Domestication of *Thunnus thynnus* – DOTT

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† Deceased December 2002

This Proceedings Volume is dedicated to the memory and work of two of our colleagues, Zarko Peric and Bart Vlaminck who died tragically in car accident in December 2002.

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Foreword

The inception of the idea to domesticate the BFT as a European drive occurred while on a short sabbatical in Woods Hole Oceanographic Institution, the Marine Policy Center in 1996. I was looking for a species or a group of species that will help close the developing gap between demand and supply of fish in the future, fish species that will serve, in times to come, as the “beef cattle” of the sea.

I was looking for a species that has a fast growth rate, good ratio of edible meat to body weight (70%) and a wide temperature range. After screening many species, I have come to considering the Blue Fin Tuna (BFT) as a candidate Species for Future Mariculture. Controlling the life cycle of the BFT will also help maintaining and enhancing its wild population in the future by developing a re-stocking program.

I aired the idea with a few colleagues (who became friends over the years) and received encouraging reactions (first stations; CIHEAM meeting in Tanger, Morocco, and then at the Univ. of Cadiz, Spain). It turned out that my idea was not an original one - a few countries had already launched national BFT domestication programs.

The initiative came on fertile soil: 55 participants, 25 different R&D institutions and industry around the Med., including Non EU members (Malta, Cyprus, Tunisia, Morocco, Croatia and Israel). We decided to apply for a Concerted Action Program in the Fifth Framework Programme under the “Quality of life and management of living resources” key action of the European Commission – Parallel to the national initiatives in Europe, which were taken place in Spain, Italy and France. The objectives of the Concerted Action program were to prepare the RTD campaign, including priorities, interdisciplinary methodology, rational interactions amongst the different scientific disciplines and the cascade of steps for implementation. Emphasis was on Integration of Fishing Industry, the BFT Farming Industry, governments and the RTD Program. We tried to draw inferences from lessons learned in other countries such as the USA, Australia, and Japan and design our program accordingly.

We put together a good proposal. However, the proposal was rejected based on it being too ambitious and too weak on the socio-economics aspects.

Well, it was a set back and a disappointment. However, we did not give up. We, resorted to the EU Accompanying Measure Program, wrote another proposal to fund a meeting in which the State of Art regarding the BFT different aspects will be told, experience of BFT farming around the world will be discussed and foundations for RTD proposals will be initiated. The proposal was accepted. We have formed a Steering Committee, which met 3 times (first to prepare the proposal, second to prepare the meeting and third, 3 weeks ago, to finalize the program of the Conference, and solve all the small problems that always crop up in the preparation of meetings like this one). We also spent many hours on the email exchanging ideas, written parts of the proposal and the conference to follow.

The first Conference on the Domestication of the Blue Fin Tuna (DOTT) which was held in Cartagena, Spain, and which this volume is presenting most of the presentations offered during the 5 days meeting, should be considered as a successful event. I, for one, am very happy with the conference itself and the outcome. We have been informed on most BFT activities around the world; state of the wild populations, BFT fisheries and landings, BFT fattening operations in different parts of the world such as Japan, Australia, Mexico, Malta, Croatia and Spain. There were very enlighten session on many of the BFT biological traits such as reproduction, larval rearing, nutrition, as well as behavioral and straddling aspects of this species. Most of the reports where given by people who have had direct experience in dealing with the BFT, however, many presentations had theoretical components in them.

Following the plenary sessions, which lasted two and a half days, the participants were divided into workshops, which dealt with a few major disciplines concerning the domestication of the BFT. Most of the workshops lasted for two sessions of a few hours each. The ultimate goal of the workshop was to come up with a program and a skeleton for a research proposal to be submitted to the EU RTD Commission in Brussels. The first workshop was held still in the plenary forum and was devoted to the reproduction of the BFT. It was done as a demonstration and guideline to the other workshops, since a proposal in this field was submitted and approved by Brussels at the time. We had four workshops on the following subjects:

1. Socio-economics and environmental aspects of farming the BFT.
2. Larval and juvenile production of the BFT
3. Husbandry and nutrition of BFT farming
4. Engineering aspects of BFT farming.

Short summaries of the above workshops can be found in the proceedings.

The closing session of the conference was devoted to drawing a set of resolutions and adopting them by the plenary forum. These resolutions are aimed at increasing the awareness of the public, the different governments and the EU Community and its administration prospects of domestication the BFT and what it involves. The amended resolutions are attached to this chapter of the proceedings.

We hope that the DOTT Conference has initiated a campaign, which will, eventually bring about the farming and restocking of the Bluefin Tuna. A worthwhile campaign, which will come to fruition in the next decade or two.

On Behalf of the Steering Committee I want to thank a couple of funding institutions and a few key people that without their efforts and hard work this event would have stayed as an unrealised dream:

The EU Commission for funding the DOTT conference

The Marine Policy Centre of Woods Hole Institution of Oceanography, Woods Hole, Mass., USA

My colleagues, who became friends, on the Steering Committee:
Antonio Garcia and, the Spanish National Coordinator and Fernando de la Gandara both from IEO, Mazarron, without whom this meeting could not have happened. Prof. Christopher Bridges from the Univ. of Düsseldorf, who tirelessly was my anchor to sanity all along the last two and a half years. Prof. Gregorio DeMetrio from the Univ. of Bari who hosted the first meeting of the, would be, Steering Committee in 1999. Prof. Joaquin Roca, from the hosting Polytechnic Univ. of Cartagena who put a lot of effort into realizing the meeting. Drs. Antonio Medina and Gabriel Mourente from the Univ. of Cadiz who were the first recruits to the DOTT crusade ever since 1998. Gines Mendez, Atunes de Mazarron, the President of ASETUN, who hosted us and encouraged our activities all along. François Rene and Christian Fauvel from IFREMER, Palavas, France, who tirelessly helped in ideas, lobbying and organization on the French side of the border. Zarko Peric from Malta who came late to the Steering committee but contributed a lot to our rational. Dr. Constantinos Mylonas from IMBC, Crete, Greece who came on board strong and creative and last but not least, David De Monbrison from CEASM, Paris, France, who all along pointed out the political, social, economics and environmental issues involved it the DOTT.

Our host, the Polytechnic Univ. of Cartagena and its president Prof.
Juan Ramón Medina Precioso, and the vice President Prof. Antonio Garcia Sánchez.

Antonio Belmonte who arranged all details of the visits to the farms and packing plants

And to Isabel Belizon from ESLABON who helped organized the
Meeting

Many other people who lent a supportive hand during the long period of preparation for the meeting, which the space is too small to mention them all.

Hillel Gordin

DOTT Coordinator May 2002

Histological and stereological assessment of batch fecundity, spawning frequency and maturation of female Atlantic Bluefin Tuna around the Balearic Islands

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SUMMARY - The average batch fecundity of female eastern Atlantic Bluefin Tuna, *Thunnus thynnus thynnus L.*, estimated from stereological quantification was of 92.8 oocytes per gram of body weight, and the spawning interval was calculated to be of 1.2 days. All the individuals from 3 years old (19 kg of total body weight, 92 cm of fork length) showed mature ovaries, with migratoy-nucleus oocytes and postovulatory follicles in most of the cases.

Key words: *Thunnus thynnus*, fecundity, stereology, maturation.

Introduction

The eastern stock of Atlantic bluefin tuna (BFT), extends from Norway southwards to the Canary Islands, into the Mediterranean Sea and further south to the coast off South Africa. This BFT stock spawns in the Mediterranean Sea, with two main spawning grounds located around the Balearic Islands and south of the Tyrrhenian Sea (Dicenta, 1977).

Recently, high fishing pressure has caused reduction in the biomass of BFT populations. The effective management of this resource requires a deep knowledge of their biology, particularly their reproduction.The aim of this study is to contribute to the understanding of the reproductive biology of Atlantic BFT.

Materials and Methods

A total of 45 female BFT, captured by purse seine in spawning grounds around the Balearic Islands from 1999 to 2001, were used for the determination of the relative batch fecundity and spawning frequency.

Gonad samples were fixed in 4% formaldehyde, dehydrated in ascending concentrations of ethanol and embedded in paraffin wax. 6-μm sections were stained with haematoxylin-eosin.

The mean spawning interval was determined by the postovulatory follicle method of Hunter and Macewicz (1985). For the estimation of the relative batch fecundity, the numerical density of migratory-nucleus stage oocytes was calculated using the stereological method (Weibel , E. R. and Gómez, D. M., 1962; Weibel, E. R., 1969). This value was raised to the whole volume of the gonad and divided by the total body weight of the animal.

Results and Discussion

The ovaries of all BFT larger than 92 cm in fork length (~ 19 kg) contained oocytes at different developmental stages, the most advanced group of oocytes being at the migratory-nucleus stage. Only in one individual were fully hydrated oocytes found. The average relative batch fecundity calculated for this specimens was 92.8 oocytes per gram of body weight. No relationship was found between batch fecundity and total body weight. The spawning interval estimated for the sample was 1.2 days.

In 2001, small tuna weighing less than 35 kg were sampled during the spawning season. Among these specimens, two distinct size classes were present. In the smaller size class (9-13 kg) all the individuals examined showed immature ovaries, while all the tuna belonging to the larger size class (19-34 kg) had spawning ovaries.

Conclusions

The simultaneous occurrence of migratory-nucleus stage oocytes and postovulatory follicles indicates the capability of BFT of spawning several batches of mature oocytes during the spawning season. This is characteristic in species with an asynchronous pattern of development of the ovaries. The estimated value obtained for the spawning interval shows that this species is able to spawn daily.

The eastern stock of Atlantic BFT appears to be able to spawn from the age of 3 years. This observation may be of interest in aquaculture, since the use of small BFT as broodstock would reduce problems of handling.

Acknowledgements

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Distribution and movements of western Mediterranean Bluefin Tuna (*Thunnus thynnus*) and implications for domestication

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SUMMARY – We tagged 84 bluefin tuna with electronic pop-up tags and released them in the Mediterranean and the Strait of Gibraltar between June 1998 and August 2000; 25 (32%) were located by the Argos satellite system. Location rates were 21% and 62%, respectively, for single-point tags (61 released) and archival tags (23 released). Most tags surfaced in the western Mediterranean and eastern Atlantic, but one archival tag transmitted from a position south of Iceland and one single-point tag transmitted from the Greenland Sea. No transatlantic migrations were observed. Most tags released in the western Mediterranean surfaced near the tagging location, suggesting local residency. Residency and spawning site fidelity (which was also indicated by our data), offer the potential for overexploitation, if the industry progressively catches more large tuna for fattening. Domestication needs to obviate this risk. PAT tag experiments were conducted in collaboration with the Tuna Research and Conservation Center, USA.

Key words: Bluefin tuna, Migrations, Tagging, Pop-up satellite tags, Pop-up archival tags, Behaviour, Feeding behaviour, Feeding migrations, Geographical distribution, Oceanography.

Introduction

Stock assessments of North Atlantic bluefin tuna are currently carried out on the assumption that there are two stocks (eastern Atlantic & Mediterranean; western Atlantic) separated by a conventional boundary at 45°W. This two-stock hypothesis is supported by the presence of small to large specimens on both sides of the Atlantic, the occurrence of spawning in the Gulf of Mexico and the Mediterranean at different times of the year, and morphometric differences between fish from different areas. Analyses of conventional tagging data, which show a low mixing rate between west and east with most tags recaptured in the area of release, also support the existence of two separate groups of bluefin tuna in the North Atlantic. Recently, however, several electronic tagging programmes have been initiated to improve our knowledge of the migrations of Atlantic bluefin tuna and investigate the occurrence of transatlantic movement (Block *et al.* 1998, 2001; Lutcavage *et al.*, 1999). In Europe, experiments with "pop-up" satellite-detected tags were carried out in the eastern Atlantic and Mediterranean between June 1998 and August 2000 as part of an EU FAIR Project (No. 97/3975). The aims of the project were: to identify and describe migrations and movements of bluefin tuna, both within the Mediterranean and between the Mediterranean and the Atlantic Ocean, in relation to spawning and nursery areas; to evaluate the practicalities of using pop-up satellite-detected tags; and to gain experience for future projects with large pelagic fish (De Metrio *et al.*, 1999, 2000).

Materials and methods

We tagged a total of 84 bluefin tuna - 52 giants, 17 smaller adults and 15 juveniles - with pop-up satellite-detected electronic tags in the Mediterranean and Eastern Atlantic, between June 1998 and September 2000. Two types of tag were used: PTT-100 single-point pop-up tags (Microwave Telemetry Inc., Columbia, Maryland, USA), which recorded a limited number of temperature measurements, and PAT archival pop-up tags (Wildlife Computers, Redmond, Washington, USA), which recorded temperature, depth and daily longitude. PAT tag experiments were conducted in collaboration with the Tuna Research and Conservation Center, Monterey, California, as part of the US co-ordinated TAG programme. We used 61 PTT-100 tags and 23 PAT tags. Three giants were tagged with PTT-100 tags, using an underwater gun, at the Stintino trap (Sardinia, Italy) in June 1998. Thirty-two fish were tagged with PTT-100 tags by underwater gun or hand-held harpoon in the large tuna trap at Barbate (Spain), to the west of the Strait of Gibraltar, in July 1998 and 1999. Twenty-two bluefin, captured in the local sport fishery, were tagged in the Bocche di Bonifacio (between Corsica and Sardinia) in September 1999 and 2000, either alongside the boat using a hand-held tagging stick and PTT-100 tags (12 fish), or on deck using PAT tags (10 fish). Fifteen fish were tagged (13 with PAT tags) by hand-held harpoon and underwater gun in aquaculture pens at Puerto Mazarron (Cartagena, Spain) in August 2000. Twelve tuna were tagged in the Aegean Sea (Greece) using a short hand-held stick. All tags were attached by a monofilament nylon leader to a nylon dart (PTT-100 tags) or a titanium anchor (PAT tags) embedded in the dorsal muscles of the fish. For the 12 fish tagged with PAT tags in the Corsican sports fishery, the titanium anchor was passed through the base of the second dorsal fin rays. A series of charts of chlorophyll-a concentration were plotted for the Tyrrhenian Sea close to Corsica and Sardinia (central Mediterranean) and the eastern Atlantic to the south of the Strait of Gibraltar, the two areas in which most of the tags surfaced. Data were extracted from the SeaWiFS database (Parrish, 1996; IOCCG Reports, 1999). Data for the first area were analysed for the period September 2000 to February 2001, obtaining a fairly homogeneous temporal coverage (about three good satellite acquisitions per month) apart from January. Some trials were made with five unused PTT-100 tags to test the ability of the Argos satellite system to detect these tags in the western Mediterranean, where there is now known to be substantial background noise and transmitter competition on the Argos radio frequency, and the eastern North Atlantic. Comparative trials were also undertaken in Madeira and Columbia, Maryland, using the same five tags.

Results and discussion

Twenty-three of the 84 pop-up tags were located by satellite, giving an overall location rate of 32% (25/78). All of these tags transmitted valid data (figure 1). Six more tags were recovered from recaptured fish. Location rates were 21% (12/57) for the PTT-100 tags and 62% (13/21) for the PAT tags, which appeared to be less influenced by the high level of background noise and high density of Argos transmitters in the Mediterranean area than the PTT-100 tags. Sporadic signals, which were too weak to allow either location or data transmission, were received from a further 6 PTT-100 tags on

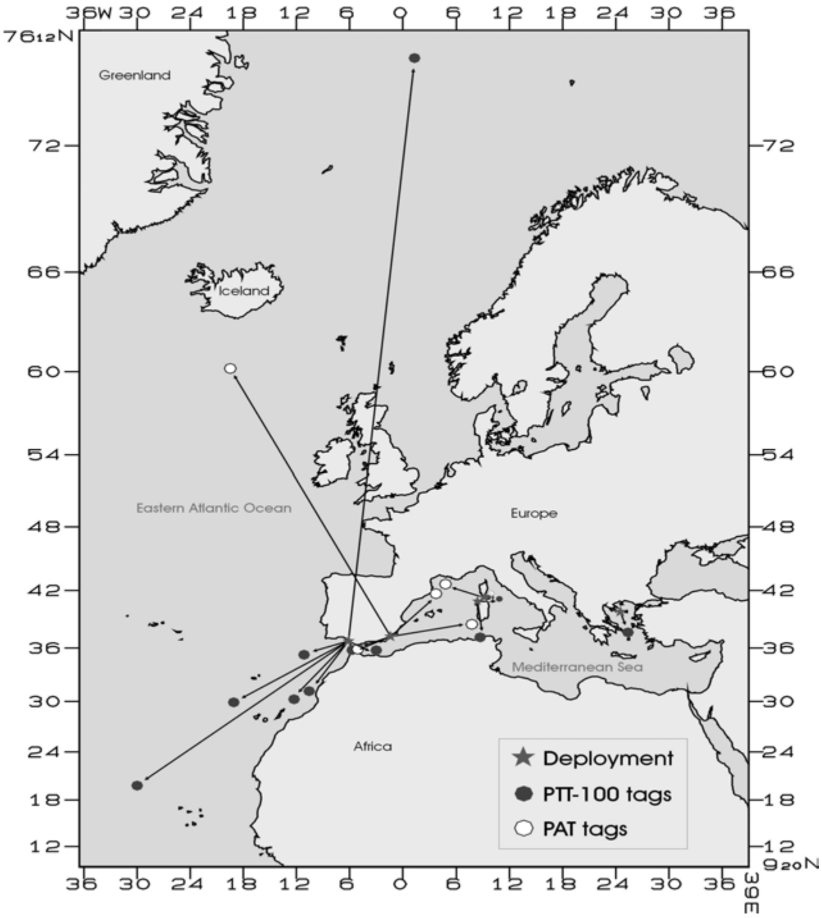

Fig. 1 - Pop-up locations of tags attached to tuna in the Mediterranean and eastern Atlantic from 1998 to 2000. Red circles, PTT-100 single-point pop-up tags; white circles, PAT archival pop-up tags

or close to the expected pop-up day, increasing the detection rate for these tags to nearly 32% (18/57) and for all the tags to 38% (31/78). Most tags were detected in the western Mediterranean or eastern

North Atlantic, off the coast of North Africa. However, one PAT tag surfaced south of Iceland and one PTT-100 tag transmitted from the Greenland Sea. No tags were detected in the western Atlantic.

Location rates of PTT-100 tags varied markedly between release sites and years. For example, in the Aegean Sea in 1998 and 1999 the location rate was only 8%, compared to 23% for Barbate in southern Spain in the same years, and 67% for releases at Stintino, Sardinia in 1998. However, only 14% (3) of the 23 tags released at Barbate in southern Spain in 1999 were located by satellite (a further tag was recovered from a recaptured fish) compared to 44% (4) of the 9 tags released from the same trap in 1998. Location rates of the PAT tags also differed markedly between release sites. Only 4 (33%) of the 13 tags deployed on bluefin tuna (11 giants and 2 smaller adults) in a holding pen at Puerto Mazarron in August 2000 were detected by satellite, although a further tag was recovered from a recaptured fish before it was due to detach from the fish. In contrast, 100% of the 10 PAT tags deployed on smaller (40-90 kg) fish in Corsica, during September 2000, were located by satellite, although no valid data were recovered from two tags that appear to have drifted ashore shortly after surfacing, and a tenth tag was recovered from a recaptured fish, again before it was due to detach from the fish. Several tags showed interesting results. One PTT-100 tag deployed near the Strait of Gibraltar was detected in the Greenland Sea; another from the same release transmitted from the eastern Atlantic close to the southern limit of the eastern bluefin stock. A PAT tag deployed in the Mediterranean, close to Cartagena, was detected in the North Atlantic south of Iceland; in contrast, most of the PAT tags deployed in the area of Bocche di Bonifacio (Corsica) surfaced in the release area. Daily longitudes recorded by the tags indicated that these fish had all remained in the area between Corsica and longitude 14°E. Maximum depths indicated that, while some fish moved off into deep water in the Tyrrhenian Sea, others remained solely in the shallow water on the continental shelf around the island. Comparison of pop-up positions with the temporal set of chlorophyll-a maps shows a correspondence with higher pigment concentration areas. In particular, the central Mediterranean and northern Tyrrhenian Sea showed higher concentrations of chlorophyll-a than other parts of the western Mediterranean and eastern Atlantic (figure 2). Given the occurrence of a persistent areas of high production in the areas where most of the tags were detected, especially to the east of Corsica, we suggest that these may be feeding areas for both pre- and post-spawning fish.

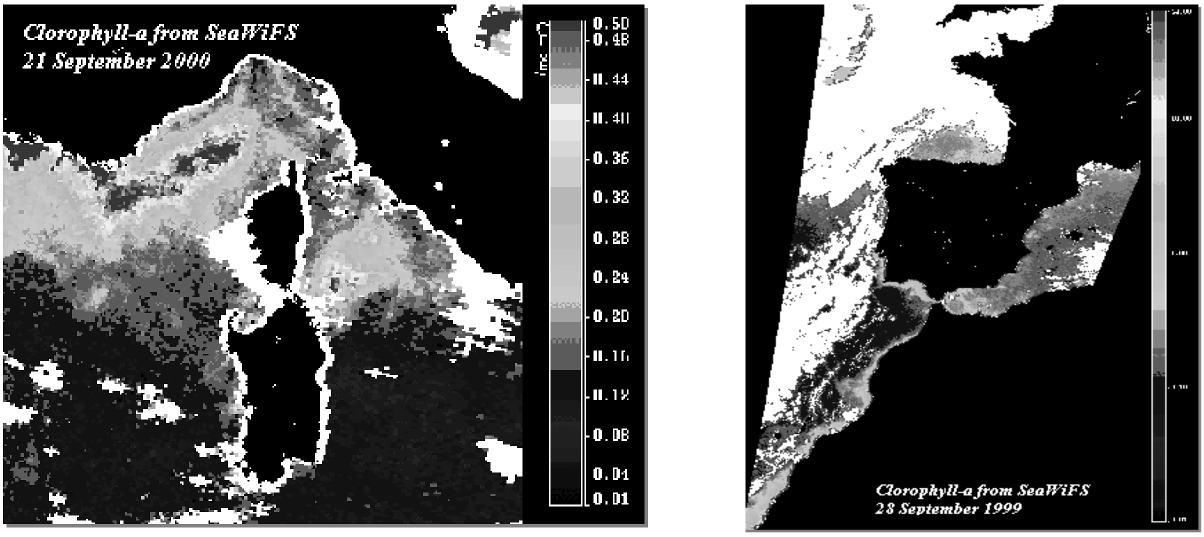

Fig. 2 - Chlorophyll-a concentration from SeaWiFS in the central Mediterranean (on the left) and western Mediterranean and eastern Atlantic (on the right).

The rate of tag detection and location was much lower than expected from previous studies with the same type of tag in the western and central North Atlantic, where rates of 56 to 93% have been reported (Block *et al.*, 1998; Lutcavage *et al.*, 1999; Lutcavage, pers. comm.). Because the difference was so large, we conducted a series of tests to compare the performance of five unused PTT-100 tags at a number of locations in Europe Madeira and the USA. The results our tests clearly indicated that there is a detection problem in parts of the Mediterranean Sea, where we expected some of our tags to surface. It seems likely, therefore, that a low signal-to-noise ratio in the affected areas may have resulted in non-detection of tags that may otherwise have successfully surfaced when programmed to detach themselves from the fish. Corroborative evidence is available from six tags, from which sporadic signals were received on, or close to, the expected pop-up day. No temperature data were obtained from these six tags and the signals were too weak, or too few, for the Argos system to determine the location of the tag.

Conclusions

Reasons for the low detection rate of the PTT-100 tags may include post-tagging mortality, fish capture, premature tag release, failure of the tag as a result of exposure to high pressure and low signal-to-noise ratio. Whilst it is difficult to quantify some of these factors, our test results clearly indicate that the strength of the transmitted signal was sufficiently low to have compromised our ability to detect tags over a significant area of the western Mediterranean and north-western Europe. According to Argos, the problem, which results from a high level of background noise and competition from more powerful transmitters is, however, confined to Europe. The ability to detect tags that surfaced in the Atlantic should therefore have been the same as that for tags attached to tuna in US waters. In this context it is interesting to note that none of the pop-up positions of our tags were located in the central or western North Atlantic, but were confined to the eastern management area with no evidence of transatlantic migrations. It was also noticeable that most of the tags deployed in the Mediterranean surfaced close to the original tagging location. This was especially true of fish released off Corsica, suggesting the existence of residency associated with the high productivity, or other environmental characteristics of this area. The recapture of a big tuna (290 kg) tagged with a PTT-100 tag at Barbate trap on July 1999 is of particular interest. This fish - to which the tag was still attached - was caught near the Balearic Islands in June 2001, suggesting fidelity to the western Mediterranean spawning area. Spawning site fidelity and Mediterranean residency clearly offer the scope for overexploitation if the industry continues to catch more and more large bluefin for fattening in cages, instead of starting to rear 'new fish' from eggs. Domestication of bluefin would need to extend to the control of all stages of the life history, including reproduction in captivity, rearing and weaning of larvae, and growth to market size, to be sure of avoiding this risk.

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Financial support provided by EU grant CFP - FAIR Project No. 97/3975 “Study on Eastern Atlantic and Mediterranean bluefin tuna (*Thunnus thynnus* L.) migrations using «Pop-up satellite tags»”.

Managing wastes in the domestication of the BFT: theoretical and practical considerations

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SUMMARY- The domestication of the BFT, amongst others, has been the target of criticism from environmental and other pressure groups with respect of the perceived impact of the industry on the environment. With its potential scope of growth, problems are likely to increase in the future unless a responsible attitude to its development is adopted. Hence, the way forward in the domestication of the BFT, is to work towards a sustainable production. This would benefit both regulatory bodies and the aquaculture industry, since they all have an interest in the preservation and enhancement of the marine environment. The aim of the present work is to frame some key considerations for the sustainability of the domestication of the BFT, by focusing on the importance of controlling and reducing wastes from marine cage fish farming. The hope is to illustrate that aquaculture, if properly implemented and managed, is the way forward.

Key words: BFT, environment, wastes, sustainability, management.

Introduction

It is doubtful if any industry has attracted as much attention as aquaculture in the last ten years. At present, aquaculture production constitutes approximately 12% of the world's fishery production, and fish farms are continuing to grow in numbers and in size. Yet despite this, it has been, and still is, the target of criticism from environmental and other pressure groups with respect to the perceived impact of the industry on the environment. As for the case of the domestication of the BFT, the same negative impacts have been put forth, and with its potential scope of growth, problems are likely to increase in the future unless a responsible attitude to its development is adopted. Hence, this new frontier for development should be approached with a degree of caution (Tisdell, 1999).

With these ideas in mind, the way forward in the domestication of the BFT is to work towards a sustainable production. This need is now globally acknowledged (Brundtland et al., 1987) and the aquaculture industry has undergone good progress in working towards achieving this fundamental goal, which can be extrapolated to the domestication of the BFT. Such sustainable production would benefit both regulatory bodies and the aquaculture industry, since they all have an interest in the preservation and enhancement of the marine environment.

The aim of the present work is to frame some key considerations for the sustainability of the domestication of the BFT, by focusing on the importance of controlling and reducing wastes from marine cage fish farming. The first part briefly highlights important ecological and economical concerns for the domestication of the BFT and covers the rationale behind achieving a sustainable development. The second part takes a look at how such theory can be put into practice. The hope is to illustrate that aquaculture, if properly implemented and managed, is the way forward.

Theoretical considerations

Environmental impacts from the domestication of the BFT

Unconsumed feed and fish faeces are the main source of solid and soluble wastes and represent the major sources of pollution. These can lead to enrichment of the benthos and a wide range of other interactions and disturbances can occur in the ecosystem in question.

In some cases production can exceed the assimilative capacity of a given site, so that its long-term viability is not assured.

In the near future, chemicals and antibiotics whilst in use during medication in fish farms, represent another source of pollution as the surrounding environment might be impacted from such practice. For example, there is a risk of toxicity to other more sensitive indigenous species.

The introduction of a pelagic species and a top-predator, as is the BFT, *Thunnus thunnus*, to a coastal environment can have profound disturbances and potentially impact the functioning and structuring of the given ecosystem.

Achieving sustainable development

The concept of sustainable resource usage remains poorly defined for aquaculture. One definition might be that sustainability incorporates social, technical, financial and ecological concerns and that the interactions between aquaculture and its ecological environment are becoming increasingly important if the demand for and supply of environmental goods are to continue indefinitely (Beveridge, Phillips and Machintosh, 1997).

The negative effects of off-farm pollution represent a cost to the coastal environment and other resource users. Waste discharges from aquaculture production suggest that the farmer is using resources inefficiently. Thus, controlling and reducing wastes will be beneficial to both the BFT aquaculture industry and the environment.

Integrating aquaculture, environmental management and product quality is the way forward: such sustainable development conserves the natural environment, reduces the potential conflicts with surrounding land or coastal users, and also enables the marketability of a product that is perceived to be safe, of the highest quality and 'environmentally friendly'.

Practical considerations

Achieving sustainable development

To date many efforts are being done to attempt to extrapolate considerations and recommendations into practical applications and outcomes to develop and achieve sustainability for the domestication of the BFT.

The major environmental effects of aquaculture generally occur as a result of day-to-day operations and management practices, so many may be reduced or ameliorated by changing these activities. Feed is the primary source of pollution, therefore minimising wastes should receive maximum attention.

At the present day, research and development in the aquaculture industry is at its peak and an integrated and multidisciplinary approach must be taken to work towards sustainability.

New technological developments

Technological advances result in an improvement in environmental performance, enhances resource efficiency and can yield economic benefits to the industry.

At the moment, improvements and developments in fish cage technology are taking place, which allow waste to be readily monitored, collected and removed from cages.

Another alternative is to shift the fish farm into offshore areas. This is being done in many areas of the world, and amongst the multiple advantages which such operations can bring about, is that of waste dilution from offshore currents and deeper water (Emerson, 1999). The necessary technological and engineering aspects that will need to proceed to achieve this offshore farming for the BFT, must be adequately planned, tested and evaluated.

