exist, there are colored boxes.

Configuring the system’s parameters

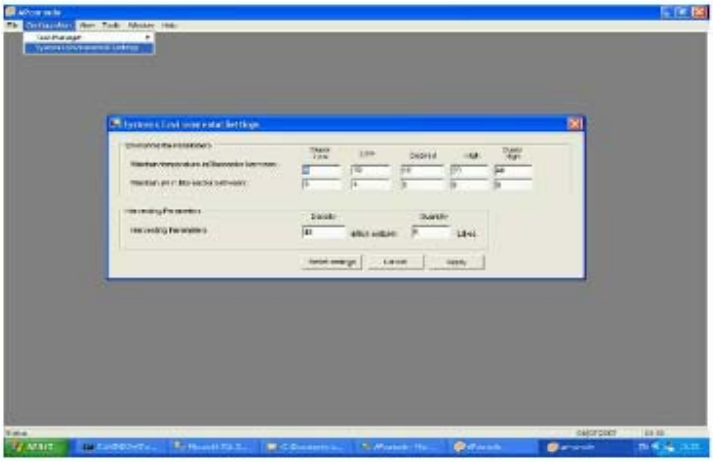


Figure 14: Configuring the system’s parameters.

The operator can configure the parameters of the system’s operation through the ‘‘System’s Environmental Settings’’ window. This window can be launched by selecting the option ‘‘Configuration’’!‘‘System’s Environmental Settings’’ from the main drop down menu. When this option is selected, the window that is shown in figure 14 is displayed.

Viewing Logged Data

The ability to view logged data which were collected by the Monitoring System is a facility offered by the Data Logging Layer. The requested data are presented in lists or graphical format by the user interface. In order to perform this action, the operator must enable the ‘‘Collected Data Viewer’’ window. This window can be launched by selecting the option ‘View’’!‘‘Collected Data’’ from the main drop down menu. When this option is selected, the window shown on the left side of figure 15 appears. This window contains controls that allow the user to define what parameters he/she wants to retrieve and for what time period. By default, all the available parameters are not selected.

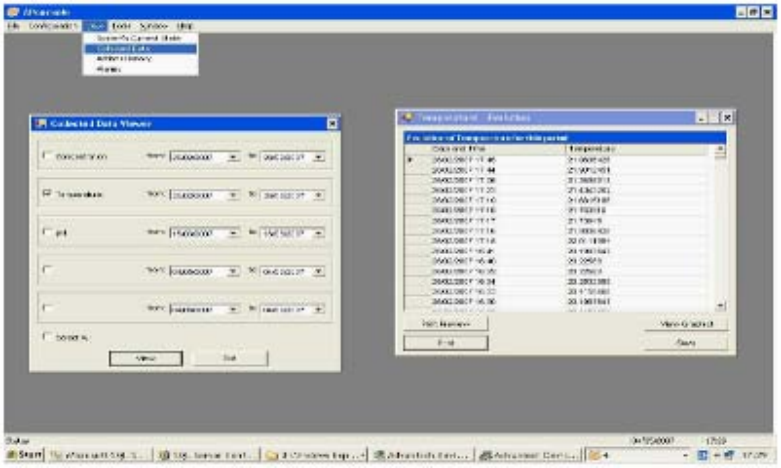


Figure 15: Creating tables of logged data.

Creating data graphs

In order to generate a data graph, the user must click on the ‘‘View Graphic!’’ button of the window which contains the data that he/she wants to view, in table format. When this is done, a graph similar to the one displayed in figure 16 is generated. The operator has then the options to preview a printing, print, save the graph in jpeg format or dismiss the window, by clicking the appropriate buttons.

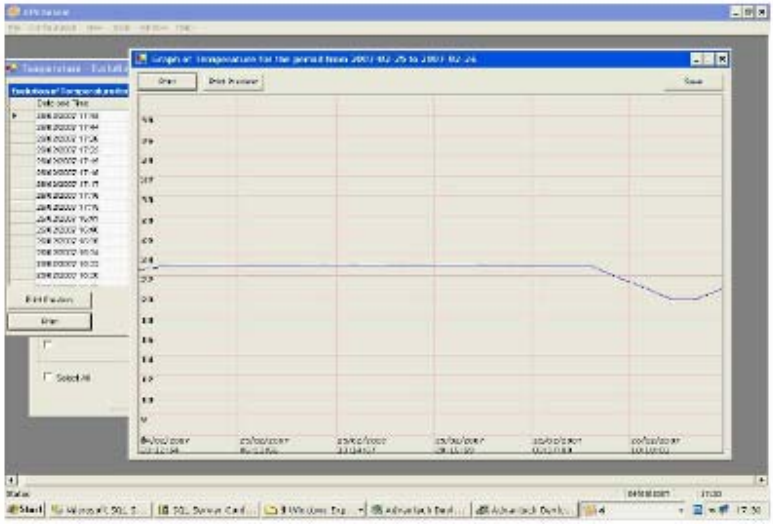


Figure 16: Creating graphs of logged data.

Viewing the Actions Log

The operator may request at any time to review the actions that were taken on the unit through the automation system. Those actions might have been taken either automatically, because of the automations, or manually, because the operator imposed them. For this reason, the user interface features a viewer of the Actions Log. The user can enable this viewer by selecting the option ‘‘View’’!‘‘Actions History’’ from the main drop down menu. Figure 17 displays one such window, for demonstrational purposes. The operator is able to see which event happened and at what moment, in the information displayed in the second and third columns of the table.

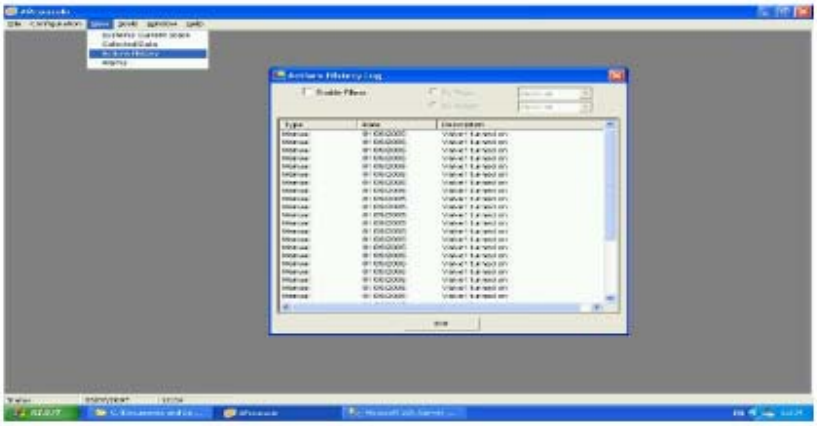


Figure 17: Viewing the log of the automatic or manual actions on the automation system.

Viewing the Alarms Log

The operator is also able to review at any time the alarms that have occurred on the unit and have been detected by the automation system. For this reason, the user interface features a viewer of the Alarms Log. The user can enable this viewer by selecting the option ‘‘View’’!‘‘Alarms’’ from the main drop down menu. Figure 18 displays one such window, for the purpose of demonstration. The operator is able to see what alarms have been detected on the system and when. This information is displayed in the second and third columns of the generated table. The first column characterizes the event as ‘‘critical’’, ‘‘important’’ or ‘‘information’’, to denote the importance of the alarm, from most to least important. The ‘‘Alarms Viewer’’ window also offers basic filtering, to allow the operator to filter out information that he is not interested in. For example, he/she might be interested to review only the alarms of ‘‘important’’ or higher importance. By default, the filters are disabled, so that no information is hidden automatically from the user.

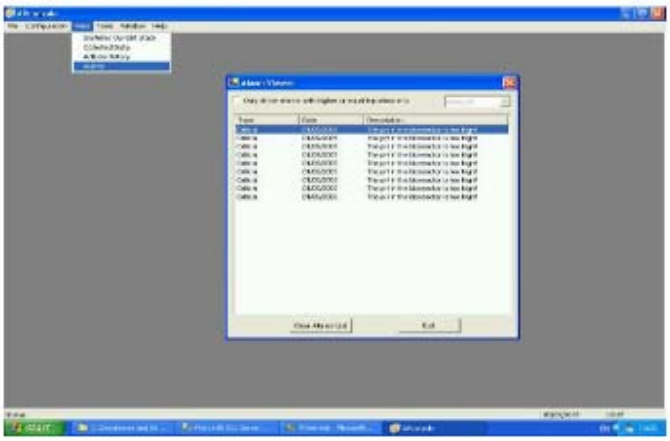


Figure 18: Viewing the log of alarms on the automation system.

Managing the sensors

The ‘‘Sensor Manager’’ allows the operator to have full control of the sensors attached to the system. The operator is able to:

- View the state of each sensor (on/off).
- Set the state of each sensor (e.g. turn it off for cleaning).
- Get the current reading of each sensor.
- Calibrate and fine-tune the sensors.

The ‘‘Sensor Manager’’ can be launched by selecting the option ‘‘Tools’’!‘‘Sensor Manager’’ from the main drop down menu. The resulting window is displayed in figure 19. This window lists the sensors attached on the system.

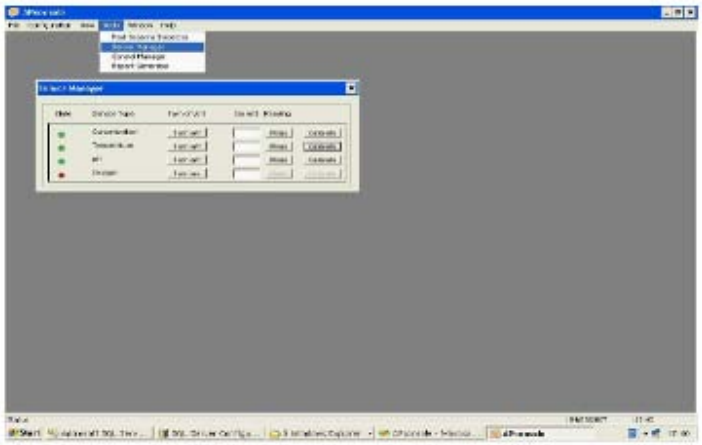


Figure 19: Managing the sensors.

Calibration of the temperature and pH sensors

The temperature and the pH sensors can be calibrated following a common approach. Typically, the sensors need to be calibrated during installation and then once every 6-12 months. In order to calibrate these sensors, the user must open the ‘‘SensorManager’’ and

click on the appropriate ‘‘Calibrate’’ button. A window similar to the one which appears in figure 20 appears.

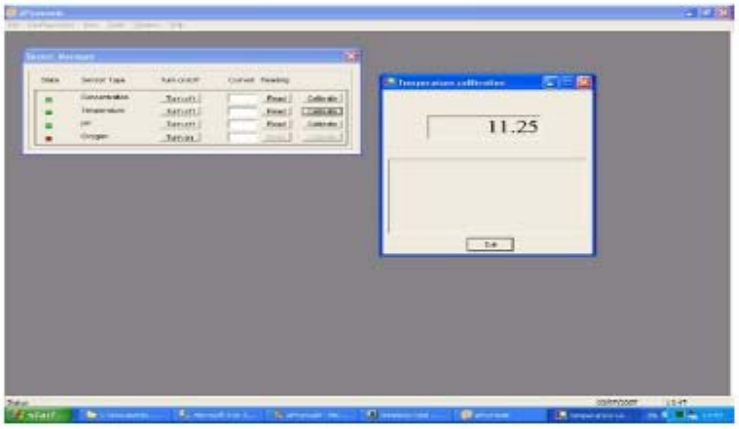


Figure 20: Calibrating the temperature/ph sensor.

Collecting data for calibrating the custom density sensor

The computer software features a mechanism for collecting data that can be used for calibrating the custom algae density sensor that has been under development by the CTI team in the frame of the ALFA project. This mechanism was necessary, so that the CTI team was able to collect calibration data in order to train the neural network structure (see Deliverable 12 ‘‘Signal Processing Unit (SPU)’’ - for more details). The algae sensor’s calibration mechanism can be started by clicking on the ‘‘Calibrate’’ button that corresponds to the density meter, from the ‘‘Sensor Manager’’ window. A window similar to the one which appears in figure 21 appears.

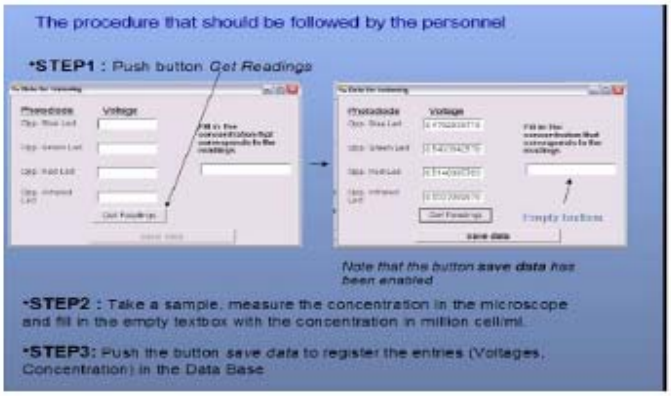


Figure 21: Calibrating the custom algae density sensor.

The Control Manager

The ‘‘Control Manager’’ is a tool that offers capabilities of immediate actions onto the system. It consists of a blueprint of the unit’s structure which includes controls each one representing a specific electrical component of the system. The operator can then take immediate actions on these actuators. This tool could be useful in cases such as:

- An emergency (eg. a need to empty the bioreactor).
- Fine-tuning of the operation of the system or in order to correct possible mistakes that have been made by the automation.

The ‘‘Control Manager’’ can be launched by selecting the option ‘‘Tools’’!‘‘Control Manager’’ from the main drop down menu. The resulting window is displayed in figure 22.

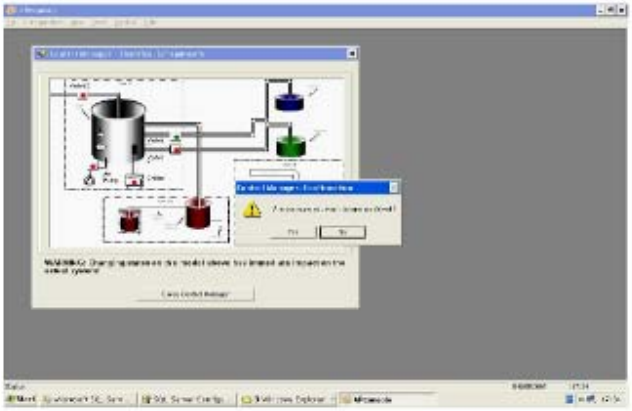


Figure 22: Using the control manager.

WORKPACKAGE 4.
OPTIMISATION AND EVALUATION OF THE CAP AND CHTP SYSTEMS.

Important findings

Task 4.1 Optimisation and testing of the CAP system

The 'Boas' CAP system was evaluated from June 26 to August 8, 2007 at the Argosaronikos facilities. Full details are given in D5 (Section III). The results of the continuous algae production were used as feedback to improve the final design with special adaptations and to design and install the final control, monitoring and harvesting systems and integrated the CAP system to the CROPS and storage units and the fry tanks with a continuous loop and special automatic refilling and cleaning systems. The results of the evaluation of the optimized CAP system were used as input to deliverable D3.

