Initial Proposal for the Research and Development of Aquatic Zooplankton (ZP), Phytoplankton (PP), and Macrophytes (M) as a Sustainable Yield Replacement for Fish Meal in Commercial Fish Feeds:

Proposal Objectives

The objectives of this proposal are summarized as follows. Basically, it is intended to generate R&D funding for the purpose of defining the commercial culture biomass of zooplankton (ZP) and duckweed, an aquatic macrophyte (M) by ulitising the blooming culture potential of phytoplankton (PP). That definition evaluates the process potential for;

a) *development of a sustainable and commercially viable fish meal substitute.*
b) *achievement of a sequential 'stepping stone' funding investment strategy based on targeted R&D result outcomes.*
c) *Evaluation of the research findings, extrapolation the potential, and establishment of a pilot scale/commercial extrusion milling process.*
d) *evaluation and costing of available commercial components and processors for the establishment of the commercial production phase.*
e) *presentation of regular findings and research result reports incidental to the directional investment strategy.*

Introduction

Aquaculture has now been identified as highly significant in terms of globally sustaining protein to feed the worlds populations. World wide, Aquaculture is making great technological advances in fish production. These include breeding and hatchery techniques, expanding species domestication and increasing efficiency of grow-out applications.

http://www.marine.csiro.au/research/aquaculture/index.html

Almost 38 million tonnes of seafood was farmed globally in 2001, representing 30% of total fisheries production. By 2030, more than half our seafood will be farmed.

Major challenges facing aquaculture industries worldwide include health management, production efficiency, genetic improvement of stock, endemic species development, and identifying alternative protein and oil sources for animal feeds.

Globally, aquaculture has increased by123% in ten years to rise from 17 million tonnes in 1996 to nearly 40 million tonnes of product worth approximately $60 billion USD in 2003-2004.

Earth Policy Institute Resources on FISH

Fish Indicator

Eco-Economy Indicators are twelve trends that the Earth Policy Institute tracks to measure progress in building an eco-economy. The world fish catch is a measure of the productivity and health of the oceanic ecosystem that covers 70 percent of the earth's surface. The extent to which world demand for seafood is outrunning the sustainable yield of fisheries can be seen in shrinking fish stocks, declining catches, and collapsing fisheries

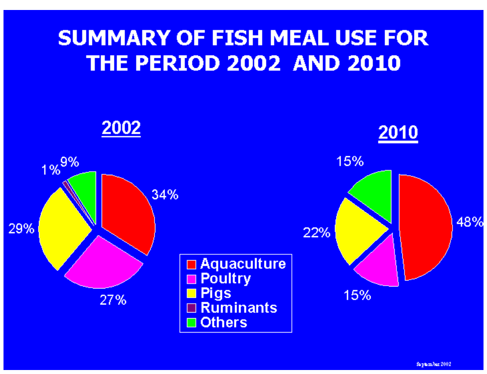
In comparison, world fisheries have been in constant return on investment decline since about 1990. This factor can be shown on almost all fish catch verses fish catch effort analysis.

Australia is a relatively small aquaculture nation with a total aquaculture production at $2.3 billion AUD in 2003-2004. However, like all other western nations Australia relies almost entirely on pelleted diets for aquaculture production. Current figures are sketchy but it is believed finfish and crustaceans make up 70% of total Australian Aquaculture production. If pellet diets make up 40% of the market price then it can be assumed that Australia uses approximately $2.3 billion x 0.7 x 0.4 = **$644 million AUD** in pelleted diets annually.

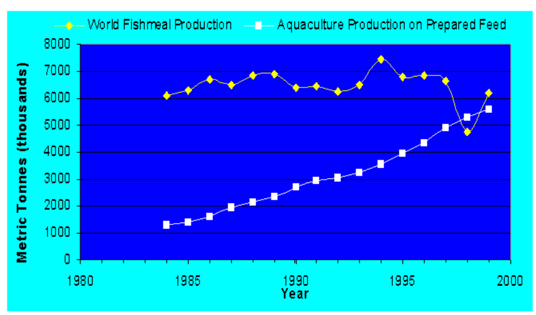
Pelleted diets vary from mill to mill but basically, they all consist of a known percentage of protein, a known percentage of lipids and a known percentage of carbohydrate (PLC). Basically, the ingredients are mixed and extruded into various sizes with varying degrees of weight/volume to suit a floating or sinking application.

Usually the ingredients are made of locally farmed grain products or grain waste products in combination with either fish meal, fisheries waste or trash and or blood meal products. Research on fish nutrition is very well established and the essential nutritional requirements of most commercial species are well documented.

In any case, the aim of the mass produced pellet diet is to produce a targeted PLC product, with adequate nutritional requirements, for a reasonable cost. However it is fairly true to say that all fish pellet diets represent a nutritional trade-off and therefore a potential reduction in fish culture performance.



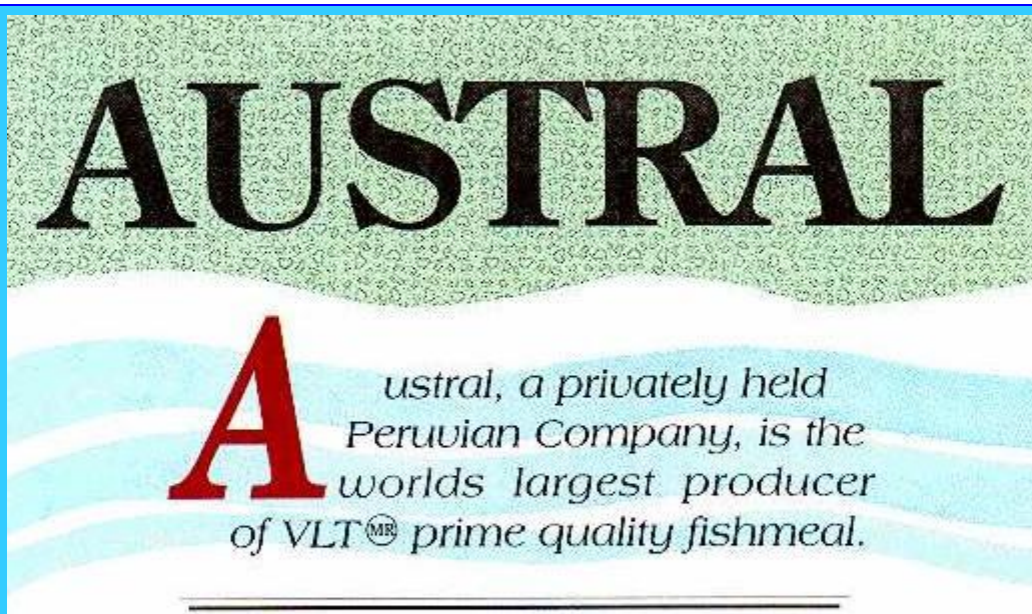
FISH MEAL PRODUCTION AND GROWTH OF AQUACULTURE



Annual time series of worldwide fish meal production and production of fed aquaculture species including salmon, shrimp, eels, marine fish, diadromous fish, and tilapia (carp, bivalves, and plants are excluded; 1999 figures are projected)

Fish meal production has not responded in any way to this growth in aquaculture production.