Conclusions

There are many issues confronting developing industries such as the domestication of the BFT, and the sustainable management of environmental resources presents yet another. Throughout the world authorities are demanding stricter environmental control, thus integrating aquaculture and environmental management is the way forward. The challenge for the domestication of the BFT is to maintain profitability and environmental compatibility. Its sustainable development would ensure the conservation of the marine environment, so that the marine life can be maintained and its potential resources be at hand for the future ahead. Furthermore, a sustainable performance would also benefit the industry itself on both short and long-timescales. Ultimately, we all benefit from a fully healthy and functional environment.

As we have seen in the sections above, the domestication of the BFT has undergone good progress in working towards sustainability, but we must acknowledge that this big project is still at its infancy and the industry is hence still at a 'learning phase'. The future lies into allocating more time, effort, resources, research and development into any area that takes part to achieve the domestication of the BFT in a sustainable manner. This demands a comprehensive, responsible, co-ordinated, multidisciplinary and unified approach to its development.

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Engineering general aspects of BFT farming

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SUMMARY – From the first phases of the DOTT (reproduction, genetics e.tc.) to the final stages in the processing plant the following tasks would, at least, be considered and studied from the Engineering point of view:

- Handling methods and techniques to keep the alive fish in good shape during catching, towing, farming, harvesting and post-harvesting operations.
- Transport cage.
- Offshore cage.
- Mooring designs and monitoring methods for these systems in the offshore cages.
- Feeding control.
- Non-invasive fish size assessment methods.
- Dead fish and feeding waste removal systems.
- Killing cage.
- Killing methods.
- System to collect fertilized eggs.
- Land base facilities.
- Processing methods.
- Installation Instrumentation and Control Systems.

Key words: offshore, cage, control systems.

Generic areas

A summary of the generic areas to be covered by the Engineering Field in the Domestication of the BFT is the following:

Cages

- Design ... New ... Upgrading (problem's solution)
- Design verification
- Mooring systems
- Hydrodynamic channel tests ... Towing tank.

Materials ... DESIGN AND TESTS ... METALLIC AND NON-METALLIC MATERIALS (PA, PES, PE, PP, NETLON, Rubbers, Nylon ...), covering all cage components: structures, floating systems, nets, chains, ropes ...

Auxiliary equipment ... Design, qualification and tests ... Cranes, pumps, filters, valves, heat exchangers, compressors, heaters, tanks ...

Auxiliary ships, barges and platforms

- New design and upgrading
- Manufacturing follow
- Hydrodynamic channel tests ... Towing tank.

Instrumentation and control ... Design and tests.
Support structures ... Design and calculations considering vibrations, corrosive environment, stress corrosion cracking ...

Welding ... Design ... Coupons tests: destructive and non-destructive.

Economic feasibility studies.

Upgrading specific areas.

In meetings with tuna grow-out private companies and R&D Institutions, the following considerations and possible upgrading areas have been identified:

Considerations.

Offshore culture.

The BFT culture should be performed in deep water, far from shore, due to the life conditions, fish behaviour and also due to environmental requirements, therefore the grow-out facilities will have to withstand the high energy (waves, currents, winds ...) of open sea. Accordingly the facilities should be designed for such conditions. These harsh conditions should be considered in the feeding equipment, fish handling, sampling and the safety problems associated to operation conditions.

Towing and transfer.

The procedure that begins after capturing the fish with pure seine nets, and follows when transferred into transportation cages, is found unsatisfactory. The towing of the alive fish after capture to final destination and the transfer to moored cages should be improved. The connections between cages for moving the fishes from cage to cage, without touching them, is an important aspect to be considered.

Cage design.

The cage design should resolve the concerns of the fish grow-out farmers:

- The depth of the cages in relation to the tuna behaviour.
- To avoid stress and mortality caused by lighting during storms.
- To consider how much this fish depends upon light for food. The research has shown that tuna see best in shaded or diffused light, and that strong summer light has a blinding effect on tuna fish as their eyes have no protection and their pupil can't expand or contract. For these reasons it seems that shaded cage may be advantageous to allow fish see well.

Handling.

The beginning of the research itself will be in cages, therefore the question of how to handle this giant fish is a major issue.

An extensive alive fish research work must be done to include taking blood samples, biopsy of their gonads, how to put them to sleep, as well as to make sure that we don't harm them at any time. Further on, work must be done on marking techniques to study the daily and seasonal growth patterns ... This is an urgent need to have a device (i.e. special hammock or cradle) to put the fish while working on it them in the water, or to move the fish out the water onto a dry platform and keep them in good shape while performing needed work. We must ensure the recover and the survival of fish when transferred back into the cage. The design of this device should consider how to reduce the stress and injury to the big tuna. This is the first priority item of the technological needs that will allow to collect information for better understanding of how tuna respond to the external (environmental) and the internal (physiological) events.

Land facilities.

One important area is the land based facilities in which we would like to keep brood stocks in order to study them and get them to spawn in captivity, including transportation methods from cages to land facilities.

The design of the tanks should consider the following parameters and controls:

- Size, form and depth.

- Temperature, salinity and light controls.
- Prevention from harming themselves by bumping into the walls.
- Indoors or outdoors.

Improved Quality.

The Technology area must also include harvesting and post-harvesting (processing) techniques. The aim of these points can be joined under Improved Quality of BFT aiming to upgrade and/or develop handling, harvesting and post-harvesting methods and techniques to maximise the colour and flesh characteristics (freshness, texture, avoid tissue damages, improve organoleptics ...) as required by the export market.

The project may also try to develop methods and techniques to process whole fish for export in which the flesh colour remains stable in spite of long transport duration while reaching consumers.

Regarding this technology area we must understand the Japanese market and to accurately evaluate existing "Killing and Handling Guidelines" as was suggested by Japanese tuna buyers. The standards and criteria should be considered in terms of their use and relevance in sushy and sashimi dishes.

Possible upgrading areas.

Methods and techniques on how to handle the alive fish and keep them in good shape during catching, towing, farming, harvesting and post-harvesting.

Transport cage design upgrading including connection between cages.

Offshore cage design with special attention to the ring materials, the depth of the cages and the light shade or diffuse systems.

Mooring designs and monitoring methods for these systems in the offshore cages.

Feeding control.

Non-invasive fish size assessment methods.

Dead fish and feeding waste removal systems.

Sacrify cage designs.

Sacrify methods ... Ultrasonic, laser, submarine magnetic chamber ... ?.

System to collect fertilized eggs from the cages.

Land based facilities design that should include the transportation methods from cages to land, the tanks and the fish moving and handling systems.

Processing method upgrade to improve the fish quality.

Description of engineering areas.

The DOTT activities to be performed in the Engineering field should be performed by the following work areas with the facilities identified here below.

Design of structural solutions for experimental facilites.

The scope in the area of structures includes:

- analysis and evaluation of design alternatives
- design of feasible structural arrangements
- selection of materials

- design of structural elements for the farm and equipment
- design of mooring, anchoring and movable elements
- design of elements for operating the farm
- design of personal devices and vehicles

The members of this group should accumulate real life experience design of complex and novel marine structures (materials, loadings and responses), mooring and anchoring systems and hydrodynamics of floating submerged and semi-submerged structures in moving waters.

The Labs and computer facilities should be well equipped for research, simulation and fabrication of prototype models, to evaluate design alternatives. Special interest will focus on the analysis of animal-friendly structural arrangements and the use of environmental safe solutions materials for structural members and coatings.

Towing tank laboratory.

The Towing Tank should have a carriage with variable speed and a regular wave generator for waves. It should also incorporate equipment for testing anti rolling tanks, and equipment for making the scaled physical models, in wood or plastic.

Other facilities are those about computational calculations, with own codes developed through years in the laboratory:

* Power prediction of floating bodies (Still water and waves).
* Pressure and velocity distributions around a vessel.
* Seakeeping in irregular seas.
* Controlability.

Another fields in which the laboratory should work:

* Instrumentation.
* Sea keeping tests in real ships.

Underwater acoustics laboratory

The laboratory should be opened to all the fields of application of the Underwater Acoustics and is oriented fundamentally toward the development, evaluation and commission of systems, without discarding the transducers and subsystems tests and trials.

With the purpose of using to the maximum the resources, this laboratory could be located in the Electrotechnics, Electronics and Systems Laboratory, by the importance that has the electronic transducer systems and signal process.

Materials group .

The materials group should have experience in subjects related with the welding and joining technologies and also in inspection (NDT) and testing of welding joining,

A very important research line should be related with environmental degradation of adhesive joints in sea water. For this purpose, the laboratory should be equipped with light and scanning electron microscopy, mechanical testing (tensile, hardness, impact, etc).

On the other hand, using non destructive testing (ultrasounds, magnetic particles, liquid penetrant, Eddy currents, radioscopy) a line of research could be established on defects of metallic and plastic materials.

Conclusions.

Considering these Engineering General Aspects, the objectives defined during the Engineering Workshop should permit to the Participants in each Cluster to establish the specific working packages to prepare a proposal for the BFT Husbandry Research.

Fishy business in the Mediterranean - Tuna, Tonnara and Testosterone

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SUMMARY - New methods based molecular biology techniques have been used for sex determination and sexual maturity staging in *Thunnus thynnus* , the Bluefin Tuna. These are based on the determination of steroid hormone concentrations and their ratios and the presence of vitellogenin in plasma and muscle. Sampling techniques for muscle are discussed and the use of such techniques for sex determination in both Bluefin tuna (BFT) and Swordfish

Keywords: Tuna, sex hormones, vitellogenin, muscle biopsy, keto-testosterone, estradiol, Swordfish testosterone, fisheries management.

In many fisheries fish are either landed already gutted or the value of the flesh prohibits ventral opening and the determination of sex and sexual maturation. If sex or sexual maturation is not determined then this can have serious consequences for future management models of stocks and their development. The objectives of our ongoing studies was to provide new molecular techniques to make sexual identification possible in species where no external sexual dimorphisms exists and to further determine the maturation state of the fish this possible and thus assist in stock assessment and management.

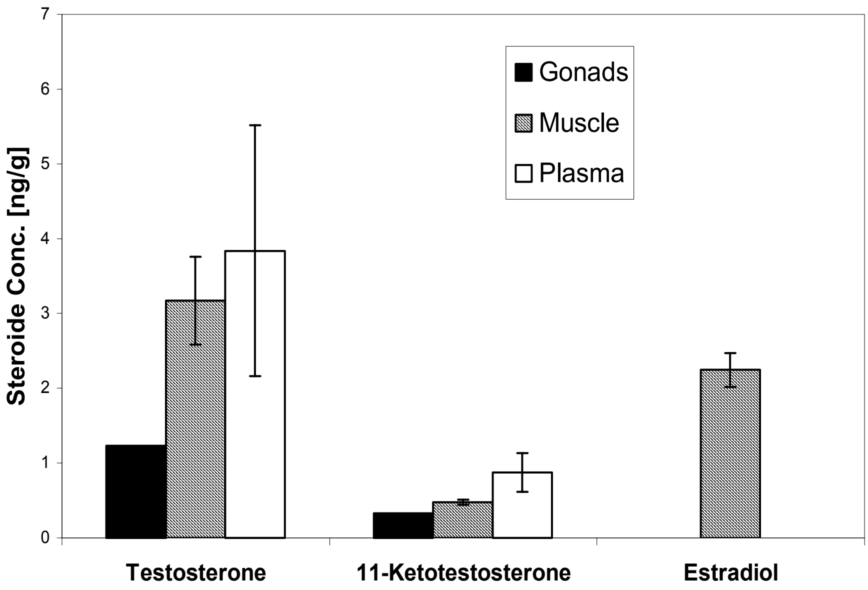

Figure 1. Steroid hormone concentrations in the various tissues of the Bluefin Tuna. Measured with standard ELISA techniques (Modified from Bridges et al., 2001).

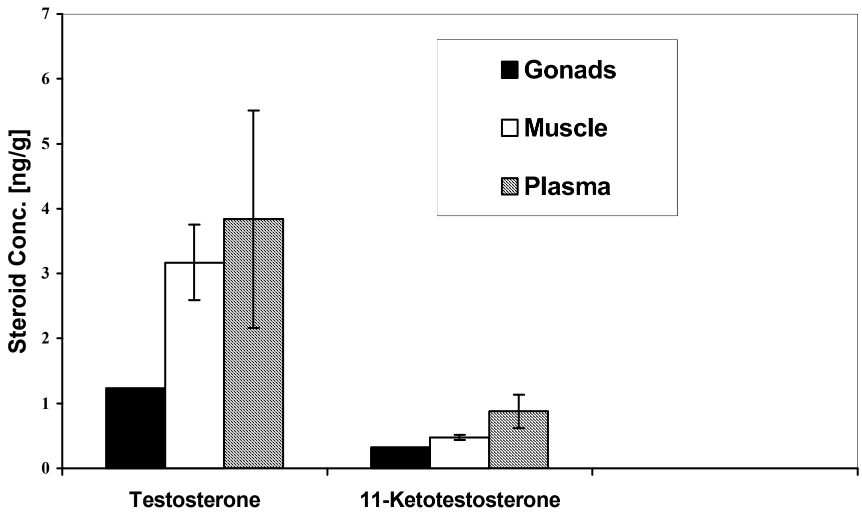

Figure 2. Steroid hormone concentrations in various tissues of *Xiphias gladius* measured with standard ELISA techniques (Modified from Bridges et al,. 2001).

To these ends concentrations of Testosterone, 11 Ketotestosterone and Estradiol were determined in BFT and Swordfish gonads, muscle and plasma from the Mediterranean. The results are shown in Figures 1 and 2. Using the Sex Steroid Ratio Formula where [(E2/11-KT)]/[T] is compared (Susca et al., 2000) males could be distinguished from female fish during the breeding season. As an alternative the presence of Vitellogenin in muscle or plasma samples can be used as in the Dot-Blot shown below which

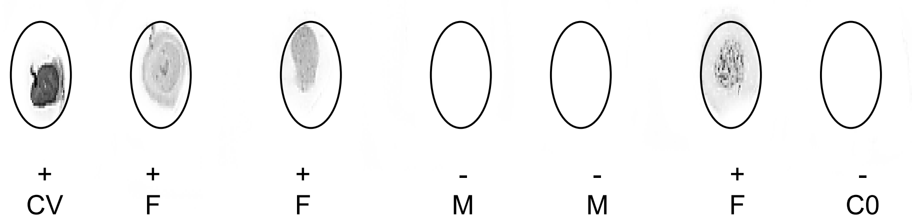

Figure 3: Use of a dot blot analysis for plasma BFT-Vtg to determine the sex. Positive reaction (+) for Vtg is detected in females in the reproductive status (F). In males (M) the test is negative (-). This simple test can be used for sex determination when the measurement of steroids gives no precise results. CV = positive control, 1 µg BFT-Vtg; C0 = negative control, 1 µg bovine serum albumin (BSA). Modified from Susca et al., (2001).

can easily distinguish between male and female fish and may be the obvious method of choice for bulk measurements as in fisheries and aquaculture applications.

Further developments include the design and testing of a semi-automatic muscle biopsy sampler where as little as 150 mg of tissue are required for sex determinations and the self-activating punch system can be used either as a "stand alone" hand-held device or mounted on a harpoon or tagging stick. This development is being considerer for sampling from live brood stock as well as for use in fish tagging programmes where it is important to have a non-lethal sex determination method (Bridges *et al*., 2000). In previous fishing seasons both plasma and muscle samples have been taken in Bluefin Tuna together with gonadal samples and full biometric data. Using this approach molecular endocrine techniques have been calibrated with histological data and are now set for market testing. This will provide a significant handling tool for aquaculture studies of the future and allow monitoring of sex and sexual maturity even at the market place. Similar developments using field trials and sampling are also underway for Swordfish management although due to the possible presence of endocrine disruption in Mediterranean fish this may not be generally applicable in Mediterranean stocks but could be used for Atlantic, Pacific and Indian Ocean stocks when available.

Acknowledgements

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Handling and manipulating Tunas in captivity: a physiologist's perspective

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SUMMARY - The Kewalo Research Facility (National Marine Fisheries Service, Honolulu Laboratory) has been routinely maintaining juvenile (1-3 kg) yellowfin and skipjack tunas in shoreside tanks for more than 40 years. Research conducted at this laboratory has shown that these high energy demand fishes are not particularly tolerant of acute reductions of ambient oxygen. Therefore, when maintaining tunas in pens for aquiculture, every effort should be made to ensure clean and well-oxygenated conditions. If for whatever reasons this can not be done, then efforts should be directed at not increasing the fishes metabolic rates (for example by not feeding them) until water quality problems can be rectified. Also, manipulation of fish should be avoided if possible. If required, anesthesia is probably not necessary for procedures that can be accomplished within a minute or two, as fish quickly removed from the water will generally remain completely quiescent for this period. If anaesthesia is required, benzocaine is preferable over MS222 as the former does not make the water acidic. Injectable anesthetics are generally not recommended as they tend to leak from the injection site, and the exact dosage given is often indeterminate.

Key words: tuna, anaesthesia, stress, aquiculture, husbandry

Introduction

The Kewalo Research Facility (National Marine Fisheries Service, Honolulu Laboratory) pioneered the practices required to acquire and maintain live tunas in shore-side tanks more than 40 years ago (e.g., Nakamura 1962). Fish are purchased from local commercial fishermen, whose boats dock literally at the laboratory's front door. The fishery employs a "live bait" technique, and tunas are returned in the vessels' 6000 l bait wells. Fish are offloaded using chamois-lined dip nets and are moved to their holding tanks in a 2700 l oval-shaped transfer tank. These procedures are intended to minimize skin and fin damage, as well as general trauma to the fish. In spite of these efforts, about 50% of tunas delivered to the laboratory die within 48 hours, the majority on the second day after arrival (Bourke et al. 1987). Fish experience up to approximately a 50% decrease in blood protein concentrations immediately following arrival in captivity (Bourke et al. 1987), and for many years it was thought that a generalized hemodilution was occurring. Being marine fish, however, tunas would be expected to show hemoconcentration because the inevitable skin damage and general stress resulting from capture and transport should result in a net decrease in total body water (i.e., a decrease in blood volume and an increase in plasma protein concentrations). Moreover, tunas recently arrived in captivity have body masses up to 10% below those calculated from length-weight equations, implying that a general loss of body water is indeed occurring.

Recent experiments have begun to shed light on this apparent paradox. It is now known tunas have capillaries "tighter" (i.e., less permeable to plasma proteins) than other fishes, except for individuals that are newly arrived in captivity whose capillaries are far "leakier" (Jones et al. 2002). Therefore, the decreases in plasma protein levels (and probably high levels of mortality) observed in recently delivered fish are most likely due to plasma proteins moving into the intercellular space at rates higher than normal. Unfortunately, procedures to minimize or to actively counteract this problem remain to be developed.

In spite of the difficulties of performing physiological experiments on live tunas, extensive research has been conducted on the energetics and performance of cardio-respiratory system. Experiments using juvenile (1-3 kg) skipjack and yellowfin tunas have elucidated the ability of tunas' cardio-respiratory system to deliver oxygen to the tissues under various reduced ambient temperature and oxygen conditions (reviewed in Brill 1994). Not surprisingly, tunas are not particularly tolerant of reductions in ambient oxygen. This problem is especially acute under circumstances where metabolic rates are elevated (e.g. during bouts of increased in swimming activity, immediately after exhaustive exercise, or following feeding). Tunas' ability to transfer oxygen from the water to the tissues is likewise impaired during exposure to particulate material in the water or any toxic algae. Under these conditions, the gills either produce excessive mucous or there is generalized swelling of the gill tissue, both of which impair oxygen transfer from the water to the blood. Therefore, the following is recommended. Tuna pens should be placed such that none is immediately "downstream" of other pen(s) to preclude any fish being exposed to significant reductions in ambient oxygen. Likewise, care should be taken to ensure there is never excessive flocculent material in the water nor significant algal blooms. Should any of these circumstances arise, it is strongly recommended that nothing be done that increases the fishes' metabolic rates, including feeding. Healthy tunas can withstand at least several weeks of starvation. Withholding food is clearly not desirable in pen rearing operations, but under circumstances where the fishes' cardio-respiratory function is likely to be impaired, this may at least help prevent excessive mortalities.

Handling and anesthesia

At first glance it may appear that manipulating tunas for biopsies, implanting sonic transmitters or archival tags, attaching physiological sensors, or other procedures should involve anesthesia. Extensive experience, both at sea and in the laboratory, has shown that tunas quickly removed from the water exhibit complete immobility and insensitivity to touch or manipulation for approximately two minutes or more (e.g., Holland et al., 1990; Musyl et al., 2002). During this period, carefully planned and quickly executed procedures can be accomplished. Moreover, induction of anesthesia is fraught with problems as it is difficult to accomplish without physical trauma to the fish (e.g., skin or fin damage), and possibly irreversible blood acidosis. Recovery from anesthesia is likewise problematic, as tunas regain their ability to struggle before they regain their ability to swim. Because they are obligate ram ventilators and negatively buoyant, tunas that cannot swim sink and rapidly suffocate. Anesthetizing tunas is, therefore, not generally recommended unless procedures cannot be accomplished without it.

If anaesthesia is necessary, the following procedures are recommended. Because tunas must be kept in large tanks, adding anesthetics directly to the holding tanks is not practical. At the Kewalo Research Facility, fish (1-3 kg body mass) to be anaesthetized are guided into a plastic bag containing 5-10 l of seawater with anesthetic. The bag is then sealed and gently rocked back and forth for about two minutes to flush anesthetic solution over the fish's gills. Following this, fish are immediately moved into the laboratory and force ventilated with a dilute anesthetic solution. With these techniques, extensive surgery and instrumentation are routinely performed (e.g., Bushnell and Brill 1992). During experiments, fish are sedated and kept from swimming by blocking spinal motor nerves with an injection of 0.1-0.3 ml local anesthetic (xylocaine) directly into the neural canal immediately behind the skull. Fish are also placed in front of a pipe delivering seawater at a velocity roughly equivalent to the fish's normal swimming speeds. With this arrangement, tunas are able to set their own ventilation volumes and respond normally to changes in ambient temperature and oxygen conditions.

Of the two most commonly used fish anesthetics, benzocaine (ethyl aminobenzoate) may be preferable to tricaine methanesulfonate (MS222, Finquel) because it is significantly cheaper yet equally effective and safe. More important, benzocaine does not make the water acidic even at the high concentrations required for the initial rapid anesthetization of tunas. Benzocaine does have one slight disadvantage. Because it is insoluble in water, it must be dissolved in ethyl alcohol first. For initial anesthesia, 1 g benzocaine per liter of seawater has been found to be safe, but also rapidly to induce immobility. A concentration 0.03 to 0.1 g/l has been found sufficient for maintenance of anesthesia. An acceptable plane of anesthesia is difficult to achieve in tunas, however, and overdosing (resulting cardiorespiratory collapse and death) is a constant threat. Therefore, during any extended anesthesia, the fish's heart rate should be monitored via ECG leads place near the heart. As soon as heart rate becomes irregular, the concentration of anesthetic in the seawater reservoir being used to ventilate the fish's gills should be reduced. Likewise, if the fish is responsive to touch, or showing overt signs of movement, the anesthetic concentration should be increased.

At first glance, injectable anesthetics might seem a way to circumvent the problems associated with water soluble anesthetics, but in actuality their use is even more difficult. Direct intravascular (IV) injection is difficult to safely achieve, especially in large fish. With intramuscular (IM) injection, the actual dose reaching the blood stream is unknown, as a variable amount of anesthetic solution inevitably leaks from the injection site. Of the injectable anesthetics, Saffan (a steroid anesthetic sold by Schering-Plough) appears to be good choice (Oswald 1978). A dose of about 0.5 ml/kg of stock solution can induce anesthesia without hyperactivity. Nembutal (sodium pentobarbital) is not recommended. It generally has such a slow induction that multiple doses can be mistakenly given eventually resulting in overdose (i.e., lethal levels being reached). Ketamine hydrochloride appears to have some promise for use with tunas where only short periods of immobility and insensitivity are required. Both induction and recovery are rapid. Unfortunately, it's use in tunas has been minimal and appropriate dosage regimes remain to be defined.

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Fattening of Bluefin Tuna (*Thunnus thynnus*) caught by the French and Spanish bait boats in the Bay of Biscay

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SUMMARY - This fattening project of bluefin tuna aims at giving a first assessment of the technical feasibility of fattening wild bluefin tuna caught by the French and Spanish bait boats in the south of the bay of Biscay. In a context of more and more restrictive fishing for this species (Total Allowable Catch and quotas, individual weight limits, drifting gill-net prohibition, PPS and licenses...), this system can constitute a way of increasing the value of the catches. The working hypothesis rests on the choice of an exploitation including a strong implication of the fishermen, both for the supply of tunas and fish food, and for the maintenance of the cages. This first study (Caill-Milly *et al*., 2001a) has been built primarily on a bibliographic research and on visits on existing Mediterranean sites (Spain and Malta). It pointed out the constraints and the advantages of the south of the bay of Biscay.

Key words: bluefin tuna, fattening, bay of Biscay, bait boat.

Zootechnical requirements

Bluefin Tuna presents demanding needs, related to exceptional zootechnical performances, among which its ability to regulate partially its internal body temperature. The requirements mainly concern steady conditions of salinity, low and steady turbidity, well oxygenated water and weak breeding load (2-4 kg/m^3). For fattening of Atlantic bluefin tuna (*Thunnus thynnus thynnus*), the temperature must be in a range of 18-26°C (over 14°C for the breeding)). According to the local conditions in the bay of Biscay, this requirements are constraint and seems unfavorable for a long production cycle.

Examination of the existing experiments of breeding

The visit on existing farms mainly showed the type of cages used (high volumes between 28 000 and 220 000 m^3, polyethylene, generally circular), the very significant quantities of food necessary daily (the main species used are mackerel, squid and horse mackerel, the daily ration during the fattening period is around 20 kg for a 200 kg tuna, the fishes are conserved frozen), the obligatory control of the Japanese market requirements (the main factor is the fat content, Caill-Milly *et al*., 2001b), as well as the importance of the investments carried out and manpower employed (between 50 and 100 salaried employees for the maintenance and the preparation of the fish before the export).

The success of these farms lies in the offer of a product presenting the optimal quality criteria for Japan (high fat content, limitation of the stress) when the demand is particularly high.

Definition of the product at the beginning of the breeding

The choice of the product in entry of exploitation was defined according to the characteristics of the fisheries practiced in the south of the bay of Biscay, the Japanese sale conditions and bluefin tuna growth data in the considered zone. In the Basque Country, the baitboat fishing season goes from June up to

November, with a maximum between July and September. In 1998, the catches respectively raised 163 and 2 736 tons for the North and the South of the Basque Country. Almost 70 % of the fishes weigh below 25 kg (45 % are below 10 kg). The demand expressed by the Japanese market mainly concern fishes above 30 kg. The highest prices are generally obtained for big tunas (over 100 kg) and for fishes proposed between the end and the beginning of the year (above 30 Euro/kg). Tuna of less than 20 kg may correspond to a demand in Japan, but also to the need of the European Asiatic communities. These outlets have to be confirmed. In the bay of Biscay, the bluefin tuna growth is very rapid from May up to November. According to information collected, the product corresponds to a fish of about 15 kilos caught from July (theoretical final weight: 25-30 kg at the end of the year).

Evaluation of 7 potential sites

In order to propose a first selection of potential sites for cages, temperature, salinity, swell, wind, currents and turbidity characteristics were taken into account. For floating cages such as those used in the Mediterranean sea, this preliminary choice concerns zones with depth of 30-50 m and sheltered from the North-West winds. Moreover, these sites have to be closed from the fishing ports so as to facilitate the maintenance, far away from the tourist areas and out of the zones influenced by river flows (responsible for high turbidity). The preliminary choice relates to seven zones: 5 on the Spanish side and 2 on the French side. Because of the high constraints to consider, another solution would be the use of offshore cages. Complementary technical and financial studies are necessary.

Conditions of bait boat fishing

With the local conditions of fishing, the monthly theoretical quantity of fishes available from the bait boats rises a hundred tons. According to the Japanese and Australian experiments, the capture with the hook for the provisioning of cage seems as much less traumatizing if the fish is small (less than 20 kg) and if the boat presents specific deck laying out (covered by fitted carpet, rubber,…). The estimated survival rate for the catch and the transport phases is 70 %. The caught tunas cannot be stored in fish ponds on the boats because of their major occupation for the bait, of the low volume available and of necessary fish recaptures. A system of floating cage collected by a specific unit appears more suitable and has to be considered.

Conclusion

The high constraints of the Basque Country are the short favorable temperature period of the sea water, the limitation of the available supply from the bait boats, the high turbidity of the water due to riverflows and the space occupation by other users (mainly for the French side). The advantages are the practice of a not very traumatizing fishing technique for small tunas, the existence of small pelagic fisheries able to provide food for the tunas and the existence of first contacts with purchasers for the Japanese market.

Under these conditions, the system that can be proposed is a very short production cycle focused on summer and autumn, with an initial tuna individual weight of approximately 15 kg (theoretical final weight: 25-30 kg), transfer of caught tunas in floating cages that have to be collected by a specific boat, settling of the tunas in offshore cages (about one mile from the coast). The technical and economic viability of the system is subjected to preliminary works which will quickly be proposed to European grants:
(i) practical studies on bait boat catches (quantity, survival rate) and the transport (floating cages);
(ii) practical experimentation of fattening small size BFT under the local conditions (growth rate, lipid);
(iii) market researches (Japanese and European Asiatic Communities);
(iv) precise studies on the hydrological conditions of selected sites;
(v) engineering studies on the design of offshore cages;
(vi) synthesis of information on the law applied to cage installation.

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Age and growth of the Bluefin Tuna *(Thunnus thynnus thynnus*) of the Northeast Atlantic

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SUMMARY- This paper studies the growth and age of East Atlantic bluefin tuna based on the observation and analysis of hard parts (fin ray sections). The different parts and types of rings observed on the fin ray sections are defined. From this study it can be deduced that hyaline rings are winter rings, which are formed between fall and winter (October-March). Areas of active growth start forming in spring and concludes their formation in the fall (March-October). For younger aged bluefin (age-classes 1 to 3), summer growth is from 3 to 4 times more in size and from 4.5 to 6 times more in weight than winter growth. Winter hyaline rings can be single (thin or thick), or double. From the growth equation obtained by combining data from the juvenile bluefin fishery of the Cantabrian Sea (north of Spain) with that from the adult bluefin fishery of the Strait of Gibraltar area, a value of L_∞=318.35 cm is obtained, which corresponds to a W_∞=615.90 kg.