Optimisation of the full-scale boa

Aeration system

The initial design had a secondary flexible perforated PVC pipe inside each coil pipe there is a which delivered fresh air mixed with CO_2 along the culture. This pipe was perforated every 1 m of its length and had an internal diameter of 8 mm.

Air input adaptor

The air pipe enters in the coil through specially formed adaptors placed on the exit and entrance manifold, Fig. 6. At the same figure both the entrance and exit manifold are shown.

Air pump

For the aeration of the culture two pumps are used. The first provides air into the coil through the air adaptors, for aeration of the culture and the second provides air directly in the tank for aeration and agitating the culture. The characteristics of each pump are given below.

Aeration pump (for Bio-coil aeration): The demand for air inside the Bio-coil, considering the prototype design, are 100 l/min for each pipe thus a total amount of 400 l/min is necessary to achieve the flow characteristics and aeration conditions met at the pilot design. The total head losses are estimated to be approx 3 m, so a pump providing 400 l/min at a pressure capable to overcome such static pressure of the water column (approximately 3.0 m) is suitable.

Aeration pump (for tank aeration): The demand for air inside the tank is estimated to be at least the half of the total volume of the tank, so a pump delivering 100 l/min at a pressure of 2.5 m is suitable.

This design was found to be probimatic during cleaning due to the internal pipe and spacers which were were difficult to remove.

Air blower pumping air directly into the boa coil pipes.

The design was changed to the pumping of air directly into each spiral pipe. This allowed a constant quantity of air to be pumped into each section. This had the effect of reducing the colume of culture fluid by about 7.5% but had the additional beneficial effect of additional friction by the air water mixture for the cleaning of the inside of the pipe.

Cooling system

There were 3 different possibilities in the design of the the cooling system

- Water bath
- Water jacket
- External shower

Water bath

This cooling design consisted of a pump which brought sea water to a specially formed bath in which the tank and the lower part of the Bio-coil is placed. Considering cooling demands and sea water temperature to be around 15-20 °C it was calculated that the pump must provide to the bath 6.5 m^3/h sea water at a pressure capable to cover the total losses in the transmit pipes depending on the piping system. This pump must be resistant to sea water, preferably with plastic components.

Water jacket and water channel

The cooling pump provides sea water through a water jacket placed in the main tank. The water jacket is a transparent pipe 0.18 m internal diameter (Fig. 7). Water passes through the jacket and is

released in the water channel. Finally sea water returns back to sea, through transmit pipes with the suitable slope.

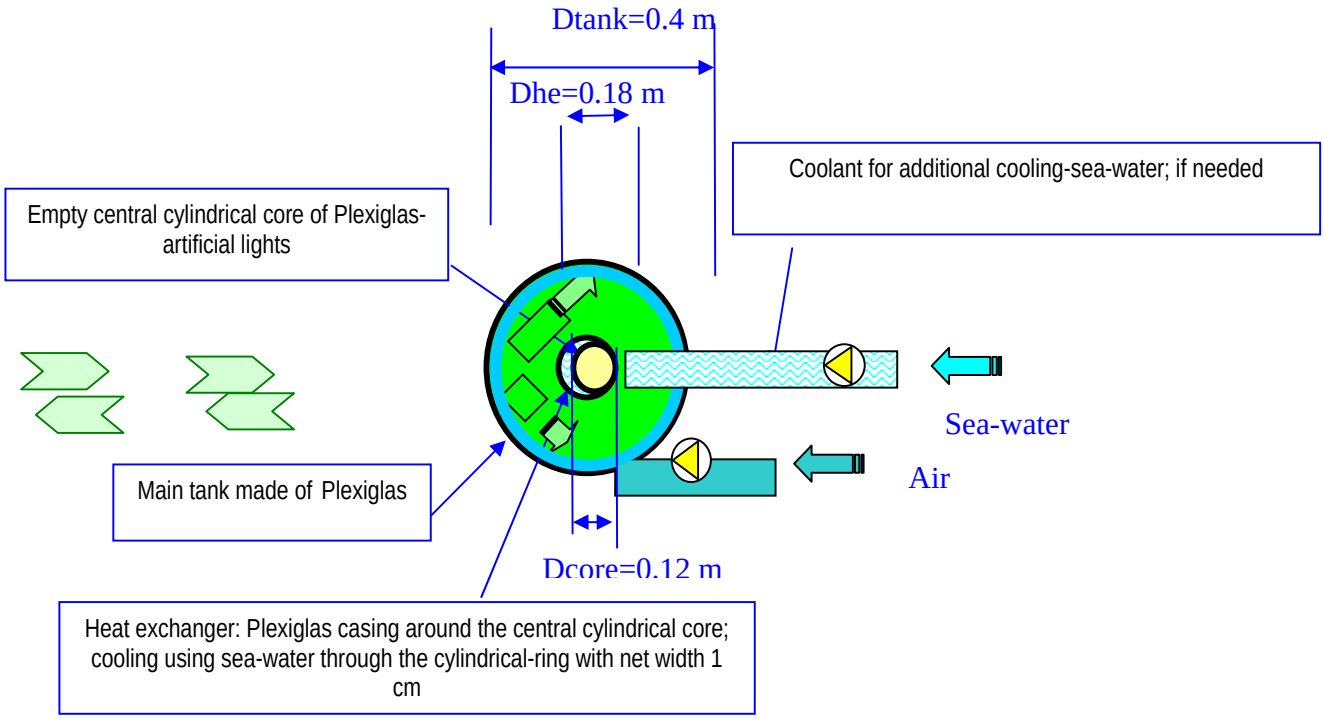
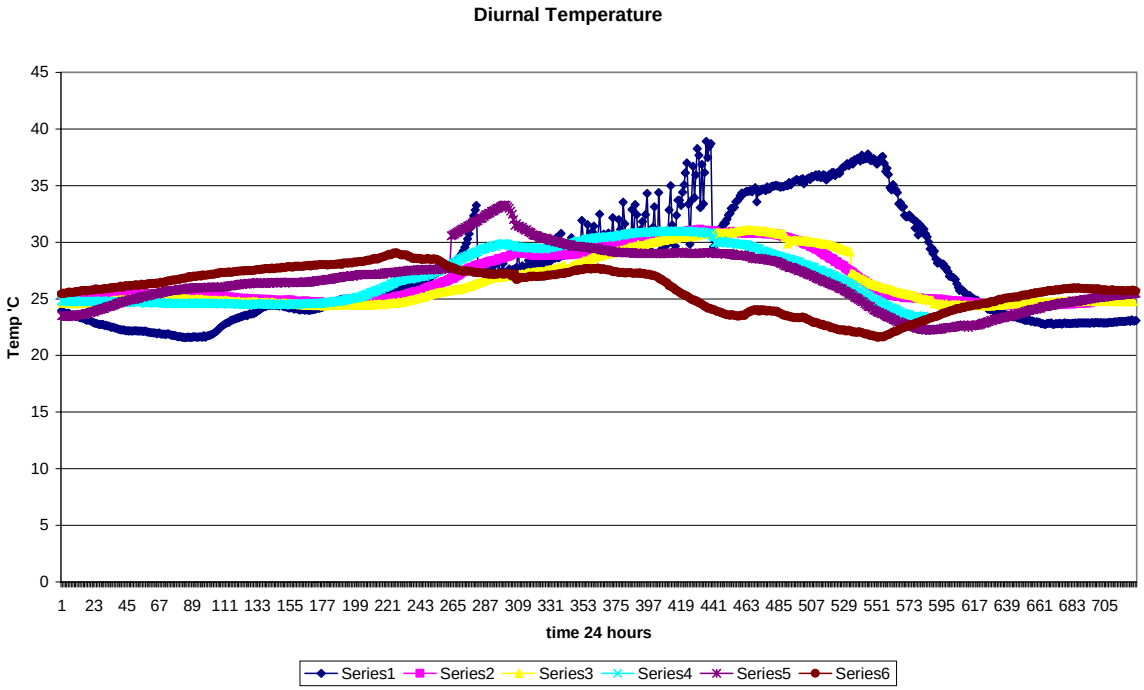


Figure 7. Cooling system water jacket

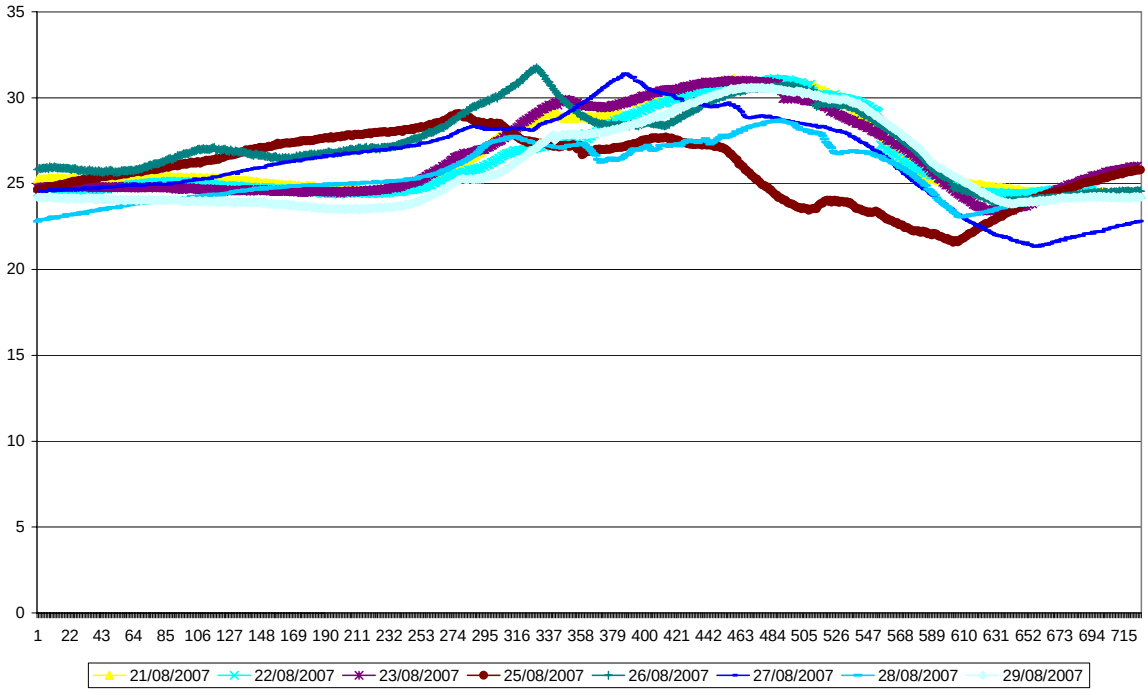
The cooling system using the internal heat exchange was found not to be able to cope with the strong heating that occurred in Greek conditions during summer. The cooling using only the internal jacket heat exchanger allowed the culture fluid temperature to rise over 35 degrees during the day during May (See series 1 in the figure below). A number of alternative cooling systems were tested and the most efficient was found tto be an external shower arrangement which was able to maintain culture fluid temperatures to a maximum of 30 ‘C.



The design was therefore changed to an external evaporative cooling system described below.

Complementary cooling with water evaporation

The external cooling system consisting of water shower-type or irrigation-type sprayers. The water is sprayed directly on the boas tubes so that cooling is achieved by the combination of convection and evaporation. The water falls into a drain below the photobioreactor.



The external evaporative cooling system was much more efficient maintaining the temperature within a differential temperature of 7 ‘C even during the hottest summer season in Greece during August.

External shower cooling.

There were 4 production trials each lasting ovwer one month.

BOA 01 Production data and analysis was undertaken between 16 May and 12 June 2007 lasting 28 days.

Productivity

- The production density increased slowly to around 80 million cells per ml at which point harvesting was stated to maintain the debnsity between 60 and 100 million cells per ml.

Specific Growth rate

- Specific growth rate was relatively high during the first 7 days (lowed density and during log phase growth) and then slowed.

Optimum production density

- The optimal production density during May/June was between 50 and 100 million cells per ml reducing in productivity at higher densities.

Environmental data

- The culture temperature was maintained at between 22 and 28 ‘C (May/June) during the production cycle. pH was kept constant at optimal levels by the addition of CO2.

Diurnal fluctuations

- The average diurnal fluctuations were an increase in temperature during daylight hours peaking at around 14.30 hrs. Density reduced during the day due to harvesting that took place at around 11.00 hrs. pH remained steady throughout the diurnal cycle.

BOA 02 Production data and analysis

The photobioreactor was then cleansd and re-inoccultated and started for a new production run. The second production run of the spiral photobioreactor was undertaken between 19 June and 8 August 2007 lasting 50 days.

Productivity

- The production density increased more quickly to around 200 million cells per ml over 12 days at which point harvesting was stated to maintain the debnsity between 180 and 300 million cells per ml.

Specific Growth rate

- Specific growth rate was relatively high during the first 12 days (lowed density and during log phase growth) and then slowed.

Optimum productivity

- The optimal production density during May/June was between a range of 200 and 250 million cells per ml reducing in productivity at higher densities.

Environmental data

- The culture temperature was maintained at between 23 and 32 ‘C (June/August) during the production cycle. pH was kept constant at optimal levels by the addition of CO2.

Automatically measured pH

- pH remained between 7 and 8.5 throughout the production cycle.

Automatically measured temperature

- Average diurnal change in temperature was 8’C. It can be noted that when the trial was stopped and there was no cooling, culture fluid reached 40’C showing the effectiveness of the cooling system.

BOA 03 Production data and analysis was undertaken between 11 August and 13 September 2007 lasting 33 days.

Productivity

- The production density increased more quickly to around 200 million cells per ml over 9 days at which point harvesting was stated to maintain the density between 200 and 350 million cells per ml.

Specific Growth rate

- Specific growth rate was relatively high during the first 12 days (low density and during log phase growth) and then slowed.

Optimum production density

- The optimal production density during August/September was between 100 and 200 million cells per ml reducing in productivity at higher densities.
- At densities of between 100 and 200 million cells per ml, the photobioreactor was producing around 40 trillion cells per day from 670 liters of culture medium (60 trillion cells/day/m3).

Environmental data

The culture temperature was maintained at between 23 and 33 ‘C (August/Sepember) during the production cycle. pH was kept constant at optimal levels by the addition of CO2.

Diurnal fluctuations

- The average diurnal fluctuations were an increase in temperature of 3 degrees during daylight hours peaking at around 14.30 hrs. Density reduced during the day due to harvesting that took place at around 11.00 hrs. pH remained steady throughout the diurnal cycle.

Automatically measured pH

- pH remained between 7 and 8.5 throughout the production cycle.

Automatically measured temperature

- The average diurnal change in temperature was again 8’C. It can be noted that before the trial was started and there was no cooling, culture fluid ranged between 22 and 40’C showing the effectiveness of the cooling system both for maintaining the temperature during the night (slight) and cooling during daylight (major).

BOA 04 Production data and analysis was undertaken between 19 September and 22 October 2007 lasting 32 days.