Fish meal protein is by far the best protein source but it is also quite expensive at approximately $1.00 - $2.00/kg landed in Australia in bulk. Because of the high fish meal cost factor, protein substitution is used to off-set the cost of pellet production. Protein substitution allows millers to supply an affordable product into the market.



http://acstradingusa.com/

What is Fishmeal?

Fishmeal is a thick powder obtained from cooking, drying, and grinding raw fish. Fishmeal is a rich protein source, and is used as an ingredient in feedstuffs in the aquaculture, dairy, and poultry industries.

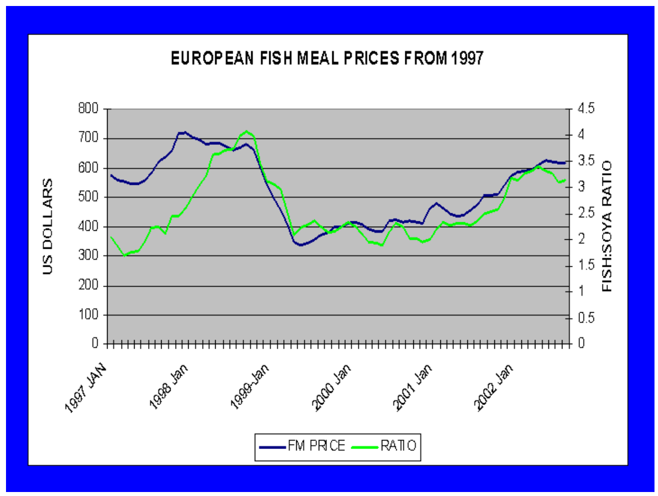
The Fishing Fleet

Our fishing fleet is the largest and newest fleet in Peru. The fleet currently totals 38

However, as mentioned above protein substitution tends to reduce growth potential as it introduces protein uptake limiting factors and other nutritional uptake and digestibility uncertainties. Added to these restraints is the potential inclusion of GM grains as well as the use of grains contaminated with insecticides and pesticides. And these are all factors of great concern for the forward development of the industry and the potential of fully organic fish feeds.

The current cost to buy an Australian made fish culture diet varies from about $1400 per tonne to approximately $2200 per tonne. Imported diets tend to be slightly more expensive but the quality and the protein source can vary considerably. Recently concerns were raised within the commercial sector about the potential to introduce disease from insufficiently processed protein meal as well as concerns about the original source of the imported protein meal.

Australia imports nearly all fish meal requirements from South America and to a lesser degree Asia. Vast seine nets are used off the coast to capture massive tonnages of schooling fish species which are all processed and sold around the world.



It is this process that makes aquaculture profitable however it is this process that makes aquaculture unsustainable.

http://www.growfish.com.au/content.asp?ContentId=7104

Intrafish says a shortage of 1,000,000 tonnes of fish meal, food fed to farm-raised salmon, is keeping down the amount of farmed salmon on the market.

Fishmeal, which has exceeded €1,265 per tonne this year, is up €790 from a year ago.

Price hikes and shortages are being blamed on an increasing Chinese demand for feed and problems in supply from South America.

It takes about 64 oz of ground up wild fish meal to generate 16 oz of farmed salmon.

Fish meal arrives in Australia, usually at the Port of Newcastle, in bulk tanker shipments. Fish meal appears as a dried and ground rough powder and is stored in huge harbour front warehouses. From there it is bulk loaded into tipper trucks and makes its way into the pet food industry, the chicken industry and the aquaculture industry.

These existing industries represent the potential markets for zooplankton

What is Zooplankton?

Zooplankton is the multitude of microscopic and semi microscopic invertebrate animals that exist in both sea and freshwater. Zooplankton includes abundant larval stages of many organisms. They feed on unicellular plants known as phytoplankton, bacteria occurring as detritus particles and other organisms of an appropriate mouth size. Mouth size equates to feed particle size and is of considerable significance in culture.

Basically though, zooplankton is an essential component of all aquatic food chains.





Currently the development of zooplankton species, as a live food source for advanced fish hatchery research, is driving the successes with fish larval survival. The end result of such work is, an increase in the number of exotic species which can be reared successfully. Examples of zooplankton species that have become synonymous with hatchery culture are brine shrimp and rotifers. (Refer to Appendix A)


Rotifer

Brine Shrimp

Historically zooplankton, as a fish culture food source, has been known to fish farmers for well over 2000 years. In China it has been an essential component of their life and so much so that in village life they are probably not aware of the significance.

To explain; village life in China has existed with an almost 'Garden of Eden' sustainability. Human waste is used to fertilise crops and ponds. Plants utilize the nutrients in both the soil and the ponds. Plants grow to crops in soil and they colour the water in ponds as phytoplankton or algae. As the algae blooms they are consumed by increasing zooplankton populations in the ponds. The zooplankton is consumed by higher organisms which are usually food fish. The humans eat the fish and the plants and then redeposit the nutrients to complete the cycle.

The Chinese example may sound a little gross to western culture but this aquatic bio-dynamic and its evolution are significant factors in how the Chinese can routinely harvest around 10 tonnes to the hectare where most western aquaculture appears limited to 5 tonnes per hectare.

In Australia significant research into Zooplankton is evident however little has been done to quantify commercial potential for the purpose of this proposal. There is considerable evidence of research work carried out during the 90's which indicates significant quantities of zooplankton biomass could be continuously harvested.

As well, supporting research studies were done on sewage effluent settling ponds in Victoria and those results indicate harvest rates approximating 2.5 tonnes per hectare per day. Those results also identified a major environmental problem in that the sewage effluent of western societies, can be high in heavy metals. And those heavy metals can be transferred to aquatic food chains via algal uptake.

Commercial trials carried out by the author confirm a potential significance for the development of several commercial applications. The basis of that work centered on hatchery and growout production of Australian freshwater species as well as survival trials of Barramundi larvae. Although the two feeding methods are significantly different and, they identify two separate culture issues, the relative results showed a vast increase in the growth rates and overall health of the cohort when compared to identical ponds cultured on standard pellet diets. Although this process was not scientifically documented it did allow the hatchery to repeatably double fingerling production to two crops per summer.

For example, silver perch grown on zooplankton reached an average of 100mm in 4 weeks, where as silver perch grown on a standard diet grew to 28mm. To achieve these results the author targeted specific water quality parameters to favour specific varieties of phytoplankton and zooplankton production. Those results yielded a significant increase in growth rate as well as a decrease in the real cost of hatchery production.

The author has also harvested considerable quantities of zooplankton over several years and has significant developments toward culture methods for the repetitive targeting of favourable zooplankton species.

Basically, all research work in the area of zooplankton, production trials and field results appears to indicate a high potential for commercial culture of certain species as an aquatic food source. However, one question remains unanswered.

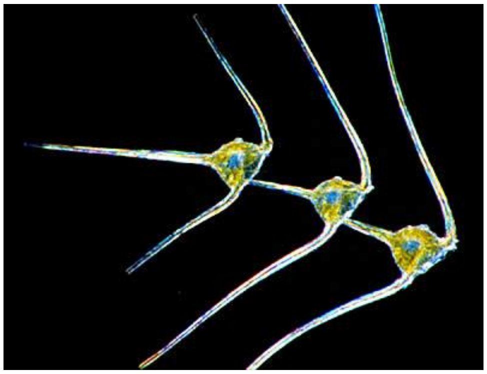
Why hasn't zooplankton been recognized as a commercially viable and sustainable alternative to sea harvesting for fish meal?