Key words: bluefin tuna, seasonal growth, fin ray sections

Introduction

The age and growth of the bluefin tuna is studied paying special attention to the interpretation of the signs manifested in some hard parts of its body (in this case, the spinal sections).

Materials and Methods

The sampling was done in northern and southern ports of Spain between the months of May and November.

The range of sizes covered is between 45 and 200 cm in the fishery of the Cantabrian Sea (northern of Spain), and between 170 and 304 cm in the traps of the Gulf of Cadiz (southern of Spain). In both cases fork length as reference was taken.

The method of extraction, preparation and the cutting of the spine, is the one described by Compeann-Jimenez & Bard (1980a; 1980b).

Following their same method, some cross sectional cuts ranging from 0.5 to 0.7 mm in thickness on the first dorsal fin using a slow rotating diamond saw were made**.**

The cuts were prepared on slides covered with a highly transparent resin, and a slide cover.

The measuring and reading of the spinal sections was carried out with a profile projector using a zoom of about 10 to 50.

The back-calculated growth size of the fish and the diameter of its spine were calculated for a sample of 300 tunas, by Rey and Cort (1984). The range of sizes in this relation is from 29 to 200 cm fork length (FL); those fish under 50 cm were from the Mediterranean Sea. Including those fish gave us a better fit to the back-calculated growth (including those fish, which gave us a better fit to the back-calculated growth). The growth model used was Von Bertalanffy (1938)

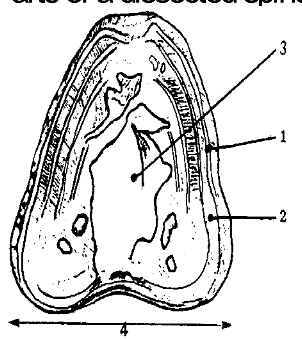
The average value by age, of bluefin tuna, from the Cantabrian Sea, between 1 and 8 years of age, was estimated from the following equation: $Y= 12.780863\ X^{0.576}$ (For 12, 24, 36. 46, 60, 72, 64 and 96; X= month). The equation is obtained by potential adjustment of the modal values of monthly size distributions (Cort, 1989).

Results

Growth in hard parts (spine sections)

The cross sectional cuts of the spines show an alternation of wide areas (active growth) and translucent bands (slow growth). The correct interpretation of these marks is fundamental in order to develop the study on growth as well as to come to conclusions on the biological and ecological aspects of this species.

A. Parts of a dissected spine



B. Different types of rings observed

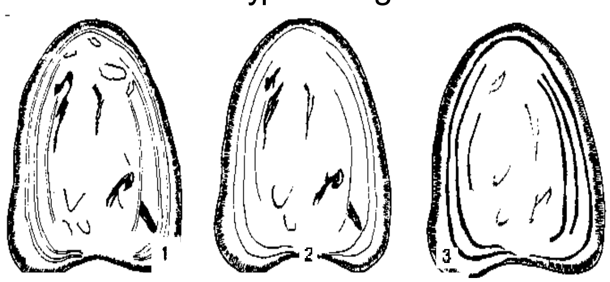


Figure1 A.Parts of a dissected spine.

(1) Translucent area of slow growth, which can be formed by two rings or just one.
(2) Opaque area of fast growth.
(3) Vascularised nucleus, which in fish over two years old has a reabsorbed part in which the transparent bands disappear, and
(4) Diameter of the spine.

Figure 1B. Different types of rings observed.

(1) Double ring (couplet)
(2) Single ring (thing ring)
(3) Single ring, thick ring

In young individuals (up to 3 years old) it is easy to find all the hyaline rings of a spine. However. in fish over 3 years the central area of the spine is reabsorbed and consequently the bands are gone. Therefore, for those fish a back calculation of the size of the fish at the moment of the formation of the first visible rings must be made.

Winter growth

For samples of the spinal sections of bluefin caught in the Cantabrian Sea during the 1985 fishing season, different observations and measurements have been carried out on the translucent rings to study the development of the sizes by age in 1 - 7 year old fish (50 to 175 cm).

The translucent rings found respond to different interpretations due to their shape and apparent composition (See Figure 1 B):

(1) In some cases two thin translucent rings can be seen separated by an opaque band. This is called couplet and the interpretation of these is as follows:

The first ring indicates the beginning of the cold season (this translucent band shown poor protein doses that causes a visible accumulation of minerals in the bonny parts) which can coincide with the outward migration from the Cantabrian Sea to the wintering areas.

The opaque band, which appears next (between the two rings that form the couplet), is the winter growth.

Finally, there is the second ring that shows the end of winter. This one, which can coincide with the migration to the areas of active feeding, has the same composition as its twin.

(2) Fine single rings.

These show that the fish scarcely grow during winter. Since the beginning of the cold season until the fish return to the active feeding area (in spring), the growth slowed down quite notably.

(3) Thick translucent rings.

This indicates that the bluefin had a more active growth than in the previous case. Here the growth is the same as in the first case, but the diet had no proteins.

After having explained the different cases of spinal rings, the growth of bluefin tuna during the winter can be shown. To do so, measurements of the diameter of the translucent rings were taken from the beginning of these (whether simple or double) till the end of them.

Using a sample of 363 bluefin tuna aged 1 to 7, the distribution of frequencies of the diameter of the winter rings were obtained. The results and their corresponding parameters are shown in Cort, 1991.

Using this equation:

$Y= 0.551 + 0.060 X$ (Rey and Cort, 1984)

Which relates Y (diameter of the spine) with X (zoological length of the tuna), the results shown in Cort, 1991 were obtained.

In these tables the size distribution back-calculated to the beginning of the winter ring are expressed as TC (1-7) and by TP (1 - 7) an the and of the winter ring. (The parameters of these back-calculated size distribution are shown in Cort (1991).

The winter growth of bluefin from the Cantabrian Sea was obtained by calculating the difference between the average values by age at the end and at the beginning of the rings (data obtained from Cort, 1991). The final results are expressed in Table I.

Table I. Winter growth of bluefin from the Cantabrian Sea by age

Age	Winter Growth (cm)
1	2.90
2	3.11
3	3.83
4	6.39
5	7.00
6	6.21
7	6.03

Summer growth

To estimate the summer growth of bluefin tuna, sampling of spines from fish of age groups 1, 2 and 3 was carried out. These fish are normally found in the Bay of Biscay from the beginning of the summer season (June), until the end of the season (October).
The idea is to follow, from the beginning, the distance from the last visible ring in the cuts of the spines to the end of the ring.
As the fishing season advances, the ring becomes farther and farther from the end of the cut. In the beginning of the season (June) this ring was at the edge of the spine.The results are shown in Cort, 1991, where the following growth can be observed.

Therefore, the average increase in length and weight for the different age classes of bluefin tuna in the Cantabrian Sea which were studied is as indicated in Table II.

Table II. Average increase in length and weight for the different age classes of bluefin tuna in the Cantabrian Sea.

Age	FL (cm)	W (kg)	FL (cm)	W (kg)
1	2.90	0.4	9.7	2.5
2	3.11	1.3	13.5	5.8
3	3.83	1.7	15.4	10.3
4	6.39	4.0	13.0*	10.5 (June-Sept.)
5	7.00	6.4	14.0**	14.8 (June-Sept.)
6	6.21	7.4		
7	6.03	8.4		

Average values taken from Cort, 1991.
(*) Between October and March.
(**) Between June and October.
Summer growth in relation to winter growth is as indicated in Table III, for the ages studied:

Table III. Summer growth in relation to winter growth, for the ages studied.

Age	Multiple of	
	Growth in size (cm)	Growth in weight (kg)
1	3.34	6.2
2	4.23	4.5
3	4.02	6.1
4	2.03	
5	2.00	

That is, for age classes 2 and 3, summer growth (in cm), is 4 times more than winter growth. For the other age classes, summer growth is at least twice as much, and in age class 1 fish it is over 3 times more. As regards weight for the first 3 age groups, summer growth is on the average, five to six times more than winter growth.

Von Bertalanffy growth model

One general model has been used for the whole species, using the age-class values (1-8) of the Bay of Biscay, and the 1984 bluefin trap fishery in the Gulf of Cadiz (age classes 9 to 19), from which fin ray spines are available (Cort, 1991).

The parameters of Von Bertalanffy equation, applying the fit of the model by Ford & Walford, are:
$L\infty$= 318.85 (cm); to = -0.97 (year); k= 0.093 (annual)

And the equation resulted was: $Lt = 318.85\ [1- e^{-0.093\ (t + 0.97)}]$

The estimated of $W\infty$ is difficult for bluefin tuna due to the numerous size/weight equations calculated in areas where there are immature and adult tunas.

Out of these, the equation by Rodriguez-Roda (1964) was selected since it has a wider range of sizes (25-279 cm, FL). This equation in as follows:

$W = 0.000019\ L^3$

Replacing in this equations: $W\infty = 0.000019\ L^3\infty$

Where $W\infty$ = 615.90 kg (for $L\infty$ = 318.85 cm)

The Von Bertalanffy weighted equation would be:

$Wt = 615.90\ [1 – e^{0.093\ (t + 0.97)}]$

The only information available on the integral growth of eastern bluefin tuna is from Rodriguez-Roda (1964), Compean-Jimenez(1980) and Compean-Jimenez & Bard (1983) although the last studies are very similar with slight variations.

Applications of the described study

Studies on bluefin growth, as noted in the preceding chapter, show a considerable metabolic activity of this species during the months they spend in the Cantabrian Sea (from the end of spring to mid-fall).

Due to the almost complete stop in growth in the months corresponding to the cold season (November to March), it is important to point out that bluefin return to the Cantabrian Sea the following year and are the same size they were when they left 7 months before (see Cort 1991).

Because of this, the applications of the size/age keys have to be done seasonally. The use of only one key for at the catches would distort the size distribution of these catches.

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Optimizing the exploitation of wild and reared Bluefin Tunas under the constraint of sustainability: the IFREMER R&D project

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During the past decade, the diversification of the Bluefin Tuna fishing industry with an emerging farming activity has been rapidly and deeply modifying the economic basis of this sector in Australia and, more recently, in Europe. Within a few years, these "catching and farming" operations dedicated to the Japanese market of sashimi became one of the key issues of the BFT resource sustainability.
France is involved in Atlantic BFT catching through its purse seiners in the Mediterranean Sea, French fleet providing around 70% of farmed BFT in Spain. Furthermore, following the Spanish example, several farming projects may be launched in the near future in our country.

Analysing this trend, IFREMER promoted, early in 200, a research project giving the priority to wild BFT farming aspects in offshore cages and to reproduction control in land based structures. After one year of preparation, our Institute has planned a multidisciplinary research project and officially announced it to the ministry representatives.

The research project aims at improving knowledge in physiological processes governing the reproduction cycle and larval and fingerling biology. The two main targets are improving fingerling production for farming operations as well as understanding the natural recruitment limitations of early stages.
Furthermore, we consider that this research project in BFT aquaculture (fattening, ongrowing of wild fish and juvenile rearing) has to be governed and enriched by the concern of the sustainability of this emerging activity within its socio economic and ecological environment.
We have also taken into account the markets new demands in terms of flesh quality, food safety and animal welfare.

In its present proposition, our research project is organised in 3 inter-related sub-projects: (i) wild fish ongrowing and/or fattening; (ii) reproduction; and (iii) fingerling production and improvement of the understanding of early stage recruitment limitations.
IFREMER and its French partnership could play a major role in 8 research topics : (i) reproduction; (ii) genetics; (iii) nutrition; (iv) adaptive ability; (v) fish quality; (vi) environmental impact; (vii) socio economics; and (viii) engineering.

This challenge in terms of scientific investigations, research infrastructures, and fields of application leads this research project to a multidisciplinary level between several research institutes from different European countries. Therefore we are strongly convinced that the Ifremer BFT R&D project has to be integrated as much as possible in this DOTT action. Although significant scientific results can be obtained within the next 5 years, we assume this action has to be politically and economically supported during the next 15 years at a regional, national and international level.

Impact of purse seine clupeoids fishery on juveniles Bluefin Tuna (*Thunnus thynnus*) in the Southern Italian seas

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SUMMARY - In the framework of the EU Project No 96/093, data concerning the accidental catches of tuna and tuna-like species in the clupeoids fishery, carried out by purse seine using light, were collected in the central Mediterranean during 1997-1998. The total amount of the bluefin tuna can be considered negligible since only a few hundreds of them were caught, especially in the South Tyrrhenian Sea and South Ionian Sea. Their catches consisted mainly of juveniles, which fork lengths ranged from 8.5 to 44.5 cm. Since the bluefin tuna was scarcely represented in the catches obtained by clupeoids purse seine, this kind of fishery does not seem to affect the stock in the southern Italian seas. Moreover, due to the great interest for the bluefin tuna domestication in the last period, it could be evaluated the possibility to use the accidental catches of juveniles bluefin tuna for aquaculture experiments.

Key words: Bluefin tuna, juvenile, by-catch, purse seine, Mediterranean Sea.

Introduction

Clupeoids fishery is generally carried out by purse seine using light. This gear may therefore catchs accidentally juveniles of large pelagic species, as tuna and tuna-like species, because they are attracted both by light and by presence of small pelagic fish which represent an important portion of their feeding. During the three-year period 1997-1999 investigations were carried out in several western, central and eastern Mediterranean areas in order to study the incidence of the aforementioned gear on the accidental catches of juveniles of tuna and tuna-like species. In the present work, the results obtained in the central Mediterranean (South Tyrrhenian Sea, South and North Ionian Sea, Channel of Otranto and South Adriatic Sea) are reported.

Materials and methods

Three categories of boats fishing clupeoids by purse seine were considered, small (8 mean gross tonnage), medium (32 mean gross tonnage) and large (84 mean gross tonnage). The fishing areas were grouped into three areas: the South Tyrrhenian and the South Ionian seas, the North Ionian Sea and the Channel of Otranto, and the Adriatic Sea. A total of 709 fishing hauls were observed: 489 on board and 220 at landing. Due to the high number of specimens, catch data were obtained only in biomass for target and other by-catch species, whereas both in biomass and number of specimens for the bluefin tuna (*Thunnus thynnus*, L.) and for the other tuna-like species. A total of 328 bluefin tuna specimens were measured and their fork length frequency distribution was calculated using a length class of 1 cm (i.e. class 5 = $5 \leq FL < 5,9$).

Results and discussion

The percentage of the target species in biomass was very high, ranging from 91.46% (total catches 392.7 MT in 1998) to 97.47% (total catches 214.2 MT in 1999) in all the areas investigated, and the percentage of bluefin tuna in biomass ranged from 0.13% (total catches 3.4 MT in 1999) to 5.84% (total catches 5.5 MT in 1997) of the whole by-catch of tuna and tuna-like species. The composition of the aforementioned catches for the whole period and areas under study is shown in figure 1. The total number of the specimens belonging to all tuna and tuna-like species and their percentage in the catches are shown in the figure 2. The presence of the bluefin tuna in the catches was more abundant in the South Tyrrhenian Sea and South Ionian Sea: the catches consisted mainly of juveniles. Bluefin tuna was also caught in a very small quantity in the North Ionian Sea and in the Channel of Otranto. No bluefin tunas were caught in the Adriatic Sea.

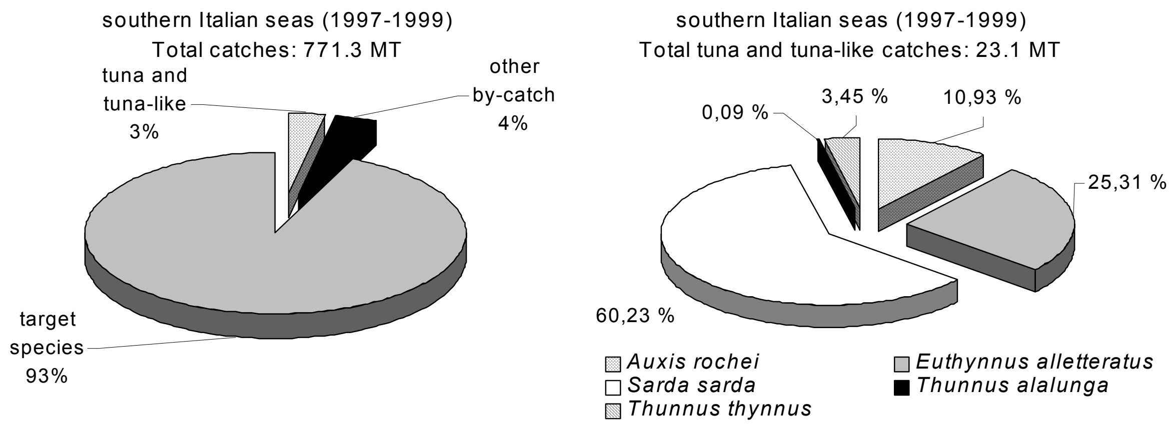

Fig. 1. Composition of the total catches and of the total tuna and tuna-like catches obtained by the purse seine clupeoids fishery in the investigated southern Italian areas from 1997 to 1999.

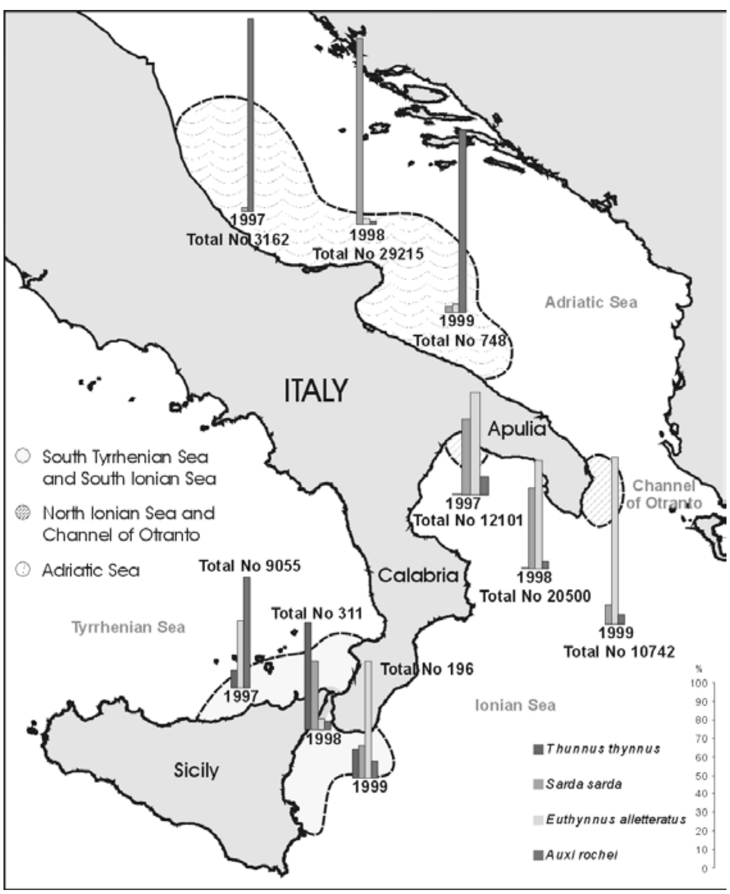

Fig. 2. Total number and percentage of tuna and tuna-like specimens caught in the southern Italian areas during 1997-1999.

The catch composition obtained by observed boats seemed to be affected by different fishing strategies according to the size of the boats. In fact, the small boats, which operated in the first grouped area, captured not only the clupeoids but also several different species, which varied with the season and the local market demand. The boats of medium tonnage, which operated in the second grouped area, were interested in several species but with a greater attention to the clupeoids. Lastly, the large boats, which operated in the Adriatic Sea, addressed their effort to catch clupeoids with great attention to the anchovies.

The fork length of the bluefin tuna caught during the whole period investigated ranged from 8.5 to 44.5 cm, in the South Tyrrhenian and in the South Ionian seas, and from 54.0 to 64.5 cm in the North Ionian Sea and in the Channel of Otranto. The monthly and annual size distributions of these specimens are shown in the figure 3.

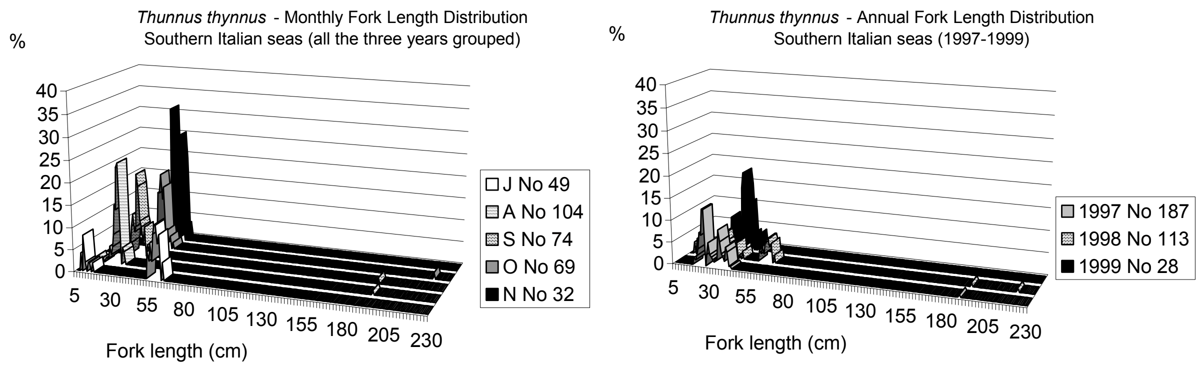

Fig. 3. Monthly and annual length frequency distribution of bluefin tuna, caught in the investigated areas by clupeoids purse seiners during 1997-1999.

Conclusions

The southern Italian boats involved in the clupeoids fishery use different strategies, according to the size of the boats, which affect the catch composition. However, the total amount of juveniles bluefin tuna can be considered negligible since only a few hundreds of them were caught almost exclusively in the South Tyrrhenian and South Ionian seas. Since the bluefin tuna specimens were scarcely represented in the total catches obtained by clupeoids purse seine, this kind of fishery does not seem to affect the stock in the southern Italian seas. The monitoring of this fishing activity could be a reliable tool for obtaining information on the recruitment of large pelagic species and, in particular, of bluefin tuna. In fact, the values of catch per unit of effort, which can be considered as an index of abundance for the recruitment evaluation, showed that the best year for the recruitment of this species was the 1997 in the South Tyrrhenian Sea. This is traditionally considered one of the most important areas in the Mediterranean for the bluefin tuna reproduction (Cavallaro *et al.*, 1996; Nishida *et al.*, 1997; Piccinetti *et al.*, 1996; Sarà, 1998) and the presence of juveniles confirms its importance also as nursery areas. Moreover, since during the last years the interest for the bluefin tuna domestication is growing up, it could be evaluated the possibility to use the accidental catches of juveniles bluefin tuna for aquaculture experiments.

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In vitro protein hydrolysis by digestive enzymes of *Thunnus thynnus*

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SUMMARY - This work presents the preliminary results of some experiments oriented to evaluate, by using the pH-stat system, the ability of *T. thynnus* digestive proteases to hydrolyse different protein sources.

Key words: inhibitor, pH-stat, proteases, protein sources, *Thunnus thynnus*..

RESUME

Mots-clés

One of the most important challenges in the rearing of tuna is to develop a kind of feed capable of substituting the general use of fresh fish. Nevertheless, the current rearing system is based on the growing of wild animals, which makes it difficult their adaptation to inert diets. But if rearing is developed from the earliest stages, such adaptation could be required. Research for tuna aquafeed should follow the same phases as those arranged for other currently cultivated marine species. On these grounds and drawing on the information contained in a previous study on bluefin tuna digestive proteases (Essed *et al*, 2002), the aim of this work is to assess by in vitro techniques tuna ability to hydrolyse different proteins in order to determine which ones could be the most suitable for its feeding. Thus, the following steps were carried out in the present work: (i) the determination of the degree of hydrolysis (DH) of proteins by digestive proteases of *T. thynnus*, (ii) a comparative study of DH with the same proteins using the digestive enzymes of another carnivorous fish, seabass (*Dicentarchus labrax*) and, (iii) the evaluation of the inhibitory effect of raw sources on digestive proteases of *T. thynnus*.

Digestive tracts of tuna (*Thunnus thynnus*) were obtained from 250-300 kg fish supplied by Tuna Farms of Mediterráneo, S.L. (Murcia). Seabass (*Dicentrarchus labrax*) were given by Predomar, S.L. (Almería). Samples of digestive tract were homogenised in distilled water (1:10 w/v). Supernatants obtained after centrifugation were stored at -20 ºC until use. Acid and alkaline protease activities were measured according to Alarcón *et al* (1998). As protein substrates, it was employed several animal and plant meals typically used in the elaboration of commercial aquafeed and provided by Proaqua, S.A. (Palencia). Casein was employed as internal control. The degree of hydrolysis of protein (DH) was determined by pH-stat titration following the method described by Alarcón *et al* (2002).

Degree of hydrolysis of proteins by *T. thynnus* digestive proteases.

The degree of hydrolysis measured in protein samples using stomach and pyloric ceca digestive extracts is detailed in *Table 1*. Mean values of DH obtained with plant proteins did not exceed 5 %, whereas those obtained with animal proteins in all cases exceeded 6 % and even reached 10.62 %.

Table 1. Values of degree of hydrolysis obtained after enzymatic digestion of different protein sources. Data are mean of duplicate measurements, (values in brackets were SD).

CS	MM	FM 1	FM 2	BM 1	FM 3	FM 4	CF	BM 2	PM 1	SC	PM 2	SM2
11.62 (0.13)	10.62 (0.39)	8.90 (0.03)	8.72 (0.36)	8.58 (0.48)	8.56 (0.19)	7.62 (0.16)	7.09 (0.25)	6.04 (0.78)	5.06 (0.29)	4.9 (0.06)	4.67 (0.19)	4.53 (0.06)

CS: casein, MM: meat meal, FM: fish meal, BM: blood meal. CF: commercial fish feed, SC: soybean concentrate, PM: plant meal, SM: soybean meal.

Comparative study with seabass.

Results in Fig 1 indicated that the action of digestive proteases of both species determines a similar profile of protein hydrolysis. However, except for a commercial aquafeed, final values of DH for *T. thynnus* were 25% higher than those for *D. labrax*.

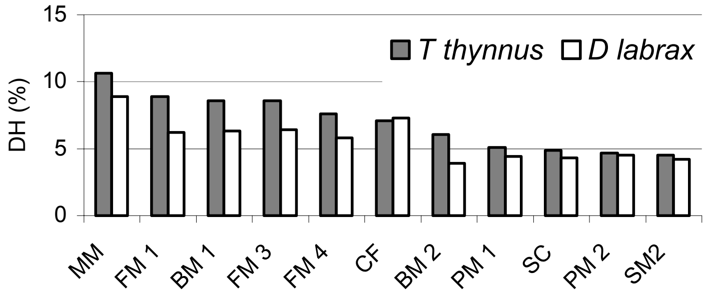

Fig 1. Degree of hydrolysis of proteins after digestion by *T. thynnus* and *D. labrax* digestive proteases.

Inhibition of *T. thynnus* digestive proteases by protein sources.

The presence of protease inhibitor within plant protein was tested by incubation of tuna extracts with a solution containing increasing amount of plant meal. Inhibition values obtained depend of type and amount of meal employed in the assay. High concentrations of meal in the inhibition assay determine high inhibition values. At the same assay concentration (75 mg of meal per unit of protease of activity), commercial soybean meal inhibits only 4% of tuna digestive protease activity whereas a concentrate of the same source reduces 27% of protease activity (Fig. 2).

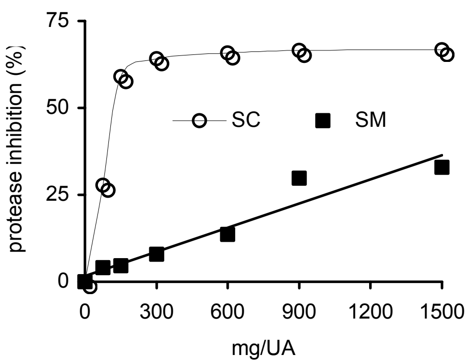

Fig 2. Inhibition of *T. thynnus* alkaline proteases using different relative concentrations of SC and SM.

The use of an in vitro digestibility test indicates that *T. thynnus* digestive proteases hydrolyse several proteins in an equal or higher degree than other carnivorous fish like seabass. The effect of soybean protease inhibitor contained in soybean meal seems to be lower than on other marine fish. This fact could allow the employment of significant amounts of such protein in future aquafeeds for the rearing of this species. However, more investigations are requested for this topic.

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Daily and annual physiological and behavioural rhythms in fish: implications for the domestication of Bluefin Tuna

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SUMMARY - Rhythms with a period of 24 h (daily) and one year (annual) represent one of the major components in the adaptation of organisms to their environment. Virtually all biochemical processes, physiological functions and behaviors are rhythmic. Photoperiod (*i.e.*, the alternation of light and darkness along a 24 h cycle) and temperature play a major role in the synchronization of the daily rhythms (Falcón, 1999; Boeuf and Falcón, 2001). As a consequence of the seasonal variations in day length and temperature, processes such as development, growth and reproduction, follow a seasonal pattern. One of the requirements to achieve domestication of any fish species, including Bluefin Tuna (BFT), is the elucidation of the mechanisms by which information from external cues is integrated; this remains far from clear. We want to emphasize here the importance of the melatonin system as a key component in the integration of external information into a temporal message involved in the synchronization of functions and behaviors (see Falcón, 1999 for extensive details).

