Mariculture Committee **ICES CM 2002/F:01**

REPORT OF THE

WORKING GROUP ON MARINE FISH CULTURE

Olhao, Portugal
11–14 March 2002

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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TABLE OF CONTENTS

Section		Page
1	PARTICIPANTS	1
2	RAPPORTEUR	1
3	AGENDA	1
4	MEETING ARRANGEMENTS	1
5	TERMS OF REFERENCE	1
5.1	ToR (a): Current status of marine fish cultivation	1
5.2	ToR (b): Technological Developments	3
5.3	ToR (c): Alternative sources of Protein and Lipid	3
5.4	ToR (d): Use of ICES Standard Reference Diets and Emulsions	3
5.5	ToR (e): Progress on the Use of Microdiets for Feeding Larval Fish	4
5.6	ToR (f): Review Fish Health Research and Diseases of Cultured Marine Fish	4
5.7	ToR (g): Procedures and Methods for Monitoring Feeding Regimes	4
5.8	ToR (h): Fish Welfare in relation to Marine Fish Culture	4
5.9	ToR (i): Develop Standard Culture Conditions	5
5.10	ToR (j): Major Recent Advances	5
6	POSSIBLE TERMS OF REFERENCE FOR THE FUTURE	5
6.1	Alternative live feeds	5
6.2	Restocking	5
6.3	Land-based recirculation	6
6.4	Selective breeding	6
6.5	Public relations	6
6.6	Single-sex fish production	6
6.7	Biosecurity	6
6.8	Growing conditions	6
7	ELECTRONIC LARVICULTURE NEWSLETTER	6
8	VENUE, TIMING AND ARRANGEMENTS FOR 2003	7
9	ICES MARICULTURE COMMITTEE PROPOSED ACTION PLAN	7
10	INTERACTION WITH OTHER MARICULTURE COMMITTEE WORKING GROUPS	7
11	PROPOSALS FOR THEME SESSIONS	7
12	NOMINATION OF A NEW WORKING GROUP CHAIR	7
13	OTHER BUSINESS	7
14	ADJOURNMENT	8
15	CONCLUSIONS (RECOMMENDATIONS FOR FUTURE ACTIVITY IN ANNEX 3)	8
	ANNEX 1: LIST OF PARTICIPANTS	12
	ANNEX 2: AGENDA	14
	ANNEX 3: RECOMMENDATIONS FOR FUTURE ACTIVITIES	16
	ANNEX 4A: PRODUCTION OF JUVENILE MARINE FISH	20
	ANNEX 4B: HARVESTED PRODUCTION OF FARMED MARINE FISH	21
	ANNEX 5: RECENT ADVANCES IN RECIRCULATION TECHNOLOGY FOR MARINE FISH CULTURE	22
	ANNEX 6: REFERENCES ON ALTERNATIVE PROTEIN SOURCE STUDIES IN MARINE FISH	27
	ANNEX 6: ALTERNATIVE PROTEIN SOURCES IN MARINE FISH NUTRITION	59
	ANNEX 7: COPEPOD CULTURE: A REVIEW	80
	ANNEX 8: THEME SESSION PROPOSALS	90
	ANNEX 9: FISH WELFARE IN RELATION TO MARINE FISH CULTURE WELFARE	91

1 PARTICIPANTS

The Working Group on Marine Fish Culture (WGMAFC) convened its third meeting under its new remit at Olhao, Portugal 11–14 March 2002. The Chair, John Castell (Canada), called the meeting to order at 09.20 hrs on 11 March. The following representatives of ICES Member Countries participated in the meeting (address and phone information in Annex 1).

Belgium:	Mr Mathieu Wille
Canada:	Dr Ed Trippel and Dr John Castell (Chair)
Ireland:	Dr Gerry Mouzakitis
Norway:	Dr Ingrid Lein, Dr Terje van der Meeren and Dr Victor Øiestad
Portugal:	Dr Pedro Pousao-Ferreira
Sweden:	Dr Hans Ackefors
United Kingdom:	Dr Ian Bricknell
United States:	Dr David Bengtson (Rapporteur)

Dr Pavlos Makridis, Dr Maria Teresa Dinis, Dr Luis Conceição, Dr Elsa Dores, and Dr Miguel Neves dos Santos (Portugal) also participated in the meeting.

Participants introduced themselves and described their research interests. The greeting from IPIMAR, host institution for the meeting, was given by Pedro Pousao-Ferreira on behalf of the Director. He explained that IPIMAR has activities in both fisheries and aquaculture, with headquarters in Lisbon and regional labs in the north, middle and south of Portugal.

2 RAPPORTEUR

David Bengtson (USA) was appointed as rapporteur.

3 AGENDA

The proposed agenda and timetable (Annex 2) for the meeting was approved, with the added recommendation from Hans Ackefors (Sweden) that we also consider the topic of a Code of Practice.

4 MEETING ARRANGEMENTS

Dr Pousao-Ferreira described the arrangements for the meeting, lunches, and tour of aquaculture facilities.

5 TERMS OF REFERENCE

5.1 ToR (a): Current status of marine fish cultivation

Report on the current status of marine fish cultivation in Member Countries and on the factors that are likely to constrain further development of the industry; graph and evaluate historical production trends for major marine finfish species and predict future development.

Participants reviewed the tables of production for juveniles (Annex 4a) and on-grown for market (Annex 4b). It was suggested that some new species should be added to the tables because there is actually production ongoing now. Examples are sole (both *Solea solea* and *Solea senegalensis*), white seabream (*Diplodus sargus*); sharpsnout seabream (*Diplodus puntazzo*); red porgy (*Pagrus pagrus*); commun dentex (*Dentex dentex*); red drum (*Sciaenops ocellatus*); Grouper (*Epinephelus guaza – Epinephelus gigas*), (red seabream (*Pagrus major*) this, at least, in Greece). These species are the most common ones that people are using for aquaculture/sea reaching purpose. Numbers should be provided even if the production is only for research purposes, because then we will have a record of the production from research level to commercial over the years and this could be valuable in the future. It was noted that the Working Group on Environmental Impacts of Mariculture (WGEIM) collects production numbers (including salmon) and that the European Federation of Aquaculture Producers also does, so it would be nice if all our numbers agreed in the end. In that regard, it is sometimes difficult to get accurate numbers from producers and, for some countries, the official government numbers may not be available yet from the previous year when we meet in March (e.g., Scottish figures are not available until later in April). The general consensus was that WGMAFC participants want to see production figures of non-ICES countries for major species (e.g., sea bass and seabream in the Mediterranean), but the tables should clearly indicate that these are non-ICES countries. Some countries are not providing production data; Dr Ackefors suggested that the WGMAFC Chair write to the ICES Secretariat to ask him to inform the country delegates that these numbers are not being provided to the WGMAFC. The usefulness of these production figures is that they help us to identify trends, especially with regard to the second part of the ToR, identifying constraints to industry development and predicting future development.

Participants tried to identify ***constraints to aquaculture*** in their own countries, or in countries in which they are working for the moment, as follows:

Belgium (Mathieu Wille) – The biggest constraint to marine fish culture is the development already of the coast. There are user conflicts for the resources of the coast.

Canada (Ed Trippel and John Castell) – Three species (halibut, cod, and haddock) are under major consideration and the constraints on each are different. Halibut production in Nova Scotia has been supplied with juveniles from Iceland; it is only now that Canadian hatcheries are going on-line and there is very little broodstock halibut outside of one company. Cod production is still in the early stages (in Nova Scotia and Newfoundland), because there were problems in the 1990s with early efforts (insufficient funding, one hatchery burned down). Much interest now exists for cod culture, but there are still questions, e.g., where to best site the cages for the optimal temperature. Haddock development has benefited from good cooperation between government and industry (government provides the juveniles and expertise, industry does the grow-out), but the industry will soon have to decide whether they will build their own hatcheries or not. Some scientific issues with haddock include a) variable fry production, b) slower growth of haddock than cod in cages, c) early sexual maturity in haddock means energy may be going to gonads rather than to marketable fillets, and d) a short growing season (May–October) due to the cold temperatures. In general, all these species need work on broodstocks and selective breeding. Good government support for aquaculture industry development exists in Canada, especially through the Aquaculture Research and Development Program. This $70-million government-industry research partnership starts with 90 % government: 10 % industry cost sharing and will move to 50 % government: 50 % industry. There is also interest in small flatfish like winter flounder, but this is still in the research stage. Canada has had a concerted program for bankers and insurers to educate them about aquaculture and this has helped to remove constraints to capitalization of the industry.

Germany (Ed Trippel) – It is difficult to obtain sites for cages in German waters; any marine fish culture will probably be done in recirculation systems.

Ireland (Gerry Mouzakitis) – Production of marine finfish in Ireland is small, with only one turbot farm. There is a big push for new species, with cod identified as the major one. Since salmon and trout are predominant, it will take a major trend for fish culture to develop in some other direction.

Norway (Terje van der Meeren and Victor Øiestad) – Money is available to cover costs of aquaculture research and development, but SND, a regional governmental development agency, had a big budget cut last year, so that may hurt development. Also, Norway cannot get EU funds to build commercial facilities like the EU countries can; fortunately Norway has 6 large companies that are investing heavily in aquaculture industry development. Scientific issues, by species, are as follows. Cod is the subject of much interest, but there is still too high mortality in the early juvenile stage (15–40 mm) just before or during weaning. In order to get funding from the government, you have to demonstrate that you have a source of fry, so fry costs are very high now. Several big hatcheries are being built, but a good protocol for stable production is still needed. Halibut juvenile production has changed from extensive to intensive, with 90 % of the juveniles last year coming from intensive production. Constraints are a) insufficient funds for building hatcheries for halibut, and therefore b) not enough farms with large broodstock producing throughout the year, c) not enough eggs in total, and d) there are still mortalities of yolk-sac stages in silos. In addition, nutritional deficiencies in live prey still cause some problems with fry quality (pigmentation, eye migration) and broodstock nutrition is under investigation. Turbot production is going well and juveniles can be produced with cheaper operational costs in Norway than in Spain. Wolffish also generate much interest, but mortality to the 1-g size is still too high.

Portugal (Pedro Pousao-Ferreira) – Major constraints are a) funding (insufficient private capital and delays in receiving EU funding), and b) the amount of bureaucracy that a start-up aquaculturist has to endure to get a license. In addition, the species are limited to sea bass, seabream and turbot; they would like to see more species developed. There have also been problems with juvenile quality, but now more captive broodstock are available.

Spain (Victor Øiestad) – The Spanish government produced a white paper in 1999 listing about 20 obstacles to aquaculture development. The biggest one is the bureaucracy; it is very difficult to get new licenses, because the autonomous regions have authority.

Sweden (Hans Ackefors) – It is very difficult to get permission for cages in the water plus the temperature and salinity conditions along the west coast are not right for marine finfish culture. One farm will try to develop cod in a land-based recirculation system. Some Arctic charr are being grown in brackish water in cages at about 4 psu and this may have some promise.

United Kingdom (Ian Bricknell) – There is a limitation on new farm sites, due to potential environmental impacts. Funding is a problem, but the government shares costs 50:50 with industry. The halibut industry suffers from variability in both the quantity and quality of fry production. Cod mania is striking (with 150,000 MT/yr demand and a catch quota of only 38,000 MT last year, great market potential exists), but there is huge variability in mortality among hatcheries. Any grow-out will probably have to be done in existing cages for salmon, as it is difficult to get licenses for new farms. One turbot farm exists in Scotland. There is interest (and research) in the diversification to new species, such as haddock, wolffish, sole and the local sea bass.

USA (Dave Bengtson) – The biggest constraint is capital investment. The government provides funding for research and demonstration projects, but then private capital must be raised to develop the industry. Pond culture of red drum along the Texas coast is the biggest production of marine finfish. Culture of summer flounder in recirculation systems along the Atlantic coast has been slow to develop because of funding. Research is continuing on several new species (southern flounder in ponds, black sea bass and mutton snapper in tanks, cod, haddock and halibut potentially for net pens).

With regard to *predictions about the future*, some participants ventured their ideas. The general thrust was that future production will move more to land-based, hyper-intensive systems for both ecological and efficiency reasons. It was noted that productivity in the salmon industry has increased about five-fold in the last decade or so to 700 MT produced per person employed in the production. By contrast, turbot productivity is about 20–50 MT/person. The more successful species will be those that can be produced at high density with automated systems in constant environmental conditions. Also, biosecurity will be a big issue, both from the point of view of preventing disease from getting to the cultured fish and also preventing selectively-bred (or even genetically modified?) fish from interacting with wild populations. Clearly, cod is the major species of interest at the moment and it was felt that there is potential here if automation can be achieved.

5.2 ToR (b): Technological Developments

Review and report on technological developments in relation to fish production and their application to various species.

Victor Øiestad and David Bengtson presented a report on recent developments in recirculation system technology (see Annex 5). Terje van der Meeren mentioned a rumour of a new compound that will convert ammonia to nitrate without using biological filters and this would be a major advance if it can be shown to be successful. Fish culture in recirculation systems has implications for other areas, such as nutrition and genetics.

5.3 ToR (c): Alternative sources of Protein and Lipid

Report on alternative sources of protein and lipid, including electronically available bibliography.

John Castell provided a bibliography (Annex 6a) and summary tables (Annex 6b) of his investigation of 129 literature references on the use of alternative protein and lipid sources in diets for marine finfish. Other authors have previously reviewed the use of these in diets for freshwater fish. It appears that size of fish is important; larger fish within a species seem to be better able to use alternative proteins than smaller fish. As one can imagine, most of these studies of marine fish used different methods, different basal diets, etc., so it may be difficult to reach any firm conclusions from a review of these studies. Nevertheless, Dr Castell will try to publish a review article based on this material in a peer-reviewed journal. It was pointed out that it would be useful to look at cost savings to the feed manufacturer and the aquaculturist from the use of these alternative materials. It was further pointed out that we should have a list of regulations for the EU and various countries about these materials, since, e.g., one is not allowed to use terrestrial animal proteins in fish feeds in the EU. We were reminded that consumer demands should also be considered, since their preferences may be even stricter than governmental regulations.

5.4 ToR (d): Use of ICES Standard Reference Diets and Emulsions

Review the use of ICES standard reference diets and emulsions in research programmes and recommend any modifications to existing formulations and procedures to publicize the availability of these diets.

Mathieu Wille (Belgium) is adding new information to the existing table on the use of ICES standard diets and emulsions. He has contacted the publishers who will be publishing papers from the Larvi 2001 meeting to see about including an announcement of the ICES reference materials with those issues. Aquaculture International has agreed as long as the announcement is included in the preface to the symposium issue; negotiations with Aquaculture are still ongoing. M. Wille will try to write a review article summarizing the use of and findings about the ICES standards in fish culture. The WGMAFC participants then discussed the philosophical issue of whether ICES standard diets and emulsions should ever change to reflect updated knowledge of nutritional requirements. For example, the current DHA:EPA ratio in the standard emulsion is probably too low and recent findings suggest that arachidonic acid should be included as well. The current formulation has existed for more than ten years. If the formulation is changed, a comparison should be conducted of the performance of the old formula vs. the new formula in order to have a benchmark. The recommendation is that we review the reference diets and emulsions and modify them if appropriate.

5.5 ToR (e): Progress on the Use of Microdiets for Feeding Larval Fish

Review progress on the use of microdiets for feeding larval fish and assess whether a reference diet/procedure can be recommended.

One session at the Larvi 2001 meeting reviewed the use and performance of microdiets. Dr Castell talked with the major investigators in this field at Larvi 2001 to see if they had any diets that could be considered for use as reference diets at this time. He also talked with other investigators who were not present at Larvi 2001. The consensus is that no diet is suitable yet, but several investigators will be willing to provide microdiets for comparative testing in the future. Thus, the WGMAFC is not able to recommend any diets to the ICES Mariculture Committee for testing this year. In the coming year, a subgroup of the WGMAFC will work on a proposal that will not only recommend microdiets for comparative testing, but also will recommend particular protocols to use (e.g., minimum number of replicates) during the testing.

5.6 ToR (f): Review Fish Health Research and Diseases of Cultured Marine Fish

Work with the WGPDMO to review fish health research and report on existing and emerging diseases of cultured marine fish, including treatments used.

Ian Bricknell, who is our liaison to the WGPDMO, has discussed this with his colleague, David Bruno, and Dr Bruno is bringing it up at the WGPDMO meeting in Copenhagen this week.

5.7 ToR (g): Procedures and Methods for Monitoring Feeding Regimes

Compile a complete list of procedures and methods for monitoring of feeding regimes.

No reports were received from anyone on this topic. We will have to re-address this in the coming year.

5.8 ToR (h): Fish Welfare in relation to Marine Fish Culture

Review current/emerging policies on fish welfare in relation to marine fish culture (country by country, FAO, EU, etc.) and prepare a report.

Dr Castell distributed to participants a compilation of reports that had been received from the various countries (see Annex 9). The comment was made that aquaculturists should be more proactive in this regard or others will dictate the writing of regulations that affect aquaculture. WGMAFC should work with WAS and EAS on this, as well as the Federation of European Aquaculture Producers, which has a statement of conduct. WGMAFC noted that the report from Denmark mentions an EU Standing Committee and we should work with that group as appropriate. We should determine what is in the literature about this. It seems likely that fish prefer to be close together more than mammalian species, that they become less stressed over generations with domestication and breeding, and that rules for fish stocking densities in net pens would not be applicable for fish in closed systems with continuous water exchange. WGMAFC identified several colleagues from outside the WGMAFC who might help us with the task of gathering information and suggested that someone prominent from the WGMAFC should serve as a representative or observer at meetings of the EU Standing Committee. In general, it was felt that this issue is too big for just the WGMAFC and that we should inform the Mariculture Committee that we would like their help as well.

5.9 ToR (i): Develop Standard Culture Conditions

Work with the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) in developing standard culture conditions under which strains, stocks or species might be tested to evaluate their performance.

WGMAFC discussed whether to recommend standard environmental conditions for each species and decided that recommendations should be about experimental protocols rather than environmental conditions. In this regard, WGMAFC discussed the issue of family effects vs. tank effects. Typically, selective breeding experiments are done by making many crosses and rearing families in individual tanks. This requires large facilities that very few laboratories have. Geneticists are concerned about the possibility that different environmental conditions will exist in the different tanks, thereby complicating the understanding of genetic vs. environmental causes of variation. Some geneticists prefer to pool all the families in one tank to eliminate the environmental effect and use modern methods such as microsatellite DNA to identify the parents of the most successful offspring. WGMAFC needs help from WGAGFM to assist in sorting out these differences, and WGMAFC could provide WGAGFM with advice on the rearing methods that would be most appropriate for marine finfish. Other issues with which help is needed are: a) the phenomenon of fish spawning in cages and producing larvae which compete with wild larvae, and b) genetics of stock enhancement (e.g., the degree of broodstock diversity required for marine finfish). WGMAFC identified a pool of people with expertise in aquaculture genetics and the recommendation is to have those people appointed to both WGAGFM and WGMAFC.

5.10 ToR (j): Major Recent Advances

Prepare a review paper on one or two interrelated major recent advances that have significantly improved marine finfish production capabilities or survival during a specific life history stage in a variety of species (e.g., technology, nutritional, physiological or disease prevention/detection).

Dr Castell will prepare a review paper on alternative proteins for use in feeds for marine fish. In discussion, it became clear that some of the WGMAFC members are writing articles for two forthcoming books, one on the use of live feeds in marine aquaculture and the other on farming of marine cold-water fish species. The first is entitled “Use of Live Feeds in Marine Aquaculture” (edited by WGMAFC member J. Støttrup, and L. McEvoy), with chapters by the following WGMAFC members: D. Bengtson (Introduction), J. Støttrup (copepods). The second one is entitled “Cultivation of Cold-water Marine Fish” (edited by E. Moksness, E. Kjørsvik and Y. Olsen), with Chapter 7 “First feeding technology” by Yngvar Olsen, Terje van der Meeren, and Kjell, Inge Reitan (the WGMAFC member is T. van der Meeren). In addition, Mathieu Wille is going to write the review article on ICES standard diets and emulsions. Thus, WGMAFC has made good progress in producing review articles on various topics. Perhaps WGMAFC can collaborate with WGAGFM and WGPDMO to produce review articles with them as well. Also, as conveners of the ICES Symposium on Gadoid Mariculture in 2004, WGMAFC will be producing a symposium volume for the ICES journal that will effectively be a review of that topic. WGMAFC recommended that a review be written on microdiets for marine fish that would appear in 2–3 years.

6 POSSIBLE TERMS OF REFERENCE FOR THE FUTURE

6.1 Alternative live feeds

Terje van der Meeren (Norway) gave a presentation of data from his studies of the variability in biochemical composition of natural zooplankton in a fertilized lagoon adjacent to the Institute of Marine Research, Austevoll Aquaculture Research Station. This information could provide the scientific basis for WGMAFC to make decisions about levels of fatty acids like EPA, DHA and arachidonic acid, as well as proportions of fatty acids appearing as different lipid classes, as we look at possible modifications to the ICES standard emulsions. Dr Castell distributed a review of copepod culture prepared by Chris Cutts (UK) (see Annex 7), along with information about a website (seabait.com) for a company that cultures polychaetes. It was pointed out that Josianne Støttrup is also preparing a review of copepod culture for her new book on live feeds. Dr Castell noted that Michael Payne in Australia has recently completed a Ph.D. on culture of both calanoid and harpacticoid copepods and is publishing very useful papers based on his dissertation research. Questions remain about the processing and storage of copepods for use as feed supplements during critical periods of development. WGMAFC concluded that it should continue to report on developments in alternative live feeds like copepods, polychaetes, trochophore larvae, etc.

6.2 Restocking

Efforts are ongoing in both Europe and North America and several specific projects were mentioned. There were reviews of this topic in a special issue of the Bulletin of Marine Science in 1998 and a book edited by former WGMAFC Chairman, Bari Howell and co-authors in 1999. Miguel Neves dos Santos gave a presentation about the artificial reef project for fishery enhancement along the Algarve coast. It will be important as we consider this topic to distinguish between stock enhancement for recreational fishers vs. commercial fishers. The first recommendation is that WGMAFC should look throughout ICES to see if other groups with responsibility in fisheries management are already working on this as a management tool. If so, WGMAFC should work with them.

6.3 Land-based recirculation

As technology develops, WGMAFC expects that marine fish culture will move increasingly to hyper-intensive land-based production for reasons of biosecurity and minimization of environmental impacts. Costs to a commercial firm for recirculation system culture are higher than for net pen culture, but it would be useful to make the economic comparison taking into account all the costs and benefits, including environmental. WGMAFC wishes to address recirculation systems on a long-term basis with the idea that marine fish culture will develop there over the next 10–15 years. WGMAFC proposed to first lay out a road map of how we expect the development process to happen and then tackle yearly projects based on that map.

6.4 Selective breeding

WGMAFC will continue the previous ToR with regard to WGAGFM.

6.5 Public relations

Many non-scientific groups are scaring the public about the quality and safety of aquacultured seafood, often with emotional arguments rather than valid scientific information. WGMAFC would like ICES and governments to provide the public with valid, scientifically based information so that consumers can make informed decisions. It was pointed out that the EU has very strict legislation on this issue of food safety. Hans Ackefors published a paper ten years ago showing less mercury in cultured fish than in wild fish and there are probably other similar comparisons out there. It was pointed out that we should not attack the fishing industry or portray them badly when we try to show the safety of aquaculture fish. A distinction was made between public relations, which is something that the aquaculture industry should do to promote their products, and public education, in which it is the role of government to provide unbiased information. We would recommend that the WGMAFC, working through ICES, should encourage governments to gather information on the safety of aquacultured food in relation to their own particular legislation and to present that information to consumers in an unbiased manner.

6.6 Single-sex fish production

There are several species in which one sex grows faster than the other and it would be useful to produce fish that are all of the faster growing sex. There are genetic, environmental and hormonal ways to accomplish this. It would be useful to identify experts in this field and have them assist in the preparation of a report on the status of this research and technology

6.7 Biosecurity

WGMAFC recommended that a review be made of methods to protect broodstock fish from disease, with Ian Bricknell to handle this task.

6.8 Growing conditions

Pedro Pousao-Ferreira pointed out that for many species, especially new ones, there is often insufficient information about the allowable and optimal environmental conditions for survival and growth of larvae. As a result, some hatcheries are poorly sited and some fail. It would be useful for ICES to provide information about rearing conditions, particularly water quality, to the industry. As a first step, WGMAFC should produce a report on existing knowledge of effects of ammonia, ozone and bromides on fish in marine hatcheries, as well as a report on hatchery microbiology and probiotics.

7 ELECTRONIC LARVICULTURE NEWSLETTER

Dr Castell explained that the Larviculture Newsletter is produced at the University of Ghent, Belgium, and it contains much useful information on marine fish larvae. WGMAFC discussed how it could be useful to WGMAFC as a means of requesting information and disseminating information. For example, the upcoming aquaculture theme sessions at the ICES ASC were announced in the Newsletter, among other places. WGMAFC could also use it to ask for input on effects of ammonia, ozone and bromides, as an example of requesting information. WGMAFC recommended that one free issue of the Newsletter be provided to those members of WGMAFC who do not now receive it and agreed that WGMAFC would use the Newsletter to collect and disseminate information.

8 VENUE, TIMING AND ARRANGEMENTS FOR 2003

It appears that WGMAFC will not be able to meet in Weymouth, UK, in early 2003, as originally hoped. WGMAFC will have to quickly make alternate plans and Vigo, Kiel, and Aberdeen were suggested as possible venues, depending on the willingness of WG members there to host the meeting.

9 ICES MARICULTURE COMMITTEE PROPOSED ACTION PLAN

WGMAFC felt that the Action Plan, as currently written, reflects a negative view of mariculture as well as the strong leaning of ICES toward fisheries and the marine environment. In general, WGMAFC would like to impress upon ICES the growing importance of mariculture and hope that they will increasingly embrace it. WGMAFC specifically would like to see the following changes to the goals of the Mariculture Committee:

Change the first goal to "Evaluate the interactions of mariculture with ecosystems";

Change the third goal to "Develop mariculture methods and protocols that are sustainable both environmentally and economically".

10 INTERACTION WITH OTHER MARICULTURE COMMITTEE WORKING GROUPS

This has already been addressed earlier in the meeting.

11 PROPOSALS FOR THEME SESSIONS

The Mariculture Committee will have four theme sessions under the "Mariculture" umbrella at the 2002 ASC in Copenhagen. The abstract deadline is May 7. We are scheduled to run an ICES Symposium on Gadoid Culture in Bergen in June 2004. Still, we need theme sessions for 2003 and beyond. (See Annex 8 for details on proposed Theme Sessions.) Dr Øiestad suggested that WGMAFC sponsor a session that will highlight for ICES the increasing importance of mariculture. Mariculture is growing and fisheries have reached their limits. If the 20th century was the century of fisheries, the 21st century may be the century of mariculture. WGMAFC should emphasize the transition of mariculture from backbencher to front-runner and point out to ICES that they are at a crossroads with regard to the prestige of fisheries vs. the prestige of aquaculture. Several possible conveners with major international stature were suggested and they will be contacted. We should also try to have a plenary speaker cover this topic in a morning plenary session at the 2003 meeting. Dr Ackefors suggested that we should have a continuing series of theme sessions (one or more per year) based on the "Latest progress in _______". The blank space could be filled in by a variety of subjects, such as Production Technology and Rearing Methods, Water Quality, Feeds and Nutrition, Marketing, Fish Health, and Genetic Selection. Another idea was to have a session on positive stories about mariculture and the environment, in which successes have been achieved in cleaning up the environment or mitigating mariculture impacts.

12 NOMINATION OF A NEW WORKING GROUP CHAIR

Since John Castell will be retiring from the Canadian Department of Fisheries and Oceans in December 2002, it is unlikely that he will be funded to attend future meetings of WGMAFC and thus a new Chair will be required. The participants at the meeting in Olhao endorsed the nomination of Dr Anders Mangor-Jensen. Dr Mangor-Jensen was contacted by phone to ask whether he would accept the nomination. He has sent an e-mail indicating in the affirmative. Thus, WGMAFC recommends to the Mariculture Committee that Dr Mangor-Jensen be considered for appointment as Chair of WGMAFC to become effective prior to the 2003 meeting of this working group.

13 OTHER BUSINESS

Though Dr Bernd Ueberschaer was not able to attend the meeting in Olhao, Portugal, he had requested that information on the LarvalBase project <www.larvalbase.org> be presented to members of the working group as a possible valuable source of information and references on larval marine fish culture.

14 ADJOURNMENT

The meeting was adjourned at 12:30 hrs on 14 March.

15 CONCLUSIONS (RECOMMENDATIONS FOR FUTURE ACTIVITY IN ANNEX 3)

The following conclusions and recommendations emerged from the discussion:

1. Fish feeds

a. **Recommendation:** It is recommended that WGMAFC compile and report on the existing regulations of individual ICES countries and the EU with regard to ingredients in fish feeds.

b. **Justification:** The increasing demands for fishmeal and fish oil by aquaculture and agricultural industries have resulted in increases in price and concerns by environmental groups. Projections indicate that the availability of fish oil will limit the aquaculture industry within 3–8 years [Waagbø, R. (2002). Fiskefor og formidler. *In* Glette, J., van der Meeren, T., Olsen, R.E., and Skilbreid, O. (eds). Havbruksrapport 2002. Fisken og Havet, særnr. 3–2002. 22–25.]. English summary of the Havbruksrapport 2002 is available at http://www.iMrno/Dokumentarkiv.php?HovedsideValgt = 11&SessionId = 2.
In addition, consumers have concerns about food safety in general and about contaminants in fishmeal and oil in particular. The high prices and the lack of opportunities to expand the capture fishery make it imperative that alternative protein and lipid sources be developed for use in feeds for aquaculture. As aquaculturists and feed manufacturers consider specific alternatives, they need to know the regulations that exist in ICES countries that may prohibit certain possible sources of feed ingredients from use.

2. ICES standard reference diets and emulsions

a. **Recommendation:** It is recommended that WGMAFC review and report on the use of ICES standard reference diets and emulsions in research programmes and recommend any modifications to existing formulations and procedures.

b. **Justification:** Enhanced standardisation of experimental procedures would facilitate comparison of growth, survival and other performance criteria of fish in nutrition experiments and thereby greatly enhance the value of work in this area. In this respect there is a need to define recommended protocols, in particular the inclusion of reference diets in the experimental design. The ICES Standard Reference Weaning Diet and enrichment emulsions are such tools that can help in this regard. As new research results identify nutritional requirements of various marine fish species, it will be possible to modify the formulation of reference diets and perhaps develop species-specific reference diets. Following recent research on the fatty acids DHA, EPA, and ARA and on phospholipids, the standard reference diets and emulsions should be reviewed and modified accordingly.

3. Fish Welfare

a. **Recommendation:** It is recommended that ICES take a proactive role in the considerations of welfare issues in aquaculture by applying for permission to appoint an ICES representative to serve on the Standing Committee for the European Convention for the Protection of Animals Kept for Farming Purposes.

b. **Justification:** There is increasing pressure to impose animal care restrictions, based on a variety of opinions but without scientific justification, that may constrain the development of sound aquaculture practices. While we are committed to proper care of cultured animals, this must be based upon species-specific knowledge of the biology and behavioural responses under culture conditions. This representative should have a world perspective on aquaculture and knowledge of fish biology and stress responses. WGMAFC will provide a list of suggested experts who would be willing and qualified to serve as the ICES representative.

4. Genetics

a. **Recommendation:** It is recommended that WGMAFC work with the Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM) to review genetic considerations in selective breeding and identify genetic applications and rearing technologies suited for selective breeding of marine fish.

b. **Justification:** Selective breeding programmes are required in order to improve and maximize the performance of cultivated marine fish. Though genetics is the topic of another MARC Working Group, WGMAFC proposes to work with WGAFGM to undertake this task. It is important to have scientific experts in molecular genetics, selective breeding and reproductive biology to communicate in order to establish appropriate strategies for the development of marine fish selective breeding programmes.

5. Microdiets review

a. **Recommendation:** It is recommended that WGMAFC compile information on the current state of the art of microdiets as a replacement for live food for larval fish with the intention to produce a review article in a peer-reviewed publication in 2–3 years.

b. **Justification:** The status of microdiets was reviewed at the Larvi 2001 meeting and some of those papers will be published, but this field is developing quickly and a new review in about three years would likely be worthwhile.

6. ICES Standard Microdiet

a. **Recommendation:** It is recommended that a subgroup of WGMAFC prepare a proposal to recommend microdiets for comparative testing and particular protocols to use during the testing.

c. **Justification:** Live foods, such as rotifers, microalgae and *Artemia*, are required to be fed to larval fish before switching to a dry weaning diet. This can limit larval production due to the high cost and the requirement for a continual supply of live food. There may also be some variation between batches of material such as *Artemia* eggs (both in quality and availability). Microdiets will have an important role in replacing live feeds but no reference diet is yet available. Several producers would be willing to provide microdiets for evaluation. A WGMAFC subgroup will investigate microdiets for evaluation and trial protocols to assess their performance, with a view to recommending an ICES reference microdiet.

7. Alternative live feeds

a. **Recommendation:** It is recommended that WGMAFC review and report on the use of live feed organisms other than rotifers and *Artemia* (alternative live feeds) that are used or considered for use in the culture of marine fish larvae.

b. **Justification:** For a number of cultured marine fish such as halibut and other flatfish, it is difficult to produce normally pigmented and properly metamorphosed fish using only enriched rotifers and/or *Artemia*. In the wild, copepods constitute a major food source for many marine fish larvae and recent advances in techniques for mass culture of copepods might be useful to marine fish culture in ICES countries. In addition, other live feeds like polychaetes and molluscan trochophore larvae might also be useful. Information on the biochemical composition of natural zooplankton could provide the scientific basis for WGMAFC to make decisions about the levels of fatty acids like DHA, EPA and arachidonic acid, and other essential nutrients for application in the enrichment of standard live feeds or in the development of diets for larval fish.

8. Restocking

a. **Recommendation**: It is recommended that WGMAFC prepare a summary of the instances where aquaculture is being used to produce fish for restocking or enhancement of wild populations in ICES Member Countries.

b. **Justification**: Though the application of aquaculture to restocking of marine fish in ICES member countries is thought to be limited, seabream are being restocked in artificial reef areas in Portugal and turbot are being restocked in the North Sea in Belgium. Programmes had been initiated as early as the late 1800s in several ICES countries. In reviewing practices, it will be important to distinguish between stocking for commercial capture fisheries versus for recreational fisheries. There should also be some guidelines on health, genetics and sources of stocked fish.

9. Land-based recirculation

a. **Recommendation:** It is recommended that WGMAFC prepare a list of technological advances that would allow land-based aquaculture, especially recirculation systems, to greatly expand in the next 10–15 years and develop a long-term set of tasks for WGMAFC to accomplish in order to aid that development.

b. **Justification:** Recirculation systems are expensive to build and operate. Nevertheless, they represent a future for marine fish culture in which a) the fish are secure (no escapes), b) the fish are less likely to fall victim to diseases or parasites normally encountered in culture in open waters, and c) environmental impacts of the culture operation are minimized. Given the potential positive impact of marine recirculation systems on the advancement of marine fish culture, much emphasis should be placed on refining these systems and especially on reducing the costs of production. WGMAFC envisions a 10–15 year process for the development of these systems and wants to prepare a road map for the Group's future tasks over a similar period.

10. Selective breeding – see item #4

11. Public education

a. **Recommendation:** It is recommended that WGMAFC, working through ICES, should encourage governments to gather information on the safety of aquaculture products in relation to their own particular legislation and to present that information to consumers in an unbiased manner based on scientific facts.

b. **Justification:** The general public still has some concerns about fish produced in aquaculture. There is a great need for governments and relevant authorities to inform people on the safety of fish and the advantages to this type of food production. This type of public education programme would benefit both the industry and the consumer. In accordance with EU and national legislation, farmed fish are fed on a diet based on their nutritional requirements produced using raw material with negligible amounts of contaminants. Furthermore, they are slaughtered just before they reach the market. The consistent supply of aquaculture products year-round is another advantage.

12. Single-sex fish production

a. **Recommendation:** It is recommended that WGMAFC prepare a report on the status of research and technology of single-sex fish production and its application to the cultivation of marine fish, based on input from experts in the field.

b. **Justification:** There are several species in which one sex grows faster than the other and it may be commercially advantageous to produce fish that are all of the faster-growing sex. A number of investigators are working in this field using a variety of techniques including the manipulation of genetics, environment and hormones. Variable success has been experienced using these different techniques and a review of the advantages and disadvantages of each method on a species-specific basis would be of assistance.

13. Biosecurity

a. **Recommendation:** It is recommended that WGMAFC compile a report on the existing methods to ensure that measures can be taken to protect broodstock and larval animals from exposure to microbial pathogens.

b. **Justification:** Many terrestrial agriculture systems have dedicated breeding units designed to avoid exposure of the broodstock and their progeny to high-risk disease organisms (e.g., pigs and swine fever, chickens and Newcastle disease). Within the EU an outbreak of a List I or II disease in a population of fish would lead to their slaughter and their loss from the breeding programme; similar control measures exist elsewhere. Outbreaks of serious diseases have occurred in cultured marine fish in the EU, e.g., VHSV outbreaks at Giga (Scotland) and Cape Clear (Ireland), which caused the compulsory slaughter of turbot and halibut. It is possible to significantly reduce the risks of these diseases occurring in broodstock and larval animals by implementing good biosecurity. This may include protecting the water supply in which the broodstock animals are held by the use of ozone or UV, vaccination of broodstock, barrier husbandry or by maintaining breeding populations in locations remote from other farms and wild fish populations where disease interaction is possible. As a first step, a report should be produced to identify disease risk reduction strategies for these high-value animals. It may then be appropriate to establish a subgroup of the WGMAFC to produce a review paper for publication.

14. Growing conditions

a. **Recommendation:** Prepare a report on existing knowledge of the effects of water quality (e.g., ozone and resulting compounds, ammonia, microbiology, and probiotics) on intensive land-based, marine fish culture.

b. **Justification:** For many species, especially newly cultivated ones, there is often insufficient information about the allowable and optimal environmental conditions for survival and growth of marine fish. As a result, some hatcheries are poorly sited and production fails. Variability of production is also experienced in well-operating farms and this may be a result of fluctuations in water quality. Identification of important water quality factors may then be of prime interest to reduce such variation. Further, the use of new technology to disinfect and stabilize water, e.g., ozonation, needs to be addressed in terms of effects on water quality.

15. Electronic Larviculture Newsletter

a. **Recommendation:** It is recommended that one free issue of the Newsletter be provided to those members of the WGMAFC who do not now subscribe and that WGMAFC use the Newsletter to collect and disseminate information.

b. **Justification:** The Electronic Larviculture Newsletter is produced by the University of Ghent, Belgium and it contains much useful information on marine fish larvae. It could also be useful to WGMAFC as a means of requesting and disseminating information. For example, the upcoming aquaculture theme sessions at the ICES ASC were announced in the Newsletter. The newsletter could also be used to solicit information on alternative live feeds, aquaculture for restocking, production statistics and most of our information-gathering objectives.

ANNEX 1: LIST OF PARTICIPANTS

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ANNEX 2: AGENDA

1 Opening, Introduction of delegates.

2 Appointment of Rapporteur.

3 Adoption of Agenda and Timetable.

4 Discussion of Meeting, Lunch, and transportation Arrangements.

5 Review of Terms of Reference.

5.1 ToR (a). Report on the current status of marine fish cultivation in Member Countries and on the factors that are likely to constrain further development of the industry; graph and evaluate historical *production* trends for major marine finfish species and predict future development. (Review of tables prepared by **Debbie Martin-Robichaud**).

5.2 ToR (b). review and report on *technological developments* in relation to fish production and their application to various species; **David Bengtson (USA) and Victor Øiestad (Norway)**will provide a review of developments in recirculation system technology for the March, 2002 meeting.

5.3 ToR (c). report on *alternative sources of protein* and lipid, including electronically available bibliography (discussion led by **Castell**).

5.4 ToR (d). review the use of ICES standard reference diets and emulsions in research programmes and recommend any modifications to existing formulations and procedures (to publicize the availability of these diets, **Matthieu Wille** will write a short announcement or article to be included in the *Aquaculture* and *Aquaculture International* issues that carry the proceedings from the Larvi '01 meeting).

5.5 ToR (e) review progress on the use of microdiets for feeding larval fish and assess whether a reference diet / procedure can be recommended (Discussion led by **Castell**).

5.6 ToR (f) work with the WGPDMO to review fish health research and report on existing and emerging diseases of cultured marine fish, including treatments used (**Ian Bricknell** and **Simon Wadsworth** will serve as the main liaison with WGPDMO).

5.7 ToR (g) compile complete list of procedures and methods for monitoring of feeding regimes (Norway (Anders Mangor-Jensen), U.K. (Simon Wadsworth), Atlantic Canada (John Castell), Pacific Canada (Craig Clarke), USA (David Bengtson), Mediterranean (Bengtson to try to identify someone)).

5.8 ToR (h) review current/emerging policies on fish welfare in relation to marine fish culture (country by country, FAO, EU, etc.) and prepare a report (Review summary of submissions from delegates by country).

5.9 ToR (i) work with Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) in developing standard culture conditions under which strains, stocks or species might be tested to evaluate their performance. Debbie Martin-Robichaud will develop a statement to explain why this topic is important. John Castell will attend the 18–20 March, 2002, meeting of the WGAGFM in Halifax, NS, to pursue this. David Bengtson will also try to talk to the WG chair at the Mariculture Committee meeting in Oslo. (Castell will meet with this WG on Monday 18 March to discuss this topic).

5.10 Prepare a review paper on one or two interrelated major recent advances that have significantly improved marine finfish production capabilities or survival during a specific life history stage in a variety of species (e.g., technology, nutritional, physiological or disease prevention/detection). (For this year it could be a review on alternative protein sources in marine fish culture?)

6 Prepare a list of possible ToR to be proposed for 2003 WG Meeting.

6.1 Alternative live feeds and systems for producing them (Chris Cutts has provided a copepod review, and website information).

6.2 Restocking and stock enhancement.

6.3 Land-based recirculation systems – governments should fund research to improve economic competitiveness of these systems and also fund demonstration projects to demonstrate their effectiveness. (Victor Øiestad and David Bengtson will handle this.)

6.4 Selective breeding – this is contingent upon our discussions with the WGAGFM. (No one identified to handle this item yet.)

6.5 Public relations – encourage governments to conduct research and prepare legislation to assure that aquacultured seafood is safe and healthy and then to make the public aware of that fact. (No one identified to handle this).

7 Discussion of the electronic Larviculture newsletter produced by the University of Ghent and how it could be useful to WGMAFC.