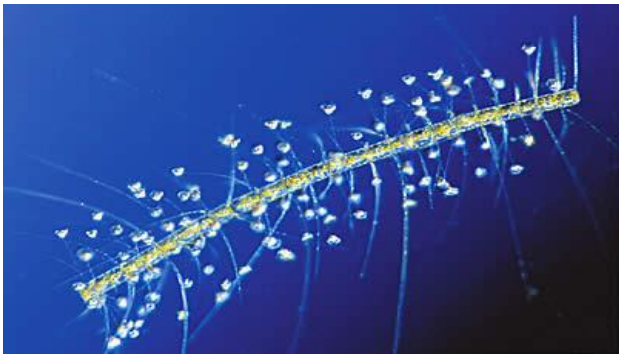
What is Phytoplankton?

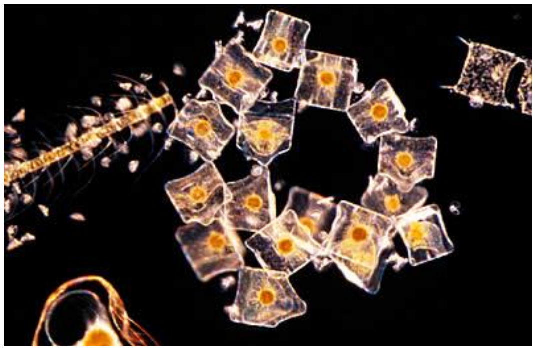
The definition of phytoplankton is microscopic unicellular plants occurring in an aquatic environment. There are several differing classifications and a multitude of species. It is the intention of this proposal to ultilise and manipulate the culture of single species (axenic or pure) and multispecies phytoplankton to stimulate zooplankton biomass production.

Phytoplankton are tiny, photosynthetic organisms. This means they can manufacture their own food using energy from sunlight, producing oxygen as a by-product. They are often referred to as tiny plants because of this ability to photosynthesise, but many species of phytoplankton are more closely related to protists and bacteria than true plants. Phytoplankton typically range in size from 0.002 mm to 1 mm and include diatoms, dinoflagellates, Radiolaria, Ciliata and Cyanobacteria (better known as 'blue-green algae').

Phytoplankton Species

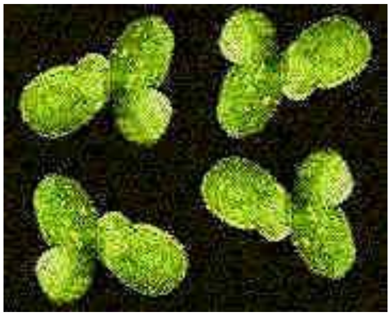






What are Macrophytes?

Macrophytes are aquatic plants, growing in or near water that are either emergent, submergent, or floating. Macrophytes are beneficial to lakes because they provide cover for fish and substrate for aquatic invertebrates. Duckweed is an important macrophyte because it can be an adequate aquatic protein resource, as an agent in achieving aquatic environmental stability and as a potential feed source in aquaculture.



Duckweed

The family of duckweeds (botanically, the Lemnaceae) are the smallest flowering plants. These plants grow floating in still or slow-moving fresh water around the globe, except in the coldest regions. The growth of these high-protein plants can be extremely rapid. *Lemna* is one of the best known of this group and has been the subject of much research.

Researchers are using these plants to study basic plant development, plant biochemistry, photosynthesis, the toxicity of hazardous substances, and much more. Genetic engineers are cloning duckweed genes and modifying duckweeds to inexpensively produce pharmaceuticals. Environmental scientists are using duckweeds to remove unwanted substances from water. Aquaculturalists find them an inexpensive feed source for fish farming.

Research on duckweed shows significant potential for culture and for use as a nutrient control on effluent drains of feed lots. Duckweed has a significant growth rate and up to 30 tonnes per Ha per year have been achieved on low level nutrient addition.

The author suspects that combinations of targeted zooplankton, duckweed and a small combination of nutrients, binders and stabilizers have the potential to produce a high quality fish feed.

Business Plan Overview

There is no doubt that zooplankton has the potential to be the basis of defining the next generation of aquaculture. It can potentially achieve this significance because zooplankton is a sustainable aquatic protein source which can occur in significant and potentially commercial quantities.

Its culture can be defined by the use of bio-effluents and inorganic fertilizers. Essentially its culture acts as a water purifier by stabilization of the excess aquatic chemical loadings and processes involved in eutrophication and it is factors in this process that can be productively manipulated.

Naturally, this raises the question as to why zooplankton has never been developed thus far. The answer to this question is complicated but in many applications, zooplankton has been developed. However to explain why there is no zooplankton meal is difficult due to the multitude of factors involved. Some of those factors are outlined below.

There are several cyclic parameters of both chemical and organic origins interacting within a variable environment. Understanding those relationships and then targeting aspects of that interaction to generate stability and induce an aquatic biomass production yield requires a considerable understanding of the variables. Then to make such a process sustainable by reducing the natural cyclic pattern requires significant experience with pond and water quality management.

The cyclic interaction can be visualized as follows. Examine any aerial photograph of a fish farm under culture. Most farms have in excess of 25 ponds set out in contoured rows. However, it is quite likely that no two of the ponds are the same colour and yet they all would have the same amount of biomass and all would receive the same amount of feed and water exchange. Why don't they all respond equally?

In the enclosed aquatic environment of an aquaculture pond there exists several cyclic parameters that are all related in complex interactions. Those interactions determine what species of algae and what species of animal dominate, and to what degree they are capable of successful and sustainable population. In aquaculture these interactions and their ability to stimulate aquatic production has been recently referred to as pond dynamics by Professor C. Boyd however, the outcomes of generated biomass dynamics have never adequately been commercially or scientifically quantified.

Basically, pond dynamics refers to the use of nutrients to increase the indeterminate productivity of a pond. Productivity is increased because the numbers of zooplankton increase and they represent a very efficient food source for the culture biomass.

Unfortunately, not all species of algae and zooplankton are beneficial as food for higher organisms. Some are toxic, some bite and cause infection and some retard the production biomass while others are nutritionally deficient but all can dominate an enclosed environment.

So, the answer to the bigger question is complex requiring skills spanning several areas of science and biology. However, in a basic summation, no research has looked at targeted pond dynamics in western aquaculture and quantified the results. Even though pond dynamics represents a considerable cost saving it appears as though the aquaculture industry is simply prepared to be reliant on available processed diets and associated cost structures.

In comparison, the amount of research compiled on the attributes of various vegetable proteins related to fish nutrition is staggering. There are absolute mountains of work covering this area. But, as it was mentioned earlier, protein substitution is a trade off. This raises further questions, as it is well known, that certain essential amino acids are only found in fish and zooplankton meals.

In summary simplification, the commercial objective of this proposal will be in consolidating a commercial application (using the sum of the R&D information) into a simple ZP & M recipe for consistent duplication. That is the basis of the work to be quantified and qualified over the next 6 to 24 months.

Sequential R&D Proposal Components

Therefore, the Commercial Focus of this proposal is to:

- **a.** define a commercial aspect of the pond dynamic complexity
- **b.** manipulate the associated parameters with documented input processes
- **c.** quantify those inputs to achieve a sustainable and commercial biomass production system
- **d.** structure R&D funding to reflect the sequential confirmations of the commercial viability of each R&D outcome.