Key words: photoperiod, temperature, melatonin, rhythms, growth, reproduction

Photoreception and melatonin production

Melatonin is produced by the photoreceptor cells of the retina and pineal organ (or epiphysis or "*third eye*"). The latter is organized as a simplified retina located above the brain hemispheres in a window below the skull and between the two eyes. Photoreceptors detect rapid changes in light environment (which results in the production of a neurotransmitter [glutamate]). They also provide information on the respective durations of the light (L) and dark (D) phases through the rhythmic production of melatonin. This usually involves circadian clocks. In other words, each photoreceptor cell is a circadian system in which the LD cycle entrains the clock, which in turn drives the expression and activity of enzymes involved in the control of melatonin secretion. Temperature as well as internal chemical transmitters and hormones also modulate the shape of the daily oscillations. These include catecholamines, cortisol, sex-steroids, and peptides, which might represent elements of a feedback loop (Fig. 1). Melatonin levels in the pineal gland and blood are higher during night than during day, whereas a reverse pattern is observed in the retina. Retinal melatonin is produced and metabolized *in situ*. There is indication that retinal melatonin might regulate a number of daily retinal rhythms such as light sensitivity, adaptative retino-motor movements, and neurotransmitters release. Pineal melatonin is released in the blood, and the profile of the rhythm varies along the annual cycle. The nocturnal melatonin increase is of high amplitude/short duration during summer, and of low amplitude/long duration during winter. Nothing is known on the visual, circadian and melatonin systems in BFT.

Role of the pineal gland and melatonin in fish

As reviewed elsewhere (Zachmann et al., 1992; Boeuf and Falcón, 2001) early physiological studies have involved the pineal gland and/or melatonin in the control of daily rhythms of (i) metabolisms (electrolytes and glucose balance, hypothalamic monoamines, lipid content) and (ii) behaviors. The latter refer to skin pigmentation, shoaling behavior, food intake, thermal preference, sleep-like status, locomotor activity and phototactic movements. Tagging studies (this meeting) indicated BFT go down at dawn and up at dusk. These dramatic daily vertical movements might reflect some special requirements of the BFT visual system, as also suggested from the observation that BFT larval mortality due tank bumping is higher at dawn (Kaji, personal communication).

There is also indication that melatonin synchronizes annual variations in fish reproduction and growth, although contradictory results were very often obtained even within the same species. For example, pinealectomy may have pro- or anti-gonadal effects or no effect at all, depending on the season the experiments were done. Similarly, growth rates are accelerated or reduced by pinealectomy, depending on the time of the year. Spawning in BFT occurs on an annual basis, but the modalities of this control are not known.

The identification and characterization of the melatonin binding sites, as well as the cloning of melatonin receptors provided a new step forward in the understanding of how external cues may affect daily and annual rhythms. Thus, it appears that the receptors display a widespread distribution in the fish brain. Brain melatonin receptors are seen in areas involved in the integration of sensory inputs (olfaction, vision, audition) and coordination of adaptative movements (cerebellum). Regarding the control of neuroendocrine functions, of great interest is the observation that functional melatonin binding sites were seen in the pituitary as well as in the hypothalamus of fish (Fig. 1). In the former, melatonin modulates the production of second messengers; we are currently investigating its involvement in the control of pituitary hormones production. In the latter, the pre-optic nuclei appear as a "key center" because they contain melatonin receptors, receive neural input from both the retina and the pineal gland, and they send projections to the pituitary (Fig. 1).

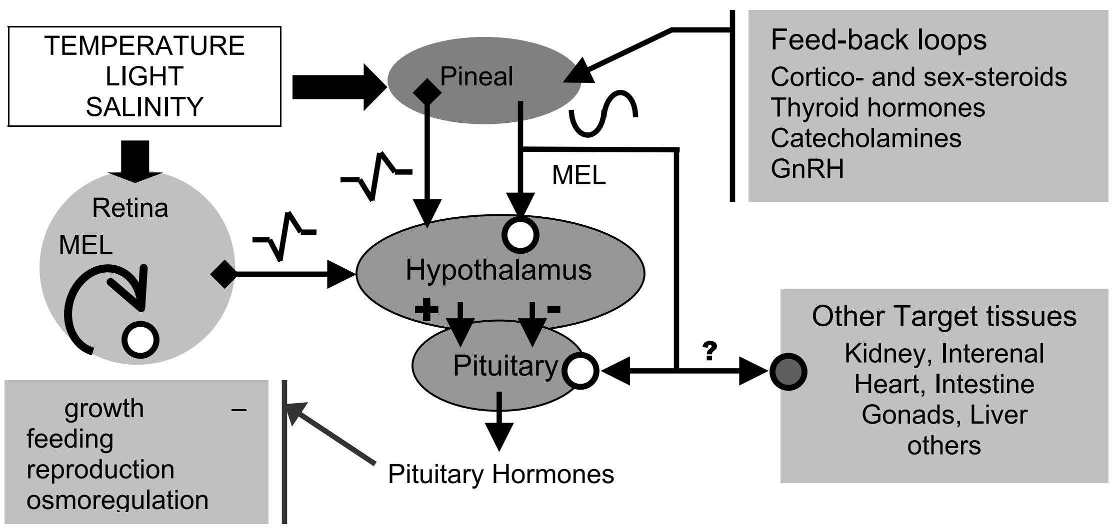

Fig. 1. Known and hypothetical (?) pathways of neuroendocrine functions control by external factors in fish. MEL = melatonin; diamonds with arrows = pineal and retinal ganglion cells; empty circles = identified melatonin receptors; filled circles = hypothetical melatonin receptors.

Conclusions and implications for the domestication of bluefin tuna

The idea that melatonin is a “conductor” that triggers the timing of daily and annual functions in fish is gaining interest (Fig. 1). However, its modes of action are far from being clearly understood and concern a very limited number of freshwater species. In addition, considering that very little is known on the physiology of BFT, and that there are great species-dependent variations, we believe there is an urgent need for basic investigations on the organization and function of the visual, circadian and melatonin systems in BFT. Such studies are of crucial importance to help defining the best photoperiod and temperature conditions for optimizing larval development, as well as adult growth and reproduction. To tackle this problem, we need to investigate, during development and along the daily and annual cycles, (i) the structure and function of the pineal gland and retina (spectral sensitivity, circadian organization, regulation of melatonin production), (ii) the factors and hormones of the hypothalamus-pituitary system (*e.g.*, FSH, LH, GH, prolactin, and corresponding receptors), and (iii) the melatonin targets and modes of action.

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Management of captive tuna: collection and transportation, holding facilities, nutrition, growth, and water quality

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SUMMARY -Tunas have been collected and maintained in captive pens and holding tanks for both commercial and research purposes for over forty years, (Brill, 1999; Harada *et al*., 1971). The Tuna Research and Conservation Center (TRCC) of the Monterey Bay Aquarium and Stanford University has maintained both yellowfin tunas, *Thunnus albacares,* starting in 1995, and bluefin tunas, *Thunnus orientalis*, for the last four years. The focus of research at the TRCC is for both physiological studies of tunas and the testing of new electronic tagging technologies. Physiologically healthy specimens are critical to both endeavors and the technology of keeping tunas in captivity is reviewed.

Key Words: Biomass, ammonia, proximate analysis

Collection and Transportation

Collection of Pacific bluefin tuna specimens is made at sea outside of San Diego, California, USA. TRCC staff and fishing vessel *Shogun's* crew, using conventional sport fishing techniques, collect and place new tuna into each of the vessel's three 8000-liter, running seawater wells. The body conventional sport fishing techniques; the number of tuna that can be transported in the wells is size dependent. All of the specimens are unloaded in San Diego, California, transported to Monterey by truck in a specially designed 11,400-liter transport tank. The number of transported bluefin tuna ranges from 5 to 15 with a total biomass of 75 to 115 kg respectively. Numbers of tuna in excess of these levels are held in a shore-side facility in San Diego for future transport. A travel time of 10-12 hours is required to cover the approximate 800-km distance. Mortality is low, less than 10 percent after the first month of captivity including transportation. Water quality samples are taken at timed intervals during transport and examined at the end of each transport event. Although total ammonium concentrations may accumulate to high levels during transport, the simultaneous decline in pH maintained concentrations of un-ionized ammonia below 0.03 mg/l. The relationship between pH and percent un-ionized ammonia is reported by (Whitfield, 1978). Long-term effects of chronic exposure to ammonia are well documented and reviewed by (Meade, 1985; Spotte, 1970). Modifications, if indicated, are made in loading (fish mass per water volume) and water temperature according to test results.

Holding Facilities

The TRCC is a land-based facility relying on life support systems, with a minimum of added new seawater, to maintain proper water quality suitable for the requirements of tunas. The facility contains four holding tanks each with its own life support system. The volumes of the four holding tanks are 327,000, two of 109,000 and one of 20,000 L. The life support systems provide seawater at rates of 4,536, 1,512 and 378 $L.min^{-1}$. These flow rates are equivalent to a turnover rate (the time required for one holding tank volume to pass through the water treatment system) of one tank volume of water every 75 minutes for the three larger tanks and one every 60 minutes for the smallest tank. The system supplies seawater 100 percent saturated with oxygen to each tank at a set temperature, and at pH values between 7.7 -8.0.

Nutrition

Nutritional requirements are met through feeding a mixed diet of fish, squid and a prepared gelatin rich in vitamins and essential fatty acids. The diet is routinely analyzed for proximate composition; the proximate analysis for different species of food items will vary according to size, age and season, Farwell, 2001. For example, large sardines, *Sardinops sagax*, will vary between 120 to 210 Kilocalories per 100 grams from winter to summer respectively. Small sardines average 100 Kilocalories per 100 grams for both winter and summer time periods. The difference may be attributed to sexual maturity and/or diet. Thus, the caloric content, percent fat and protein are not constant over time and the food ration must be adjusted accordingly to insure that the tuna receive a consistent level of Kilocalories per unit body mass. The food ration is also adjusted over time to account for growth in the captive population of tunas.

Growth

Growth is measured through frequent fork-length measurements of individually tagged specimens. These measurements are made over time and during regimes of constant temperature and food rations. With regression analysis, estimates of change in size, mm day^{-1} can be applied to the population of captive tunas. Measurements of 0.63 mm day^{-1} have been obtained for Pacific bluefin tuna held at 20 ^{O}C, preferred temperature, (Kitagawa *et al*., 2000; Marcinek *et al*., 2001)and fed a diet equivalent to 30 Kcals kg^{-1}day^{-1}. Temperature effects on growth of farmed southern bluefin in sea pens in South Australia has been shown to virtually cease when the water temperature dropped below 15°C. (Glencross *et al*., 2000; Kitagawa *et al*., 2000). References for estimates of growth and age for wild bluefin include (Bayliff, 1993b; Foreman, 1996; Yukinawa and Yabata, 1967). Mass is calculated from published weight-length tables, length is converted to mass using published weight-length relationships, [Anonymous, 1974b; Bayliff, 1993; Davidoff, 1963; Hennemuth, 1961; Wild, 1986]

Handling

In-tank handling of all specimens is accomplished through the use of a soft, pliable vinyl barrier, which allows the isolation of individual specimens, which can then be caught by hand and placed into a water-filled sling. The tuna at this point will remain motionless and can be moved to new holding areas or used for both invasive and non-invasive procedures with a minimum of stress to the tuna. These techniques have allowed TRCC to move and handled over 1000 tunas with minimal damage or mortalities.

Water Quality

Biomass and the corresponding food ration influence water quality in the holding tanks. Routine measurements for Ammonium ion, Nitrite, Nitrate and pH are taken bi-weekly in TRCC to insure that proper water quality standards are met as listed by (Spotte, 1970) and (Meade, 1985). Recovery to normal values after a feeding can extend beyond 24 hours depending on the biomass held in each system. For example, with a biomass exceeding the design parameters of one kilogram per cubic meter of water by 50 percent, recovery time to normal values can exceed 36 hours with the bio-filters unable to digest 100 percent of ammonia passing through in one cycle. (Phillips *et al*., 1998).

Research Activities

Research activities, including current research comprise comparative physiological studies, (Altringham and Block, 1997; Ellerby *et al*., 2000; Freund, 1999; Marcinek *et al*., 2001). Methodology for archival tag implantation surgery and Pop-up Satellite Tag attachment to living tuna was perfected under laboratory conditions (Block *et al*., 1998a; Block *et al*., 1998b). Measurement of Standard Dynamic Action (heat production associated with digestion) for food items of different caloric content, cardiac performance at different temperatures and testing of the most current electronic tag hardware used in studying tuna migration is on-going.

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A screening of lipophilic antioxidants in muscle tissue of cage reared Bluefin Tuna (*Thunnus thynnus thynnus* l.): a potential tool to assess oxidative stress

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SUMMARY - Oxidative stress research in aquaculture is particularly important in the assessment of both the health of farmed fish and the quality of sea food. Lipophilic antioxidant levels in relation to fatty acid patterns of total lipids in muscle tissue of *Thunnus thynnus* were investigated. Significant differences in ubiquinol/ubiquinone and α-tocopherol content are well characterising white and red muscle, in relation to their different energetic metabolism. Variations in lipophilic antioxidant levels may represent a specific oxidative stress marker in relation to alteration in swimming behaviour.

Key words: Ubiquinol/ubiquinone, Vitamin E, molecular markers, oxidative stress, confinement, Bluefin Tuna.

Introduction

Oxidative stress research in aquaculture is particularly important in the assessment of both the health of farmed fish and the quality of sea food. Moreover stressed fish have been demonstrated to be more vulnerable to diseases due to an impairment of antioxidant defence systems (Sakai *et al.*, 1998). Oxidative stress is characterized by an overload of oxidant species and/or a significant depletion of antioxidants (Gutteridge & Halliwell, 1994). As in Mammalians, Vitamin E (d-RRR α–tocopherol) and reduced and oxidized forms of ubiquinones (CoQ_nH_2 –CoQ_n) are the main lipophilic antioxidants in fish tissues and constitute powerful defence tools against ω-3 HUFA oxidation (Filho, 1996; Di Marco *et al.*, 2001). Ubiquinone are ubiquitous and essential for life, so they exist in all body cells and support cellular energy production by helping in the generation of adenosin triphosphate (ATP). It is well known that CoQ_n, in addition to its function as an electron and proton carrier in mitochondria, acts as a powerful antioxidant in its reduced form ubiquinol (CoQ_nH_2), by preventing both the initiation and the propagation steps of lipoperoxidation in biological membranes. Furthermore, it is able to sustain the chain breaking antioxidant capacity of Vitamin E efficiently, by regenerating it from the α-tocopheryl radical.

In this study lipophilic antioxidant (ubiquinol/ubiquinone and α-tocopherol) levels in relation to fatty acid patterns of total lipids in muscle tissue of *Thunnus thynnus* were investigated. The aim was to evaluate if these antioxidant compounds may be useful tool to assess oxidative stress in Bluefin Tuna under rearing conditions.

Materials and methods

White and red muscle portions of 14 wild Bluefin Tuna specimens (TL=137.4±11.2 cm; BW=32.1±6.0 Kg), which were reared in sea cages (Favignana island, TP, Italy) for about 4 months, were analysed. Samples were collected, 6 hours after fish death, and stored at –80°C.
Analyses were performed by liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) techniques (Passi *et al.,* 1998; De Luca *et al.*, 1999).

Statistical analysis of the antioxidant levels were performed by the Student Test; differences between groups were considered statistically significant at $p<0.05$.

Results

Bluefin Tuna has $CoQ_{10}H_2/CoQ_{10}$ as coenzyme Q. From HPLC analyses it resulted that $CoQ_{10}H_2$ is fully oxidised and the values obtained should be considered as the total content of ubiquinone. Though, ubiquinol depletion may only partially be related to the oxidation process that took place during the time between fish death and sampling. Our previous work (Di Marco *et al*., 2001), on ω-3 PUFA degradation in muscle tissue of Sea Bass during a storage period of 7 days at 4°C, indicated that ubiquinol was oxidised *post mortem*; however it was fully oxidised to ubiquinone within 3 days. In the same way, Vitamin E values are probably underestimated, because this compound also degrades.

Lipophilic antioxidant levels were significantly different ($p<0.01$): in red muscle there are higher levels of both ubiquinone (13,66±6.46 μg/g) and α-tocopherol (15,87±13.18 μg/g), in spite of 8,01±2.57 μg/g and 4,14±1.25 μg/g respectively in white muscle. These significant differences may be related to the different contractile and metabolic activity of white and red muscle tissue. The former is involved in short and fast contractions, the second in the long ones and for resistance in swimming activity. Thus, a higher energetic metabolism is needed for red muscle functions (red muscle is in fact characterised by a high number of mitochondria). Consequently, high ubiquinol/ubiquinone and α-tocopherol levels are required for the ATP production and the antioxidant activity.

The total lipid content was very low and similar in white and red muscle tissue, 2.36% and 2.94% respectively. These values may be related to the long period of starvation during rearing confinement.
A high level of polyunsaturated fatty acid (PUFA) (40-60%), in particular of eicosapentaenoic acid (C20:5 ω-3) (7.9-5.9%) and docosahexaenoic acid (C22:6 ω-3) (40.8-27.7%), in white and red muscle tissue was measured, confirming the high nutritional value of this species. The saturated and monounsaturated fatty acids percentages were higher in red (33.4%; 25.0%) than in white muscle (23.0%; 17.3%). Given the oxidation of a saturated or a monounsaturated fatty acid produces more energy, i.e. a higher number of ATP molecules, in spite of the same polyunsaturated fatty acid oxidation, this result would seem to depend on the stronger energetic activity of red muscle.

Conclusions

In literature there is a lack of reference values on lipophilic antioxidants in tuna fish, thus our results can not be used presently to quantify the oxidative status of reared tuna compared to wild ones. However, as the complete degradation from ubiquinol to ubiquinone, measured in white and red muscle tissue, is only partially related to the oxidation *post mortem*, our results may indicate an oxidative stress status in Bluefin Tuna, probably related to rearing conditions.

Significant differences in ubiquinol/ubiquinone and α-tocopherol content are well characterising white and red muscle, in relation to their different energetic metabolism. Since cage rearing may induce some chronic stress responses in pelagic fish, e.g. alteration in swimming behaviour, variations in lipophilic antioxidant levels may represent a specific oxidative stress marker.

On applying aspects, lipophilic antioxidants such as ubiquinols, ubiquinones and Vitamin E represent powerful defence tools against ω–3 PUFA oxidation in fish and their degradation can be considered to be a reliable marker of sea food quality.

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The Atlantic Bluefin Tuna: a global perspective

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SUMMARY - This communication presents a global overview of Atlantic bluefin tuna biology, exploitation and management. The comparison between bluefin tuna and all other tuna species do show that this species is ranking number one among other tunas world wide in a wide range of its resources and fisheries characteristics. Bluefin tuna is for instance the gold winner in the category of fisheries (about 10.000 years of documented fisheries, the highest price in the market, the largest tuna taken), of its peculiar biology (large migration, homing, growth, etc) and in the category of risks presently faced by the resource, because of its multiple scientific uncertainties still pending, its increasingly serious statistical problems and the heavy political context in the management of the stocks.

Key words: fisheries, sustainability, over-fishing, migration, life history trait

Bluefin tuna (BFT) is a species which has been extensively studied for centuries by multiple persons (from Aristotle to the king Don Carlos of Portugal during the nineteenth century) and international teams of scientists (e.g. Aristote, IVth BC; Sella, 1929; Rodriguez-Roda, 1964; Mather et al., 1995). Among other research, the very spectacular and successful recent development of archival and pop up tags on bluefin tunas should be noted as a major progress in fishery science (e.g., Lutcavage et al. 1999; Block et al., 2001). Migration of the three bluefin population world wide (Atlantic BFT: *Thunnus thynnus thynnus*; Southern BFT: *Thunnus macoyii* and Pacific BFT: *Thunnus thynnus orientalis*) are quite similar. BFT displayed very large migration patterns between small spawning strata (located in warm waters) and large feeding zones (located primarily in cold waters and reaching sub-polar areas, see e.g. Mather et al., 1995; Polovina, 1996; Gunn and Block 2001). These migration patterns probably imply a homing behaviour, allowing each individual adult bluefin to target in due time its precise spawning strata.

Surprisingly and despite of this active research, many serious mysteries remain upon this species. For instance the stocks structure itself and the mixing rates between the Eastern and Western Atlantic is still poorly known or quantified (ICCAT 2001). Furthermore, BFT migrations seem to vary between years and some fractions of the population may be quite sedentary in some areas (Block et al. 2001; Fromentin, 2002a). Among the other major problems still pending for the Atlantic BFT, the once major North Sea BFT fishery as well as its sudden collapse in the 1960's still remain poorly understood (Tiews, 1978). In parallel, the BFT fishery that took large quantities of giant bluefin tunas in the warm waters of Brazil during the early sixties also remains a mystery. More recently, the surprising migration patterns off Bermuda shown by several pop-up tags were hard to understand.

Another category in which bluefin tuna is a definite winner will be of course the value of this species in the Japanese sashimi market. However, it should be kept in mind that this rocketing price of bluefin in the Japanese sashimi market has been observed only after the development of deep freezing, e.g. during the seventies. This present high value of bluefin is an increasing problem, recently worsened by farming, as it allows the BFT fisheries to make profit in this fishery even at low levels of biomass. It term of exploitation, it appears that Atlantic BFT stocks are also probably facing nowadays the worst risk of over-fishing, a risk which was already demonstrated for the Southern BFT in the 1980's. The recovery of such long lived species is slow and takes many years. Concerning bluefin tuna stocks, one should also keep in mind the potential risk of genetic erosion due to over-fishing. As the stock may be built with various sub-populations (each one with its own given spawning strata and peculiar genome), the over-fishing may tend to eliminate some of the more fragile (or more easily caught) populations. In such a case, the over-fishing may have irreversible effects on the short term productivity of the stock.

BFT fishery has also among the longest fishery history (Doumenge, 1998). The first evidences indicated that BFT was caught in the Mediterranean Sea seven thousand years before Christ. In the Antiquity, especially during Roman time, BFT fishery was an intensive activity (the most common gears being hand line and large beach seine). At the end of the Middle Ages, the first traps appeared in the Mediterranean Sea and became progressively dominant. The analysis of the trap data from the XVIth to the XXth centuries showed that the historical total catch over the Western Mediterranean and Gibraltar area, was estimated in average about 15,000 tons/year (Ravier and Fromentin, 2002). Furthermore, catches displayed long-term fluctuations of about 100-120 years that were synchronous between all the different locations and countries (Ravier and Fromentin, 2001). Whatever the origin (e.g., changes in recruitment and biomass or in migration patterns), these fluctuations suggest that the size of the stock varies over time (BFT stock could be more productive in some periods than others). On a fisheries point of view, this means that the biological reference points, such as the Maximum Sustainable Yield, and so the quotas, should also change over time in relation to the productivity of the stock (Ravier and Fromentin, 2001).

Considering the Atlantic BFT, two major events occur during the last two centuries. Firstly the appearance of a baitboat fishery in the Bay of Biscay in the mid-XIXth century. Secondly the development of long liner and purse seiner fisheries, in the North Atlantic and the Mediterranean Sea during the second part of the XXth century (e.g. Farrugio, 1981). In the last decade, catches reached an historical level of about 50,000 tonnes, which is about two to three times larger than catches taken by the trap fisheries or the Nordic fisheries (ICCAT, 1999). Despite various uncertainties that we mentioned before, ICCAT scientists have concluded, on the basis of modelling studies, that these large catches are not sustainable (ICCAT, 1999). To understand better this diagnosis, it is worthwhile to look at two other gold medals: one related to BFT life history and the other to the political context.

Atlantic BFT is indeed an impressive fish in size (larger than 3m and 600 kg), in longevity (can live over 20 years) and in fecundity (up to 45.000.000 eggs/ female). It is among the most efficient endothermic animals and can keep its body temperature around 20-25°C within a wide range of water temperatures (between 5°C and 30°C). It is also an impressive swimmer that can cross the Atlantic within 50 days (Lutcavage et al., 2000). However, BFT life history has also some weak points. The major ones are: a late age-at-maturity, a short spawning season and a relative slow juvenile growth (Fromentin and Fonteneau, 2001). This is very different from the life history traits of a tropical tuna, such as the skipjack, which is small, has an early age-at-maturity, a continuous spawning all over the year and a rapid growth. These differences make BFT more fragile to exploitation than tropical tunas (Fromentin and Fonteneau, 2001). Because short live species (tropical tunas) are more productive and have a higher population turn-over, they are indeed more resistant to exploitation than long-lived species (BFT). Furthermore, the long-term fluctuations in BFT stock and yields make more difficult to detect overfishing and depletion risks. Finally, the occurrence of fishing on juveniles also reinforces the fragility of BFT to exploitation because it greatly increases the risk of recruitment over-fishing (Fromentin and Fonteneau, 2001). Fishing BFT juveniles is thus a bad strategy, on both a fisheries (i.e. less productive) and conservation viewpoints and current measures on size limit should be better controlled and reinforced.

The second gold medal that makes bluefin more fragile too exploitation is related to the fact that bluefin is an emblematic example of a shared stock and this creates a tricky political context (Fromentin, 2002b). Along its yearly migration pattern, a bluefin tuna can be caught by many different fisheries, such as the Canadian, Croatian, French, Greek, Italian, Japanese, Lybian, Moroccan, Portuguese, Spanish, Tunisian, Turkish and US fleets. Because of this highly migratory pattern, bluefin assessment and management cannot be local or even regional. It can only be made at the scale of the whole North Atlantic (including the Mediterranean Sea), so at an international level. This is actually done by ICCAT and its scientific committee that evaluates the bluefin tuna stock every 2 years since the late seventies. The assessment is based on an age-structure population model, which uses catch and fishing effort data from all the countries to estimate the whole population size. Then, the committee evaluates whether the present stock size is upper or under the sustainable level (see ICCAT, 1999). If it is under, then a catch limit is recommended and the commission implements a TAC, which is the case since 1996. Other management measures have been also implemented, such as a size limit (at 6.4 kg, i.e. 2 years), a closure season and a measure about the planes used to locate tuna schools.

However, the scientific committee has serious concerns about the quality of the official catch data (ICCAT, 2001). Firstly, because of the poor reporting by the non-ICCAT member countries and of the illegal fishing (concerning mainly very young fish, <1 year old). Secondly, because of the increasing under-reporting of the ICCAT-members. The situation of 1998 perfectly illustrated this problem. At this time, the European Community became a contracting party of ICCAT. The new and old European members asked for a revision of the statistics. This request was actually due to the implementation of a TAC two years before and to the fact that the quotas are proportional to the historical catches. Not surprisingly, this revision lead to an 20% increase of the total catches, demonstrating that the official statistics of the contracting parties were and/or are doubtful (Fromentin, 2002b). The present situation has not been improved and the official catches reported to ICCAT (especially those of the three last years) are likely to be widely underestimated (ICCAT, 2001). Furthermore, some information has been recently lost due to the caging system, because the number, size, weight and origin of fish going into cages are rarely transmitted to ICCAT.

In conclusion, BFT is a fascinating animal and this is really the fish of all the awards. However, we still lack a sufficient knowledge about some major biological and ecological processes. Therefore, there is an urgent need of more active international scientific research programs, such as large-scale tagging experiments, modelling works, scientific surveys, studies on genetic, fingerprint and about the impact of the environmental variations. However, the main challenge for the conservation and management of this stock is nowadays more political than scientific. The priority is indeed to get accurate and trustworthy fisheries information and a real control of the statistics and the current management measures.

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First experience on adult Bluefin Tuna (BFT), *Thunnus thynnus*: transportation from rearing cages to inland facilities

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SUMMARY - For the first time, BFT weighing 40 kg. has been conveyed from rearing cages at sea to facilities on land, achieving survival for up to 6 days. This article describes the methodology used for the capture, conveyance and stabling of tuna, noting the difficulties observed and the potential application of results.

Keywords: Bluefin tuna, transportation, aquaculture, new finfish species

RESUME - C'est la première fois que des thons rouges pesant 40 kg ont été transportés dès cages en mer aux installations en terre, avec un succès de survie de six jours. Cette article décrit la méthodologie utilisée pour la capture, le transport et la stabulation des thons, notant les difficultés observées et l'application potentielle des résultats

Mot-clés: Thon rouge, transport, aquaculture, nouvelle poison especes

Introduction

Considerable development of BFT farming and fattening in floating sea cages has occurred in the Murcia Region (SE Spain) over the last five years. This activity entails capturing large sized animals off the Eastern Spanish coast, in the course of their reproductive migrations. From the fishing grounds, BFT are conveyed in mobile floating cages towed by ships to the rearing sites where floating cages or net enclosures are placed close to the shore. It is here where BFT are stabled for 6-8 months, fed on low cost fish (sardine, mackerel, squid) to increase the fat content of their meat. Practically all the production is forwarded to the Japanese market, where BFT is highly appreciated by the consumer, attaining high prices during the winter months.

In the Murcia Region, there are currently five BFT farms, producing around 5,000 tonnes per year, providing a considerable number of local direct and indirect jobs. Due to the fact that, for several reasons, the BFT stock is severely depleted, the future of this activity would seem, by necessity, to require controlled reproduction. As it is difficult to collect spawning eggs from the floating rearing cages, it seems more appropriate to convey mature animals to facilities on land where they can be more easily handled.

In order to check the viability of this procedure, an initial experience was carried out involving capturing and conveying mature BFT from the rearing facilities of the company Atunes de Mazarrón S.L., located off Puntas de Calnegre (Lorca, Murcia, SE Spain), to the facilities on land of the Spanish Institute of Oceanography (IEO) in Mazarrón (Murcia, SE Spain).

Materials and methods

The work involved three phases: 1) capture, 2) conveying by boat; and 3) transporting overland from the boat to the inland facilities. Capture was performed by means of a sardine-baited line with a barb less hook (Photo 1). The line was thrown to the middle of the circular cage (50 m in diameter and 22 m depth) from the ship located alongside the cage. The large sized fish caught (over 100 kg) were released since the objective was to transport the smaller animals. Once the BFT had bitten on the hook, they were brought to the ship's side (Photo 2) where several divers placed them on a plastic sheet attached to the ship's crane for hoisting on board. Once aboard, the hook was removed and fish was introduced into a 12 cubic meter onboard tank, with a constant supply of pumped seawater (Photo 3).