Productivity

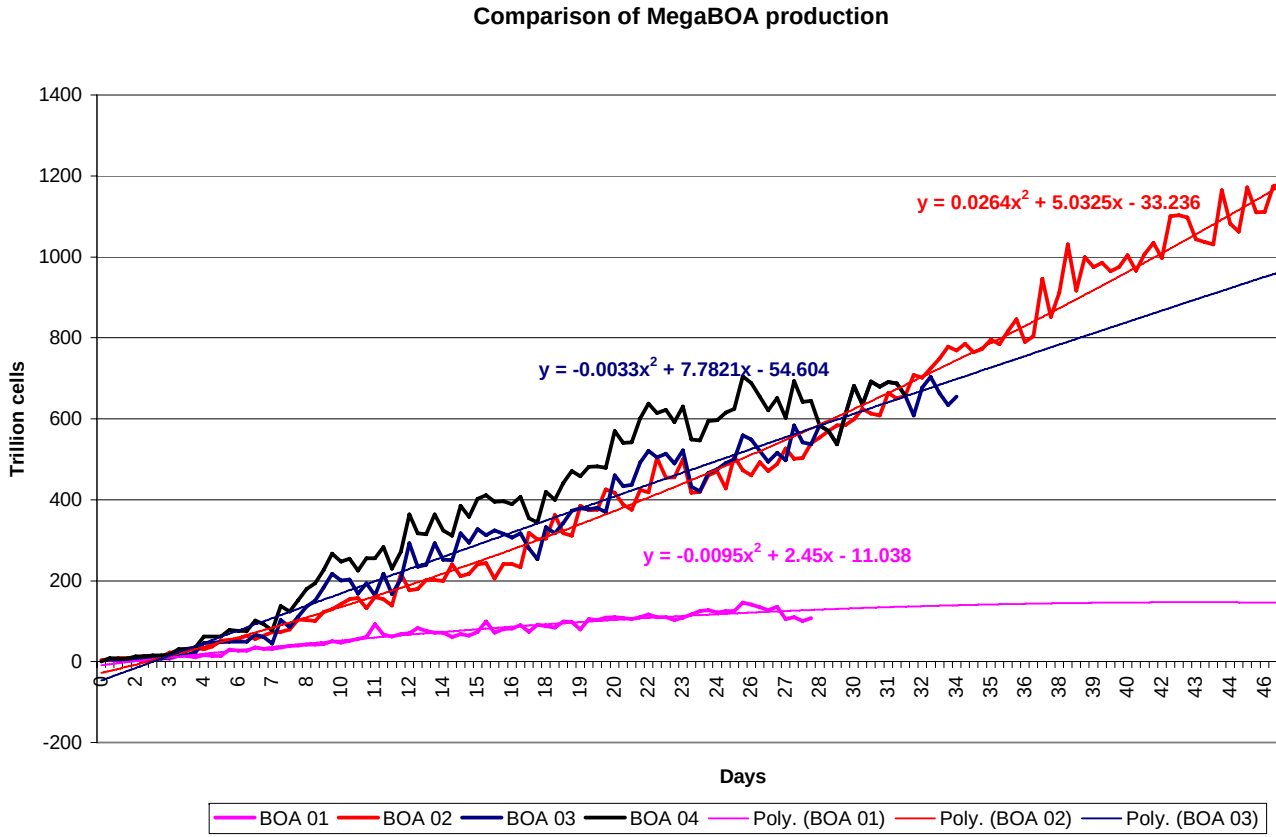
- The production density increased more quickly to around 350 million cells per ml over 7 days at which point harvesting was started to maintain the density between 300 and 450 million cells per ml. However, density and productivity decreased later in the production run.

Optimum productivity

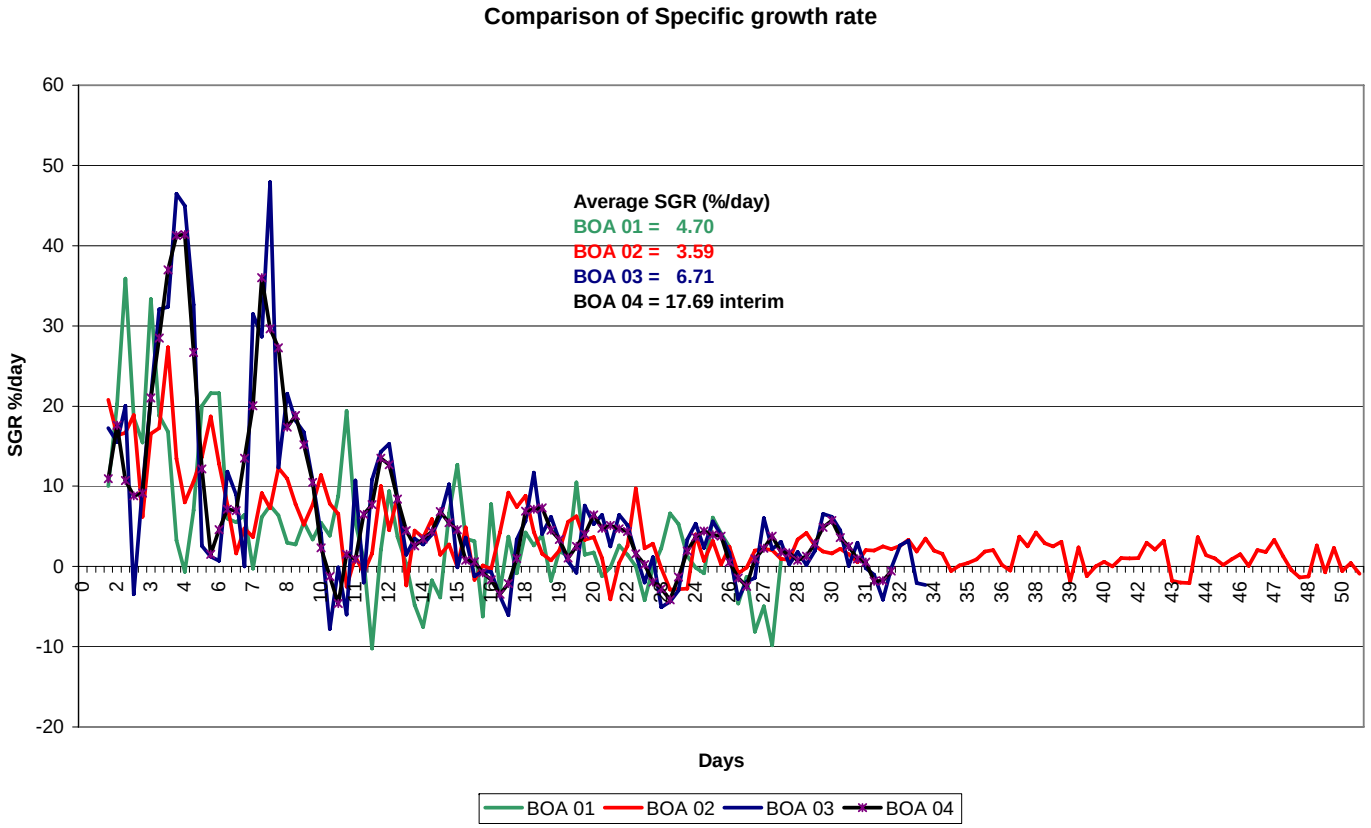
- The optimal specific growth rate during August/September was at densities between a range of 50 and 100 and 300 and 400 million cells per ml reducing in productivity at higher densities.

Comparison of production

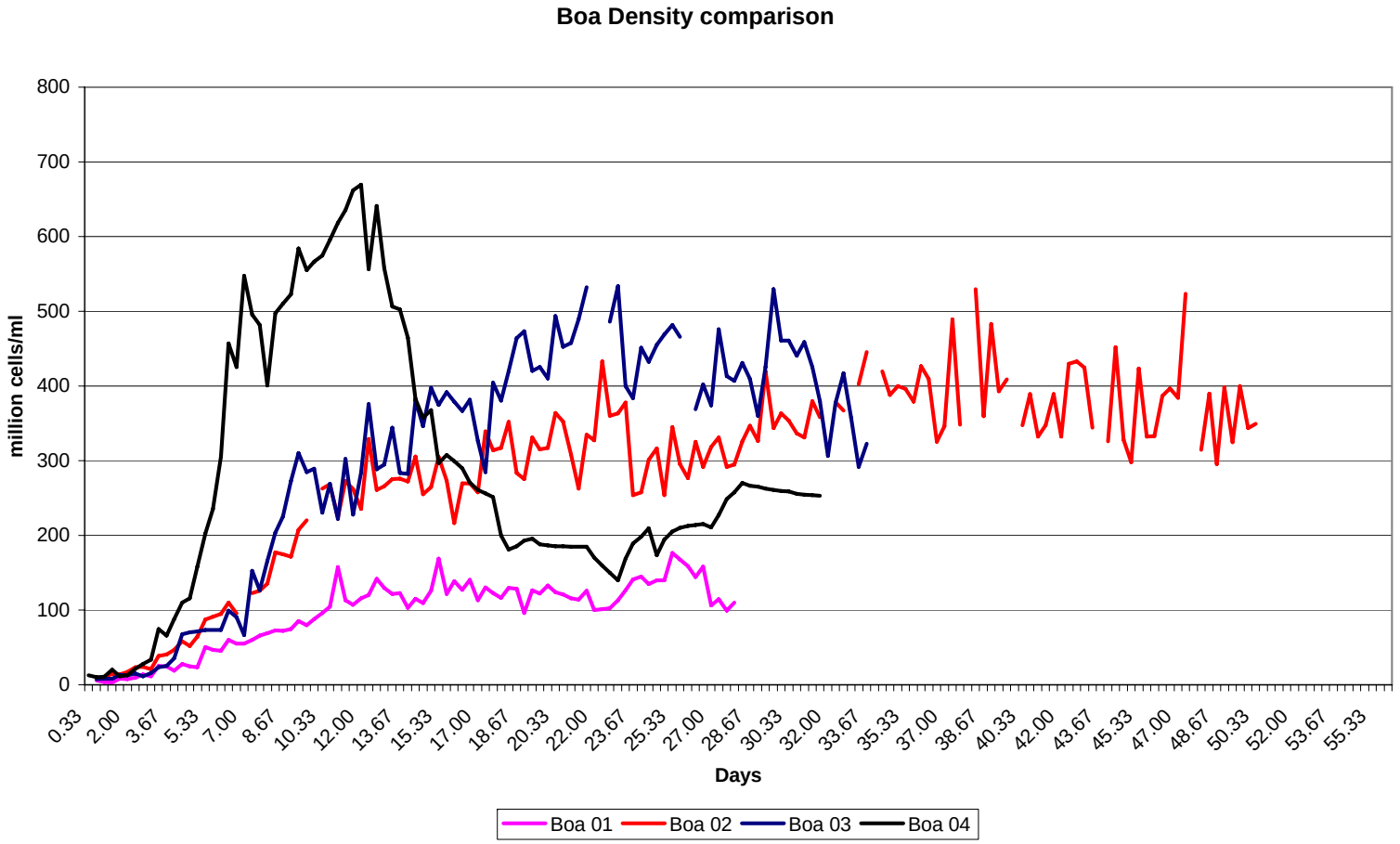
Productivity increase with each successive production run illustrating the optimisation of production over time. Typical production runs were of 30 days to allow improvements to be made between the runs. The longest run was 50 days with continuing increase in production showing that there is no reduction in production with time.



In all production runs, specific growth rate was fastest at the start (and lowest algal density) but reducing with time. However production in terms of trillion cells per m2 culture medium per day was highest at the higer algae densities.



Increase in cell density was faster in each successive production run. The fastest growth occurred in the last production run Boa 04 with densities of over 600 million cells/ml recorded. However the densities then reduced.


Boa Density comparison
million cells/ml
800
700
600
500
400
300
200
100
0
0.33
2.00
3.67
5.33
7.00
8.67
10.33
12.00
13.67
15.33
17.00
18.67
20.33
22.00
23.67
25.33
27.00
28.67
30.33
32.00
33.67
35.33
37.00
38.67
40.33
42.00
43.67
45.33
47.00
48.67
50.33
52.00
53.67
55.33
Days
Boa 01
Boa 02
Boa 03
Boa 04

Discussion and conclusions on the optimising of the spiral MegaBoa for live algae production

Comparision of results from the different production runs

Production results	BOA 01	BOA 02	BOA 03	BOA 04	Average
Average Density tot	96.0	291.5	305.2	321.7	306.1
Max density (million/ml)	176.4	529.6	533.8	669.5	577.6
Days to reach average	10.3	14.3	10.0	5.0	9.8
Average density (millions/m)l	125.5	340.8	392.8	381.2	371.6
Standard deviation	18.8	59.5	74.9	133.6	89.3
Average SGR	4.7	3.6	6.7	5.7	5.3
Cumulative production	107.1	1234.8	654.7	691.3	860.3
Days of production	28.0	50.0	33.7	32.0	38.6
Production per day (trillions/day)	3.8	24.7	19.4	21.6	21.9
BOA Volume	585.0	627.5	670.0	670.0	655.8
Average harvest per day (lits/d)		77.0	62.1	49.0	62.7
% harvested/day		12.3	9.3	7.3	9.6
Production (trillions/m3/day)	6.5	39.4	29.0	32.2	33.5

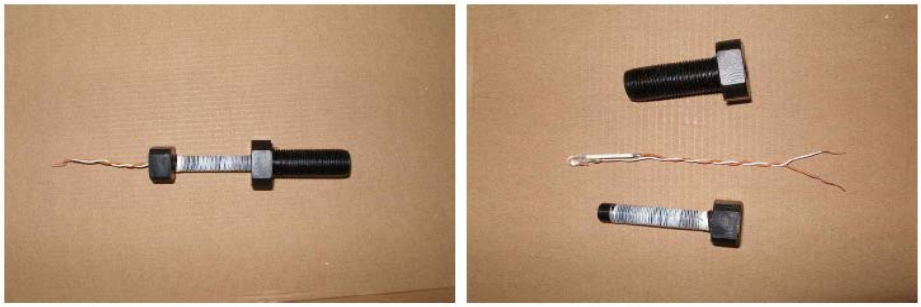
Each successive production run gave better results.interms of speed to reach averge density and maximum density. The original hypothesis was that the higher average density (400 million cells/ml) with a medium cropping rate (7-9%/day) would give the highest productivity. However, high densities reduce the specific growth rate and so it would seem that it is better to run at lower densities (350 million/ml or less) and higher cropping rate (12%/day) give the highest production of cells/day.

Task 4.1 Optimisation and testing of the CAP and CHTP system

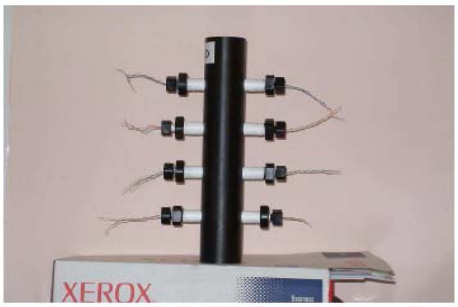
During this task the hardware and software that was designed and developed under WP2 & 3, have been installed in an experimental prototype scale at the hatchery of ARGOSARONIKOS at Salamis, Greece. The system operated under real conditions. In this way the software and hardware were debugged and optimised. The user-friendliness of the new system also tested while the hatchery personnel were trained in the use of the new equipment. In the following paragraphs we will describe the optimization procedure that leaded to the final version of the systems. This procedure was based on the matters emerged during the testing of the experimental prototype scale at the hatchery of ARGOSARONIKOS and also on observations emerged from the lab's prototypes. Finally, we describe the two-tubular version of the software. This software was designed to meet the necessary requirements of the system which is going to be installed at Sagafjord Fish Farms at Norway.

