Restricted – Commercial (When completed)

FC0909

LINK AQUACULTURE - PROJECT COMPLETION FORM

Please enter details in the boxes below:

Name of LINK Programme: AQUACULTURE

Name of LINK Project: Preserved microalgae as an alternative diet in aquaculture

Project Ref. No.	Project Start Date	Project Completion Date
ALG02	15/09/1997	14/09/2000

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Project Costs (£)/Effort

	Government Department	Research Council	Industry	Total
Approved Spend	[redacted]	[redacted]	[redacted]	[redacted]
Actual Spend	[redacted]	[redacted]	[redacted]	[redacted]
Approved Staff Input*	[redacted]	[redacted]	[redacted]	[redacted]
Actual Staff Input*	[redacted]	[redacted]	[redacted]	[redacted]

* *Staff years*

Participants
List all project participants by the following categories:

Industry		Research Base		Other
Large Enterprises	*Small and Medium Sized Enterprises**	*Higher Education Institutes*	*Other Research Base Partner*	*Other*
	Seasalter Shellfish, The Harbour, Whitstable, Kent	School of Ocean Sciences, University College of North Wales, Menai Bridge	CEFAS Weymouth Laboratory, Barrack Road, Weymouth, Dorset	
	Mainland Salmon Ltd., Frotoft, Rousay, Orkney			

* *Less than 500 employees and with an annual turnover of less than £30 million*

Section 2 – Objectives

1. To develop protocols to preserve algae cultures by concentration by centrifugation, spray-drying and freeze-drying.

2. To examine the effect of these processes on the physical and biochemical characteristics of the cells, and to assess any changes with time of storage.

3. To determine and compare the nutritional value of algae preserved by these three methods as diets for bivalve molluscs, and to assess any changes with time of storage.

4. To determine and compare the nutritional value of algae preserved by these three methods as diets for zooplankton used as fish larval diets, and to assess any changes with time of storage.

5. To evaluate preserved diets in commercial hatchery systems, using information obtained from the above experimental approaches.

Milestones		Target Date	Milestones Met?	
Number	Title		In full	On time
01	Establish cultures and develop preservation and regeneration methods	March 1999	For 7 species	Yes
02/01	Compare food value of preserved and live algae for bivalve molluscs.	March 2000	Yes	Yes
02/02	Compare food value of preserved and live algae for growth and enrichment of zooplankton.	June 2000	Yes	Yes
02/03	Evaluate Artemia enriched with preserved diets as food for Atlantic Halibut larvae	June 2000	A few industry trials and laboratory experiments with Clownfish	No
03	Estimate practical value and cost-effectiveness of preserved diets. Prepare final report.	Sept 2000	Yes	Yes

Executive Summary of Research and Results *(Include targets and objectives indicating progress made towards achieving them, or reasons for those not achieved. Also include highlights, outputs, deliverables and any unexpected benefits)*

1. Algae preservation

Methods for concentrating and preserving seven species of microalgae (T-ISO, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros ceratosporum*, *C. calcitrans*, *Rhinomonas reticulata* and *Nannochloropsis oculata*) were evaluated. All species concentrated well when centrifuged at speeds of 3500 rpm, with excellent recovery rates.

Storage life of the concentrates at 4-6 ^{0}C varied with species. Some algae, e.g. T-ISO and *Chaetoceros* sp. began to smell 'off' after 2/3 days and T-ISO smelt strongly even after 24 hours. This was improved by bubbling the concentrate overnight instead of holding in a fridge. Concentrated pastes stored at 4 -6 ^{0}C have a much shorter shelf life than other preservation methods, with an average of about 2 weeks, although sometimes longer for species such as *N. oculata*.

It was found that freezing (at -20 ^{0}C) can preserve cells of some species, and protocols were developed for this method. *T. suecica* (≈ 60% cells recovered), T-ISO (≈ 45% cells) and *N. oculata* (100% cells) could all be preserved by freezing. For other species, a soup-like substance with very few intact algal cells resulted.

Techniques for spray drying of the concentrates of all species were established. The resultant powder after spray-drying the algae concentrate contains 5-7% of algae by dry weight, the rest of the powder comprising of salts, and with 55-71% recovery in terms of total algae dry weight from the concentrate. The best method for re-hydration of the cells was found to be by adding the spray-dried powder to filtered seawater and blending for around 20 seconds in a domestic blender. This aided the breaking up of clumps of cells, especially for the larger species. When examining the re-hydrated cells under a light microscope, some physical changes could be seen. In *Chaetoceros* sp. the cells appeared very shrunken and shrivelled with many damaged cells. With T-ISO, *P. lutheri*, and *R. reticulata* the cells appeared shrunken, although the majority remained intact. *T. suecica* was less affected and *N. oculata* cells did not appear to be any different after spray drying. Shrinkage of the dried cells was quantified, using a Coulter, to the following percentages: *P. lutheri*, T-ISO - 24%, *R. reticulata* - 18%, *T. suecica* - 7%.

2. Biochemical changes

There appeared to be a decrease in total carbohydrate content of the cells on spray drying. Also, spray drying generally reduces the essential PUFAs (20:5w3 and 22:6w3) content of the cells. This is especially severe for the diatoms. It would explain why food value of dried algae is broadly similar (and poor) irrespective of species (see below). With the other algae tested the 20:5w3 seems to be more easily lost on drying than the 22:6w3. There was no significant loss of cell numbers of spray dried or frozen species over time (18 weeks). With frozen algae, biochemical changes differed between species. For example, T-ISO retained 22:6w3 whereas *N. oculata* did not.