8 Venue, timing and Arrangements for 2003 meeting of the WG

9 Discussion of Mariculture Committee Workplan.

10 Developing plans for interactions with other Mariculture Committee Working Groups.

10.1 Working Group on the Application of Genetics in Fisheries and Mariculture: Recommendations.

10.2 Working Group on Pathology and Diseases of Marine Organisms: Recommendations.

10.3 Working Group on Environmental Interactions of Mariculture.

10.4 Discussion.

11 Proposals of Theme Sessions for 2003 and 2004 ASC and Consideration of Workshops, Mini-symposia and Symposia.

12 Nomination of new Working Group Chair.

13 Other New Business.

13.1 LarvalBase project www.larvalbase.org

14 Adjournment.

ANNEX 3: RECOMMENDATIONS FOR FUTURE ACTIVITIES

The **Working Group on Marine Fish Culture** [WGMAFC] (new Chair: A. Mangor-Jensen, Norway) will meet for five days in March 2003 in a venue to be decided to:

a) report on the current status of marine fish cultivation in Member Countries and on the factors that are likely to constrain further development of the industry; graph and evaluate historical production trends for major marine finfish species and predict future development;

b) compile and report on the existing regulations of individual ICES Member Countries and the EU with regard to ingredients in fish feeds;

c) review and report on the use of ICES standard reference diets and emulsions in research programmes and recommend any modifications to existing formulations and procedures;

d) work with the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) to review genetic considerations in selective breeding and identify genetic applications and rearing technologies suited for selective breeding of marine fish;

e) compile information on the current state of the art of microdiets as a replacement for live food for larval fish with the intention to produce a review article in a peer-reviewed publication in 2–3 years;

f) prepare a proposal to recommend microdiets for comparative testing and particular protocols to use during the testing;

g) review and report on the use of live feed organisms other than rotifers and *Artemia* (alternative live feeds) that are used or considered for use in the culture of marine fish larvae;

h) prepare a summary of the instances where aquaculture is being used to produce fish for restocking or enhancement of wild populations in ICES Member Countries;

i) prepare a list of technological advances that would allow land-based aquaculture, especially recirculation systems, to greatly expand in the next 10–15 years and develop a long-term set of tasks for WGMAFC to accomplish to aid that development;

j) working through ICES, encourage governments to gather information on the safety of aquaculture products in relation to their own particular legislation and to present that information to consumers in an unbiased manner based on scientific facts;

k) prepare a report on the status of research and technology of single-sex fish production and its application to the cultivation of marine fish, based on input from experts in the field;

l) compile a report on the existing methods to ensure that measures can be taken to protect broodstock and larval animals from exposure to microbial pathogens;

m) prepare a report on existing knowledge of the effects of water quality (e.g., ozone and resulting compounds, ammonia, microbiology, and probiotics) on intensive land-based marine fish culture;

n) use the Larviculture Newsletter to collect and disseminate information.

WGMAFC will report by ?? for the attention of the Mariculture Committee.

Supporting Information

Priority:	This is an important aspect to the future development of mariculture in the sea and in land-based operations.
Scientific Justification:	a) This provides a continuing mechanism for focusing the WG activities. In addition to providing data on the most recent year's production by country for each marine fish species being cultured, it would be useful to have a multi-year graphical perspective to more clearly see trends that are developing. The 2001 Report has provided some preliminary historical pictures but the accuracy of some of the data is in doubt, and the WGMAFC will review and revise these data and figures. b) The increasing demands for fishmeal and fish oil by aquaculture and agricultural industries has resulted in increases in price and concerns by environmental groups. Projections indicate that the availability of fish oil will limit the aquaculture industry within 3–8 years [Waagbø, R. (2002). Fiskefor og formidler. *In* Glette, J., van der Meeren, T., Olsen, R.E., and Skilbreid, O. (Eds.). Havbruksrapport 2002. Fisken og Havet, særnr. 3–2002. 22–25.]. In addition, consumers have concerns about food safety in general and about contaminants in fishmeal and oil in particular. The high prices and the lack of opportunities to expand the capture fishery make it imperative that alternative protein and lipid sources be developed for use in feeds for aquaculture. As aquaculturists and feed manufacturers consider specific alternatives, they need to know the regulations that exist in ICES countries that may prohibit certain possible sources of feed ingredients from use. c) Enhanced standardisation of experimental procedures would facilitate comparison of growth, survival and other performance criteria of fish in nutrition experiments and thereby greatly enhance the value of work in this area. In this respect there is a need to define recommended protocols, in particular the inclusion of reference diets in the experimental design. The ICES Standard Reference Weaning Diet and enrichment emulsions are such tools that can help in this regard. As new research results identify the nutritional requirements of various marine fish species, it will be possible to modify the formulation of reference diets and perhaps develop species-specific reference diets. Following recent research on the fatty acids DHA, EPA and ARA and on phospholipids, the standard reference diets and emulsions should be reviewed and modified accordingly. d) Selective breeding programmes are required in order to improve and maximize the performance of cultivated marine fish. Though genetics is the topic of another Working Group, WGMAFC proposes to work with WGAFGM to undertake this task. It is important to have scientific experts in molecular genetics, selective breeding and reproductive biology to communicate in order to establish appropriate strategies for the development of marine fish selective breeding programmes. e) The status of microdiets was reviewed at the Larvi '01 meeting and some of those papers will be published, but this field is developing quickly and a new review in about 3 years would likely be worthwhile. f) Live foods, such a rotifers, microalgae and *Artemia*, are required to be fed to larval fish before switching to a dry weaning diet. This can limit larval production due to the high cost and the requirement for a continual supply of live food. There may also be some variation between batches of material such as *Artemia* eggs (both in quality and availability). Microdiets will have an important role in replacing live feeds but no reference diet is yet available. Several producers would be willing to provide microdiets for evaluation. A WGMAFC subgroup will investigate microdiets for evaluation and trial protocols to assess their performance, with a view to recommending an ICES reference microdiet. g) For a number of cultured marine fish such as halibut and other flatfish, it is difficult to produce normally pigmented and properly metamorphosed fish using only enriched rotifers and/or *Artemia*. In the wild, copepods constitute a major food source for many marine fish larvae and recent advances in techniques for

	mass culture of copepods might be useful to marine fish culture in ICES countries. In addition, other live feeds like polychaetes and molluscan trochophore larvae might also be useful. Information on the biochemical composition of natural zooplankton could provide the scientific basis for us to make decisions about the levels of fatty acids like DHA, EPA and arachidonic acid, and other essential nutrients for application in the enrichment of standard live feeds or in the development of diets for larval fish. h) Though the application of aquaculture to restocking of marine fish in ICES Member Countries is thought to be limited, seabream are being restocked in artificial reef areas in Portugal and turbot are being restocked in the North Sea in Belgium. Programmes had been initiated as early as the late 1800s in several ICES countries. In reviewing practices, it will be important to distinguish between stocking for commercial capture fisheries versus for recreational fisheries. There should also be some guidelines on health, genetics and sources of stocked fish. i) Recirculation systems are expensive to build and operate. Nevertheless, they represent a future for marine fish culture in which a) the fish are secure (no escapes), b) the fish are less likely to fall victim to diseases or parasites normally encountered in culture in open waters, and c) environmental impacts of the culture operation are minimized. Given the potential positive impact of marine recirculation systems on the advancement of marine fish culture, much emphasis should be placed on refining these systems and especially on reducing the costs of production. WGMAFC envisions a 10–15 year process for the development of these systems and wants to prepare a road map for our future tasks over a similar period. j) The general public still has some concerns about fish produced in aquaculture. There is a great need for governments and relevant authorities to inform people on the safety of fish and the advantages to this type of food production. This type of public education programme would benefit both the industry and the consumer. In accordance with EU and national legislation, farmed fish are fed on a diet based on their nutritional requirements produced using raw material with negligible amounts of contaminants. Furthermore, they are slaughtered just before they reach the market. The consistent supply of aquaculture products year-round is another advantage. k) There are several species in which one sex grows faster than the other and it may be commercially advantageous to produce fish that are all of the faster-growing sex. A number of investigators are working in this field using a variety of techniques including the manipulation of genetics, environment and hormones. Variable success has been experienced using these different techniques and a review of the advantages and disadvantages of each method on a species-specific basis would be of assistance. l) Many terrestrial agriculture systems have dedicated breeding units designed to avoid exposure of the broodstock and their progeny to high-risk disease organisms (e.g., pigs and swine fever, chickens and Newcastle disease). Within the EU an outbreak of a List I or II disease in a population of fish would lead to their slaughter and their loss from the breeding programme, similar control measure exist elsewhere. Outbreaks of serious diseases have occurred in cultured marine fish in the EU, e.g., VHSV outbreaks at Giga *[check spelling]* (Scotland) and Cape Clear (Ireland), which caused the compulsory slaughter of turbot and halibut. It is possible to significantly reduce the risks of these diseases occurring in broodstock and larval animals by implementing good biosecurity. This may include protecting the water supply in which the broodstock animals are held by the use of ozone or UV, vaccination of broodstock, barrier husbandry or by maintaining breeding populations in locations remote from other farms and wild fish populations where disease interaction is possible. As a first step, a report should be produced to identify disease risk reduction strategies for these high-value animals. It may then be appropriate to establish a subgroup of the WGMAFC to produce a review paper for publication.

	m) For many species, especially newly cultivated ones, there is often insufficient information about the allowable and optimal environmental conditions for survival and growth of marine fish. As a result, some hatcheries are poorly sited and production fails. Variability of production is also experienced in well-operating farms and this may be a result of fluctuations in water quality. Identification of important water quality factors may then be of prime interest to reduce such variation. Further, the use of new technology to disinfect and stabilize water, e.g., ozonation, needs to be addressed in terms of effects on water quality. n) The University of Ghent, Belgium, produces the Electronic Larviculture Newsletter and it contains much useful information on marine fish larvae. It could also be useful to WGMAFC as a means of requesting and disseminating information. For example, the upcoming aquaculture theme sessions at the ICES ASC were announced in the Newsletter. The newsletter could also be used to solicit information on alternative live feeds, aquaculture for restocking, production statistics and most of our information-gathering objectives.
Relation to Strategic Plan:	Responds appropriately to goals and activities.
Resource Requirements:	None required, other than those provided by the host institution.
Participants:	WGMAFC members
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	There are no direct linkages to the advisory committees.
Linkages to other Committees or Groups:	WGPDMO, WGAGFM, WGEIM
Linkages to other Organisations:	

ANNEX 4A: PRODUCTION OF JUVENILE MARINE FISH

Table 4a.1. Production ('000s) of juvenile marine fish in ICES Member Countries in 2001 (values for 2000 in parenthesis).

Country **Species**	**Canada**	**Denmark**	**France**	**Iceland**	**Norway**	**Portugal**	**Spain**	**U.K.**	**U.S.A.**	**TOTALS**
Sea Bass (*Dicentrarchus labrax*)			(20,950)	220 (140)		(4,500)	(9,300)	(170)		
Seabream (*Sparus aurata*)			(18,920)			(11,340)	(42,400)			
Turbot (*Psetta [Scophthalmus] maximus*)		645 (643)	(3,500)	25 (30)	300 (150)	(140)	(2,100)			
Cod (*Gadus morhua*)	80 (20)			8 (2)	975 (530)			70 (5)	100 (2♣)	
Atlantic Halibut (*Hippoglossus hippoglossus*)	96 (55.2)			350 (400)	450 (250)			145 (70)		
Pacific Halibut (*Hippoglossus stenolepis*)										
Flounder (*Platichthys flesus*)		70.2 (60.5)								
Winter Flounder (*Pleuronectes americanus*)	(4)									
Yellowtail Flounder (*Limanda ferruginae*)										
Summer Flounder (*Paralichthys dentatus*)									10	
Lemon Sole (*Microstomus kitt*)										
Red drum (*Sciaenops ocellata*)			125 (?)						34,908°	
Tautog (*Tautoga oritis*)										
Sablefish (*Anoplopoma fimbria*)	10 (12.1)									
Haddock (*Melanogrammus aeglefinus*)	160 (90)								(2♣)	
Eel *(Anguilla anguilla)*										
Mutton snapper *(lutjanus analis)*									3	
Spotted Seatrout (*Cynoscion nebuluosus)*									3,823°	
Brown Rockfish *(Sebastes auriculatus)*									0.8♣	
Striped Bass *(Morone saxatilis)*									4,000	
Cobia *(Rachycentron canadum)*									26	
Spotted Wolffish (*Anarhichas minor*)					45					
TOTALS										

No data were available for Belgium, Estonia, Finland, Germany, Ireland, Latvia, Netherlands, Poland, Russia or Sweden. No data were available from Portugal for year 2000. No data were available for turbot juvenile production for the UK. Cod and bass production data were from demonstration units rather than commercial operations.

♣Produced in the US for research purposes
° Fish were released

ANNEX 4B: HARVESTED PRODUCTION OF FARMED MARINE FISH

Table 4b.1. Harvested production (tonnes) of farmed marine fish in ICES Member Countries in 2001 (values for 2000 are in parenthesis).

	Canada	Croatia	Denmark	France	Greece (observer)	Iceland	Italy	Norway	Portugal	Spain	Sweden	Turkey	U.K.	U.S.A.	TOTALS
Sea Bass (*Dicentrarchus labrax*)				3,500* (3,020)	(23,000*)	20 (15)	(8,800*)		(719)	3,295 (2,702)		(8,000*)			
Seabream (*Sparus aurata*)				1,300* (1,180)	(33,000*)		(6,200*)		(1,352	12,972 (10,090)		(6,000*)			
Turbot (*Psetta [Scophthalmus] maximus*)				1,000* (908)		3		200 (200)	(378)	3,974 (3,683)					
Cod (*Gadus morhua*)						70^ (15)		600^+ (150)					(10)		
Atlantic Halibut (*Hippoglossus hippoglossus*)	10					93 (30)		450 (450)					(2)		
Pacific Halibut (*Hippoglossus stenolepis*)	(44[11])														
Flounder (*Platichthys flesus*)															
Winter Flounder (*Pseudopleuronectes americanus*)															
Yellowtail Flounder (*Limanda ferruginae*)															
Summer Flounder (*Paralichthys dentatus*)														19.8 (5.5)	
Lemon Sole (*Microstomus kitt*)															
Red drum (*Sciaenops ocellata*)				2 (36)										590 (1,000)	
Tautog (*Tautoga onitis*)															
Sablefish (*Anoplopoma fimbria*)	0.7														
White Seabass *(Atractoscian nobilis)*														11	
Black Sea Bass *(Centropristis striata)*														$0.455^{\#}$	
Lingcod *(Ophiodon elongatus)*														7.5♣	
Spotted Wolffish *(Anarhichas minor)*								1							
Haddock (*Melanogrammus aeglefinus*)	1.5 (0.08)														
Eel (*Anguilla anguilla*)			(2,500*)						(200*)	450 (350*)	(250*)				
TOTALS															

*Values reported on the Federation of European Aquaculture Producers Website. $^+$300 from intensive produced fry, and 300 from wild catch then further fed.
$^{\#}$ Wild-caught fish grown in recirc system. [11] Live captured adults held in cages for up to nine months ♣ Fish produced for research purposes ^ongrowing of wild captured fish

ANNEX 5: RECENT ADVANCES IN RECIRCULATION TECHNOLOGY FOR MARINE FISH CULTURE

A report prepared for the ICES Working Group on Marine Fish Culture (WGMAFC) meeting in Olhao, Portugal, 11–14 March 2002

David Bengtson (USA) and Victor Øiestad (Norway)

Introduction

Recirculation systems minimally consist of a fish-rearing unit (usually a tank) and a treatment unit for removal of biological wastes. The treatment unit consists of a container of some medium (plastic beads, sand, etc.) that harbors nitrifying bacteria, which convert the ammonia that is produced by fish as a metabolic waste into nitrite and then nitrate (a process called nitrification). Ammonia and nitrite are toxic to fish if they are not removed by water flushing (in a flow-through system) or by bacteria (in a recirculation system). Nitrate can be toxic to fish at very high levels, but these are normally not seen in typical culture conditions. Such a treatment unit, known as a biofilter, can be of several types, e.g., rotating biological contactor, bead filter, trickling filter, fluidized bed filter, etc., all of which have been used for years. The chemical reactions involving the bacteria include the production of hydrogen ions (thereby lowering the pH of the water) and the consumption of oxygen. Thus, the design of a recirculation system requires consideration not only of physical conditions and processes, but also of biological and chemical processes. Based on the estimated production of ammonia by the fish, one designs the treatment unit to process that amount of ammonia. One can find considerable information about this and the topics below in books by Timmons and Losordo (1994) and Timmons *et al.* (2001).

Few recirculation systems rely just on the minimum fish-rearing and treatment units. Normally, a settling tank is placed immediately after the fish-rearing tank to collect settleable solids, such as feces and uneaten feed. In a freshwater system, these can be used as manure for agriculture, but the inclusion of salt in the wastes of a marine system makes this option more difficult. Screen filters are usually used in the system to remove suspended solids. Foam fractionators may also be used to remove the dissolved solids. The aforementioned components are usually quite capable of adequately filtering the water. Other components of the system are used for control of dissolved gases. Depending on the density of fish in the system, the operator may rely just on aeration (introducing atmospheric gas to the water) or on oxygenation (introducing pure oxygen), through the use of components such as bubble contactors, spray towers, packed columns, and others. Finally, control of pathogens in the system is normally done by ultraviolet (UV) filters to kill bacteria. Ozonation, which was originally incorporated as a means of removing fine particles, is also very effective at killing bacteria and viruses; however, the ozone must be destroyed before the water enters the fish-rearing unit because it is toxic. Ozonation has been problematical in marine recirculation systems because of the bromides produced as a by-product.

Recirculation systems are, as one might imagine from the list of components, expensive to build and operate. Nevertheless, they represent a future for marine fish culture in which a) the fish are secure (no escapes), b) the fish are less likely to fall victim to diseases or parasites normally encountered in culture in open waters, and c) environmental impacts of the culture operation are minimized. Given the potential positive impact of marine recirculation systems on the advancement of marine fish culture, much emphasis should be placed on refining these systems and especially on reducing the costs of production.

We have surveyed people in both Europe and North America to identify <u>recent</u> advances in recirculation technology for marine systems and have found very few. We identify them specifically below.

1) The Cyclo-biofilter – This is a fluidized bed biofilter in which the water is introduced tangentially, rather than through manifolds, so that it runs at reduced operating pressure and therefore uses less electricity. In addition, the flow pattern generated means that the filter is less susceptible to channeling (channels form in the medium to allow water to pass untreated). This product is made by Marine Biotech, Inc. in the United States.
2) Recent research by Dr Thomas Losordo (personal communication) using a moving bed biofilter in Australia confirms the findings of Nijhof and Bovendeur (1990) that nitrification rates in marine systems are only about one-tenth of the rates in freshwater systems.

Recirculation systems are used primarily for the culture of freshwater fish, but interest in their use for marine fish culture is increasing. In almost every issue of *Fish Farming International* you will see articles about successful use of recirculation system for marine fish culture. In the February 2002 issue, *FFI* reported on on-growing turbot in recirculation in Scotland (by *Seafish Authority*) and in the January 2002 issue they reported on juvenile turbot in recirculation in Denmark (by *Maximus*). So seemingly there is a growing activity also in sea water after a decade with significant increase in recirc activity in fresh water with eel and African catfish in countries like Italy and Netherlands. But what is the actual status in ICES countries? Details of marine fish culture in recirculation systems in Europe are given in Table A5.1, though the over-view is far from complete. In North America, some pilot systems have been tested for the production of summer flounder, but these are on the scale of only 10 tonnes per year. Interestingly, a considerable quantity of hybrid striped bass is produced in freshwater recirculation systems by two companies in the United States (mentioned because FAO considers these to be marine fish even though virtually all production is in fresh water).

Table A5.1. General information about marine fish species in recirculation systems in Europe (not complete).

Company/institute and country	Species (stage)	Quantity (year)	Remarks
Maritech, IFREMER - France	Sea bass (ongrowing)	< 100 t (2001)	
Maki - Iceland	Sea bass (ongrowing)	< 100 t (2001)	
Mistral-Mar - Iceland	Sea bass (ongrowing)	> 1,000 t (2003?)	
APIAN/Puraq - Italy	Sea bass (ongrowing)	> 100 t (1997)	No production in 2001 Built by Puraq
Frence Turbot, Brittany - France	Turbot (all stages)	> 100 t (2001)	
Frence Turbot, Noirmoutier - France	Turbot (all stages)	> 100 t (2001) Minimum 3 million juveniles/year	
Aquacria Piscicola, Puraq - Portugal	Turbot (ongrowing	> 100 t (2001)	Capacity 270 tonnes
Aquacria Arousa, Puraq - Spain	Turbot (ongrowing)	> 100 t (2002)	No sales in 2001; capacity 500 tonnes
IAT-Selonda - UK	Turbot (ongrowing)	> 100 t (2002)	
Aquascot - UK	Turbot	< 10 t	
Seafarm- Netherlands	Turbot	25 t (2001)	
Fish Farm Yerseke - Netherlands	Turbot	Production start 2001	
Ecomares - Germany	Turbot	Production start 2001	100 tons capacity
Maximus - Denmark	Turbot (pre-ongrowing)	Minimum 500,000 juveniles/year	
Seafish Authority - UK	Turbot (ongrowing)		Pilot scale
RIVO – Netherlands	Sole		Pilot scale

Juvenile production

Almost all juvenile production of sea bass, seabream and turbot in France takes place in recirculation systems. Probably the situation is similar in other European countries. Most of the juvenile cod production units now under construction in Norway will also rely on this technology as they mainly copy European sea bass and seabream technology.

Ongrowing

Sea bass

Ongrowing of sea bass has been tested in recirculation systems within the frame of the project MARITECH run by French (Mediteranée Pisciculture near Perpignan in the South of France together with IFREMER) and Icelandic interests (Maritech – Maki hf). They have gradually increased their facilities step-by-step from 2–5 tonnes via 60–100 tonnes to the present 1,000–2,000 tonnes, an activity now under realisation on Iceland (EU project Mistral-Mar). A facility constructed by Puraq in Italy for sea bass for 180 tonnes, was completed in 1996 (Apian). After a test production, the plant has since been out of use.

Turbot

Ongrowing of turbot has been in full scale operation in a recirculation system in Portugal (close to Porto, Aquacria Piscicola) and in Galicia, Spain (Aquacria Arousa). The two plants were constructed by Puraq and are run by the company. In 2001 the production in Portugal was 110 tonnes. The production in 2002 is expected to be about 250 tonnes in total from the two plants although their capacity is about 700 tonnes altogether. The growth performance of the turbot was initially fairly low, probably due to a combined effect of an inappropriate feeding regime and variable and often low water quality. The situation is improving.

France Turbot has one farm in Brittany using recirculation on 85 % of its production and they have another farm on Noirmoutier already 75 % equiped with recirculation and soon 100 %. Probably their fish production per year is higher than for the two Puraq farms.

In northern Wales, IAT (Selonda group; Blue Water FF) has a turbot farm from which minor information is available. Seemingly they achieve a mean weight of 1 kg in eleven months (starting with 5 grams); they have crystal-clear water in the round tanks indicating a very sophisticated behind-screen water filtration system (and they have received excellent test panel results on the produced fish). They are about to start harvesting turbot in quantities; planned production is 220 tonnes in full production.

There is another turbot farm in Scotland (AQUASCOT) with no information available. There are two turbot farms in Netherlands (Yerseke and Seafarm) and one in Germany (Ecomares), also with minor information at hand.

Sole

There is a recirculation test unit for sole (*Solea solea* and *S. senegalensis*) at RIVA in Netherlands.

Opinions on present status and the future

***D. Leclerque* from France** Turbot sees a big future for recirculation, allowing for a stable environment and culture conditions. He acknowledges that recirculation is still an experimental technique. He has in an interview claimed that future fish farms of marine species may even occur in Paris as the need for new water may be insignificant.

In a statement from ***Patrick White*** (Akvaplan-niva; Athens office) who is at the moment preparing an internal report on the state-of-the-art of recirculation system, he claims:

“My view so far is that the best concepts are:

- Octagonal tanks for self cleaning and quick solids separation (AquaOptima type)
- Double sump system for particle separation (AquaOptima type)
- Dewatering of suspended solids - swirl tank then bag filter (tank and mechanical filter effluents)
- Mechanical filter conveyor type for gentle separation of suspended solids (MegaFisch, Dryden or Hydrotech)
- Biofiltration - trickle type for ease of use - Kaldnes moving bed-type for efficiency
- Denitrification - Blue label type
- Foam fractionator with Ozone injection
- pH control - Dryden MagpHlow media (expensive, but efficient)
- degassing and re-aeration (vacuum degasser)
- pure oxygen injection – in-line saturator
- new inlet water - low dose of ozone followed by UV sterilisation to get rid of free radicals.
- effluent water – “end of pipe treatment”

State-of-the-art

Puraq - SunFish

The system applied by Puraq in their newest farm, Aquacria Arousa, with a planned production of 500 tonnes/year and a prospective standing biomass of 300 tonnes, is still under modification. At the present stage, with a biomass of about 120 tonnes (mixed populations ranging in weight from 100 g to 1500 g and with a mean weight of about 400 g) and a daily feed supply of 600 kg (0.5 %/day), the water quality parameters are typically as shown in Table A5.2. Briefly described: turbot is ongrown in a 5 m deep tank, formed as a "doughnut" 50 m in diameter and 12 m wide with the centre as a water treatment unit 26 m in diameter. Longitudinal current in the doughnut is created by three propellers and spiral-rolling flow-pattern by intensive aeration along the outer rim of the tank resulting in heavy foaming. The foam is removed by numerous vacuum skimmers and stored in a sludge depot (volume 2,000 m^3), removing 60–80 % of all organic suspended solids in the on-growing basin. The volume of water in circulation is about 6,700 m^3 and this water passes the replicated biofilter 10–12 times per day and the ozonation unit 5–6 times per day (3,000 m^3/h pass through the water treatment channel). The two biofilter tanks each have 120 m^3 of Kaldnes moving bed bodies; the ozone unit produces 1.4 kg of ozone/h (after correcting for 70 % efficiency). Typical redox values in the main basin have been about 200. New water is coming in at a daily rate of 15–20 %; new water passes the ozone unit. Bioblocks (100 m^3) are located along the outer rim of the basin in a length of 150 m; they contribute significantly as biofilters. A mechanical fine-mesh filter has been temporary removed due to minor cleaning effect as a result of particles being too small for this purpose. At some few points along the bottom are installed settling sites for sediments; these are pumped at time intervals. The former pre-ongrowing tank, designed for keeping the juveniles from 5 g to 50 g, has been replaced by four circular shallow raceways with a total bottom area of 560 m^2 (compared to the former area of 250 m^2) where the fish now will be kept to 200 g or more.

In addition to a bottom of the main tank of 1200 m^2, the fish shall gradually colonize nine shelves in each of 35 racks. Each shelf has a frame of 3 m × 7 m (21 m^2) covered with fine-meshed net adding another 6,600 m^2 of surface for the fish to settle on. When fully colonized the 7,800 m^2 will have a mean fish density of 40 kg/m^2, a fairly modest density for fish with an expected mean weight of 600 g.

Table A5.2. Illustration of the WQ observations made in Main Basin, *Aquacria Arousa*, in January 2002, together with some data on mortality and feeding. First row 7 days' average; second row 30 days' average. Coloured values when outside recommended (= set) level.

Susp.s.	Turbidity	Transmission		pH	Alkalinity	[NH_4]	[NO_2]
mg/l		400 nm	500 nm			mg/l	mg/l
26.00	1.15	96.10	98.10	7.38	55.00	0.80	0.50
39.43	1. 93	94.91	97.14	7.42	63.71	0.54	0.57

[NO_3]	[O_2]	Temp	Salinity	[H_2S]	ORP	Ozon	Feed	Dead	TCBS
mg/l	mg/l			mg/l	mv	mg/l	Kilo	No	no/ml
35.00	8.79	14.50	35.00	0.00	196.00	0.00	0.00	0.00	0.00
40.00	8.97	14.14	35.43	0.00	185.14	0.00	400.86	127.29	2071.43

For further information

For more information about specific companies that produce fish in recirculation systems, or produce recirculation systems or components thereof, the reader is referred to the following web sites:

1) THE SUN FISH SYSTEM (http://www.puraq.no) Oslo, Norway;
2) RIVO-Netherlands Institute for Fisheries Research – P.O.Box 68 – 1970 AB – IJmuiden –The Netherlands (http://www.rivo.dlo.nl);
3) AquaOptima recirculation system (http://www.aquaoptima.com) Trondheim, Norway;

4) DIAT – (http://www.danishaquaculture.com) - Denmark;
5) IAT (http://www.iat.uk.com) Bluewater - Great Britain;
6) Hibo-Dan Ltd (http://www.hibo-dan.com) Montrose, Asgard Road, Howth Co. Dublin;
7) Advanced aquaculture systems, INC (http://www.aquasystems.co.uk) - UK;
8) Dryden Aqua (http://www.drydenaqua.com) Edinburgh – Scotland;
9) Hesy Bergambacht BV (http://www.Hesy.com) Netherlands;
10) Marine Biotech (http://www.marinebiotech.com) Beverly, Massachusetts, USA;
11) Kent SeaTech (http://www.kentseatech.com) SanDiego, California, USA;
12) Fins Technology (http://www.finstechnology.com) Turners Falls, Massachusetts, USA.

ANNEX 6: REFERENCES ON ALTERNATIVE PROTEIN SOURCE STUDIES IN MARINE FISH

Compiled by

John Castell and Troy Lyons

Department of Fisheries & Oceans, Biological Station, 531 Brandy Cove Road, St. Andrews, NB. E5B 2L9, Canada

1) Aksnes, A. 1997. The effect of fishmeal quality on voluntary feed intake. *In*: Houlihan, D., Kiessling, A., Boujard, T. (Editors). Voluntary food intake in fish., 1. COST 827 Workshop, Aberdeen (UK), 3–6 Apr 1997 p.18. The quality of fishmeal varies both due to the freshness of the raw material used and due to the processing conditions. These factors will affect the nutrient value and the palatability of the fishmeal. In two growth experiments with Atlantic halibut, four pilot plant-produced fishmeals were used to investigate the effect of raw material freshness and processing temperature, respectively. In experiment I, two fishmeals were produced from fresh and stored raw material and these fishmeals were used in four experimental diets. Freshness was evaluated as TVN in the raw material and as biogenic amines (e.g., cadaverine) in the fishmeals. In experiment II, two fishmeals were produced from fresh raw material at different processing temperatures. The two fishmeals differed in protein digestibility as measured by mink and were used in four experimental diets. The diets were fed to Atlantic halibut in two growth experiments to record growth, feed efficiency and feed intake. The experiments were performed as long-term experiments to investigate any habituational effects. *Dr A. Aksnes, Norwegian Herring Oil and Meal Industry Research Institute, 5033 Fyllingsdalen, Norway.*

2) Aksnes, A., Izquierdo, M. S., Robaina, L., Vergara, J. M., and Montero, D. 1997. Influence of fishmeal quality and feed pellet on growth, feed efficiency and muscle composition in gilthead seabream (*Sparus aurata*). Aquaculture, 153: 251–261. All groups showed good growth and feed efficiency, with daily specific growth rates (SGR) of 0.90–1.00 % and feed efficiencies from 0.58 to 0.66. The fish fed with low quality fishmeal showed significantly poorer feed efficiency than other groups, and there was a significant correlation between feed efficiency obtained in seabream and the protein digestibility as measured with mink. No difference was observed among groups fed the extruded diets, for growth, fillet composition or amount of liver or viscera, although a significant correlation was obtained between SGR and protein digestibility in mink. Fillet lipid content was higher in fish fed the extruded feed, compared with those fed the corresponding pelleted diet. *Dr A. Aksnes, Norwegian Herring Oil and Meal Research Institute, N 5033 Fyllingsdalen, Bergen, Norway.*

3) Aksnes, A., and Mundheim, H. 1997. The impact of raw material freshness and processing temperature for fishmeal on growth, feed efficiency and chemical composition in Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture, 149: 87–106. Experimentally produced fishmeals were compared with commercial fishmeals to investigate quality factors in long-term growth experiments with Atlantic halibut. The effect of fishmeal quality on growth, feed efficiency and chemical composition of the fish were studied. The first experiment used two meals made from raw material in four diets with varying protein digestibility. The second experiment used two meals produced at the same processing conditions but varying in raw material freshness. The third experiment used 6 commercial fishmeals varying in protein digestibility and cadaverine. Generally the fish showed good growth, high feed efficiency and good survival. The first experiment showed a linear correlation between protein digestibility and specific growth rate (SGR) and feed efficiency (FE). Similarly, Experiment 2 revealed a negative relation with the fourth exponential of cadaverine in those diets both for SGR and FE. The experiment with commercial fishmeals also showed dependencies both on protein digestibility and dietary level of cadaverine. Both raw material freshness and processing conditions, as measured by effects on protein digestibility, are useful and important in assessing fishmeal quality for halibut. Reduced fishmeal quality due to digestibility was compensated for by increased feed intake. This was not the case when fishmeal quality was reduced due to raw material spoilage. *Dr Anders Aksnes, Norwegian Herring Oil and Meal Research Institute, N 5033 Fyllingsdalen, Bergen, Norway.*

4) Alexis, M. N. 1997. Fishmeal and fish oil replacers in Mediterranean marine fish diets. *In:* Tacon, A., Barsureo, B. (Editors). Feeding tomorrow's fish. Proceedings of the workshop of the CIHEAM network on technology of aquaculture in the Mediterranean. CIHEAM, Zaragoza, Spain. 22: 183–204. A number of experiments were performed in order to evaluate different feedstuffs as fishmeal and fish oil replacers in Mediterranean marine fish diets, including the European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). Apparent crude protein digestibilities were relatively high for most feedstuffs, while energy digestibilities varied over a great range. Selected feedstuffs were further tested concerning their potential to replace white fishmeal protein in gilthead bream diets. Meat and bone meals performed well up to 40 % substitution of fishmeal protein. Good quality poultry meal could replace 100 % of white fishmeal without a significant loss in fish performance. However, feather meal could be tolerated at lower levels. Different soya products were also tested. Good results were obtained using adequately heated full fat soya substituting 35 % of fishmeal protein while soybean meal could be tolerated at lower levels. A soya protein concentrate, tested at 35 % inclusion level, provided significantly lower growth. Plant oils do not cover EFA requirements of Mediterranean fish and result in severe histological lesions if used singly as an oil supplement. Adequate amounts of n-3 PUFA, supplied by fish oils, are required to

suppress histological lesions. Tissue lipids reflect dietary lipid composition and could possibly contribute to the taste of fish. Comparison of tissue fatty acid composition of wild and cultured seabream and sea bass revealed certain differences wild fish containing higher levels of arachidonic acid than cultured fish. In view of the importance of arachidonic acid for various physiological functions adequate enrichment of diets might be necessary for optimum fish performance. *National Centre for Marine Research, Aghios Kosmas, Helliniko, GR 16604, Athens, Greece*.

5) Alliot, E., Pastoreaud, A., Hudlet, J. P., and Métailler, R. 1979. Utilisation des farines végétales et des levures cultivées sur alcanes pour l'alimentation du bar (*Dicentrarchus labrax*). *In:* Halver, J. E., Tiews, K. (Editors). Finfish Nutrition and Fishfeed Technology. Heenemann, Berlin Vol. II pp. 229–238.

6) Amerio, M., Costa, M., Mazzola, A., and Crisafi, E. 1989. Use of extracted soybean meal in diets for sea bass. *In:* de Pauw, N., Jaspers, E., Ackefors, H., Wilkins, N. (Editors). Aquaculture - A Biotechnology in Progress. European Aquaculture Society, Bredene, Belgium. pp.605–608.

7) Amerio, M., Vignali, C., Castelli, L., Fiorentini, L., and Tibaldi, E. 1998. Vegetable protein sources, protein evaluation indices and "ideal protein" of seabream (*Sparus aurata*). [Materie prime vegetali, indici di valutazione proteica e valori previsionali della 'proteina ideale' nell'orata (*Sparus aurata*)] Rivista italiana di acquacoltura. Verona, 33: 135–145. Most protein sources, other than fishmeal, have one or more negative characteristics that limit their use in fish feeding. Amino acid profile and antinutritional factor levels are the first elements to be tested in evaluating plant protein sources before assessing palatability and nutrient value in fish. In the present study, the chemical composition of several plant feedstuffs was determined by standard laboratory procedures and compared to the nutrient dietary allowances of seabream (*Sparus aurata*), in order to obtain a data group as well as preliminary criteria for selecting possible dietary substitutes for fishmeal in practical feeding of this species. Feeds evaluated were: three derived by oil extraction (debulled solvent extracted soybean meal SBM, rapeseed meal RSM, oil extracted sunflower meal SFM); three by industrial treatment of legumes (soy protein concentrate SPC, pea meal PM and lupine seed meal LSM); 3 by starch extraction (maize gluten meal CGM, wheat gluten meal WGM and potato protein concentrate PPC). The biological value of the protein of each feedstuff was evaluated by some protein evaluation indexes: Oser's Essential Amino Acid Index (EAA index) (Sheffner, 1963), the chemical score by Mitchell and Block (Sheffner, 1963), and the protein efficiency ratio DC-PER (Satterlee *et al.*, 1982) and by comparing the essential amino acid (EAA) profile of each feedstuff with the EAA requirements of seabream like "ideal protein" (Wilson, 1994). The most lacking AA in the EAA comparison was: lysine in RSM, CGM, WGM and SFM, arginine in CGM and WGM, threonine in WGM, methionine in RSM, PM, SBM, WGM, LSM and SPC, tryptophan in CGM. *Dr M. Amerio, Istituto di Scienze degli Alimenti e della Nutrizione, Facolta di Agraria, U.C.S.C. Via E. Parmense 84, 29100 Piacenza Italia.*

8) Amerio, M., Vignali, C., Castelli, L., Fiorentini, L., and Tibaldi, E. 1998. Chemical and nutritional evaluation of vegetable protein sources as possible dietary ingredients for seabream (*Sparus aurata*). VIII International Symposium on Nutrition and Feeding of fish. Recent Advances in Finfish and Crustacean Nutrition. Las Palmas de Gran Canaria, Spain June 1–4, p. 145. The chemical composition of several plant feedstuffs was determined in order to obtain a data base. Rapeseed meal (RSM), peas meal (PM), dehulled solvent extracted soybean meal (SBM), soy protein concentrate (SPC), oil extracted sunflower meal (SFM), lupine seed meal (LSM), maize gluten meal (CGM), wheat gluten meal (WGM) and potato protein concentrate (PPC). The anti-trypsin activity was: 0.53 mg trypsin/g (w.w) for PM, 1.71 mg try/g (w.w.) for SBM, 3.29 mg try/g (w.w.) for SPC and 0.48 mg try/g (w.w.) for PPC. The most lacking AA in the EAA comparison were: lysine in RSM, CGM, WGM and SFM, histidine in SFM, arginine in CGM, SFM and WGM, valine in SFM, methionine in PM, SBM, WGM, SFM, LSM and SOC, thryptophan in CGM and SFM, isoleucine, leucine, and phenylalanine in SFM. *Dr M.Amerio, Istituto degli Alimenti e della Nutrizione. Facolta di Agraria, UCSC via E, Parmense 84, 29100 Placenza, Italy.*

9) Aoki, H., Watanabe, T., Tsuda, H., and Sakamoto, H. 1996. Use of defatted soybean meal as a substitutive protein source for fishmeal in a newly developed high energy diet for red seabream. Suisanzoskohu, 44: 345–351.

10) Bai, S.C., Choi, S-M., Kim, K-W., and Wang, X.J. 2..1. Apparent protein and phosphorus digestibilities of five different dietary protein sources in Korean rockfish, *Sebastes schlegeli* (Holgendorf). Aquaculture Research, 31(Suppl. 1): 99–105. A 6-week feeding trial was conducted to determine the apparent protein and phosphorus digestibilities in order to evaluate five dietary protein sources in juvenile Korean rockfish. Five diets containing fishmeal analog (BAIFA-M™), white fishmeal (WFM), rockfish muscle (RM), soybean meal (SM) or corn gluten meal (CGM) were prepared by mixing a basal diet (BD) with one of five test ingredients at the ratio of 7:3. As a reference diet, BD contained three different protein sources, such as casein, gelatin and rockfish muscle. Fish averaging 3.1 ± 0.03 g were distributed to each aquarium as a group of 20 fish reared in the recirculating system. Fish of triplicate groups were fed one of six experimental diets (BD + five test diets). Apparent digestibilities of dry matter, protein and phosphorus were determined by the chromic oxide method. After six weeks, apparent protein digestibilities of RM, WFM, CGM, SM and BAIFA-M™ were 92.6, 87.5, 79.1, 76.2 and 70.6 %. Apparent phosphorus digestibilities were 77.5, 59.6, 22.2, 12.6, and 66.9 %, respectively. The protein efficiency ratio ranked with RM> EFM, BD> BAIFA-M™, CGM and SM. Protein efficiency ratio was positively correlated with apparent protein digestibility. *Dr Sungchul, C., Bai, Department of Agriculture, Pukyong National University, Pusan 608–737, Korea. E-mail: scbai@mail.pknu.ac.kr.*

11) Bai, S. C., and Kim, K-W. 1997. Effects of dietary animal protein sources on growth and body composition in Korean rockfish, *Sebastes schlegeli*. Journal of Aquaculture, 10: 77–85. (in Korean, with English Summary).