Optimization of the Density Sensor and the sensor driving circuits

Two density sensors have been constructed according to the design that was presented in Task 2.3 (see Fig. 1). One should note that special care has been taken to minimize the effort for sensor maintenance. The Leds & Photodiodes can be easily replaced since they are threaded on the sensor body with the use of a spanner and the Led housing length has been increased, to allow the user to reduce even more the distance between Leds & Phototdiodes.



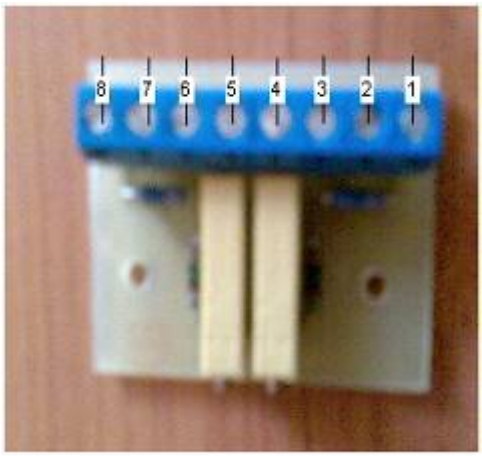
(a) Led housing assembled (b) Led housing disassembled

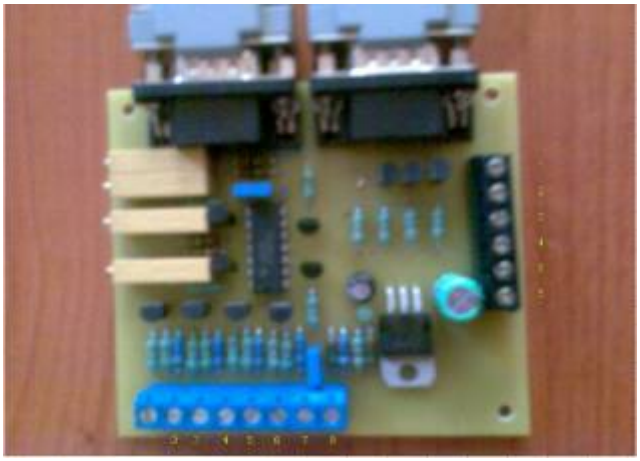


(c) sensor assembled

Figure 1: Pictures of the new density sensor.

During this Task the driving circuits for interconnecting the sensors (pH, temperature, algae density) to the PC were constructed from a small Greek company that designs and implements custom made boards. The new are optimized in terms of size, accuracy and maintenance. The new driving circuits are presented in the figure below.





Harvesting and Washing Software unit version II

AUA optimized the harvesting and washing unit and provided us with an updated design of the harvesting and washing sub-system. This design is depicted in figure 2. We modified the part of the software which used this sub-system.

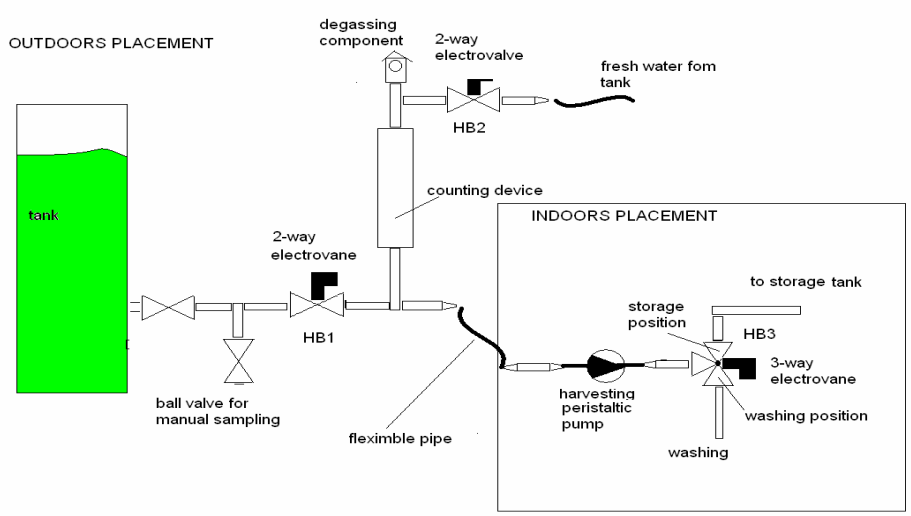


Figure 2: The updated version of the harvesting – washing sub-system

To meet the desired specifications, several changes were made in the software. We must note that the electro - valve 1 of the first design was replaced by an electro – vane. The electro-vane must be operated in a different way, unlike with the electro-valves which working with this simple manner; an electro-valve allows the flow of a liquid if in its coil is applied and maintained the appropriate Volts (220/24/12 – depending on the model and the manufacturer). The electro-vane opens if the appropriate Volts are applied only for some seconds in the first of its two coils. Then, the electro-vane closes if we apply the appropriate volts in its second coil. Thus, we had to include this procedure in the parts of the software that concern the opening and closing of V1 (electro-vane 1). Apart from this change, the only important difference that one can note in the algorithms is related to the quantity of algae that we harvest every time the concentration of the tank exceeds the pre-defined value. Due to the washing procedure, some quantity of algae is lost and since in the first approach the refilling procedure follows only after the harvesting procedure, if the tank reaches the specified concentration and it had lost match liters, we bypass the harvesting procedure and initiate the refilling procedure (see task 3.4 for more information). In the second approach, after every washing procedure, we refill the tank with the appropriate amount of water and so, the previous procedure is no longer necessary. In the second approach the harvesting procedure is simpler, and it can be better illustrated with the flow diagram of figure 3.

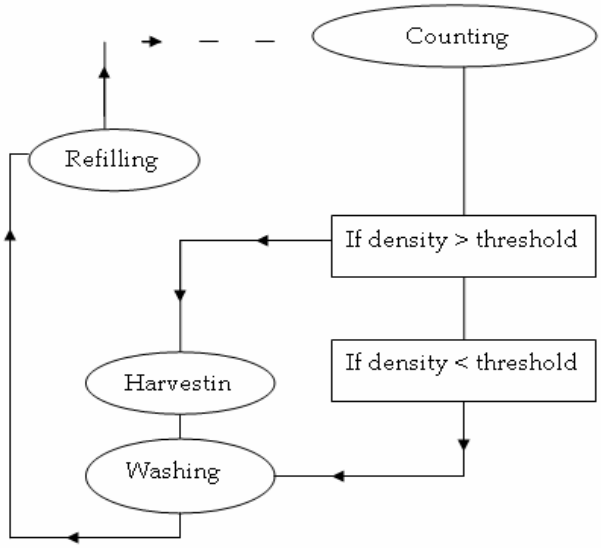


Figure 3: Flow diagram of the harvesting algorithm.

Final version of the mega-boa system

During the period that the experimental unit was working at Salamis, we noticed some facts that we took into account in the designing of the final version o the system. The first versions of the system have continuous flow of the algae via the concentration sensor, which continuously measured the concentration of the tank. As described in task 2.3, the concentration sensor consists of 4 LED and 4 Photo–diodes. We had noticed that continuous exposure of the photo-diodes to the algae lead to photo-diode destruction. As a consequence in the final version of the system, algae flow through the sensor only when we desire to get a measurement of the concentration. So now, the software gives two options to the user:

1. Instantly start the measurement procedure via the pressing of a button
2. Pre-define the number of measurements that the system will initiate automatically during the day.

AUA, also decided to design a final system that the harvesting procedure was done manually and independently of the automated system. Thus, the system now has automated only the washing and refilling procedures. So, AUA provided us, the designs for the washing and refilling sub-systems depicted in figures 4 and 5 respectively.

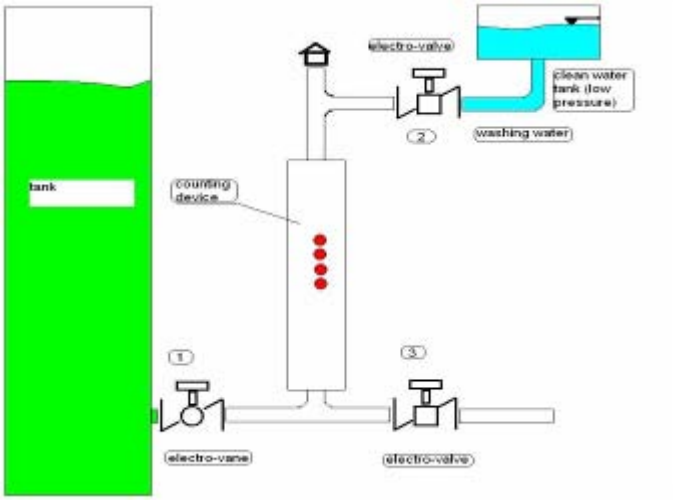


Figure 4: Washing sub-system

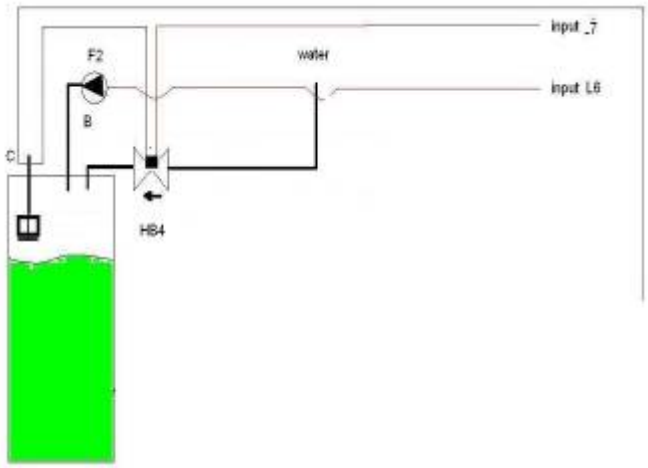


Figure 5: Refilling System

To support these functions several changes had to be done in the user's interface part of the software. The final version of the Bioreactor state window is depicted in figure 6. As we can see now the PH and temperature values are still continuously updated whereas the concentration value has the most recent value, and the date-time this value was measured. By pushing the button Get concentration, the process of the measurement of the concentration starts. In figure 7 we illustrate the upper part of the main window. Here we can see three buttons. The button with the label "Add fertilizer" opens the fertilizer's pump for the time that the user had specified in the field "Feeding time" of environmental settings. This variable can be set by the user and its value is saved in the variable Feeding_Time of table 1 of the database. The updated version of environmental settings can be seen in figure 8. Here we can note also and another change, the field "Counting Times". This value is correlated with the variable Measurements of the table and it concerns the number of programmed measurements done during the day. It can also be defined by the user.

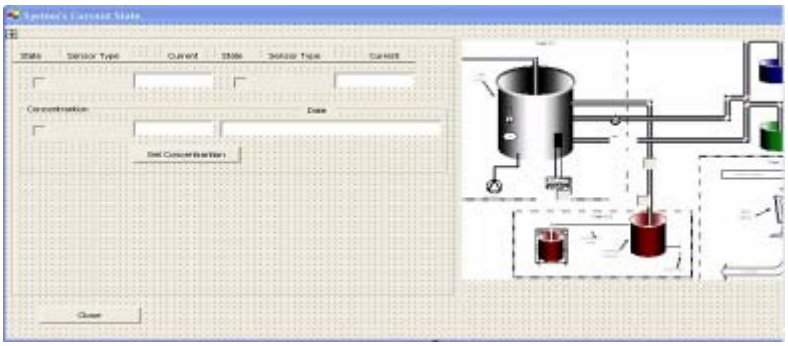


Figure 6: Bioreactor state window

In figure 7 the other 2 buttons concern the PH regulation and the measurement procedure. By pressing these buttons we can enable/disable the regulation of PH and measurement procedure respectively. These buttons replace the "control manager" of the first version. We should also mention that during this optimization phase of the software we examined the case of adding network support to the software. Finally we decided not to include network support to the software, due to the lack of internet connection in the Salamis SME.

Figure 7: Regulator settings window.

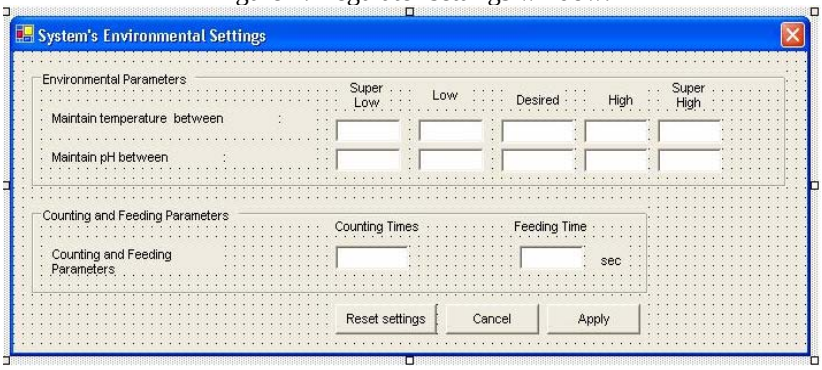
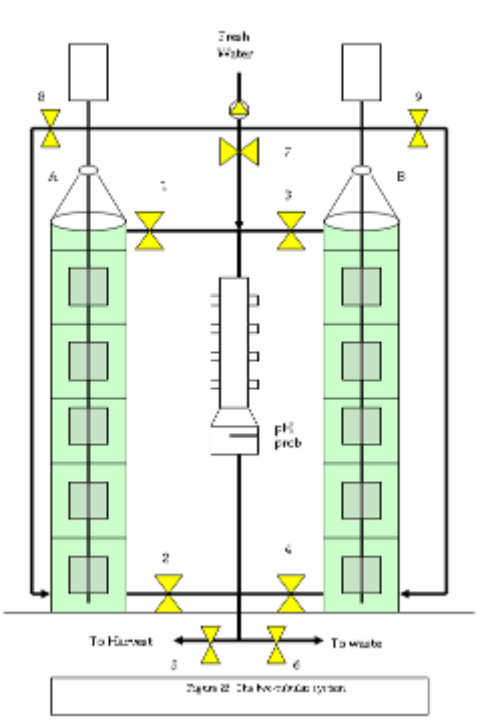


Figure 8: Environmental settings window.

The two-tubular system

The designs of the two-tubular system are depicted in figure 23. This system is going to be installed in Sagafjord Fish Farms at Norway. The basic interconnection of the relays, ADVANTECH cards, PC and sensors is exactly the same with the mega-boa system and it follows the diagrams of figures 1 and 2. The software architecture of the system is similar to that of figure 3, but each software layer has different functionality than these of the mega-boa system. The two – tubular system has a completely different way to use the relays, since now before every action(monitoring, harvesting, etc.) , the software must first choose the desired tube, in which the action is going to be applied. This is done due to the fact that there is a single monitoring and control unit for the two tubes. So, each process must be done in a time-scheduling manner for each tube. Moreover, the harvesting and cleaning algorithms are different. In the next paragraphs we are going to present each layer of figure 3, emphasizing, where differences exist in the two-tubular system.