Therefore, the Sequential R&D Outcomes for zooplankton and duckweed are to:

1…identify and quantify sustainable yield potential for both fresh and saltwater pond production. **(This phase represents the first 6 month trial period)**

2…qualify the ingredient potential for fish meal replacement and publish the outcomes.

3…design and develop a continuous harvesting system

4…produce a defined pellet based product.

5…complete hatchery, pond and tank culture growth research data to support the product and publish those results

6…document the production process for potential 'zooplankton farm franchise'.

7…define and establish the manufacturing processes.

8… identify other potential sales of ZP meal and examine the potential short-term and long-term return on investment.

Zooplankton Production Proposal Example

Assumptions: The simplified production proposal example assumes a 50% production loss on calculated pond production to account for farming misadventure, climate variations, rainfall etc.

1…Existing research into zooplankton production discusses **2.5 tonnes per day per hectare wet weight** production.

2…The dried weight of ZP meal is expected to represent approximately **18%** which equates to **450 kg of ZP dried meal/day/Ha.** (published dried weight varies from 5% to 21%)

3…ZP meal will have a market value of approximately **$1.50 per kg.**

4…Therefore the per day per Ha production gross is **$675**

5…Therefore the potential per day per Ha production gross on 25 Ha is **$16875**

6…Therefore the potential return per annum equates to $16875 x 365 / 2 = **$3,079,687.50**

7…It is assumed that a 25 Ha ZP farm would require approximately 3 people to run the pond management system. The management of the feed production system would require 3 to 4 people and therefore the total labour component would approximate **6.5 staff**.

8…Duckweed can be cultured to 30 tonne dry weight per ha per year = 576kg/week

9…Duckweed is cultured extremely well on animal manures and can be incorporated on existing animal feed lots

10…There is no cost structure associated with duckweed as a meal component however its protein levels are about 40%.

R&D Business Structure

Any business has to have defined goals and defined business structure from the outset. And that fact is probably more important with such a venture proposal as outlined in these pages. This factor is significant as the business method and commercial return on investment are yet to be defined. Aspects of the initial focus may change to suit the identified commercial focus and the costing structure may alter to suit that purpose as well. Indeed, it is highly likely that the business may go into commercial aquaculture production during the second year.

Generally, aquaculture tends to be a variable market focus and a profitable business needs to stay ahead of the current 'market fashion' as these fashions tend to attract over supply which consequently end in lower profits. However, a protein meal product has many advantages over normal aquaculture products in that it is not the end product and it is not limited to one industry. And further, there is a significant potential in the application of constant crop turn-over and associated cash flow which is not a normal aspect of aquaculture. Combine these factors with the initial business structure, running and production costings, which indicate a potential investment return above 27%, and the proposal begins shows commercial merit of investigation. However, such grand extrapolations need caution and proper evidencing based on the due diligence of commercially relevant data of applied application. In summary however, the demand for fish meal products far outstrips supply and will continue to do so for the next 30 years. The time is right for commercial investigation.

From the proposal discussion, it can be assumed that a considerable amount of existing experience and academic expertise has been applied to identify the commercial strategies as outlined above. That work has taken several years of observation, trial and scientific analysis to achieve a point of confident proposal presentation.

The author has continued researching and refining the processes involved in zooplankton production for many years and that knowledge and development insures a defined low risk and staggered investment based on the commercial potential of the sequential results as outlined above.

Therefore, it is proposed that such a business should contain a fare and equitable portion of technical input and business management. And as such, the start up components should reflect that in all aspects. To that point the idea of an R&D company structure with an equitable **60%** holding between the technical and scientific resources and **40%** equity for the financial support resources. The author has discussed this point with several friends and professional associates to achieve an equitable beginning and these suggestions would appear to be a considered and logical first step.

From the Business plan below, we can see the expenditure budget over one year will equal approximately $200,000 if the sequential results indicate commercial potential. It is anticipated the second year budget will consist mainly of wages as the R&D equipment will have been basically accounted for.

During the initial 12 -24 months of R&D activities, investment would, as mentioned, be sequentially staggered as a form of investment security and injected to suit the results of the R&D Outcomes and budget requirements.

These months will provide the credible research and development to provide the potential for significant business investment potential. The R&D will also provide duplicated farm system technology for potential franchise options.

The Business Proposal anticipates that at some point during the second year, the R&D component will cease and the commercial component will commence. Therefore, it is anticipated that the overall R&D budget will not exceed $400,000 and therefore that is the relative figure of the financial resources component commensurate with the proposed 40% equity within the business structure.

The financial structure of the commercial component will be further financed with investments based on the qualified R&D results and the anticipated attractive return on investment. The author notes that positive publications should be written and made available to the press and associated journals to stimulate professional and commercial interest for the longer term investment.

From the proposal, we can see that a considerable amount of business acumen will be required to maintain the focus on the goals and tasks. There will be problems. Indeed, there will always be problems as that is the nature of commercial aquaculture development. It is the belief of the author that the request for financial support is based in solid fact and that the as a team the new company can be of significant community and commercial benefit.

Footnote to R&D Business Structure

1…Once the initial test results have been concluded, the R&D ponds can be secured for potential use over the next 2 years on a lease basis. This includes 4 ponds on 2 farms which are approximately 20 kilometers apart.

2…One farm has agreed to allow access for wet lab facilities and some storage areas. They have agreed to allow access to hatchery facilities and are open to reasonable requests. The author has agreed to assist in their breeding program with production advice if required as it will be important to have relevant data on hatchery survival based on a zooplankton high protein micro-diet.

3…In terms of when such a project should commence is a matter of proposal analysis financial analysis and the finalization of the working business structure.

4…It is anticipated that a utility vehicle will be essential and the author has secured both a Holden utility and a Toyota Dyna tray back.

5…The other staff member has been secured and will be an asset to the project.

Business Proposal Costing

Business Proposal Setup Costing for Initial 6 Month R&D Assessment Period

1a…During the writing of this proposal, discussions and collaborations with relevant industry representatives has created a degree of skepticism in regard to the efficiencies of lower order proteins and amino acids available from zooplankton and duckweed. This raised concern is difficult to validate or discount from available university research searches and on-line publications.

Although this concern seems insignificant to the author, to be certain of all facts and potential success of the proposal an initial 6 month R&D assessment period would seem appropriate.

Unfortunately, the concept of targeted phytoplankton, zooplankton and macrophyte species culture has no research comparison which accounts for the conservative opinion.

The initial 6 month period will be sufficient time to quantify target cultured zooplankton species and associated duckweed. Independent analysis can then confirm relevant protein, lipid and carbohydrates levels as well as the type and quantity of amino acids.

Business Proposal Setup Costing for First 12 Month R&D Period

1…The R & D components will take 12 months to evaluate seasonal fluctuations however that may extend well into the second year. The R&D process is unlikely to extend past 24 months based on existing research and development.

2…No price structure has been given for commercial extrusion equipment at this stage. It is anticipated that at the correct time a site development proposal, for feed manufacture, will follow. However initial investigations indicate a commercial mill rated at 1.5 tonnes per hour can be purchased for approximately $2 million AUD sourced from China.

All feed milling and processing will be of a research nature until the actual profitability and feasibility of each process has been commercially evaluated. It will be necessary to acquire pelletising and batching equipment of a research nature for initial ingredient processing (as outlined in the costing).