12) Ballestrazzi, R., Lanari, D.,Dagaro, E., and Mion, A. 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*). Aquaculture, 127(2–3): 197–206. Three hundred sea bass (75.8 ± 5.8 g) were randomly distributed among 12 tanks and fed for 168 days, at 1.0 % live weight, with one of 6 experimental diets in a 3 × 2 factorial design [3 protein levels: 44, 49 and 54 % crude protein (CP) on a dry matter basis; 2 protein sources: herring meal and herring meal plus corn gluten meal] with 2 replicates for each treatment. Final live weight and relative weight gain were affected by the protein level ($P < 0.05$) but not by the protein source. The moisture and crude protein of fish increased with dietary protein level while fat and energy contents decreased accordingly ($P < 0.05$). Fish fed the 44 % CP herring meal diet had the highest energy, protein and lipid retention. Total ammonia excretion increased linearly and significantly with the protein level of the diet. Diets containing corn gluten gave lower reactive phosphate concentration in the effluent water than those with herring meal (55.1 vs. 113.9 mg PO_4/kg/day). *Dr D. Lanari, Università segli Studi di Udine, Dipartimento di Scienze Produzione Animale, Via S. Mauro 2, 33010 Pagnacco, Udine, Italy.*

13) Berge, G.M., Grisdale-Helland, B., and Helland, S.J. 1999. Soy protein concentrate in diets for Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture, 178(1–2): 139–148. Four experimental diets were fed to triplicate groups of Atlantic halibut in a 2 × 2 factorial design, testing the effects of protein source and the use of an attractant. The diets contained 61 % fishmeal (FM) or 37 % FM and 28 % soy protein concentrate (SPC) with or without a coating of squid meal. In the SPC diets, 44 % of nitrogen was supplied from the SPC. The mean initial fish weight was 633 g, and at the end of the 12-week trial, the mean fish weight was 874 g. There was no significant effect of dietary treatment on specific growth rate (0.43 ± 0.02 % body weight d^{-1}, mean ± S.E.M.). Feed efficiency ratio was significantly lower in the group fed the SPC diet (1.22 ± 0.02) compared with those fed the FM diet (1.28 ± 0.02), Fish fed the SPC diets had a higher feed intake (0.64 ± 0.02 %) than those fed the FM diet (0.59 ± 0.02 %). Addition of squid had no effect on feed intake. The groups fed diets not supplemented with squid were used for determination of whole body chemical composition, and the remaining fish from these groups were subsequently stripped for feces for digestibility estimations. There were no effects of protein source on digestibility or protein or energy retention in whole fish. The fish fed the diet containing soy protein had a slightly higher dry matter (DM) content ($P < 0.05$), than the fish fed the diet containing only fishmeal. *Dr G. M. Berge, Institute of Aquaculture Research (AKVAFORSK), N 6600 Sunndalsora, Norway.*

14) Boonyaratpalin, M., Suraneiranat, P., and Tunpibal, T. 1998. Replacement of fishmeal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. Aquaculture, 161(1–4): 67–78. Five isonitrogenous and isocaloric diets were formulated to contain all fishmeal (control diet), 21.0 % solvent extracted SBM, 27 % extruded full-fat SBM, 28.5 % steamed full-fat SBM and 27.5 % soaked raw full-fat SBM as substitutes for 15 % fishmeal in the control diet. Fish fed the control diet grew significantly better than fish fed extruded full-fat SBM, steamed full-fat SBM or soaked raw full-fat SBM diets, but not significantly different from that of fish fed the solvent-extracted SBM diet. Feed efficiency and survival did not differ significantly among treatments except for fish fed the soaked raw full-fat SBM diet. Fish fed the soaked raw full-fat SBM had significantly lower weight gain, feed efficiency, protein efficiency and survival. Fish fed this diet also showed some histological changes in their gastrointestinal tract. The apparent protein digestibility of diets containing all fishmeal, solvent-extracted, extruded, steamed and soaked raw full-fat SBMs were 92.8, 94.2, 92.3, 94.4 and 73.7 %, respectively. The lower growth obtained in fish fed the extruded or steamed full-fat SBM diets as compared to that of the control fishmeal diet could not be attributed to protein digestibility, but it could be due to the lower feed intake during the first two weeks suggesting that palatability was possibly a factor. Soaked raw full-fat SBM is a poor protein source for seabass diet. Solvent-extracted SBM is more palatable for small seabass (1.30–3.50 g) than extruded or steamed full-fat SBM. Thus, this study suggests that, if palatability of the diet can be improved, 37.5 % of protein from fishmeal or 15 % fishmeal in diet for seabass can be replaced by solvent-extracted SBM, extruded full-fat SBM or steamed full-fat SBM. *Dr M. Boonyaratpalin, Department of Fisheries, Feed Quality Control & Development Division, Bangkok 10900, Thailand. E-mail: omab@nontri.ku.ac.th*

15) Burel, C., Boujard, T., Kaushik, S.J., Boeuf, G., Vander-Geyten, S., Mol, K.A., Kuhn, E.R., Quinsac, A., Krouti, M., and Ribaillier, D. 2000. Potential of plant-protein sources as fishmeal substitutes in diets for turbot (*Psetta maxima*): growth, nutrient utilisation and thyroid status. Aquaculture, 188(3–4): 363–382. An experiment was conducted in order to assess the incorporation in diets for juvenile turbot of extruded lupin (*Lupinus albus*) and heat-treated (RM1) or untreated (RM2) rapeseed meals (*Brassica napus*) (26 and 40 μmol glucosinolate/g DM, respectively). The level of incorporation of 30 % for each plant-protein, as well as 46 % for RM1 and 50 % for lupin was tested and compared with a fishmeal-based control diet. Triplicate groups of turbot (initial body weight of 66 g) were fed by hand with isonitrogenous and isoenergetic experimental diets, twice daily and to visual satiety, during 63 days. Extruded lupin can be incorporated in diets of turbot up to a level of 50 % without adverse effects on growth performance and body composition. Rapeseed meal can only be incorporated at levels up to 30 %, but a preliminary heat treatment of RM is necessary in order to improve its nutritional quality. In turbot fed the RM-based diets, plasma T-4 levels were reduced with low dietary content in glucosinolate breakdown products (3.6 μmol/g), but no decrease in plasma T-3 levels was observed with the higher level of toxic compounds (4.4

µmol/g). A significant deiodinase type II compensatory effect, leading to an increase of the conversion of T-4 to T-3, was observed *in vitro* in the liver of turbot fed RM1-based diets. The intake of lupin-based diets also had an effect on thyroid status with an increase of plasma T-3 levels and of deiodinase type I activity in liver and kidney, suggesting an increase in the degradation of rT(3) and in the conversion of T-4 to T-3. *Dr T. Boujard, INRA, IFREMER, Unité Mixte, Station d'Hydrobiologie, Laboratoire de Nutrition des Poissons, BP 3, F 64310 St Pée Sur Nivelle, France. E-mail: boujard@st-pee.inra.fr.*

16) Burel, C., Boujard, T., Tulli, F., and Kaushik, S.J. 2000. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture, 188(3–4): 285–298. Apparent digestibility coefficients (ADC) of nutrients and energy of extruded peas, extruded lupin and rapeseed meals were determined in juvenile rainbow trout and turbot. Extruded lupin was found to be a promising substitute for fishmeal in the diets of trout and turbot, with an acceptable digestibility of its dry matter (70 % in trout and 81 % in turbot) and a high digestibility of its protein (96 % in trout and 98 % in turbot) and its energy (77 % in trout and 85 % in turbot). Extruded peas had a lower digestibility of its protein in trout (88 %) than in turbot (92 %), and the ADC of energy, mainly supplied as starch, was relatively low (69 % in trout and 78 % in turbot). The digestibility of rapeseed meal was improved by a thermal treatment. Without thermal treatment, rapeseed meal had a low digestibility of its dry matter (57 %) and energy (69 %) in turbot. The availability of phosphorus was higher for extruded lupin (62 % in trout and 100 % in turbot) compared to the other plant-ingredients. When compared to a solvent-extracted meal, the availability of phosphorus from rapeseed meal was improved by heat treatment in both species (42 % vs. 26 % in trout and 65 % vs. 49 % in turbot). *Dr S. J. Kaushik, INRA, IFREMER, Unité Mixte, Station d'Hydrobiologie, Laboratoire de Nutrition des Poissons, BP 3, F 64310 St Pée Sur Nivelle, France. E-mail: kaushik@st-pee.inra.fr.*

17) Caballero, M.J., López-Calero, G., Socorro, J., Roo, F.J., Izquierdo, M.S., and Férnandez, A.J. 1999. Combined effect of lipid level and fishmeal quality on liver histology of gilthead seabream (*Sparus aurata*). Aquaculture, 179(1–4): 277–290. Effects of eight diets comparing three different lipid levels (15, 22 and 27 %) and two fishmeal qualities were studied on growth and liver histology. Fishmeal quality was judged by the content of biogenic amines and temperature processing techniques. The experiment included a comparison of pelleted feed with extruded feed for the 22 % lipid diet. A total of 1140 gilthead seabream of 70 g average initial body weight were randomly stocked in 500-litre fiberglass tanks in duplicate groups of 60 fish. After 2 months of experiment, the fish were transferred to 1-m^3 tanks. Fish were fed twice a day to apparent satiation for 6 months until they reached about 400 g (commercial size). Fish fed diets containing high quality fishmeal showed, in general, a higher growth than those fish fed with low quality fishmeal. For diets containing high quality fishmeal, the fish fed 22 and 27 % dietary lipid had significantly higher growth than those fish fed 15 % dietary lipid. On the contrary, in diets containing low quality fishmeal, only fish fed 27 % dietary lipid showed significantly the higher growth rate. Fish fed the pelletized diets showed a lower growth than those fish fed extruded diets. Livers from fish fed diets containing high quality fishmeal and 27 % lipid showed foci of swelling hepatocytes that were not found for low quality fishmeal at the same dietary lipid content. Ultrastructurally, these foci were characterized to present irregular nuclei displaced to the periphery of hepatocytes and large lipid droplets in the cytoplasm. Livers from fish fed high and low fishmeal qualities with 22 % lipid showed similar morphological characters of hepatocytes to those that fed 15 % lipid, but the difference was observed in the nuclei displacement. *Dr M. J. Caballero, University las Palmas de Gran Canaria, Department of Biology, Campus University Tafira, Las Palmas Gran Canaria 35017, Canary Isl, Spain. E-mail: mjcaballero@iccm.rcanaria.es*

18) Cahu, C.L., and Zambonino Infante, J.L., Quazuguel, P., and LeGall, M.M. 1999. Protein hydrolysate vs. fishmeal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. Aquaculture, 171(1–2): 109–119. In diets H19, H38 and H58, 25, 50 and 75 % of the fishmeal was replaced by commercial fish protein hydrolysate (CPSP). The highest survival was obtained in the group fed H19 (47%). Final weights of larvae fed H0 and H19 diets were significantly higher than those of larvae fed H38 and H58. At day 41, the highest trypsin secretion levels were obtained in groups fed H0 and H19, suggesting a proper maturation of pancreas digestive function. The incorporation of 19 and 38 % hydrolysate in diets induced a high level of two membranous enzymes of intestine, alkaline phosphatase (AP) and aminopeptidase (lap) as early as day 20. The cytosolic enzyme leucine-alanine peptidase was also assayed. The ratios of AP/leu-ala and lap/leu-ala revealed that the development of the intestine was more advanced in the H19-fed group than in the others. The proper onset of intestinal digestive function was associated with good larval survival. The experiment showed that the incorporation of a moderate dietary level of fish protein hydrolysate facilitates the onset of the adult mode of digestion in developing fish. *Dr C. L. Cahu, IFREMER, INRA, Unite Mixte Nutr Poissons, BP 70, F 29280 Plouzane, France. E-mail: ccahu@ifremer.fr.*

19) Calcedo-Juanes, E. 1989. Growth and feed intake of turbot (*Scophthalmus maximus* L.) on a silage-based feed. *In* Beardsell, M.F. (Editor). Proc. Aquaz 1988: A national Conference on Aquaculture, Wellington, 6–8 September 1988. N.Z. Fish. Occas. Publ. Vol. 4 p.115. Silage fed fish showed poorer feed conversion efficiency and growth than those fed a control diet. *Ministry of Agriculture and Fisheries, Wellington, New Zealand.*

20) Cowey, C. B., Adron, J. W., and Blair, A. 1970. Studies on the nutrition of marine flatfish. The essential amino acid requirements of plaice and sole. J. Mar. Biol. Assoc. UK, 50: 87–95. Group-0 plaice and sole of 2–3 g wet weight were each given intraperitoneal injections of [U-14C]glucose. They were kept under controlled conditions

and the metabolism of glucose in the whole animal was followed by measuring the rate of release of 14-CO_2 and other labelled metabolites into the environment. After six days the animals were sacrificed and fractionated into trichloracetic acid-soluble compounds, lipids, nucleic acids and protein. The protein was hydrolysed and the constituent amino acids isolated by ion-exchange chromatography and finally purified by partition chromatography. There was little or no incorporation of 14-C into arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine and phenylalanine. It was inferred that plaice and sole cannot synthesize these amino acids from ordinary available materials; the fish thus have an absolute dietary requirement for them. Radioactive carbon was incorporated into aspartic and glutamic acids, cysteine, serine, glycine, alanine, and proline. It is likely that these amino acids are not indispensable dietary constituents in these fish. Radioactive carbon from glucose was not incorporated into tyrosine. Tyrosine is probably derived solely by the hydroxylation of ingested phenylalinine. *Natural Environmental Research Council, Fisheries Biochemical Research Unit, University of Aberdeen; Aberdeen, Scotland, U.K.*

21) Cowey, C. B., Pope, J. A., Adron, J. W., and Blair, A. 1971. Studies on the nutrition of marine flatfish. Growth of the plaice *Pleuronectes platessa* on diets containing proteins derived from plants and other sources. Marine Biology, 10: 145–153. The growth of 0-group *Pleuronectes platessa* L. on diets containing plant proteins was examined. Diets were made acceptable to the fish by including in them cod flesh which had been pre-digested with proteolytic enzymes, as well as a proportion of air-dried cod meal. A leaf-protein concentrate was fed at 6 levels ranging from 0 to 35 % of the total dietary protein; B. P. protein concentrate (Toprina) and soybean meal were examined at a single level - about 45 % of the dietary protein. The remainder of the protein in these diets was animal protein, cod meal or cod flesh. Analysis of the weight gains after 12 weeks' feeding period and of the growth curves showed that, as the proportion of the leaf protein in the diet increased, the growth rate diminished, the effect becoming more pronounced when leaf protein reached 40 % or more of the dietary protein. Growth on the diet containing B. P. protein concentrate was superior to that on the diet containing soybean meal and was comparable with diets containing low levels of leaf protein. The protein efficiency ratios (PER = g live weight gain/g protein fed) for the different diets showed similar trends to the growth rates. Diets containing 40 % or less of leaf protein and the B. P. protein diet had PERs of 1.4 to 1.5. The soybean meal diet and diets containing more than 40 % of the protein as leaf protein had PERs of 1.0 or less. The results are considered in the context of production diets for flatfish. The leaf protein was prepared from rye grass. *Natural Environmental Research Council, Fisheries Biochemical Research Unit, University of Aberdeen; Aberdeen, Scotland, U.K.*

22) Davis, D.A., Jirsa, D., and Arnold, C.R. 1995. Evaluation of soybean proteins as replacements for menhaden fishmeal in practical diets for the red drum *Sciaenops ocellatus*. Journal of the World Aquaculture Society, 26(1): 48–58. A series of growth trials was conducted to evaluate the use of soy protein as a replacement for fish protein in isonitrogenous practical diets for juvenile red drum *Sciaenops ocellatus*. Feeds were offered at or in excess of satiation to juvenile red drum maintained at 26–28°C and a salinity of 25–35 ppt. In the first growth trial, red drum were offered one of four diets containing graded levels of menhaden fishmeal, replacing solvent-extracted soybean meal and soy-protein isolates. Differences in weight gain, survival and feed efficiency ratios of the fish corresponded to increases in fishmeal content of the diets. Due to poor performance of the fish maintained on the low (15 %) fishmeal diet, a methionine supplement was introduced into this diet at the midpoint of the growth trial. A positive increase in growth indicated a dietary deficiency of methionine and/or total sulfur amino acids in the unsupplemented diet. A positive response to dietary fishmeal also occurred in the second growth trial despite the supplementation of L-methionine in the test diets. In low fishmeal diets, the utilization of solvent-extracted soybean meal or a soy-protein isolate resulted in similar growth responses. Hence, the presence of an antinutrient did not likely cause reduced growth rates. In the third feeding trial, weight gain also increased with increasing fishmeal content of the diet despite the equalization of digestible protein and selected amino acids. There were no significant differences in whole-body compositions, which indicated similar biological value of the diets (protein digestibility, amino acid balance and energy availability). The singular deletion of fish-solubles, glycine, lysine and methionine from the diet containing the lowest level of fishmeal (10 g/100 g diet) did not result in significant changes in weight gain. This indicated that these components did not add to the nutritive value and/or palatability of this formulation. The final experiment was designed to evaluate the response of red drum to a control diet (high fishmeal) as compared to a low fishmeal diet with and without potential attractants/palatability enhancers. Weight gain and feed efficiency ratios of fish offered the low fishmeal diet supplemented with seafood flavor or fish flavor #2 were not significantly different from the control (high fishmeal diet). Based on the results of this study, with suitable formulation restrictions, soy protein is acceptable for inclusion in practical diet formulations for red drum. However, soy protein itself does not appear replete in sulfur-containing amino acids and does not have acceptable palatability properties. Consequently, feeds containing reduced levels of marine proteins could require suitable attractants and/or amino acid supplements. *Dr D. A. Davis, University of Texas at Austin, Marine Science Institute, P.O. Box 1267, Port Aransas, TX 78373, USA.*

23) Davis, S. J., Negas, I., and Alexis, M. 1993. Partial substitution of fishmeal with different meat meal products in diets for seabream *Sparus aurata*. *In:* Kaushik, S. J., Luquet, P. (Eds.) Fish Nutrition in Practice; Meeting, Birritz, France, June 24–27, 1991. INRA (Institut National de la Recherche Agronomique): Paris, France. ISBN 2–7380–0449–0. Colleques de L'INRA Vol. 61: 907–911. Feed conversion ratios (FCR) ranged from 1.5–1.6, and specific growth rates (SGR) together with protein utilization indices were found to be similar for all diets. It was concluded

that meat and bone meal can replace up to 40 % of the fishmeal component in practical diets for seabream. *Fish Nutrition Unit, Department of Biological Sciences, Polytechnic South West, Drake Circus, Plymouth, Devon PL4 8AA, UK.*

24) Day, O.J., and Gonzalez, H.G.P. 2000. Soybean protein concentrate as a protein source for turbot *Scophthalmus maximus* L. Aquaculture Nutrition, 6(4): 221–228. In the first of two experiments, the effect of a gradual substitution of dietary fishmeal with soybean protein concentrate (SPC) on growth, feed consumption and protein digestibility was examined in 13 g turbot *Scophthalmus maximus*. Five isonitrogenous and isoenergetic diets (50 % protein and 22 kJ/g) containing SPC at protein replacement levels of 0, 25, 50, 75 and 100 % were offered by hand twice daily. Growth rates of fish fed diets with zero and 25 % replacement were not significantly different, with SGRs of 2.47 and 2.28, respectively. At higher replacement levels, growth rates decreased significantly with SGRs of 2.00, 1.33 and 0.68, respectively. Feed conversion ratios increased with soya replacement, with values of 0.68, 0.75, 0.89, 1.27 and 2.32, respectively, although there was no significant difference between the first two. Feed consumption rates remained constant up to 50 % replacement, above which they decreased significantly, possibly because of reduced diet palatability. Apparent protein digestibility (APD) was not affected by the incorporation of SPC and ranged from 82.8 to 87.5 %. Results suggest that protein catabolism increases in SPC-rich diets, possibly because of rapid assimilation and utilization of the methionine supplement. In the second experiment, the importance of amino acid supplements and the beneficial effects of protecting these, either by coating them in protein or incorporating them in a protein-lipid emulsion, was investigated. Growth data provided some indication that the utilization of SPC may be improved by incorporating the methionine and lysine supplement in a protein-lipid emulsion prior to diet preparation, although this finding was not found to be statistically significant ($0.1 < P < 0.2$). Dr O. J. Day, Conwy Lab, Centre for Environmental Fisheries and Aquaculture Science, Conwy, Wales.

25) DeSilva, S.S., Gunasekera, R.M., and Gooley, G. 2000. Digestibility and amino acid availability of three protein-rich ingredient-incorporated diets by Murray cod *Maccullochella peelii peelii* (Mitchell) and the Australian shortfin eel *Anguilla australis* Richardson. Aquaculture Research, 31(2): 195–205. In this study, the apparent dry matter (ADM), protein (PD) and energy (ED) digestibility, and the amino acid availability (essential, EAAA; nonessential, NEAAA; total, TAAA) of diets incorporated with one of three protein-rich ingredients (soybean meal, shark meat meal waste and meat meal) were evaluated for Murray cod *Maccullochella peelii peelii* (Mitchell) and the Australian shortfin eel *Anguilla australis* Richardson. The reference diets (RDs) used for Murray cod and shortfin eel had 50 % and 45 % protein, and 10 % and 15 % lipid, respectively. The test diets consisted of 30 % ingredient and 70 % RD, and digestibility estimations were made using chromic oxide as a marker. In both species, the highest ADM and PD of the test diets were observed for shark meat meal (73.1 ± 1.58 and 87.5 ±1.27) and soybean meal (70.6 ± 0.82 and 86.5 ± 0.49) diets respectively. The PD of meat meal-incorporated diets was the lowest and. In shortfin eel, significantly so compared with all the experimental diets. For any one species, the ED of the diets did not differ significantly. The above observations were also reflected in dry matter and nutrient digestibility of ingredients. Significant differences ($P < 0.05$) in EAAA among diets were evident in both species. In shortfin eel, TEAAA for the meat meal-incorporated diet (50.5 ± 4.25) was significantly lower than for all the other diets. In Murray cod, TEAAA, TNEAAA and TAAA (89.7 %, 82.1 % and 85.2 % respectively) were significantly higher (except for TNEAAA) for the meat meal-incorporated diet than for the reference diet (84.5 %, 77.6 % and 80.6 % respectively), and all essential amino acids of this diet were available in excess of 82 %. The results indicate species differences in the utilization of ingredients. The present data on PD and EAAA, combined with previously published data, indicate a close correlation between these two parameters, suggesting that PD may provide a fairly reliable indication of the amino acid availability. *Dr S. S. De Silva, Deakin University, School of Aquatic Science & Natural Resources Management, POB 423, Warrnambool, Vic 3280, Australia.*

26) El-Sayed, A.F.M. 1994. Evaluation of soybean meal, spirulina meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. Aquaculture, 127(2–3): 169–176. The present study was designed to evaluate the use of soybean meal (SBM), spirulina meal (SM) and chicken offal meal (COM) as protein sources for silver seabream fingerlings. SBM and SM were incorporated into isonitrogenous (40 % protein), isocaloric (450 kcal GE/ 100 g) diets to replace 25, 50, 75 and 100 % of the fishmeal (FM) protein in the control diet, while COM was included at 25, 50 and 75 % levels. The results revealed that silver seabream utilized SM more efficiently than either SBM or COM. Growth rates and feed utilization efficiency of fish fed on SBM and COM at 25 % substitution level were not significantly different from those fed the FM-based diet. Further increase in SBM and COM in the diets resulted in growth retardation. The growth of fish fed SM up to 50 % level was not significantly different from, while feed utilization efficiency was superior to, those offered the FM diets. At 75 % level, growth rates were significantly reduced, while feed utilization efficiency was still comparable to the control diet and sharply reduced at 100 % level. Body protein was negatively correlated with SBM, SM and COM levels in the diets. Body lipid and ash contents were positively correlated with dietary COM and SBM, respectively, and were not significantly affected by other protein sources. This study demonstrated that SBM, COM and SM could replace 25, 25, and 50 %, respectively, of FM protein in practical diets of silver seabream without any adverse effects on fish growth. *Dr A. F. M. El-Sayed, University of Qatar, Faculty of Science, Dept. of Marine Science, PO Box 2713, Doha, Qatar.*

27) Elangovan, A., and Shim, K.F. 2000. The influence of replacing fishmeal partially in the diet with soybean meal on growth and body composition of juvenile tin foil barb (*Barbodes altus*). Aquaculture, 189(1–2): 133–144. Juvenile *Barbodes altus* (0.90 ± 0.02 g body weight) were fed 42 % crude protein diets in which dietary protein was supplied by brown fishmeal (FM) (60 % crude protein) or an isonitrogenous mixture of soybean (defatted, with hulls, 44 % crude protein) and PM. Diet 1 was a control diet containing 73 % FM (100 % fishmeal protein (FP)) as a protein source and without any soybean meal (SP). Diets 2–4 contained 27 %, 37 %, and 52 % SBM with FM (ratios of FP to soybean meal protein (SBM) were 3:1, 2:1, and 1:1, respectively). Fish fed the diet containing FP/SP ratio of 1:1 had significantly ($P < 0.05$) lower weight gain and feed efficiency than fish fed with the other diets. There was no significant difference in body weights of fish fed with the other three diets. When compared to the control (Diet 1), fish fed with the other three diets did not show any significant difference in body protein content. However, body fat content was significantly higher in fish fed with the control diet than the fish fed with diets containing FP/SP ratios of 2:1 and 1:1. Whole-body ash content was significantly lower in fish fed with a diet containing fish-protein/soy-protein ratio of 1:1 than in those fed the other diets. The results of the present study indicate that SBM may be included in the diet up to 37 % as a substitute for FM, replacing about 33 % of FP. *Dr A. Elangovan, National University of Singapore, Department of Biological Sciences, Nutrition Lab., 10 Kent Ridge Crescent, Singapore 119260, Singapore.*

28) Eusebio, P. S., and Coloso, R. M. 2000. Nutritional evaluation of various plant protein sources in diets for Asian sea bass *Lates calcarifer*. Journal of Applied Ichthyology, 16: 56–60. A biological assay was conducted to evaluate the suitability of various leguminous seed meals and leaf meals as dietary protein sources for Asian sea bass, *Lates calcarifer*. In the growth experiment, fish (initial mean weight + standard error (SE) of 3.8 ± 0.5 g) were fed isonitrogenous and isocaloric diets containing test ingredients to replace 13–18 % of the diet. The same diet formulations were used in a digestibility experiment, except that 1 % Cr20, was added as an external indicator. The growth of the control fish was comparable to fish fed leguminous seed meal-based diets, and better than those given leaf meal-based diets. The control diet had the highest apparent protein digestibility (APD) value. No significant differences were observed between the APD of white cowpea (*Vigna unguiculata*), green mungbean (*V. radiata*) and Oapaya (*Carica papaya*) leaf meal-based diets. However, the cassava (*Manihot esculenta*) leaf meal-based diet had the lowest APD value. The present findings suggest that white cowpea and green mungbean meals can be used as protein sources in practical diets to replace 18 % of the sea bass diet without affecting their growth. *Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Phillipines.*

29) Francis, G., Makkar, H. P. S., and Becker, K. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199(3–4): 197–227. The use of plant-derived materials such as legume seeds, different types of oilseed cake, leaf meals, leaf protein concentrates, and root tuber meals as fish feed ingredients is limited by the presence of a wide variety of antinutritional substances. Important among these are protease inhibitors, phytates, glucosinolates, saponins tannins, lectins, oligosaccharides and non-starch polysaccharides, phytoestrogens, alkaloids, antigenic compounds, gossypols, cyanogens, mimosine, cyclopropenoid fatty acids, canavanine, antivitamins, and phorbol esters. The effects of these substances on finfish are reviewed. Evidently, little unanimity exists between the results of different studies as to the specific effects of antinutrients, since most studies have been conducted using an ingredient rich in one particular factor and the observed effects have been attributed to this factor without considering other antinutrients present in the ingredient, or interactions between them. Tentatively, protease inhibitors, phytates, antigenic compounds, and alkaloids, at levels usually present in fish diets containing commercially available plant-derived protein sources, are unlikely to affect fish growth performance. In contrast, glucosinolates, saponins, tannins, soluble non-starch polysaccharides, gossypol, and phorbol esters, are more important from a practical point of view. The effectiveness of common processing techniques such as dry and wet heating, solvent extraction and enzyme treatment in removing the deleterious effects of antinutrients from feed materials is discussed. More insights into the nutritional, physiological and ecological effects of antinutrients on fish need to be accumulated through studies using purified individual antinutrients and their mixtures in proportions similar to those in alternative nutritional sources in fish feeds. Such studies would provide data useful for designing optimum inclusion levels of plant-derived materials and treatment methods that would neutralise the negative effects of the antinutritional factors. *Dr Klaus Becker, Department of Animal Nutrition and Aquaculture, Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim (480), D 70593 Stuttgart, Germany. E-mail: kbecker@uni-hohenheim.de.*

30) Gaylord, T. G., and Gatlin, D. M., III. 1996. Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). Aquaculture, 139: 303–314. The availability of nutrients and energy in feedstuffs to fish may vary considerably, depending on a variety of factors including fish species, ingredient quality and processing conditions. The red drum is an emerging aquaculture species for which information concerning nutrient and energy availability is needed to more precisely formulate diets to meet their requirements and to enable effective substitution of ingredients. This study was conducted with red drum to determine apparent organic matter, protein, lipid, and gross energy digestibility coefficients and apparent phosphorus availability of the following ingredients: select (low temperature) menhaden fishmeal; regular-quality menhaden fishmeal, poultry by-product meal, meat and bone meal, dehulled soybean meal, cottonseed meal, and wheat. Test diets consisting

of a 70:30 mixture of reference diet to test ingredient were utilized with chromic oxide as the non-digestible marker. Organic matter digestibility of ingredients generally decreased as the nitrogen-free extract fraction increased, ranging between 94 % for select menhaden fishmeal to 47 % for wheat. Crude protein digestibility was high for most ingredients, ranging from 77 % for regular menhaden fishmeal to 97 % for wheat, with the exception of poultry by-product meal, which was 49 %. Lipid digestibility coefficients ranged from 59 % for poultry by-product meal to 88 % for wheat. Digestible energy coefficients were generally high for the animal meals, ranging from 72 % for poultry by-product meal to 95 % for select menhaden fishmeal; however, digestible energy coefficients for plant feedstuffs were considerably lower, from 62 % for wheat to 70 % for cottonseed meal. Phosphorus availability from animal products was variable, with a low of 27 % for poultry by-product meal and a high of 66 % for meat and bone meal. Phosphorus availability from soybean meal and cottonseed meal was 47 % and 40 %, respectively. Wheat had the highest phosphorus availability at 79 %. Data from this study indicate red drum can digest and absorb the nutrients in animal products more completely than those from plant products. This difference presumably reflects their inability to effectively digest the nitrogen-free extract portion of plant products. These data provide more precise information concerning nutrient and energy utilization of red drum and will allow ingredient substitutions in practical diet formulations based on levels of available nutrients. *Dr Delbert M. Gatlin III. Department of' Wildlife and Fisheries Sciences and Faculty of Nutrition, Texas A &M University System, College, Station, TX 77843, USA. E-mail: d-gatlin@tamu.edu.*

31) Geurden, I., Coutteau, P., and Sorgeloos, P. 1997. Increased docosahexaenoic acid levels in total and polar lipid of European sea bass (*Dicentrarchus labrax*) postlarvae fed vegetable or animal phospholipids. Marine Biology, 129(4): 689–698. A 6-week feeding trial was conducted with 44-d-old European sea bass (*Dicentrarchus labrax* L.) in order to examine the effect of various dietary phospholipid (PL) sources on the incorporation of n-3 highly unsaturated fatty acids (HUFA) in tissue lipids. From weaning onwards the fish received diets prepared by coating different lipid fractions (7.5 % diet) on an extruded basal diet (92.5 % diet). The two PL-free control diets contained 0.5 and 2 % of an emulsifier blend, respectively. Seven other diets contained 2 % FL, differing by their purity and origin (vegetable or animal). All diets were rendered isolipidic by the addition of hydrogenated coconut oil. Feeding the PL-supplemented diets, except the diet containing hydrolyzed soybean PL (lyse FL), resulted in a higher survival and a 10 to 30 % better growth as compared to the PL-free diets. No difference according to the PL origin was observed. The sea bass final lipid content increased with increasing body weight. Also the lipid class composition of the fish was clearly correlated with the final weight gain. Total neutral lipid increased from 51 % of total lipid (initial fish) to 76 % for fish fed the PL-free diets, and up to 88 % for fish fed the sunflower FL. Weaning the fish on the experimental diets induced important changes in their fatty acid profiles characterized by a decrease in 18:3 n-3, 20:5 n-3 and 20:4 n-6 and an increase in saturated fatty acids and 22:6 n-3 (DHA). According to the fatty acid composition of both total and polar lipid, the weaned fish could be divided into three groups reflecting the dietary fatty acids: a group fed the vegetable FL, a group fed the animal PL and a PL-deprived group. An effect of dietary PL on the incorporation of dietary n-3 HUFA, more particularly DHA, was noticed. For a similar supply of DHA through the neutral lipids in the diet, fish fed PL-supplemented diets (except for the lyse PL diet) had 10 to 25 % higher DHA levels in total and polar lipid than PL-deprived fish. This PL effect was already clear at the end of the weaning and was not related to the presence of n-3 HUFA in the PL source, as suspected in a previous study when feeding egg yolk FL. A better incorporation of DHA was not obtained by replacing the PL by an emulsifier or by lyso PL with higher emulsifying properties. Present results confirm a role of dietary PL in the absorption of dietary neutral lipids, by a mechanism other than emulsification. *Dr I. Geurden, State University of Ghent, Laboratory of Aquaculture, Rozier 44, B 9000 Ghent, Belgium.*

32) Gomes da Silva, J., and Oliva-Teles, A. 1998. Apparent digestibility of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. Aquatic Living Resources, 11: 187–191. A number of feedstuffs were tested to obtain the apparent digestibility coefficients (ADC) of dry matter, protein, energy and phosphorus for sea bass juveniles (individual mean weight of 40 g). The effect of the inclusion level (15 and 30 %) of a feedstuff in a reference diet on the ADC was also evaluated. The feedstuffs tested in this trial were two fishmeals, a fish protein hydrolysate, blood meal, meat meal, soybean meal and yellow dextrin. The ADC of dry matter was consistently lower than those of energy, but there was a significant positive correlation between them. The ADC of protein was high (range 89 %–97 %) in all feedstuffs. The ADC of energy of animal feedstuffs (range 86 %–96 %) and dextrin (93 %) were also high, while that of soybean was lower (70 %). The ADC of phosphorus of animal feedstuffs averaged 81 % whereas that of soybean was only 38 %. There were no significant differences in the ADC of protein and energy of each feedstuff at the two inclusion levels tested. *Dr A. Oliva-Teles, Fac. Ciencias., Dept. Zool. & Antropol., P 4050 Oporto, PORTUGAL. E-mail: aoteles@fc.up.pt.*

33) Goto, T., Takagi, S., Ichiki, T., Sakai, T., Endo, M., Yoshida, T., Ukawa, M., and Murata, H. 2001. Studies on the green liver in cultured red seabream fed low level and non-fishmeal diets: Relationship between hepatic taurine and biliverdin levels. Fisheries Science, 67(1): 58–63. The aim of this study was to investigate the cause of green liver in red seabream fed substitute protein diets. Red seabream *Pagrus major* was given either of the following diets for 28 weeks: (1) control diet (50 % fishmeal), (2) low level fishmeal diet (15 % fishmeal), and (3) non-fishmeal diet (0 % fishmeal). The green liver was observed in all groups tested, but the incidence was much higher in the experimental diet groups. The feeding of substitute protein diets reduced plasma triglyceride and cholesterol levels. However, there was no significant difference in plasma hepatic enzyme activities and plasma bile salts

concentration among the treatments. Fish fed the substitute protein diets showed low hepatic taurine levels with an appearance of a biliverdin in the liver. Moreover, the proportion of ditaurobilirubin to total biliary bile pigments was significantly lower in fish fed the substitute protein diets. These data indicate that feeding of substitute protein diets did not induce any cholestatic hepatobiliary obstructions and that the low hepatic taurine level was one of the probable factors responsible for the occurrence of green liver in red seabream fed substitute protein diets. Dr T. Goto, Numazu College of Technology, Department of Chemistry and Biochemistry, Shizuoka 4108501, Japan.

34) Gouveia, A., and Davies, S.J. 1998. Preliminary nutritional evaluation of pea seed meal (*Pisum sativum*) for juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture, 166(3–4): 311–320. An 84-day feeding and digestibility trial was conducted to evaluate the use of a pea seed derived meal in experimental diets for European sea bass fingerlings of initial weight 10 g. It was demonstrated that up to 40 % pea seed meal inclusion was feasible in diets allowing for a 12 % reduction in fishmeal content and a 25 % substitution of carbohydrate content without appreciable loss in growth performance of juvenile sea bass or diet utilization. The data showed no difference in final weight and specific growth rate measurements, and feed utilization was not compromised by the supplementation of the reference fishmeal basal diet with pea seed meal. The diets were formulated to be essentially isonitrogenous and isocaloric and the results of the digestibility trial confirmed that the availability of the protein and energy was consistent between the different dietary treatments. However, carbohydrate digestibility was markedly affected by the nature of the pea seed meal starch matrix and contributed to a significant reduction in digestible energy. It was also evident that this affected carcass composition with respect to energy deposition (lipid content), but not overall growth of fish in the relatively short duration of this trial. *Dr A. Gouveia, Fac Ciencias, Dept Zool & Anthropol, P 4050 Oporto, Portugal.*

35) Gouveia, A., and Davies, S.J. 2000. Inclusion of an extruded dehulled pea seed meal in diets for juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture, 182(1–2): 183–193. A feeding trial and subsequent digestibility trial were performed on juvenile European sea bass. The diet formulation allowed for the incorporation of up to 30 % of a highly processed commercial pea seed meal as a replacement for both fishmeal and the non-protein energy component of the diet (mainly starch). After eleven weeks, it was observed that a positive but non-significant trend existed for both growth and feed utilization with increasing incorporation of pea seed meal in the diets. There were, however, other nutritional parameters such as protein efficiency ratio (PER) and nitrogen deposition that increased according to graded levels of pea seed meal indicating that a high inclusion of this ingredient was beneficial to protein assimilation. Digestibility coefficients for protein, lipid and carbohydrates were not appreciably affected by the inclusion of this ingredient which was also reflected with respect to the overall carcass composition of sea bass at the end of the study. *Dr A. Gouveia, Fac Ciencias, Dept Zool & Antropol, P 4099002 Porto, Portugal.*

36) Grisdale-Helland, B., and Helland, S.J. 1998. Macronutrient utilization by Atlantic halibut (*Hippoglossus hippoglossus*): diet digestibility and growth of 1 kg fish. Aquaculture, 166: 57–65. A nine-week growth trial, followed by digestibility determinations, was conducted to study the effects of replacing dietary protein with fat and/or carbohydrate on diet utilization and growth of 1 kg Atlantic halibut. The exchange of fishmeal for wheat had small, negative effects on fat digestibility. The increase in wheat content from 8 to 17 % resulted in a decrease in starch digestibility from 84 to 53 %. There were no significant effects of diet composition on protein digestibility. The specific growth rates (0.30 % body weight/d) and the feed efficiency ratios (1.02 g gain/g dry feed) were not significantly affected by the reduction in dietary protein content from 61 to 48 %. A minimum of 338 g digestible protein and 18 MJ digestible energy was used by the halibut/kg gain. Diet composition did not significantly affect the amount of protein lost in the feces per kg gain. Organic matter loss in the feces however, was greatest from the fish fed diets containing the highest level of carbohydrate. *Dr B. Grisdale-Helland, Akvaforsk, Institute of Aquaculture Research, N 6600 Sunndalsora, Norway.*

37) Haiqing, S., and Xiqin, H. 1994. Effects of dietary animal and plant protein ratios and energy levels on growth and body composition of bream (*Megalobrama skolkovii* Dybowski) fingerlings. Aquaculture, 127: 189–196. Isonitrogenous, practical diets with varying levels of fishmeal (6–24 %) and energy (351–381 kcal/100 g) were fed to triplicate groups of bream fingerlings for 60 days to determine the amount of plant protein that could be substituted for fishmeal protein in formulated diets. Results indicate that specific growth rate increased and feed conversion decreased as the inclusion level of fishmeal increased. However, the growth performance among the fish fed diets containing 12–24 % fishmeal did not differ significantly ($P > 0.05$). Low-energy diets were superior to high-energy diets. The optimum protein/energy (P/E) ratio for bream fingerlings was found to be 88–90 mg protein/kcal. The optimum dietary lipid level was found to be 6–8 %. No significant effect of varying dietary fishmeal or energy content on body composition was found. *Dr Sheng Haiqing, Institute of Reservoir Fisheries, Ministry of Water Resources, Chinese Academy of Science, Wuha, Hubei, 430073, People's Republic of China.*

38) Hara, K., Yamamoto, R., and Ishihara, T. 1986. Nutritive effect of pig blood as a protein source in diet for red seabream – I. Experiment of long period feeding. Bull. Fac. Fish. Nagasaki Univ./Chodai Suikenpo, 60: 61–69. Studies on the nutritive effect of the dried powder from pig blood in the culture of fish were attempted, aiming at the protein efficiency of the blood as protein source, in which protein content as dry matter is widely known to be high. Red seabream were divided into four groups and fed (1) minced sardine, (2) minced sardine containing commercial compound feed (1:0.25 W/W) (3) minced sardine containing pig blood powder (1:0.25), (4) minced

sardine containing commercial compound feed and pig blood powder (1:0.125:0.125). There were no significant differences between the four groups as to the blood hemoglobin content, hematocrit value, plasma protein content, plasma cholesterol content or glutamic oxaloacetic transaminase activity of plasma. The groups 3 and 4 showed good growth and higher feed efficiency than groups 1 and 2, but with low protein efficiency of group 3.

39) Ishida, R., and Shimma, Y. 1979. Effects of single cell protein feeds on the growth and fatty acid composition of au, *Plecoglossus altivelis*. Bull. Tokai Reg. Fish. Res. Lab. 100: 161–167. In all trials, the control fish grew better than the SCP fish. In the SCP fish, amounts of 17:1 acid were 4.3 % in trunk flesh, 3.8 % in gonad, and 2.6–3.0 % in liver due to its high level (34 %) in the yeast lipids, while amounts of 20:5 and 22:6 acids were lower than those of the control.

40) Jeon, Y.J., Byun, H.G., and Kim, S.K. 2000. Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. Process Biochemistry, 35(5): 471–478. Enzymatic hydrolysis was applied for the efficient recovery of the protein sources from the fish processing by-product, cod frame. The enzyme used for the hydrolysis was crude proteinase extracted from tuna pyloric caeca. The resultant hydrolysate, cod frame protein hydrolysate (CFPH), was separated based on the molecular weight of the peptides in the hydrolysate and several functional properties were examined, including physicochemical properties (emulsifying and foaming property) and bioactivities (antioxidative and angiotensin I converting enzyme (ACE) inhibitory activity) to determine its potential functions. CFPH was processed through a series of ultrafiltration (UF) membranes with molecular weight cut-off (MWCO) of 30, 10, 5 and 3 kDA, and four types of permeates including 30-K (permeate from 30 kDA), 10-K (permeate from 10 kDA), 5-K (permeate from 5 kDA) and 3-K hydrolysate (permeate from 3 kDA) were obtained. 10- and 30-K hydrolysates showed excellent emulsion properties and whippability. The 10-K hydrolysate showed high antioxidative activity, while the 3-K hydrolysate had excellent ACE inhibitory activity. In terms of all functional properties tested, the fractionated hydrolysates were superior to the original non-separated hydrolysate. These results suggested that separating hydrolysates enhanced several functional properties. *Dr S. K. Kim, Pukyong National University, Department of Chemistry, Pusan 608737, South Korea.*

41) Kalogeropoulos, N., Alexis, M.N., and Henderson, R.J. 1992. Effects of dietary soybean and cod-liver oil levels on growth and body composition of Gilthead bream, *Sparus aurata*. Aquaculture, 104(3–4): 293–308. Gilthead bream of 1 g mean weight were fed six purified diets supplemented with 12 % lipid composed of different proportions of soybean oil (SBO) and cod-liver oil (CLO). The experiment lasted for five months. Fish performance and body composition, as well as the fatty acid composition of liver phospholipids, were studied. Fish performance improved with increasing dietary CLO content, approaching an optimum at about 6 % CLO. Higher values for liver fat content and hepatosomatic index were observed for diets containing less than 6 % CLO, suggesting a deficiency of essential fatty acids in these diets. Liver fatty infiltration with low CLO diets was high although a certain degree of fatty infiltration was apparent in livers for all the dietary treatments. Body composition of fish was also affected by dietary lipid composition. Higher protein and lower fat contents were observed for the fish fed low levels of CLO. The eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content of liver phospholipids was high, approaching a constant value after inclusion of 6 % CLO. The levels of 20:4 n-6 were also found to approach a plateau at this level. Saturated and n-9 fatty acid content remained almost stable, while the level of 18:2 n-6 in liver phospholipids reflected the amount of SBO in the diet. *National Centre for Marine Research, Aghios Kosmas, Helliniko, GR 16604, Athens, Greece.*

42) Kikuchi, K. 1999. Use of defatted soybean meal as a substitute for fishmeal in diets of Japanese flounder (*Paralichthys olivaceus*). Aquaculture, 179(1–4): 3–11. Feeding experiments were conducted to examine the potential use of defatted soybean meal (SBM) in combinations with blood meal (BM), corn gluten meal (CGM), and freeze-dried meat of blue mussel as a partial replacement of fishmeal in the diet of Japanese flounder. Juvenile fish of about 5 g in initial body weight were fed diets to satiation twice daily, 6 days per week for 8 weeks at 20 degrees C. The control diet contained 75 % fishmeal as the protein source, while 35 or 45 % fishmeal was replaced with other sources of protein in the experimental diets (replacing approximately 45 or 55 % of fishmeal protein in the control); 25, 30, or 40 % SBM, 10 % BM or CGM, and 5 % freeze-dried meat of blue mussel. Diets containing 25 % SBM in combination with BM or CGM and the blue mussel (replace 47 or 44 % of fishmeal protein) resulted in best growth and feed utilization among all dietary groups tested. The final body weight, weight gain, and protein efficiency ratio in these dietary groups were higher than those for the control ($P < 0.05$). The weight gain of fish fed diets containing 40 % SBM (replace 43 %), and 30 % SBM in combination with 10 % BM or CGM (replace 47 or 45 %), were comparable to that of the control, however, feed efficiency and protein efficiency ratios were significantly lower except the feed efficiency for the 30 % SBM and 10 % BM diet ($P < 0.05$). Diets containing 40 % SBM and 10 % BM or CGM (replace 59 or 57 %) showed inferior growth and feed utilization compared to the control ($P < 0.05$). There were no marked differences in hematological characteristics together with proximate composition of the whole body of cultured fish fed the dietary treatments. These results indicate that about 45 % of fishmeal protein can be replaced with SBM in combination with other protein sources in the diet of juvenile Japanese flounder. *Dr K. Kikuchi, Central Research Institute for the Electric Power Industry, Abiko Research Laboratory, 1646 Abiko, Chiba 2701194, Japan.*

43) Kikuchi, K. 1999. Partial replacement of fishmeal with corn gluten meal in diets for Japanese flounder *Paralichthys olivaceus*. Journal of the World Aquaculture Society, 30(3): 357–363. Feeding experiments were conducted to evaluate corn gluten meal (CGM) as an alternative protein source for fishmeal in the diet of Japanese Bounder *Paralichthys olivaceus*. A diet containing 75 % white fishmeal as a sole protein source was the control, and 20, 40, and 60 % of fishmeal protein was replaced with CGM protein in the experimental feeds. Juvenile fish of about 8 g initial body weight were fed each diet to apparent satiation twice a day, 6 d per week for 8 wk at 20° C. Survival rates of fish ranged from 98 to 100 % and were not significantly different ($P > 0.05$) among treatments. Final body weight, weight gain, feed efficiency, and protein efficiency ratio of fish fed the diets containing CGM up to 40 % substitution levels were not statistically different from those of fish fed the control diet. All production parameters for fish fed the diet replacing 60 % of fishmeal protein were significantly lower than the control (P less than or equal to 0.05). Supplements of crystalline amino acids to the CGM diet improved the nutritive value of the diet. Since substitution up to 40 % did not adversely affect hematological and hematochemical parameters as well as whole body composition of the cultured fish, it is suggested that up to 40 % of fishmeal protein can be replaced with CGM in the diet of juvenile Japanese flounder. *Dr K. Kikuchi, Center for Research Inst. Elect. Power Industry, Abiko Research Lab., Chiba 2701194, Japan.*

44) Kikuchi, K., Furuta, T., and Honda, H. 1994. Utilization of feather meal as a protein source in the diet of juvenile Japanese flounder. Fisheries Science, 60(2): 203–206. Feeding experiments were conducted at 20°C with diets containing 0, 12, 25, 37, and 50 % of feather meal to examine the potential of feather meal as a substitute for fishmeal in the diet of Japanese flounder. Juvenile fish, about 3 g in initial body weight, were fed on each diet to satiation twice a day for 6 days a week for 8 weeks. The weight gain of fish fed on the diets containing 12 and 25 % of feather meal did not differ from that of fish fed on the control diet containing 80 % of white fishmeal, however, fish fed on the 37 and 50 % feather meal diets gained less weight. The feed conversion efficiency and protein efficiency ratio of fish fed on the 12 % feather meal diet were almost the same as in the control group, however, these efficiencies decreased as the proportion of feather meal in the diet increased from 25, 37 to 50 %. Supplements of crystalline amino acids to the feather meal diet improved its nutritive value slightly. There was little difference in the proximate composition of the whole body together with the hematological and hematochemical parameters among the dietary groups tested. The present study showed that 12 to 25 % of feather meal is an appropriate substitute for fishmeal in the diet of juvenile Japanese flounder. *Dr K. Kikuchi, Central Research Institute for Electrical Power Ind., Abiko Res Lab, Abiko, Chiba 27011, Japan.*

45) Kikuchi, K., and Furuta, T., Honda, H. 1994. Utilization of soybean meal as a protein source in the diet of juvenile Japanese flounder, *Paralichthys olivaceus*. Suisanzoshoku, 42: 601–604.