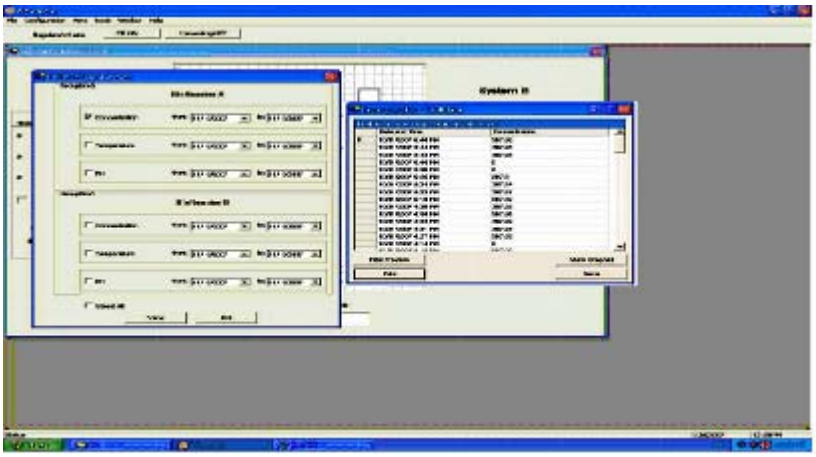


The data logging unit

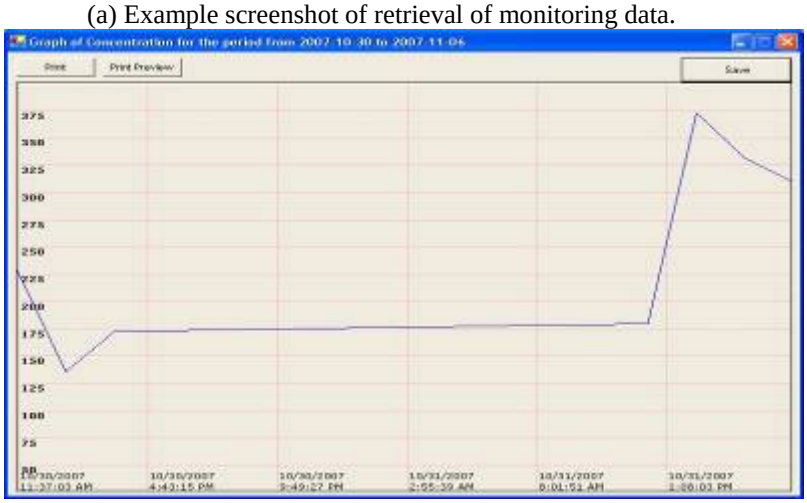
The Data Logging Unit of the tubular system has exactly the same organization as in the mega-boa system.

The Data Logging Layer

The data logging layer has the same structure and in the tubular system. The difference is that, some functions of the Data Logging Layer of the mega-boa system correspond to two functions in the two-tubular system. Each couple of functions in the two-tubular system has the names X() and X2() respectively. The first function logs data in the database that emerged from the first tube and the second function logs data in the database that emerged from the second tube. The rest functions are working with exactly the similar way as in the mega-boa system. The facilities that are used to retrieve logged monitoring data and to present them in lists or graphical format by the user interface are modified with a similar manner. The functions which are used for the retrieval of the data are the same with those of the mega-boa but now we have again two same functions for each tube named with the same way X and X2(). In figure 24(a), one such sample list with retrieved data is displayed. The operator selected which parameter(s) and for what time period(s) should the data refer to, and the results were included in a listbox. The figure 24(b) shows a similar case, where the data are presented visually. Note in the figures, that now we have the option to retrieve the data for each of the tubes, as the data for each tube are organized in the different groupboxes.



(a) Example screenshot of retrieval of monitoring data.



(b) Example screenshot of plotting of monitoring data.

Figure 24: Example screenshots of data retrieval and presentation, using the facilities provided by the Data Logging Layer.

Signal Processing Layer

Exactly the same and in the two–tubular system.

The main control thread

The main control thread is the thread with the most differences. It is consisted again of the same four sub-threads but the functionality of the threads is modified to meet the desired requirements of the two-tubular system. More specifically:

The Monitoring sub-thread

The temperature, pH and algae concentration of each tube are monitored and the values are logged in a database, through the Data Logging Layer. The difference in the tubular system is that before each measurement, a tube must be selected. This process is done interchangeably, and every 2 minutes we update the measurements for each tube. The selection of each tube can be done with the following configuration of the valves (See figure 23):

To sample Tube A

Valves open 1 and 2

Valves closed 3, 4, 5, 6, 7, 8, 9

After the selection of the tube, the Monitoring sub-thread executes a specific algorithm in order to perform the measurement (the same as in the mega-boa case):

Relay Output Layer
Same, as in the mega-boa case.

PH regulator
In the two-tubular system we are using two PH regulator threads, one for every tube. The functionality of these threads is exactly the same with the one PH regulator thread of the mega-boa system. More information can be found in the paragraph that corresponds to the mega-boa system.

Temperature regulator
According to the designs that we were provided, the two-tubular system does not use some kind of a temperature regulation procedure, so we have not implemented temperature regulators for the tubular system.

Description of Harvesting and Washing Software unit
The two-tubular system uses a very simpler algorithm for the harvesting procedure. For each tube, if the current concentration exceeds a predefined limit, then the software initiates the harvesting procedure for this tube. The harvesting procedure lasts until the concentration decreases bellow the desired limit. Then the system initiates the cleaning procedure of the pipes and the sensors. The harvesting of each tube and the washing can be done with the following configuration of the valves (See figure 23):

To harvest Tube A

Valves open 8, 1 and 5 (until set density)

Valves closed all others

To harvest Tube B

Valves open 9, 4, and 5 (until set density)

Valves closed all others

User Interface

The user interface of the software was redesigned in order to provide the necessary functionalities to the user of the two-tubular system. The following paragraphs explain in similar manner with the mega-boa system the options that are available to the user.

INSTALLING AND RUNNING THE APPLICATION

The installation of the software is exactly the same and in the tubular case. In order to launch the application, the program ‘‘APConsole.exe’’ must be executed. Normally, this program is configured to start automatically, as soon as the computer system completes its boot sequence. When the application starts, during its initialization phase, a splash screen is displayed as in figure 12. After this phase, the main window, the main menus and the Current Status window appear, as in figure 25.

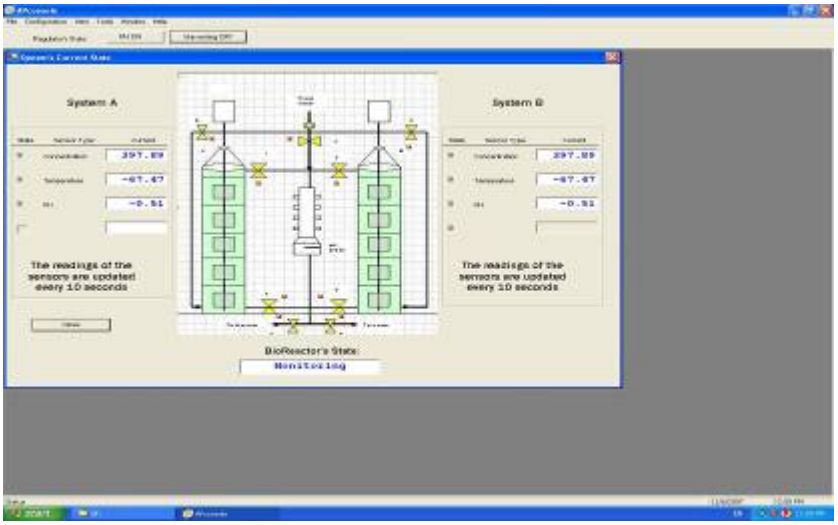


Figure 25: Monitoring the current state of the automation system (the values are for demonstration purposes only).

MONITORING OF THE CURRENT STATUS

In order to monitor the current status of the automation system, the operator must open the ‘‘System’s Current State’’ window. As it can be seen, on the left part of the window the operator can see the current readings of the monitoring system for the first tube. On the right part of the window, the operator can see the current readings of the monitoring system for the second tube and in the centre of the window a sketch of the system is displayed. At the points where actuators exist, there are labels. A label with a text “off” indicates that this actuator is currently turned off, while a label with a text “on” indicates that the respective actuator is currently operating. At the bottom of ‘‘System’s Current State’’ window we can see a text field entitled “Bioreactor’s state”. In this textbox is indicated the current state of the reactor. At the upper centre of the window we can also see a bar with the title “Regulator’s state”. This bar has to buttons that can be at state “on” or state “off”. The one button corresponds to the PH regulator and the other corresponds to the harvesting regulator.

Configuring the system's parameters

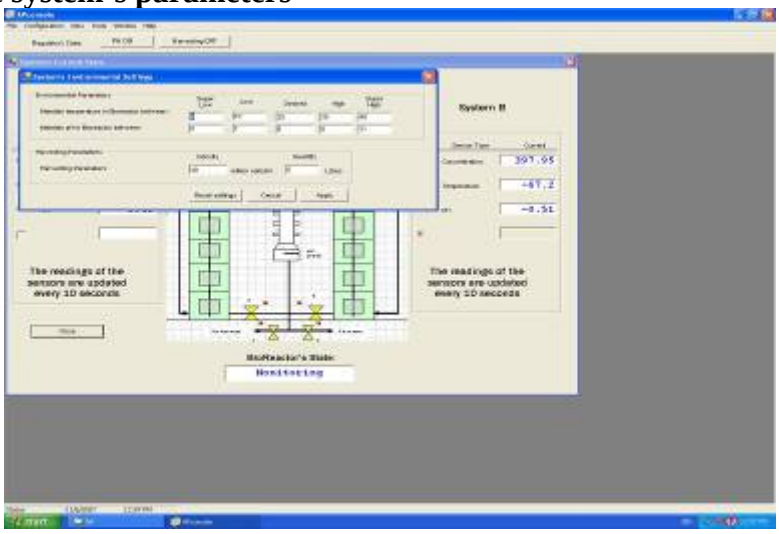


Figure 26: Configuring the system's parameters.

The operator can configure the parameters of the system's operation through the ''Configuration " → "System's Environmental Settings'' window. This window can be launched by selecting the option ''Configuration''!''System's Environmental Settings'' from the main drop down menu. When this option is selected, the window that is shown in figure 26 is displayed.

Viewing Logged Data

The ability to view logged data which were collected by the Monitoring System is a facility offered by the Data Logging Layer. The requested data are presented in lists or graphical format by the user interface. In order to perform this action, the operator must enable the ''Collected Data Viewer'' window

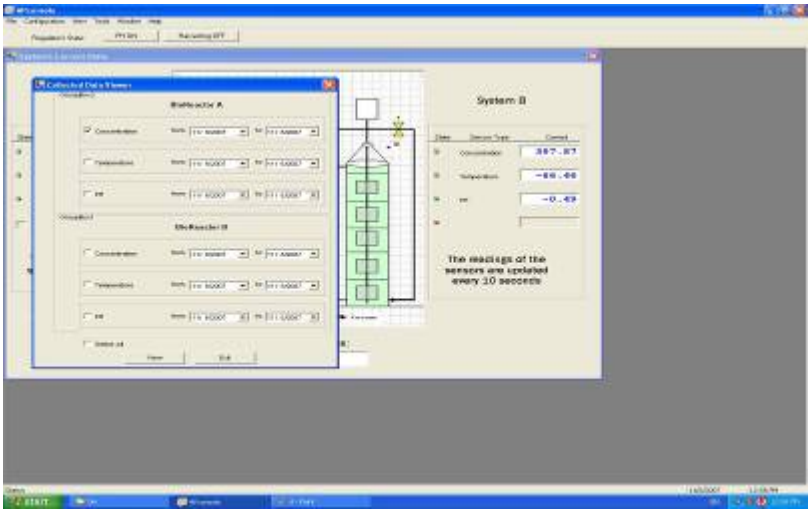


Figure 27: Selection of the desired parameters and the corresponding period.

This window can be launched by selecting the option ''View''!''Collected Data'' from the main drop down menu. When this option is selected, the window shown on the left side of figure 27 appears.

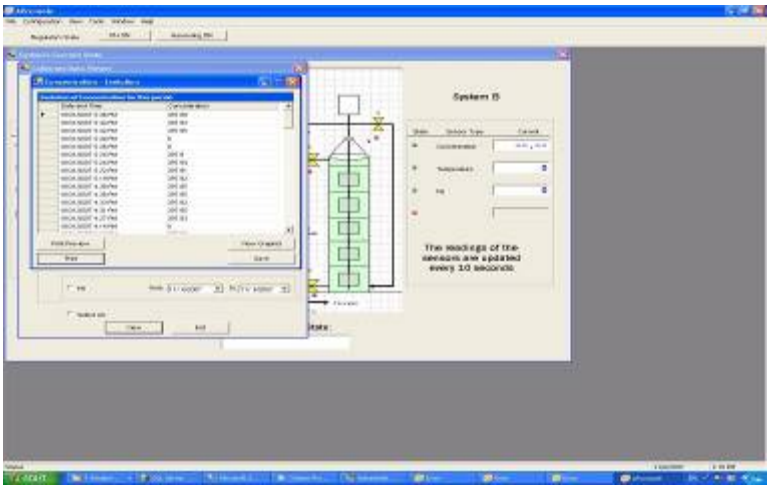


Figure 28: Creating tables of logged data.

Creating data graphs

In order to generate a data graph, the user must click on the ‘‘View Graphic!’’ button of the window which contains the data that he/she wants to view, in table format. When this is done, a graph similar to the one displayed in figure 29 is generated. The operator has then the option to save the graph, by clicking the “save” button. After the save button is pressed an image is created at “C:/” having the name “test.jpg”.

MANAGING THE SENSORS

The ‘‘Sensor Manager’’ allows the operator to have full control of the sensors attached to the system. The operator is able to:

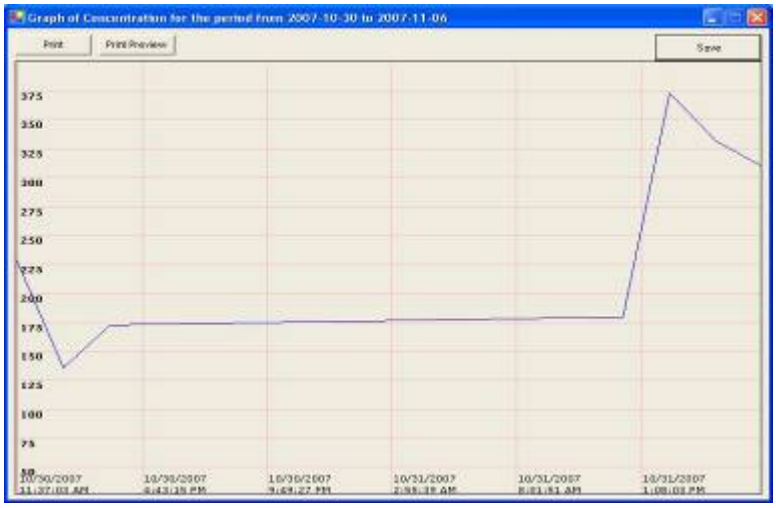


Figure 29: Creating graphs of logged data

- View the state of each sensor (on/off).
- Get the current reading of each sensor.
- Calibrate and fine-tune the sensors.

The ‘‘Sensor Manager’’ can be launched by selecting the option ‘‘Tools’’!‘‘Sensor Manager’’ from the main drop down menu. The resulting window is displayed in figure 30.