3…It is anticipated that most of the first 12 months will be concerned with quantitative and qualitative analysis of both fresh and seawater pond potential for ZP and DW meal production.

Business Proposal Setup Costing for Initial 6 Month R&D Assessment Period

Initial 6 Month R&D Assessment Period

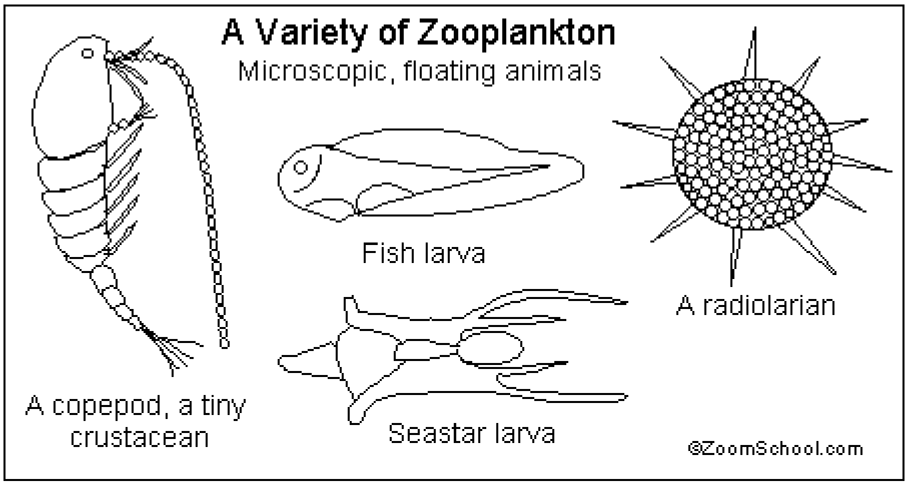
Item	Sundry Details				Sundry Total	Weekly Totals	Annual Total
Labour	Kel Gordon	40hrs	$25/hr	1,000.00			
				1,000.00			
	Labour Sundry Costs at 18%			180.00		**1,180.00**	**$30,680.00**
Test Equipment							
	Palintest						
	Microscope			800.00			
	Water Testing Meter						
	Pond Test Kits			1,000.00			
	Biological Test Equipme		P/L/C	1,000.00			
	Lab Equipment Sundry			500.00	3,300.00		**$3,300.00**
Pond Equipment							
	Harvest Units			500.00			
	Fertilizer/Nutrients			500.00			
	Initial Pellet Extrusion Equipment			0.00			
	Pond Rental	Salt		0.00			
		Fresh		0.00			
	Remote Data Logging			0.00			
				1,000.00			
	Sundry at 15%			150.00	1,150.00		**$1,150.00**
Buildings	Wet Lab			0.00			
	Container			0.00			**$0.00**
Aquarium Trials							
	Sample Nets			200.00			
	Micron Mesh			200.00			
	Aquariums			500.00			
	Pumps Lights etc			0.00			
				900.00			
	Sundry 15%			135.00	1,035.00		**$1,035.00**
Fuel							**$0.00**
Sundry Office							
	Computer						
	Internet						
	Printing						
	Phone						
	Furniture						**$0.00**
							$36,165.00

Business Proposal Setup Costing for First 12 Month R&D Period

Setup & Running Costing for the First12 Months							
Item	**Sundry Details**				**Sundry Total**	**Weekly Totals**	**Annual Total**
Labour	Kel Gordon	60hrs	25/hr	1,500.00			
	Aquaculturist	40hrs	18.50/hr	740.00			
				2,240.00			
	Labour Sundry Costs at 18%			403.00		**2,643.00**	**$137,436.00**
Test Equipment							
	Palintest			3,500.00			
	Microscope			800.00			
	Water Testing Meter			2,500.00			
	Pond Test Kits			1,000.00			
	Biological Test Equipme		P/L/C	5,000.00			
	Lab Equipment Sundry			2,000.00	14,800.00		**$14,800.00**
Pond Equipment							
	Harvest Units			2,000.00			
	Fertilizer/Nutrients			3,000.00			
	Initial Pellet Extrusion Equipment			5,000.00			
	Pond Rental	Salt		10,000.00			
		Fresh		4,000.00			
	Remote Data Logging			6,000.00			
				30,000.00			
	Sundry at 15%			4,500.00	34,500.00		**$34,500.00**
Buildings	Wet Lab			10,000.00			
	Container			3,000.00			**$13,000.00**
Aquarium Trials							
	Sample Nets			200.00			
	Micron Mesh			200.00			
	Aquariums			1,500.00			
	Pumps Lights etc			1,500.00			
				3,400.00			
	Sundry 15%			510.00	3,910.00		**$3,910.00**
Fuel						$50.00	**$2,600.00**
Sundry Office							
	Computer				1200		
	Internet				600		
	Printing				200		
	Phone				400		
	Furniture				500		**$2,900.00**
							$209,146.00

APPENDIX A

Plankton



Plankton are microscopic organisms that float freely with oceanic currents and in other bodies of water. Plankton is made up of tiny plants (called **phytoplankton**) and tiny animals (called **zooplankton**). The word plankton comes from the Greek word "planktos" which means "drifting."

Phytoplankton: Phytoplankton are primary producers (also called autotrophs). As the base of the oceanic food web, they use chlorophyll to convert energy (from sunlight), inorganic chemicals (like nitrogen), and dissolved carbon dioxide gas into carbohydrates.

Zooplankton: Zooplankton are microscopic animals that eat other plankton.

- Some zooplankton are larval or very immature stages of larger animals, including mollusks (like snails and squid), crustaceans (like crabs and lobsters), fish, jellyfish, sea cucumbers, and seastars (these are called **meroplankton**).
- Other zooplankton are single-celled animals, like foraminifera and radiolarians.
- Other zooplankton are tiny crustaceans, like Daphnia. (If you include krill and copepods, which can swim, this group constitutes about 70 percent of all plankton)

APPENDIX B

PLANKTON HARVEST: RECYCLING WASTE NUTRIENTS TO IMPROVE YIELD AND SUSTAINABILITY

Keywords: plankton, harvest, polyculture, pond, biomass

Presented as: BIO-MECHANICAL PLANKTON HARVEST: A CONCEPTUAL MODEL FOR THE CULTURE OF MULTIPLE SPECIES. Aquaculture 2001 -- The international triennial conference and exposition of the World Aquaculture Society, the National Shellfisheries Association and the Fish Culture Section of the American Fisheries Society. Book of Abstracts, p. 704. (click here for Slide Show)

William A. Wurts
Kentucky State University CEP
www.ca.uky.edu/wkrec/Wurtspage.htm

Polyculture is practiced throughout the world. Several species of aquatic animals are stocked simultaneously to take advantage of the different food niches available in the pond environment. In many systems, the primary culture species is fed a prepared diet. The resultant wastes stimulate large phytoplankton populations, which in turn support several species of zooplankton. On a dry weight basis, plankton can account for almost half of the standing biomass in a culture pond (900-1000 kg/ha). Phytoplankton and zooplankton represent the largest niches of surplus food available in commercial production ponds. As such, filter feeding animals, or planktivores, are stocked as additional species in polyculture ponds. This increases production efficiency overall and minimizes the loss of waste nutrients.