46) Kikuchi, K., Honda, H., and Kiyono, M. 1993. Effect of dietary protein sources on growth and nitrogen excretion of Japanese flounder (*Paralichthys olivaceus*). *In*: Carrillo, M, Dahle, L., Morales, J., Sorgeloos, P., Svennevig, N., Wyban, J. (Editors) From Discovery to Commercialization. EAS Special Pub. 19: 400. Rearing experiments employing diets containing white fishmeal, cuttlefishmeal, soybean meal, feather meal, brewers yeast and egg white albumin as sole sources of protein were conducted to examine the effects of dietary protein source on growth of Japanese flounder. Dietary crude protein levels ranged from 41 to 51 %. Rearing trials were conducted for eight weeks with 2 g and five weeks for 25 g fish. Effects of protein source on nitrogen excretion were evaluated in 7 and 21 g fish. The results suggest that a large proportion of the fishmeal used in Japanese flounder diets can be replaced with soybean meal. *Dr K. Kikuchi, Central Research Institute for Electrical Power Ind., Abiko Res Lab, Abiko, Chiba 27011, Japan*.

47) Kikuchi, K., Honda, H., Kiyono, M., and Miyazono, I. 1993. Total replacement of fishmeal with other protein sources in the diet of Japanese flounder. Suisanzoahoku, 41: 345–351. *Central Research Institute of Electric Power Industry, Abiko, Chiba, Japan.*

48) Kikuchi, K., and Sakaguchi, I. 1997. Blue mussel as an ingredient in the diet of juvenile Japanese flounder. Fisheries Science, 63(5): 837–838. The essential amino acid composition of blue mussels comparable to white fishmeal certainly brought good growth and feed performance in this and previous studies. Because the meat of these mussels has a high protein content, it is considered that they are an excellent alternative ingredient for fishmeal in the pellet diet of juvenile Japanese flounder, one of the carnivorous fish species. The final body weight and the weight gain of fish fed blue mussel diets were higher than those in the control, because of greater feed consumption. It may be possible that there is a feeding stimulant in the blue mussel *Mytilus galloprovincialis* even after freeze-drying. *Dr K. Kikuchi, Central Research Institute of Electric Power Industry, Abiko Research Laboratory, Abiko, Chiba 27011, Japan.*

49) Kikuchi, K., Sato, T., Furuta, I., Sakaguchi, I., and Deguchi, Y. 1997 Use of meat and bone meal as a protein source in the diet of juvenile Japanese flounder. Fisheries Science, 63: 29–32. Feeding experiments were conducted at 20ºC with diets containing 0, 9, 18, 36, and 44 % of meat and bone meal (MBM) to examine the potential of MBM as a substitute for fishmeal in the diet of Japanese flounder. Juvenile fish, 2 to 5 g in initial body weight, were fed on each diet to satiation twice a day, 6 days per week, for 4 and 7 weeks (trials 1 and 2). In both trials, the final body weight of fish, the weight gain, feed efficiency (FE), and protein efficiency ratio (PER) of fish fed MBM diets tended to decrease as the proportion of MBM in the diet increased. The FE of fish fed the diet containing 44 % of MBM as well as PER of those fed 36 and 44 % MBM diets were significantly lower than those

of the control diet (0 % MBM diet). The weight gain of fish fed 36 and 44 % MBM diets was also lower than that of the control in trial 2. No significant differences in these parameters were observed among dietary groups containing 0, 9, and 18 % of MBM except the final body weight in trial 2. There was little difference in the proximate composition of the whole fish body and the hematological and hematochemical parameters between the fish fed 18 % MBM diet and the control group. *Dr K. Kikuchi, Central Research Institute of Electric Power Industry, Abiko Research Laboratory, Abiko, Chiba 27011, Japan.*

50) Kikuchi, K., Sato, T., Furuta, T., and Sakaguchi, I. 1997. Use of meat meal as a protein source in the diet of juvenile Japanese flounder, *Paralichthys olivaceus*. *In:* Hershberger, W., Mancebo, V., Chew, K. (Editors) Abstracts. World Aquaculture 1997. February 19–23, 1997. Seattle, Washington. World Aquaculture Society, LSU, Baton Rouge. LA. P. 252. The control diet contained 80 % of white fishmeal as a sole protein source. Twenty, 40, 60, 80 and 100 % of the fishmeal protein were replaced by meat meal protein with additions of crystalline methionine, lysine, and tryptophan. Another group, which did not contain crystalline amino acids, was prepared for 40 % substitute group. The weight gain and protein efficiency ratio of fish fed the diets substituting meat meal up to 60 % level were not different from those of fish fed the control diet. However, these values of fish fed the 80 and 100 % levels were significantly lower than control group. The feed efficiency of fish fed 20 and 40 % substitution levels were almost the same as in the control group, however, it decreased as the meat meal in the diet increased from 60 to 100 %. Supplements of crystalline amino acids to meat meal diet improved its nutritive value slightly. Meat meal can substitute for 60 % of fishmeal protein in the diet of juvenile Japanese flounder. *Abiko Research Laboratory, Central Research Institute of Electric Power Industry, Abiko, Chiba, 270–11, Japan.*

51) Kissil, G.W., and Lupatsch, I. 1998. Use of rapeseed (RPC) and soy protein concentrates (SPC) as partial or complete replacements for fishmeal in gilthead seabream (*Sparus aurata*) diets. *In:* VIII International Symposium on Nutrition and Feeding of fish. Recent Advances in Finfish & Crustacean Nutrition. Las Palmas de Gran Canaria, Spain, June 1–4, 1998. Page 146. Food costs can range from 35–60 % of running expenses with the protein fraction of the food accounting for about half of this expense. Protein concentrates from rapeseed (CP 59 %) and soybean (CP 61.73 %) substituted fishmeal in practical diets for gilthead seabream from 30–> 100 % (26–> 100 % on a protein basis). Small seabream (2.65 g av. wt.) grown to 19 g, a decrease in specific growth rate (3.09–> 2.48) and poorer FCR (1.15–> 1.48) with an increase in the level of RPC in the diet (only 30–> 60 % replacement used in this trial). SGR and FCR were only significantly ($p < 0.05$) affected when more than 39 % of fishmeal protein was replaced by RPC protein. Larger seabream (12 g av. wt.) grown out to 40–> 50 g displayed the same decrease in SGR (2.51–> 1.63) and poorer FCR (1.08–> 1.2) with increased RPC, but the effect was only significant ($p < 0.05$) above 53 % protein substitution for SGR and 100 % ($p < 0.05$) substitution for FCR. The effect of increased SPC was similar to that of RPC with SGR decreasing (2.41–> 1.92) and poorer FCR values (1.09–> 1.29) both being statistically significant ($p < 0.05$) from a 53 % level of protein substitution. Both concentrates were equally effective as substitutes for fishmeal in the fish size range of these trials and could partially replace fishmeal. Complete replacement would mean a 23.5 % (SPC)–> 35 % (RPC) loss in SGR and 19–> 11 % loss in FCR. An evaluation of the economic feasibility of using these concentrates in seabream feed is presented. *Dr G.W. Kissil, National Center for Mariculture, Israel Oceanographic & Limnological Research Ltd., P.O. Box 1212, Eilat 88112, Israel.*

52) Kissil, G.W., Lupatsch, I., Higgs, D.A., and Hardy, R.W. 2000. Dietary substitution of soy and rapeseed protein concentrates for fishmeal, and their effects on growth and nutrient utilization in gilthead seabream *Sparus aurata* L. Aquaculture Research, 31(7): 595–601. Soy and rapeseed protein concentrates (SPC and RPC) were evaluated as fishmeal substitutes in gilthead seabream *Sparus aurata* L. diets. The protein concentrates were used to replace 30 %, 60 % and 100 % fishmeal, and effects on feed intake, weight gain and feed gain ratio were determined in a 56-day growth trial. Some groups were then grown beyond 56 days, until all reached an average weight of 50 g. A comparison of body composition at 50 g showed no significant differences in protein and ash content among all fish, while lipid and energy contents were different. The 100 % RPC and 60 % and 100 % SPC replacement diets had lower body lipid and energy contents compared with those of the control diet. Feed intake and weight gains were inversely related to inclusion levels of plant proteins. Feed intake dropped to 52–72 % of that of the control treatment and weight gain to 46–61 %. Energy retention followed this same trend, decreasing from ERV values of 53 to 44 with an increase in dietary plant protein content. With the exception of 100 % SPC substitution (PPV = 35), protein retention among treatments was similar (PPV = 37–39). These results suggest that both SPC and RPC may be promising protein sources for inclusion in seabream diets. The relative palatability of these plant proteins could be a limiting factor in their use. *Dr George W. Kissil, Israel Oceanography & Limnology Research, National Center for Mariculture, POB 1212, IL 88112 Eilat, Israel.*

53) Kissil, G.W., Lupatsch, I., Higgs, D.A., and Hardy, R.W. 1997. Preliminary evaluation of rapeseed protein concentrate as an alternative to fishmeal in diets for gilthead seabream (*Sparus aurata*). Israeli Journal of Aquaculture Bamidgeh, 49(3): 135–143. This study was undertaken to assess the potential for including rapeseed protein concentrate (RPC) as a partial replacement for locally available fishmeal (Chilean origin) in diets for gilthead seabream cultured in Israel. Fifteen groups of seabream of 2.6 g mean weight held in 26 degrees C sea water on a natural photoperiod were each fed one of five isonitrogenous (390 g digestible protein/kg) and isoenergetic (16.3 MJ digestible energy/kg) diets twice daily at 3.5 % of their biomass for 60 days. Chilean

fishmeal in the control diet was replaced progressively (30 %, 45 %, and 60 %) by RPC so that the latter protein source replaced 26 %, 38.9 % and 53.3 % of the fishmeal protein in diets 2, 3, and 4, respectively. A fifth diet contained a blend of premium quality fishmeal, Norse LT-94(R), which replaced the Chilean source of fishmeal at the level of diet 3, and RPC. This was done to determine whether the available levels of nutrients (e.g., essential amino acids and minerals) in the premium quality meal were more complementary to those in RPC for performance of seabream relative to the local fishmeal source. Weight gains and specific growth rates of seabream after sixty days were noted to be inversely related to the dietary level of RPC when the source of fishmeal was Chilean. Fish fed diet 5, however, grew as well as those fed the control diet even though 38.9 % of the fishmeal protein in diet 5 was replaced by protein from RPC. The trend for feed utilization paralleled that for growth. The results suggest that there is potential for including high levels of RPC in diets for seabream if this protein source is blended with premium quality fishmeal. The poorer performance of seabream fed diets based upon combinations of RPC and Chilean fishmeal may indicate deficiencies of one or more essential amino acids and minerals that possibly can be corrected through appropriate dietary supplementation. *Dr G.W. Kissil, Israel Oceanology & Limnological Research, National Centre for Mariculture, POB 1212, IL 88112 Eilat, Israel.*

54) Kissil, G. Wm., and Lupatsch, I. 2000. Growth performance and nutrient utilization of gilthead seabream (*Sparus aurata*) fed a transgenic lupin (*Lupinus angustifolius* L) with enhanced methionine. Abstracts of the Ninth Inter. Symposium on Nutrition and Feeding in Fish, May 21–25, 2000, Miyazaki, Japan 155 (P66) As part of an ongoing effort to develop alternative protein sources to fishmeal, the following study was carried out with the objectives of assessing the potential for including lupin meal as a partial replacement for fishmeal in seabream diets and testing the potential growth improvement in seabream using a genetically improved lupin with higher methionine content. A digestibility study using 0.8 % chromic oxide as the inert marker and fecal collection by stripping provided apparent digestibility coefficients for the fishmeal (82 % for protein; 80 % for energy) and two lupin seed meals Warrah (92 % protein; 54 % energy) and the genetically improved TG (94 % protein; 62 % energy) used as protein sources in the growth trial. Five isonitrogenous (~450 g kg^{-1} crude protein) and isoenergetic (~ 20 MJ kg^{-1}) diets were formulated using these protein sources and each diet was fed to triplicate groups of 28 g (initial weight) seabream over 86 days. Four diets replaced 36 % of the fishmeal protein with each of the lupins and half of these diets were supplemented with crystalline DL-methionine and compared to the control all FM protein feed. Diets containing lupin meal were well accepted by seabream with no apparent feed rejection being observed. After an average 250 % weight gain in all treatments, no significant differences could be detected in weight gain (68–72 g $fish^{-1}$), feed intake (95–106 g $fish^{-1}$) or feed/gain ratio (1.4–1.5). The same was true for energy retention (44–48) and protein retention values (30–33) which were calculated on a digestible nutrient basis. The high protein digestibility and lack of feed rejection suggest that lupins could be incorporated in seabream feeds as partial replacements of FM. No advantage could be detected from either the supplementation of methionine or the use of the methionine-enhanced TG lupin. *Dr G. Wm. Kissil, Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O.Box 1212, Eilat 88112, Israel.*

55) Kolkovski, S., Tandler, A. 2000. The use of squid protein hydrolysate as a protein source in microdiets for gilthead seabream *Sparus aurata* larvae. Aquaculture Nutrition, 6: 11–15. The protein source (freeze-dried squid powder) was hydrolysed with protease (trypsin and pancreatin). Different levels of raw squid protein and hydrolysate (100 % protein, 50 % protein/50 % hydrolysate, 100 % hydrolysate) were added to the microdiets to produce a dietary protein level of 65 %. For comparison, cofeeding of *Artemia* nauplii and microdiet as well as microdiet supplemented with pancreatin were also offered to the larvae. The final average dry weights of 32-day-old larvae were 1.65± 0.04, 1.38 ± 0.06 and 1.13 ± 0.1 mg, respectively, for larvae cofed 0 %, 50 % and 100 % hydrolysate microdiets and *Artemia* nauplii. Survival of larvae was not affected by protein source. The survival of larvae cofed *Artemia* nauplii and microdiet was significantly higher than that of larvae fed exclusively on microdiet (68 % and 80 %, respectively). These results suggest that the use of hydrolysate (at 50 % or greater) as a protein source in diets for seabream larvae is not to be recommended. *Dr Sagiv Kolkovski, Fisheries Western Australia, I Fleet Street, Fremantle W. A. 6160, Australia. E-mail:* skolkovski@fish.wa.gov.au

56) Kotzamanis, Y.P., Alexis, M.N., Andriopoulou, A., CastritsiCathariou, I., and Fotis, G. 2001. Utilization of waste material resulting from trout processing in gilthead bream (*Sparus aurata* L.) diets. Aquaculture Research, 32(Suppl 1): 288–295. Fish processing creates a large amount of waste of high nutrient content which, if not properly processed for use in human or animal nutrition, is likely to be deposited in the environment creating pollution problems. Waste parts from rainbow trout processing for smoking, consisting of heads, bones, tails and intestines, were used as feed ingredients for gilthead bream diets. Heads, bones and tails had similar compositions, their weighed mean indicating about 700 g kg^{-1} moisture, 150 g kg^{-1} protein and 110 g kg^{-1} fat. Intestines contained higher lipid (350 g kg^{-1}) and lower moisture (560 g kg^{-1}) and protein content (80 g kg^{-1}). Seasonal changes in composition indicated significant differences. Three experimental diets were formulated having the same proximate composition on a dry weight basis. The control diet (A) contained fishmeal as the main protein source and fish oil as the oil supplement. In diet B part of the protein and most of lipid was provided by trout waste and in diet C most of the lipid was provided by trout intestines, Gilthead bream fingerlings of 4 g initial weight were fed to apparent satiation for 72 days, at a temperature of 20 degrees C to an average final weight of 19 g. All diets were fed in a dry form. The experiment was performed in duplicate. Growth and feed utilization data were high and similar among groups. The body composition of the resulting fish did not show any difference among dietary

treatments, Differences in liver lipid and fatty acid content were found between all dietary treatments. The growth and body composition data from this preliminary experiment indicated that trout waste could be used successfully as a dietary ingredient of seabream diets. *Dr M. N. Alexis, National Center for Marine Research, Ag Kosmas, GR-16604 Athens, Greece.*

57) Kureshy, N., Davis, D. A., and Arnold, C. R. 2000. Replacement of fishmeal in practical diets for juvenile red drum, *Sciaenops ocellatus*. Abstracts of Aquaculture America 2000, New Orleans, Louisiana, February 2–5, 2000. World Aquaculture Society, Baton Rouge, LA, USA. P.141. The practice of feeding cultured fish with products derived from finite fishery resources jeopardizes the long-term sustainability of aquaculture systems. To reduce long-term dependence upon fishery resources, research needs to be conducted on the utilization of by-products arising from the terrestrial agricultural production sector. The objective of this study was to evaluate the effects of replacement of fishmeal in practical diets for juvenile red drum (*Sciaenops ocellatus*). A six-week feeding trial was conducted in which ten diets (basal diet and 9 test diets) were formulated on an iso-nitrogenous (44 % crude protein) and iso-lipolitic basis. The primary sources of protein in the basal diet were fishmeal and soybean meal, which were incorporated at 30 and 24.8 %, respectively. Replacement of fishmeal in the test diets ranged from 16.6 to 66.7 %. Low ash meat-and-bone meal (MBM), flash-dried poultry by-product meal (PBM), and enzyme-digested poultry by-product meal (EPM) were used to replace the fishmeal. Fish maintained on all four MBM diets had significantly ($P< 0.05$) lower percent weight gain as compared to fish maintained on the basal diet. Feed efficiency (FE) and protein conversion efficiency (PCE) were not significantly different for the 16.6 % MBM diet and the basal diet, but higher levels of MBM produced significantly lower FE and PCE values. The PBM and EPM diets did not show any significant differences in percent weight gain, FE, or PCE in comparison to the basal diet. Based on these results, the use of high quality poultry by-product meals is a viable option in practical diets for juvenile *S. ocellatus*, whereas the MBM utilized in this study was not acceptable as a replacement for fishmeal. *University of Texas/Marine Science Institute/Fisheries and Mariculture Laboratory, 750 Channel View Dr, Port Aransas, TX 78373, USA.*

58) Kureshy, N., Davis, D.A., and Arnold, C.R. 2000. Partial replacement of fishmeal with meat-and-bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. North American Journal of Aquaculture, 62(4): 266–272. To reduce long-term dependence upon fishery resources, we evaluated selected terrestrial by-products as alternatives to fishmeal for rearing juvenile (mean weight, 2.3 ± 0.1 g) red drum *Sciaenops ocellatus*. Over a 6-week feeding trial, 10 test diets (basal diet and 9 test diets) were formulated on an isonitrogenous (44 % gross protein) basis. The primary protein sources in the basal diet were fishmeal and soybean meal, which were incorporated at 30 and 24.8 g/100 g of diet, respectively. Replacement of fishmeal in the test diets ranged from 16.6 % to 66.7 %. Low ash meat-and-bone meal (MBM), flash-dried poultry by-product meal (PBM), and enzyme-digested poultry by-product meal (EPM) were used to replace the fishmeal. All four MBM diets produced significantly lower weight gain compared with fish maintained on the basal diet. Feed efficiency (FE) and protein conversion efficiency (PCE) were similar for the 16.6 % MBM diet and the basal diet, but higher levels of MBM produced significantly lower FE and PCE values. The PBM and EPM diets did not show any significant differences in percent weight gain, FE, or PCE compared with the basal diet. Based on these results, the use of high quality poultry by-product meals is a viable option in practical diets for red drum, whereas the MBM used in this study was not acceptable as a replacement for fishmeal. *Dr D. A. Davis, Auburn University, Department of Fisheries & Allied Aquacultures, 203 Swingle Hall, Auburn, AL 36849 USA.*

59) Lanari, D., Yones, M., Ballestrazzi, R., and D'Agaro, E. 1998. Alternative dietary protein sources (soybean, rapeseed and potato) in diets for Sea bass. *In:* VIII International Symposium on Nutrition and Feeding of fish. Recent Advances in Finfish and Crustacean Nutrition. Las Palmas de Gran Canaria, Spain June 1–4, 1998. p. 148. Diets containing only animal protein and 7 diets (S1, S2, "S" soybean meal; R1, R2, "R" rapeseed meal; P1, P2, "P" potato protein concentrate and M2, a mix of S, R and P) plant protein which replaced 25 % (1) or 50 % (2) of animal protein. After 97 d fish fed S1 and R1 had growth performance similar to C whereas diets S2 and R2 had lower growth rates and worse feed utilization. Fish fed diets containing P had a substantial reduction (P1 and M2) and an inhibition (P2) of growth because of low palatability and feed intake. Final energy body concentration (adjusted for body weight) were not influenced by dietary treatments. Apparent digestibility coefficients (%) were: single ingredients, fishmeal (FM), 83.19A; S, 82.87AB; R, 77.61C; P, 76.52CD for DM; FM, 91.38A; S, 91.0AB; R, 89.75C; P, 51.6D for CP ($P< 0.01$); experimental diets, C, 87.5A; S1, 86.58AB; S2, 85.418BC; R1, 79.08D; R2, 78.58DE; P1, 76.75FG; P2, 77.83DEF; M2, 85.83BC for DM; C, 92.25A; S1, 91.35AB; S2, 90.55BC; R1, 90.33AB; R2, 89.43BC; P1, 57.0E; P2, 55.5F; M2, 85.71D for CP ($P< 0.01$). ANFs (phytic acid. TIA, glucosinolates, alkaloids) were also determined in single ingredients and in the experimental diets. Soybean meal and rapeseed meal can substitute 25 % of animal protein in sea bass diets without negative effects on fish performance. *Dr D. Lanari, Dipartimento di Scienze della Produzione Animale; Universita degli studi di Udine, Via S. Mauro 2, 33010 Pagnaco. (UD) Italia.*

60) Lee, S. M., Kang, Y. J., and Lee, J. Y. 1991. The effect of soybean meal as partial replacement for white fishmeal in diets for yellowtail (*Seriola quinqueradata*). Bulletin of the National Fisheries Research Development Agency, 45: 247–257. A feeding experiment was conducted to study the feasibility of using soybean meal to replace white fishmeal as a protein source for yellowtail (*Seriola quinqueradiata*) feed containing 52 % dietary protein. Yellowtails of 20 g in initial average weight were stocked in 16 net cages and fed on 8 isonitrogenous and

isocaloric experimental diets for 38 days. Amino acids (L-lysine 0.98 % and DL-methionine 0.5 %), phosphorus or attractant were supplemented in 40 % soybean diet. There was no significant adverse effect on growth, feed efficiency and nutrient utilization up to 30 % soybean diet group. However the feeding performances of 40 % soybean diet groups were significantly lower than those of other dietary groups. The supplementation of amino acids, phosphorus and attractant did not have beneficial effect on fish performance. The poor effects of 40 % soybean diets, with or without nutrient supplements, were discussed in relation to anti-nutritional factors, requirements and dietary levels of amino acids and phosphorus.

61) Laining, A., Rachmansyah, R., Ahmad, T., and Williams, K. 2002. Apparent digestibility of selected feed ingredients for humpback grouper, *Cromileptes altivelis*. Aquaculture. (submitted). The dry matter (DM), crude protein (CP), and gross energy (GE) apparent digestibility of nine feed ingredients locally available in South Sulawesi, Indonesia were determined with juvenile (~20 g) humpback grouper *Cromileptes altivelis*. In each of three 4 × 4 latin square experiments, humpback grouper were fed a reference diet and three other diets in which test feed ingredients were substituted at rates of either 40 % or 30 % for animal or plant sources, respectively. Chromic oxide was used as an indigestible marker. Faeces were collected by sedimentation at 3 h intervals. Each experiment entailed four collection periods with each diet being randomly changed between the tanks. The apparent digestibility of GE could not be determined (nd) for all feed ingredients as the amount of collected faeces was insufficient for energy analyses in some instances while in others the derived value was negative. The derived DM, CP and GE apparent digestibility coefficients (mean ±SD) for the test feed ingredients were, respectively: shrimp head meal, 61.8±2.09, 80.1±0.87 and 23.7±2.47; soybean meal, 56.3±1.33, 77.7±0.83 and nd; palm oil cake meal, 53.6±1.39, 81.5±0.55 and nd; oven-dried blood meal, 53.0±2.35, 83.5±6.92 and nd; formic acid-fermented blood meal, 59.0±1.79, 86.5±0.50 and nd; propionic acid-fermented blood meal, 60.1±1.09, 72.3±1.21 and nd; imported sardine fishmeal, 70.4±1.82, 91.3±0.28 and 83.2±2.35; local fishmeal, 65.9±0.70, 86.8±0.55 and 69.7±0.55; and rice bran meal, 48.3±0.65; 86.1±0.75 and nd. The protein of both plant and animal feed ingredients was well digested by humpback grouper while the DM and GE of the protein-rich animal feed ingredients were more digestible than for the carbohydrate-rich plant feed ingredients.

62) Lopez-Bote, C.J., Diez, A., Corraze, G., Arzel, J., Alvarez, M., Dias, J., Kaushik, S.J., and Bautista, J.M. 2001. Dietary protein source affects the susceptibility to lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*) muscle. Animal Science, 73: 443–449. This study was designed to explore the effect of protein source on muscle susceptibility to lipid peroxidation in two representative species of fish farmed for human consumption: the freshwater rainbow trout and the seawater European sea bass. Four isoproteic diets (digestible protein in the range 366 to 392 g/kg for rainbow trout and 391 to 415 g/kg for European sea bass) were formulated to contain one of the following as the main protein source: fishmeal, warm water alcohol-extracted or toasted soya protein concentrates or maize gluten meal. Highest daily growth indices were always achieved using the diets based on fishmeal as the main source of protein ($P < 0.05$). Fish of both species given diets containing maize gluten and the toasted soya protein concentrate showed slowest growth. The depressant growth effect of the vegetable protein concentrates was greater in sea bass than in rainbow trout. Dietary treatment was not correlated with any significant effect on whole-body composition or intramuscular fat content except for ash concentration in European sea bass. Under conditions of forced peroxidation *in vitro* for 240 min, muscle specimens of trout and sea bass given diets containing fish protein as the main source of protein showed the highest peroxidation levels ($P < 0.05$); while the lowest peroxidation values were found in fish given maize gluten-containing diets ($P < 0.05$). In the present case, the partial substitution of fishmeal with vegetable proteins in diets led to a lower susceptibility of fish flesh to peroxidation. This finding may have applications in the production of fish of improved quality and longer shelf life. *Dr J. M. Bautista, Universidad Complutense Madrid, Fac Vet, Departamento Bioquímica y Biología Molecular IV 28040 Madrid, Spain.*

63) Lopez-Calero, G., Vergara, J.M., Robaina, L., Caballero, M.J., Montero, D., Izquierdo, M.S., and Aksnes, A. 1998. Growth, feed utilization and fish quality of gilthead bream, *Sparus aurata*, fed increased lipid levels combined with different fishmeal qualities up to commercial size. *In:* VIII International Symposium on Nutrition and Feeding of fish. Recent Advances in Finfish & Crustacean Nutrition. Las Palmas de Gran Canaria, Spain June 1–4, 1998. p. 88. Different protein to lipid ratios (44/14, 44/20 and 44/27), with two different fishmeal qualities. Results showed a clear effect of fishmeal quality on specific growth rate, being significantly higher for the high quality meal. Feed conversion ratios obtained for the high quality were significantly lower than those observed for the low quality meal for the same dietary lipid contents. According to fishmeal qualities, values for FCR obtained for the high quality meal were significantly lower than those observed for the low quality meal at the same dietary lipid contents. Significant differences in growth rates were observed in diets containing different lipid levels at any of the fishmeal qualities assayed, being highest and lowest in fish fed diets 44/20 and 44/14, respectively. FCR followed the opposite trend and thus lowest values were obtained with diet 44/20. *Dr G. Lopez-Calero, Instituto Canario de Ciencias Marinas, Gobierno de Canarias, P.O. box 56, 35200, Telde, Las Palmas, Canary Island, Spain.*

64) Lupatsch, I., Kissil, G. Wm., Sklan, D., and Pfeffer, E. 1997. Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. Aquaculture Nutrition, 3: 81–89. Apparent digestibility of crude protein ranged from 79 to 90 %, lipids from 83 to 95 % and energy from 72 to 88 % in different feed ingredients (fish, poultry, meat, blood and squid meals, extracted soybean and wheat

flower). Apparent digestibility of amino acids was higher than that of crude protein and differences were found among digestibilities of individual amino acids. Ingredient digestibility was additive for protein, amino acids, lipids and energy, whereas the digestibility of carbohydrates in the compounded feeds was slightly lower than predicted. *Dr Ingred Lupatsch, National Center for Mariculture, POB 1212, 88112 Eilat, Israel.*

65) Masumoto, T., Ruchimat, T., Ito, Y., Hosokawa, H., and Shimeno, S. 1996. Amino acid availability values for several protein sources for yellowtail (*Seriola quinqueradiata*). Aquaculture, 146(1–2): 109–119. Brown fishmeal, casein, meat meal, soy protein concentrate, corn gluten meal and full-fat soybean meal were incorporated at 40–72 % of the diet as the only source of protein to yield 30–36 % crude protein diets. The apparent average amino acid availability and apparent protein digestibility values were similar: 89.3 and 88.7 %, 96.7 and 95.4 %, 78.4 and 80.3 %, 87.5 and 87.3 %, 46.8 and 49.7 %, 78.1 and 83.2 %, respectively. True average amino acid availability and true protein digestibility values were also similar: 92.7 and 94.2 %, 100.0 and 99.0 %, 82.0 and 85.7 %, 90.8 and 92.9 %, 50.9 and 55.1 %, 82.4 and 90.2 %. All essential amino acid availability values were not significantly different from comparable values based on protein digestibility data ($P < 0.05$) except for histidine in full-fat soybean meal. Protein digestibility values can be used to approximate available amino acid values in diets. However, precaution should be taken because some amino acids may have lower availability than predicted based on protein digestibility values, such as histidine in full-fat soybean meal found in the present study. *Dr Toshiro Masumoto, Kochi University, Laboratory of Fish Nutrition, Nanko Ku, Kochi 780, Japan.*

66) McMeniman, N.P., and Williams, K.C. 2002. The digestion and utilization of some protein meals by barramundi (*Lates calcarifer).* Aquaculture (submitted). A series of five digestibility experiments was carried out with barramundi to determine the apparent digestibility of nine test protein meals using substitution procedures. Faecal samples were obtained by manual stripping of the fish and Yb was used as the digestibility marker. The determined apparent digestibility coefficients (mean ± sem) for dry matter (DM), protein and energy of the meals were respectively: meat meal (34 % DM ash), 22.2 ±10.32, 53.9±3.92 and 58.2±6.50; meat meal (24 % DM ash) 42.6±12.58, 63.5±3.39 and 66.5±3.37; poultry offal meal, 49.0±10.80, 78.8±3.50 and 76.7±5.58; solvent soybean meal, 55.8±1.79, 86.0±0.80 and 69.4±1.68; microwave roasted full-fat soybean meal, 69.0±6.88, 84.8±3.84 and 75.9±7.76; peanut meal 62.7±11.40, 91.9±8.02 and 68.7±5.01; canola meal, 48.9±81.0±2.34 and 56.1±2.96; dehulled lupin meal, 60.6±1.68, 98.1±1.34 and 61.5±1.79; and wheat gluten,100.6±101.9±1.61 and 98.8±3.13. Four comparative slaughter nutrient retention growth assay experiments of 4 to 6 weeks duration were carried out with barramundi fingerlings to assess the N utilization efficiency of diets containing the test protein meals as the main source of dietary protein. In each of three of these experiments, the biological value of two test protein meals and that of a reference protein (either a casein and fishmeal mixture or fishmeal alone) was determined by regressing N retention against N intake when fish were fed each diet at four rationed amounts of 1, 1.5, 2 and 2.5 % of body weight. Due to marked refusal by fish for diets containing soybean meal or canola meal, a summit dilution procedure was employed in the fourth experiment to enable N and energy utilization efficiencies for solvent and full-fat soybean meals to be determined. The efficiency of N utilization for diets in which protein was provided predominantly by the test protein meal varied from 0.33 for the high ash meat meal to 0.47 for poultry offal meal; that for low ash meat meal (0.46) and fishmeal (0.42) were intermediate. Using summit dilution procedures, the derived N and energy utilization efficiencies for full-fat soybean meal were 0.36 and 0.35 while those for solvent soybean meal were 0.35 and 0.34, respectively. The potential of terrestrial protein meals as replacements for fishmeal in grow-out diets for barramundi is discussed.

67) McGoogan, B.B., and Gatlin, D.M. 1997. Effects of replacing fishmeal with soybean meal in diets for red drum *Sciaenops ocellatus* and potential for palatability enhancement. Journal of the World Aquaculture Society, 28(4): 374–385. Two 8-wk feeding trials were conducted with juvenile red drum to determine the maximum levels of soybean meal that may replace fishmeal in diets containing 38 % crude protein, without reducing weight gain. In the first experiment, fish fed diets containing up to 90 % of the protein from soybean meal gained as much weight as fish fed a diet with 100 % of protein from fishmeal, but fish fed the diet with 100 % of its protein from soybean meal gained significantly ($P < 0.05$) less. Supplementation of glycine and fish solubles individually at 2 % (as-fed basis) in diets containing 90 % of their protein from soybean meal tended to increase weight gain of fish compared to those fed a similar diet without supplementation. Similar results were obtained in the second experiment, as fish fed diets containing 90 % of their protein from soybean meal gained as much weight as fish fed a diet with 100 % of its protein from fishmeal. Fish fed diets with 95 % and 100 % of their protein from soybean meal gained significantly less weight than those fed the diet with all of its protein from fishmeal. Supplementation of glycine at 2 % in the diet containing 95 % of its protein from soybean meal significantly improved weight gain of fish relative to those fed a similar unsupplemented diet. Supplementation of fish solubles at 5 % of diet on a dry-matter basis provided a non-significant increase in weight gain compared to that of fish fed a similar unsupplemented diet. In both experiments there was greater observed consumption of the soybean-meal-based diets than diets with all of their protein from fishmeal. A minimum of 10 % of protein from fishmeal appears necessary in practical diets containing most of their protein from soybean meal to prevent impaired growth and feed efficiency of red drum. *Dr D. M. Gatlin, III, Texas A&M University, Department of Wildlife & Fisheries Science, College Station, TX 77843 USA.*

68) Meilahn, C. W., Davis, D. A., and Arnold, C. R. 1996. Effects of commercial fishmeal analogue and menhaden fishmeal on growth of red drum fed isonitrogenous diets. Progressive Fish-Culturist, 58: 111–116. Four diets were formulated to determine the effectiveness of supplementing a commercially available protein source (Pro-Pak® from H. I. Baker and Brothers, Inc.) for menhaden fish protein (Special Select™ from Zapata Haynie Corp.), in diets for red drum *Sciaenops ocellatus*. The diets were formulated to be isonitrogenous, each contained 45 % total protein. Protein from soybean meal and wheat gluten was kept constant; Pro-Pak replaced 0, 25, 50, or 75 % of the menhaden fishmeal on an equal protein basis, In the first 8-week growth trial, which used satiation feeding, positive results from high fishmeal diets were observed; percent weight gain ranged from 630 to 830 %, and there was near 100 % survival. In a basal diet containing 40 % fishmeal, the replacement of 50 % of the fish protein with Pro-Pak resulted in minimal changes in growth and feed consumption. The replacement of 75 % of the fish protein in the basal diet (diet 4) resulted in reduced performance of the fish. The second growth trial confirmed reduced growth of fish fed diet 4 under fixed feeding conditions, confirming nutritional differences between the test ingredients. Based on the results of this study, with appropriate restrictions, Pro-Pak should be considered an acceptable ingredient in practical diets for red drum. *Dr D. A. Davis, The University of Texas at Austin, Marine Science Institute, Fisheries and Mariculture Laboratory, 750 Channelview Drive, Port Aransas, Texas 78373–1267, USA.*

69) Millamena, O. M. 2002. Replacement of fishmeal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides*. Aquaculture, 204(1–2): 75–84. A feeding trial was conducted to evaluate the potential of replacing fishmeal with processed animal by-product meals, meat meal and blood meal (4:1 ratio), in practical diets for juvenile grouper (*Epinephelus coioides*). Eight isonitrogenous diets were formulated to contain 45 % protein and 12 % lipid. Fishmeal was replaced by 0 %, 10 %, 20 %, 30 %, 40 %, 60 %, 80 %, and 100 % of meat meal and blood meal (4:1) mixture (diets 1–8). The diet with 100 % fishmeal (diet 1) or trash fish as feed (diet 9) were used as controls. Grouper juveniles were reared in 250-litre circular fiberglass tanks maintained in a flow-through seawater system. Each dietary treatment was tested in quadruplicate groups of 25 fish per tank arranged in a completely randomized design. Fish were fed the diets twice per day at a daily feeding rate of 5–6 % of biomass and trash fish at 10–12 % of biomass for 60 days. Percentage weight gain, specific growth rate (SGR), survival, feed conversion ratio (FCR) and body composition of grouper juveniles were measured. There were no significant differences ($P > 0.05$) in growth performance among fish fed diets 1–7 (0–80 % fishmeal replacement) with those fed diet 9 (trash fish as feed). However, fish fed diet 3 had significantly higher ($P < 0.05$) growth than those fed diet 8 (100 % fishmeal replacement). Survival among fish fed the experimental diets did not significantly differ (96–100 %) but was significantly higher ($P < 0.05$) than survival (90 %) of fish fed trash fish. These results showed that up to 80 % of fishmeal protein can be replaced by processed meat meal and blood meal coming from terrestrial animals with no adverse effects on growth, survival, and feed conversion ratio of *E. coioides* juveniles. *Dr, Oseni M. Millamena, Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021, Philippines. E-mail: oseni@aqd.seafdec.org.ph*

70) Millamena, O.M., and Golez, N.V. 2001. Evaluation of processed meat solubles as replacement for fishmeal in diet for juvenile grouper *Epinephelus coioides* (Hamilton). Aquaculture Research, 32(Suppl. 1): 281–287. Feeding experiments were conducted to determine the efficacy of low fish-meal-based diets for juvenile grouper *Epinephelus coioides* (Hamilton). A diet containing 44 % protein was formulated using fishmeal as the major protein source. Processed meat solubles, a rendered by-product of slaughter houses, was tested as a replacement for fishmeal at increasing percentages from 0 to 100 % in isonitrogenous diets. Eight dietary treatments representing fish-meal replacements were arranged in a completely randomized design with four replicates per treatment. Twenty-five fish were reared in circular fibreglass tanks of capacity 250 L, maintained in a flow-through seawater system and fed at 5–6 % of total biomass, provided daily at 08:00 and 16:00 for 60 days. Results indicate that processed meat solubles can replace 40 % of fish-meal protein with no adverse effects on weight gain, survival and or feed conversion ratio of *E. coioides* juveniles. Higher inclusion levels resulted in a significant decline in growth performance and inefficient feed conversion ratios, which may partly result from the lack of essential nutrients such as essential amino acids in meat solubles. This study has shown that the use of processed meat solubles substantially lowers the level of fishmeal required in juvenile grouper diet and can be an efficient means of turning by-products from slaughterhouses into a useful feed resource. *Dr, Oseni M. Millamena, Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021, Philippines. E-mail: oseni@aqd.seafdec.org.ph*

71) Mohammed Suhaimee, A. M., Che Utma, C. M., Mohamid Khan, B., and Daud, A. 1999. Substitution of fishmeal in sea bass (*Lates calcarifer*) diet with soybean meal. Fri Newsletter, 4(1): 16–17. An 8-week feeding trial was conducted to evaluate the effect of partial substitution of fishmeal with soybean meal (SBM), as a protein source on the growth of juvenile sea bass, *Lates calcarifer*. The inclusion levels tested were 0 % SBM t (control diet), 10 %, 20 %, 30 %, 40 % and 50 % SBM resulting in a final substitution levels of 0 %, 15 %, 29 %, 41 %, 51 % and 60 % of the fishmeal protein in the diets. All the diets were kept isonitrogenous and isolipid at around 47 % protein and 12 % lipid, respectively. The trial was conducted in glass-aquarium tanks. Sea bass juveniles with mean and coefficient of variation of body weight around 9.17 g and less than 25 %, respectively, were randomly stocked into the tanks at a stocking density of 10 pieces/tank. The results showed that the 50 % SBM diet was significantly different ($p < 0.05$) compared to the control, 20 %, 30 % and 40 % SBM diets on specific growth rate