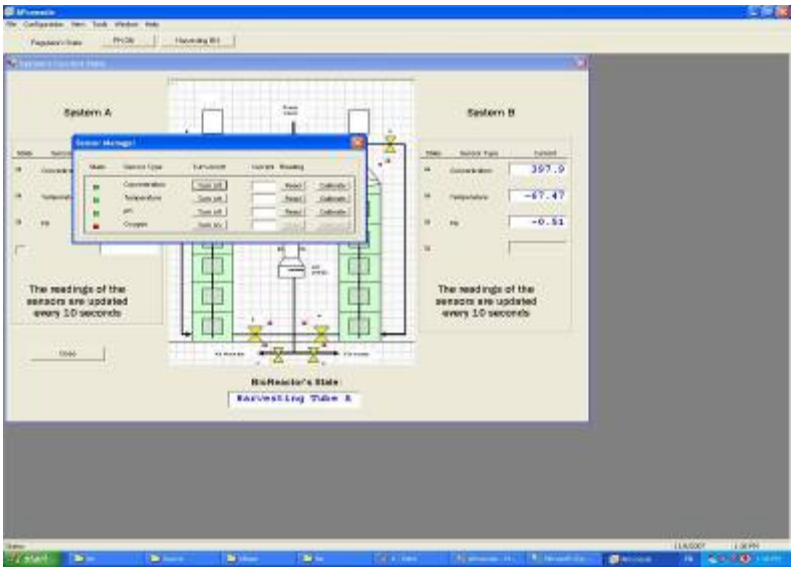


Figure 30: Managing the sensors.

Calibration of the temperature and pH sensors

The temperature and the pH sensors can be calibrated following a common approach. Typically, the sensors need to be calibrated during installation and then once every 6-12 months. In order to calibrate these sensors, the user must open the ‘‘SensorManager’’ and click on the appropriate ‘‘Calibrate’’ button. A window similar to the one which appears in figure 31 appears.

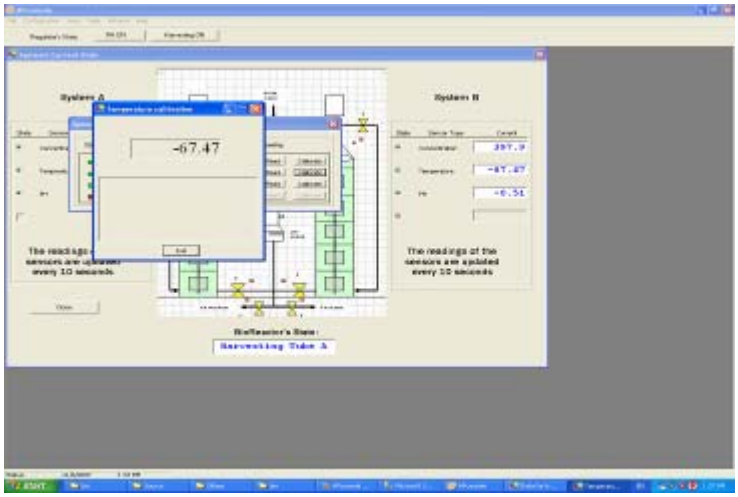


Figure 31: Calibrating the temperature/ph sensor.

Collecting data for calibrating the custom density sensor

The computer software features a mechanism for collecting data that can be used for calibrating the custom algae density sensor that has been under development by the CTI team in the frame of the ALFA project. This mechanism was necessary, so that the CTI team was able to collect calibration data in order to train the neural network structure (see Deliverable 12 ‘‘ Signal Processing Unit (SPU)’’ for more details). The algae sensor’s calibration mechanism can be started by clicking on the ‘‘Calibrate’’ button that corresponds to the density meter, from the ‘‘Sensor Manager’’ window. A window similar to the one which appears in figure 32 appears.

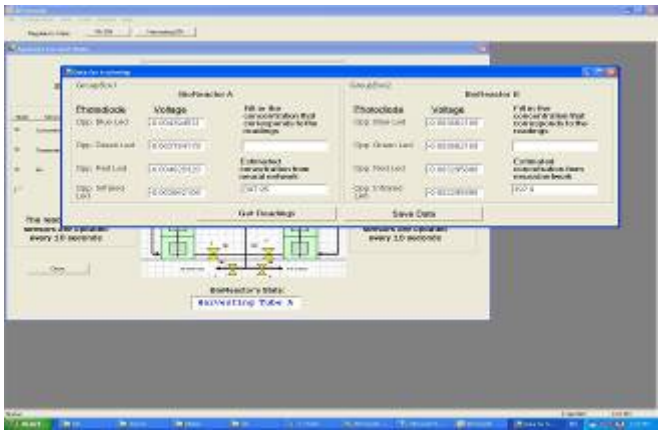


Figure 32: Calibrating the custom algae density sensor.

Visits to the SMEs

In the frame of task 4.2 of the Annex we visited ARGOSARONIKOS at Salamis, Greece several times in order to install and update the system, train the personnel and take data for the training of neural network used by the concentration sensor. We present some pictures of the system parts we had installed during our visits.

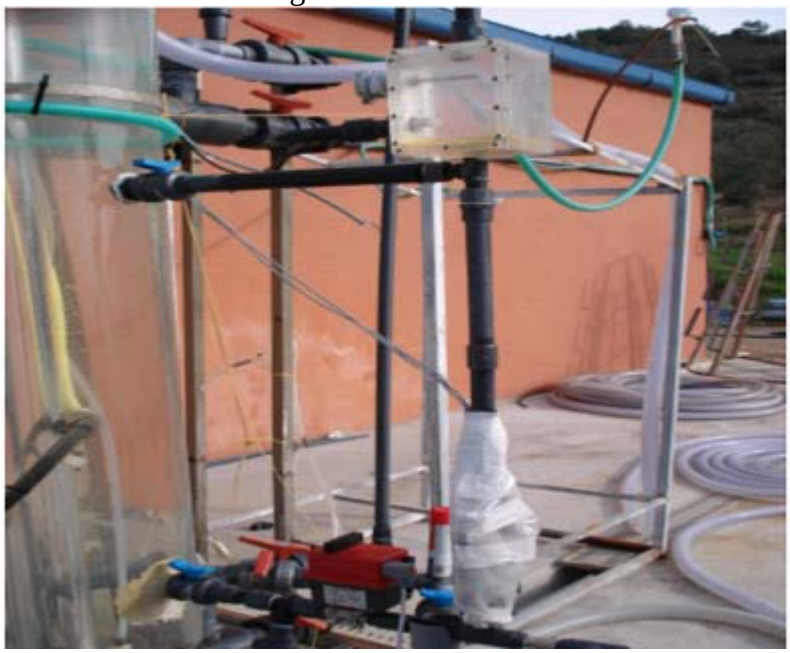


Figure 9: Sensors and part of the washing and refilling system at Salamis

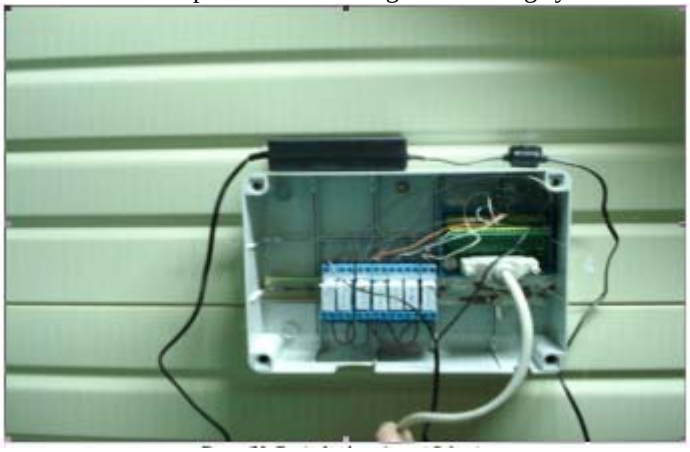


Figure 10: Control sub-system at Salamis

During November we have visited and SagaFjord Fish farms at Norway. In this SME it was decided to be installed the two-tubular system (see task 3.4). Unfortunately, when we visited the farm, the tubular system was not installed, so we only installed the control and monitoring system and when the tubular system is installed, it could be completely functional. We also trained the staff of the SME of how to use the software and how to maintain the control and monitoring system. The following picture, illustrates the system he had installed in SagaFjord.

Training the neural network

As we already described, the concentration sensor evaluates the concentration of the algae using a neural network which has as inputs the voltages of the pins of sensor's led.

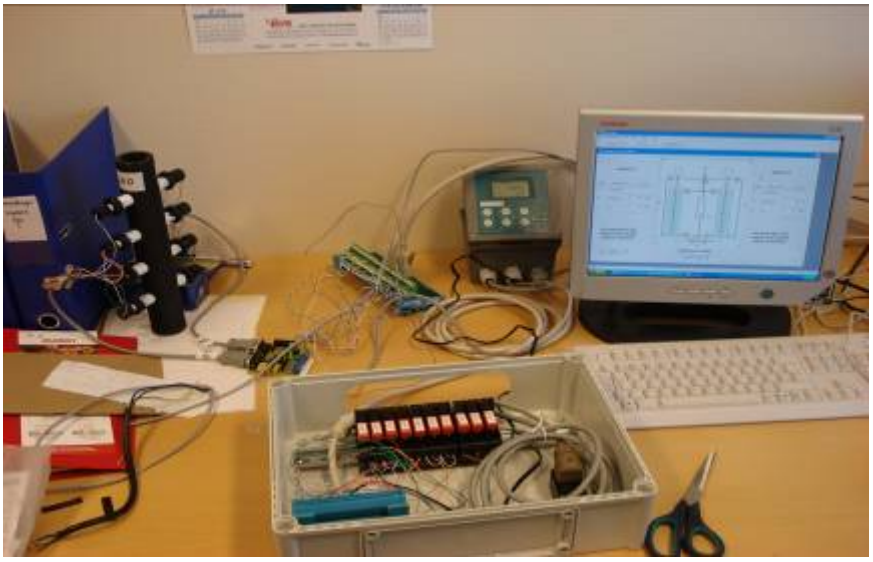


Figure 11: Monitoring and control system at Norway.

The neural network needs to be trained with real data, in order to estimate correctly the real concentration. In the lab, we had successfully trained the sensor, but with data that emerged from our measurements with algae paste. These results are presented in the "signal processing layer" paragraph of task 3.4. The algae used in Salamis were of different culture, so we had to re-train the neural network, in order to get the desired estimates. Unfortunately, due to the problems we had with the photo-diodes function, which we had described in a previous section, and the delayed installation of the cleaning system we were not able to collect sufficient number of data, so to successfully train the neural network. We were able to get only a small number of appropriate measurements at the range of 0 to 150 million cells. Despite the small number of measurements, we will be able to present the functionality of the concentration sensor at this small range of values. With the data we have collected, we tested in the lab several architectures of the neural network with different number of hidden layers and neurons per hidden layer. We conclude to 3 hidden layers with 20 neurons at each one and one hidden layer with 10 neurons. From the 53 measurements we had collected, we used only the half to train the neural network (sampling them at equally spaced points, so to have coverage of all the range of the values) and then we used all the points to evaluate the neural network.

In figure 12 we show the performance of the training of the neural network, and in figure 13 we present the absolute error between the real concentration and in the concentration emerged from the neural network we had calculated. As we can see the error is always below 8 million cells, an acceptable error, taking into account the needs of the application. We should again

mention that we could have gotten even better results if we had a bigger number of data. We should also notice that if we had more data it will probably sufficient a neural network with only one hidden level as the one we have calculate in the lab's experiments (see paragraph "signal processing unit" of task 3.4)

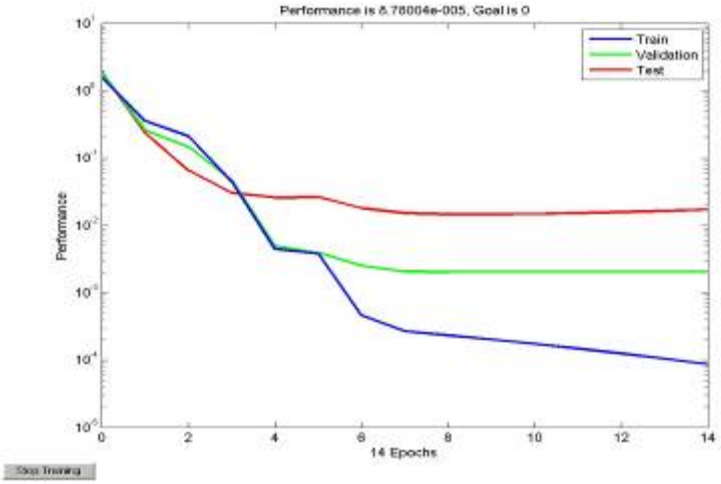


Figure 12: Performance of training the neural network.

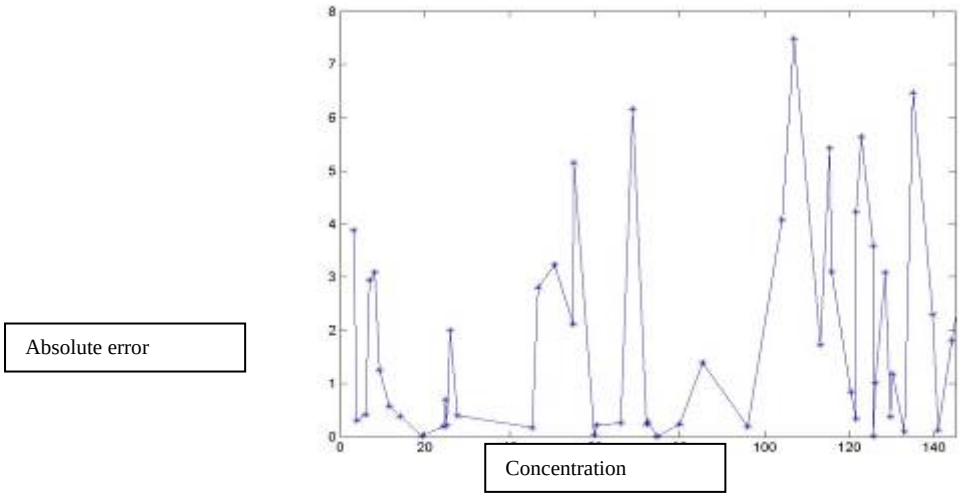


Figure 13: The absolute error between the real and the estimated values of the concentration.

Manuals

In order to make easy the installation and use of the control and monitoring system we wrote some manuals. More specifically we wrote a connections guide for the two-tubular system, in which we describe all the necessary connections between the special purpose hardware which is used in the system we had designed so it can be easily installed. We also wrote a user's guide of the software in which we describe the procedure that must be followed in order to install the software in a new PC. In this manual are also described the main functions and the options that are available to the user of the two-tubular version of the software. Finally, we wrote a presentation in MS PowerPoint format in which the main functions and the options that are available to the user of the two-tubular version are presented. This presentation can be used in order to train the staff of the SMEs.