Each of the filter feeding species stocked is permitted to graze simultaneously on the same plankton populations. Essentially, these animals feed parallel to one another. Filter feeders screen planktonic plants and animals non-selectively from the water column on the basis of particle size. These planktivores consume phytoplankton and/or zooplankton. By filtering small particles, such as phytoplankton and minute zooplankton, herbivorous planktivores would remove larger plankton as well and would negatively impact the numbers of large-size zooplankton, decreasing zooplankton populations overall (total productivity and standing biomass). The filtering activity of animals that rely primarily on phytoplankton for subsistence would reduce the harvest biomass of planktivores that depend solely on intermediate and large plankton (particles) for food.

To improve the efficiency of plankton harvest, filter feeders should be placed in a series arrangement, flowing plankton rich waters past animals that feed on the largest plankton first and to those that consume the smallest plankton last. Each of the different planktivores should be compartmentalized according to the size of the particles they filter. Pond water could be pumped, from one enclosure into the next, through a series of floating or land-based chambers. Removing plankton sequentially, big particles first and small particles last, would improve net filtration efficiency and increase the (potential) biomass of planktivores at harvest. Careful selection and segregation of filter feeders for an aquaculture system could, in theory, double the harvest biomass in a production pond without significant deterioration of water quality. Waste nutrients would be recycled indirectly through the planktivores.

For related information click on the topics below:

MODIFIED POLYCULTURE
In: Reviews in Fisheries Science, 8(2): 141-150
SUSTAINABLE AQUACULTURE IN THE TWENTY-FIRST CENTURY
Reviews in Fisheries Science, 8(2): 141-150

(back to On-Line Literature page)

APPENDIX C

Research and Development

USDA SBIR Grant entitled "High Frequency Wash Airlift Bioclarifier Using Modified Floating Media for Recirculating Systems". Phase I & Phase II Funding. Period of Performance September 1, 1996 to August 31, 1999.

Project Abstract:

Recent research findings during Phase I of this project have led the research team to conclude that optimum nitrification performance in floating bead filters will only occur in filters subjected to high frequency washing, in which the adverse impacts of solids accumulation are virtually eliminated. The solids not only break down to produce ammonia (Matsuda et al., 1990), but also encourage rapid heterotrophic bacteria growth that competes with the nitrifiers for space, potentially limiting nutrients, and oxygen (Bovendeur et al, 1990). The beads will have to be re-formed to store increased volumes of nitrifying bacteria at loadings in excess of 2 Lbs/day-ft^3 without a reduction in hydraulic conductivity. The beads will also have to provide for abrasion protection in aggressively washed formats, but, excessive protection should be avoided. In summary, this project proposes to continue the development of a highly specialized bioclarifier which will reduce the water reconditioning costs associated with the holding, breeding, or production of aquatic animals in recirculating aquaculture systems.

Collaborators:

- Louisiana State University - Dr. Ron Malone
- TilTech Aquafarm
- Archer Daniels Midland

Results:

- Data substantiating increased nitrification of Propeller-Washed Bead Filters using EN Bead Media.

End Products:

- Enhanced Nitrification Bead Media
- Automated Bead Filter Controllers
- Rotational Molded 3 ft^3 Propeller-Washed Bead Filter

DOC/NOAA SBIR Grant entitled "Continuous Culture Zooplankton System for Marine Aquaculture Feed Production". Phase I Funding only. Period of Performance June 30, 1998 to December 30, 1998.

Project Abstract:

Declining natural harvests are driving the development of the marine aquaculture industry. Growth of this industry will come through vertical integration of facilities. At the bottom of the pyramid is live feed production. Enhanced production of zooplankton is critical to reducing feed costs for the marine aquaculture industry. The current bottleneck for many marine finfish culture facilities is providing live feed to larval stages. Lack of

large-scale, advanced technologies forces most facilities to place larvae in outdoor ponds containing natural zooplankton populations, usually resulting in tremendous mortality rates. Computerized, integrated algal/zooplankton culture systems, which improve culture stability and reliability and reduce labor costs, are needed to fully control the "base of the food chain". The overall goal of this research will be to investigate and refine an automated, integrated algal/zooplankton system. The Phase I objectives will be to develop design and operational protocols for the zooplankton component of the integrated system. Preliminary production capacities will be examined and an initial economic analysis will be performed to examine full-scale economic feasibility.

Collaborators:

- Louisiana State University - Dr. Kelly Rusch

Results:

- Confirmation that rotifers can be cultured using an "approximated plug flow" reactor comprised of a series of completely mixed reactors (CFSTR's).

End Products:

None to date

DOC/NOAA SBIR Grant entitled "Development of an Extremely Low Water Loss Floating Bead Filter for Biofiltration and Solids Capture on Recirculating Marine Aquaculture Systems". Phase I & Phase II . Period of Performance October 1, 1998 to December 1, 2001.

Project Abstract:

The shortfall in worldwide marine fisheries landings are aiding the development of aquaculture technologies on many fronts. However, the interest and use of recirculating systems for production of marine organisms have lagged behind their freshwater counterparts. A floating bead filter which is corrosion resistant (no metal parts), operating with minimal water loss, and can be automated to backwash with minimal electronics is needed to enhance the economic feasibility of marine recirculating systems. Phase I proved that the drop filter concept works. Phase II will address the scientific issues and conduct system evaluations needed for the production of a commercial-scale MRBF that is a compatible with projected integrated marine system designs based on airlift or pumped recirculation.

Collaborators:

- Louisiana State University - Dr. Ron Malone
- TilTech Aquafarm - Tilapia
- Archer Daniels Midland - Tilapia
- Kent SeaTech - HSB
- Auburn University - Dr. Ron Phelps - Red Snapper
- Texas A&M University - Dr. Tzachi Samocha - Shrimp
- Carters Fish Hatchery- Jeff Carter - Tropical Fish

Anticipated Results:

- Determination of performance parameters including the volumetric nitrification rate for MRBF.

Anticipated End Products:

- Marine Recirculating Bead Filters ("Drop Filter")
- 3 ft^3 Rotationally Molded MRBF
- 25 ft^3 Fiberglass MRBF

USDA SBIR Grant entitled "Development of Production and Nutritional Characteristics of HISTAR: Beta II Evaluation ". Phase I & Phase II Funding. Period of Performance May 15, 1999 to August 31, 2003.

Project Abstract:

Commercial microalgal production still relies heavily on batch culture methods. Recent investigations have been successful in the development of enclosed photobioreactors, though mostly still at the research level. Due to several factors, production costs of continuous culture methods remain high and thwart the adoption within the commercial aquaculture sector. This project will investigate the use of greenhouses and control algorithms to enhance production and reduce energy input in a Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR). Phase I results indicate that HISTAR is capable of sustained production and contaminant mitigation under varying temperature and irradiance regimes seen in greenhouses. The objective of this Phase II project is to refine the HISTAR technology at AST, develop design, production and operational criteria, determine nutritional consistency, determine profitability and execute Beta II testing at three commercial sites. A suite of 1.5-month studies will be performed over the two-year project period to collect the productivity data required for modeling and economic analysis efforts. The data will also be compared to performance data collected historically on HISTAR operated under artificially illuminated conditions. Systems will be installed at three commercial sites in the second year and evaluated using the same protocols developed for the AST system. Successful results will position the HISTAR technology for commercial adoption within one year following the end of this project.