To avoid any possible aggressive behaviour in BFTs, anaesthetic was dissolved in the ship's water tank (4 ppm of clove oil, 1/10 of the dose required for full anaesthesia in other fish species). Ship travel time to the harbour was approximately one hour. BFT was conveyed to the IEO facilities by lorry, in a tank fitted with a pump, which provided water flow through a nozzle specifically designed for the BFT mouth (Photo 4). The trip from the harbour to the IEO laboratory took 10 minutes. Once there (Photo 5), fish were stocked in an 80 cubic meter concrete tank, sufficient to guarantee a near-saturation level of oxygen, fitted with a tangential water inlet (Photo 6).

Photo 1

Photo 2

Photo 3

Photo 4

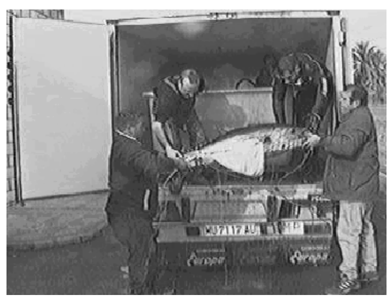

Photo 5

Photo 6

Results and discussion

Two transportation trials were conducted on 3 BFT, with an average of 40 kg body weight and 1 m length, in January-February 2000. Although, the first initial reaction of the BFT after biting the hook was violent and swam quickly, after being immobilised and turned on its belly by the divers, it remained apparently calm, thus making it possible to hoist them aboard ship. Air exposure elapsed time from the sea to the tank was about 2 minutes. Use of anaesthetics was dismissed, after noting clear signs in one individual of an acute response to anaesthesia. At this point, all the water inside the tank was replaced and a water hose was inserted near the mouth in order to reanimate. After five minutes, fish returned to their normal behaviour, swimming in circles around the tank. The rest of the BFT showed no signs of struggle to escape, swimming calmly in the tank. At the entrance to Puerto de Mazarrón fishing harbour, water flow inside the tank was shut off to avoid any influx of polluted seawater. Oxygen concentration in the water was kept at a suitable level by using pure oxygen administered by a diffuser.

From the ship, BFT were removed from the tank with the help of a purpose-built stretcher. No resistance was noted, and they were then introduced into a tank mounted on the lorry. A nozzle was then fitted over the BFT mouth, providing sufficient water flow through the gills to meet oxygen requirements. On arrival at the facilities on land, BFT were again removed and placed on the stretcher with no resistance.

In the first trial, once inside the tank, the two BFT specimens transported started to swim in circles and counter-clockwise, near the walls, drawing to a halt when obstacles were found in the way (i.e., overflow pipes). Due to their inability to ventilate gills when static (the tuna is a ram ventilator fish), BFT died by suffocation 12 hours after their arrival. It was further noted that the lighting system over the tank was inadequate, probably causing a degree of nervousness.

In order to solve these problems, prior to the second attempt, modifications were incorporated into the tank. Hydrodynamism was improved by fitting air-lift pipes shielded on the corners by curved PVC plates to remove all interference inside the tank. Also, a system was installed for turning the light on and off with a dimmer control. As a result of this, the BFT specimen transported in the second trial remained alive inside the tank for 6 days. Death occurred by injuries inflicted due to constant scraping of the right side against the tank walls, when swimming in circles at a relatively constant speed (approximately 1 m per second) counter-clockwise.

Previous similar experiences with this and other Tunid species, conducted mainly by aquariums, such us Monterrey Bay (USA), Tokyo Sea Life Park (Japan) and Two Ocean Aquarium (South Africa), in all cases involved individuals of less than 12 kg (Farwell *et al*., 1997; Garrat *et al*., 1997; Masuma and Oka, 1997; Sakurai, *et al*., 1997). Observing similar problems on handling the fish (mortality caused by skin abrasions).

Conclusions

It can be concluded from these preliminary results that the transportation of live BFT from sea-based structures to facilities on land is feasible and relatively simple, the use of anaesthetics being unnecessary and inadvisable. When keeping BFT in facilities on land, there is an evident need for large sized, circular tanks, to avoid scraping of individuals against the walls.

The time that BFT was kept alive inside the tank in the second attempt leads to the possibility of obtaining controlled spawns of small size mature fish at facilities on land, since in terms of experience with other marine finfish species, 2 or 3 days are sufficient for hormonal induction of spawning in mature animals (Donaldson and Hunter, 1983; Abraham, 1988), usually kept in farming cages from June to August. So, studies on artificial fertilisation and larval rearing may be started for this commercially important species

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Macronutrient composition of food for bluefin tuna (*Thunnus thynnus thynnus*) fattening.

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SUMMARY - To study accurately the food utilization (digestibility, growth, waste production, etc) of Bluefin Tuna (BFT), is necessary a replicated analysis of food samples along the fattening process. In this work we present some results of macronutrient composition of fishfood used in BFT fattening in Southeast Spain. The aim was not to know the variations of macronutrient composition of fishfood, but to know their composition for further studies, as mentioned above.

Key words: *Thunnus thynnus*; fattening; fishfood; macronutrient composition.

Introduction.

Body composition of fishes are submited to a high variability because of (Fig. 1) endogenous and exogenous factors (Shearer, 1994), which may have influence in some key aspects for BFT fattening, as lipid levels of final product, which depend enormously on lipid content of food (Doumenge, 1996). In order to assess the macronutrient composition and nutritional properties of a fish which will be used as food for tuna, it seems to be more accurate the periodical and replicate analysis of samples than simple analysis or scarce and vague bibliographical data.

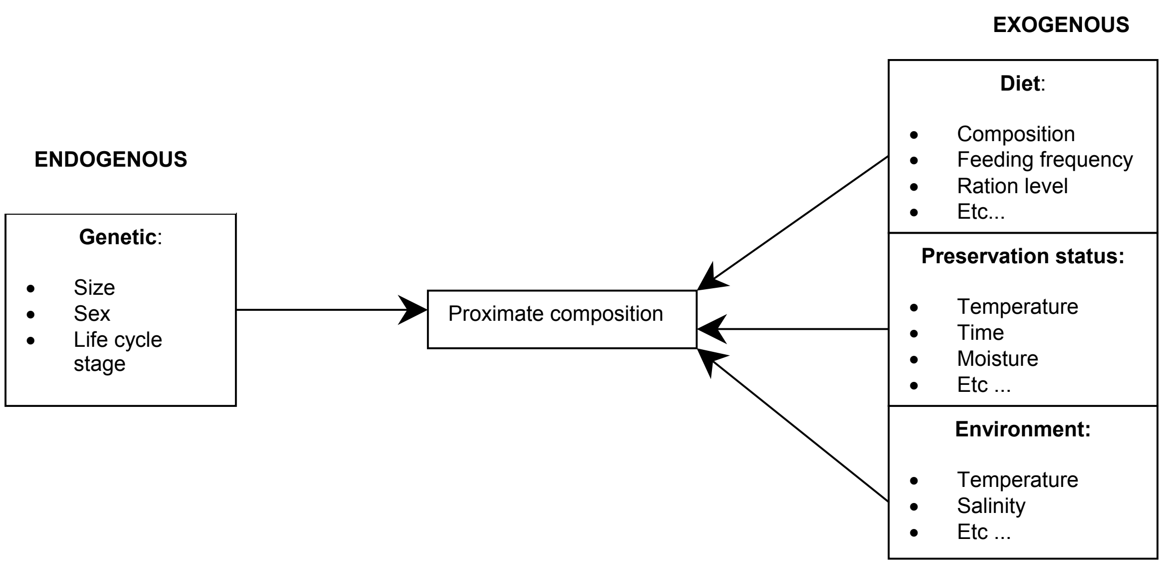

Fig.1: Factors affecting the proximate composition of fishes (adapted from Shearer, 1994).

Materials and methods.

In Southeast Mediterranean, BFT fattening starts in summer, and along autumn tunas are slaughtered. Fish food for tuna fattening were defrosted before supplying. Samples were taken monthly in july, august and September 2001. Catch data of fish food and preservation conditions prior to its utilization were unknown. Each fish food specie was analysed three times (sub-samples), and three (individuals) of each one were done. Previously, samples were crushed and homogenised. For each diet, the protein and total nitrogen, lipid, total phosphorous and moisture content were analysed. The protein and total N content was obtained by the Kjeldhal method, using 6.25 as conversion factor for protein. The lipid content was obtained by ethylic ether extraction (SOXTEC System-HTC). The moisture was obtained by desiccation (105 ± 1 ºC, 24 h.) until constant weight. Total P was obtained by vanadium-molybdinate method.

Results and discussion.

Table 1 shows the average results of macronutrient composition of BFT fattening fish food. Moisture, protein, total N and total P did not change significantly from one month to another for each specie. Nevertheless, lipid content experience sensible variations. This factor seems to be more influenced by different aspects as environmental temperature, life cycle stage, feeding or preservation. Lipid content of final BFT product is very important (Giménez *et al*., 1999) and generally the accumulation of lipids notably depends on food lipid content (Kaushik, 1997; Nakagawa, 1997) in fishes. *Clupea harengus* always showed the higher lipid content than the rest of species, while *Ilex coindetii* showed the lesser.

Table 1: Average values (%) of macro and micronutrient composition from fish food for tuna fattening (D.S.: dirty substance).

%	Moisture	Lipid	Lipid D.S.	Protein	Protein D.S.	Total Nitrogen	Total N D.S.	Total Phosph. D.S.
JULY 2001								
Sardinella aurita	72.57	4.37	15.77	18.53	67.88	2.94	10.86	0.21
Clupea harengus	66.86	15.33	46.19	15.70	47.49	2.51	7.59	0.12
Scomber japonicus	72.85	6.63	24.23	17.72	65.56	2.83	10.48	0.17
Ilex coindetii	75.51	0.72	2.96	17.35	70.88	2.77	11.34	0.25
Boops boops	68.60	7.73	24.61	20.37	65.02	3.25	10.40	0.14
AUGUST 2001								
Sardinella aurita	70.89	8.08	27.35	16.95	58.89	2.71	9.42	0.15
Clupea harengus	73.45	2.90	10.80	18.07	68.12	2.89	10.90	0.15
Scomber japonicus	71.73	8.51	29.21	17.69	63.61	2.83	10.17	0.11
SEPTEMBER 2001								
Sardina pilchardus	75.60	0.99	4.11	17.57	72.05	2.81	11.52	0.17
Clupea harengus	66.88	12.55	37.35	18.07	55.06	2.89	8.80	0.17
Scomber japonicus	73.08	5.44	18.82	18.64	70.88	2.98	11.34	0.17

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Perspectives for tuna farming in Madeira archipelago

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SUMMARY - The archipelago of Madeira appears to present the physical, environmental and economical conditions to support the sustainable development of tuna farms. However, it is thought that feed for the fattening of tuna will have to be imported. A pilot project is necessary in order to evaluate and demonstrate technical and economical viability of this venture.

Key words: Tuna farming, tuna culture, bluefin, bigeye

Introduction

The archipelago of Madeira, situated in the North Eastern Atlantic, has a long tradition in tuna fishing using the pole and line method, a selective technique which catches mid size tuna from the surface (Carvalho *et al.*, 1983; Gouveia, 1986).

Since 1997 there has been a dramatic decrease of most tuna species landings with a significant increase in bluefin tuna *Thunnus thynnus* catches - BFT (Gouveia *et al*, 2000).

Concerns about the status of the local fishery and the recent developments of BFT culture in the Mediterranean Sea have raised local interest in tuna farming.

Madeiras potential for tuna farming

Madeira presents suitable physical and environmental conditions for the development of tuna farms, namely:

-Clean open waters;

-Seawater temperatures ranging from 17 to 24^{o} C;

-Although the ocean currents are weak, the steep seashore, strong spring tides and meteorological conditions concur for a good dispersal of wastes in fish farms.

-The south coast of Madeira island is more sheltered and adequate for the installation of sea cages (Andrade, 1996).

Tuna farming is dependent upon the availability of two main supplies: tuna fish and food. In Madeira the main tuna fishing grounds are located only a relatively short distance (within 40 miles) from the south coast (Gouveia *et al.*, 2000) and also the best farm sites. A significant number (about 6 % of total catch) of the bluefin tuna caught locally are over 180 cm in length, (Fig. 1), the size targeted by the Mediterranean fish farms for growing on (Nuno Gouveia, pers. obs.).

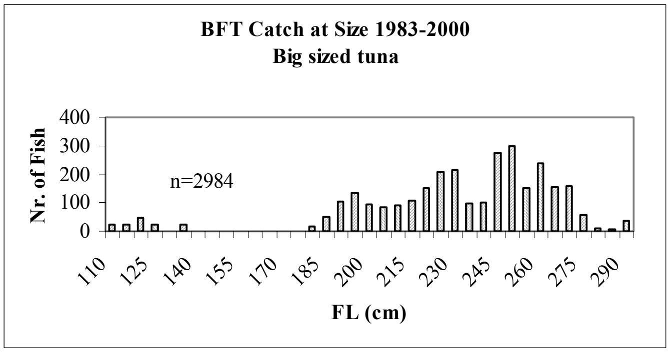

Fig. 1. Size composition of big sized bluefin tuna caught in Madeira archipelago.

However, low catches and limited stocks of small pelagic species limit their potential exploitation as a food source for the tuna under culture. Madeira also possesses the technical and social-economical requirements for supporting this potential industry:

- -Areas pre-selected for fish farm installation;
- -Previous experience in offshore fish farming (of spariids);
- -Extensive services providing technical support to private fish farms;
- -Financial incentives through national and EU funding.

Conclusions

The archipelago of Madeira presents many favourable conditions for establishing fish farms to fatten tuna fish. However, such ventures would depend on the importation of food for the tuna. An experimental pilot project is necessary in order to evaluate the technical and economical potential for tuna farming in Madeira using BFT or other tuna species locally available (such as Big eye *Thunnus obesus*).

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Market and domestic production of cultured tuna in Japan - cultured tuna in the Japanese market

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SUMMARY- A short summary is given of the occurrence and extent of bluefin tuna farming in Japan. The methods used are described and compared with Mediterranean farms and the influence of this cage culture on Japanese markets reviewed.

Keywords: bluefin tuna farming, Japanese tuna market, cages, survival rates, TORO

Bluefin Tuna Farming in Japan

In Japan, there are about 20 bluefin tuna farming and research stations. From their farms, 300 to 500 MT are harvested per year. Their farms are located in central and southern Japan, nearly Wakayama, Ehime, Kochi, Nagasaki, Amami (Kagoshima) and Okinawa Pref.

Bluefin tuna farming in Japan starts in July or August when 150-500g tunas are caught and stocked into the cages. These small tunas are caught by the fishermen and sold to the farmers. They are reared for 3 to 4 years until they grow to a body weight of about 30kg to 70kg.

According to the method followed by Kinki University, the fish are reared for the first 4 or 5 months in 12m X 12m square cages. After this period they are transferred to large cages. For example: 50m diameter circular cages or 20 to 40 X 40 to 60m rectangular cages; without a frame using just floats to hold the net.

The farming method in Japan is different from that used in the Mediterranean, because; for example if a 20kg tuna is caught in Japan, the fisherman can get better price at the fish market immediately, rather than if sold to the farms. This is why Japanese tuna farmers prefer to stock smaller tuna. Also, the small tuna which are stocked in cages show a better survival rate than if they were left in the wild.

In the cages, tunas are fed on fresh or frozen sand eel, anchovy, sardines, mackerel, jack mackerel and squid. To them, a vitamin mix is also added. FCR up to 40kg is 10 to 14, over 60kg is 14 to 20, on a wet matter basis.

In the case of Wakayama, Kinki University, growth is as following for this broodstock data. A 150g-500g small tuna reaches to 3 to 8kg after one year, and 10 to 30kg after two years, and maximum weight of 50kg after 3 years.

In comparison, tunas farmed at the research station JASFA in Okinawa, reached 100kg within 4 years, probably because of higher average water temperature. Also seawater currents and stocking density, may be factors that affect growth rate.

In the large cages, survival rates are 90% or higher. But in 3 to 4 years, feed expenses, labor costs, equipment costs etc. create a very high cost. So, for the farm to make a profit when selling to the fish market, the price must be at least 5,000 yen/kg. Consequently, main sales focus on large cities such as Tokyo and Osaka.

On the other hand, in terms of color, fat content and taste, generally Japanese farmed tuna are superior to Mediterranean farmed tuna.

However, there is a limit for the consumption of this expensive tuna, so if the yearly production of Japanese cultured tuna is to increase, then the production cost must drop but the market price will also drop.

Japanese tuna market

The supply of tuna (total production of domestically culture, wild-caught, imported fresh and frozen fish) indicates values that range from 451,000MT to 507,000MT per year during the last four years. It should be noted that the ratio of fish with a high product value, known as TORO in Japanese, is decreasing. TORO forms only approximately 30% of wild-caught tuna, so the figures for 2001 indicate that only 137,000MT of TORO were obtained. On the other hand, almost all cultured tunas are TORO and although this has only a 4% share of all tuna provided to the Japanese market, it increases the supply of TORO products by 15%. Moreover, to obtain the same quantity of TORO provided by 20,000MT of cultured tuna, it would be necessary to catch 70,000MT of wild tuna. The advantages of cultured tuna are that it could be supplied at a third or half the price of wild-caught bluefin or Southern Bluefin Tuna, and that its available at supermarkets, fresh fish shop or KAITEN-ZUSHI restaurants throughout the year.

Then, nearly all of the cultured tuna consists of the high quality TORO, the supply of TORO has recently increased, so the price of TORO may drag down in the future. In addition, the demand for cultured bigeye and yellowfin tuna is expected to increase, because the fatty meat of albacore, TORO-BINCHOU, is also accepted by consumers. And fatty but cheap tuna´s (bigeye and yellowfin) lay down a further potential demand for tuna on the market as valuable intermediate products between conventional cultured tuna and TORO-BINCHOU.

However, farmers must think wisely about this, as the supply of the TORO of these two species will also drag down the price of bluefin TORO.

Bluefin tuna larval rearing and developmentstate of the art

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SUMMARY-In Japan, trials for mass seed production of Pacific bluefin tuna *Thunnus thynnus* have been conducted vigorously in recent years. There are three major problems concerning the seed production: (i) early mortality during first the 10 days after hatching; (ii) cannibalism around 20 days after hatching; and (iii) collisions against the tank wall observed from juvenile period. Studies on the developmental biology of bluefin tuna larvae have been published intensively over the last several years, using laboratory-reared specimens. Developmental characteristics of bluefin tuna are summarized in the second part of the present paper.

Key words: digestive system, RNA/DNA, larvae, juvenile, growth, enzyme

Introduction

Since the Pacific bluefin tuna *Thunnus thynnus* (BFT) is one of the most expensive fish in Japan, the fish has received considerable attention as an important candidate for future aquaculture and stock enhancement. Spontaneous spawning of BFT in captivity was first recorded in 1979 at Kinki University, Wakayama, Japan (Kumai, 1997). Since then, the seed production of BFT has been attempted vigorously in some hatcheries. Although there remain many challenges before a rearing technique can be established, efforts are beginning to yield increased survival rates of BFT larvae and early juveniles.

To establish suitable seed production techniques and facilities for finfish, it is indispensable to accumulate fundamental knowledge about species-specific early life history traits. In particular, results of seed production trials of BFT strongly suggest that they have unique eco-physiological characteristics during their early life history (Miyashita, 2001). The basic developmental biology of hatchery-reared BFT, therefore, has been studied and published intensively over the last several years, by taking advantage of the ability to use laboratory-reared BFT larvae (Kaji *et al*., 1996; Takii *et al*., 1997; Miyashita *et al*., 1998; Miyashita *et al*., 1999; Ito *et al*., 2000; Kaji, 2000; Miyashita *et al*., 2000a, b; Sawada *et al*., 2000; Hattori *et al*., 2001; Masuma *et al*., 2001; Miyashita, 2001; Miyashita *et al*., 2001; Shimizu and Takeuchi, 2002).

The first part of this paper briefly introduces the present state of larval rearing of BFT. Development of BFT larvae is summarized in the second part in terms of morphological, physiological and biochemical data (Kaji, 2000).

Rearing

The present state of mass seed production for BFT larvae in Japan was well described by Kumai (1997), Miyashita (2001) and Tezuka (2001). They noted that there are three major mortality stages during the seed production. The first stage is the first ten days after hatching (DAH). The second one is from 10 DAH to the juvenile, caused by active cannibalistic behavior. The third one is from the juvenile to the young stage, caused by collisions against the tank and the net-pen wall.

The mechanism of these collisions have been investigated by several researchers, because it is unique and the most serious problem for BFT culture (Miyashita *et al*., 2000; Miyashita, 2001; Masuma *et al*., 2001). Miyashita *et al.* (2000) and Miyashita (2001) investigated the development of the external morphology concerning swimming performance and found that BFT attain remarkable propulsion power at the juvenile period to young stage, but their control system for swimming develops later, resulting in inability to avoid the tank wall. Masuma *et al*. (2001) carried out retinomotor response experiments using cultured bluefin tuna juveniles and concluded that visual disorientation due to the incompatibility of the retinal adaptation with the change in the ambient light intensity at dawn is one of the causes for colliding with the net-pen wall.

Development

All the fish described in this part were reared at the Amami and Yaeyama Stations, Japan Sea Farming Association. Results were mainly based on Kaji (2000), and are summarized in Fig. 1. To describe the early ontogeny of BFT, the developmental phases proposed by Kendall *et al*. (1984) were used in the present study. Briefly, the larval period was divided into 3 phases based on the state of notochord tip flexion (preflexion phase: first feeding to onset of notochord flexion, flexion phase: to completion of notochord flexion, postflexion phase: to completion of fin formation.

Digestive System

The BFT larvae initially fed on rotifers from 3 DAH, concurrent with the establishment of a primitive larval type digestive system. The gastric glands and pyloric caeca first appeared during the flexion phase, indicating early formation of the juvenile-type digestive system. The number of gastric glands and pyloric caeca and volume of the gastric blind sac increased markedly as larvae developed toward the juvenile period. When compared to the other marine fish larvae hatched from pelagic eggs (Tanaka, 1973), the development of the digestive system of BFT larvae appeared to be precocious, supporting early shift to piscivory and resultant rapid somatic growth.

Digestive Enzymes

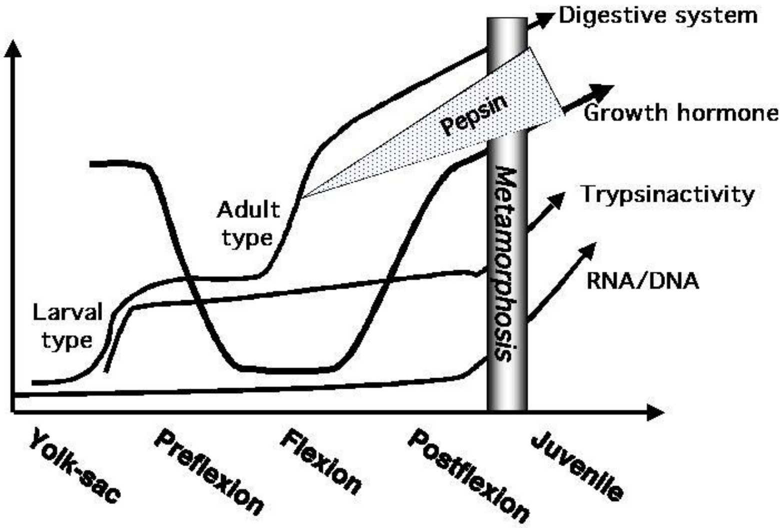
Specific activities of trypsin of BFT larvae were determined individually by the methods reported by Ueberschär (1988). The specific activity exhibited three peaks, 3, 14, and 25 DAH, which coincided with first feeding, flexion phase, and transformation phase to juvenile, respectively. Pepsinogen synthesis in the stomach, detected by immunohistochemistry, started just before the flexion phase, and activated as larval development proceeded. These results suggest that the digestive capability increase markedly from the flexion phase.

Growth Hormone

Growth hormone (GH) immunoreactive cells in the pituitary were first detected 3 DAH at first feeding. Percent GH, defined as the ratio of GH cell-mass volume to pituitary volume, was very high during a few days after initiation of feeding. Then it rapidly decreased, remained at the lowest level throughout the flexion phase, and began to increase from the postflexion phase to the juvenile period. Such a V-shaped ontogenetic pattern of %GH was also observed in yellowfin tuna *T. albacares* larvae (Kaji *et al*., 1999). The %GH of tuna larvae was considerably higher than those of the other marine fish species previously examined throughout the larval period.

RNA/DNA Ratio

RNA/DNA ratio is used as an index of protein synthesis and the nutritional condition of marine fish larvae. Thus, the RNA/DNA ratios were determined individually using an ethidium bromide technique (Clemmesen, 1993, Sato *et al*., 1995). The ratios remained at a constant low level during most of the larval period with a temporal decrease around first feeding. Then the ratios increased steeply from 20 DAH at metamorphosis, suggesting drastic increase of protein synthesis capacity.



Developmental characteristics of Bluefin Tuna

Fig. 1. Schematic drawing of the early development of bluefin tuna *Thunnus thynnus* reared in the laboratory. Data are based on Kaji (2000). Developmental phases proposed by Kendall *et al*. (1984) were used in the present figure.

Altricial Yolk-sac and Preflexion Larval Phases

Histological, physiological, and biochemical data suggest that BFT undergo an altricial and immature early larval period from hatching until the end of the preflexion phase. Overall developmental features during this period, e.g. primitive body structure with normal-size mouth and larval-type digestive system structure and function, are similar to those of common coastal marine teleosts hatched from small pelagic eggs.

The existence of a "critical period" around first feeding, represented by slow growth and high mortalities under rearing conditions, was confirmed consistently by histological, physiological, and biochemical data. Because of tuna's high fecundity and batch spawning mode offshore, they could be a representative group with a life history pattern characterized by very high annual production of larvae and subsequent high mortality at sea. Vulnerability of tuna larvae revealed in the laboratory-reared fish could be common to in the open ocean. This mode of spawning may also force larvae to undergo an altricial larval period offshore in which the densities of available food organisms are relatively low. Primitive body structure of tuna at an early larval period would be adaptive to utilize small but abundant invertebrate zooplankton, such as copepod nauplii (Uotani *et al*., 1990).

Flexion Phase as a Turning Point of Development

The flexion phase should be noted as a "turning point" in tuna larvae. External morphology common to most fish species transformed into a specialized form of scombrid larvae characterized by a disproportionately large head with large mouth and eyes. Although the other body structures are still of the less-advanced larval type, only the digestive system attained the adult type; differentiation of gastric glands in the stomach followed by differentiation of pyloric caeca. Compared to other marine fish larvae, apparently precocious development of the digestive system coupled with an enlarged head and mouth allow the larvae to shift the survival strategy from "altricial larval life with planktivory" to "large prey-fast growth (Hunter, 1981)" with a potential piscivorous food habit.

Large Prey-Fast Growth During Postflexion Phase to Juvenile

Histological, immunohistochemical and physiological data, as well as such morphological features as posterior shift of the anus position, demonstrated that the structure and function of the digestive system developed in an extremely quantitative way during the period from postflexion phase to the juvenile period. At the transition to juvenile, fins completed and vertebral column ossified, which are generally observed in the metamorphosis of many marine teleosts, indicating marked increase in swimming ability which enable the tuna juvenile to actively exploit prey fish larvae at relatively low densities in their offshore habitat. Therefore, the developmental feature of BFT at postflexion phase and juvenile period can be described as "large prey-fast growth", as demonstrated by the steep increase of %GH and RNA/DNA ratio. In fact, Miyashita *et al*. (2001) noted that the growth of BFT was markedly accelerated from about 2 weeks after hatching, while the growth during first 10 days was low and similar to those of common marine fish larvae previously examined.

In summary, the early development of BFT divides into two contrasting periods; altricial larval period and rapidly developing postflexion phase to juvenile. The flexion phase intervenes between these two periods as a turning point.

Further studies on ecological traits of wild tuna larvae, as well as a comparative study among other scombrid species, are required to understand the early survival strategy of tuna.

Acknowledgements

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Catch and effort of the bluefın tuna bluefın tuna purse seıne fıshıng ın Turkısh waters

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SUMMARY - Data on technical specifications, numbers, catch amounts, fishing power per boat and CPUE of purse-seining boats currently operated for tuna fishery in the Turkish waters between 1994-2000 period, are presented. The number of the purse-seining boats operating during the study period varied between 22 and 62. Larger fishing boats and powerful equipments in terms of boats engine, fishing nets, etc., intensive use of echosounders, as well as bird radars in recent years were most important factors which caused a significant increasing in the tuna fishery.

Key words: bluefin tuna, fishery, purse seine, CPUE, Turkey

Introductıon

In Turkey, the traditional fishing of the bluefin tuna has been carried out by means of fish traps and hand lines in the 1920s (Devedjian, 1926). The major fish traps for bluefin tuna have been set in the Sea of Marmara and in the Bosphorus Strait. Using those fish traps for catching bluefin tuna lasted until 1987. In Turkish seas, the bluefin tuna purse-seine fishery was started for the first time in the Sea of Marmara in 1950s (İyigüngör, 1957). Due to the significant development of the purse-seining boats particularly in the late 1980s nowadays, purse-seining is commonly used for catching bluefin tuna.

According to the FAO records, the catch of the bluefin tuna by Turkish fishing fleets was 3466 metric tons (MT) in 1994 and it increased to 5899 MT in 1998, however, this value decreased to 1407 MT in 1999 and 1070 MT in 2000.

Materıals and methods

Data on technical specifications, numbers, catch amounts and days of fishing per year of bluefin tuna purse-seiners were collected from the boat owners and by the field surveys between 1994 and 2000. Fishing effort and catch for unit of effort were computed according to following formulae. In the present study, fork length (FL) and weight (TW) of 6171 bluefin tunas were recorded.

Effort (E) = number of fishing days for each of the n boats
CPUE (MT) = catches / E
CPU = catches / vessel

Results

Bluefin Tuna Fishing Fleet

The number of the purse-seining boats operated in the bluefin tuna fishery varied between 22 to 62. Those boats were 22 to 62 m in length, 24 to 694 grosstons, and powered by 300 to 2610 HP enginees (Table 1). The presence of echosounder and sonar devices, and the recent using of bird radars on purse-seining boats make possible to locate the schools of bluefin tunas precisely.

The length and the height of the bluefin tuna nets depending on the boat size varied between 756 and 1980 m and 108 and 270 m, respectively.