2. SECTION 3. CONSORTIUM MANAGEMENT

Consortium management

The Project Management of the the project was given a separate WP. The main aim is to co-ordinate the overall development of the project, to assess the accomplishment of tasks allocated to each participant, to discuss the results obtained during different phases of the project, to discuss means of recovering the time lost due to technical or other difficulties (if any) in order to keep the targeted goals on time, to review the milestones for each phase in order to make any adjustments, to submit internal reports that will make the bases of the periodical reports to be submitted once a year to the European Commission, and to fix deadlines for submission of manuscripts for publication, and for submission of results for the elaboration of Period Reports. The management structure with the RTD coordinating the work of 'their' (i.e. local responsibility) SME's proved to work well in the first reporting period.

To day no major changes have been made in the running of the project. In the first reporting period the following meetings took place:

Project meetings during the first reporting period

Date	Nature	Participants	Location
April 2005	Preliminary meeting	AUA, APN, CTI	Athens
June 2005	Kick off meeting	All	Cyprus
November 2005	Interim Meeting	All	Portugal
May 2006	Mid term	All	Norway

Two kick-off meetings was scheduled to discuss and establish experimental protocols. Interim meetings were used for presentation and discussion of results and discussion on the next steps within the scientific work as well as discussion on administrative and practical matters.

A number of telephone conference meetings were undertaken using VOIP (Skype and Festoon) to deal with technical matters.

Date	Nature	Participants
27 January 2006	Technical meeting	AUA, APN, DFMB, SF, VVN
8 March 2006	Technical meeting	AUA, DFMB, SF, APN, CTI, HESY
11 April 2006	Technical Meeting	AUA, DFMB, APN, CTI
4 May 2006	Technical Meeting	AUA, DFMB, APN, CTI, VVN
19 June 2006	CROPS Meeting	APN, VVN, HESY

During the second reporting period the following meetings were held:

Date	Nature	Participants	Location
November 2006	Interim meeting	All	Athens and Salamis
April 2007	Final meeting	All	Athens and Salamis

Contractors

No changes have been made in the main responsibilities of the project contractors. Below we will comment on the contributions of the partners in each WP.

Role of partners in the work packages.

Role of Partners in WP 1. Economic and operational analysis of hatcheries

AUA was the participant with the main responsibility for this work package. Akvaplan was the participant with the responsibility to provide technical information concerning the current state-of-the-art technology in hatchery operation, while the participating SMEs contributed with their hatchery production data and practical experience.

Task 1.1 Build a baseline case model for the current hatchery operation against which to compare any proposed technological modifications.

The model was developed and validated by AUA, based on literature and data and information provided by all other participants in this task. The SME participants Nos. 1, 2, 3 and 4 provided production, economic and performance data for analysis. Akvaplan-niva provided cost data for other algae production systems in use by the hatchery industry.

Task 1.2 Build a model for the best designed process using the new technology.

The model was developed by AUA based on the new proposed designs using the laboratory and pilot test results to validate the model and guide / predict the productivity and operational regime of the scaled-up commercial units. Akvaplan contributed information on topics related to aquaculture production techniques, capital costs, data for the algae intermediate storage system and costs of production for other continuous algae production systems. The SME participants Nos. 1, 2, 3 and 4 provided production, economic and performance data for analysis.

Task 1.3 Technology evaluation and decision criteria.

The models of the new technologies built on task 1.2 were validated with the data from the operations of the commercial units provided by partners 1 and 2 and the capital cost provided by 5. Partner 6 provided data on the control and automation system while partner 8 provided data on the storage system. Using this information, AUA validated the model and used it to provide a detailed economic evaluation of the new technology with direct comparison with existing benchmarked technologies. AUA conducted sensitivity analysis and strategic investment analysis using the model and the information provided by the participating partners. The SME participants Nos 1, 2, 3 and 4 provided input on the tactical and strategic considerations in the design and operation of the CAP and CHTPs. SME participant No. 5 provided information on the assembly and construction considerations for the new equipment. In particular, SMEs contributed their experience from workpackage 4.

Role of Partners in WP2 Design and development of a Continuous Algae Production (CAP) system

Akvaplan coordinated the activities of WP2 and had the main responsibility for integrating the technologies developed through the project for the development of a CAP system. DFMB had the main responsibility for task 2.1, while AUA had the main responsibility for task 2.2 and CTI had the main responsibility for task 2.3. SMEs contributed their practical experience in hatchery operation throughout WP2. Akvaplan contributed to tasks 2.1 to 2.3 by providing information on existing technology and backstopping and trouble shooting technical and production problems during the start up of the prototype photobioreactors.

Task 2.1 Biology of algal species suitable for aquaculture – Definition of biological requirements for high-density Continuous Algae Production

This task was undertaken by DFMB, which also had the main responsibility for the algal biology research work into light/dark effects and LED light colour comparisons, assisted by Akvaplan. The SMEs provided detailed information on the algal species used, production performance of these species and quality of resulting fry. AUA also performed a series of laboratory tests to define key design parameters of the biological requirements for high-density CAP for the selected species.

Task 2.2 Design optimisation of a CAP

This task was coordinated by AUA. Four prototype CAPs were designed, constructed and tested by AUA with the help of APN for start-up and operational matters. This work was also supported by parallel laboratory tests performed by AUA under Task 2.1. Two optimised CAPs have been developed by AUA based on the two most successful prototype bioreactors (BOA-type, tubular with agitation) following a series of pilot-scale experiments. The SMEs have reviewed the proposed designs with respect to operational aspects.

Task 2.3 Monitoring and Control of the CAP system

This task was coordinated by CTI. The algal density sensor was developed and tested by CTI. The integrated monitoring system of the new CAP was developed by a research team consisting of researchers of CTI, AUA, Akvaplan, and DFMB. AUA and HESY gave advice on construction materials and methods and the other SMEs supplied information on the fluctuations in the environmental parameters in their existing algal production systems and they also contributed their existing know-how and previous experience.

Role of Partners in WP 3. Design and development of a Computerised Harvesting, Transferring and Packaging (CHTP) system for algae.

CTI coordinated WP3 as well as task 3.4. AUA had the main responsibility for designing and constructing (with the technical support of Akvaplan and HESY Bergambacht BV) the structural and mechanical components of the interface system (task 3.1) in connection with the work performed under WP1, while CTI supported the design of the electronic equipment relevant to task 3.1. Akvaplan together with HESY and VVN had the main responsibility for adapting existing rotifer production systems to the new CAP technology (task 3.2). Akvaplan also supported task 3.1 by providing the ichthyological expertise needed for designing and constructing the CHTP system. HESY Bergambacht BV had the main responsibility for developing the Concentration and Storage Unit (task 3.3), where also DFMB contributed by evaluating the quality of preserved algae. The CHTP was based on the system presently in use by SF. All SMEs provided their practical experience in designing and reviewing the CHTP system.

Task 3.1 Design and development of a harvesting, temporal storage, and transferring system linking the new CAP system with the other modules of the hatchery

This task was coordinated by APN. The harvest, storage and transfer system in SF was analysed and APN designed the CHTP system with respect to its topological arrangement, capacity of buffers and mechanical parts. CTI assisted in the selection of the necessary compatible electronic equipment to be used in task 3.4. AUA and APN designed and supervised the construction of an automatic harvesting-cleaning system and an automatic refilling-feeding system, in cooperation with CTI, for the CAP installed at the facilities of SARONIC. HESY Bergambacht BV, AUA, SF and DFMB assisted the selection of the suitable equipment components for building the system based on ichthyologic requirements. The hatchery SMEs were involved in defining the logistical problems of live food movement and reviewing the solutions developed. SARONIC was the site for the prototype and had a higher involvement in this task than the others. They also contributed their existing know-how and previous experience.

Task 3.2 Linking CAP with existing production systems for rotifers adapted to cold, temperate and tropical conditions

This task was coordinated by Akvaplan. Akvaplan-niva undertook a literature review and state of the art analysis of existing continuous rotifer production systems. Akvaplan assisted by Hesy and VVN designed the new rotifer production system and it was constructed by HESY and tested by VVN. The CROPS was then transferred to Saronikos and operated and optimized by APN and Saronikos. AUA and CTI supported the design so the new system was compatible with the CHTP system. APN undertook a literature review of biosecurity issues that were addressed in the design. The SMEs were involved by providing detailed information on the rotifer productivity and performance of their existing systems.

Task 3.3 Development of a Concentration and Storage Unit (CSU) for phytoplankton

This task was coordinated by HESY Bergambacht BV. A literature review was undertaken by APN on algae harvesting techniques and algae preservation. A research team consisted of researchers from Akvaplan, DFMB, and SF developed and tested the CSU at Saronikos. DFMB had a crucial role in testing the quality of the preserved algae. Following satisfactory results in maintenance of algal quality, HESY was the main SME involved with the development of the packaging and storage systems. The DFMB and Saronikos tested algae that had been stored for 6 weeks for quality.

Task 3.4 Development of a Control System for managing the algae production, harvesting, and transferring process

This task was coordinated by CTI. CTI prepared some outline designs for the new automation system (hardware and software) and developed these further assisted by AUA on the mechanical-operational design. The hatchery SMEs provided details of existing monitoring systems used in their hatcheries and contributed their existing know-how and previous experience. HESY Bergambacht BV provided information about existing electronic equipment which could be adapted for achieving the objectives of this task.

Role of Partners in WP 4. Optimisation and evaluation of the CAP and CHTP systems

WP4 was coordinated by DFMB. The RTD partners supported the construction and installation of the CAP system at the SME hatcheries and the integrated system at Saronic. The participating SME-hatcheries contributed their expertise in operating the new equipment, optimising operational procedures and suggested improvements.

Task 4.1 Optimisation and testing of the CAP system

This task was coordinated by SF. AUA designed the up-scaled CAP systems and provided all technical details for their construction, including materials, connections, mechanical equipment and operational guidelines. The SMEs took the responsibility for constructing, testing and optimizing the prototype photobioreactors. They collected production performance data, environmental data and provided regular feed back to the RTDs to allow further improvement. AUA supervised the installation of a CAP system at the facilities of SARONIC, with the advice of Akvaplan. AUA tested the automatic harvesting and refilling systems in cooperation with CTI. The RTD-performers contributed to the evaluation of the systems. APN together with Saronikos optimized production. DFMB undertook the analysis of samples.

Task 4.2 Optimisation and testing of the CHTP system

This task was coordinated by SARONIC. SARONIC and APN took the responsibility for testing the integrated system and provided information on performance and feedback on strengths, weaknesses and potential areas for improvement supported by the RTDs. The RTD-performers contributed to the data analysis and evaluation of the system.

EXPLOITATION, DISSEMINATION AND USE

A. Exploitation of the results

HESY is the leader of the SME committee for exploitation and the following was agreed.

- A European patent would be taken out on the photobioreactor design (CAP) and the Algal cell density sensor. The patent would be sold to HESY to the benefit of the SME partners.
- HESY would manufacture and sell the CAP as a complete unit and make it available for resale by major aquaculture suppliers such as CATVIS, Aquatic Ecosystems, Tropical Marine, Dryden, etc.
- HESY would manufacture the sensor box that would include the algal density sensor together with the monitoring and control software separately from the CAP.
- CTI would trouble shoot and update the computer control system on a fee paying basis.

Overview table

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
1. Optimised photobioreactor	Technical knowledge	Aquaculture	2008	Decision not to patent	Owners SMEs and manufacturer HESY
2. Algal cell density sensor	Technical knowledge	Aquaculture	2007	Decision not to patent	Owners SMEs and manufacturer HESY

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
3. Monitoring and control software	Technical knowledge	Aquaculture	2007	IPR protection	Owner CTI but made available under licence
4. Continuous rotifer production system	Technical knowledge	Aquaculture	2007	NA	No Owner but manufactured by HESY
5. Algae Biology Dark/light, LEDs	Biological knowledge	Aquaculture	2007	NA	General knowledge and no specific owner
6. Live food transfer systems	Technical knowledge	Aquaculture	2007	IPR protection	Owner SMEs and manufactured by HESY

1. Continuous algae production photobioreactor (CAP)

The two different CAP designs are stand alone pieces of equipment that can be installed into existing hatchery facilities. The control system bot both is the same with computerised monitoring and self-control through feedback sensor systems. The CAPs are modular allowing hatcheries to operate one or more of the modules to satisfy demand for algae. The algal density sensor will be an integral part of the CAP.

The decision of the SME partners was not to patent the design as it could be reverse engineered and copied with some minor changes circumventing the patent. It was decided that HESY would manufacture the systems commercially and sell the system within the aquaculture and algae production markets at a reasonable cost . It would maintain the lead in the design by continually improving the design based on feedback from customers. The SMEs gave permission to the RTDs to patent their designs if they so wished. The AUA is considering the patenting of the tubular bioreactor at their own cost (not project)

These photobioreactors can be used for continuous production of quality algae for;

1. Marine fish hatcheries requiring algae
2. Companies producting algae for health products, cosmetics, etc.
3. Rotifer production systems ultilising live algae as feed or enrichment.
4. Larvae tanks using the green water techniques
5. Way of using waste CO2 from industry

2. Algae density sensor.

A sensor that is capable of measuring cell density and that can be calibrated for different algal species and and is not greatly affected by turbidity. This sensor is sensitive to light frequencies absorbed by Chlorophyll. The sensor should be able to be trained to identify quality of algae in the system (not enough time in the project to test this).

The SME partners decided not to patent the design of the sensor as the sensor housing containing the LEDs and photodiode were to easy to copy but that the monitoring and controlling software that are essential for the operation of the sensor would be protected by IPR. It was agreed that HESY would manufacture and sell the sensor at a reasonable cost within the aquaculture and algae production markets.

This sensor can be used for measuring algal density levels in;

- Algal production systems (batch or continuous) and CAP (see above)
- Rotifer production systems ultilising live algae or algae paste as feed or enrichment.
- Larvae tanks using the green water techniques
- Algal density monitoring for naturally occurring algal blooms (marine and freshwater)

3. Continuous rotifer production system (CROPS)

The continuous rotifer production system was successfully integrated into the CAP utilising the live algae produced. However it was also tested using algae paste so that it could be operated independently of the CAP.

It is not the intention to patent the design of CROPS as it is based on previous continuous rotifer productions sytems but with improvements made and optimised for live algae and algae paste. However HESY will manufacture and sell the system at a reasonable cost to the aquaculture markets.

CROPS can be used for producing quality rotifers for;

- Marine fish and crustacean hatcheries

4. Integrated system control software

The integrated monitoring and controlsystem will have a computer interface that will be partitioned into the following categories; *configuration* tools, *monitoring* tools, mechanisms for *manual operations* and tools for managing *older information*. This control software will have modules for the CAP, CROPS and transfer system which can be operated independently or fully integrated.

CTI produced the monitoring and control software and will provide the software together with the CAP, CROPS and/or CHTP as a licenced product. CTI will provide support services for the software with upgrades for a small annual fee. However HESY will provide the software as part of the systems that it sells to the aquaculture markets.

5. Integrated live food production system

The fully intergrated live food production system comprise the optimised photobioreactors (MegaBoa or tubular), CROPS, transfer system and harvesting and storage system. This is all controlled by a computerised monitoring and control system. The modules will be fully integrated to work together.

6. Scientific Knowledge on Algae Biology, Dark/light, LEDs

A number of scientific trials have been undertaken during the course of the project to clarify certain issues to allow informed design of the photobioreactor.