Collaborators:

- Louisiana State University - Dr. Kelly Rusch
- Louisiana State University - Dr. john Supan -Oysters
- Auburn University - Dr. Ron Phelps - Red Snapper
- Sea Perfect - Knox Grant - Clams

Anticipated Results:

- Reduce Cost of Micro algal Production
- Document Nutritional Characteristics of Several Algal Species produced using HISTAR
- Confirmation of HISTAR Controller Operation

Anticipated End Products:

- Manufacture and Sale of Turnkey HISTAR Systems and/or major system components such as the proprietary system controller.

USDA SBIR Grant entitled "Development of a Dissolved Oxygen and Carbon Dioxide Control System for Large Scale Intensive Marine Commercial Aquaculture Recirculating Systems". Phase I Funding only to date. Period of Performance May 15, 2001 to November 30, 2001.

Project Abstract:

Commercial-scale marine aquaculture has the potential to close the gap between an increasing demand for high quality seafood products and a marine fisheries near maximum sustainable yields. Intensive recirculation systems are regarded as one critical component of a commercially viable marine aquaculture development program. However, the application of recirculating technology for the production of marine organisms has lagged behind freshwater applications, due in part to the lack of design criteria for system components operating in a marine environment. To fully realize the economic potential of intensively stocked marine recirculating systems, a gas exchange system capable of transferring oxygen into the water, while removing carbon dioxide is critical. This research project will modify a multi-stage low head oxygenator (MS-LHO) and its supporting gas transfer simulation program to operate efficiently in a marine system over a range of salinities with minimum energy input. Dissolved carbon dioxide removal capability will be added by coupling a low profile packed-bed stripping column to the MS-LHO as a pretreatment step. Overall system performance will be optimized using simulation programs for gas transfer and field experience with prototype systems. Finally, the economic feasibility of producing full-scale marine grade MS-LHO units coupled with stripping columns will be determined.

Collaborators:

- Freshwater Institute - Dr. Brian Vinci and Dr. Jim Ebeling
- Louisiana State University - Dr. Steven Hall

Anticipated Results:

- Determination of key design data over a range of salinities to calibrate supporting model.
- Design of a scalable MS-LHO/Carbon Dioxide Stripping Unit.
- Evaluate and Optimize performance of MS-LHO Stripper and comparison to results predicted by model.

Anticipated End Products:

- Manufacture and Sale of MS-LHO Stripping Units for marine RAS's.

Journal of Plankton Research

Journal of Plankton Research Vol. 26 No. 6 © Oxford University Press 2004; all rights reserved

Spatial and temporal patterns of sexual reproduction in a hybrid Daphnia *species complex*

Piet Spaak[1,*], Angelika Denk[1,4], Maarten Boersma[1,2] and Lawrence J. Weider[1,3]

Department of Limnology, EAWAG, Überlandstrasse 133, 8600 Dübendorf, Switzerland, [1] Max-Planck-Institut für Limnologie, Postfach 165, D-24302 Plön, Germany, [2] Alfred-Wegener-Institut für Polar und Meeresforschung, Biologische, Anstalt Helgoland, Postfach 180, 27483 Helgoland, Germany and [3] Department of Zoology and The University of Oklahoma Biological Station, HC-71, Box 205, Kingston, OK 73439, USA [4] Present Address: Research Centre for Ornithology of the Max-Planck-Society, Reproductive Biology and Behaviour, Postfach 1564, D-82305 Starnberg/Seewiesen, Germany

*Corresponding Author: spaak@eawag.ch

Evidence for extensive interspecific hybridization among species of the genus *Daphnia* has been accumulating on a global scale. Although there is evidence for limited gene flow between taxa via hybridization, many species still maintain discrete morphological and molecular characteristics. We studied temporal and spatial patterns of sexual reproduction within the *Daphnia galeata–hyalina–cucullata* hybrid species complex in a lake (Plußsee), located in northern Germany. Allozyme electrophoresis allowed us to track seasonal changes in taxon composition as well as the quantification of back-crosses. Sexually-reproducing animals (ephippial females and males) were mainly found in autumn. The simultaneous presence of sexual morphs of *D. galeata* and *D. galeata* x *hyalina* with the dominant *D. hyalina* taxa makes recent hybridization, as well as back-crossing, plausible. Males and ephippial females of *D. hyalina* were not back-crossed as were the parthenogenetic females. The low number of sexual clones of the hybrid *D. galeata* x *hyalina* might reflect its reduced fertility, although these few clones were detected in high densities. Only hybrid-clones that had a back-cross genotype (towards *D. hyalina*) exhibited ephippial females and males. This indicates that male and ephippial female production within the *Daphnia* taxa is not random, which might increase the chance for the parental *Daphnia* species to remain distinct.

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R.R. Bidigare, F.D. King, and D.C. Biggs
Glutamate dehydrogenase (GDH) and respiratory electron-transport-system (ETS) activities in Gulf of Mexico zooplankton
J. Plankton Res., 1982; 4: 895 - 911.
......two natural **zooplankton** assemblages...than 80% of the **total** GDH and ETS...suggesting a strong **zooplankton**-phytoplankton...**zooplankton protein** biomass was 3-fo...of gelatinous **zooplankton** and fish from...ETS activity. **Total** enzyme activity...per unit of **zooplankton protein** biomass by the **total** amount of extractable......

Abstract
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ORIGINAL ARTICLES:
Kathryn L. Cottingham, Susan E. Knight, Stephen R. Carpenter, Jonathan J. Cole, Michael L. Pace, and Amy E. Wagner
Response of phytoplankton and bacteria to nutrients and zooplankton: a mesocosm experiment
J. Plankton Res., August 1997; 19: 995 - 1010.
......chlorophyll a, **total zooplankton** biomass, and...2 ug H day-1 **Total zooplankton1** Daphnia Other...are ? 1 SD. (A) **Total** phytoplankton...et at unsieved **zooplankton** treatments (P...incorporation into **protein**, was significantly......

Abstract
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ORIGINAL ARTICLES:
Juan Diaz Zaballa and Raymond Gaudy
Seasonal variations in the zooplankton and in the population structure of *Acartia tonsa* in a very eutrophic area: La Habana Bay (Cuba)
J. Plankton Res., July 1996; 18: 1123 - 1135.
......a periodic decrease in the **total zooplankton** density due to the flushing...variations in abundance of **total zooplankton**. A.ionsa. Oikopleura sp. and...reflect the dilution of the **total zooplankton** population provoked by the......

Abstract
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ORIGINAL ARTICLES:
S Hwang and R Health
Zooplankton bacterivory at coastal and offshore sites of Lake Erie
J. Plankton Res., Apr 1999; 21: 699 - 719.
......accounted for 56 and 71% of **total zooplankton** bacterivory at the coastal...cladocerans accounted for 23% of the **total zooplankton** abundance (ind. l ?1) each...respectively. Bacterial C flux to **total zooplankton** (MICZ + MACZ) at the coastal......

Abstract
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ORIGINAL ARTICLES:
P. Carrillo, I. Reche, and L. Cruz-Pizarro
Quantification of the phosphorus released by zooplankton in an oligotrophic lake (La Caldera, Spain): regulating factors and adjustment to theoretical models
J. Plankton Res., 1996; 18: 1567 - 1586.
......percentage of the **zooplankton**, an evaluation...phosphate (SRP), **total** dissolved phosphate...percentage of the **zooplankton**, an evaluation...phosphate (SRP), **total** dis- solved...biomass of the **zooplankton**, the final concentration of **total** and dis- solved......