3. Food value for bivalve molluscs

Feeding trials were carried out using Pacific oysters (*Crassostrea gigas*) and king scallops (*Pecten maximus*). Oyster larvae fed dried diets gave very little growth and showed high mortalities. Spat fed dried diets, in three-week trials, survived and increased in organic weight, but at only 20% of the rate with the live algae control. Mixed live:dried diets gave intermediate results, and supplements of dried algae to lower rations of live algae significantly improved growth of spat. An experimental concentrated paste diet (heterotrophically-grown *Cyclotella cryptica* supplied by Liverpool John Moores University) was slightly more successful, with oyster growth rates of 50% of that with a live diet.

Seasalter have further developed improved methods for large-scale algae production, in both extensive (algal ponds) and intensive systems. The latter includes continuous bag culture and evaluation of an AAPS vessel with Addavita Ltd. Algal pond water from Seasalter (Walney) was transported to Conwy for concentration to a paste. The bulk of the paste was taken back to Walney where it was successfully used immediately as food for oyster spat. Samples were also stored at Conwy for 24 weeks at 4 ^{0}C and frozen. Food value (for scallop spat) of the 4 ^{0}C sample deteriorated during this period, but that of the frozen sample was similar to preserved (dried or frozen) samples of cultured species.

Executive Summary of Research and Results/continued:

4. Food value for zooplankton

Algae pastes supplied to Mainland Salmon to evaluate as diets for rearing *Artemia* for feeding to Halibut larvae were used successfully. There has been some uptake at this and other hatcheries of commercially available equivalents, which have become available during the course of this work. Pastes are also being used for green-water techniques.

Protocols were established for feeding experiments providing preserved algae diets for rotifers (*Brachionus plicatilis*). Comparisons included algae pastes from Biosynthesis (a company producing preserved *T. suecica* and *N. oculata* as food for zooplankton in fish farms). Preserved algae generally gave good results; a rotifer culture was maintained for 24 days using spray dried *Tetraselmis suecica* alone, although live algae gave the greatest increase in animal numbers. About 80% of live *N. oculata* could be replaced with spray dried *N. oculata* and approx. 65% of live *T. suecica* could be replaced with the spray dried alternative, before rotifer numbers decreased significantly. The *C. cryptica* paste was not suitable, as it encouraged bacterial contamination, resulting in death of the animals. Spray-dried algae and frozen algae appeared to be of similar nutritional value, and further experiments focussed on spray dried samples of the species that preserve well.

Artemia nauplii and adults were observed to feed upon spray dried algae and successfully reared for 7 days on spray dried *T. suecica, R. reticulata* and T-ISO. All diets showed greater survival rates than *Artemia* that remained unfed.

5. Larval fish feeding experiments

It was decided to use Clown fish as a test species, instead of Atlantic Halibut, as this work could be carried out at Menai Bridge, with the closure of the CEFAS Conwy Laboratory.

A successful system for maintaining clownfish (*Amphiprion clarkii, A. oscillaris* and *A. perygidium*) was available at the University of Wales Bangor, but there had been only limited success with larval spawning. The system was adopted and modified to attempt to gain frequent larval batches. There was some success with the system and the addition of potassium iodide into the water supply induced adults to lay eggs. Of the 6 pairs of adult clownfish only one yielded eggs that we were able to use, but never settled into the expected cycle of reproduction, in which eggs are produced every 2-3 weeks.

Hatching larvae from eggs was successful at each attempt and from these a system was set-up to allow the larvae to be reared firstly on rotifers enriched on spray dried diets and then onto *Artemia*, also enriched on the diets. It was intended to grow around 40 larvae in each trial. However, the larvae did not survive beyond the first week on either spray dried diets or live diets. It is thought that problems with water quality have been the main contributor to the high mortality rate, as the system has been used successfully as described in the literature.

Future R&D resulting from this project *(Include any other non-tangible benefits and state any Teaching Companies Scheme action if appropriate)*

This project has seen collaborative work with commercial companies developing preserved algae diets, and there is scope for this to be developed further, with science partners evaluating the food value of the diets in controlled laboratory experiments. This would best be achieved through formulating a standard protocol, possibly by further developing the Clownfish model.

The problem of abnormal pigmentation of fish continues to be a concern in fish hatcheries and it is thought that this might be diet-related. Preserved algae diets would provide a useful tool for further investigation of this problem, and could include studies on dietary enhancements.

The spray dryer has been transferred to SAMS in Dunstaffnage for extending findings from studies on preserved diets to rearing sea urchins.

Industrial relevance and plans for future commercial exploitation

During this study preserved algae diets, both frozen and dried, have become commercially available and there has been significant uptake by the fish farming industry. The results from this work have contributed to developments in this field.

We have shown that preserved diets cannot fully replace live algae in shellfish hatcheries but have some application as supplemental diets and could prove beneficial when the supply of algae within a hatchery is interrupted for any reason.

There is considerable potential for preserved algae diet products in fish hatcheries.

Patents and Publications (Including those pending)

Bennet T. (1998) 'Preserved microalgae diets' Shellfish News No. 5 (May 1998), pp.13-15.
Syvret M. and Rawlinson L. (1999) 'CEFAS casts new light on larval nutrition' Fish Farmer Volume 22 Number 1, pp 46-48.
Laing I. and Rawlinson L. (2000) 'Preserved microalgae diets' Poster presentation for Link Aquaculture Conference, SEC, Glasgow, March 2000.
Ward N. (2000) 'Pacific oysters grown with preserved algae diets' Shellfish News No. 10 (November 2000, in press)

To be completed by the Project Leader

Declaration

I declare that the information given has been approved by all the project participants and is correct to the best of the best of my knowledge and belief I understand that the information contained in this form may be held on a computer system.

Signed:  Position:Contract Leader Date: 22/12/00

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