These trials included

- The influence of light and dark phases on algae productivity
- The use of different types of light on lagae productivity
- The use of LEDs and different colours of LEDs on algae productivity
- The use of ultrasound for concentrating live algae
- Different light/darkphases on algal productivity
- Preservation of live algae at different temperatures and different types of plastic bags.

The SME partners have authorised the RTDs to publish some of this scientific knowledge including the following.

Papers in International Journals

1. D. Briassoulis, P. Panagakiș, M. Chionidis, ‘Optimised Design of a Helical-Tubular Photobioreactor for Continuous Production of Nannochloropsis sp.’, submitted for publications, Aquacultural Engineering
2. Hiskakis M., Mistriotis A., Briassoulis D., (2008). "A Novel Cylindrical Photobioreactor with Axial Agitation for Live Algae Production", to be submitted in Aquaculture Engineering.
3. A. Mistriotis, “A Growth Model for Nannochloropsis Occ. in Batch Culture” submitted in the 11th International Conference on Applied Phycology, Galway, Ireland, June 21-27, 2008
4. Jacobsen A, White P et al. 2008. Difference in nutritional quality of stored Nannochloropsis sp. when stored in different containers. To be submitted to Aquaculture

6. Live food transfer systems

The live food transfer and distribution systems are integrated into the CAP and CROPS systems allowing controlled dosing of algae to rotifer tanks, larvae tanks and excess algae to the concentration and temporary stoage system.

It is not the intention to patent the design of live food transfer system as it is based on typical systems found in marine fish hatcheries. However it is a system that can be integrated into the monitoring and control software and therefore will be sold as a component of the integrated live food production system. HESY will manufacture and sell the system as a component of the integrated live food production system into the aquaculture markets.

7. Exploitation plan

HESY has prepared a sales hand out for the two types of bioreactors (see appendix 1) that was disseminated at the AquaNOR conference and exhibition in Norway in August 2007. HESY are preparing an article and advert for the bioreactors that will be published during the first half of 2008 in the Fish Farming International (http://www.fishfarminginternational.com/heighway/home.htm?site=ffi) and also in Hatchery International Magazine (http://www.hatcheryinternational.com/)both of which have wide international readership.

HESY will continue to advertise the final CAP for sale in the major aquaculture magazines and try to "sell the story" of the development of the equipment with and article and advert. In addition, the web will be used to market the equipment using the ALFA web site www.alfa.eu.com (which will continue to be maintained for a further 2 years) and the HESY web site http://www.hesy.com/ .

Potential markets in Europe

The main marine hatchery species (2006) that utilise algae are seabream (519.7 million), seabass (350.6 million), cod (estimated 5 million), turbot (6.8 million) and meagre (1.5 million).

Table: Production ('000 of fry),

PRODUCT	COUNTRY	2001	2002	2003	2004	2005	2006
European Seabass - Juveniles	CROATIA				7,000	5,000	5,000
	CYPRUS	3,000	4,500	3,000	5,600	3,337	4,000
	FRANCE	22,000	23,000	29,000	28,000	33,000	35,000
	GREECE	100,000	110,000	120,000	130,000	140,000	130,000
	ITALY	50,000	50,000	45,000	50,000	50,000	52,000
	PORTUGAL	4,900	8,500	10,000	10,000	10,000	10,000
	SPAIN	8,000	8,000	13,500	19,200	23,228	24,600
	TURKEY	20,000			100,000	110,000	90,000
European Seabass - Juveniles Total		**207,900**	**204,000**	**220,500**	**349,800**	**374,565**	**350,600**
Gilthead Seabream - Juveniles	CROATIA					2,000	2,000
	CYPRUS	30,000	15,000	14,000	9,000	8,086	9,000
	FRANCE	23,500	21,000	19,500	24,000	34,000	36,000
	GREECE	130,000	170,000	160,000	142,500	207,000	230,000
	ITALY	40,000	40,000	30,000	40,000	45,000	54,000
	PORTUGAL	13,900	18,000	20,000	20,000	20,000	20,000
	SPAIN	53,000	53,000	64,200	48,300	56,235	68,700
	TURKEY	3,000			35,000	75,000	100,000
Gilthead Seabream - Juveniles Total		**293,400**	**317,000**	**307,700**	**318,800**	**447,321**	**519,700**
Sturgeon Juveniles	FRANCE	1,000					
Sturgeon Juveniles Total		**1,000**					
Turbot Juveniles	FRANCE	4,000	5,200	5,200		1,878	1,355
	SPAIN					4,915	4,920
Turbot Juveniles Total		**4,000**	**5,200**	**5,200**		**6,793**	**6,275**
Meagre - juveniles	FRANCE			1,300	1,100	1,700	1,500
Meagre - juveniles Total				**1,300**	**1,100**	**1,700**	**1,500**
Grand Total		**506,300**	**526,200**	**534,700**	**669,700**	**830,379**	**878,075**
% increase per year			3.93	1.62	25.25	23.99	5.74

Source: http://www.feap.info/production/euproduction/juveniles_en.asp

The main counties with marine hatchery production were Greece (360 million) Turkey (190.0 million), Italy (106 million), Spain (98 million), France (78 million), Potrugal (30 million), Cyprus (13 million), Norway (estimate 10 million -cod) and Croatio (7 million).

In addition there are overseas markets such as Japan, Taiwan, China, Australia and Chile. The production of marine fry are more difficult to estimate for these countrties

Table: Production ('000 of fry),

PRODUCT	2001	2002	2003	2004	2005	2006
European Seabass - Juveniles				7,000	5,000	5,000
Gilthead Seabream - Juveniles					2,000	2,000
Total Croatia	**0**	**0**	**0**	**7,000**	**7,000**	**7,000**
European Seabass - Juveniles	3,000	4,500	3,000	5,600	3,337	4,000
Gilthead Seabream - Juveniles	30,000	15,000	14,000	9,000	8,086	9,000
Total Cyprus	**33,000**	**19,500**	**17,000**	**14,600**	**11,423**	**13,000**
European Seabass - Juveniles	22,000	23,000	29,000	28,000	33,000	35,000
Gilthead Seabream - Juveniles	23,500	21,000	19,500	24,000	34,000	36,000
Sturgeon Juveniles	1,000					
Turbot Juveniles	4,000	5,200	5,200		1,878	1,355
Meagre - juveniles			1,300	1,100	1,700	1,500
Total France	**50,500**	**49,200**	**55,000**	**53,100**	**70,578**	**73,855**
European Seabass - Juveniles	100,000	110,000	120,000	130,000	140,000	130,000
Gilthead Seabream - Juveniles	130,000	170,000	160,000	142,500	207,000	230,000
Total Greece	**230,000**	**280,000**	**280,000**	**272,500**	**347,000**	**360,000**
European Seabass - Juveniles	50,000	50,000	45,000	50,000	50,000	52,000
Gilthead Seabream - Juveniles	40,000	40,000	30,000	40,000	45,000	54,000
Total Italy	**90,000**	**90,000**	**75,000**	**90,000**	**95,000**	**106,000**
European Seabass - Juveniles	4,900	8,500	10,000	10,000	10,000	10,000
Gilthead Seabream - Juveniles	13,900	18,000	20,000	20,000	20,000	20,000
Total Portugal	**18,800**	**26,500**	**30,000**	**30,000**	**30,000**	**30,000**
European Seabass - Juveniles	8,000	8,000	13,500	19,200	23,228	24,600
Gilthead Seabream - Juveniles	53,000	53,000	64,200	48,300	56,235	68,700
Turbot Juveniles					4,915	4,920
Total Spain	**61,000**	**61,000**	**77,700**	**67,500**	**84,378**	**98,220**
European Seabass - Juveniles	20,000			100,000	110,000	90,000
Gilthead Seabream - Juveniles	3,000			35,000	75,000	100,000
Total Turkey	**23,000**	**0**	**0**	**135,000**	**185,000**	**190,000**

Source: http://www.feap.info/production/euproduction/juveniles_en.asp

However there are specific markets that are more likely to take up new technology quickly. These markets were identified as Turkey, Norway, Australia and Chile.

Consortium agreement on exploitation

The ownership and use of the intellectual property is set out in the Consortium agreement as follows.

The SME Partners having jointly the ownership of the Knowledge of:

- The design of the continuous algae production photobioreactor (CAP)
- The design of the algae density sensor.
- The design of the improved continuous rotifer production system (CROPS)
- The design of the integrated live food production system

This knowledge will be available for use by each of the participating SME for their own uses. The knowledge will be commercially exploited by HESY and a share of the profits will be returned to the other participating SMEs

Details of the exploitation and dissemination are given in Deviverable 19.

Profiting from the Knowledge
The SME consortium (all SME partners) will jointly own the knowledge and HESY Bergambacht BV will construct and sell the equipment modules. A royalty will be set a side from all sales of the modules and this will be distributed equally to all members of the SME consortium at the end of every year. The SMEs will be able to purchase additional modules (for their own use) at cost directly from HESY Bergambacht BV. The SMEs will act as agents for HESY Bergambacht BV regarding the sale of the modules and will receive a commission for any sales.

Royalty Payments
A royalty is a payment will be made by HESY Bergambacht BV to the European Patent holders (the SME consortium) for the permission to use their invention.

Royalty Rates
For the Period 2008-2016. For eligible equipment based on the European patent resulting from the project made by HESY Bergambacht BV during the period beginning on October 1, 2006, and ending on September 30, 2016, the royalty rate shall be a percent of HESY Bergambacht BV gross revenues for eligible equipment sold during such period.

Payment of Royalties
For the Period 2006-2016. The balance of any royalty amounts which has not already been paid, shall be paid in one instalment due September 30 each year.

Notice and Recordkeeping
Reports to Be Provided. For year, HESY Bergambacht BV will keep records and make available to each SME, the following records:

(1) Number of eligible items sold;
(2) Value of eligible items sold;
(3) Computation of royalty amounts.

Definitions
As used in these Rates and Terms, the following terms shall have the following meanings:

(a) The term "*eligible equipment*"—equipment based on the two different types of bioreactor resulting from the project
(b) The term "*gross revenues*"—Invoiced ex-factory price for the said equipment

Details of the IPR and exploitation of the knowledge are given in Deliverable 19.

B. Dissemination of knowledge

Dissemination of the results and transfer of the technology

In the first half of the project, dissemination of information about the project was limited to the consortium partners as this was the development phase. During the second half of the project, articles will be published in scientific journals as well as in more industrially oriented magazines. These publications will include all scientific results in areas of the project that will not affect the patenting and protection of knowledge. The project coordinator will coordinate the publication effort of the consortium. The SMEs, Universities and Research Institutes will allow the free use of scientific results obtained through the proposed project after their presentation through publications, international conferences and open workshops. These results will be available to all EU aquaculture industry.

Publications (peer review journals, PRJ)

In cooperation with the partner SMEs, data from both small scale laboratory trials and large scale production trials will be published, when feasible and agreed between all partners, in acknowledged scientific journals. Submission of manuscripts to journals will occur at the end of the project.

Papers in International Journals

- D. Briassoulis, P. Panagakiș, M. Chionidis, 'Optimised Design of a Helical-Tubular Photobioreactor for Continuous Production of Nannochloropsis sp.', submitted for publications, Aquacultural Engineering
- Hiskakis M., Mistriotis A., Briassoulis D., (2008). "A Novel Cylindrical Photobioreactor with Axial Agitation for Live Algae Production", to be submitted in Aquaculture Engineering.
- A. Mistriotis, "A Growth Model for Nannochloropsis Occ. in Batch Culture" submitted in the 11th International Conference on Applied Phycology, Galway, Ireland, June 21-27, 2008
- Jacobsen A, White P et al. 2008. Difference in nutritional quality of stored Nannochloropsis sp. when stored in different containers. To be submitted to Aquaculture

Conferences and exhibitions

Data obtained in the project that are not subject to limitations, i.e. industrial protection, may be presented orally or as posters in national (within each country in question) and international conferences in the future.

Overview table

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
2005, 2006, 2007, 2008	Web site	Interested public and scientists	International		APN
August 2007	Marketing brochure at AquaNOR in Trondheim, Norway	Farm operators	Norway primarily but also international	2,000	APN and HESY
March 2008	Article and advert in Fish Farming International	Farm Operators	International	5,000	APN and HESY
May 2008	Article in Hatchery International	Hatchery operators	USA and International	2,000	APN and HESY

Website

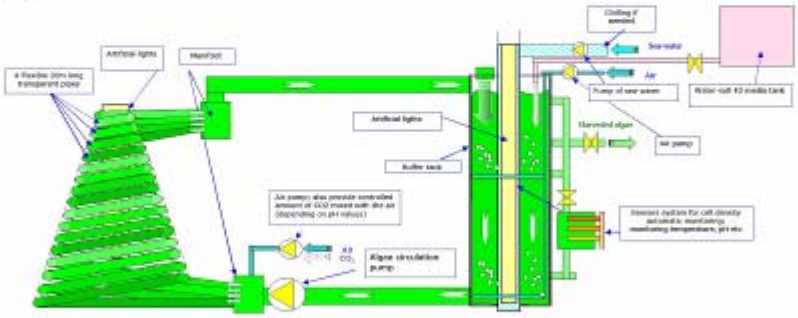
The web site www.alfa.eu.com was set up and operated by Akvaplan-niva It became operational in September 2005. It has been continued to be managed by Akvaplan-niva throught the project and will be maintained for a further 2 years.

The site has a public section and a password protected private section with all ALFA documentation. There is a comprehensive searchable bibliography on algae in the private section of the web site that will be moved to the public side in the next reporting period.

Appendix 1 Promotional sales brochure produced for AquaNor in August 2007.

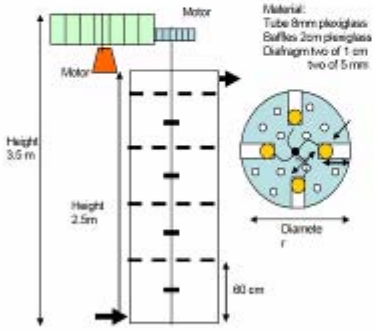
Automated live algae production for aquaculture hatcheries

HESY are the commercial partners of the EU funded CRAFT research project to design a fully automated live algae photobioreactor for the fish and shellfish hatcheries in Europe.



Optimal growth
These systems provide optimal nutritional conditions, both natural and artificial illumination and a feedback controlling system for the temperature, the nutrient content, the pH and the CO2 concentration of the water.

Continuous Algae production
Two reactors were developed and are now available commercially through HESY. Both reactors are fully automated with feedback control systems. One is based on medium bore tubes and the other on a large bore cylinder with internal rotors for mixing the algae.



Fully tested
Both reactors have been tested at a commercial hatchery in Greece. They can operate in outdoor conditions (with cooling) or indoors (close to a window). They have been tested with different algae species such as Nannochloropsis, Tetraselmis and Isochrysis

Advantages of the systems

- Small footprint
- High output
- High density culture
- Fully automated
- Low staff requirements
- Harvested at log phase
- High nutritional quality
- 24 hours light (sunlight and artificial)
- Modular

Quotations
Quotations can be obtained from HESY at the contact details below.

HESY Aquaculture B.V.
Bovendijk 35-Z, 2295 RV Kwintsheul, The Netherlands

Phone: +31 (174) 220140, Fax: : +31 (174) 226864, Mail: office@hesy.com

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