Abstract
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Yong C. Park, Edward J. Carpenter, and Paul G. Falkowski
Ammonium excretion and glutamate dehydrogenase activity of zooplankton in Great South Bay, New York

Abstract

J. Plankton Res., 1986; 8: 489 - 503.

......be utilized for **protein** synthesis of **zooplankton**...pool inside of **zooplankton**. However, this...finmarchicus Mixed **zooplankton** Mixed **zooplankton** (GSB) Mixed **zooplankton** (LIS) 10 25 3-27...averaged 20% of the **total** GDH activity found......

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ORIGINAL ARTICLES:

S Beaulieu, M Mullin, V Tang, S Pyne, A King, and B Twining

Using an optical plankton counter to determine the size distributions of preserved zooplankton samples

J. Plankton Res., Oct 1999; 21: 1939 - 1956.

......changes in transparency of **zooplankton** after preservation...between live and preserved **zooplankton** samples. Wieland et...crustaceans in formalin tans **protein** (Steedman, 1976). Thus...Although the gelatinous **zooplankton** (A.aurita medusae...why the estimates for **total** biovolume based on OPC......

Abstract

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ORIGINAL ARTICLES:

Thomas R. Anderson

Modelling the influence of food C:N ratio, and respiration on growth and nitrogen excretion in marine zooplankton and bacteria

J. Plankton Res., 1992; 14: 1645 - 1671.

......the growth of marine **zooplankton** is commonly carbon...gains (DeMott, 1989), **zooplankton** should, according to...evidence suggests that **zooplankton** may maximize nitrogen...that copepods maximize **total protein** ingestion, and when......

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R.C. Hart

Aspects of the feeding ecology of turbid water zooplankton. *In situ* studies of community filtration rates in silt-laden Lake le Roux, Orange River, South Africa

J. Plankton Res., 1986; 8: 401 - 426.

......microphagous **zooplankton**. These include...significantly to the **total** biomass of **zooplankton**...animals) is the **total** body burden of the **zooplankton** collected, d...than 33 % of die **total** biomass, although...dominance of die **zooplankton** community is......

Abstract

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ORIGINAL ARTICLES:

D Dobberfuhl and J Elser

Use of dried algae as a food source for zooplankton growth and nutrient release experiments

J. Plankton Res., May 1999; 21: 957 - 970.

......Berberovic, 1991). **Protein** was quantified...and Gill, 1964). **Total** lipids were extracted...rapid method of **total** lipid extraction...excretion rates of **zooplankton** from the eutrophic...analysis of the **zooplankton**-phytoplankton interaction...for estimating **proteins**. Anal. Biochem...mediom for algae and **zooplankton**. Hydrobiologia......

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ORIGINAL ARTICLES

Use of dried algae as a food source for zooplankton growth and nutrient release experiments

D Dobberfuhl and J Elser
Department of Biology, Arizona State University, Tempe, AZ 85287-1501, USA

Zooplankton growth and nutrient recycling are key processes in the operation of pelagic food webs. Most studies investigating these processes rely on complex methods and often require extensive laboratory facilities. Here we introduce a technique for preserving algae by rapid drying for later use in laboratory- or field-based growth and nutrient recycling experiments. Chemostat-grown *Scenedesmus acutus* was rapidly dried for later experiments evaluating its nutritional composition, suitability for animal growth and potential for use in nutrient release experiments. Reconstituted dried algae had slightly lower nitrogen (N), Phosphorus (P) and **protein** content (% dry weight) than fresh algae, but lipid content did not differ and elemental ratios were in the range considered to indicate favorable food quality. These elemental and biochemical differences did not appear functionally important, as *Daphnia magna* grew identically on fresh and dried food. Freeze-dried *S.acutus* did not work as an alternative to oven drying as it resulted in 100% mortality. NH4 and PO4 concentrations did not change over 24 h when dried algae were resuspended in normal media or boiled lake water. However, concentrations of PO4 decreased over 24 h, suggesting chemical adsorption of PO4 to the dried algae and reinforcing the need for animal-free controls in nutrient release experiments using this approach. N and P release rates for *D.magna* and natural **zooplankton** communities were estimated using dried algae, and values were comparable to published ones. Thus, dried algae may be a useful, simple technique for studying food quality and nutrient release in environments where maintaining active algal cultures may not be practical and a constant supply of consistent quality food is needed.

Seasonal variations in the zooplankton and in the population structure of Acartia tonsa *in a very eutrophic area: La Habana Bay (Cuba)*

Juan Diaz Zaballa and Raymond Gaudy[1]

Instituto de Investigaciones del Transporte Apartado 17029, zona postal 17, Ciudad de La Habana, Cuba [1]Centre d'Océanologie de Marseille Station marine d'Endoume, rue de la Batterie des lions, F-130007 Marseille, France

Received on August 15, 1995; accepted on January 29, 1996 Seasonal changes in the abundance of **total zooplankton** and of main components were studied at a fixed station in La Habana Bay. Special attention was given to the dominant species. *Acartia tonsa*, which constituted 89% of the **total** number of **zooplankton**ic organisms. The variations in some of its population parameters (eggs, nauplii, copepodites, adults, sex ratio, number of eggs per female) were analysed in relation to temperature, salinity and chlorophyll. The abundance of eggs was independent of the environmental factors, particularly of chlorophyll which was always present in excess. The number of eggs per female showed several peaks, but was also independent of environmental factors. Nauplii were poorly correlated with egg numbers, but depended on the abundance of chlorophyll in particles of size <55 µm. This suggested that the part of edible food suitable for them in size or quality was not always in excess in the available seston, provoking a temporary increase in naupliar mortality. The temporal evolution of the following successive stages was nearly synchronous: thus, the changes observed in their abundance were not caused by fluctuations in the reproductive potential of females. but appeared to be related to a periodic decrease in the **total zooplankton** density due to the flushing effect of inland water in the bay.

-

Fish Meal and Alternate Sources of Protein in Fish Feeds Update 1993

Gary L. Rumsey

Tunison Laboratory of Fish Nutrition, U.S. Fish and Wildlife Service, 3075 Gracie Road, Cortland, NY 13045; 607/753-9391, Fax: 607/753-0259

Abstract.—Close to 12% of the world's 6.5 million metric tons of fish meal is used for aquaculture feeds. If current trends continue, roughly 20% to 25% of total world fish meal production could be used for aquaculture by the year 2000. Fish stocks used in fish meal reduction, however, appear to be in worldwide decline. A growing fish farming industry and a stagnating, if not diminishing, supply of fish meal have sobering economic and technologic implications for fish culture. Unless suitable alternate protein sources are found or other animal feeds begin to rely less on fish meal, fish production costs can be expected to increase dramatically. A precedent was set by the poultry industry, the most economically successful and competitive of the animal agriculture industries. Twenty years ago, the poultry industry consumed up to 80% of fish meal supplies. Through deliberate and well-organized research into alternate protein sources, the industry now uses less than 40% of supplies, and the trend is toward complete independence from fish meal. A comparable research effort is needed for aquaculture. Considering the biotechnologies available, plant proteins, processed to remove enzyme inhibitors and other antinutritional factors and properly supplemented with essential amino acids and minerals where needed, could produce results at least equivalent to those obtained with fish meal.

top