Table 1. Some of the technical specifications of bluefin tuna purse-seiners between 1994 and 2000.

Year	Number of Purse seine	Boat length (m)	GRT	HP
1994	51	19,4–55	24,5–496	300-1720
1995	60	24–55	48,5–496	435-1720
1996	61	25,15–62	76–694	470-2028
1997	62	28–62	148–694	800-2028
1998	62	25–62	92–694	400-2028
1999	22	20-62	90–694	470-2610
2000	26	28-62	120-694	470-2610

Fishing Area and Fishing Period

The main fishing grounds of bluefin tuna are the eastern Mediterranean Sea, and northern and central Aegean Sea; occasinal catches of bluefin tunas were also recorded from the Sea of Marmara. Fishing activities are carried out close to the coast-line, as well as 30 to 40 miles off the shore.

Technically, the bluefin tuna fishery lasts all year round in Turkish seas, however, with the termination of the fishing season of bonito, anchovy and bluefish, the main fishing season for bluefin tuna begin in February and lasts untill June.

Fishing Effort and CPUE

Catch amounts, catch per unit of effort and fishing effort data are given in Table 2.

Table 2. The nominal CPUE of bluefin tuna in Turkish seas by purse-seine fishery.

Year	Catch (MT)	Effort (Fishing days)	CPUE (MT/F.day)	CPU (MT/vessel)	Mean FL (cm) ± SE	Mean Weight (kg) ±SE
1994	3466	526	6,59	67,96	145,42 ± 1,74	60,20 ± 2,06
1995	4220	925	4,56	70,33	137,15 ± 1,04	48,31 ± 0,98
1996	4616	997	4,63	75,67	108,42 ± 0,49	23,77 ± 0,39
1997	5093	841	6,06	82,15	125,05 ± 0,57	36,44 ± 0,56
1998	5899	806	7,32	95,15	127,53 ± 0,72	39,52 ± 0,76
1999	1407	210	6,7	63,95	138,84 ± 2,23	57,81 ± 4,39
2000	1070	332	3,22	41,15	121,02 ± 0,76	35,00 ± 0,83

CPUE values varied between 3.22 to 7.32 MT, with a mean of 5.58 MT per year. The linear regression analysis of the obtained data indicates a decreasing in the CPUE data as 0.112 MT per year. CPU values show a decreasing of 2.633 MT per year (Figure 1). A variation is seen in the numbers of days of fishing per year.

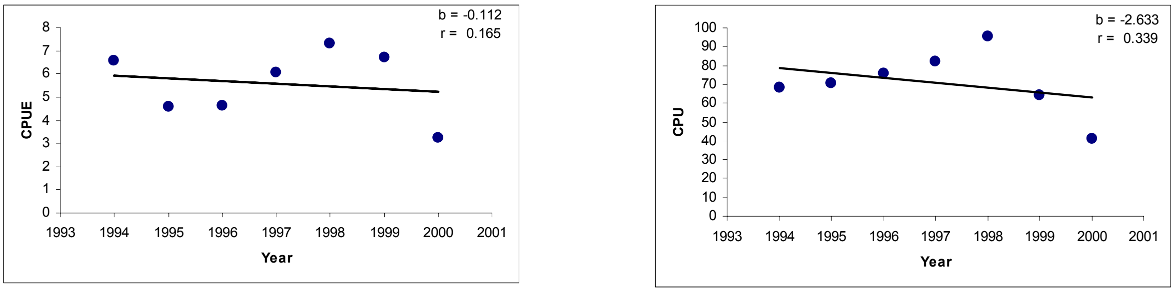

Figure 1. Annual CPUE and CPU values in purse-seine fishery of bluefin tuna.

The annual catch amounts of the bluefin tuna showed an increasing trend between 1994 and 1998 (5899 MT), while they exhibited a significant reduction in 1999 (1407 MT) and 2000 (1070 MT). Similarly, the CPUE showed a decrease since 1998. Paralell incresings and decreasings were observed in the numbers of the boats and days of fishing (Figure 2). Days of fishing and CPUE were inversely proportioned. The value of CPUE decreases as the days of fishing increase (Figure 3).

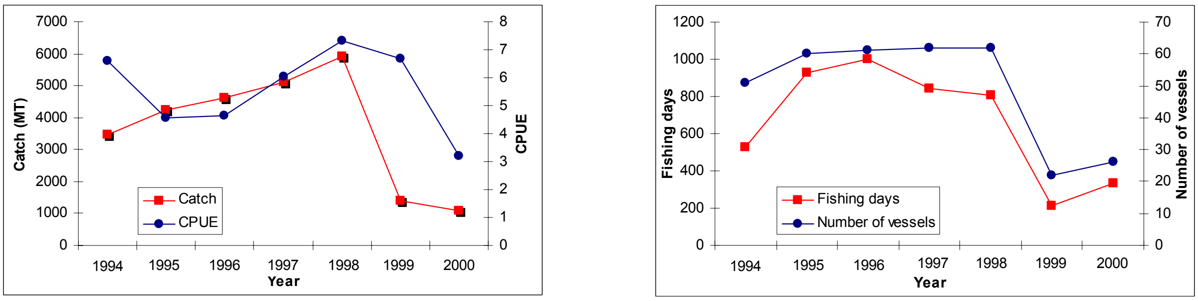

Figure 2. Catch amounts and fishing effort, days of fishing and number of vessels for bluefin tuna purse-seine fishery

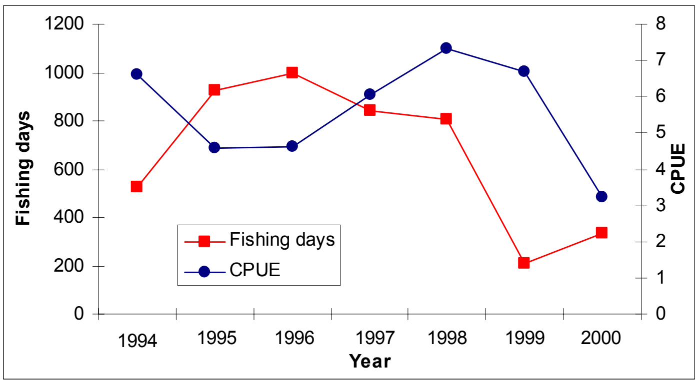

Figure 3. Days of fishing and CPUE for bluefin tuna purse-seine fishery

Catch-at-size

Mean lengths and mean weights of bluefin tunas with regards to years, are given in Table 2. Maximum value of the mean weight (60.2 kg) was recorded in 1994 and the minimum value (23.77 kg) recorded in 1996.

Conclusıons

In Turkey, the bluefin tuna fishery is commonly carried out by means of purse-seining. Furthermore, hand-liners and swordfish harpooning boats also capture some bluefin tunas. The pelagic long-lining is not used for bluefin tuna fishery in Turkish seas.

The rapid development of the bluefin tuna fishery caused an increase in the size and enginee power of the purse-seining boats, as well as an development in their fishing nets. Echosounder and sonar devices, and most recently the bird radars are commonly used by the purse-seiners.

Very close coorporation is existed among the bluefin tuna fishermen. Locating the schools of bluefin tunas and entrappment operations are carried out collectively. During the fishing in 30 to 40 miles offshore, all of the captured bluefin tunas are transferred to one of large purse-seiners and placed in its' cold-storage, and this boat immediately returns to fishing port for landing the catch. This collective fishing strategy allows the other boats pursuing the schools of bluefin tunas without waste time.

Due to the drastic reduction in stocks of the bluefin tuna, ICCAT set some quotas for export. Following this, Turkey has received a letter of warning which concerns the overexploitation of bluefin tunas. The bluefin tuna is an important item of export in Turkey and, therefore, the Ministry of Agriculture and Rural Affairs has set new fishing regulations for protecting this pelagic fish against overfishing. Following this new regulations, the annual catch amount of the bluefin tuna decreased to 1407 MT in 1999 and the observed reductions in the values of CPU, CPUE and days of fishing in that year resulted from this reason.

According to the regulations set by the Ministry of Agriculture and Rural Affairs, catching bluefin tunas smaller than 90 cm (15 kg), and catching bluefin tunas by purse-seining boats between June 1st and September 1st are strictly forbidden (Anonymous, 2000). Most of the fishermen respect these regulations. In comparison with the recommendations of ICCAT (1999) which aim to prevent the catch of bluefin tunas smaller than 6.4 kg in the Mediterranean Sea, the limitations in Turkey seem more realistic.

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Rearing of small bluefin tunas (*Thunnus thynnus* l.) in the Adriatic sea – preliminary study

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SUMMARY - The objective of this paper was to describe cage rearing of small bluefin tuna (*Thunnus thynn*us L.) in the Adriatic Sea. Approximately 6200 fish ($W_{avg.}$=11.2 kg), captured by purse seine were stocked in a 50m diameter cage. After two weeks of acclimatisation, tunas were fed raw, defrosted small pelagic fish, dominated by herrings with 87.9%. Feeding experiment was initiated July 9 and monitored up to October 31st 1999. Feed was hand distributed six days/week, twice a day. Tunas were daily fed with the quantity of food equal to 5.1% of their biomass within the temperature range from 18° to 24°C and the oxygen saturation was above 90%. The highest mortality rate (2.1%) was noticed during the first month of adaptation, while in the following months it was significantly lower (0.6%). Some specimens were measured and tagged, with aim to estimate their daily growth rate over 540 days rearing period.

Key words: fish farming, bluefin tuna, growth-out cages, tagging, feeding, Adriatic Sea

Introduction

In Australia, in 1992, tuna fishermen and Japanese consultants commenced a project for fattening young southern bluefin tuna, *Thunnus maccoyii* caught by purse-seine boats out in the ocean (Mourente and Pascual, 2000). The Croats living in Australia transferred the experiences gathered during these initial trials in tuna farming to the Adriatic Sea. Thus, in the year 1996, these new activities in aquaculture, concerning the northern bluefin tuna (BFT), *Thunnus thynnus* (L.) farming, took place on the eastern part of the Adriatic Sea. In the last few years, the rapid development of this practice of the bluefin tuna farming in the Adriatic has been noticed.

Purse seined tunas caught in the central Adriatic are transferred into a floating cage. After having been filled up with certain quantity of tunas, the floating cage is tugged by tugboat toward near shore waters, and tunas are transferred into an anchored growth-out cage. The tunas in the cages are fed with raw, defrosted small pelagic fish, such as herring, sardine, anchovy, sprat and cephalopods. The tunas are usually reared from summer to winter, when they are sold to Japan (Ticina, 1999).

The tuna has traditionally supported an important fishery in Croatia and elsewhere. Large decline in catches over recent years has emphasised the need for information on biology and ecology of the species. The fact that the wild caught tunas are normally of variable quality (being dependent on many uncontrolled factors) and that sushi market requests specific harvesting process and better quality control, has led to increased subjection of tunas to fattening in the cage before being marketed.
However, the smallest specimens usually remain in the cages to grow during the period of two or three years. The aim of this practice is better usage of limited fishing quota, and the improvement of the value added. The practice of tuna transferring into cages allows better quality control during the harvesting process that improves prices and utilisation of existing natural resource without increasing the fishing mortality. Furthermore, it represents a needed step from "wild" bluefin tuna fisheries towards domestication in captivity with full control on the entire production cycle that will eventually decrease future pressure on the natural stocks. Additionally, the fact that small tunas dominate in the catch composition in the Adriatic Sea over the most part of fishing season (Ticina *et al.,* 2002), and the reduction in quotas has further stimulated the development of a tuna on-growing industry in Croatia. In most cases 1-2 year old juveniles are grown to more profitable weight and condition. However, it should be also taken into consideration that this practice could cause difficulties in the catch statistics of ICCAT and concurrence of catch data with trade data. Therefore, the aim of this preliminary research was to give some information relevant to further research in both mariculture and tuna fisheries. The data described in this paper provide indications about survival rate of purse seined tuna during the adaptation period in the cage and the growth rate and feed consumption of bluefin tuna when reared in the cages.

Material and methods

Feeding experiment

Juvenile BFT were captured by purse-seine in the off-shore waters of the central part of the Adriatic Sea during June 1999. After that, tunas were transferred into Bridgestone sea cage modified for towing and towed to Zadar's archipelago coastal area. The restocking of the fish into grow-out floating cage situated by the southern coast of a small island Iz (Fig. 1) was completed July 1st. Approximately 6200 tunas were stocked into one 50 m diameter cage with sac 20 m in depth.

The average size of the fish in the cage was 11.2 kg, ranging from 3.5 to 25 kg thus giving an initial biomass that was estimated up to 69,626 kg. During the rearing period, from July to October, sea temperature and oxygen concentration were measured in the cage.

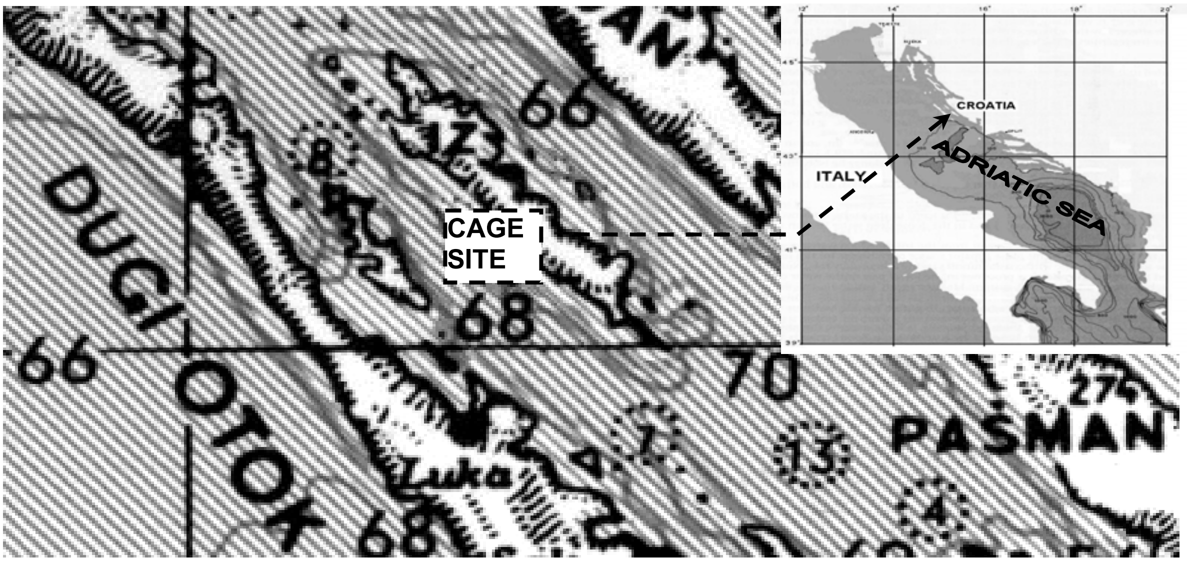

Fig. 1. Map of the area where BFT growth-out cage was located

During the rearing period, morts were collected and frozen (95 specimens in total), and used for initial samples. Tunas in the grow-out cage were fed for the first time nine days after being stocked. The raw, defrosted herring, *Clupea harengus* was major feed component (87.9%), which is currently used by all commercial tuna farms. The remaining feed was composed of raw defrosted sardines, *Sardina pilchardus* (6.7%) and cephalopods (5.4%). The feed was given to satiation twice per day, in the morning and in the late afternoon, approximately six days per week. The daily feeding records include information about water temperature (°C), oxygen concentrations (mg/l) in the cage, mortality, diet composition and feed quantity. Quantity of food was expressed as percentage of the total fish biomass estimated when tunas entered the cage.

Growth experiment

At the beginning of the feeding experiment, 25 tuna were randomly sampled from the cage. Specimens were line caught, lifted from the cage onto the 10 m^3 tank with anaesthetic added (benzocaine) into ambiental sea water. Tuna were taken from the tank by means of a lifting device made of plankton nets. Fish were measured in fork length, weighted and tagged with a conventional tag and transferred back into the rearing cage. Tagged fish were reared in the same cage for 540 days and recaptured in December 2000.

Statistical analysis

To test significance of relation between daily growth rates and initial size of the fish, linear and power regression analyses were performed. Differences between two length-weight relationships of wild bluefin tuna taken directly from the purse-seine catches in the open sea and fish reared in the cages were tested using paired t-test. In all these statistical analyses, P-value was calculated and significance tested at 0.05 α-level (Sokal and Rohlf, 1997).

Results and discussion

Mortality and feeding

It was noticed that mortality rate of the tuna specimens in the growth-out cage was not constant during the entire rearing period. The highest mortality rate (2.1%) was noticed during the first month (July) when 132 fish died out of 6200 fish stocked. From August to October mortality rate was significantly lower (0.6%) and only 35 fish died during next three months period (Fig. 2.).

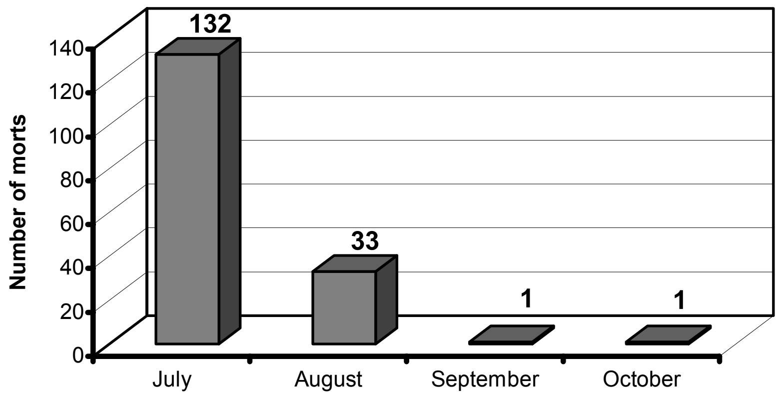

Fig. 2. Mortality of the bluefin tunas in the growth-out cage (July-October).

The total weight of 132 fish that died during July was 826.5 kg. Average weight of these tunas was 6.26 kg. In August, 33 dead fish weighted all together 260 kg, giving an average of 7.88 kg/fish. The difference between the average size of tunas that died in July and August can be explained by weight increase of these fish in the cage. However, it should be pointed out that both values of average size of dead fish were significantly lower than average size of the fish that were put in the cage. This fact indicates that post harvesting mortality is stress related and that smaller juveniles seem to be more sensitive to stress when compared to more advanced stages.

The feeding was initiated on the 9^{th} day after transferring fish into growing cage with 50 kg of mixed raw small pelagic fish. The daily feeding rate has gradually increased until the end of July when tunas were fed with feed quantity equal to 7.70% of their biomass. According to the daily feeding records (feed composition and quantity) that cover feeding period of 155 days, it was calculated that tunas were fed with 399,005 kg of feed in total. During the entire feeding experiment from July 9^{th} to October 31^{st} 1999, feed was distributed approximately six days/week, or more precisely in 137 out of 155 days (Fig. 3).

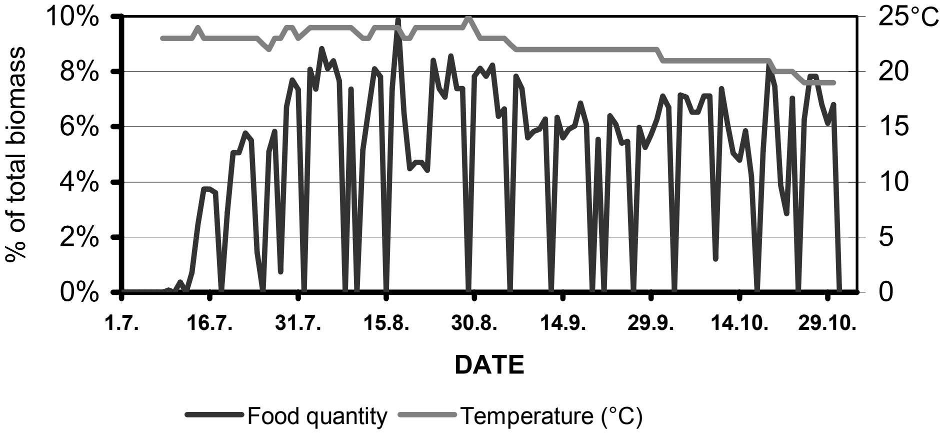

Fig. 3. Feed consumption of BFT reared in the cages over 155 days as related to temperature

Gradual temperature decrease from August to October was noticed. The oxygen concentration varied within a range from 6.8 to 7.6 mg/l, which means that the water was well oxygenated and often even saturated with oxygen. Even at the lowest value during the entire period (6.8 mg/l O_2 at 20°C and salinity 37.0‰) the oxygen saturation level was 93.3%. The maximum daily feeding rate was equal to 9.8% of the estimated fish biomass in the cage, but average daily feeding rate was approximately equal to 5.1% (std = 0.028) of total fish biomass

Growth characteristics

By analysing tag-recapture data obtained in this experiment, it was found that juvenile bluefin tuna of estimated age 1+ year weighing averagely 12 kg reached body weight of approximately 45 kg after 540 days rearing period. During the same period, smaller juvenile tunas with estimated age of 0+ year and 5 kg average body weight increased their weight up to 25-30 kg. This means that 1+ year old tunas weighing approximately 12 kg in average have increased their weight three to four times after 540 days rearing period. However, in the same time, smaller juvenile tunas with estimated age of 0+ year and approximately 5 kg body weight showed an increase of nearly 600% regarding to their initial weight.

Regarding the length-weight relationship of these bluefin tuna reared in the cages (Katavic *et al.,* 2002), the positive alometry was noticed (coefficient of condition b>3). However, Ticina (1994) calculated length-weight relationship of wild bluefin tuna caught in the Adriatic Sea with coefficient b<3 that describe a negative alometry. When compared these two models of length-weight relationships (Fig. 4), we found that there were no significant differences (t-test, P=0.308) within size range from 60 to 110 cm in fork length or up to 25 kg. However, a significant differences were observed for the fish >110 cm in fork length that correspond to the fish above 25 kg in weight (t-test, P=0.026). This could explain the fact that improving condition factor of tuna above 40 kg increase prices on the sushi market.

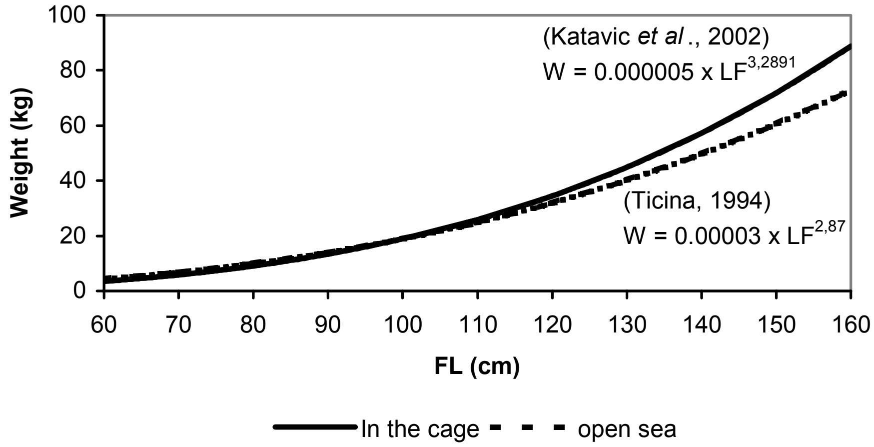

Fig. 4. Comparison of two different length-weight relationships of wild and farmed bluefin tunas.

Conclusions

This preliminary research on farming juveniles of bluefin tuna provided the following indications: No significant decline of oxygen saturation was recorded in the cage even during the maximum feeding intensity from July to October. Within the temperature range from 18° to 24°C mean daily feeding rate was approximately 5.1% of fish biomass. Stress related mortality in conjunction with injures during seining and transporting procedures may cause heavy commercial risks. Smaller juveniles seems to be much more sensitive to the stress than more advanced stages. However, on the other hand, daily growth rates of small juveniles (approx. 5 kg in weight) were found significantly higher compared to the yearly fish. The fattening of the tunas in the cage has resulted in a gradual increase of condition factor of the fish above 25 kg in weight. Further research and the industry collaboration concerning the growth performances of different size class of cultured bluefin tunas in the cages, additionally focusing in diet improvement, product quality and environmental management should be continued.

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Bluefin tuna *(Thunnus thynnus* l.) farming on the Croatian coast of Adriatic sea – present stage and future plan

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SUMMARY - In Croatia, during the last five years, bluefin tuna (BFT) farming reached around 2500 metric tons per year of harvested fish. The industry consists of 6 commercial companies using 9 lease sites. It was based on fishing BFT by purse seine and growing them in the 50 m diameter cages for a few months up to a couple of years. The BFT are fed small pelagic fish by hand, six days per week, twice per day. Farm mortality is about 3 to 5% per year, while stress related mortality during adaptation period is around 2%. Daily feed consumption was in average around 5% of biomass, within the temperature range from 18° to 24°C. Because of some poorly planed and managed BFT operations had negatively impacted natural environment. The improvement of the environmental performances in BFT farming is of the highest importance for this industry in Croatia.

Key words: Bluefin tuna, purse seine, grow-out in cages, environmental impact

Introduction

Commercial activities in fattening of captive bluefin tuna (BFT) *Thunnus thynnus* has been recently undertaken in Croatia. It has been based on fishing BFT in their natural habit of the Middle Adriatic and/or Eastern Mediterranean and fattening them in floating cages, located semi-offshore, within a rearing period that could be from a few months up to couple of years.

The first development program was established in 1996 and 39 metric tons of gutted and gilled fish were exported to Japan. The transfer of farming technology originated from southern BFT (*Thunnus maccoyii*) in Australia and investments by Croats living in Australia made enable a rapid increase of farmed BFT. The export increased from 390 tons in 1997 to 1,090 tons in 2000 according to Croatian Statistical Documents. Recently, (January, 2002) six mediums to large farms are operating at nine leased sites, with installed capacity of about 3,000 tons.
An overview of the data and experience collected over the previous five years farming caged tuna is provided as well as the danger of over fishing and the perspectives of BFT farming are discussed.

Fishing methods and techniques

Purse seine is a principal fishing gear used for fishing bluefin tuna in the Adriatic. The number of active purse seine fishing vessels increased from 19 in 1999 to 30 in 2000, showing an increased interest for tuna farming among Croatian fishermen. However, the international quota system allocated to Croatia (876 tons only) is not meeting growers' needs, and some BFT farmers are obtaining greater sized fish caught in the Mediterranean by buying portion of EU quota.
The BFT are caught predominantly from June to October when they are present in the deep waters of central Adriatic. The BFT schools are found, seined and transferred via connections from nets to towing cages. About 80 to 100 tonnes of 5 to 25 kg BFT are towed at about 1.2 knots to the farm areas. However, adult fish that are caught in the Mediterranean usually during June and first half of July are towed several weeks what involves some feeding of the fish. By means of special hook some specimens are weighted, and then, using underwater video cameras they are counted as they are transferred into 50m diameter and 20m deep cages. In this way the total biomass in the cages is estimated as to adjust feeding strategy. Most of BFT farms are placed in a relatively shallow semi-offshore marine environment of the eastern central Adriatic Sea (Fig. 1).

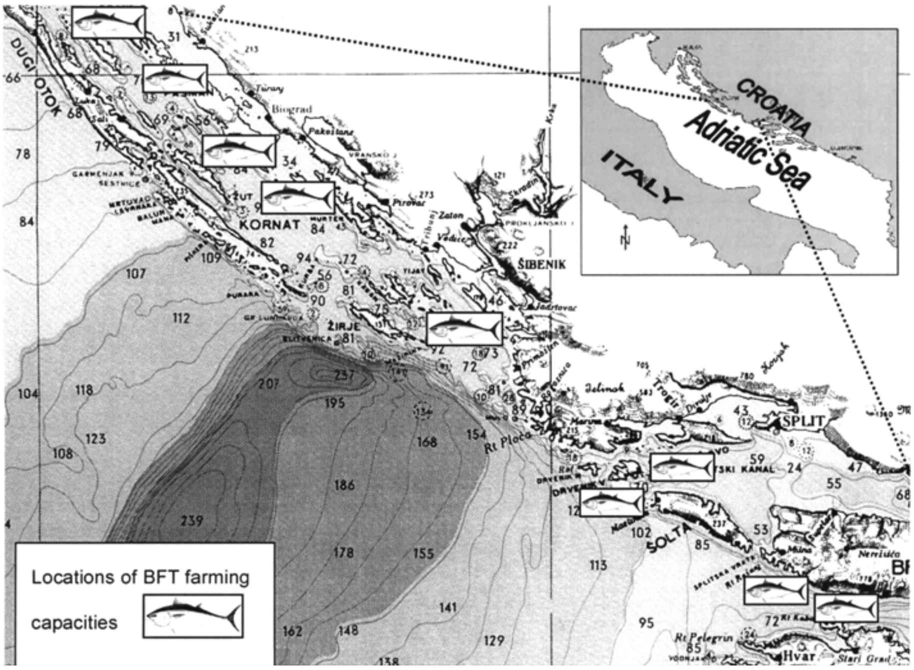

Fig. 1. Locations of bluefin tuna grow-out cages on the central part of eastern Adriatic Sea.

The size composition of fish stocked into the cages correspond to the catch size composition (Fig. 2.) Analysed catch data for the period of 1999 to 2001, showed a progressive increase of the proportion in the number of small BFT specimens.