(SGR) when the comparison of means was made by using Student-Newman-Keuls Test (SPSS Statistical Package, USA). The 10 % SBM diet did not differ significantly with all treatments. However, there were no significant differences (p> 0.05) on the final weight, weight gain, growth rate, survival rate and feed. *Dr A. M. Mohammed Suhaimee, Brackishwater Aquaculture Research Centre Gelang Patah, Johore Malaysia.*

72) Moyano-López, F.J., Diaz, I.M., Lopez, M.D., and Lopez, F.J.A. 1999. Inhibition of digestive proteases by vegetable meals in three fish species; seabream (*Sparus aurata*), tilapia (*Oreochromis niloticus*) and African sole (*Solea senegalensis*). Comparative Biochemistry and Physiology B Biochemistry & Molecular Biology, 122(3): 327–332. The inhibitory effect of three different vegetable foodstuffs (defatted soybean meal, corn gluten meal and wheat bran) on alkaline protease activity of seabream, tilapia and sole was evaluated. Protease inhibition on crude digestive extracts was assessed using different relative concentrations of plant meals and represented by constructing inhibition curves. SDS-PAGE zymograms were utilised to obtain further details in the characterisation of sensitivity of some fish enzymes to protease inhibitors. Tilapia showed the greatest sensitivity to protease inhibitors present in the assayed meals. A high resistance of sole digestive proteases to inhibition produced by soybean meal or corn gluten meal was detected, although they were sensitive to protease inhibitor activity in wheat bran. *Dr F. J. M. Lopez, University of Almeria, Escuela Politecn Super, Dept. Biol. Aplicada, Almeria 04120, Spain. E-mail: fjmoyano@ualm.es.*

73) Nakagawa, H., Kasahara, S., and Sugiyama, T. 1986. Influence of *Ulva* meal supplement to diet on plasma lipoprotein of Black Seabream. Journal of the Faculty of Fisheries and Animal Husbandry, Hiroshima. University 25: 11–17. Effect of *Ulva pertusa* meal supplement to diet on improvement in lipid metabolism was determined with regard to plasma lipoprotein in black seabream. Nine lipoproteins were electrophoretically detected. These lipoproteins were classified into albumin and globulin by salting-out. A lipoprotein classified as albumin having 150,000 in molecular weight accounted for 57 % in the total lipoproteins of control group The *Ulva* meal increased its proportion to 67.6 %. Molecular weight of lipoproteins in globulin fraction was more than 700,000. While the lipid class composition of the albumin fraction did not change, that of the globulin fraction was highly variable under the influence of the dietary *Ulva* meal. Winter without feeding caused great changes in electrophoretic behavior of lipoprotein. However, no differences were found in lipoprotein composition between both groups after wintering. *Dr Heisuke Nakagawa, Faculty of Applied Biological Science, Hiroshima University, Fukuyama, Japan.*

74) Nengas, I., Alexis, M.N., and Davies, S.J. 1999. High inclusion levels of poultry meals and related by-products in diets for gilthead seabream *Sparus aurata* L. Aquaculture, 179(1–4): 13–23. The feasibility of replacing fishmeal protein at high levels of 75 and 100 % with a high quality poultry meat meal was assessed in diets for gilthead seabream. A combined mixture of poultry meat meal and feather meal was also tested at high inclusion levels of 75 and 100 %. Finally, two lower grade poultry by-product meals produced for the Greek industry were tested at various levels for comparison. The diets were isocaloric containing 18 MJ/kg of gross energy, isonitrogenous (CP:45 %) and had a lipid content of 13 % on an as-fed basis. The experiment was carried out in a semiclosed rearing system and its duration was 84 days. The groups of fish fed 75 and 100 % poultry meat meal showed a slight reduction in growth parameters compared to fish fed the control diet containing white fishmeal but was not statistically significant ($P < 0.05$). Similar results were obtained for the fish fed the poultry and feather mixture. Feed efficiency, protein and energy utilisation followed the same trends. One of the locally produced meals at substitution levels of up to 50 % produced no significant reduction in growth of seabream ($P < 0.05$). At the level of 75 % of the protein, however, the material caused a severe decrease in growth performance, feed efficiency, protein efficiency ratio and apparent net protein utilisation. Inferior quality was also demonstrated by the other poultry meal available on the Greek market. Protein digestibility coefficients were measured for all diets and essential amino acid indices and chemical score values were calculated. *Dr I. Nengas, National Center for Marine Research, GR 16604 Athens, Greece.*

75) Nengas, I., Alexis, M.N., and Davies, S.J. 1998. High inclusion levels of poultry meals and related by-products in diets for gilthead bream, *Sparus aurata* L. VIII International Symposium on Nutrition and Feeding of fish. Recent Advances in Finfish & Crustacean Nutrition. Las Palmas de Gran Canaria, Spain June 1–4, 1998. Page 87. The groups of fish fed 75 and 100 % poultry meat meal showed a slight reduction in all growth parameters compared to the control white fishmeal-based formulation which, however, was not statistically significant. Feed efficiency and growth parameters followed the same trend. Similar results were obtained for the fish fed the poultry and feather mixture. One of the lower grade meals, at substitution levels of up to 50 %, produced no significant reduction in growth of seabream. At the level of 75 % of the protein, the material caused a severe decrease in growth performance, feed efficiency, protein efficiency ratio and apparent net protein utilization. Inferior quality was also demonstrated by the other low grade product. Protein and energy digestibilities were measured for all ingredients and essential amino acid indices and chemical score values were calculated. Carcass analysis showed statistically significant differences among the experimental groups. *Dr I. Nengas, National centre for Marine Research, Agios Kosmas, Helliniko, GR 16604, Athens, Greece.*

76) Nengas, I., Alexis, M. N., Davies, S. J., and Petichakis, G. 1995. Investigation to determine digestibility coefficients of various raw materials in diets for gilthead seabream, *Sparus aurata* L. Aquaculture Research, 26: 185–194. The apparent protein, lipid and gross energy digestibility of poultry by-product meal, feather meal,

herring meal, meat and bone meal, poultry meat meal, lipromel, skimmed milk, blood meal, sunflower meal, cottonseed meal, soybean meal, tomato pulp meal, wheat middlings, flaked maize, corn gluten, full-fat soya meal and corn gluten feed for gilthead bream, *Sparus aurata* L., were tested *in vivo* under controlled conditions. Protein digestibilities were generally high, with values between 60 % and 96 % being observed for most of the materials studied. However, some exceptions were apparent in materials of both animal and plant origin. Lipid digestibilities also ranged between 50 % and 90 %. Values for energy digestibilities indicated a much greater variation, from 6 % to 80 %. These observations are discussed in relation to the nature and source of ingredients and to the type of processing technology employed. *Dr M. N. Alexis, National Centre for Marine Research, Aghios Kosmas, Hellinikon, Athens 16604, Greece.*

77) Nengas, I., Alexis, M. N., and Davis, S. J. 1996. Partial substitution of fishmeal with soybean meal products and derivatives in diets for the gilthead seabream *Sparus aurata* (L.). Aquaculture Research, 27: 147–156. Two growth trials were conducted to evaluate the nutritional quality of several soybean products as constituents in diets for the gilthead seabream, *Sparus aurata* (L.). In a preliminary experiment, the fish were fed six diets containing different levels of solvent-extracted soybean meal as a replacement for white fishmeal at four substitution levels: 10, 20, 30 and 40 % of the fishmeal protein component. The diets supported less growth as the inclusion of soybean meal increased. However, significant reductions in growth were apparent at the 30 % substitution level. All growth parameters followed the same trend. In the second experiment, six diets with 35 % of the total protein contributed from differently processed soybean meal were tested. The products included three industrial full-fat meals heat processed for different periods, a solvent-extracted meal and a soya protein concentrate. Protein digestibility coefficients were measured for all the experimental diets. All growth parameters of the fish fed the unheated full-fat meal, solvent-extracted meal and soya concentrate were significantly lower than the control group. Protein digestibility coefficients were similar, with no statistical differences ($P< 0.05$). *Dr Maria N. Alexis, National Centre for Marine Research, Aghios Kosmas, Hellinikon, Athens 16604, Greece.*

78) Oliva-Teles, A., Cerqueira, A. L., and Conçalves, P. 1998. The utilization of diets including high levels of fish protein hydrolysate by turbot (*Scophthalmus maximus*) juveniles. *In:* VIII International Symposium on Nutrition and Feeding of fish. Recent Advances in Finfish & Crustacean Nutrition. Las Palmas de Gran Canaria, Spain June 1–4, 1998. p. 96. No. 151. Diets included standard fishmeal (Diet A) or low temperature fishmeal (Diet B) as the only protein sources, or standard fishmeal and 5, 15 or 25 % of a fish protein hydrolysate (Diets C, D and E). The diets were formulated to have 56 % protein and 14 % lipid. The initial weight averaged 58.5 g. The final weight of turbot fed Diet B (173 g) was higher than that of fish fed the other diets (126–155 g). Food conversion ratio ranged between 0.9 and 1.2 and was not significantly different among groups. Also ADC of dry matter and energy were not significantly different among groups. The ADC of protein of Diet B (77.8 %) was higher than the other groups (74.2–75.4 %), but it was only significantly higher than that of Diet C. Partial replacement with fish protein hydrolysate did not improve growth or feed utilization. *Department de Zoología e Antropolpgie e CIMAR (Centro de Investigaçcaõ Marinha e Ambiental), Faculdade de Ciências do Porto, Portugal.*

79) Oliva-Teles, A., Cerqueira, A.L., and Goncalves, P. 1999. The utilization of diets containing high levels of fish protein hydrolysate by turbot (*Scophthalmus maximus*) juveniles. Aquaculture, 179(1–4): 195–201. A feeding trial was carried out to compare growth and feed utilization of turbot juveniles (mean initial weight of 58.5 g) fed diets including standard fishmeal (diet A) or LT fishmeal (diet B) from Denmark as the only protein sources, or standard fishmeal and 5, 15 or 25 % of a fish protein hydrolysate (diets C, D and E, respectively) as the protein source. The diets were formulated to contain 56 % protein and 14 % lipid and were prepared as dry pellets. At the end of the trial, the final weight of turbot fed diet B (173 g) tended to be higher than that of fish fed the other diets (126–155 g) but this difference was not statistically significant. Feed conversion ratio ranged between 0.9 and 1.2 and was not significantly different among groups. Furthermore, N retention (% N intake) was not significantly different among groups. Energy retention (EI) was significantly higher in fish fed diet B than in those fed diet C. Regarding whole body composition, at the end of the trial there were no significant differences in proximate composition among groups. Energy content was, however, significantly higher in fish fed diet B than in those fed diet C. ADC of the diets were determined in a separate trial, using settling columns for feces collection. The ADC of dry matter and energy were not significantly different among groups. The ADC of protein of diet B (77.8 %) was higher than in the other groups but it was only significantly higher than that of diet C (74.2). It is concluded that turbot juveniles performed better with a diet including LT fishmeal than standard fishmeal as the only protein source. Partial replacement of fishmeal with fish protein hydrolysate did not improve growth or feed utilization. *Dr A. Oliva-Teles, Universidade do Porto, Faculdade de Cîencias, Departamento de Zoologia e Anthropologia, Pr. Gomes Teixeira, P 4050 Porto, Portugal.*

80) Oliva-Teles, A., and Gonçalves, P. 2001. Partial replacement of fishmeal by brewers yeast (*Saccaromyces cerevisae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture, 202(3–4): 269–278. A trial was conducted to test the effect of partial replacement of fishmeal (Danish LT fishmeal - the only protein source in the control diet) by brewers yeast, in isonitrogenous (48 % CP) and isoenergetic (22 MJ/kg) diets for sea bass juveniles with an initial average weight of 12 g. Diets were formulated to include 0 %, 10 %, 20 %, 30 % or 50 % of dietary N from yeast (diets D0, D10, D20, D30, D50, respectively); another diet supplemented with methionine (diet D50M) was also prepared. Each diet was distributed by hand to satiation to duplicate groups of 25 fish and the growth trial lasted 12 weeks. During the trial, feed intake (g kg^{-1} day^{-1}) was identical in all groups. At the end

of the trial, growth rate was not significantly different among groups, except for the D50M diet, which was significantly lower than diet D30. Feed conversion was better for diets D10, D20 and D30, containing yeast than for the control diet. N retention (% N intake) was significantly higher in fish fed diets containing yeast (except for the D50M diet) than in those fed the control diet. There were no significant differences among groups in energy retention (% E intake). The protein content of the fish was significantly higher in fish fed diets containing yeast (except for the D50M diet) than in those fed the control diet. Apparent digestibility of the diets was determined in a separate trial with fish weighing 62 g. Feces collection was performed according to the Guelph system. Apparent digestibility coefficients of dry matter and energy significantly decreased with the increase of dietary yeast level. ADC of protein was significantly lower for the D50 diet than for the other diets. Results of this trial indicate that brewers yeast can replace 50 % of fishmeal protein with no negative effects in fish performance. Moreover, the inclusion of up to 30 % brewers yeast in the diet improved feed efficiency. There was no beneficial effect of supplementing the brewers yeast diets with methionine. *Dr Aires Oliva-Teles, Departamento de Zoologia e Antropologia e CIIMAR (Centro de Investigação Marinha e Ambiental), Faculdade de Ciências da Universidade do Porto, 4099–002 Porto, Portugal.*

81) Oliva-Teles, A., Pereira, J.P., Gouveia, A., and Gomes, E. 1998. Utilisation of diets supplemented with microbial phytase by sea bass (*Dicentrarchus labrax*) juveniles. Aquatic Living Resources, 11(4): 255–259. A trial was carried out to compare the growth performance, body composition and apparent digestibility coefficients (ADC) of diets by sea bass, with an initial weight of 13.5 g, fed diets including fishmeal (68.6 % of the dietary protein) or soybean meal (65.6 % of the dietary protein) as the main protein sources. Microbial phytase was added to the soybean meal-based diet (1000 and 2000 IU/kg) and to the fishmeal-based diet (2000 IU/kg) in order to study its effect on phosphorus utilisation. Results of this trial showed that growth rate, feed conversion and nitrogen retention were significantly better in fish fed the fishmeal-based diet. Energy retention was similar in both groups. ADC of protein were similar among groups. ADC of phosphorus was significantly higher in the fishmeal-based diet (63 %) than in the soybean meal based diet (25 %). Supplementation of the fishmeal-based diet with microbial phytase did not improve the ADC of phosphorus, while in the soybean meal-based diet the inclusion of 1000 and 2000 IU/kg of microbial phytase significantly increased the ADC of phosphorus to 71.5 % and 79.8 %, respectively. It is concluded that for practical purposes supplementation of diets with microbial phytase may prove valuable in diets including high levels of plant feedstuffs. *Dr A. Oliva-Teles, Fac. Ciencias., Dept. Zool. & Antropol., P 4050 Oporto, PORTUGAL. E-mail: aoteles@fc.up.pt*

82) Quartararo, N., Allan, G.L., and Bell, J.D. 1998. Replacement of fishmeal in diets for Australian snapper, *Pagrus auratus*. Aquaculture, 166(3–4): 279–295. The potential for replacing fishmeal with soybean and poultry offal meals in the diets of Australian snapper was assessed. Four diets with 50 % crude protein were formulated using fishmeal, soybean meal and poultry offal meal as sources of protein. The control diet had 64 % fishmeal. The other three diets had 30, 20 or 10 % fishmeal, with the remaining crude protein contributed by a combination of soybean meal and poultry offal meal. Weight gain of juvenile snapper (77 g mean initial weight) decreased as the amount of fishmeal decreased below 30 %. The relationship was best described by the equation $Y = AX/(X + B)$ where Y is weight gain, X is fishmeal content (%) and, A and B are fitted constants. Similar results were achieved when snapper were reared under improved conditions for growth, i.e., at water temperatures 3–5 degrees C above ambient. At the higher water temperatures, growth rate almost doubled. Apparent digestibility coefficients for energy, phosphorus, crude protein and amino acids were determined for the diets with 64 and 30 % fishmeal. Small but significant ($P < 0.05$) differences were found for energy, phosphorus and methionine availability. However, the differences in digestible energy and available nutrients were negligible since both diets satisfied published requirements for warmwater fishes. The data on growth and FCR were used in a simple economic model, which considered fishmeal content of the diet, asymptotic growth rate and relative production costs. This model predicted that relative production costs were: (1) reduced substantially when asymptotic growth rates increased from 0.4 to 0.8 g per day, and (2) relatively insensitive to replacing up to 50 % of fishmeal in the diets by soybean meal and poultry meal. *Dr G. L. Allan, NSW Fisheries, Port Stephens Research Center, Taylors Beach, NSW 2316, Australia.*

83) Quartararo, N., Allen, G. L., and Bell, J. D. 1992. Fishmeal substitution in a diet for Australian snapper, *Pagrus auratus*. *In:* Allan, G. L., Dall, W. (Editors.). Proceedings of the Aquaculture Nutrition Workshop, Salamander Bay, 15–17 April, 1991. NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia. pages 125–126. Four diets were compared. One diet formulated for snapper contained 60 % high-quality Chilean fishmeal, the second had 10 % of this fishmeal and soybran and poultry meal to give the same protein level, the third diet was a commercial Atlantic salmon starter diet, and the last was a starter diet for barramundi. Fish fed the high fishmeal diet were significantly heavier. There were no significant differences among fish fed the other three diets. *NSW Fisheries, Fisheries Research Institute, P. O. Box 21, Cronulla, NSW 2230, Australia.*

84) Quartararo, N., Bell, J. D., and Allan, G. L. 1998. Substitution of fishmeal in a diet for the carnivorous marine fish *Pagrus auratus* (Bloch and Schneider) from Southeastern Australia. Asian Fisheries Science, 10(4): 269–279. Two commercial diets for other carnivorous species and two experimental diets for snapper were compared. Fishmeal was the predominant source of protein for one experimental diet (60 % content), while the other contained only 10 % fishmeal with the remainder of the protein sourced from soybean meal and poultry offal meal. Replacement of fishmeal gave a lower growth rate and higher apparent food conversion ratio compared to fishmeal. The growth

rate and FCR obtained with the soybean meal and poultry offal meal diet was similar to that of the two commercial diets tested. Fatty acids analysis of the two experimental diets indicated that concentrations of EFA exceeded those reported to reduce growth of snapper. Concentration of lysine in the experimental diets associated with reduced growth was lower than that in the diet based on fishmeal. *NSW Fisheries, Fisheries Research institute, P. O. Box 21, Cronulla, NSW 2230 Australia.*

85) Ramsay, J. M., Castell, J. D., Anderson, D. M., and Hebb, C. D. 2000. Effects of fecal collection method on estimation of digestibility of protein feedstuffs by winter flounder. North American Journal of Nutrition, 62: 168–173. An accurate method for estimating digestibility of nutrients is essential when developing diets suitable for aquaculture species. Two methods of fecal collection (stripping and sedimentation) were evaluated for estimating crude protein and gross energy digestibility of three protein sources by juvenile (150 ± 8.48 g) winter flounder *Pleuronectes americanus* in a 3 × 2 factorial design experiment (protein × method) with three replicates of each diet. Fishmeal (FM), canola protein concentrate (CPC) and soybean meal (SBM) were included at 15 % of a marine fish diet. Chromic oxide (0.5 % of dry matter) was added as an inert marker. Crude protein digestibilities determined for stripped feces were significantly higher than for feces collected by sedimentation. Gross energy digestibilities based upon stripped fish were also higher than those from the sedimentation methods. No significant differences in digestibility occurred among the diets containing the different protein sources. Crude protein and gross energy digestibility in FM, CPC and SBM were similar. The difference in digestibility using different fecal collection methods might be the result of uneven movement of chromic oxide through the digestive tract. *Dr John D. Castell, Department of Fisheries & Oceans, Biological Station, 531 Brandy Cove Road, St. Andrews, New Brunswick, Canada E5B 2L9. E-mail: castellj@mar.dfo-mpo.gc.ca.*

86) Regost, C., Arzel, J., and Kaushik, S.J. 1999. Partial or total replacement of fishmeal by corn gluten meal in diet for turbot (*Psetta maxima*). Aquaculture, 180(1–2): 99–117. The effect of corn gluten meal (CGM) as a partial or total replacement of fishmeal was studied in turbot (*Psetta maxima*). Five experimental diets containing a gradient of CGM were fed to triplicate groups of turbot (initial body weight of 65 g) over 9 weeks at 17 degrees C. Turbot fed a diet containing 20 % of CGM had comparable growth performances as those fed a fishmeal-based diet. Total replacement of fishmeal adversely affected growth. Digestibility of nutrients and energy of the diets decreased with the increase in levels of dietary corn gluten meal. Supplementation of arginine and lysine in diets containing CGM induced an improvement of the availability of these two amino acids. The incorporation of CGM in diets significantly affected plasma cholesterol and triglyceride concentrations, while no effect was observed on plasma levels of thyroid hormones. These results suggest that protein from CGM can replace one third of fishmeal protein in the diets for turbot. *Dr S. J. Kaushik, IFREMER, Centre Brest, Unite Mixte INRA, Fish Nutr Lab, F 29280 Plouzané, France.*

87) Reigh, R.C., and Ellis, S.C. 1992. Effects of dietary soybean and fish-protein ratios on growth and body composition of red drum *Sciaenops ocellatus* fed isonitrogenous diets. Aquaculture, 104(3–4): 279–292. 34 % crude protein diets, protein supplied by soybean (soy) meal, menhaden fishmeal or an isonitrogenous mixture of soybean and fishmeal: 1) 100 % soy protein, 2) 100 % soy protein with a methionine supplement, 3) 75 % soy protein: 25 % fish protein, 4) 50 % soy protein:50 % fish protein, 5) 25 % soy protein:75 % fish protein, and 6) 100 % fish protein. Diets containing only soy protein or soy protein plus a methionine supplement were poorly consumed by red drum. Approximately half of the fish that received these diets died during the first 4 weeks of the growth trial and the remainder lost weight. The 100 % soy and 100 % soy + methionine diets were therefore considered unacceptable for practical use and both treatments were terminated after 4 weeks. Red drum fed diets containing only fish protein or soy-protein:fish-protein ratios of 1:3 and 1:1 had higher growth rates than fish fed a 3:1 soy-protein:fish-protein ratio. Feed efficiency ratio was highest in fish fed a 1:1 mixture of soy and fish protein. Feed consumption was lower in red drum fed a 1:1 soy-protein:fish-protein ratio than in those fed 3:1 and 1:3 SP:FP ratios, or 100 % fish protein. Superior cost:benefit ratio for the 1:1 soy-protein:fish-protein mixture in a 34 % -crude-protein diet for fingerling red drum. *School of Forestry, Wildlife & Fisheries, Louisiana Agricultural Experimental Station, Louisiana State University Agricultural Centre, Baton Rouge, LA 70803–6202, USA.*

88) Robaina, L., Corraze, G., Aguirre, P., Blanc, D., Melcion, J.P., and Kaushik, S. 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (*Dicentrarchus labrax*) fed pelleted or extruded diets with or without wheat gluten. Aquaculture, 179(1–4): 45–56. The effects of diet processing technology on digestibility, postprandial ammonia excretion rates and plasma concentrations of ammonia, glucose and cholesterol (CHOL) were determined in European sea bass. Four dietary treatments were compared: diet EO (extruded basal fishmeal diet); diet PO (pelleted basal fishmeal diet); diet E30 (extruded, 70 % basal diet plus 30 % wheat gluten) and diet P30 (pelleted, 70 % basal diet plus 30 % wheat gluten). Each dietary treatment was assigned to triplicate groups of fish (body weight: 200 g). Levels of ammonia-N excretion from fish fed the different diets were measured in water samples taken from culture tanks every 2 h. At the end of the experiment, plasma samples from 3 fish per diet, taken in alternative tanks every 2 h during 24 h, were assayed for ammonia, glucose and cholesterol determination. Nutrient digestibility was not affected by the diet processing methods used. Wheat gluten showed high apparent digestibility coefficient (ADC) values for protein, energy and organic matter. Postprandial ammonia excretion values showed maximum values between 4 and 10 h after the meal depending on treatment. Daily cumulative total ammonia excretion rates were lower in fish fed fishmeal-based diets than in those fed diets containing 30 % wheat gluten. Irrespective of dietary formulation, fish fed extruded diets showed

lower ammonia excretion than those fed the dry pelleted diets. Postprandial values for plasma ammonia ranged between 3 and 8 mg/ml and the pre-feeding values were reached 24 h after feeding in all treatment groups. Maximal levels of plasma glucose (120–180 mg/ml) appeared within 6 to 10 h after feeding. Plasma cholesterol levels were lower in sea bass fed diets containing 30 % wheat gluten than in those fed diets containing fishmeal alone as the dietary protein source. *Dr L. Robaina, Univ Las Palmas Gran Canaria, Dept Biol, Las Palmas Gran Canaria 35017, Canary Isl., Spain.*

89) Robaina, L., Izquierdo, M. S., Moyano, F. J., Socorro, J., Vergara, J. M., Montero, D., and Fernández-Palacios, H. 1995. Soybean and lupin seed meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. Aquaculture, 130(2–3): 219–233. The use of vegetable protein sources in diets for freshwater fish has been studied in more detail than for marine fish species. Two experiments were conducted to compare the effect of the partial substitution of fishmeal by two different vegetable protein sources, soybean and lupin seed meals. Mean feed intake and growth were not significantly influenced by type or level of plant protein in the diet. Feed utilization indexes such as feed efficiency, protein efficiency ratio and protein productive values were not significantly affected by the type of plant protein in the diet, although a general reduction of these values was observed with increased inclusion of soybean meal. Histological studies showed an increased deposition of lipid and decreased glycogen deposits in the liver with increased levels of dietary soybean meal. Protein digestibility coefficients for lupin seed meal diets were similar to the control and 10 % higher than those for the soybean meal diets. A significant reduction in trypsin activity was observed in fish fed the lupin seed meal diets, and for soybean meal diets when the substitution level reached 30 %. Diets including plant protein showed a higher peak of ammonia excretion rate, which appeared 2 h later than that of the fishmeal diet. Highest values of dissolved ammonia were registered in fish fed a soybean meal-based diet. These results suggest that properly treated lupin meals could be an important alternative dietary protein source for gilthead seabream. *Dr M. S. Izquierdo, Dpto. Biología, Univ. de Las Palmas de G.C., Campus Univ. Tafira 35017, Las Palmas de Gran Canaria Canary Islands Spain.*

90) Robaina, L., Izquierdo, M.S., Moyano, F.J., Socorro, J., Vergara, J.M., and Montero, D. 1998. Increase of the dietary n-3/n-6 fatty acid ratio and addition of phosphorus improves liver histological alterations induced by feeding diets containing soybean meal to gilthead seabream, *Sparus aurata*. Aquaculture, 161(1–4): 281–293. In a previous study, several histological alterations were found in the liver of gilthead seabream fed with a diet containing 30 % soybean meal (SBM). In the current study, SBM containing diets were supplemented with either potassium phosphate, zinc sulfate or phytase (*Aspergillus ficuum*), or increasing the dietary n-3/n-6 fatty acid ratio to meet that of a fishmeal-based diet. Diet composition did not affect fish growth, feed efficiency (FE) or protein productive value (PER). Phosphorus supplementation significantly reduced hepatosomatic indexes (HSI), although it did not alter liver lipid content. Both phosphorus supplementation and correcting the dietary n-3/n-6 fatty acid ratio significantly altered the lipid and protein content in fish muscle, Only these two treatments, and principally the corrected dietary n-3/n-6 fatty acid ratio, improved the liver histological alterations observed in fish fed with SBM-based diets. *Dr L. Robaina, University of Las Palmas GC, Department of Biology, Las Palmas 35017, Canary Islands, Spain.*

91) Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M., and Montero, D. 1997. Corn gluten and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus aurata*): Nutritional and histological implications. Aquaculture, 157(3–4): 347–359. Two experiments were conducted to compare the effect of the partial substitution of fishmeal by two different protein sources, corn gluten (CGM) and meat and bone meals (MBM) in diets for juvenile gilthead seabream. Growth, feed efficiency, protein efficiency ratio and protein productive values were not significantly affected by the source of dietary protein, although higher values were observed with diets containing increasing levels of meat and bone meal. No differences were found in fish proximate composition at the end of the experiment. Histological studies of liver tissue showed no liver alteration in fish fed diets including increasing levels of corn gluten meal. On the contrary, an increased deposition of lipids, nuclei polarization and isolated necrotic focus were found in hepatocites of fish fed diets exceeding 20 % of meat and bone protein. Apparent protein digestibility (ADC) in diets containing CGM showed similar results to those obtained with the control diet. However, a significant reduction in digestibility was observed when MBM was used as partial substitute of fishmeal protein. A negative correlation between dietary ash content and protein digestibility was observed. Higher amounts of nitrogen were excreted as levels of CGM and MBM increased in the diets, being significantly higher in the case of 40 % substitution either with CGM or MBM protein. *Dr M. S. Izquierdo, University Las Palmas GC, Dept. Biol., Campus Univ. Tafira 35017, Las Palmas, Canary Islands, Spain.*

92) Sanz, A., Gallego, W.G., and De la Higuera, M. 2000. Protein nutrition in fish: protein/energy ratio and alternative protein sources to fishmeal. Journal of Physiology and Biochemistry, 56(3): 275–282. Those interested in the design and manufacture of feeds for intensive fish farming face the basic concern of formulating mixtures for the best yield at the lowest costs. Of the macronutrients in the feed, protein has and continues to receive special consideration because fish present high and specific needs for this constituent. Traditionally, protein has been supplied primarily by fishmeals. This paper presents a synthesis of the efforts made and the lines explored to achieve an effective reduction of the amount of fishmeal in the feeds for fish, following two strategies: reduction

of the protein in the feeds and the use of new raw materials to replace fishmeal. *Dr A. Sanz, Univ Granada, Fac Ciencias, Dept Biol Anim & Ecol, Granada 18071, Spain. E-mail: anasanz@goliat.ugr.es.*

93) Sato, T., and Kikuchi, K. 1997. Meat meal as a protein source in the diet of juvenile Japanese flounder. Fisheries Science, 63(5): 877–880. The diet contained 80 % of white fishmeal as sole protein source as control and 20, 40, 60, 80, and 100 % fishmeal protein replaced by meat meal. Survival rates of fish ranged from 93 to 100 % and were not significantly different among all treatments. The final body weight, weight gain, feed efficiency and PER of fish fed diets containing up to 60 % substitution levels were statistically identical to those of fish fed the control diet, except that the feed efficiency in the 60 % substitution diet was lower. Feed performances in diets replacing 80 and 100 % of the fishmeal protein were significantly inferior to those in the control group. Supplements of crystalline amino acids to the MM diet did not improve the nutritive value of the diet. Since substitution up to 60 % did not adversely affect the hematological and hematochemical parameters or body composition, it is considered that about 60 % of white fishmeal protein can be replaced by meat meal in the diet of Japanese flounder. *Dr K. Kikuchi, Central Research Institute of Electric Power Industry, Abiko Research Laboratory, Abiko, Chiba 27011, Japan.*

94) Sheng, H.Q., and He, X.Q. 1994. Effects of dietary animal and plant protein ratios and energy levels on growth and body composition of bream (*Megalobrama skolkovii* Dybowski) fingerlings. Aquaculture, 127(2–3): 189–196. Isonitrogenous, practical diets with varying levels of fishmeal (6–24 %) and energy (351–381 kcal/100 g) were fed to triplicate groups of bream fingerlings for 60 days to determine the amount of plant protein that could be substituted for fishmeal protein in formulated diets. Results indicate that specific growth rate increased and feed conversion ratio decreased as the inclusion level of fishmeal increased. However, the growth performance among the fish fed diets containing 12–24 % fishmeal did not differ significantly ($P > 0.05$). Low-energy diets were superior to high-energy diets. The optimum protein/energy (P/E) ratio for bream fingerlings was found to be 88–90 mg protein/kcal. The optimum dietary lipid level was found to be 6–8 %. No significant effect of varying dietary fishmeal or energy content on body composition was found. *Dr H. Q. Sheng, Chinese Academy of Science, Ministry of Water Resources, Institute of Reservoir Fisheries, Wuhan 430073, Peoples Republic of China.*

95) Shimeno, S., Hosokawa, H., Kunon, M., Masumoto, T., and Ukawa, M. 1992. Inclusion of defatted soybean meal in diet for fingerling yellowtail. Nippon Suisan Gakkaishi, 98(7): 1319–1325. A feeding trial was performed with the fingerling yellowtail *Seriola quinqueradiata* to evaluate several kinds of defatted soybean meal as a partial substitute for fishmeal in the diet. Fish fed on diets including up to 20 % commercially available defatted soybean meal showed just as good growth and feed efficiency as those fed on the control diet free from soybean meal and high in fishmeal, while feeding with a 30 % soybean meal diet appreciably depressed the performance. No marked differences were observed in the general composition of the fish body and the hematological characteristics among the groups, although the concentration of plasma zinc decreased as the soybean meal levels of the diets increased. *Fish Nutrition, Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi 783, Japan.*

96) Shimeno, S., Hosokawa, H., Masumoto, T., Ruchimat, T., and Kishi, S. 1996. Addition of combined defatted soybean meal, malt protein flour, and meat meal to yellowtail diet. (Burishiryo ni taisuru daizuyukasu, bakugatanpaku oyobi mitomiru no heiyotenka). Nippon Suisan Gakkaishi, 62(2): 243–247. In order to reduce fishmeal in fish diets by combined addition of several alternative protein sources, fingerling yellowtail *Seriola quinqueradiata* were fed for 30 days on single moist pellet diets including 0–20 % of defatted soybean meal (SBM) or meat meal (MM) together with a fixed amount of 20 % malt protein flour (MPF). The combination with 20 % SBM reduced growth and feed efficiency, probably due to both the poor amino acid profile and low digestibilities of dietary protein and carbohydrate. On the other hand, the combination with up to 20 % MM or 10 % SBM exhibited comparable growth to single MPF inclusion. Furthermore, there were no marked differences in the body composition, hematological characteristics, and nutrient retentions in all groups tested. These results indicate that combined additions of MPF and MM could replace half of the fishmeal in yellowtail diet without any deleterious effects. *Fish Nutrition, Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi 783, Japan.*

97) Shimeno, S., Hosokawa, H., Yamane, R., Masumoto, T., and Ueno, S-I. 1992. Change in nutritive value of defatted soybean meal with duration of heating time for young yellowtail. Nippon Suisan Gakkaishi (Bulletin of the Japanese Society of Scientific Fisheries), 58(7): 1351–1359. In order to clarify changes in the nutritive value of defatted soybean meal with the duration of heating time, the general and amino acid composition and trypsin inhibitor (TI) activity of several types of meal heated at 108 degree C for 0–40 min were determined, and the growth, feed conversion, protein digestibility, and body composition of young yellowtail *Seriola quinqueradiata* were also compared by feeding them with diets containing about 20 % of each meal. The nutritive value of raw defatted soybean meal was extremely low: it had a high TI activity and high nitrogen solubility index, resulting in a low protein digestibility; its feeding caused weight loss in fish with inferior hematological characteristics and body composition. The nutritive value of the meal was markedly improved by heating it for 20 min. *Fish Nutrition, Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi 783, Japan.*

98) Shimeno, S., Kanetaka, Y., Ruchimat, T., and Ukawa, M. 1995. Nutritional evaluation of several soy proteins for fingerling yellowtail. Bulletin of the Japanese Society of Fisheries Science (Nippon Suisan Gakkaishi) 61(6): 919–926. The nutritive values of several soy proteins were evaluated for fingerling yellowtail *Seriola quinqueradiata* on the basis of chemical analysis and feeding experiments with moist pellet diets containing 30 % of each protein

source. Trypsin inhibitor (TI) activity and antigen concentration tended to decrease with heating a raw soybean meal (SBM) and with extruding heated SBM. The protein content increased and the sugar content together with TI activity decreased with the purification and enzymatic hydrolysis of SBM. However, the amino acid composition hardly changed with these treatments. Dietary supplementation with raw SBM caused weight loss in the fish with quite poor hematological characteristics, while that of heated SBM improved the growth and feed conversion, but it is inferior to a control group fed a diet without soy protein. Dietary supplementation of soy protein concentrate, soy protein separate or soy protein peptide showed similar better growth performance and was comparable to the control group. The results indicate that the nutritional value of soy protein is markedly improved by purification together with heating. It is also suggested that the heating treatment is essential and alcohol purification is sufficient to use SBM for the fish feed. *Dr Sadao Shimeno, Laboratory of Fish Nutrition, Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi 783, Japan.*

99) Shimeno, S., Kumon, M., Ando, H., and Ukawa, M. 1993. The growth performance and body composition of young yellowtail fed with diets containing defatted soybean meal for a long period. Bulletin of the Japanese. Society of Scientific Fisheries, 59(5): 821–825. A long-term feeding experiment was carried out with young yellowtail *Seriola quinqueradiata* to examine the possibility of practical use of formulated diets with single moist pellets (SMP) and extruded pellets (EP) containing 20 and 30 % defatted soybean meal (SBM) as a partial substitute for fishmeal. The fish fed with the SMP and EP diets with SBM for 12 weeks showed similar growth, feed efficiency, and protein efficiency ratio to those fed with the control diet. Though feeding SBM diets tended to enlarge their digestive tract appreciably, there were no marked differences in body composition or hematological or serum characteristics between groups throughout the experimental period. Furthermore, similar retentions of dietary protein, fat, and energy in the body were observed in all groups regardless of the dietary types and SBM levels tested. These results indicate that commercial SBM can substitute for fishmeal for up to 30 % of practical diets for yellowtail. *Laboratory of Fish Nutrition, Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi 783, Japan.*

100) Shimeno, S., Masumoto, T., Hujita, T., Mima, T., and Ueno, S. 1993. Alternative protein sources for fishmeal in diets of young yellowtail. Nippon Suisan Gakkaishi/Bulletin of the Japanese Society of Scientific Fisheries, 59(1): 137–143. Meat meal, meat-and-bone meal, corn gluten meal, and rapeseed meal were evaluated individually as partial replacement for fishmeal in single moist pellet diets for young yellowtail *Seriola quinqueradiata*. Brown fishmeal in the diet was replaced isonitrogenously and isocalorically with 10, 20, and 30 % of each source. Better growth and feed efficiency were found in fish fed on diets with replacement of 10 % of all the protein sources used, and the performance further increased as the replacement with meat meal was increased up to 30 %. On the other hand, the performance tended to decrease as the replacement of other sources was increased to levels of more than 10 %, and the worst performance together with diminished blood and serum characteristics was found in fish fed on the diet with 30 % rapeseed meal. These results show that fishmeal can be replaced with several cheaper proteins such as meat meal and corn gluten meal within limited amounts, and that the adequate addition of these protein sources to fishmeal-based diets improves not only the dietary cost but also their performance. *Laboratory of Fisheries Nutrition, Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi 783, Japan.*

101) Shimeno, S., Mima, T., Imanaga, T., and Tomaru, K. 1993. Inclusion of combination of defatted soybean meal, meat meal and corn gluten meal to yellowtail diets. Nippon Suisan Gakkaishi/Bulletin of the Japanese Society of Scientific Fisheries, 59: 1889–1895. In order to evaluate large amount replacements of fishmeal by the inclusion of combinations of soybean meal with several protein sources, juvenile yellowtail *Seriola quinqueradiata* were fed on single moist pellet diets including 0–30 % of meat meal (MM) or corn gluten meal (CGM) together with 20 % defatted soybean meal (SBM). Though the inclusion of a combination with the highest amount of CGM slightly reduced the growth and feed efficiency, probably due to its poor amino acid profile, a combination with up to 20 % MM or CGM showed comparable growth performance to single SBM inclusion. The best performance was found in the group fed on the diet including the combination with 10 % MM, which was superior to that in not only the single SBM-including feed group but also the SBM-free high-fishmeal diet group. Furthermore, there were no marked differences in the body composition and hematological characteristics of any of the groups tested. These results have indicated that the inclusion of SBM with an adequate combination of several abundant and cheaper protein sources can replace larger amounts of fishmeal in yellowtail diets without any adverse effects. *Laboratory of Fish Nutrition, Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi 783, Japan.*

102) Shimeno, S., Mima, T., Kinoshita, H., and Kishi, S. 1994. Inclusion of malt protein flour to diet for fingerling yellowtail. Nippon Suisan Gakkaishi, 60(4): 521–525. The malt protein flour (MPF) used is a newly developed protein source from brewer's spent grain. MPP has lower protein and higher carbohydrate contents, as well as higher phenylalanine and lower lysine contents than fishmeal. Its nutritive value and biological availability were evaluated by chemical analysis and feeding trials. Fingerling yellowtail *Seriola quinqueradiata* were fed with fishmeal-based moist pellet diets containing 0–40 % MPF in 0.8 tonne aquaria for 30 days. Dietary inclusion of MPF tended to depress the growth rate and feed efficiency as its level increased. The inclusion of more than 30 % MPF significantly lowered these values and the body fat content and hematological characteristics, probably due to its poor digestibility and amino acid profile. However, dietary inclusion of less than 20 % MPF resulted in a comparable growth performance and hematological characteristics to the MPF-free control diet. These results indicate that MPP is useful as an alternative protein sources, and can substitute for fish up to 20 % in the diet for

fingerling yellowtail without any adverse effects. *Dr Sadao Shimeno, Laboratory of Fish Nutrition, Faculty of Agriculture, Kochi university, Monobe, Nankoku, Kochi 783, Japan.*

103) Shimeno, S., Seki, S-I., Masumoto, T., and Hosokawa, H. 1994. Post-feeding changes in digestion and serum constituent in juvenile yellowtail force-fed with raw and heated defatted soybean meals. Nippon Suisan Gakkaishi (Bulletin of the Japanese Society of Scientific Fisheries), 60(1): 95–99. In order to clarify differences in the digestion and absorption process of raw and heated defatted soybean meals (SBM) in juvenile yellowtail *Seriola quinqueradiata*, the fish were force-fed with the two kinds of SBMs as well as raw fish and fishmeal as control, and post-feeding changes in digesta quality, serum constituent concentrations, and protein digestibility were determined. The intestinal digesta contents were higher in the fish fed with either SBM than those in the fish fed with raw fish during the experimental period. The digesta in the raw SBM-fed group passed rapidly through the stomach and intestine at a similar speed as in the raw fish-fed group, but the solubility and digestibility of the intestinal digesta protein and serum free amino acid concentration were lowest among the four protein sources, probably due to the activity of trypsin inhibitor in raw SBM. On the other hand, the digesta in the heated SBM-fed group passed slowly through the digestive tracts, and the solubility of the intestinal digesta protein was higher than that in the raw SBM group. *Dr Sadao Shimeno, Laboratory of Fish Nutrition, Faculty of Agriculture, Kochi university, Monobe, Nankoku, Kochi 783, Japan.*

104) Tacon, A. G.J., and Jackson, A. J. 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. *In:* Cowey, C. B., Mackie, A. M., Bell, J. G. (Editors) Nutrition and Feeding of Fish. (c) 1985. Academic Press, London. Pages 119–145. Despite the wide variety of animal and plant feeds which have been nutritionally evaluated for fish, the selection of foodstuffs by the commercial fish feed compounder is based on their market cost, nutritional value, availability and the ultimate market value of the farmed fish. As attempts are made to use greater amounts of novel or foreign (plant) proteins within compounded fish feeds, the problem of feed acceptability or palatability becomes greater. Exogenous dietary feeding stimulants or attractants are therefore necessary with certain fish species. Supplements which have been found to enhance the palatability of a soybean-based trout diet include marine fish oil (sprayed onto the outside of the pellet), liver meal, vinasse (derived from beet-molasses) and a variety of animal and plant concentrates (branded under such titles as “fish aroma”, “meat aroma” and “soup aroma”) (Tacon, Unpublished subject area). The development of a whole series of high quality protein sources, in addition to fishmeal, will not necessarily reduce the cost of the finished feed to the fish farmer, but will reduce reliance of the fish feed manufacturing industry upon fishmeal. By so doing, the feed manufacturer in the next decade will be able to ensure that the fish farmer receives a relatively stable and high quality ration, not subject to the shortcomings in the supply, quality and fluctuating cost of a single commodity - fishmeal. *Dr Albert G. J Tacon, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK.*

105) Takagi, S., Hosokawa, H., Shimeno, S., Maita, M., Ukawa, M., and Ueno, S. 1999. Utilization of soy protein concentrate in a diet for red seabream *Pagrus major*. Suisanzoshoku, 47: 77–87.

106) Takagi, S., Hosokawa, H., Shimeno, S., and Ukawa, M. 2000. Utilization of corn gluten meal in a diet for red seabream *Pagrus major*. Nippon Suisan Gakkaishi, 66(3): 417–427. The utilization of corn gluten meal (CGM) as a substitute for fishmeal (FM) in red seabream *Pagrus major* diet was evaluated with yearlings and juveniles. Yearling fish weighing 280 g on average, and juvenile fish weighing 53 g on average, were fed diets containing 0–52 % CGM (replacing 0–100 % of FM) for 232 days and 40 days, respectively. In yearling fish, growth performance was not affected by inclusion of up to 36 % CGM, but it decreased with increase of CGM above 47 %. Similar feed conversion was observed in the fish fed diets with less than 47 % CGM to the control fish fed the CGM-free diet, but it was inferior in the fish fed a diet with 52 % CGM and without FM. In juvenile fish, the growth and feed conversion together with feed intake were decreased with increase of CGM in diet, although they were not affected by the inclusion of 15 % CGM. In both the fish, apparent protein digestibilities of the diets were not affected (90–95 %) by CGM content in the diets. These results indicate that FM in diet for red seabream could be replaced up to 70 % by the inclusion of 36 % CGM in yearling fish, and could be replaced up to 30 % by the inclusion of 15 % CGM in juvenile fish, without supplementation of essential amino acids. *Dr S. Takagi, Ehime Prefecture Fisheries Experimental Station, Uwajima, Ehime 7980104, Japan.*

107) Takagi, S., Hosokawa, H., Shimeno, S., and Ukawa, M. 2000. Utilization of poultry by-product meal in a diet for red seabream *Pagrus major*. Nippon Suisan Gakkaishi, 66(3): 428–438. The utilization of poultry by-product meal (PBM) as a substitute for fishmeal (FM) in red seabream *Pagrus major* diet was evaluated with yearlings and juveniles. Yearling fish weighing 280 g on average, and juvenile fish weighing 54 g on average were fed diets containing 0–59 % PBM (replacing 0–100 % of FM) for 232 days and 60 days, respectively. In yearling fish, the growth performance and feed conversion of fish fed diets containing up to 59 % PBM were superior or similar to the control fish fed a PBM-free diet. In juvenile fish, the growth performance of fish fed diets containing up to 41 % PBM was similar to that of the control fish, but that in fish fed diets containing more than 53 % PBM was lower. Feed conversion was similar or slightly decreased by dietary inclusion of up to 41 % PBM, but it was inferior in diets with more than 53 % PBM. These results indicate that FM in red seabream diet could be completely replaced by the inclusion of 59 % PBM in yearling fish, and could be replaced up to 70 % by the inclusion of 41 % PBM in juvenile fish, without supplementation of essential amino acids. *Dr S. Takagi, Ehime Prefecture Fisheries Experimental Station, Uwajima, Ehime 7980104, Japan.*

108) Takagi, S., Takeda, M., Hosokawa, H., Shimeno, S., Ukawa, M., and Alitsu, M. 1995. Practical inclusion levels of soybean meal in mash for Oregon moist pellets for yearling yellowtail. Suisanzoshiku, 43: 57–65.

109) Takii, K., Kita, E., Nakamura, M., Kumai, H., and Tagi, T. 1999. Evaluation of rapeseed protein concentration as protein source of diet for red seabream. Fisheries Science, 65(1): 150–154. Red seabream were fed diets containing 0, 10, 20 or 30 % rapeseed protein concentrate (RPC) for 52 days. Glucosinolates, sinapine and phytic acid content of RPC were 0.58 µmol/g, 0.2 % and 4.4 %, respectively. Growth of fish fed the 20 and 30 % RPC diets were lower than fish fed the 0 and 10 % RPC diets. No significant differences among the dietary treatments were found in daily feed intake, feed efficiency, protein efficiency ratio, hematology, and whole body fell, but that of the hepatopancreas increased with increase in diet RPC levels. Hematological character and plasma total protein, albumin, cholesterol, Mg and Zn concentrations did not differ among the dietary treatments. However, plasma P concentration of fish fed the 0 and 10 % RPC diets were higher than of fish fed the 30 % RPC diet. Moreover, apparent protein and carbohydrate digestibilities fell slightly with an increase in dietary RPC content. A practical substitute limit with RPC for dietary fishmeal replacement is about 10 % in red seabream. *Dr Kenji Takii, Fisheries laboratory, Kinki University, Uragami, Nachikatsuura, Wakayama 649–5145, Japan.*

110) Takii, K., Maoka, T., Seoka, M., Kondo, T., Nakamura, M., Kitano, H., and Kumai, H. 1999. Preliminary assessment of dietary yeast, *Saccharomyces cerevisiae*, protein for red seabream. Suisanzoshoku, 47(1): 71–76. Red seabream having a mean bw of 11 g were fed diets containing yeast protein at 0, 17, 25.5 and 34 % for 30 days. Both final mean weight and HSI showed significant differences among the treatments and increasing trends with the increase in dietary yeast protein. Feed efficiencies and PER of fish fed 17 and 25.5 % YP diets were slightly lower than those fed 0 and 34 % YP diets. No significant differences among the dietary treatments were found in hematology, stomach- and intestine-somatic indices, proximate compositions of whole body or the hepatopancreas. Also apparent protein and sugar digestibilities appeared to have no significant differences between 0 % and 17 % YP diets, respectively. Yeast protein is a potential substitute for brown fishmeal in red seabream diet. *Dr Kenji Takii, Fisheries laboratory, Kinki University, Uragami, Nachikatsuura, Wakayama 649–5145, Japan.*

111) Takii, K., Shimeno, S., Nakamura, M., Itoh, Y., Obatake, A., Kumai, H., and Takeda, M. 1990. Evaluation of soy protein concentrate as a partial substitute for fishmeal protein in practical diet for yellowtail. The Current Status of Fish Nutrition in Aquaculture. The Proceedings of the Third International Symposium on Feeding and Nutrition in Fish. *In*: Takeda, M., Watanabe, T. (Editors). The Current Status of Fish Nutrition in Aquaculture. The Proceedings of the Third International Symposium on Feeding and Nutrition in Fish. Aug 28–Sep 1, 1989, Toba, Japan. pp. 281–288. The feasibility of using soy protein concentrate (SPC) to replace brown fishmeal as a protein source in yellowtail (*Seriola quinqueradiata*) feeds was investigated with young fish having 3 different body weights, 35, 129 and 189 g. The larger 2 groups of fish, fed diets containing up to 20 % SPC with supplemental amino acids (SPCA diets), showed the same performance as fish fed the BFM diet (control), but fish fed the 30 % SPCA diet and the smaller-sized fish fed the SPCA diets showed slightly inferior performance and hematological characteristics. The relative intestinal digesta weight was maintained at a higher level in the largest group of fish fed 20 % SPCA diet until 7 h-post feeding when compared to the control group, even though similar growth rates were observed. *Fisheries Laboratory, Kinki University, Uragami Nachikatsuura, Wakayama 649–51, Japan.*

112) Takii, K., Ukawa, M., Nakamura, M., and Kumai, H. 1996. Apparent protein digestive coefficient of some meals in tiger puffer diet. Bulletin of the Fisheries Laboratory, Kinki University 5: 9–124.

113) Teskeredzic, Z., Higgs, D.A., Kosanjh, B.S., McBride, J.R., and Teskeredzic, E. 1992. Utilization of plant proteins in feeding of rainbow trout, *Oncorhynchus mykiss* Rich. Ribarstvo, 47(1–2): 13–23. Three sources of rapeseed protein concentrate, (RPC; undephytinized, untreated control; undephytinized, solvent treated control; dephytinized). Rainbow trout growth rate, appetite, feed efficiency, mortality and health were not compromised when either the undephytinized control or dephytinized RPC sources were substituted for 66 % of the herring meal protein in the basal diet. Replacement of all the fishmeal protein with each RPC source did not depress appetite, but did significantly reduce growth rate and feed efficiency. *Department of Fisheries & Oceans, 4160 Marine Drive, West Vancouver, BC V7V 1N6, Canada.*

114) Tibaldi, E., and Tulli, F. 1998. Partial replacement of fishmeal with soybean products in diets for juvenile sea bass (*D. labrax*). VIII International Symposium on Nutrition and Feeding of fish. Proceedings of the VIII International Symposium on Fish Nutrition and Feeding. Recent Advances in Finfish & Crustacean Nutrition. Las Palmas de Gran Canaria, Spain June 1–4, 1998. p. 149. Solvent-extracted soybean meal (SBM: crude protein, CP, 50 % DM; Trypsin Inhibitor Activity (TIA); 1.5 mg/g) and soy protein concentrate (SPC: CP, 71 % DM; TIA, 2.8 mg/g) supplemented with L-methionine. Diets SBM20, SBM40, SBM60 in which SBM replaced 20, 40 and 60 % respectively of the FM protein, diet SPC60 where 60 % of FM protein was replaced by SPC. Partial replacement of FM for SPC gave similar apparent digestibility coefficients for CP (96 %) and energy (92 %). In diets containing SBM, the ADC of CP was slightly reduced at the highest substitution rate (SBM60, 91 %), while the ADC of energy declined linearly from 87 to 77 % as the level of dietary SBM increased. Fish groups given diets SBM40 and SBM60 had higher daily feed intakes than those fed diets FM100, SBM20 or SPC60. Specific growth rate (SGR), feed efficiency (FE), gross protein and energy retentions (GPR, GER) were only depressed in the fish fed diet SBM60 (SGR, 1.55 vs. 1.65; FE, 0.71 vs. 0.81; GPR, 29.2 vs. 32.3 %; GER 33.1 vs. 36.7 %, $p < 0.05$).

SPC and SBM (supplemented with L-methionine), could replace 60 and 40 %, respectively, of dietary FM protein without adverse effect on growth performance of juvenile sea bass. *Dr E. Tibaldi, Dipartimento di Scienze della Produzione Animale, Universita di Udine, bia S. Mauro, 2 1–33010 Pagnacco (UD), Italia.*

115) Tucker, J. W., Jr., Lellis, W. A., Vermeer, G. K., Roberts, D. E., Jr., and Woodward, P. N. 1997. The effects of experimental starter diets with different levels of soybean or menhaden oil on red drum (*Sciaenops ocellatus*). Aquaculture, 149: 323–339. With 0.67 % EPA+DHA in the basal diet, no EFA-deficiency signs were observed. Dietary menhaden oil (MHO) was utilized up to 12.7 %, but fish fed diets with more than 1.5 % soybean oil (SBO) had lower growth rates and increased feed conversion ratios (especially with 9.0 and 12.7 % SBO). Diets containing 1.5 % SBO or MHO produced leaner fish (70.5–71.6 % protein, 13.0–13.1 % lipid). Diets containing 9.0 and 12.7 % MHO produced the fattest fish (64.5–66.0 % protein and 19.1–19.9 % lipid). The liver lipid was affected by dietary lipid level, with highest content in fish fed 5.2 % SBO (41.4 %), followed by those fed 9.0 % MHO (36.5 %) and 12.7 % MHO (35.4 %) and lowest in fish fed the 1.5 % MHO diet (24.0 %). All fish in this study had fatty livers to some degree, and their liver histological features differed from those of wild fish. *Harbour Branch Oceanographic Institution, 5600 Old Dixie Highway, Fort Pierce, Florida 34936, USA.*

116) Ukawa, M., Takii, K., Nakamura, M., and Kumai, H. 1994. Utilization of soybean meal for red seabream diet. Suisanzoshoku, 42(2): 335–338. Diets containing 0–40 % commercially available soy bean meal (SBM) as a partial substitute for fishmeal in practical diets. No remarkable differences were detected between growth and feed efficiency of fish fed on diets containing 0–25 % SBM. Though those on diet with 40 % SBM were lower than other dietary groups. Red seabream can utilize SBM effectively and the maximum dietary SBM level for getting good growth performances comparable to those on the BFM diet was about 25 %. *Technical Research Center, Marubeni Shiryo Co, Ltd, 1292 Otaru, Shinbe, Ono, Hyogo 675–13, Japan.*

117) Ukawa, M., Takii, K., Nakamura, M., and Kumai, H. 1996. Utilization of some protein sources in single moist pellet of tiger puffer. Suisanzoshoku, 44: 511–516.

118) Ukawa, M., Takii, K., Nakamura, M., and Kumai, H. 1997. Utilization of soybean proteins for single moist pellet of tiger puffer. Suisanzoshoku, 45: 547–553.

119) Vassallo-Agius, R., Imaizumi, H., Watanabe, T., Yamazaki, T., Satoh, S., and Kiron, V. 2001. Effect of squid meal in dry pellets on the spawning performance of striped jack *Pseudocaranx dentex*. Fisheries Science, 67(2): 271–280. This study was carried out to investigate the effects of dietary squid meal or a combination of squid meal and krill meal as part of the protein source in dry pellets on the spawning of striped jack *Pseudocaranx dentex*. Five months prior to spawning, 7-year-old fish were divided into three groups of 10 fish each (male:female ratio, 5:5). The control group was fed a raw fish mix (RF) and the other two groups were fed either steam-dry pellets with squid meal replacing 50 % of their fishmeal (fs-DP) or steam-dry pellets containing equal proportions of fish, squid and krill meals (fsk-DP). Feeding was carried out once every other day in 5 × 5 × 5 m floating net cages and the fish were transferred to 65 m^3 indoor tanks for spawning. Eggs and yolksac larvae produced were evaluated for their quality and those obtained during the first 2 weeks of spawning were sampled for chemical analysis. The fish had an average bodyweight of 3.5±0.4 kg at spawning. Although egg production of the RF group was significantly higher ($P< 0.05$) than that of the dry pellet groups, the fs-DP group produced the best quality eggs with higher fertilization and hatching rates. The fsk-DP group had the lowest egg production and quality. Lipid classes and fatty acid compositions of eggs and yolksac larvae were dependent on the broodstock diets. These results show that the combination of fishmeal and squid meal in dry pellets for striped jack improved egg quality but not production, whereas the combination of fishmeal, squid meal and krill meal was not effective. *Dr T. Watanabe, Tokyo University of Fisheries, Department of Aquatic Bioscience, Laboratory of Fish Nutrition, Tokyo 1088477, Japan.*

120) Verakunpiriya, V., Watanabe, K., Mushiake, K., Kawano, K., Kobayashi, T., Hasegawa, I., Kiron, V., Satoh, S., and Watanabe, T. 1997. Effect of krill meal supplementation in soft-dry pellets on spawning and quality of egg of yellowtail. Fisheries Science, 63(3): 433–439. This study was conducted to clarify whether krill meal contained in the soft-dry pellets (SDP) was responsible for the improved spawning performance and egg quality of yellowtail broodstock. After the termination of a previous study, broodstock from the experimental lots fed on raw fish, moist pellets and soft-dry pellets were separately kept on a commercial SDP for about 5 months. Thereafter, the fish which had been fed entirely on SDP were offered a test diet without krill meal supplementation; whereas broodstock which had been fed moist pellets or raw fish were fed test diets containing 20 and 30 % krill meal, respectively, all for about 5 months prior to spawning. The total egg production, the hatching rate among the fertilized eggs and the rate of normal larvae were the highest in the eggs obtained from the broodfish fed SDP without krill meal supplementation. These figures decreased relatively with the increase of krill meal in the diets. The rates of buoyant and fertilized eggs among the total eggs produced were not markedly different. It appears that higher levels of krill meal in the SDP was not beneficial for yellowtail broodfish. *Dr V. Verakunpiriya, Tokyo University of Fisheries, Department of Aquatic Bioscience, Lab Fish Nutrition, Tokyo 108, Japan.*

121) Vergara, J.M., Lopez-Calero, G., Robaina, L., Caballero, M.J., Montero, D., Izquierdo, M.S., and Aksnes, A. 1999. Growth, feed utilization and body lipid content of gilthead seabream (*Sparus aurata*) fed increasing lipid levels and fishmeals of different quality. Aquaculture, 179(1–4): 35–44. The present study shows the effects of feeding gilthead seabream, 70 to 400 g, with nine diets containing three different lipid levels (15, 22 and 28 % on a

dry weight basis), combined with two types of fishmeal of different quality. In addition, feed processing effects (extruded vs. pelletized) were also compared for the diet containing 22 % lipid. The diets prepared with the high quality fishmeal promoted better growth than those prepared with the low quality one. Protein utilization was enhanced by a sparing effect of dietary lipid at 22 %. This lipid level needed to be increased up to 28 % with low quality meal to effectively spare dietary protein. A significant increase in fish growth with higher dietary lipid levels, in both high and low quality fishmeal diets, was only related to a significant increase in total body lipid content in high quality fishmeal diets, and an excess of lipid may have been the cause of hepatocyte abnormalities in fish fed the highest lipid levels. The effect of pellet processing was apparent in low quality fishmeal diets, where a significantly better growth performance was obtained in fish fed the extruded diet. There were no significant differences among all treatments in feed intake, and values were similar to those suggested by commercial fish feed producers, for similar fish size and water temperatures. Values for feed conversion ratio (FCR) were lower than 1.6 for all diets. *Dr J. M. Vergara, Inst Canario Ciencias Marinas, Gobierno Canarias, POB 56, Las Palmas Gran Canaria 35200, Canary Island, Spain*.

122) Viyakarn, V., Watanabe, T., Aoki, H., and Tsuda, H. 1992. Use of soybean meal as a substitute for fishmeal in newly developed soft-dry pellet for yellowtail. Nippon Suisan Gakkaishi, 58: 1991–2000. The availability of defatted soybean meal (SBM) as a substitute for fishmeal (FM) in a newly developed soft-dry pellet (SDP) was evaluated by feeding SDP containing 0–50 % SBM to both juvenile and adult yellowtail. Palatability or acceptability of the experimental SDP was not influenced by the inclusion of SBM up to 50 %. Growth and feed gain ratios were highest in both sizes of fish fed on the control FM diet but decreased corresponding to the elevation of SBM levels. This might be due to the reduced levels of protein and energy in the SBM diets resulting from the difference in protein and lipid contents between FM and SBM, as the diets were not iso-nitrogenous and iso-caloric. This was also supported by the result that the weight gain per digestible energy or protein intake did not differ greatly among the experimental groups. A diet with 25 % SBM and l5 % corn gluten meal (CGM) showed better feed performances than the 40 % SBM diet, suggesting the availability of CGM as a protein source in combination with SBM. These results together with hemochemical assessments and rheological properties have shown that SBM can be included as a protein source up to 30 % in place of FM in SDP for yellowtail without any adverse effects.

123) Voss, B. 1988. Replacement of fishmeal in a diet for 0-group turbot by a single cell protein, probion, and a mixture of poultry offal-feather meal and hydrolyzed feather meal respectively. Arch. Fischerweiss, 38: 203–214. None of the components (mixture of poultry offal-hydrolysed feather meal or bacterial protein Probion) were able to substitute the fishmeal component of the diet as evidenced by growth rate, PER, PPW.

124) Watanabe, T., Aoki, H., Viyakarn, V., Miata, M., Tamagata, Y., Satoh, S., and Takeuchi, T. 1995. Combined use of alternative protein sources as a partial replacement for fish in a newly developed soft-dry pellet for yellowtail. Suisanzohoku 43: 511–520.

125) Watanabe, T., Aoki, H., Watanabe, K., Maita, M., Yamagata, Y., and Satoh, S. 2001. Quality evaluation of different types of non-fishmeal diets for yellowtail. Fisheries Science, 67(3): 461–469. Two feeding experiments were conducted to evaluate the feed quality of non-fishmeal diets having the same protein ingredient composition but prepared as different types, and to determine the supplemental effect of crystalline essential amino acids (EAA) on feed utilization by young yellowtail, *Seriola quinqueradiata*. Non-fishmeal diets formulated with soy protein concentrate, defatted soybean meal, corn gluten meal, meat meal, and krill meal were prepared as either soft dry pellets (SDP) or extruded pellets (EP) by using a large- or a small-sized twin screw extruder under different preparation conditions; or as a single moist pellet (SMP), each with and without EAA mixtures. Commercial yellowtail SDP was used as the control diet. Fish weighing 134 g and 237 g on average were reared with the experimental diets, for 93 (net cages) and 44 (aquariums) days, respectively. The fish fed both the control and test diets were found to have a good appetite. Growth rate and feed gain ratio were highest in the control diet group. The physiological condition of fish fed the control diet was evaluated as superior compared to those on the non-fishmeal diets. Among the non-fishmeal diet groups, the best performances were obtained for fish fed the SDP-type diet with EAA supplement, and performance parameters excelled in the order of SDP, EP and SMP both among the diets with and without supplemental EAA. This suggests that the nutritional quality of non-fishmeal diet was affected by the diet preparation method. It also indicates that supplementation of EAA could improve the quality of non-fishmeal diets, irrespective of the diet type, probably as a result of the enhancement of feed protein utilization. *Dr T. Watanabe, Tokyo University of Fisheries, Department of Aquatic Bioscience, Laboratory of Fish Nutrition, Tokyo.*

126) Watanabe, T., Viyakarn, V., Aoki, H., Tsuda, H., Sakamoto, H., Maita, M., Satoh, S., and Takeuchi, T. 1994. Utilization of alternative protein sources as substitute for fishmeal in newly developed soft-dry pellet for yellowtail. Suisanzohoku, 42: 499–506.

127) Watanabe, T., Viyakarn, V., Kimura, H., Ogawa, K., Okamoto, N., and Iso, N. 1992. Utilization of soybean meal as a protein source in a newly developed soft-dry pellet for yellowtail. Nippon Suisan Gakkaishi (Bull. Jpn. Soc. Sci. Fish.), 58(9): 1761–1773. Palatability and acceptability in both sizes of fish were not affected by the inclusion of SBM in SDP, while growth and feed/gain ratio were slightly reduced at higher SBM levels, probably due to the nitrogenous and caloric imbalance of the diets. There were no marked differences in proximate composition and

rheogical properties of muscle together with hemochemical parameters in either juvenile or adult yellowtail fed on SDP containing 1–30 % SBM. Digestibility of crude protein in the experimental SDP and SBM was about 85 % regardless of dietary SBM levels, while that of crude starch was reduced by the elevation of dietary SBM levels. *Department of Aquatic Bioscience, Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan.*

128) Williams, K., Barlow, C., and Rodgers, L. 2001. Efficacy of crystalline and protein-bound amino acids for amino acid enrichment of diets for barramundi/Asian seabass (*Lates calcarifer* Bloch). Aquaculture Research, 32(Suppl. 1): 415–429. The efficacy of either crystalline (C-) amino acids (AAs) or casein as sources of protein-bound (P-) AAs for AA enrichment of either high- (540 g kg^{-1}, dry matter) or low- (390 g kg^{-1}, dry matter) protein diets was examined in two 6-week experiments with barramundi *Lates calcarifer* (Bloch). The AA profile of a lysine (Lys) deficient gluten-rich basal diet was enriched incrementally in five steps either by using a mixture of C-AAs (predominantly Lys) at the expense of starch or by serial substitution of the gluten with casein (a rich source of Lys). These substitutions had a minimal effect on the protein and energy composition of the diet but enriched the Lys content (and that of other critically low essential AA) of the basal diet from 15 to 31 g kg^{-1} in Experiment 1, and from 12 to 18 g kg^{-1} in Experiment 2. A high fishmeal control diet was included in both experiments. In each experiment, 12 diets were compared using 48 tanks of fish held in a freshwater recirculation system maintained at 28 degrees C and with a 12:12 h light-dark photoperiod and fed once daily to satiety. Fish growth rate and feed conversion ratio improved quadratically with AA enrichment; the response was most marked for the low-protein diets. Efficacy of AA enrichment was dose dependent. At low dietary supplementation rates (< 3.3 g Lys kg^{-1} for the high-protein diets and up to 6 g Lys kg^{-1}) for the low-protein diets), C-AAs were utilized as effectively as P-AAs. No further enhancement of fish productivity was induced by higher rates of C-AA supplementation with the high-protein diets. *Dr K. Williams, CSIRO Marine Research, POB 120, Cleveland, Qld 4163, AUSTRALIA. E-mail: Kevin.Williams@marine.csiro.au*

129) Williams, K.C., Barlow, C.G., Rodgers, L.J., and Ruscoe, I. 2002. Potential of meat meal to replace fishmeal in extruded dry diets for barramundi *Lates calcarifer* (Bloch): I Growth performance. Aquacult. Res. (in press). Juvenile barramundi (~220 to 280 g start weight) were fed extruded dry-pelleted diets containing varying amounts of fishmeal and meat meal in three experiments (E). E1 and E2 were each 66-d farm studies utilising 16 floating cages (400 fish $cage^{-1}$) in an aerated freshwater pond. E3 examined the same diets as fed in E2 but under controlled water temperature (28±0.7ºC) and photoperiod (12:12) laboratory conditions in a 42-d study involving 24 aquaria (8 fish/aquarium). In all studies, the same 430 g kg^{-1} crude protein (CP), 15 kJ g^{-1} digestible energy (DE) control (Ctl) diet (containing 35 % Chilean anchovy fishmeal) was compared with two high-inclusion meat meal diets and a proprietary diet. The meat meal diets used in E1 evaluated a high-ash (260 g kg^{-1}) meat meal that contained 520 g kg^{-1}, CP and a low-ash (140 g kg^{-1}) meat meal that contained 600 g kg^{-1} CP when included at either 450 or 400 g kg^{-1} respectively, in combination with 100 g kg^{-1} Chilean fishmeal in diets that were isonitrogenous and isoenergetic with the Ctl diet. Growth rates and feed conversions were similar ($P > 0.05$) for all diets. In E2 and E3, the 520 g kg^{-1} CP meat meal was included at 500 g kg^{-1} without any marine protein source in diets formulated to provide either 15 or 16.2 kJ g^{-1} DE and the same CP:DE (29 mg kJ^{-1}) as the Ctl diet. Fish performance ranking of diets was similar in both experiments with the 16.2 kJ g^{-1} DE diet supporting better ($P < 0.05$) growth rates than the Ctl diet and feed conversion ratios equivalent to the Ctl diet but better ($P < 0.05$) than all other diets. *Dr K. Williams, CSIRO Marine Research, POB 120, Cleveland, Qld 4163, Australia. E-mail: Kevin.Williams@marine.csiro.au*

130) Williams, K.C., and McMeniman, N.P. 2002. Methods of measuring *in vivo* digestibility of diets consumed by barramundi *Lates calcarifer* (Bloch). Aquaculture. (submitted). Five experiments were carried out with barramundi to investigate alternative methods for recovering faeces and various digestibility markers for determining the apparent digestibility (AD) of feeds. AD derived using faecal samples collected by sedimentation after 3, 16 or 24 h was unsatisfactory because of the rapid and quantitatively large amount of nitrogenous compounds lost from the faeces into the surrounding waters. As much as 53 % of the dry matter (DM) and 62 % of total N of the faeces was present in the liquid phase after a period of 24 h suspension in water. Proportionally more of the total N was associated with the suspended fraction than with the sediment (59.9 % vs 29.7 % of the original sample). The composition of the N in the supernatant and in the sediment was different, the sediment containing a high proportion of precipitable N (41.9 % of original) compared to the supernatant (13.9 % of original). This suggests that the N in the supernatant, as expected, is predominantly soluble. Collection of faeces recovered after 3 h sedimentation resulted in AD estimates that were far higher ($P < 0.05$) than those derived using digesta taken from the terminal intestine (ileal): DM, 69.6 vs 58.2 %; N, 90.9 vs 80.3 %; and energy 81.3 vs 72.5 %, respectively. The intestinal dissection procedure gave reliable digestibility estimates but the small faecal sample recovered and high costs of labour and sacrificed fish were serious disadvantages which mitigated against its use for routine determination of AD. Faecal samples collected by manual stripping of large (~500 g) barramundi produced more reliable AD estimates than those using anal suction procedures and the former is advocated as the procedure of choice for faecal recovery in barramundi. All three digestibility markers produced reliable digestibility estimates but Yb acetate was preferred because of its solubility (facilitated even dispersion in the diet), precise analytical measurement and safety of use.

131) Williams, K.C., Paterson, B.D., Barlog, C.G., Forde, A., and Roberts, R. 2002. Potential of meat meal to replace fishmeal in extruded dry diets for barramundi *Lates calcarifer* (Bloch): II Organoleptic characteristics and fatty

acid composition. Aquacult. Res. (in press). The organoleptic quality of barramundi fed for 66 d on pelleted diets containing varying amounts of fishmeal and meat meal was determined in two experiments (E). Each compared four diets: a 430 g kg^{-1} crude protein (CP) control (Ctl) diet (containing 35 % Chilean anchovy fishmeal); two diets that contained high inclusions (40 % or more) of meat meal; and a proprietary barramundi diet. In E1, the two meat meal diets contained 10 % Chilean fishmeal, whereas the two meat meal diets in E2 had no marine protein ingredients. Panelists identified and rated the colour of flesh, and scored odour, flavour and texture characteristics and overall liking on structured graphic line scales (0 to 100). Fish fed the high-meat meal diets were sweeter and firmer than those fed the high-fishmeal Ctl diet in E1 ($P< 0.05$). Scores for fishy flavour were also highest for the meat meal diets and lowest for the proprietary diet. In both E1 and E2, scores were high (> 60) for overall liking and low (< 10) for undesirable odours and tastes. Exclusion of all sources of marine protein from the diet in E2 did not detract from the sensory value of the fish. The influence of diet on the fatty acid profile of the fish was examined in E2. Compared to fish fed the Ctl diet, the neutral lipid fraction of those fed the meat meal diets had higher proportions of saturated and short-chain monounsaturated fatty acids at the expense of longer chain fatty acids, especially 22:6 n-3. Polar lipids showed only subtle dietary effects that were confined to the long-chain unsaturated fatty acids. *Dr K. Williams, CSIRO Marine Research, POB 120, Cleveland, Qld 4163, Australia. E-mail: Kevin.Williams@marine.csiro.au.*

132) Yamamoto, T., Unuma, T., and Akiyama, T. 1998. Apparent availabilities of amino acids from several protein sources for fingerling Japanese flounder. Bull. Batl. Res. Inst. Aquacult, 27: 27–35. Apparent availabilities of amino acids from white fishmeal (FM), defatted soybean meal (SBM), malt protein flour (MPF), and corn gluten meal (CGM) were determined. Test diets contained test protein sources at 40 % crude protein. The APD and the mean AAAA of each protein source were similar: 91 and 94 % for FM: 80 and 81 % for SBM; 69 and 71 % for MPF; and 42 and 46 % for CGM. The individual AAAA of FM were similar and almost approximated the APD, while intravariations of the AAAA for the three plant protein sources were larger than that for FM. Japanese flounder does not utilize protein and amino acids from plant sources as effectively as from fishmeal. *Dr T. Yamamoto, National Research Institute of Aquaculture, Inland Station, Nutrition Section, Tamaki, MIE 51904, Japan.*

133) Yamamoto, T., Unuma, T., and Akiyama, T. 1995. Utilization of malt protein flour in fingerling Japanese flounder diets. Bull. Natl. Inst. Aquacult, 24: 33–42. Two feeding experiments were conducted to evaluate the nutritional value of malt protein flour (MPF) as an alternative protein source for fishmeal in the diets of Japanese flounder. In experiment 1, 10 to 50 % of white fishmeal protein was isonitrogenously replaced by MPF and in Experiment 2, 20 to 40 % of whitefishmeal protein was replaced by a combination of MPF and defatted soybean meal at a protein ratio of 1:1. These diets were fed to fingerling flounder with a mean initial weight of about 15 g for 4 weeks at 20°C. Weight gains and feed conversion efficiencies of fish fed diets containing 10 and 20 % malt protein flour were not significantly different from those of the control group. The performance of fish fed diets containing 30 and 40 % malt protein flour was poorer than that of fish fed a combination of malt protein flour and soybean meal. The results suggest that MPF can substitute 20 % of white fishmeal protein in a fingerling flounder diet, and that use in combination with soybean meal would be one way to improve the performance of the fish fed malt protein flour diets. *Dr T. Yamamoto, National Research Institute of Aquaculture, Inland Station, Tamaki, MIE 51904, Japan.*

134) Yamamoto, T., Unuma, T., Akiyama, T., and Kishi, S. 1996. Utilization of malt protein flour in the diets for fingerling red seabream. Fisheries Science, 62(1): 59–63. A 6-week feeding experiment was conducted to evaluate the nutritional value of malt protein flour (MPF) as an alternative protein source for fishmeal in the diets for fingerling red seabream. In the control diet, white fishmeal (WFM) was used as the sole protein source to provide approximately 52 % crude protein. In the test diets, 10 to 50 % of the WFM protein was isonitrogenously replaced by MPF. A diet containing defatted soybean meal (SBM) at 20 % replacement level was also prepared. These diets were fed to satiation to the fish with an initial mean weight of 10 g at 23°C. The dietary feed consumption rate decreased as the MPF level in the diets increased, whereas the consumption rate of the SBM diet was almost the same as that of the control diet. No difference was observed between weight gains of the fish fed the control diet and the diets containing MPF at the 10 to 30 % replacement level or SBM. Furthermore, the highest feed efficiency and retentions of protein and energy were noted in the fish fed the diet with 30 % MPF level, and the lowest values in the fish fed the control and the SBM diets. The findings suggest that MPF can substitute 30 % of white fishmeal protein with the most effective utilization of dietary protein. *Dr T. Yamamoto, National Research Institute of Aquaculture, Inland Station, Tamaki, MIE 51904, Japan.*

135) Yone, Y., Hossain, A., Furuichi, M., and Kato, F. 1986. Effect of fermented and fermented-resteamed scrap meals on growth and feed efficiency of red seabream. Bulletin of the Japanese Society of Scientific Fisheries, 52(3): 549–552. Two attempts were conducted to examine the effect of fermentation on proximate composition, POV, TBA, and VBN values of fish waste, and the effects of fermented and fermented-resteamed scrap meals on the growth of red seabream and feed efficiency. Fermentation by a combination of *Aspergillus oryzae*, *Aspergillus sojae*, *Saccharomyces cerevisiae*, and *Bacillus subtilis* decreased the lipid content, POV, and TBA in fish waste, and increased VBN. However, VBN decreased by resteaming after fermentation. The growth and feed efficiency of fish fed on the fermented scrap meal diet was superior to those of the nonfermented scrap meal diet group. However, both groups were significantly inferior to the white fishmeal diet group. On the other hand, no

significant difference was recognized between the fermented-resteamed scrap meal diet group and the white fishmeal diet group in the growth and feed efficiency. These findings indicate that the removal of both toxic oxidized lipids and protein degenerates is essential in the utilization of fish waste as a dietary protein source and that fermentation and steaming are effective for the removal. *Dr Yasuo Yone, Fisheries Research Laboratory, Kyushu University.*

ANNEX 6: ALTERNATIVE PROTEIN SOURCES IN MARINE FISH NUTRITION

Table prepared by John Castell and Troy Lyons

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Sparus aurata	Gilthead seabream	45	Poultry by-product meal (51 % CP; 29 % fat)				81.8 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Poultry by-product meal (65 % CP; 14 % fat)				89.9 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Feather meal (82 % CP; 2 % fat)				24.9 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Feather meal (81 % CP; 6 % fat)				57.5 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Meat and bone meal (59 % CP; 9 % fat)				72.2 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Skimmed milk (32 % CP; 0.4 % fat)				95.5 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Blood Meal (92 % CP; 0.5 % fat)				46.3 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Soybean meal (44 % CP; 1 % fat)				90.9 %	Protease inhibitors, phytohaemaglutinins, glucosinolates, phytic acid, saponins, oestrogenic factors, flatulence factor, anti-vitamin E, anti-vitamin A, anti-vitamin D, anti-vitamin B12, possible mycotoxin contamination		Alexis, 1997
Sparus aurata	Gilthead seabream	45	Soybean meal (full-fat (34 % CP; 23 % fat)				75.7 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Corn gluten meal (66 % CP; 5 % fat)				90.0 %	Protease inhibitors, phytic acid, oestrogenic factors, invertase inhibitor, possible mycotoxin contamination		Alexis, 1997
Sparus aurata	Gilthead seabream	45	Corn gluten feed (19 % CP; 7 % fat)				65.3 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Corn flakes (8 % CP; 4 % fat)				60.3 %			Alexis, 1997
Sparus aurata	Gilthead seabream	45	Sunflower meal (32 % CP; 1 % fat)				86.2 %	Protease inhibitors, tanins, arginase inhibitor, possible mycotoxin contamination		Alexis, 1997

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Sparus aurata	Gilthead seabream	45	Cottonseed meal (42 % CP; 4 % fat)				75.4 %	Phytic acid, ostrogenic factors, gossypol, anti-vitamin E, cyclopropenoic fatty acids, possible mycotoxin contamination		Alexis, 1997
Sparus aurata	Gilthead seabream	45	Tomato pulp meal (21 % CP; 8 % fat)				20.1 %			Alexis, 1997
Dicentrarchus labrax	Sea bass	25	Poultry by-product meal (51 % CP; 29 % fat)				84.5 %			Alexis, 1997
Dicentrarchus labrax	Sea bass	25	Feather meal (81 % CP; 6 % fat)				81.5 %			Alexis, 1997
Dicentrarchus labrax	Sea bass	25	Meat and bone meal (59 % CP; 9 % fat)				92.2 %			Alexis, 1997
Dicentrarchus labrax	Sea bass	25	Soybean meal (44 % CP; 1 % fat)				88.4 %			Alexis, 1997
Dicentrarchus labrax	Sea bass	25	Corn gluten meal (66 % CP; 5 % fat)				93.8 %			Alexis, 1997
Dicentrarchus labrax	Sea bass	25	Sunflower meal (32 % CP; 1 % fat)				91.3 %			Alexis, 1997
Dicentrarchus labrax	Sea bass	25	Cottonseed meal (42 % CP; 4 % fat)				87.0 %			Alexis, 1997
Sparus aurata	Gilthead seabream	1.1	Poultry meal (high fat)		> 20 %	20 % of fishmeal			No difference from fishmeal control	Alexis, 1997.
Sparus aurata	Gilthead seabream	1.1	Poultry meal (defatted)		> 35 %	35 % of fishmeal			No difference from fishmeal control	Alexis, 1997.
Sparus aurata	Gilthead seabream	1.1	Poultry meal (high quality poultry meat)		> 50 %	20, 35, 50 % of fishmeal			Replacing fishmeal with high quality poultry meat meal resulted in improved growth, feed efficiency and apparent net protein utilization.	Alexis, 1997.
Sparus aurata	Gilthead seabream	5	Meat and bone meal		32.9 % of diet	16.5, 32.9 % of diet (47–50 % protein diets)		Excessive ash	Diets performed well up to highest inclusion level in spite of high ash.	Alexis, 1997.
Sebastes schlegeli	Korean rockfish	3.1 g	Fishmeal analog (BAIFA-M) (70.6 % CP, 11.8 % fat, 7.85 %	55.4 %		30 % of diet	70.6 %			Bai *et al.*, 2001.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
			ash)							
Sebastes schlegeli	Korean rockfish	3.1 g	White fishmeal (71.7 % CP, 6.96 % fat, 15.12 % ash)	55.9 %		30 % of diet	87.5 %			Bai *et al.,* 2001.
Sebastes schlegeli	Korean rockfish	3.1 g	Rockfish muscle (78 % CP, 14.7 % fat, 4.72 % ash)	57.9 %		30 % of diet	92.6 %			Bai *et al.,* 2001.
Sebastes schlegeli	Korean rockfish	3.1 g	Soybean meal (47.6 % CP, 2.06 % fat, 7.32 % ash)	49.6 %		30 % of diet	76.2 %		Decreased growth, feed efficiency	Bai *et al.,* 2001.
Sebastes schlegeli	Korean rockfish	3.1 g	Corn gluten meal 64 % CP, 0.72 % fat, 1.97 % ash)	55.0 %		30 % of diet	79.1 %		Decreased growth, feed efficiency	Bai *et al.*, 2001.
Dicentrarchus labrax	Sea bass	75.8 ± 5.8	Corn gluten (60 % CP)		35 %	31–35 % of dietary protein			No negative impact on growth or feed efficiency, corn gluten resulted in reduced phosphorus discharge in effluent water	Ballestrazzi *et al.,* 1994.
Hippoglossus hippoglossus	Halibut	663	Soy protein Concentrate		45 %		85.5±1.1 %		Growth was not affected but feed efficiency was reduced by soybean protein concentrate supplementation	Berge *et al.*, 1999
Lates calcarifer	Sea bass	1.3	Soybean meal (extruded full-fat)		37.5 %	37.50 %		Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, allergens	Reduced palatability	Boonyaratpalin *et al.*, 1998.
Lates calcarifer	Sea bass	1.3	Soybean meal (soaked raw full-fat)		0 %	37.50 %		Trypsin inhibitor, lectins, phytic acid, saponins, phytoestrogens, antivitamins, allergens	Reduced growth, feed efficiency and protein efficiency ratio, poor protein digestibility, reduced feed consumption, reduced survival, atrophic changes and loss of normal structure of the pancreas	Boonyaratpalin *et al.,* 1998.
Lates calcarifer	Sea bass	1.3	Soybean meal (solvent estracted)		37.5 %	37.50 %			Reduced palatability	Boonyaratpalin *et al.,* 1998.
Lates calcarifer	Sea bass	1.3	Soybean meal (steamed full-fat)		37.5 %	37.50 %			Reduced palatability, reduced feed intake	Boonyaratpalin *et al.*, 1998.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Scophthalmus maximus	Turbot	50–65	Rapeseed meal (heat treated 91.5 % CP)		30 %			Glucosinolate	Reduced T_4 hormone production	Burel *et al.,* 2000a
Scophthalmus maximus	Turbot	50–65	Lupin meal (92.8 % CP)		50 %			Protease inhibitors, saponins, phytoestrogens, alkaloids		Burel *et al.,* 2000a
Scophthalmus maximus	Turbot	50–65	Rapeseed meal (93.7 % CP)		< 30 %			Protease inhibitors, glucosinolates, phytic acid, tannins, possible mycotoxin contmination	Hyperactivity of thyroid function, reduced (growth, protein retention, energy retention, feed efficiency)	Burel *et al.,* 2000a
Scophthalmus maximus	Turbot	110	Rapeseed meal (heat treated 91.5 % CP)		30 %		88.5±1.5 %	Protease inhibitors, glucosinolates, phytic acid, tannins	Reduced dry matter digestibility, heat processing improved protein and energy digestibility	Burel *et al.,* 2000b
Scophthalmus maximus	Turbot	110	Lupin meal (92.8 % CP)		> 30 %		96.2±0.7	Protease inhibitors, saponins, phytoesterogens, alkaloids	High protein digestibility	Burel *et al.,* 2000b
Scophthalmus maximus	Turbot	110	Pea meal (90.9 % CP)		> 30 %		87.9±0.2	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, antivitamins		Burel *et al.,* 2000b
Scophthalmus maximus	Turbot	110	Rapeseed meal (solvent extracted, 93.7 % CP)		< 30 %	50 %	90.9±2.3 %	Glucosinolate, protease inhibitors, phytic acid, tannins	Reduced protein, energy, dry matter digestibilities	Burel *et al.,* 2000b
Dicentrarchus labrax	Sea bass (larvae)	0	Fish protein hydrolysate		25 %	25, 50, 75 %			Highest levels of hydrolysate suppressed growth, however, replacing 25 % of fishmeal resulted in advanced development of alkaline phosphatase and aminopeptidase in larvae intestine	Cahu *et al.,* 1999.
Scophthalmus maximus	Turbot	5	Fish silage			44 % of diet			Live weight gain was significantly lower in silage fed fish	Calcedo-Juanes, 1988.
Pleuronectes platessa	Plaice	4.5–6.5	Soybean meal (47 % CP)		< < 50 %	50 %			Decreased growth, feed efficiency	Cowey *et al.,* 1971