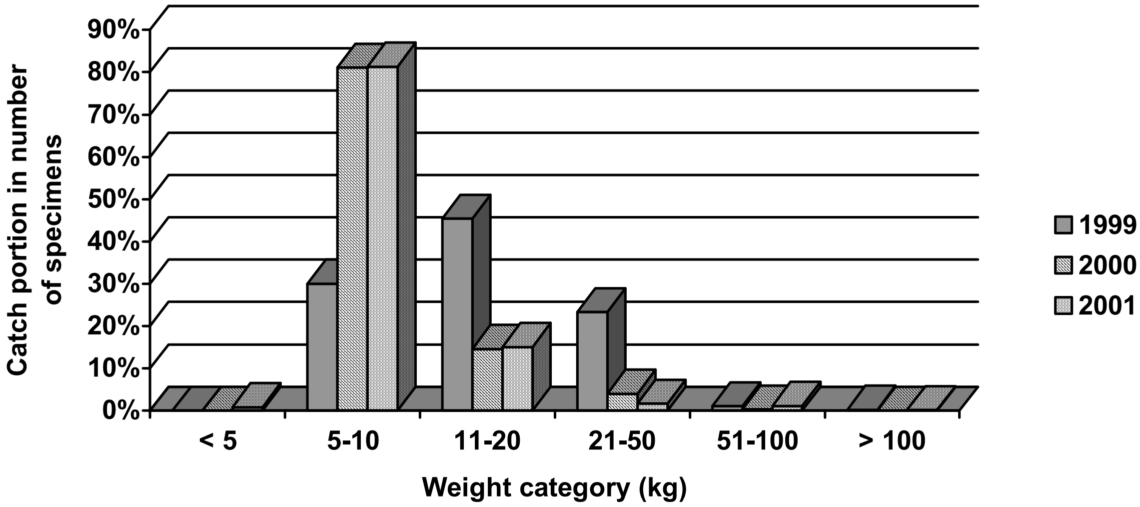

Fig. 2. The catch size composition of the bluefin tuna in the Adriatic by year (1999-2001).

Feeding of BFT in the cages

While being fattened the fish are fed with defrosted small pelagic fish such as herrings (*Clupea harengus*), pilchard (*Sardina pilchardus*) and round sardinella (*Sardinella aurita*) and also shortfin squid six days per week, twice a day, generally done by hand. In 2001 about 15,000 tonnes of baitfish were used, sourced from North Sea (i.e. herrings) and locally.

Typically, during the fattening season, bluefin tunas are overfed. The food conversion ratios are about 15 to 20 : 1. The highest feed consumption occurred at 23-25ºC, which may be up to 10% of body biomass. It may reduce twofold at 20ºC, and at 18ºC daily feed consumption is not exceeding 2 to 3% of body weight (Fig. 3). It is obvious that tuna can tolerate a wide range of temperatures. The anatomical, physiological and biochemical features makes this fish to maintain its body balance even during the wintertime when the fish were taking food at 11°C. During the adaptation period, due to the stress and injures mortality is about 2% while the farm mortalities are about 3-5%.

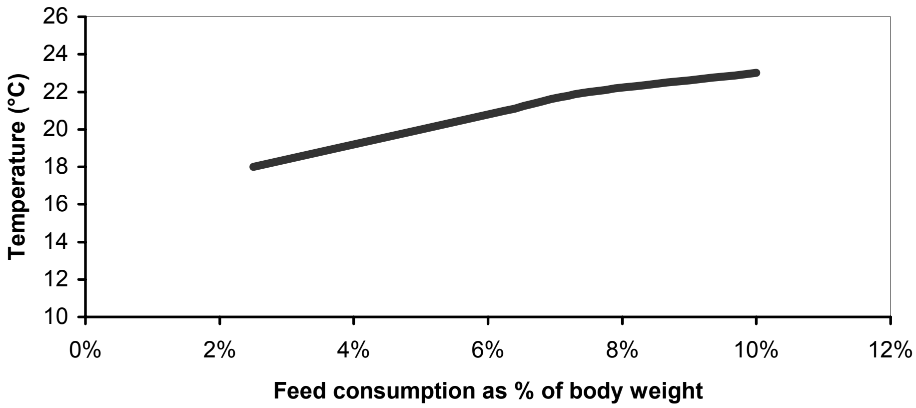

Fig. 3. Relation between sea water temperature and feed consumption of BFT in the cages.

Daily husbandry practices include diving inspections for regular checking of mortalities, moorings and integrity of nets. Cleaning of nets and floating infrastructure is usually undertaken on shore when all BFT are harvested.

Fattening of adult bluefin tunas take four to six months to get required quality and appreciable price. However, the majority of the fish caught in Adriatic are 5 to 25kg. Therefore, these tunas for value-adding purpose, usually remain in the cages for couple of years. It should be noticed that this new practice could cause difficulties in terms of the catch statistics and concurrence of catch data with trade data.

Harvesting

Harvesting strategies vary predominantly with size of the harvested fish. Typically BFT are crowded into a small area using a net where divers capture them by hand. The divers kill tunas with a spike to the brain, and then swim with them to the harvesting point with the pontoon where tunas are pulled out from the water. At the slaughtering table they are cored, a wire passed through their neural canal and left bled. The post harvests activities depend on whether they are send to Japan market by plain or sea cargo. That may include fresh, chilled or frozen product to be marketed. In any case, methods of killing more than anything else influence the quality of tuna meat. The fish that straggle during the slaughter have a less pink or reddish meat that makes the market price inferior.

Environmental issues

Rearing sites

Experiences have shown that environmental planning for BFT farming was inadequate and a more comprehensive approach to sitting these farms has to be used. Minimisation of conflicts and environmental impact requests careful planning of such farms by avoiding conflicts with other users.

Up to the present there were no any damages caused by the environment-related phenomenon in Croatia. The experience gained from tuna farming up to now particularly emphasises the importance of choosing a suitable location for tuna farming, which should comply with the following requirements:

- a suitable rearing site must not be affected by muddy waters from rivers or any other inflow of waters from the land that may clogged the gills and cause mass mortality (Lee, 1998);

- ensuring the open sea dominant influence on the dynamics of water masses having high transparency and high dissolved oxygen to sustain their physiological maintenance and continuous swimming costs.

Environmental impact of the BFT farming

While being very sensitive to environmental impact, tuna farming, such as most of the other economic activities, may have an undesirable impact on the marine environment. Environmental studies were carried out on several locations where the cages have been installed. The most common problems are caused by overfeeding and smell pollution during the summer season. Uncollected fat skim on the sea surface may be widespread much outside of concessioned zones and have disastrous effects on the beaches. This is a very important aspect of BFT management causing a negative reactions in the major tourist destinations. The next problem is associated with tuna killing and dressing the fish, and a method of removing waste (head, intestine, blood) to a safe deposit on farms when some waste may be deposited. Several hundreds of tons of waste have not been used yet, even though it can be a profitable business operation.

So far, there has been no functional, environmental monitoring of key water quality parameters and sediment chemistry at the tuna farms. However, some poorly planned and managed bluefin tuna operations have resulted in negative impacts on ecosystems and communities. Because of great public oppose of placing tuna farms into the coastal and island areas there is an urgent need to improve environmental performances of tuna farming. A comprehensive area plan for coastal zone must be worked out as soon as possible in order to allocate areas for the various aquaculture activities with particular reference to BFT farming.

Economic and social issues

When dealing with economic issues of tuna farming at this stage one must consider two approaches. One is fattening of adult stock through four to six months that gives quick revenue of capital investment and before tax profit from 15 to 40%. This profit depends on the initial cost of fish stocked in the cages (e.g. Mediterranean vs. Adriatic BFT) and the integration of the BFT project into compatible and supporting fisheries activities. However, the production costs of small tunas of different sizes through several years is still to be evaluated. Based on some data that comes from some Croatian growers that fattened young BFT through two or more years, it is clear that such production is feasible and justifiable, but economically inferior to the farming of adult fish.

It is obvious that such a grow-out operations may negatively affect on accuracy of ICCAT bluefin tuna statistical document (BTSD), and make disagreements between the final production, export records and catch reported.

Bluefin tuna farming and farming related activities generated a large number of jobs and a very significant income. Employment on the heavily depopulated Croatian islands is very important social issue, and tuna business in Croatia has enabled about 300 on farm jobs and much more through rather high multiplication factor. It is even more important as it is practiced around Croatian islands where new industries and jobs are needed badly.

BFT farming vs. traditional fisheries

Bluefin tuna farming in Croatia has an important impact on the traditional fisheries in whole. As 98% of BFT have been caught by purse seine, and the rest by long lines and hooks. Farming activities attract a number of fishermen to become active partners, either as suppliers or tuna farmers. So, the number of purse seine vessels that actively fishing bluefin tunas has increased from 19 in 1999 to 30 in 2000. This facts show a growing importance of tuna farming for fishermen that own large fishing vessels equipped with purse seines for fishing on large pelagic species. The remaining big fishing vessels are employed in fishing small pelagic fish to feed tuna in cages. This fact makes a lot of troubles to fish processing factories in obtaining necessary row material at the acceptable price level.

Further positive effect to Croatian demersal fisheries is a fact that 30 trawlers found interest to be fully integrated into tuna farming operations, either in transporting or delivering feed to the fish on farm. This will reduce fishing pressure on already over-exploited demersal fish resources.

Research programme on bluefin tuna in Croatia

Due to the increased interest in the growing of BFT, and because of necessity to continue fattening operation of majority of captured fish up to a couple of years, the growing performances of various size of BFT reared in the cages are studied. Some provisional information on growth of BFT held in cages has been reported (Katavic *et al*., 2002) in addition to the age composition of BFT catches in Adriatic (Ticina *et al.,* 2002). Morphological characteristics and feeding of wild bluefin tuna were studied earlier (Ticina, 1994). Study on growth patterns of BFT when reared in the floating cages, including conversion factors for tuna products originating from the cages, will be carried out within framework of the ICCAT Bluefin Year Program (BYP).

Perspectives

With all limitations we have learned that the BFT project is growing in Croatia, and it is commercially successful. It is clear that the major problem in expanding BFT farming is and will be a further decline in quota of eastern BFT stock. Recent analyses as presented at the 2001 SCRS Meeting in Madrid (Spain) indicate that current catch estimated at 31,935 tons or more is not sustainable. A reduction to 75% of the 1994 level was not sufficient to prevent a continuing decline in spawning biomass. A catch of 25,000 tones, or less, may be acceptable. However, it should be noted that even these quantities might be to optimistic since they assume that future recruitment continues at average level observed so far. There is a great concern about the high percentage of small individuals in catches (fish <10 kg), that will seriously reduce the long term potential yield, especially if such a stock is not used for further, at least two to three years growing period in cages. Namely, catching of small tuna make sense only if the aim is to increase tuna production in a quantity.
Various measures that has been taken by ICCAT such as minimum size tolerance in number of fish and by not allowing any tolerance with respect to age 0+ fish, weighted less than 3.2 kg. In addition to this protective measure, the prohibition of purse seine fishing in the Mediterranean from 16 July to 15 August while in the Adriatic during May, were designed to protect juveniles. However, reservations on the effect of these new measures have to be expressed. Recent abrupt increase of catches of large fish in the Mediterranean during the spawning season is likely to influence spawning stock biomass and future reduction in recruitment is expected. Massive expansion of tuna farming makes pressure on juveniles that needs to be properly evaluated and regulated under the ICCAT management strategies. It is not clear enough whether such a practice is interfering with existing conservation measures or BFT farming reduces the pressure on wild stocks. The fact is that in recent years the tuna industry stepped from the conditioning of the wild caught fish before being marketed towards growing them up to a couple of years. The practice of tuna growing affords further opportunities of the utilisation of an existing natural resource. It represent also intermediate phase from wild fisheries to fully controlled farming as has happened with other species now well established in mariculture. In any case, it is obvious that such a grow-out operations will affect the accuracy of ICCAT bluefin tuna statistical document (BTSD), and make disagreements between the export records and catch reported.
The fact is that progress in successful rearing of BFT does not contribute significantly to basic problems that are the reproduction control and captive breeding of the different life stages of the BFT. Having in mind all what was said, the closing the life cycle of the BFT in captivity is the only guaranty that sustainable BFT production will be established. This will eventually lead to decreased pressure on the natural stocks as has happened with other cultivated marine species.

Conclusive remarks

Bluefin tuna farming in Croatia has yielded an economic benefit to the fisheries industry with some 50,000,000 EUR income realised in 6 commercial companies using 9 lease sites. Improvement in environmental performances of BFT farming is needed to reduce the environmental pressure to the companies involved and to avoid further conflicts with other coastal users. It is a general opinion that zoning for tuna culture and development of an offshore technology is needed to facilitate environmental compatibility of BFT farming industry. Rapid and innovative low cost environmental impact assessment programme, as well as, an acceptable environmental monitoring based on key environmental performance indicators will be highly beneficial to the future BFT farming programme. There will be need to establish a full feeding control over tuna farming that includes the optimisation of the feeding frequency, amount of the feed and feeding techniques to be adjustable to BFT behaviour.

By minimizing waste release, dispersal and accumulation of organic matter there will be benefit to both, BFT farming economy and environmental quality. Harvesting and post harvesting methodology and techniques should be also improved as to minimise pollution in the cage area and to meet requirements of export market.

A multidisciplinary approach focused on growth, nutrition, environmental issues and full control of reproduction may lead to the sustainability of BFT farming industry and decrease of pressure on the natural stocks.

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Considerations on the development of Bluefin Tuna aquaculture

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SUMMARY – Fishing industry has undoubtely the legitimacy to take part in the process of the development of bluefin aquaculture. First because of the pre-eminent role of fishermen in bluefin tuna fishing, specially in Mediterranean sea, and also because of their capacity to adapt to fishing-based aquaculture by using new practises of fattening. A partnership is necessary with fishermen in order to reinforce what has already been achieved with fishing-based aquaculture (fattening) and also to answer to many questions of the fishing industry concerning the possible development of a more advanced form of bluefin tuna aquaculture that would be independent of fishing.

Key words : bluefin, fishery, aquaculture, fattening.

RESUME – Le secteur de la pêche dispose incontestablement d'une légitimité pour prendre part au processus de développement de l'aquaculture du thon rouge, tout d'abord en raison de la primauté des pêcheurs dans l'exploitation de cette espèce, notamment en Méditerranée, ensuite, grâce à la capacité dont ils ont su faire preuve pour évoluer vers les pratiques récentes de l'engraissement du thon rouge. Un partenariat est nécessaire avec les pêcheurs pour permettre de sécuriser les acquis actuels de ce type d'aquaculture directement tirée de la pêche (engraissement) et pour répondre aux nombreuses interrogations du secteur à l'égard du développement éventuel d'une forme plus aboutie d'aquaculture du thon rouge, indépendante de la pêche.

Mots-clés : thon rouge, pêche, aquaculture, engraissement.

The legitimate role of the fishing industry in the development of Bluefin Tuna aquaculture.

The pre-eminent role of fishermen :

The legitimate role of the fishing industry professionals in the development of bluefin tuna aquaculture is firstly the result of their presence in fishing over several decades, not to speak of the recent past, which saw the appearance in France of bluefin tuna fishing with purse seine nets at the beginning of the 60s.

Over the years, the French fleet of purse-seiners established itself in the western Mediterranean and modernised its ships by taking advantage of the constantly improving technology that enabled the conditions of capture and storage aboard to be improved[1].

Progressively, in addition to the requirements of the European market for fresh fish, the French fleet showed it was capable of meeting the stringent demands of the Japanese market ; today this market has become essential to achieve profitability for the fishing industry.

The fishermen and the fish merchants are the main actors in the bluefin tuna sector ; this position now enables them to claim a genuine pre-eminence in leading the sector in the eventuality of a move

[1] In France, bluefin tuna fishing in the Mediterranean is generally carry out with purse-seiners (20 to 45 meters long, 10 to 15 men on board) operating for about 8 months of the year, from march to november, over a total of maximum 164 days at seas. Fishing is strictly monitored by satellites and controlled through a system of licences witch sets a limit of 43 vessels authorized to catch bluefin tuna with purse-seine in Mediterranean.

toward aquaculture. In particular, it fully justifies their demand to be the main beneficiaries in terms of the access to sites set aside for aquaculture, which is the pre-condition for future development.

This legitimate role of the industry is also based on the fact that, faced with the heavy constraints of the Common Fisheries Policy (CFP) which limit the possibility of the movement of fleets and the size of the boats, and faced with the introduction of quotas by ICCAT[2], it has managed to cope and to innovate, together with the fish merchants, by moving toward an aquaculture based on the new practices of fattening bluefin tuna.

The ability of fishermen to carry through a transition from fishing to aquaculture: fattening bluefin tuna :

For approximately 5 years now, a part of the tuna captured by fishing boats have been transferred directly from the nets to boats equipped with large tanks and placed in cages ; the fish are fed for several months to allow them to grow in size and weight and, especially, to increase their fat content.

Today, the French tuna fishing boats are the main suppliers of bluefin tuna to Spanish fish merchants, who own the cages in Spain.

This change, which corresponds to a form of fishing-based aquaculture, undoubtedly constitutes a qualitative leap in terms of stock management and works in favour of a reduction in the capture of juveniles ; it also enables a better development of a resource which is still taken in its entirely from a natural stock whose limits are not really known.

It also reinforces the position of fishermen in their request, as direct witnesses to the change in the industry and with the confidence gained from their experience on the sea and empirical observations, that an independent scientific study be carried out to estimate the biomass of the bluefin tuna stock in the Mediterranean.

Since it is recognised that the influence of the environment, probably due to climate changes, may affect the abundance of the resource, we believe it is urgent to put an end to the extremely restrictive estimations that use mathematical models based only on capture data [3].

+ What about data concerning the natural mortality of bluefin tuna ocean-wide and in the Mediterranean basin not related to fishing? + the influence of factors other than fishing, such as for example the effect of alluvial deposits from the coastal regions of the Mediterranean ?

Large numbers of small tuna have recently been observed in the Mediterranean, so it may also be useful to carry out research on reproduction in fattening cages and to try to measure the potential effects of this on the level of stocks.

Conditions necessary for a partnership with the fishing industry

Reinforcing what has already been achieved in fishing-based aquaculture (fattening):

The partnership of the fishing sector would appear to have been accepted in principle when the ambition to develop bluefin tuna aquaculture aims to ensure the future of fishing in a healthy complementarily between the two activities.

In this respect, it can already be seen that present practices for fattening large tuna (80 kg to + 150 kg and more) and growing up smaller tuna (10-15 kg to 30-40 kg) are already contributing to ensure this complementarily between ‘fishing’ and ‘aquaculture’ within an operational commercial sector where the fisherman no longer appears simply as a predator but as a breeder or a farmer of the sea.

Fishermen and fish merchants have shown their capacity to develop an original “savoir-faire” **;** today, the bluefin tuna is the only species where the fishermen has managed the transition to aquaculture.

[2] International commission for the conservation of the Atlantic tunas (ICCAT).
[3] The last evaluation of bluefin tuna stock was made in 1998, the time at which the ICCAT introduced quotas.

This is undeniably a major advance which fully justifies the fact that research is contributing to the development and the consolidation of this kind of activity where the benefits in terms of jobs and local economic development can already be seen.

However, the development of this type of fishing-based aquaculture has also raised many questions, particularly as regards the crucial choice of the most appropriate sites whether from a legal (legal status of the water, management of the uses …), environmental (quality of the water, impact of the waste …), or socio-economic viewpoint (transport links with the transformation, the logistics of transport …).

A cartography of the most appropriate potential sites in the Mediterranean should be produced rapidly, together with a guide for setting up and implementing good practices for bluefin tuna aquaculture.

In order to consolidate fishing-based tuna farming, a form of aquaculture which has the advantage of already existing, the fishing industry also expects of this research that it consolidates what has already been achieved and advances our knowledge in certain areas, for example feeding tuna - its nutritional requirements are not well understood-, the prevention of illnesses or the improvement of slaughtering techniques etc.…

This progress is necessary if we are to set the present day practices of growing and fattening on the path to a responsible and sustainable aquaculture entirely respectful of the environment and which meets the demands of consumers in terms of the quality of the products, of course, but also ethical aspects such as the well-being of the animal.

Questions on making aquaculture independent from fishing :

The prospects for the development of a bluefin tuna aquaculture independent from fishing, requiring that cages be stocked with fish produced from a broodstock entirely bred in captivity, have inspired a certain scepticism within the fishing industry.

Although such a profound change is not expected to occur - in the opinion of some scientists - for another 10 or 15 years, it is important to begin now to look for the main potential risks for the future of the fishing industry.

Here, the questions are many, derived for the most part from experience acquired in the aquaculture of bass and sea bream in the Mediterranean, where the fish produced were directly in competition with fish captured in the sea.

Generally speaking with aquaculture of this kind, independent from natural production, the fisherman, unless he has the necessary capital available, can no longer be entirely responsible for his own development.

The first questions are of a socio-economic nature and obviously concern the impact of an uncontrolled aquaculture production on the bluefin tuna market and the sector as it is today : price setting, commercialisation circuits, the present-day structure of the sector and of course the place of fishermen within a 'bluefin tuna' sector which would be dramatically different and where there would clearly be a radical change in the size and the origin of the capital required. All of these elements will need to be carefully examined and accurate simulations performed.

Other questions concern the measurement of the real impact of this type of aquaculture on the environment, particularly in terms of the localisation of the cages and the stocking density : what will be the impact of the waste, of the use of chemical products, antibiotics and feed products ?

Again, these are all questions that will require answers, not only for the fishermen but also for the community as a whole.

There's no reason to believe that an aquaculture which controls the reproductive cycle of the bluefin tuna cannot also contribute to restocking the populations of wild fish : + what would be the effect of 'sea ranching' on the fisheries, on the integrity of the species and on the biodiversity of the surrounding ecosystems ? These parameters will also have to be examined carefully on the basis of the best scientific information available.

Finally, nothing can be achieved without transparency and a dialogue with the main actors : to nourish the debate and help in the decision-making it will also be necessary to carry out studies on the conditions in which the space required for this aquaculture is to be acquired, in close collaboration with the others who also use the sea along the coast (tourism, fishing, navigation etc..).

Conclusions

In France, the fishing industry has managed to develop, in a partnership with the those downstream in the sector, a form of aquaculture of the bluefin tuna dependent on fishing and based on growing and fattening ; these activities –which exist thanks to fishing- must be encouraged since :

- they guarantee the future of fishing and its jobs, while contributing to a better management of the stock ;
-they also guarantee a supply of wild fish- the veritable wealth of the Mediterranean- whose natural quality is improved by the fattening which is carried out to meet the demands of the Japanese market.

This development will not transform today's fisherman into tomorrow's aquaculture farmer ; it simply shows the capacity for diversification of the sector, the capacity to move toward a complementary activity which will still be dependant on the natural environment.

It also provides an additional justification for the industry to ask for a scientific evaluation of the biomass of the stock of bluefin tuna in the Mediterranean to be carried out independently of the fishing industry.

The development of an aquaculture independent from fishing through the control of the reproduction 'right from the egg', would still appear to be unlikely; nevertheless, if such a prospect were to be envisaged, it should be remembered that the French fishermen are, and intend to remain, the main suppliers of broodstock, and that France -which has a recognised scientific expertise in this field- must take a central role in this specialised research.

Here again, the industry has defined as a precondition that a large series of impact studies be undertaken both on the current 'bluefin tuna' sector and on the marine environment and the territories that this new kind of activity intends to appropriate.

Distribution and movements of Alantic Bluefin Tuna from electronic tagging

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SUMMARY - Since 1997, fishery independent pop-up satellite tags have been used to identify the migration paths and behavior of giant bluefin tuna released in New England and Canada. So far, all of the fish tagged in this region were in the central north Atlantic during their presumed spawning period, and 30-58 % had tags that reported from the eastern management area. These results were compared with conventional tagging studies and bluefin tagging studies in other regions.

Key Words: Atlantic Bluefin Tuna, pop-up satellite tags, migration

Since 1981, Atlantic bluefin tuna have been managed by ICCAT as two biological units separated by a management line at 45 deg. W. The biological basis of this management division is based on presumed separate and exclusive spawning grounds (in the Gulf of Mexico and the Mediterranean Sea), different ages of first reproduction, and an assumed low transfer rate (2-7% annually). Beginning in 1997, in an international collaboration between scientists and fishermen, our research group conducted electronic (satellite) tagging of spawning size class Atlantic bluefin tuna (ABFT) in New England and Canada (Lutcavage et al., 1998). The single point pop-up tags (PSAT), developed by Microwave Telemetry, Inc., and successfully tested and deployed on medium sized fish off North Carolina (Block et al., 1997) consist of a radio transmitter, environmental sensors, and a data logger that jettison from the fish after a predetermined release date. Data is relayed to orbiting satellites and distributed by Service Argos, Inc. to researchers via the Internet. The goals of our program were to determine the long-term movements, origins and behavior of the giant AFBT assemblages found on the New England shelf in summer and fall. We initially targeted adult fish comprising spawning size classes (>200 cm SFL) and programmed the majority of PSAT's to detach from the fish over their presumed spawning period (April - July).

From 1998 onwards, several independent tagging studies in the US, Canada, and the Mediterranean utilized new PSAT tags, which shortly thereafter incorporated light sensing and full geolocation capabilities. All studies soon produced surprising results, including data returns from eastern Atlantic fish (DeMetrio et al., 2001). From 1997-2001, all successfully released tags from our ABFT program reported from the central Atlantic roughly between Bermuda and the Azores. Each year, from 30-58% of tags on New England fish reported from east of the 45 ^{o} W. stock division line and none were in or near known spawning grounds in the Gulf of Mexico or Mediterranean Sea (Lutcavage et al., 1999; 2000). This raised the possibility that some ABFT spawn in warm waters of the central north Atlantic, along the edges of current boundary systems, a possibility raised by previous investigators (e.g., Mowbry, 1952; Hamre, 1963; Suzuki, 1991; Suzuki and Ishizuka, 1991:Mather et al., 1995), but deemed unlikely by others (reviewed in Bakun, 1996).

A separate tagging study on medium sized fish off North Carolina by Block and colleagues that utilized implanted archival tags and PSAT's produced similar findings. Their results from the North Carolina archival tagging (and elsewhere) provided additional information on longer-term movements and behavior (1-3.4 yrs, Block et al, 2001). In 2001, data returned from several implanted archival tags from fish captured in the Mediterranean confirmed migration of fish (tagged in the western Atlantic) to the Mediterranean and back. However, Block and colleagues suggested that while ABFT may mix extensively on feeding grounds, they believed that they exhibit spawning site fidelity (to the Gulf of Mexico or Mediterranean), and may not spawn every year. In contrast, our ongoing studies on the energetic and reproductive status of adult ABFT in the Gulf of Maine so far do not support the hypothesis that mature ABFT are not annual spawners.

Despite divergent views on some aspects of Bluefin behavior, new electronic tagging studies confirm the linkage between western Atlantic, eastern Atlantic and Mediterranean ABFT. Unfortunately, we are also left with great gaps in knowledge regarding their long term mixing rates, reproductive habits, Atlantic wide movements, and natal origins. In 2000, with others we established an international scientific steering committee to plan a series of cruises for biological sampling of ABFT in the central north Atlantic. Specific recommendations call for a multi-year study, with international participation by oceanographic research vessels, long-line sampling vessels, and full financial support for oceanographic data collection and analysis of samples. Future goals of international ABFT research teams should include expansion of electronic and conventional tagging to all areas of the north Atlantic, and elucidation of crucial components of the Bluefin Tuna's life history.

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Electrofishing techniques: slaughtering and quality analysis for BFT

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SUMMARY - Fresh water electrofishing techniques have been adapted in order to be used in sea water for electrofishing of some fish species such as the Blue Fin Tuna (BFT). The effect of the current flow from the harpoon through the spine may damage it, decreasing the final quality of the obtained meat. This paper describes a project that approaches the development of a non-conventional electronic power converter that allows to study different waveforms and its effect on the quality of the product, as well as an automatic system of visual inspection for the objective evaluation of shape and the quality of tuna meat.

Key words: Tuna Farming, Electronic systems, Multivariate quality control, Power Systems, Computer vision, Food processing

Introduction

Nowadays, shotgun slaughtering is the most widely used technique, but this method has two main drawbacks: On the one hand, the slaughtering is very stressful and on the other hand, scuba divers are required to stay inside the cage in order to “strain” the nets, working under risky and unsafe conditions. In order to avoid these annoyances, the managers of the G. Méndez España S.L. enterprise, adopted the electrofishing at their farms. This technique has been widely used for electrofishing in fresh water (Reynolds, 1996), which little conductivity facilitates the application of high voltage fields through the body of the fish.

Problems associated with electrofishing

The main problem that limited its use in sea water (high conductivity 50 mS · cm-1) was overcome by means of an underwater harpooning system connected to an electronic power converter installed onboard (registered at the owned EU patent nº 005001131.8).

The main goal of this project is concerned about improving the knowledge of the nature and the problematic associated to the use of the electro-slaughtering technique used at this time, by the development of a specially designed equipment for the research in this field, to carry out a scientific analysis of data to be collected.

Equipment Development

Electronic power converter

The research has involved the development of three different stages for the electronic system (Fig. 1):

1. Electronic Power Converter
2. Arbitrary Signal Generator
3. Security System

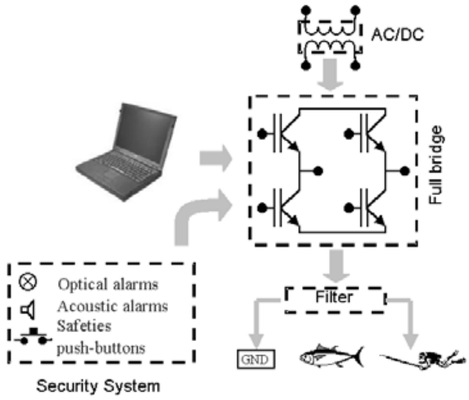

Fig.1. Electronic power converter.

Visual inspection system

In this respect is being developed an Automatic System of Visual Inspection with which is tried to realize a study of the quality of the meat of tuna, acting as feedback inside the process of analysis of the ideal waveforms to obtain the sacrifice of the tuna with the best qualities regarding of meat color and non break of dorsal thorn (bone); both determinant parameters in the evaluation of the BFT.

The above mentioned system of inspection will use samples of meat of tuna proceeding from the central zone and distal zone just before the tail (Fig. 2).

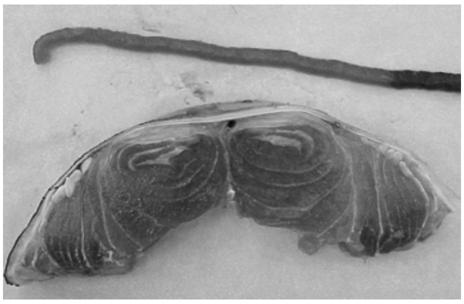

Fig. 2. Tuna meat samples.