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Pleuronectes platessa	Plaice	4.5–6.5	Leaf protein (from rye grass, 60 % CP)		40 %	0–63 %			When leaf protein exceeded 40 % of total protein growth was significantly depressed	Cowey *et al.,* 1971
Pleuronectes platessa	Plaice	4.5–6.5	Single cell protein (B. P., 60 % CP)		45 %	45 %			No growth depression with 45 % fishmeal replacement with B. P. single cell protein source	Cowey *et al.,* 1971
Sparus aurata	Gilthead seabream	5	Meat and bone meal (59.4 % CP)		40 %	20, 40 %				Davies *et al.,* 1991
Sparus aurata	Gilthead seabream	5	Meat and bone meal (49 % CP)		40 %	40 %				Davies *et al.,* 1991
Sparus aurata	Gilthead seabream	5	Meat and bone meal (46 % CP)		40 %	40 %				Davies *et al.,* 1991
Sparus aurata	Gilthead seabream	5	Enzymatically hydrolyzed animal protein and emulsified animal fat (Liprome 50 % CP)		15 %	15 %				Davies *et al.,* 1991.
Sciaenops ocellatus	Red drum	0.2, 0.4 and 2.5	Soybean meal (solvent estracted)			50, 75, 84, 92 %			With smallest fish all levels of soybean meal or soy protein resulted in reductions in growth. Feed attractants and methionine supplements improved performance of soybean meal diets.	Davis *et al,*. 1995.
Scophthalmus maximus	Turbot	13	Soybean protein concentrate (68 % protein)		25 %	0, 25, 50, 75, 100 %	82.8–87.5 %		Decreased feed consumption and feed conversion ratios above 50 % replacement, decreased growth rates above 25 % replacement	Day *et al.,* 2000.
Scophthalmus maximus	Turbot	13	Soybean protein concentrate (68 % protein) + lysine and methionine		< 60 %	60 %			Growth improved by lysine and methionine supplementation (most effective when amino acids coated in gelatin), but still inferior to fishmeal control	Day *et al.,* 2000.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
									diet	
Maccullochells peelii peelii	Murray cod	100	Meat meal				78.3±1.92			De Silva *et al.,* 2000.
Maccullochells peelii peelii	Murray cod	100	Shark meat waste meal				87.5±1.27			De Silva *et al,.* 2000.
Maccullochells peelii peelii	Murray cod	100	Soybean meal				86.5 ± 0.49 %			De Silva *et al,.* 2000.
Barbodes altus	Tin foil barb	1	Soybean meal		33 %	25, 33, 50		Protease inhibitors, lectins, phytic acid, saponins		Elangovan and Shim, 2000.
Rhabdosargus sarba	Silver seabream	1.45	Poultry offal meal (full-fat, 59.4 % CP)		25 %	0, 25, 50, 75 %			Reduced growth above 25 %	El-Sayed, 1994.
Rhabdosargus sarba	Silver seabream	1.45	Soybean meal (dehulled, defatted, solvent extracted, heated 1 hr at 100C, 47.7 %CP)		25 %	0, 25, 50, 75, 100 %		Protease inhibitors, lectins, phytic acid, saponins	Reduced growth above 25 %	El-Sayed, 1994.
Rhabdosargus sarba	Silver seabream	1.45	Spirulina meal (commercial powder, 61.9 % CP)		50 %	0, 25, 50, 75, 100 %			Reduced growth above 50 %, improved feed efficiency	El-Sayed, 1994.
Lates calcarifer	Sea bass	3.8 ± 0.5	White cowpea meal	45.6	18 % of diet	18 % of diet	91.2 ± 0.2 %		Equivalent to defatted soybean meal control, all diets contained 36 % Peruvian fishmeal)	Eusebio and Coloso, 2000.
Lates calcarifer	Sea bass	3.8 ± 0.5	Green mungbean meal	46.4	18 % of diet	17.45 % of diet	91.2 ± 0.2 %		Equivalent to defatted soybean meal control, all diets contained 36 % Peruvian fishmeal)	Eusebio and Coloso, 2000.
Lates calcarifer	Sea bass	3.8 ± 0.5	Papaya leaf meal	46.3		14 % of diet	91.2 ± 0.2 %		Growth depressed compared with defatted soy meal in control diet (all diets contained 36 % Peruvian fishmeal)	Eusebio and Coloso, 2000.
Lates calcarifer	Sea bass	3.8 ± 0.5	Cassava leaf meal	46.5		13.5 % of diet	89.2 ± 0.5 %		Growth depressed compared with defatted soy meal in control diet (all diets contained 36 % Peruvian fishmeal)	Eusebio and Coloso, 2000.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Sciaenops ocellatus	Red drum	150	Cottonseed meal (40.8 % CP)				84.5±4.1			Gaylord and Gatlin, 1996.
Sciaenops ocellatus	Red drum	150	Dehulled soybean meal (53.2 % CP)				8.6±4.7			Gaylord and Gatlin, 1996.
Sciaenops ocellatus	Red drum	150	Meat and bone meal (55.0 % CP)				78.9±6.7			Gaylord and Gatlin, 1996.
Sciaenops ocellatus	Red drum	150 g	Poultry by-product meal (62.2 % CP)				48.7±5.3			Gaylord and Gatlin, 1996.
Sciaenops ocellatus	Red drum	150 g	Wheat (18.1 % CP)				96.8±2.7	Protease inhibitors, phytohaemaglutinins, phytic acid, oestrogenic factors, flatulence factor, amylase inhibitor, dihydroxyphenylalanine, possible mycotoxin contamination		Gaylord and Gatlin, 1996.
Dicentrarchus labrax	Sea Bass	40 g	Fishmeal (74.9 % CP, 13.7 % fat, 11.4 % ash)	57.70 %			95.3–96 %		Digestibilities tested at the 15 and 30 % replacement levels in diet	Gomes da Silva and Oliva-Teles, 1998.
Dicentrarchus labrax	Sea Bass	40 g	Fishmeal (70.1 % CP, 11.5 % fat, 18.3 % ash)	56.0 %			89.5–90.5 %		Digestibilities tested at the 15 and 30 % replacement levels in diet	Gomes da Silva and Oliva-Teles, 1998.
Dicentrarchus labrax	Sea Bass	40 g	Fish Protein hydrolysate (71.7 % CP, 21.3 % fat, 4.5 % ash)	56.5 %			96.5–97.8 %		Digestibilities tested at the 15 and 30 % replacement levels in diet	Gomes da Silva and Oliva-Teles, 1998.
Dicentrarchus labrax	Sea Bass	40 g	Blood Meal (97.1 % CP, 1.0 % fat, 1.9 % ash)	64.2 %			90.6–93.1 %		Digestibilities tested at the 15 and 30 % replacement levels in diet	Gomes da Silva and Oliva-Teles, 1998.
Dicentrarchus labrax	Sea Bass	40 g	Meat Meal (75.1 % CP, 14.7 % fat, 10.2 % ash)	54.5 %			91.0–92.0 %		Digestibilities tested at the 15 and 30 % replacement levels in diet	Gomes da Silva and Oliva-Teles, 1998.
Dicentrarchus labrax	Sea Bass	40 g	Soybean Meal (51.6 % CP, 2.5 % fat, 7.5 % ash)	50.5 %			88.6–89.8 %		Digestibilities tested at the 15 and 30 % replacement levels in diet	Gomes da Silva and Oliva-Teles, 1998.
Pagrus major	red seabream	260 g	(Soybean meal + corn gluten meal) or (soybean meal + corn gluten meal + poultry meal)			15 %			Green liver associated with soybean and corn gluten meal diet, reduced weight gain, reduced feed efficiency and	Goto *et al.*,. 2001.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
									PER	
Dicentrarchus labrax	Sea bass	10	Pea meal (20.57 % Crude protein)		> 20 %	10, 20 %	89.1±1.8	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, antivitamins		Gouveia and Davis, 1998.
Dicentrarchus labrax	Sea bass	6	Extruded dehulled pea meal (24.66 % CP)		> 15 %	5, 10, 15 %	93.96–94.25		No negative effects on growth, feed conversion, NPU, might be limiting in methionine if replaced at higher levels	Gouveia and Davis, 2000.
Megalobrama skolkovii	freshwater bream	101–103	Peanut cake + rapeseed cake + wheat meal + rice +chaff meal			75, 81, 87 % of diet			Decreased growth, feed efficiency	Haiqing and Xiqin, 1994.
Pagrus major	red seabream	41	Pig blood		56 %	32 and 56 %			Improved weight gain,	Hara *et al.,* 1986.
Plecoglossus altivelis	ayu	5 to 9	Sincle cell protein (hydrocarbon-grown yeast)			27 %			Reduced growth, reduced EFA content in fish lipid	Ishida and Shimma, 1979.
Paralichthys olivaceus	Japanese flounder	8	Corn gluten meal		40 %	20, 40, 60 %			Growth and feed efficiency depression with 60 % fishmeal replacement	Kikuchi, 1999a
Paralichthys olivaceus	Japanese flounder	4.6	Defatted soybean meal, SBM + (corn gluten meal or blood meal)		45 %	45, 55 %			Reduced growth and feed utilization, addition of blood meal improved performance	Kikuchi, 1999b
Paralichthys olivaceus	Japanese flounder	3	Feather meal	53.2–55.6 %	40 %	20, 40, 60, 80 %			Above 40 % fishmeal replacement there was reduced growth, feed efficiency, hematocrit, increased whole body water content	Kikuchi *et al..,* 1994.
Paralichthys olivaceus	Japanese flounder	2.6–4.3	Meat and bone meal	49.1–50.6 %	20 % of diet	10, 20, 37, 45 % of diet			Reduced growth, feed efficiency and protein efficiency ratio inferior to defatted soybean meal	Kikuchi *et al.,* 1997a.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Paralichthys olivaceus	Japanese flounder		Meat meal		60 %	0, 20, 40, 60, 80, 100 %			Supplements of crystalline amino acids to meat meal diet improved its nutritive value slightly	Kikuchi *et al.,* 1997b.
Sparus aurata	Gilthead seabream	12.1 ± 0.5	Rapeseed meal	49.3–50.4 %	30 %	30, 60, 100 %			Reduced feed consumption, reduced growth	Kissil *et al.,* 2000.
Sparus aurata	Gilthead seabream	12.1 ± 0.5	Soybean meal	49.1–50.7 %	30 %	30, 60, 100 %			Reduced feed consumption, reduced growth	Kissil *et al.*, 2000.
Sparus aurata	Gilthead seabream		Rapeseed protein concentrate		39 % if Norse LT-94(R) fishmeal is used rather than Chilean fishmeal	26, 38.9, 53.3 %			Weight gain was inversely proportional to rapeseed meal inclusion rate	Kissil *et al.*, 1997.
Sparus aurata	Gilthead seabream	28	Lupin meal	45 %	36 %	36 %	92 %		No significant differences were detected in weight gain, feed intake, feed/gain ratio, energy retention or protein retention	Kissil and Lupatsch, 2000.
Sparus aurata	Gilthead seabream	28	Lupin meal (genetically improved with higher methionine content)	45 %	36 %	36 %	94 %		No significant differences were detected in weight gain, feed intake, feed/gain ratio, energy retention or protein retention	Kissil and Lupatsch, 2000.
Sparus aurata	Gilthead seabream	larvae	Squid protein hydrolysate		< 50 %	0, 50, 100 %			Reduced growth at both levels of substitution	Kolkovski and Tandler, 2000.
Sparus aurata	Gilthead seabream	4 g	Trout offal (heads, skeletons, tails) (14.5 % CP, 11.1 % fat, 3.3 % ash, 70 % water)	42.2 %		31.6 % of diet			No negative effects on growth or fish health reported.	Kotzamanis *et al.,* 2001
Sparus aurata	Gilthead seabream	4 g	Trout offal (intestines) (7.9 % CP, 35 % fat, 0.6 % ash, 56.3 % water)	42 %		22.2 % of diet		Possible fish pathogens as offal was not heat processed or pasturized.	No negative effects on growth or fish health reported.	Kotzamanis *et al.*, 2001
Sciaenops ocellatus	Red drum	2.3	Enzume-digested poultry by-product meal	44 %	67 %	66.7 %		Possible fish pathogens as offal was not heat processed or pasturized.	No negative effects on growth, feed conversion, or protein conversion efficiency	Kureshy *et al.*, 2000.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Sciaenops ocellatus	Red drum	2.3	Flash-dried poultry by-product meal	44 %	67 %	16.6, 33.3, 50, 66.7 %			No negative effects on growth, feed conversion, or protein conversion efficiency, for any of the levels tested	Kureshy *et al.,* 2000.
Sciaenops ocellatus	Red drum	2.3	Low-ash meat and bone meal	44 %	17 %	16.6, 33.3, 50, 66.7 %			Reduced growth, feed efficiency, and protein conversion efficiency	Kureshy *et al.,* 2000.
Dicentrarchus labrax	Sea bass		Potato protein concentrate			25, 50 %	51.60 %	Protease inhibitors, phytohaemaglutinins, cyanogens, oestrogenic factors, amylase inhibitor, invertase inhibitor, cholinesterase inhibitorphytic, glucosinolates, alkaloids	Reduced growth even at 25 % replacement	Lanari *et al,.* 1998.
Dicentrarchus labrax	Sea bass		Rapeseed meal		25 %	25, 50 %	89.75 %	Phytic acid. TIA, glucosinolates, alkaloids	Reduced growth at 50 % replacement	Lanari *et al.,* 1998.
Dicentrarchus labrax	Sea bass		Soybean meal		25 %	25, 50 %	91 %	Phytic acid. TIA, glucosinolates, alkaloids	Reduced growth at 50 % replacement	Lanari *et al.,* 1998.
Dicentrarchus labrax	Sea bass		Corn gluten meal						Reduced growth for all replacement levels	Lopez-Bote *et al.,* 2001
Dicentrarchus labrax	Sea bass		Soybean meal						Reduced growth for all replacement levels	Lopez-Bote *et al.,* 2001
Sparus aurata	Gilthead seabream	250–350	Meat meal (64 % CP, 12.7 % fat, 17.3 % ash)	62.6 %	50.8–52.1 %	100.0 %	79.0 %		Digestibility study	Lupatsch *et al.,* 1997.
Sparus aurata	Gilthead seabream	250–350	Poultry meal (67.4 % CO, 15.4 % fat, 15.6 % ash)	67 %	50.8–52.1 %	100.0 %	80.0 %		Digestibility study	Lupatsch *et al.,* 1997.
Sparus aurata	Gilthead seabream	250–350 g	Solvent-extracted soybean meal (48.4 % CP, 2.2 % lipid, 6.2 % ash)	43.4 %	50.8–52.1 %	100.0 %	86–88 %		Digestibility study	Lupatsch *et al.,* 1997.
Sparus aurata	Gilthead seabream	250–350 g	Squid meal (75.3 % CP, 10.2 % fat, 8.7 % ash)	74.4 %	50.8–52.1 %	100.0 %	87±1.4 %		Digestibility study	Lupatsch *et al.,* 1997.
Sparus aurata	Gilthead seabream	250–350 g	Wheat flour (13 % CP, 1.6 % fat, 1.5 % ash)	56.8 %	50.8–52.1 %	100.0 %	82.0 %		Digestibility study	Lupatsch *et al.,* 1997.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Sparus aurata	Gilthead seabream	250–350 g	Blood meal (89.7 % CP, 2.6 % fat, 4.7 % ash)	76.6 %	50.8–52.1 %	100.0 %	90.0 ± 0.7 %		Digestibility study	Lupatsch *et al.,* 1997.
Seriola quinqueradiata	Yellowtail	98–109 g	Corn gluten meal (63.1 % CP, 5.1 % fat)	35.9 % control fishmeal was 35.4 % protein		100 %	49.7 %		Digestibility study	Masumoto *et al.,* 1996.
Seriola quinqueradiata	Yellowtail	98–109 g	Full-fat soybean meal (45.2 % CP, 19.1 % fat)	29.5 % control fishmeal was 35.4 % protein		100 %	83.2 %		Digestibility study	Masumoto *et al.* 1996.
Seriola quinqueradiata	Yellowtail	98–109 g	Meat meal (50.5 % CP, 13.8 % fat, 20.6 % ash)	36.1 % control fishmeal was 35.4 % protein		100 %	80.3 %		Digestibility study	Masumoto *et al.*, 1996.
Seriola quinqueradiata	Yellowtail	98–109 g	Soy protein concentrate (62.0 % CP, 0.5 % fat)	34.9 % control fishmeal was 35.4 % protein		100 %	87.3 %		Digestibility study	Masumoto *et al.,* 1996.
Sciaenops ocellatus	Red drum (low salinity [7ppt] may not be marine fish)	220–444 g	Soybean meal (51.3 % CP; 1.9 % fat)	40 %	90 %	90, 95, 100 %			Growth reduction only when 100 % anchovy meal replaced by soybean meal, glycine or fish solubles improved growth with 95 % soybean meal replacement of fishmeal	McGoogan and Gatlin, 1997.
Sciaenops ocellatus	Red drum	220 g	Fishmeal (menhaden)				95.9 ± 3.0 %			McGoogan and Reigh, 1996.
Sciaenops ocellatus	Red drum	220 g	Blood meal				100.1 ± 1.6 %			McGoogan and Reigh, 1996.
Sciaenops ocellatus	Red drum	220 g	Sorghum, grain				77.1 ± 2.5 %			McGoogan and Reigh, 1996.
Sciaenops ocellatus	Red drum	220 g	Meat and bone meal				74.1 ± 1.4 %			McGoogan and Reigh ,1996.
Sciaenops ocellatus	Red drum	220 g	Corn, grain				81.6 ± 2.0 %			McGoogan and Reigh, 1996.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Sciaenops ocellatus	Red drum	220 g	Soybean meal				80.2 ± 2.2 %			McGoogan and Reigh, 1996.
Sciaenops ocellatus	Red drum	220 g	Cottonseed meal (42.2 % CP)				76.4 ± 4.1 %			McGoogan and Reigh, 1996.
Sciaenops ocellatus	Red drum	220 g	Wheat middlings				87.4 ± 1.9 %			McGoogan and Reigh, 1996.
Sciaenops ocellatus	Red drum	220 g	Rice bran				77.2 ± 0.2 %			McGoogan and Reigh , 1996.
Sciaenops ocellatus	Red drum (27.8 ppt salinity)	12.6 g	Pro-Pak (a blend of animal by-products, 62.6 % CP; 9.63 % fat)	45 %	50 %	0, 25, 50, 75 %			At 75 % replacement there was reduced feed consumption, reduced growth	Meilahn *et al.,* 1996.
Epinephelus coioides	Grouper	2 g	Meat solubles (minced, pressed, sterilized at 133 C and spray-dried, CP 72–75 %)	43–44 %	40 %	0, 10, 20, 30, 40, 60, 80 and 100 %			Weight gain was depressed at 60 % replacement and above, there was no difference among groups in survival, replacing 20–40 % of fishmeal resulted in lower levels of methionine, phenylalanine and threonine in the diet. At 60 % replacement, lysine also became lower.	Millamena and Golez, 2001.
Epinephelus coioides	Grouper	6.1 ± 0.55	Meat meal and blood meal (4:1)	43.3–44.8 %	80 %	0, 10, 20, 30, 40, 60, 80, 100 %			Up to 80 % fishmeal can be replaced with no significant effect on growth, feed conversion ratio or body composition	Millamena, 2002.
Lates calcarifer	Sea bass	8–10 g	Soybean meal	47 %	60 %	0, 15, 29, 41, 51 and 60 %			No growth depression, reduction in feed efficiency or impact on survival for any of the soybean meal levels tested	Mohammed Suhaimee *et al.*, 1999.
Solea senegalensis	African sole		Wheat bran					protease inhibitors	Very sensitive to alkaline protease activity inhibition of wheat bran	Moyano-López *et al.,* 1999.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Solea senegalensis	African sole		Corn gluten meal					protease inhibitors	Highly resistant to alkaline protease activity inhibition by corn gluten meal	Moyano-López *et al.,* 1999.
Solea senegalensis	African sole		Defatted soybean meal					Protease inhibitors	Highly resistant to alkaline protease activity inhibition of soybean meal	Moyano-López *et al.,* 1999.
Sparus aurata	Gilthead seabream		Wheat bran					Protease inhibitors	Protease not sensitive to low concentrations of wheat bran, response increased linearly up to 40 % of total activity at high concentrations of wheat bran	Moyano-López *et al.,* 1999.
Sparus aurata	Gilthead seabream		Corn gluten meal					Protease inhibitors	Quite resistant to alkaline protease activity inhibition by corn gluten meal	Moyano-López *et al.,.* 1999.
Sparus aurata	Gilthead seabream		Defatted soybean meal					Protease inhibitors	Very sensitive to alkaline protease activity inhibition of soybean meal	Moyano-López *et al.,.* 1999.
Acanthopagrus schlegeli	Black seabream	24	*Ulva* meal			10 % of diet			No effect on growth but increased blood lipoprotein globulin pholipids, and decreased blood lipoprotein TG/PL ratios	Nakagawa *et al.,* 1986.
Sparus aurata	Gilthead seabream	45	Blood meal (92.1 % CP)	61 %			46.3±6.8 %			Nengas *et al,.* 1995.
Sparus aurata	Gilthead seabream	45	Corn flake (8.1 % CP)	21 %			60.3±2.8 %			Nengas *et al.,* 1995.
Sparus aurata	Gilthead seabream	45	Corn gluten feed (19.0 % CP)	26 %			65.3±3.4 %			Nengas *et al.,* 1995.
Sparus aurata	Gilthead seabream	45	Corn gluten meal (65.7 % CP)	50 %			90.0±2.6 %			Nengas *et al.,* 1995.
Sparus aurata	Gilthead seabream	45	Cottonseed meal (42.2 % CP)	34 %			75.4±2.7 %			Nengas *et al.,* 1995.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Sparus aurata	Gilthead seabream	45	Feather meal (81.2 % CP)	58 %			57.5±0.4 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Feather meal (82.1 % CP)	54 %			24.9±5.0 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Full-fat soybean meal (34.4 % CP)	34 %			75.7±1.9 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Hydrolyzed feather meal; Lipomel (55.2 %CP)	50 %			65.6±11.4 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Meat and bone meal (46.0 % CP)	46 %			43.8±8.7 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Meat and bone meal (56.1 % CP)	44 %			35.4±2.8 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Meat and bone meal (59.4 % CP)	51 %			72.2±2.9 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Poultry by-product meal (51.7 % CP)	44 %			81.8±1.6 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Poultry meat meal (65.3 % CP)	52 %			89.9±1.3 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Skimmed milk powder (32.8 % CP)	30 %			95.5±0.3 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Soybean meal (43.7 % CP)	39 %			90.9±1.1 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Sunflower meal (32.1 %CP)	35 %			86.2±1.3 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	45	Tomato pulp meal (21.4 % CP)	27 %			20.1±0.9 %			Nengas *et al.*, 1995.
Sparus aurata	Gilthead seabream	6	Solvent-extracted soybean meal (43.1 % CP)	48 %	20 %	10, 20, 30, 40 %		Insoluble fibre, trypsin inhibitor, saponins. Lectins, tannins, phytic acid	Reduced growth at 30 % and 40 % substitution	Nengas *et al.*, 1996.
Sparus aurata	Gilthead seabream	6	Full-fat soybean meal cooked for 5 min at 110° C	45 %		35 %		Insoluble fibre, trypsin inhibitor, saponins. Lectins, tannins, phytic acid	Slightly reduced growth	Nengas *et al.* , 1996.
Sparus aurata	Gilthead seabream	6	Full-fat soybean meal cooked for 20 min at 110° C	47 %		35 %		Insoluble fibre, trypsin inhibitor, saponins. Lectins, tannins, phytic acid	Heat processing resulted in growth equal to control diet	Nengas *et al.*, 1996.
Sparus aurata	Gilthead seabream	6	Full-fat soybean meal cooked for 45 min at 110° C	45 %		35 %		Insoluble fibre, trypsin inhibitor, saponins. Lectins, tannins, phytic acid	Heat processing resulted in growth equal to control diet	Nengas *et al.*, 1996.

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Sparus aurata	Gilthead seabream	6	Solvent extracted soybean meal cooked at 100° C	45 %		35 %		Insoluble fibre, trypsin inhibitor, saponins. Lectins, tannins, phytic acid	Reduced growth	Nengas *et al.,* 1996.
Sparus aurata	Gilthead seabream	6	Soybean protein concentrate	45 %		35 %		Insoluble fibre, trypsin inhibitor, saponins. Lectins, tannins, phytic acid	Reduced growth	Nengas *et al.,* 1996.
Sparus aurata	Gilthead seabream	1.6	High quality poultry meat meal	45 %	100 %	75, 100 %			Limiting essential amino acid was methionine	Nengas *et al.,* 1999.
Sparus aurata	Gilthead seabream	1.6	Low grade high-fat poultry by-product meal	45 %	< 40 %	40 %			Reduced growth at 40 % substitution, reduced feed efficiency, NPU and PER	Nengas *et al.,* 1999.
Sparus aurata	Gilthead seabream	1.6	Low grade poultry by-product meal	45 %	50 %	35, 50, 75 %			Reduced growth at 75 % substitution, limiting essential amino acid was histidine	Nengas *et al.,* 1999.
Sparus aurata	Gilthead seabream	1.6	Mixed poultry meat and feather meal	45 %	75 %	75, 100 %			Reduced growth at 100 % substitution, limiting essential amino acid was methionine	Nengas *et al.* , 1999.
Scophthalmus maximus	Turbot	12	Fish protein hydrolysate	56.2–57.2 %		7, 21, 34 %	74–75 %		No significant difference among treatments	Oliva-Teles *et al.,* 1999.
Dicentrarchus labrax	Sea bass	58	Brewers yeast	49 %	50 %	10, 20, 30, 50 %	Decreased to 86.7 from 92.8 when yeast replaced 50 % of fishmeal protein		Up to 30 % protein replacement the yeast improved growth rates and feed efficiency	Oliva-Teles and Gonçalves, 2001. 1999.
Pargrus auratus	Australian snapper	77 ± 24	Soybean meal + poultry offal meal	50.7–53.9 %	50 %	53, 69, 84 %			Significant growth depression at the two highest substitution levels	Quartararo *et al.,* 1998a.
Pargrus auratus	Australian snapper	5 to 20 g	Soybean meal + poultry offal meal	35.1–53.75 %		70 % of diet			Reduced growth, increased feed conversion	Quartararo *et al.,* 1998b.
Pleuronectes americanus	Winter flounder	159.6 ± 8.5	Soybean meal (48.0 % CP)	48 %			91.1–93.8 %			Ramsey *et al.,* 2000.

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Pleuronectes americanus	Winter flounder	159.6 ± 8.5	Canola meal (49.2 % CP)	49.20 %			91.5–94.9 %			Ramsey *et al.* , 2000.
Scophthalmus maximus	Turbot	130.8 ± 1.2	Corn gluten meal	49 to 53.53 %		20, 40, 57 % of diet	Caused reduced dietary protein and energy digestibility		At greater than 20 % of the diet corn gluten meal caused significant reduction of growth and feed efficiency proportional to dietary level, all levels of supplementation reduced PER	Regost *et al.,* 1999.
Sciaenops ocellatus	Red drum (low salinity [7ppt] may not be marine fish)	7.4 ± 0.1	Solvent-extracted soybean meal	33.3 to 34.4 %	50 %	0, 25, 50, 75, 100 %			Reduced growth, but improved feed efficiency and improved apparent net protein utilization, increased body moisture content	Reigh and Ellis, 1992
Sparus aurata	Gilthead seabream	38	Lupin seed meal	55.3 to 57.1 %	30 %	10, 20, 30 %	86.2 to 93.6 % of dietary protein		Trypsin activity retarded at 30 % level	Robaina *et al.,* 1995.
Sparus aurata	Gilthead seabream	38	Soybean meal	52.9 to 56.3 %	30 %	10, 20, 30 %	93.0 to 95.6 % of dietary protein		Trypsin activity retarded, lipid deposition in liver at high inclusion levels	Robaina *et al.,* 1995.
Sparus aurata	Gilthead seabream	40	Corn gluten meal (85.4 % CP)	42.8 to 45.2 %	30 %	20, 30,40 %	No effect on protein digestibility of diet, 88.6 to 92.0 % of dietary protein		No negative effects on growth, feed efficiency, PER, HIS, body composition, or histology.	Robaina *et al.,* 1997.
Sparus aurata	Gilthead seabream	40	Meat and bone meal 64.1 % CP)	42.0 to 44.3 %	20 %	20, 30,40 %	Reduced protein digestibility of diet, 75.0 to 91.5 % of dietary protein		Meat bone meal above 30 % protein produced lipid deposits in liver	Robaina *et al.,* 1997.
Dicentrarchus labrax	Sea bass	200 g	Wheat gluten	48 or 58 % for extruded or pelleted form of diet	30 %	30 %	100 %		Ammonia excretion was lower in fish fed fishmeal-based diets compared to those fed 30 % wheat gluten diets. Lower excretion rates were recorded in fish fed extruded diets compared with those fed	Robaina *et al.,* 1999.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
									pelleted diets.	
Paralichthys olivaceus	Japanese flounder	2.9 to 3.5 g	Meat meal (80 % CP)	52.0 to 55.6 %	60 %	20, 40, 60, 80, 100 %			Reduced growth, feed efficiency, PER at 80 and 100 % replacement	Sato and Kikuchi, 1997.
Seriola quinqueradiata	Yellowtail	230 g	Defatted soybean meal, defatted (47.2 % CP, 1.2 % fat)	46.0 to 48.6 %	30 % of diet	0, 20 and 30 % of diet, with 72, 60, 50 % brown fishmeal, respectively			20 % soybean meal increased growth compared with fishmeal control	Shimeno *et al.,* 1993 a
Seriola quinqueradiata	Yellowtail	38 g	Meat meal (67.9 % CP, 12.5 % fat)	48.9 to 49.3 %	> 30 % of diet	0, 10, 20 or 30 % of diet, with fishmeal at 73, 66, 59 and 52 % of diet respectively			Meat meal increased gain with each increase in content	Shimeno *et al.*, 1993 b
Seriola quinqueradiata	Yellowtail	38 g	Meat and bone meal (56.2 % CP, 10.7 % fat)	47.8 to 49.3 %	20 % of diet	0, 10, 20 or 30 % of diet, with fishmeal at 73, 63, 53 and 43 % of diet respectively				Shimeno *et al.,* 1993 b
Seriola quinqueradiata	Yellowtail	17.2 g	Malt protein flour (49.0 % CP)	48.2 to49.7 %	20 % of diet	0, 10, 20 or 30 % of diet, with fishmeal at 73, 63, 53 and 43 % of diet respectively			Each increase in malt protein meal decreased weight gain with 30 and 40 % levels being most pronounced.	Shimeno *et al.*,1994.
Seriola quinqueradiata	Yellowtail	38 g	Corn gluten meal (66.5 % CP, 0.9 % fat)	49.3 to 50.5 %	20 % of diet	0, 10, 20 or 30 % of diet, with fishmeal at 73, 63, 54 and 44 % of diet respectively				Shimeno *et al.,* 1993 b
Seriola quinqueradiata	Yellowtail	38 g	Rapeseed meal (37.5 % CP, 1.8 % fat)	47.8 to 49.3 %	10 % of diet	0, 10, 20 or 30 % of diet, with fishmeal at 73, 67, 62 and 56 % of diet respectively				Shimeno *et al.,* 1993 b
Seriola quinqueradiata	Yellowtail	21 g	Soybean meal (44.2 % CP, 1.8 % fat) with corn gluten meal (70.3 % CP, 1.7 % fat)	48.8 to 52.0 %	20 % of diet corn gluten with 20 % of diet soybean meal	0 or 20 % soybean meal with 0, 10, 20 or 30 % corn gluten meal, 73, 60, 51, 41 or 31 %				Shimeno *et al.*, 1993 c

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
						fishmeal, respectively.				
Seriola quinqueradiata	Yellowtail	21 g	Soybean meal (44.2 % CP, 1.8 % fat) with meat meal (65.3 % CP, 14.8 % fat)	48.8 to 50.1 %	20 % of diet corn gluten with 20 % of diet meat meal	0 or 20 % soybean meal with 0, 10, 20 or 30 % meat meal, 73, 60, 50, 39 or 30 % fishmeal, respectively.			Diet with 20 % soybean meal and 10 % meat meal gave best growth, 30 % meat meal depresed growth	Shimeno *et al.,* 1993 c
Seriola quinqueradiata	Yellowtail	18.0 g	Raw defatted soybean meal (43.% CP, 1.2 % fat)	50.20 %	> > 30 % of diet	30 % of diet			Great growth depression, reduced hematocrit, greatly reduced serum protein, free amino acids, triglycerides, phospholipid, zinc and calcium levels	Shimeno *et al.,* 1995.
Seriola quinqueradiata	Yellowtail	18.0 g	Extruded soybean meal (44.0 %CP, 1.6 % fat)	52.50 %	> 30 % of diet	30 % of diet			Moderate growth depression, reduced hematocrit	Shimeno *et al.,* 1995.
Seriola quinqueradiata	Yellowtail	18.0 g	Heated full-fat soybean meal (37.9$ CO, 18.8 % fat)	49.90 %	> 30 % of diet	30 % of diet			Moderate growth depression, reduced hematocrit, reduced serum protein, phospholipid, zinc and calcium.	Shimeno *et al.,* 1995.
Seriola quinqueradiata	Yellowtail	18.0 g	Heated defatted soybean meal (45.9 % CP, 1.8 % fat)	52.00 %	> 30 % of diet	30 % of diet			Moderate growth depression, reduced hematocrit, reduced serum protein, phospholipid, zinc and calcium.	Shimeno *et al.,* 1995.
Seriola quinqueradiata	Yellowtail	18.0 g	Soybean protein concentrate (61.9 % CP,.07 % fat)	50.30 %	> 20 % of diet	20 % of diet			No growth depression	Shimeno *et al.*, 1995.
Seriola quinqueradiata	Yellowtail	18.0 g	Soy protein peptide (81.0 % CP, 0.04 % fat)	52.40 %	> 16 % of diet	16 % of diet			No growth depression	Shimeno *et al.*, 1995.
Seriola quinqueradiata	Yellowtail	18.0 g	Soy protein separate (82.4 % CP, 0.7 % fat)	51.70 %	> 16 % of diet	16 % of diet			Slight growth depression	Shimeno *et al.,* 1995.

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Pagrus major	Red seabream yearlings	280	Corn gluten meal (75.2 % CP)	46.6 to 48.8 %	70 %	0, 30, 50, 70, 90, 100 %	70.0 to 91.0 % of dietary protein		Growth depression and reduced feed efficiency above noted fishmeal protein replacement levels	Takagi *et al.,* 2000a.
Pagrus major	Red seabream juveniles	53	Corn gluten meal (75.2 % CP)	46.6 to 48.8 %	30 %	0, 30, 50, 70, 90, 100 %	90.6 to 95.3 % of dietary protein		Growth depression and reduced feed efficiency above noted fishmeal protein replacement levels	Takagi *et al.,* 2000a.
Pagrus major	Red seabream yearlings	280	Poultry by-product meal (57.1 % CP)	46.0 to 52.0 %	100 %	0, 28, 48, 68, 89, 100 %				Takagi *et al.,* 2000b.
Pagrus major	Red seabream juveniles	54	Poultry by-product meal (57.1 % CP)	46.0 to 52.0 %	70 %	0, 28, 48, 68, 89, 100 %	88.3 to 95.5 %		Protein digestibility decreased with each increase in poultry by-product meal	Takagi *et al.*, 2000b.
Pagrus major	Red seabream	11.5 g	Yeast *(Saccharomyces cerevisiae)* (45 to 56 % CP, 1.0 to 2.0 % fat)	51.7 to 54.5 %	40 %	20–40 %	94.4 ± 0.99		Yeast replacement enhanced growth up to maximum replacement tested	Takii *et al.,* 1999a
Pagrus major	Red seabream	18.5 g	Rapeseed protein concentrate (56.6 % CP, 3.5 % fat)	50.9 to 51.9 %	10 % of diet	0, 10, 20 and 30 % of diet with 60, 52, 44, and 35 % brown fishmeal, respectively.		Glucosinolates, erusic acid, sinapine, phytic acid	Growth was depressed with the 20 and 30 % RPC.	Takaii *et al.,* 1999b
Seriola quinqueradiata	Yellowtail	35, 129, 189	Soy protein concentrate (71 % CP)	57 %	20 %	15–45		Phytic acid	Reduction in hematocrit and hemoglobin levels with increased levels of soy protein concentrate, increased growth with EAA supplementation	Takii *et al.,* 1989
Pagrus major	Red seabream	14 g	Soybean meal	48.6 to 51.1 %	< 33 %	17–50		Protease inhibitors, lectins, phytic acid, saponins	Reduced growth above 25 % inclusion (33 % of fishmeal replacement)	Ukawa *et al.,* 1994

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Pseudocaranx dentex	Striped jack	3.6 +/- 0.4 kg	Squid meal or squid meal plus krill meal	47 %		31 % of diet squid meal, or 12 % squid and 21.5 % krill meal			Squid meal improved egg quality but not production; krill meal was not effective.	Vassallo-Agius *et al.*, 2001.
Seriola quinqueradiata	Yellowtail	5 to 8 kg	Krill meal	51.9 to 55.2 %	0 % of diet	0, 20 and 30 % of diet, with 67, 47 and 37 % sardine meal, respectively			Krill meal was not beneficial for egg production in yellowtail broodstock	Verakun[iriya *et al.*, 1997.
Seriola quinqueradiata	Yellowtail	13.5 g	Soybean meal	36.8–49.5 %	55 %	36–91		Protease inhibitors, lectins, phytic acid, saponins	Reduced growth above 30 % of diet (55 % fishmeal replacement), lipid deposition in liver increased proportionally to soybean inclusion, A diet with 25 % SBM and 15 % corn gluten meal showed better feed performance than 40 % SBM.	Viyakarn *et al.*, 1992
Seriola quinqueradiata	Yellowtail	350 g	Soybean meal	37.7–49.5 %	55 %	36–91		Protease inhibitors, lectins, phytic acid, saponins	Reduced growth above 30 % of diet (55 % fishmeal replacement), lipid deposition in liver increased proportionally to soybean inclusion	Viyakarn *et al.,* 1992
Seriola quinqueradiata	Yellowtail	160 and 1,200 g	Defatted soybean meal (46 % CP)	36.1 to 43.6 %	30 % of diet	1, 10, 20, and 30 % of diet, Fishmeal 56, 46, 36 and 26 % of diet	84–86 %		Growth and feed gain ratio were slightly reduced at 30 % soybean meal, but no marked differences in proximate composition of rheological properties of muscle or hemochemical parameters.	Watanabe *et al.*,1992.
Seriola quinqueradiata	Yellowtail	134 g	Defatted soybean meal	45.8–48.0 % dm	54 %	18–54 %	86 %			Watanabe *et al.,* 2001a
Seriola quinqueradiata	Yellowtail	1,200 g	Defatted soybean meal	45.8–48.0 % dm	45 %	45 %				Watanabe *et al.*, 2001a

Species	Common name	Average Initial Weight (g)	Alternative Protein	Dietary protein level (%)	Acceptable % protein replacement	% Fish meal protein replacement tested	Protein digestibility	Antinutrient	Physiological impact	Reference
Seriola quinqueradiata	Yellowtail	135 and 237 g	Soy protein concentrate/defatted soy meal/corn gluten meal/ meat meal/ krill meal						Varied feed processing technologies, soft dry pellets were best	Watanabe *et al.,* 2001b
Paralichthys olivaceus	Japanese flounder	15 g	Malt protein flour	57.6–58.1 % dm	20 %	10 to 50 %				Yamamoto *et al.,* 1995.
Pagrus major	Red seabream	10 g	Malt protein flour	54 %	30 %	10, 20, 30, 40, 50 %	89.30 %		Feed consumption decreased as MPF in the diet increased. There was no difference, however, in weight gain. Thus MPF improved feed efficiency.	Yamamoto *et al.,* 1996.
Paralichthys olivaceus	Japanese flounder	25 g	Defatted soybean meal (44.5 % CP, 2.1 % fat)	42.90 %		86.5 % of diet	79.90 %			Yamamoto *et al.,* 1998.
Paralichthys olivaceus	Japanese flounder	25 g	Malt protein (51.4 % CP, 13.3 % fat)	41.40 %		72.7 % of diet	69.40 %			Yamamoto *et al.,* 1998.
Paralichthys olivaceus	Japanese flounder	25 g	Corn gluten meal (65.4 % CP, 3.0 % fat)	44.30 %		58.6 % of diet	42.10 %			Yamamoto *et al.,* 1998.
Pagrus major	Red seabream	40 g	Non-fermented fish scrap	44.90 %		100 %			Caused decreased growth	Yone *et al.,* 1986
Pagrus major	Red seabream	40 g	Fermented fish scrap	45.50 %		100 %			Fermentation improved growth perofrmance	Yone *et al.,* 1986
Pagrus major	Red seabream	40 g	Fermented-resteamed fish scrap	46.10 %		100 %			Fermentation and resteaming improved growth and feed efficiency even more than just fermentation	Yone *et al.,* 1986

ANNEX 7: COPEPOD CULTURE: A REVIEW

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Abstract

The suitability of copepods as prey for marine fish larviculture, in terms of both nutrition and ease of culture, is reviewed. Harpacticoid copepods are favoured over calanoids, since harpacticoids (due to their benthic ecology) can be reared at much higher densities. However, their benthic nature also makes mass culture more difficult, due to their requirements for large surface areas. Within harpacticoida, *Tisbe* spp. seems most favourable due to their overall high fecundity, and positive phototaxis of the nauplii. Harpacticoids can biosynthesise *de novo* several nutritionally important essential fatty acids (EFA), making them desirable as marine fish prey items. However, a diet rich in EFAs (e.g., animal-derived feed) will improve the productivity of copepod cultures, suggesting that biosynthesis is rate-limiting for reproduction. Substrate is also important in maintaining a good population, since copepod biomass is more dependent on surface area than volume of a culture. Heterogeneous substrates can support large cultures due to their high surface area, but efficient cleaning methods must be devised. Frequent harvesting of populations will maintain good water quality and an overall low density of sexually mature copepods, raising naupliar productivity overall, but over-harvesting will naturally deplete the population. Harpacticoids are generally tolerant of environmental fluctuations but temperature and salinity optima do exist, and these will be species- and strain-dependent. These copepods are nutritionally superior to *Artemia*, due to their ability to biosynthesise EFAs such as DHA, EPA and AA. However, the nauplii are energetically poor but appear to have an appetite-stimulatory effect. Furthermore, uneaten nauplii will grow within the rearing tank and graze on the walls, thus maintaining their own nutritional value and tank hygiene.

Introduction

This paper is a review of the intensive culture of copepods. It will synthesise the findings of previous reports on their suitability as food for marine fish larvae, in terms of nutritional quality and ease of culture. It also summarises the different techniques used in copepod culture (e.g., diet and rate of harvest), and investigates the suitability of different species for the intensive culture of live feed on the basis of their life history.

The larviculture of most marine fish species depends on the provision of live prey during the larval stage. The success of mass culture techniques for both rotifers and *Artemia* has resulted in their widespread use as food for larvae. Both species provide a wide size range suitable for most marine fish larvae, but are inherently nutritionally inadequate. Specifically, essential fatty acids such as docosahexaneoic acid (22:6 n-3, DHA), eicosapentaneoic acid (20:5 n-3, EPA) and arachidonic acid (20:4 n-6, ARA) are important for normal development in several marine species. Both the absolute levels of these fatty acids and the dietary ratios between them are known to be important in maintaining larval viability. Although enrichment regimes to improve the nutritional value of rotifers and *Artemia* are well established, the rearing success of species such as Atlantic halibut (*Hippoglossus hippoglossus*) is still limited.

Evidence suggests that copepods may serve as superior prey for fish and crustaceans in intensive systems (Watanabe *et al.*, 1983; Sun and Fleeger, 1995; Støttrup and Norsker, 1997). Of the order copepoda, the sub-classes calanoida and harpacticoida have been most studied in this respect. For example, when offered a mixture of prey items simultaneously, turbot larvae (*Scophthalmus maximus*) selectively ingested the nauplii of harpacticoids (Van der Meeren, 1991). Furthermore, copepods are known to have greater digestability (Schipp *et al.*, 1999) and a relatively high caloric content per unit weight (Kahan *et al.*, 1982; Sun and Fleeger, 1995). They are also more nutritional than some strains of *Artemia*. Specifically, harpacticoids are rich in essential fatty acids, most notably 22:6ω-3 and 20:5ωn-3 (Leger *et al.*, 1986; Norsker and Støttrup, 1994; Nanton and Castell, 1998*a*), which are vital for marine larval development (McEvoy *et al.*, 1998; Shields *et al.*, 1999). In addition, both calanoids and harpacticoids, from first nauplius to adult, exhibit a broad spectrum of prey sizes (80 to > 900 μm in length and 3–5 μg in dry weight). This makes them suitable for ingestion by a similarly broad range of developing fish sizes (Gee, 1989; Sun and Fleeger, 1995; Schipp *et al.*, 1999). For example, *Acartia* has been sucessfully fed to red snapper (*Lutjanus argentimaculatus*) since rotifers are too large (Schipp *et al.*, 1999), and Kahan *et al.* (1982) has suggested that *Artemia* may be too large for larvae with especially small gapes. In addition to their nutritional and physical superiority as live feed, they are also highly suitable for culture due to their eurythermal and euryhaline characteristics. This gives them tolerance to large environmental fluctuations (Miliou and Moraitou-Apostolopoulou, 1991a; Carli *et al.*, 1995).

Although copepods in general make good prey items, it is important to consider certain inter-species characteristics if they are to be intensively cultured in sufficiently large numbers. The life histories of calanoids and harpacticoids are fundamentally different. Calanoid copepods are entirely planktonic, so their cultivation environments are homogeneous and relatively easy to operate on different scales (Støttrup and Norsker, 1997). However, calanoid production is limited by difficulties in maintaining broodstock at high densities. Stress caused by overcrowding decreases fecundity in the calanoid *Centropages typicus* (Miralto *et al.*, 1996), and cannibalism of nauplii by adults can also occur (Ohno *et al.*, 1990). Conversely, harpacticoids are benthic or epibenthic copepods that sometimes enter near-bottom waters. They often serve as obligate prey for wild marine fish larvae (Sun and Fleeger, 1995). Since they are benthic, their population growth rate depends on the area of solid substrate but they can be produced in volumetrically much denser cultures than calanoids. However, such rearing environments are not homogeneous and are correspondingly harder to scale-up and manage (Støttrup and Norsker, 1997). The two contrasting life histories in culture are emphasised in two separate studies, where a large volume culture of the calanoid *Acartia tonsa* produced 530 eggs.L^{-1} (Støttrup *et al.*, 1986), and a small volume culture of the harpacticoid *Tisbe holothuriae* produced 100,000 nauplii.L^{-1} (Støttrup and Norsker, 1997).

Irrespective of life history, desirable characteristics for the mass culture of copepods are high reproductive potential, short turnover time (from egg to egg), and fast individual and population growth rates. Other requirements are a diet flexible enough to allow good growth on a variety of food sources and a tolerance of a wide range of environmental factors such as temperature and salinity (Sun and Fleeger, 1995). Although *Acartia* species (calanoida) have been cultured successfully for many generations in the laboratory (Støttrup *et al.*, 1986) and in large outdoor tanks (Ohno *et al.*, 1990), the low culture densities make calanoids inappropriate for intensive mass cultivation. Harpacticoid species are therefore the preferred organisms for the development of an intensive copepod culture system (Støttrup and Norsker, 1997). Several harpacticoid species have been studied in this respect, under a variety of regimes. This paper presents a review of the different species and rearing techniques used, with regard to species' life history (reproductive potential, turnover time, etc.). The influences of different diets and substrate are summarised, as are the relative merits of batch *versus* continuous culture and the effects of the rearing environment (e.g., salinity and temperature). Data on optimal harvesting techniques are also investigated. Furthermore, the suitability of harpacticoid copepods as a diet for marine fish larvae is also reviewed.

Harpacticoid life histories

After stocking trays with 40,000 adult *Tisbe holothuriae* harpacticoids (predominantly oviger females), Støttrup and Norsker (1997) harvested a daily average yield of 300,000 nauplii per tray (see below for details of the cultivation system), or 125 nauplii.cm^{-2}.d^{-1}. This relatively high production is due to the high reproductive capacity and short life-cycle of *Tisbe*. For example, Miliou and Moraitou-Apostolopoupou (1991a,b) reported reproductive characteristics for *Tisbe* of 6.14 d for larval development, 1.40 d for egg sac maturation, up to 76 offspring per female, and a longevity of as little as 14.67 d. Due to longevity and the time for larval development, inter-generation time was on average, 8.11 d. Gaudy and Guerin (1982) reported similar findings.

However, species of *Tigriopus* are considered by some to be more suitable for mass culture. Lee and Hu (1981) reported that female *Tigriopus japonicus* can produce up to 204 nauplii in two weeks, and Harris (1973) reported a total production of 301 eggs per female. Therefore, *Tigriopus* is more fecund than *Tisbe*. However, generation time for *Tigriopus* is longer than for *Tisbe* (ca. 14 d) (Takano, 1971; Carli *et al.*, 1995). Although more fecund, the longer generation time of *Tigriopus* means that *Tisbe* can achieve greater maximum yields in mass culture (Miliou and Moraitou-Apostolopoupou, 1991b). Furthermore, within the *Tisbe* genus, *T. holothuriae* has higher values of intrinsic rate of natural increase (r_m) than other *Tisbe* species (r_m is the replacement rate of ovigerous females by their female progeny over time). There is also experimental evidence that *Tigriopus* spp. show maternal hatching inhibition at high population densities (Kahan *et al.*, 1988). As well as evidence of hatching inhibition from *Tigriopus*, there is evidence of sex ratio regulation in *Tisbe*. Zhang and Uhlig (1993) discuss possible causes of sex ratio (the female percentage) regulation:crowding may cause changes in the sex ratio, with low female percentages occurring at high stocking densities.

In addition to favourable reproductive characteristics, *Tisbe* also demonstrate changes in behaviour which make them suitable as prey for marine fish larvae. Although harpacticoids are benthic, *Tisbe* nauplii will collect in the upper water layers of first-feeding tanks after ca. 17 h post-introduction. Prior to this they exhibit benthic behaviour and are found predominantly at the tank walls. This change in behaviour makes them available to foraging fish larvae (Stottrup and Norsker, 1997). Furthermore, *Tisbe* nauplii exhibit positive phototaxis, making them relatively easy to harvest from the upper water layers of a culture. Adult and copepodite *Tisbe* exhibit more benthic behaviour, and are negatively phototactic (Støttrup and Norsker, 1997).

However, the negative phototaxism of adults and copepodites has also been used as a harvesting tool in a third genus, *Amphiascoides*. Sun and Fleeger (1995) collected older stages of copepods by harvesting from the dark end of a rearing tank. They also reported harvests of 70 copepods.$cm^{-2}.d^{-1}$ from their culture system. This respectable harvest included all stages of the copepod life cycle, but no effort was made to maximise yield, so many more could probably have been collected. However, the initial population was much larger than the starting population reported by Støttrup and Norsker (1997): four million compared to 40,000. Despite longer generation times than *Tisbe*, this population of *Amphiascoides* produced a consistently good harvest. This may be due in part to the larger initial population, which was also allowed to grow for one month unharvested.

Sexual dimorphism is apparent in many harpacticoid species. For example, male *T. carolinensis* are 50 to 60 % of the female length (Lee *et al.*, 1985). This will influence the choice of food particle size offered to a culture of harpacticoids. Since males are smaller, with smaller feeding appendages, they will benefit from efficient use of food under a regime of small food particles. Dimorphism will reduce intraspecific competition for food sources, but may also account for differential sex mortality under particular dietary regimes (Lee *et al.*, 1985).

The effects of diet on harpacticoid nutritional quality and population growth

Harpacticoids are widely distributed in coastal waters and particularly abundant in the near-shore benthic marine environment (Hicks, 1980). In their natural habitat, they feed on algae and settled organic particles (Coull and Wells, 1983). In culture, a combination of different algae has been shown to be a better diet than monoxenic algal cultures, presumably due to the mix of vitamins, minerals and trace elements that are vital for the survival, growth and reproduction of animals (Lee *et al.*, 1985). Furthermore, as mentioned above, a range of food sizes will reduce differential sex mortality. However, copepod diet (in culture) is not necessarily limited to algal species. Carli *et al.* (1995) compared *Tigriopus fulvus* culture performance between an algal (*Monochrysis lutheri*, 70,000 cells.ml^{-1}) and yeast diet (*Saccharomyces cerevisiae,* 0.1 mg.ml^{-1}). The study found that yeast-fed copepods had a lower daily production of nauplii than copepods fed algae, but naupliar production was spread over a longer time period. Algae-fed copepods also exhibited lower survival and a higher incidence of infertile females. The authors concluded that both diets were beneficial: algal food provided a high daily naupliar production, but yeast-fed copepods had higher overall production over a longer period of time. Therefore, diet can have a big influence on the reproduction and life history of harpacticoids.

However, it must be stressed that copepod productivity is directly correlated with several life-history parameters: survival, sex ratio, the number of egg-sacs per female, the number of offspring per female and the development rate. Furthermore, different diets may favourably affect different parameters. For example, Miliou and Moraitou-Apostolopoulou (1991b) tested the influence of different diets on several life-history parameters. They reported that the seaweed *Ulva* increased the developmental rate of nauplii and copepodids, also increasing the survival of nauplii, whereas the artificial compound feed Fryfood® (Waterlife) increased offspring production and increased the survival of copepodids.

Generally, the suitability of different diets depends on their digestibility and how they fulfil the nutritional requirements of the species. The free amino acid content does not seem to be very important in the effectiveness of food, since the diet richest in amino acids tested by Miliou and Moraitou-Apostolopoulou (1991b) was also the least efficient. Despite this, free amino acids are generally very abundant in copepods (Fhyn, 1989). However, total protein content does seem to be important. Lee *et al.* (1985) found that the algal diet with the highest protein content provided the best copepod reproductive performance. Similarly, Guidi (1984) found survival and naupliar development rate related to the protein content of the diet. The paucity of information on proteins and amino acids in copepod diet reflects the importance of essential fatty acids (EFA) in marine fish nutrition, with a corresponding interest in their role in copepod nutrition.

It is well known that cold-water marine fish larvae have a nutritional requirement for live food with high concentrations of the long-chain n-3 essential fatty acids such as eicosapentaenoic acid (EPA; 20:5 n–3) and docosahexaenoic acid (DHA; 22:6 n–3) (Watanabe, 1982). Arachidonic acid (10:4 n–6; AA) is also considered to be essential for marine fish, but at a lower demand (Castell *et al.*, 1994). Marine fish require these fatty acids since they lack the desaturase enzymes necessary to convert short-chain fatty acids into their long-chain EFA end-products (Tocher, 1989). Harpacticoid copepods can produce significant amounts of EPA and DHA when fed diets deficient in these EFA. Furthermore, copepods have a high proportion of DHA in their polar membrane lipids, as opposed to their neutral lipids (cf. *Artemia*). Polar lipids are involved in the formation of lipid emulsions which facilitate lipid digestion. The increased amounts of DHA in the polar lipid fraction therefore increases the digestibility of DHA in marine fish larvae fed copepods (Nanton and Castell, 1999). Although copepods are capable of *de novo* fatty acid biosynthesis, it ceases in the presence of a substantial input of dietary fatty acids. However, the fatty acid content of food will influence copepod productivity, namely improving copepodid survival and egg number (Miliou and Moraitou-Apostolopoulou, 1991b). Therefore, animal-derived feeds have proved to be more efficient than compound vegetarian feeds (e.g., soya and yeast), and

Guérin and Gaudy (1977) have suggested that *T. holothuriae* demonstrates a higher productivity and lipid content when fed on animal-derived compound artificial diets. *Tisbe* species are attractive live food organisms for marine fish culture since their high desaturase activity ensures that they produce large quantities of favourable fatty acids (such as DHA) even if the EFA composition of their diet is poor (Nanton and Castell, 1999). Since fatty acid biosynthesis ceases under a large input of dietary fatty acids, the fatty acid distribution in copepods largely reflects that of their diet (Norsker and Støttrup, 1994). Biosynthesis of EFAs may be rate-limiting for reproduction, so the dietary supply of fatty acids would enhance naupliar production (see above). Furthermore, diets rich in animal-derived fatty acids increase the carbon content, C:N ratio, and the energy content of *T. holothuriae* (Miliou, 1996). Despite improved productivity under such regimes, final body size remains almost constant. It is hypothesised that harpacticoids, since they are not normally subjected to periodic variation in food supply, can utilise excess energy for reproduction at the expense of storage. This will keep final body size constant (Miliou, 1996).

It must be stressed that the suitability of diet does not rely on chemical composition alone. The seaweed *Ulva*, as well as improving developmental rate, increases the substratum available to *Tisbe*, and an increase in the surface to volume ratio in rearing tanks can result in an increase in production (Miliou and Moraitou-Apostolopoulou, 1991b). Furthermore, live seaweed can supply oxygen and absorb toxic compounds (Harlin, 1978). As mentioned earlier, a large range of particle sizes is necessary to match the changing size of copepod mouthparts during development. The success of a diet will also rely on its capability of allowing bacteria to proliferate. Harpacticoids will assimilate bacteria, and bacteria will also serve as food to microzooplankton on which the copepods feed (Rieper, 1978; Miliou and Moraitou-Apostolopoulou, 1991b). Finally, the optimum diet should be cheap and simple, such as an easily available animal-derived compound diet, used in conjunction with an algae (rich in fatty acids) or a seaweed to increase surface area (Guérin and Gaudy, 1977; Miliou and Moraitou-Apostolopoulou, 1991b).

The effects of different substrates on harpacticoid populations

The importance of substrate has already been stressed above. Choosing a suitable substrate is vital for good productivity in a copepod culture. Since harpacticoids are benthic, a large surface area to volume ratio is vital. Støttrup and Norsker (1997) investigated two different methods for achieving this. One method employed large, shallow trays, to which fresh sea water and algae (*Rhodomonas baltica*) were added daily, after removing *Tisbe* nauplii through a mesh. This is a batch culture method, and is rather labour intensive. Therefore, Støttrup and Norsker (1997) designed a continuous culture system, to compare efficiency and productivity of that system with batch culture. The continuous culture system consisted of a closed bioreactor continuously supplied with algae. Positively phototactic nauplii were harvested from the upper water layers. In order to provide a large surface area, the bioreactor was filled with polypropylene balls. All the balls developed a thin biofilm of sediment. By calculating the surface areas of both systems, the authors deduced that productivity in the bioreactor was only 32 $mg.m^{-2}.d^{-1}$, compared with 100 $mg.m^{-2}.d^{-1}$ in the tray system. The authors surmised that the relatively poor productivity was due to insufficient capacity of the algal dosing system. The addition of more food did improve productivity in the continuous system (Støttrup, unpublished data).

It is precisely their preference for sand or mud substrates of a particular size, texture and faunal composition which precludes the easy cultivation of harpacticoids in a laboratory (Hockin, 1981; Chandler, 1986). Although *Tisbe* spp. are semi-planktonic, a substrate providing a large surface area will be important for the adults. Chandler (1986) experimented with mud and sand substrates and found that cleaned and sorted mud was superior because all the sediment particles were less than 0.125 mm. Adult and late-stage copepodites are usually larger than 0.125 mm, allowing them to be easily separated in a sieve. Care must be taken to prevent excessive bacterial films overgrowing the substrate, entrapping and killing the copepods. Chandler (1986) prevented fouling by not feeding cultures until accumulated food on the substrate disappeared.

Although most studies attempting large-scale harpacticoid production have eliminated the use of natural substrates, and use glass or plastic surfaces, Sun and Fleeger (1995) used limestone cobbles in a system that produced in excess of one million individuals (5 g dry weight biomass) of *Amphiascoides atopus* per day. They covered a surface area of 2 m^2 with cobbles (1 cm in diameter) to 2 cm in depth. Air lifts were used to increase gas exchange in the limestone. Sun and Fleeger (1995) attributed the large daily harvests to the large surface area available for growth, and to the biofilms and bacteria coating the limestone cobbles. The system only ran for 17 weeks, and the authors stressed the need to devise a low-maintenance method to clean the culture and prevent the build-up of faeces and decomposing algae. Kahan *et al.* (1982) used a novel technique, by-passing the use of trays or natural substrate. They employed floating net-bottomed trays with an appropriate pore size, allowing *Tisbe* nauplii to pass directly into the fish tank. This system allowed close observation and control of conditions, particularly of excessive bacterial growth. The total number of nauplii produced per tray (200 cm^2) per day was 132,000, giving a density of 10 nauplii per ml for the whole tank volume. The floating trays also removed the need to harvest copepods and then offer them to fish larvae.

Effects of harvesting

Harvesting, or the regular exploitation of copepod cultures, will act on the biomass and the production of the population. Therefore, a good knowledge of the conditions of exploitation is required to guarantee the maintenance of a good yield. Using *T. holothuriae*, Gaudy and Guerin (1982) maintained populations for 70 days, and found that mean production was highest in the most frequently harvested culture (weekly exploitation, with 50 % of the tank volume being removed). The frequent harvesting increased the ovigerous rate in the population (the percentage of females with egg sacs), demonstrated by the high production. By citing a paper by Alessio (1974), where a population of *T. furcata* were exploited daily at 40 % with no decrease in yield, Gaudy and Guerin (1982) surmised that the maximum yield of their own cultures was probably not reached. However, they caution that too rapid a frequency will decrease the yield, citing a study on *A. tonsa* (Heinle, 1970). This study reported that a harvesting frequency of four days was too rapid to maintain the population level. By also varying the amount of water removed at harvest, Gaudy and Guerin (1982) hypothesised that the increase in production was due to the improvement in water quality and the decrease in density brought about by the harvest. Crowding can lower naupliar production and survival (Zhang and Uhlig, 1993). Sun and Fleeger (1995) also reported no population decline, even with a high level of exploitation (more than one million individuals per day).

These studies complement earlier work by Hoppenheit (1975, 1976). He reported low naupliar mortality at high exploitation rates, attributable to the subsequent low densities. He also proposed that sex ratio was influenced by high exploitation rates, or low densities: a surplus of males was found at an exploitation rate of 90 %, while more females were found at an exploitation rate of 10 %. However, the paucity of explicit studies on the effect of exploitation rates on standing biomass demonstrates that this important factor has been relatively neglected.

Environmental effects on harpacticoid quality and growth

It has already been stated that the density of a population can affect population growth by modulating survival, development and fecundity. This is an important observation, especially since the mass culture of *Tisbe* spp. is related to the available substrate area, rather than the available water volume (Zhang and Uhlig, 1993). Female fecundity is influenced by both the density of breeding females and nauplii. It has been suggested that complex chemical compounds may be produced by the animals as a result of crowding, allowing them to perceive and respond to different crowding levels (Zhang and Uhlig, 1993). Alternatively, it has also been hypothesised that direct close encounter may change the behaviour of copepods, and even their development (Brand, 1985). Since fecundity decreases with increasing density, Zhang and Uhlig (1993) suggested a density of 40 female *T. holothuriae* per cm^2 to achieve maximum daily yield of nauplii.

In addition to biotic environmental factors, abiotic factors will also affect the population dynamics of harpacticoids. Harpacticoids are very tolerant to environmental fluctuations, but discerning the most favourable conditions for mass culture is paramount. Using a Greek strain of *T. holothuriae*, Miliou and Moraitou-Apostolopoulou (1991) found an optimum salinity of 38 ‰ for offspring productivity. The decreased fecundity beyond 38 ‰ suggested inhibition of an enzymatic mechanism in salinities different from those to which the strain is genetically acclimated. Furthermore, in extreme salinities (20 and 48 ‰), the strain tested was not able to survive. Interpopulation tolerance differences are therefore of considerable importance, both for their ecology and for mass culture. Generally *Tisbe* has limited euryhalinity, and can only reach its maximum development in culture within a narrow range of salinity (Miliou and Moraitou-Apostolopoulou, 1991). Moreover, sub- and supra-normal salinities will cause a decrease in total body length. Extreme salinities require extensive adjustments in osmoregulation and will affect structural properties of aquatic invertebrates (Miliou, 1996). Estuarine harpacticoids, such as *Amphiascoides subdebilis*, are able to survive in a wider range of salinities, but will still show a preferred optimal salinity for fecundity and longevity (Ingole, 1994). Salinity appears to affect mainly longevity and the number of offspring per egg sac. Temperature appears to have a more pronounced effect (Miliou and Moraitou-Apostolopoulou, 1991).

Miliou and Moraitou-Apostolopoulou (1991) state an optimal culture temperature of 19 ^{o}C for their strain of *T. holothuriae*. Lower temperatures caused longer development and maturation, but an increase in the number of offspring per egg sac. Higher temperatures caused an acceleration of development and maturation, but a reduction in the number of offspring per egg sac. In both cases the number of egg sacs and offspring decreased. Therefore, temperature had a more pronounced effect than salinity. There is a well-documented inverse relationship between temperature and body size of zooplankton (McLaren, 1965; Corkett and McLaren, 1978). Fecundity increases with increasing body length (McLaren, 1965). The increase in total body length of *T. holothuriae* at low temperatures is associated with an increase in egg production per egg sac (Miliou and Moraitou-Apostolopoulou, 1991). The two factors of temperature and salinity act on populations independently, as there was no significant interaction between the two variables (Miliou, 1996).

In addition to affecting population variables, temperature can also have a marked effect on the nutritional quality of copepods. Nutritionally important long-chain EFAs, such as DHA, EPA and AA, are incorporated into the cell membranes of animals, where they help maintain membrane fluidity. Nanton and Castell (1999) hypothesised that in response to lower temperatures, copepods would increase the amounts of long-chain EFA to maintain a standard membrane fluidity (homeoviscous adaptation; Sinensky, 1974), since they can synthesise fatty acids *de novo*. Using two genera of harpacticoid, *Amonardia* and *Tisbe*, the authors indeed found higher levels of DHA, EPA and AA at 6 °C compared with 15 °C. However, DHA and EPA were also high at 20 °C. This may have been due to the selective oxidative catabolization of neutral lipids rather than phospholipids, raising the relative amounts of DHA and EPA accordingly. Although satisfying the hypothesis of homeoviscous adaptation, the high levels of DHA and EPA (and high DHA:EPA ratio) at the higher temperature precludes the need to cold-water-enrich these harpacticoids.

Harpacticoids such as *Tisbe* are semi-planktonic, so it is worth noting the effects of turbulence on copepod productivity. The first limiting step for feeding and meeting is the initial encounter of prey or mate. Under turbulence, the contact rates are higher than predicted when only densities and relative velocities of predator and prey are considered (Alcaraz, 1997). Although food intake rates can be increased by turbulence, it can also decrease the number of eggs laid per female. This is due to an increase in metabolic rate caused by the turbulent conditions, and a concomitant decrease in the energy allocated to egg production (Saiz and Alcaraz, 1992). Although this work was carried out on calanoid copepods, it will apply to zooplankton in general. Working on *Acartia grani*, Alcaraz (1997) observed a reduction in the development time for the different instars under turbulent conditions. Life-span was also shortened, and both effects may be due to higher metabolic expenses. Although feeding is increased, turbulence tends to reduce overall biomass due to the decrease in size and fecundity. Therefore, the turbulence characteristics of a culture can be a significant factor in the modulation of copepod populations.

Harpacticoids as prey for marine fish larvae

In a study comparing wild copepods with enriched *Artemia*, McEvoy *et al.*, (1998) found much greater proportions of polar lipids in the copepods (66 % compared with 36 % in the enriched *Artemia*). This was reflected by significantly higher proportions of polar lipids in the copepod-fed fish. It has been suggested that polar lipids are more readily digested by larvae, and may also facilitate the digestion of other lipids in the digestive tract of larval fish (Koven *et al.*, 1993). Furthermore, halibut larvae fed copepods were heavier from 30 days post first-feeding than their *Artemia*-fed conspecifics (McEvoy *et al.*, 1998).

Harpacticoids such as *Tisbe* have great potential as live food, since they contain large amounts of EFAs and have consistently high DHA:EPA ratios even when fed on EFA-poor food such as yeast. Furthermore, newly hatched nauplii are 90 µm long and reach 2 mm as adult females, so they could potentially replace both rotifers and *Artemia* throughout the first-feeding stage of marine fish (Nanton and Castell, 1998a). However, a potential drawback would be their benthic nature. *Tisbe* tends to stay near the sides and the bottom of the tank, rather than swimming freely where the larval fish can feed upon them (Nanton and Castell, 1998a). Nevertheless, a later study by the same authors demonstrated improved growth in haddock larvae (*Melanogrammus aeglefinus*) fed on *Tisbe* at 19 days post-hatch, compared with those fed rotifers (Nanton and Castell, 1998b). Furthermore, Støttrup and Norsker (1997) demonstrated that, despite their benthic nature, *Tisbe* nauplii were available to fish larvae. Nauplii were present in the water column from ten hours post-introduction, and successful first-feeding of turbot larvae could be established with *Tisbe* nauplii alone. *Tisbe* nauplii are half the size of rotifers, and have a caloric content of 0.00147 J per individual, compared with 0.0036 J for rotifers. Despite this, the introduction of *Tisbe* nauplii seemed to have an appetite-stimulatory effect, and fish co-fed with *Tisbe* and rotifers grew better than those fed rotifers alone (Støttrup and Norsker, 1997). A study using larval Dover sole (*Solea solea*) showed similar results. Including *Tisbe* in the dietary regime resulted in improved appetite and growth rate compared to sole reared on *Artemia* alone (Heath and Moore, 1997). Furthermore, pigmentation is improved in both halibut and sole larvae, possible due to the high levels of DHA present in copepod diets (Heath and Moore, 1997; McEvoy *et al.*, 1998).

As well as providing a superior diet, harpacticoid nauplii which are not eaten would be able to find nourishment in fish-rearing tanks by feeding on detritus, the biofilm, and bacteria, maintaining their nutritional value as well as keeping the tank clean. These are both important factors in the successful rearing of marine larvae (Norsker and Støttrup, 1994).

Conclusions

By comparing harpacticoid life histories, *Tisbe* spp. seem more suitable for mass culture due to their short generation time and intrinsic rate of natural increase. Although *Tigriopus* spp. are generally more fecund, their longer generation time precludes their suitability as species for mass culture. Although benthic, *Tisbe* nauplii will exhibit positive phototaxis, enabling their easy harvest and availability to foraging fish larvae. In terms of suitable diet for mass harpacticoid culture, the importance of a large size range of food particles must be stressed, since sexual dimorphism occurs, as does changing mouthpart size during copepod development. Of several diets tested, animal-derived compound feed seems to perform best. Although harpacticoids can biosynthesise nutritionally important essential fatty acids, their productivity is rate-limited by this biosynthesis, so cultures fed with EFA-rich diets usually show enhanced productivity. However, seaweed diets, as well as providing food, also provide a large area of substratum. This is an important point, as harpacticoid productivity is related more to surface area than volume, and seaweed will supply oxygen and absorb toxins. Although most cultures have been raised using glass or plastic surfaces, good results can be obtained using more natural substrates, such as limestone cobbles. This technique provides a large surface area for copepod growth, and also for biofilm and bacteria deposition. However, effective methods of cleaning are still to be developed.

Harvesting will also affect productivity, by modulating the standing density of the culture. Frequent harvesting (with the addition of fresh sea water to replace that abstracted by the harvest) maintains a low standing density and high water quality, with subsequent high naupliar productivity. However, too frequent a harvest will eventually reduce the yield, by lowering the population level. A density of 40 female *T. holothuriae* per cm^2 has been suggested to achieve a maximum yield of nauplii. Although *Tisbe* are tolerant of environmental fluctuations, optimal salinities and temperatures of 38 ‰ and 19 ^{o}C have been suggested for *Tisbe* cultures, although there will be inter-strain differences in performance. Temperature will also influence the amounts of EFA in copepods, with high amounts at low temperatures. However, there seems to be no need to cold-water-enrich harpacticoids, as high levels of EFA are also present at higher temperatures due to selective oxidative catabolization of neutral lipids. Turbulence will also affect productivity in contrasting ways. Although it can increase feeding rates, turbulence can also increase metabolic expenses, lowering productivity and life-span in copepod cultures. Finally, harpacticoids seem suitable as a diet for marine fish larvae due to their relatively high levels of essential fatty acids, even when fed on an EFA-poor diet. The nauplii are available to fish larvae in the water column, although the adults are benthic. Furthermore, although small in comparison to rotifers, *Tisbe* nauplii appear to have an appetite-stimulatory effect on fish larvae when co-fed with rotifers. Uneaten harpacticoids will also graze on rearing tank walls, maintaining their own nutritional value and tank hygiene.

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ANNEX 8: THEME SESSION PROPOSALS

Proposed Theme Session for ICES Annual Science Conference
Mariculture Committee
Working Group on Marine Fish Culture

Water treatment in intensive fish cultures

Synopsis

The development of intensive methods for the cultures of marine fish requires in most cases pretreatment of the water entering the fish tanks. In many places open systems for intensive cultures are still commonly used, although closed, recirculated systems have received increasing attention over the past years.

Traditional methods for water treatment in open systems have more or less been restricted to filtration of particles, maybe in combination with UV-treatment. These systems have been shown to be vulnerable for pathogens entering the system through the general water supply. An example of this is the VER-disease in larval halibut cultures caused by a NODA-virus. To improve this situation, new techniques including protein skimming and ozone, have been employed with good results. In most cases, open systems demand temperature adjustment, which opens the possibility for gas supersaturation. Although recognized, very little is known about the physiological impact low tension gas supersaturation exercises on the organism.

Closed systems have the advantage of high controllability and stability. However, buildups of metabolic wastes like nitrate compounds, and unfavourable bacterial communities may still be problematic. Ammonium and nitrite may represent severe hazards to the fish, and need to be controlled. Biofilters represent an additional culture that may cause problems, both in sustainability and in efficiency. The use of ozone in combination with protein skimmers has been shown to be very efficient in removal of suspended particles. Oxidized residuals (e.g., bromate) due to high redox potentials is suspected to have a long-term negative impact on the fish.

This theme session will be devoted to examination of methods for water treatment in both open and closed systems for intensive fish culture, and the physiological response of fish to environmental factors resulting from the culture conditions.

Proposed conveners:

Anders Mangor-Jensen
Terje van der Meeren
Torstein Harboe
Uwe Waller
Ed Trippel

ANNEX 9: FISH WELFARE IN RELATION TO MARINE FISH CULTURE WELFARE

Belgium:

Information supplied by Peter Coutteau,

That is an easy one: There is no marine fish culture in Belgium except for the experiments at ARC.

Canada:

Submitted by Gordon Doonan, AAFC and a long-standing member of ECFAWB.

Aquaculture industry representatives have attended the annual meeting of the Canada Committee on Animals on past occasions, although that committee does not appear to be involved in aquaculture either.

Both of the above are committees of the Canadian Agri-Food Research Council (CARC), which also administers the Recommended Codes of Practice for the Care and Handling of Farm Animals. Derek Anderson is the Chair of CARC's Livestock Codes of Practice Steering Committee and also of the Canada Committee on Animals.

CARC has been considering the possibility of developing an aquaculture code of practice. Under the Office of the Commissioner for Aquaculture Development's "Aquaculture Partnership Program", a Canadian Aquaculture General Code of Practice may be produced. This General Code would reflect the numerous aquaculture codes currently in use or in preparation. I am not aware of the status of this General Code.

An eventual "Recommended Code of Practice for the Care and Handling of Farm Animals – Aquaculture" under CARC could become the animal welfare component of the Canadian Aquaculture General Code of Practice.

Denmark:

Information supplied by Josianne Støttrup, Danish Institute for Fisheries Research, Department for Marine Ecology and Aquaculture, Charlottenlund Castle, DK-2920 Charlottenlund, Denmark [jgs@dfu.min.dk].

In Denmark there are no specific rules or regulations concerning animal welfare in fish culture. The "Standing Committee of the European Convention for the protection of animals kept for farming purposes" is in the process of writing up recommendations within the EU. It was hoped that these recommendations concerning fish would have been approved in 2001, but it seems more likely that they would be approved during the first half of 2002.

It would then be up to each country to implement parts of or the recommendations relevant to their industry.

Regulations for the protection of animals exist in Denmark but do not specifically mention fish.

France:

Information provided by: Jeannine Person-Le Ruyet, IFREMER, Centre de Brest, Laboratoire de Physiologie des Poissons, BP 70, 29280 Plouzané (jperson@ifremer.fr).

For France, there are no specific rules or regulations concerning animal welfare in fish culture. The "Standing Committee of the European Convention for the protection of animals kept for farming purposes" is in the process of writing up recommendations within the Conseil de l'Europe (Strasbourg). For France the Ministry of Agriculture and Fisheries (Food directory, DGAL) is the executive official Authority in charge of welfare "project". DGAL has been cooperating with INRA and IFREMER and fish farmers associations on this topic for about three years.

DGAL represents, with a delegate from INRA, the French point of view at Conseil de l'Europe (Strasbourg) where recommendations are prepared. Concerning fish welfare, a general recommendation for fish culture and specific recommendations for any cultivated fresh water and sea water fish species are still under discussion for approbation by Conseil de l'Europe. When accepted, these recommendations will be submitted to European Parliament (Bruxelles). At the end of the process, European Directives will be published and applied as European regulations.

For your information INRA IFREMER welfare group on rainbow trout (in fresh water) has carried out some experiments focused on interactions of stocking densities with water quality and/or access to food (no data published yet). For sea water species, we just started to look for relevant indicators of fish welfare in sea bass and turbot.

Iceland:

There is no specific act on fish welfare in Iceland but it will fall under The Animal Welfare Act No. 15/1994. The following is a rough translation of the most relevant points:

This act applies to all animals, but is especially aimed at animals held for farming purposes, such as horses, cattle, sheep, etc. It applies to the general public but is not specifically aimed at controlling scientific experimentation. It says that all animals must be treated well. It is not allowed to hurt or harm animals. An attempt should be made to avoid testing their strength and endurance. The animals must be provided with satisfactory accommodation, feed, drink and caretaking. They must have sufficient room for movements according to general practice and knowledge. Animals must be protected against too much noise. The owners or the caretakers of the animals are responsible for their health and must do what is necessary to avoid their suffering. Inspection by police, animal welfare committees and official veterinarians may be carried out at any time.

Reykjavík, December 3, 2001, Björn Björnsson, Marine Research Institute

Norway:

Information supplied by Terje van der Meeren.

In Norway, fish welfare in research and commercial culture is regulated by several laws related to fish welfare, fish disease control, and fish breeding. The primary law is the "*Animal Welfare Act*" (20 Dec.1974, No. 73), which includes most aspects of animal welfare in vertebrates and crustaceans. In addition, there are a number of regulations that detail specific areas of the act. Some of these regulations are covering research or commercial culture of aquatic organisms, e.g., "*Regulation on transport of aquatic organisms*" (20 Feb. 1997, No. 193) and "*Regulation on experiments with animals*" (15 Jan. 1996, No. 23). In the latter, crustaceans are defined as decapods. Other regulations are more specific to freshwater fish culture or salmonids. The executive official authority for the *Animal Welfare Act* is shared by local governmental Animal Welfare Committees and the Norwegian Animal Health Authority (a directorate under the Ministry of Agriculture), the latter often represented by the District Veterinary officers. The Police are also allowed to inspect animal livestock.

Norway has ratified the European Convention for the Protection of Vertebrates used for Scientific Purposes. In Norway, the National Animal Research Authority (a separate official authority under the Ministry of Agriculture) approves institutions and laboratories for experimental work with animals (vertebrates and decapods). Compulsory education in laboratory animal science is required for all users of laboratory animals in Norway. The National Animal Research Authority has to approve all experiments with animals, but may also nominate a local responsible authority to do this.

Another law that affects animal welfare in fish culture is the "*Fish Disease Act*" (13 June 1997, No. 54). Also under this act there are a number of regulations. These are made to ensure good hygienic conditions and procedures in all part of the production cycle. The most important actions are of a preventive character and include obstruction of pathogen transfer between the different production steps of fish and other aquatic organisms. The act also states a demand for authorised permission to establish, extend or move fish farms. As for the *Animal Welfare Act*, the executive official authority for the *Fish Disease Act* is the Norwegian Animal Health Authority, represented by the Regional Veterinary officers and the District Veterinary officers. For some areas of the act regarding slaughtering and processing of fish and aquatic organisms, the executive official authority is the Directorate of Fisheries (Ministry of Fisheries).

Finally, the "*Act no.68 of 14 June 1985, relating to the breeding of Fish, Shellfish etc.*" contributes to the welfare of aquatic organisms by demanding that fish farms must be licenced. This controls among others farm size, location, environmental monitoring, and competence of the fish farmer. One of the most important regulation under the Act is the "*Regulations relating to Establishment, Operation and Disease-Prevention Measures at Fish Farms*" (18 Dec. 1998, No. 1409). This regulation states that "fish farms shall be established and operated in accordance with the requirements set forth in the licences and relevant rules and in other respects in such a manner that they are technically, biologically and environmentally acceptable". It includes demands for a management plan, health control, fish density (maximum 25 kg/m^3), environmental impacts, and record keeping (stocking density, feeding and food consumption, escapes, health situation, use of drugs and chemicals, etc.). The executive official authority for the Act is the Directorate of Fisheries.

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Sweden:

A compilation by Hans Ackefors, Swedish Board of Agriculture

Responsibilities

The Swedish Board of Agriculture is the Government's expert authority in the field of agricultural and food policy, and the authority responsible for the sectors agriculture, horticulture and reindeer husbandry. Its responsibility therefore includes monitoring, analysing and reporting to the Government on developments in these areas, and implementing policy decisions within its designated field of activities.

One major task of the Board of Agriculture is the administration of the Common Agricultural Policy (CAP) of the European Union. The Board works for simplification of the CAP legislation, and to promote an efficient and environmentally adapted agricultural policy in the EU. The Board shall also strive to promote rural development.

The Swedish Board of Agriculture is also the chief authority for Sweden's district veterinarians and the authority responsible for food supply within the civilian defence of the total defence system.

The Common Agricultural Policy

The CAP is applied within the European Union. This means, for example, that agricultural produce is traded freely within the EU, and that price and support policies are determined jointly by Member States. The CAP is based on maintaining the price level within the EU borders by means of border protection and export refunds. Lowered price levels within the Union are in part compensated for by direct support to farmers. The CAP also includes various kinds of environmental, regional, and structural policy measures.

Regarding CAP administration, the Swedish Board of Agriculture has a central coordinating role among the central and regional authorities involved. This means that the Board's main activities are to a great extent concerned with administering and implementing EU legislation and support measures. The Board also participates in EU committee work together with the Ministry of Agriculture, Food and Fisheries. The Board gives effect to agricultural policy by monitoring developments, proposing changes, coordinating activities, disseminating information, managing compensatory and regulatory systems, and by engaging in follow-up and control.

The Board is the decision-making authority for measures in the fields of intervention, export refunds, special beef premium, national support, milk quotas and premium rights. Decisions concerning the majority of the direct aids to farmers are made by the County Administrative Boards.

Furthermore, the Swedish Board of Agriculture is the only Swedish agency paying support and subsidies financed by the European Agricultural Guidance and Guarantee Fund (EAGGF).

Animal welfare and sustainable agriculture

The Government has laid down that Swedish agriculture is to be both ecologically and economically sustainable. Ecological sustainability implies that the sector is to conserve its resources, and that farming should be conducted in an environmentally adapted and ethically acceptable way.

The Swedish Board of Agriculture promotes animal health by sharpening animal welfare requirements, and by combatting and preventing the spread of contagious animal diseases. Through the organisation of district veterinarians, the Board is required to provide cost-efficient emergency veterinary care in all parts of the country at all hours, and to work with preventive animal health care.

The Swedish Board of Agriculture works to promote a rich and varied agricultural landscape with biological diversity and with as little negative impact as possible on the environment. The Board is also responsible for combatting plant pests.

Preparedness

As the authority responsible for food preparedness, the Board is to ensure that the population has sufficient access to food in the event of a national emergency. In addition the Board shall prevent or limit the consequences for the agri-food sector in the event of severe peacetime crises.

United Kingdom:

Scotland

Provided by: Andrew Voas, Veterinary Adviser

Legislation on fish welfare in scotland

1. The main legislation concerning the welfare of farmed fish in Scotland is the Welfare of Farmed Animals (Scotland) Regulations 2000, which are made under the Agriculture (Miscellaneous Provisions) Act 1968. These Regulations apply to any animals, including fish, which are kept for the production of food or for other farming purposes. The Regulations require keepers of animals to take all reasonable steps to ensure that the animals are not caused any unnecessary pain, suffering or injury. They also make provision for access by persons attending animals to statutory welfare codes (which are still being developed for fish) and allow an authorised person to serve a notice on the person in charge of the animals requiring them to take action to improve the situation when animals are being kept in a way which is likely to cause unnecessary pain, suffering or injury.

2. I hope this is useful for you. The other legislation worth noting is the Agriculture (Miscellaneous Provisions) Act 1968 itself which makes it an offence to cause or permit unnecessary pain or distress to livestock on agricultural land. This does not specifically mention fish but I think we have obtained legal advice in the past which was that the Act would in fact apply to fish held for the production of food in most cases.

United States of America:

Provided by: Dr. Lawrence J. Buckley, Director URI/NOAA CMER Program, University of Rhode Island, Graduate School of Oceanography, Narragansett, RI 02882.

There is no single set of rules governing animal welfare in federal government laboratories. Regulations and reporting requirements vary among agencies. Marine fishes are particularly susceptible to poor water quality, culture and handling practices. Consequently, it is always in the researcher's best interest to maintain marine fishes under optimal conditions.

At the grower level the rules governing animal welfare are rather vague or non-existent. There are no requirements *per se* at the commercial culture level (except possibly in CA). This may be due to the fact that the commercial grower needs the fish to grow and remain healthy until harvest. If conditions are poor, the commercial entity will most likely not perform well and in fact probably solve the animal welfare problem by going out of business. Commercial producers do have reporting requirements for the fish, but these are limited to numbers of animals produced and sold.

At the retail level there is great concern for animal welfare and how fish are displayed. Some states and municipalities have regulations and guidelines in place related to display of fish for market. These vary from state to state. The majority of regulations deal with post-harvest and processing (i.e., HAACP) and not with the living animal.

Aquaculture and experimental work with marine fishes at U.S. universities fall under the oversight of two federal agencies, the Public Health Service (the parent agency of NIH) and the Department of Agriculture. The guidelines and procedures are specific for fish in general and do not address species differences. Universities receiving federal funding for research with vertebrates must have formal oversight committees in place to approve the work and protocols in advance and to monitor compliance with guidelines.