These samples are taken in the initial stage of the line factory processing of the tuna. To the images obtained from these samples were applying Artificial Vision techniques, based on the analysis and modeling of color and texture, together with the utilization of other technologies of analysis.

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Reproductive status of Bluefin Tuna during migration to Mediterranean spawning grounds through the Straits of Gibraltar

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SUMMARY - The sexual maturation stage of adult bluefin tuna caught at the Strait of Gibraltar (Barbate, southern Spain) during migration to Mediterranean spawning grounds was assessed by histological and stereological studies of the gonads. The gonad weight and gonadosomatic indices (GSI) of these migrant tuna were several times lower than those found in tuna from Mediterranean spawning grounds (Balearic Islands). The testes of migrant male tuna contained gametes at all stages of spermatogenesis; there were no marked histological differences between them and male spawners from Baleares, though the gonad size and GSI were significantly higher in the latter. A small proportion of female bluefin tuna caught off Barbate (~ 8%) were immature, whereas most of them (~ 92%) were non-spawning mature. The ovaries of spawning tuna from the Balearic Islands contained 5-fold more vitellogenic oocytes than did tuna from Barbate. The average fecundity per spawning estimated from stereological quantification of migratory-nucleus oocytes in the specimens collected from Balearic waters was some 13 million eggs (93 oocytes per g of body weight), and the inter-spawning interval in this area was calculated to be 1.2 days. In specimens from Barbate a fecundity per spawning of around 13 million eggs (relative batch fecundity: 96 g^{-1}) was estimated using counts of late vitellogenic oocytes. These estimates are close to those calculated for other tuna species.

Key words: Reproduction, sexual maturation, bluefin tuna, *Thunnus thynnus*, gonads, stereology.

Introduction

Thunnus thynnus (the northern bluefin tuna) and the closely related *Thunnus maccoyii* (the southern bluefin tuna) are unique among the tuna species in that they live mainly in cold waters and move into warmer waters to spawn (Lee, 1998). The eastern stock of the Atlantic northern bluefin tuna (BFT) spawns in the Mediterranean Sea, the two main spawning grounds being located around the Balearic Islands and south of the Tyrrhenian Sea, between Sicily and Sardinia (Dicenta, 1977). Therefore, every spring BFT from the eastern stock migrate from different locations in the Atlantic to the Mediterranean to spawn in waters where conditions are optimal for the offspring survival. Migrant BFT have then to pass through the Strait of Gibraltar, where fishermen can catch them by trap.

A number of reasons exist to recommend the development of technologies specific to the culture of *Thunnus thynnus*. One such reason is the high fishing pressure to which this species is being subjected in recent years, which has provoked a worrying reduction in the biomass of natural populations (Sissenwine *et al.*, 1998; Forés *et al.*, 1999). To support the sustainability of the resource an appropriate management of tuna fisheries should be accompanied by the development of effective aquaculture technologies for stock enhancement. The high commercial value and impressive growth rate of the bluefin and other large tunas point to these species as ideal candidates for a profitable and ubiquitous aquaculture industry.

Prior to de development of the Atlantic bluefin tuna aquaculture technology it is essential to understand the reproductive biology of the species, and more particularly to know the reproductive potential of the stock that can be used as broodfish. In this paper we aim to contribute to the understanding of the reproductive biology of BFT from the coasts of southern and eastern Spain with a view to improving the knowledge on the biology of the species thus establishing the basis for further development of the aquaculture of *Thunnus thynnus*.

Materials and methods

Animals

Adult pre-spawning BFT were caught by trap off Barbate de Franco (Cádiz, southern Spain) from late April to early June, 1999, 2000 and 2001. Spawners were fished by purse seine around the

Balearic Islands in June. At the moment of sampling, the total body weight, gonad weight and gonad volume of each specimen were recorded, and the gonadosomatic index calculated according to the equation: GSI (%) = (gonad weight / total body weight) × 100.

Histology (light and electron microscopy)

Samples of gonad tissue were fixed for 48-96 h in 4 % formaldehyde in phosphate buffer, 0.1 M, pH 7.2. After dehydration in ascending concentrations of ethyl alcohol and clearing in xylene, they were embedded in paraffin wax. 6-μm sections were stained with haematoxylin-eosin for examination on the light microscope (Medina *et al.*, 2002).

Small tissue samples (~ 1 mm^3) were fixed for 3-4 h in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer (pH 7.2). Following two 30-min washes in cacodylate buffer for 1 h, they were postfixed for 1h at 4ºC in cacodylate-buffered osmium tetroxide, rinsed several times in the buffer, dehydrated in acetones, and embedded in Spurr's resin (Abascal *et al.*, 2002). Thin sections (~ 80 nm thick) were picked up on copper grids, doubly stained with uranyl acetate and lead citrate and examined in a Jeol JEM 1200 EX electron microscope.

Stereology

For estimation of the number of the different oocyte types contained in the gonads, the stereological method of Weibel and Gómez (1962) was applied to paraffin sections of the ovaries. N_V (number of oocytes per unit volume), was calculated for each oocyte developmental stage according to the formula (Weibel and Gómez, 1962; Weibel *et al.*, 1966):

$$N_V = \frac{K}{\beta} \frac{N_A^{3/2}}{V_V^{1/2}},$$

where β and *K* are coefficients related to shape and size distribution, respectively (Weibel, 1969; Williams, 1977). In our samples, the estimated value of β ranged from 1.4 to 1.52, whereas *K* varied between 1.01 and 1.19 (Medina *et al.*, 2002). N_A is the number of transections of oocytes per unit section area, and was calculated as the number of oocyte profiles lying within the stereological test system divided by the test area. V_V (the volume fraction occupied by oocytes of a given category) was calculated by the superimposition on to the micrographs of a test system (Weibel and Gómez, 1962; Weibel *et al.*, 1966; Weibel, 1969; Williams, 1977) consisting of a 14 × 22 cm square lattice in which the unit area was 1 cm^2, representing an actual area of 2,500 μm^2 in the histological samples.

Results and discussion

Males

The histological study did not reveal significant qualitative differences between males from the Strait of Gibraltar (Barbate) and spawning grounds in the Mediterranean (Balearic Islands). An intense spermatogenetic activity was observed in both cases, with large masses of spermatozoa accumulating in the seminiferous tubules. Nevertheless, as seen in Table 1, there was an evident quantitative difference in the gonad development, with an average GSI in male tuna from the Balearic Islands that was more than 4-fold higher than the GSI in Barbate specimens. Hence, the spawners produced a volume of sperm several times larger than the BFT captured during migration to the spawning grounds. Consequently, the specimens sampled in Barbate were not usually fluent and milt could not be obtained by conventional methods. When suspended in seawater, sperm removed from the testes had a low motility rate (around 30%) in comparison with that observed in spermatozoa obtained by cannulation from tuna reared in cages in Mazarrón (Murcia), which showed over 70% motility rate (pers. observ.). These observations reveal a marked spatio-temporal difference in the degree of male gonad maturation between the Strait of Gibraltar and the Mediterranean spawning grounds, and suggest a relatively quick development of the testis in the reproductive season.

Table 1. A comparison of the gonadosomatic indices (GSI) between male BFT from Barbate and the Balearic Islands. The values are expressed as mean ± SD; N is the number of individuals examined.

Sampling locations	Years	GSI	N	Total GSI	N
Barbate	1999	1.2	17	1.1	45
	2000	1.3	10		
	2001	1.0	18		
Balearic Islands	1999	5.6	5	4.7	35
	2000	3.6	10		
	2001	5.0	20		

The mature sperm (Fig. 1) of *Thunnus thynnus* have the typical ultrastructure of the perciform type II spermatozoon, which is characterised by the asymmetrical insertion of the flagellum and the centriolar complex located outside of the nuclear fossa (Mattei, 1970). These and other morphological features, such as a) the shallow nuclear fossa forming a groove over the proximal segment of the axoneme, b) centrioles located perpendicularly to each other, c) deep and narrow cytoplasmic canal, and d) flagellum lacking lateral fins, determine a consistent homogeneity in the sperm ultrastructure within the family Scombridae (Mattei, 1991; Hara and Okiyama, 1998; Abascal *et al.*, 2002).

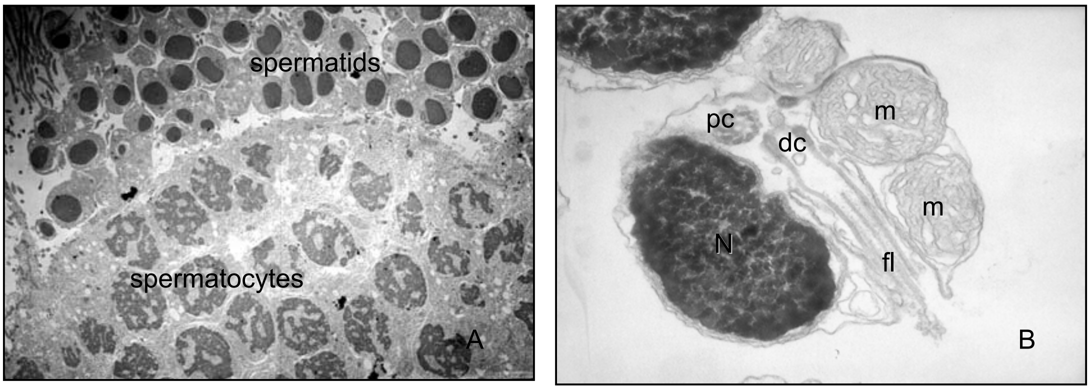

Fig. 1. Transmission electron micrographs of tuna testes from Barbate. (A) partial view of a tubule showing spermatocytes and spermatids; (B) sagittal section of spermatozoon; dc: distal centriole, fl: flagellum, m: mitochondria, N: nucleus, pc: proximal centriole.

Females

Histology

Most female BFT caught off Barbate (92%) had maturing ovaries in which the most advanced group of oocytes (MAGOs) were vitellogenic (stage 3) oocytes (averaging 380 micrometers in diameter) that contained yolk globules and lipid droplets. Only 8% were immature: all the oocytes were previtellogenic oocytes at the perinucleolar stage (stage1) or at lipid stage (stage 2). The mean GSI in specimens from the Strait of Gibraltar was 1.23. In contrast, all BFT from the Balearic Islands had spawning ovaries in which the MAGOs were migratory-nucleus oocytes (stage 4) measuring over 500 micrometers in diameter, the GSI being as high as 4.19. In 83% of the histological samples examined postovulatory follicles were found, which indicates an inter-spawning interval of 1.2 days according to the method of Hunter and Macewicz (1985).

BFT fished off Barbate in May 2000 were reared in seacages for 6 weeks and sampled in spawning time. After this short confinement, vitellogenic oocyte resorption had ocurred concomitant with a significant decline of the GSI to 0.99. The experience was repeated in 2001, but in this case GnRHa implants of the sustained-release delivery system type (see review by Zohar and Mylonas, 2001) were injected to many of these fish. The histological structure of the gonad and the GSI (2.27) were now close to or even higher than that observed in wild tuna from Barbate. These results appear to confirm the applicability of GnRHa implant treatments in the BFT aquaculture, though they should be taken with caution as the number of experimental specimens used was low and the maturation pattern of the species could vary between years.

The cytoplasm of previtellogenic oocytes at the perinucleolar stage is poor in organelles, microvilli are short and scarce, and the vitelline envelope is thin. No sign of endocytosis is evident (Fig. 2A). At the lipid stage there are no significant ultrastructural changes except that lipid droplets become apparent in the cytoplasm. Vitellogenic oocytes (Fig. 2B) show a dense brush border embedded in the multilayered vitelline envelope. The well-developed microvilli as well as the presence of abundant coated pits and vesicles in the cortical cytoplasm of vitellogenic oocytes suggest a selective uptake of extracellular material, which, as shown by Susca *et al.* (2001), most probably consists of vitellogenin. The inner ooplasm of vitellogenic oocytes displays lipid droplets and yolk granules whose size increases gradually towards the centre of the oocyte. In migratory-nucleus (stage 4) oocytes the lipid droplets coalesce to form the single globule, the nucleus move to the oocyte animal pole, and hydration begins. The absence of fully hydrated oocytes in our samples of spawning ovaries must be due to the time of the catch and to the fast hydration process, which is known to occur very shortly before spawning (Farley and Davis, 1998).

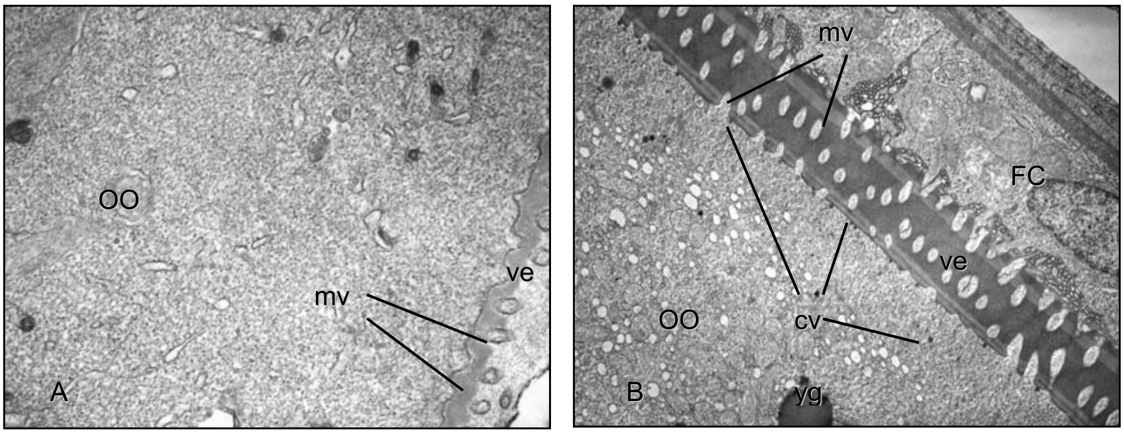

Fig. 2. Transmission electron micrographs of BFT oocytes. (A) partial view of a previtellogenic oocyte (stage 1); (B) cortical region of vitellogenic oocyte (stage 3); cv: coated pits and vesicles, FC: follicle cells, mv: microvilli, OO: oocyte, ve: vitelline envelope, yg: yolk granule.

Stereology

The application of the stereological method to histological sections allowed us to estimate for each oocyte category the volume density, numerical density, total no. of oocytes per individual, and no. of oocytes per gram of body weight. These values did not differ significantly in BFT from Barbate either throughout the migratory season within a year or between years 1999 and 2000, but significant differences did become evident when these values were compared with those from BFT caught off the Balearic Islands (Table 2). For instance, though the estimated number of previtellogenic oocytes (stages 1 and 2) was similar in both cases, BFT from the Balearic fishing area produced five-fold more vitellogenic oocytes than did tuna sampled off Barbate. Therefore, the considerable growth undergone by BFT ovaries in an apparently short period of time (between the Strait of Gibraltar and Balearic samplings) is largely accounted for by an active production of yolky (vitellogenic and migratory-nucleus) oocytes from the previtellogenic oocyte stock, which appears to remain constant throughout the reproductive cycle (Medina *et al.*, 2002). As commented in the histological description, migratory-nucleus oocytes were present only in the Balearic specimens.

From the stereological data presented in Table 2 the average fecundity per spawning estimated from counts of migratory-nucleus oocytes is around 13 million eggs (equivalent to some 90 eggs per gram of total body weight).

Table 2. Comparison of stereological data (V_v and N_v not shown) from tuna caught off Barbate and around the Balearic Islands during the reproductive season in 1999 and 2000. The values are expressed as mean ± SD; *n* is the number of individuals examined in each case (modified from Medina *et al.*, 2002).

	Barbate	Balearic Islands
Stage 1 oocytes		
No. per individual ($\times 10^6$)	1080 (*n* = 59)	970 (*n* = 24)
No. per g of BW (g^{-1})	6410 (*n* = 37)	6470 (*n* = 24)
Stage 2 oocytes		
No. per individual ($\times 10^6$)	101 (*n* = 59)	127 (*n* = 24)
No. per g of BW (g^{-1})	578 (*n* = 37)	821 (*n* = 24)
Stage 3 oocytes		
No. per individual ($\times 10^6$)	15 (*n* = 59)	66 (*n* = 24)
No. per g of BW (g^{-1})	96 (*n* = 37)	442 (*n* = 24)
Stage 4 oocytes		
No. per individual ($\times 10^6$)	0 (*n* = 59)	13 (*n* = 24)
No. per g of BW (g^{-1})	0 (*n* = 37)	93 (*n* = 24)

Conclusions

BFT caught in the Strait of Gibraltar during their migration towards spawning grounds in the Mediterranean are at a relatively early stage of sexual maturation. Fully mature gametes cannot be obtained from these fish, whereby artificial fertilisation is probably unviable or at least difficult.

The BFT is a multiple, highly fecund spawner in which the average fecundity per spawning around the Balearic Islands was estimated in ~ 13 million eggs (~ 90 eggs g^{-1} total weight). The inter-spawning interval has been estimated in about 1.2 days.

BFT reared for several weeks in seacages off Barbate undergo resorption of vitellogenic oocytes. The use of GnRHa implants could be of help to stimulate sexual maturation, especially in adverse environmental conditions.

Acknowledgements

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Age estimation of juvenile Bluefin Tuna, *Thunnus thynnus*, from the Mediterranean sea

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SUMMARY - The sagittae of juvenile bluefin tuna captured in the Mediterranean Sea were prepared using sectioning and immersion in Methylbenzoat reinst. Counts of presumed daily growth increments were performed. Estimated ages ranged from 15 to 162 days of fish ranging in size from 8.5 to 55.5 cm fork length. A growth rate of 4.5 cm.day^{-1} for the range of size of the fish was estimated. Most of the back-calculated spawning days were found in June, July and August.

Key words: age, growth, otoliths, bluefin tuna, Mediterranean

Introduction

Skeletal hard parts such as fin spines, otoliths and vertebrae have been used to study age and growth of Atlantic bluefin tuna (Compeán-Jimenez & Bard, 1983; Lee et al., 1983; Cort, 1991) however, information on age estimates of the early growth stages of this fish is limited (Brothers et al., 1983; Radtke & Morales, 1989; Megalofonou and De Metrio, 2000). In this study, we used the otoliths to estimate the age and growth of juvenile bluefin tuna sampled from the Mediterranean Sea and test if estimated spawning dates are consistent with the observed times of gonad maturation and occurrence of bluefin tuna larvae in the plankton.

Materials and methods

Juveniles of bluefin tuna were collected from purse seine boats fishing small pelagic fishes in the Aegean, Adriatic, Ionian and Tyrrhenian Seas. Fork length (FL) measurements were taken to the nearest millimeter and weight (RW) to the nearest gram. Place and date of capture were also recorded. Sagittae were removed from a total of 106 specimens, weighed to the nearest µg and measured for length and width to the nearest µm. Then, they were prepared for age reading following two methods: a) observation after sectioning and b) observation after immersion in Methylbenzoat reinst ($C_8H_8O_2$, Merck) for three days. Counts of presumed daily growth increments performed at 250 to 630 magnification were used to estimate age and growth. Otolith ages were corrected by adding 4 days to total counts (Brothers et al., 1983). This correction implies that the first counted increment was formed 4 days after spawning or fertilization. The birthday of each individual was back-calculated from the date of capture and the estimated age.

Results and discussion

Bluefin tuna ranged from 8,5 to 55,5 cm in fork length and weighed from 7,5 to 3000 g. Their sagittae were small, quite complex calcified structures with elongated form. Summary statistics of sagittae weight, length and width are shown in Table 1. Preliminary examination under the light microscope revealed the optically dense core near the sulcus, surrounded by fine, apparently daily, increments. Immersion in methyl benzoate or sectioning enhanced the light microscopy images of sagittae. The age of individual fish was determined by counting the increments and assuming that each corresponded to a day. The estimated ages were from 19 to 164 days. The average growth rate for each individual, calculated from length at estimated age was 4,8 ± 1,1 mm per day and the average growth rate, calculated from weight at estimated age was 17,3 ± 8,4 g per day.

Table 1. Summary statistics of sagittae measurements.

		N	Mean	S.D.	Min	Max
Left Otolith	Length (mm)	49	6,11	1,74	1,74	8,08
	Width (mm)	51	2,05	0,36	1,11	2,56
	Weight (mg)	50	6,96	3,44	0,10	13,50
Right Otolith	Length (mm)	49	6,42	1,47	1,86	8,08
	Width (mm)	52	2,02	0,28	1,09	2,34
	Weight (mg)	54	8,56	10,15	0,10	78,00
All	Length (mm)	98	6,27	1,61	1,74	8,08
	Width (mm)	103	2,03	0,32	1,09	2,56
	Weight (mg)	104	7,79	7,70	1,10	78,00

The relationship between estimated ages and length of fish was studied. A sigmoid regression model of age versus fork length (Fig.1A) yielded the best fit (FL=exp(4,3-46,3/Age) r^2=0,86).

The estimated spawning dates indicated that most of the specimens were born during June, July and August (Fig. 1B). These dates coincide with increased gonadal activity in adult bluefin tuna (EU project 97/029) and the appearance of bluefin larvae in the Mediterranean Sea (Piccinetti & Piccinetti Manfrin, 1993).

A B

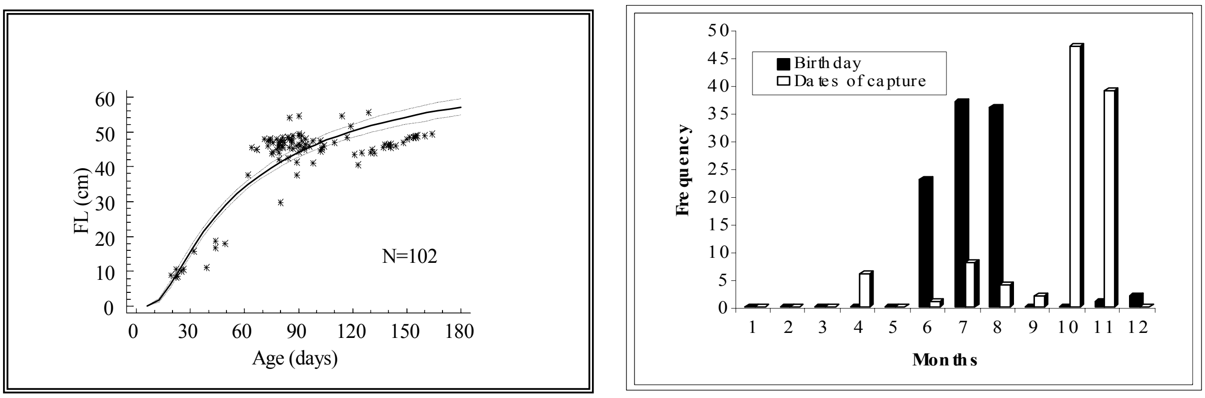

Fig.1. A. Relationship between estimated ages (days) and fork length (FL) of bluefin tuna.
B. Dates of capture and back calculated spawning dates.

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Preliminary study for DOTT symposium Bluefin Tuna (BFT) impacts on local development a socio-economic approach

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SUMMARY : the study presents BFT fattening socio-economical impacts on Mediterranean major BFT fishing fleet and the major fattening region. Impacts on fishermen are mainly: BFT price increase, modernisation, concentration of the fleet and integration of BFT fatteners. Positive impacts on Murcia region are observed on small scale fisheries, exportation and regional economy while impacts on tourism, coastal management and environment are detailed. Is BFT aquaculture complete cycle production a future sustainable activity? In the context of world competition the question is even more acute as BFT fattening seems to pass from a pioneer profitable phases to a less profitable one with increasing risks. Common priorities of socio-professionals and regional officers are presented.

Keywords: preliminary study, integrated coastal zone management, research priorities

RESUME : L'étude d'aide à la décision présente quelques élements de précision sur les impacts socio-economiques du développement rapide de l'embouche de Thon Rouge sur la principale flotte thonière et sur la première région d'embouche de Méditerranée. La modernisation et la concentration de la flotte ainsi que l'intégration des capitaux espagnols dans les flotilles sont les principales conséquences de ce développement. Les impacts économiques sur la région de Murcia sont nombreux et ceux sur l'environnement ou le tourisme, sont présentés. Est-ce qu'une production aquacole de Thon rouge est économiquement durable à l'avenir? Dans un contexte de compétition internationale exacerbée, la question est très prégnante d'autant plus que la filière engraissement de Thon Rouge semble passer d'une phase pionière lucrative à une phase plus incertaine où les risques augmentent. Les priorités de recherche communes aux socio-professionnels sont présentées.

Mots clés : étude préliminaire, développement intégré des zones côtières, recherches prioritaires

Introduction and objectives

This preliminary study is part of a DOTT seminar as a support tool for decision making. Its objective is to give some concrete socio-economics indicators and involve, from the beginning, representatives and organisations that will not be able to express themselves in the seminar. The aim is also to present some impacts of Blue Fin Tuna (BFT) fattening in Mediterranean context and synthesis of opinion of various stakeholders of the coastal area concerned by the results of a potential ambitious Bluefin Tuna (BFT) European Research Program: i.e. fishermen and producers organisations, environmentalists and consumers, regional policy makers (environment, tourism, fisheries offices).
The study also presents some recommendations concerning sustainable fishery and aquaculture in a context of integrated and harmonious development of coastal areas.

Methodology

The study is built around the comparison of two exemplary sites in France and Spain :
(i) Murcia region (Spain) is a pioneer territory in Mediterranean Sea where BFT fattening exists for 5 years. The main interests consist of measuring the impacts on local economy, employment and environment, new interactions (tourism, small fisheries), new activities and future perspectives;
(ii) Languedoc-Roussillon Region (France): This region has the largest tuna fleet in the Mediterranean Sea but does not produce BFT in cages. The main interest is to present perspectives of interactions between fattening and BFT fisheries in a context of Tuna fisheries restrictive stocks.

In each of the two sites, CEASM collected and summarised existing data concerning the impacts of BFT fattening on the local economy (PIB) and employment, the coastal environment, the interactions with other users. In each of the two sites, CEASM collected and summarised the points of view of various structures and regional officers through the following questions: What are the indirect impacts of BFT and the perspectives of development for the fattening production channel? What are the perspectives of development for a sustainable BFT aquaculture in the Mediterranean Sea? What are the breaks, the needs and the priorities for a future BFT research program?

Languedoc Roussillon BFT Production

With 32 boats permanently specialised for Tuna fishing. BFT fisheries production represents 21% of total value for the fishery production field. BFT French fishing fleet major production is centred on the Balearic Islands (70-75% of regional BFT production) during the months of May-July, targeting 117-126 Kg/ fish on average. The French fleet is seeking as well smaller BFT, in the Golf of Lion, during the early season (March-May : 28 kg/fish) and the late season (August- October : 14 Kg/fish).

BFT catches from Balearic Islands (estimation of 4800 kg in 2001) are transferred alive from fishing nets to cages and pulled to the Murcia region, where they are fattened in a short-term cycle (3-6 month). It is assumed from the data and interviews that between 1997 and 2001, the quantity of catches transferred alive to cages increased from 20 % to almost 95 % of Balearic captures. Nowadays, BFT Spanish fatteners are also seeking BFT resources in southern Mediterranean countries (Malta, Italy, Tunisia, Libya) (15-50 days trip) (CEPRALMAR, 1999).

Impacts of BFT fattening on French fishing fleet

No data could be collected on BFT fattening effects on regional PIB and fishing companies accounts since most of the data are kept private and secret.

Prices increase for BFT primary product: the prices for “BFT livestock” are 50 to 100% higher than the “dead tuna” since fishermen have to spend longer time between fishing runs. In addition prices increased (+30% average) during the last 5 years and 65% for live tuna increasing from 4 to 6 euros/kg.

Modernisation of French fleet and strategic changes : the rapid rise of BFT fattening demand, and the increase in prices supported technical, and comfort improvements onboard. Since BFT is valorised as « live BFT », the target for producers is a better access to BFT resources and is less focussed on storing techniques (freezing units, etc). The consequences of BFT fattening on French fleet are :
(i) Specialisation on BFT : before 1995, some of the fleet was partly fishing small pelagic fish. During the period 1995-2000, specialised BFT fleet increased from 21 to 32 boats in the region ;
(ii) Increase detection efficiency (sonar, planes) : the fleet bought 2 new sonars/year in the years 1990-1993 and 6 to 7 new ones/year in the last 5 years. For BFT school detection in the Balearic islands, fatteners rented 2 planes in 1993 and this increased to 22 planes in 2000 to support the fishermen, introducing aerial traffic problems. In 2001, planes were forbidden in July ;
(iii) Increase speed (size) : the priority is not any longer to store the fishes (since they are transferred to cages alive) but to reach BFT resources as fast as possible, thus, increasing speed and length of the boats. In 1992 all the boats were below 27 m length, in 2001 the newest boats (less than 10 years old) have an average size of 35 m;
(iv) Diminution of investment on quality facilities : new freezing equipments on fishing boats are less and less important since alive tuna are purchased. In the first years of fattening, Spanish fish processing industry rented “cargo: swimming pool boats” from Atlantic side (Russia, Galicia, Scotland) to improve storage capacity for dead fish. In 1997, they bought almost 20 carriers ;
(v) Changing capture techniques : increase in number of skiffs, platforms to lift the skiffs onboard, increase net sizes in order to catch bigger schools and store the fish better (waiting for the tugboat and the transfer to the cage). Some producers start to imagine inflated cages (stored onboard) that would allow to stock fishes allowing pursuing new schools instead of waiting for the tugboats ;
(vi) Improving security, communication, comfort : desalinisation systems, security systems, etc…) ;

Concentration of the fleet : The French fleet is becoming more and more concentrated to compete with other countries and fishermen. In France, 3 major fishermen and fish processing companies are leading the sector (3 to 5 BFT fishing boats each). Recently individual boat owners developed a shared company in order to face competition and allowing fatteners to participate in fishermen's boat capital;

Diminution of pressure on BFT resources : Catching *"live fish"* could induce reduction on fishing effort (reduction of fishing time) since the fishing boats have to wait for arrival of tugboats (often hours) in order to proceed to after transfer to transport cages. Thus they are not seeking new schools. Better studies have to be conducted to determine the precise impact on captures;

Impacts of BFT fattening on French fish processing companies

5 companies out of 23 processing fish companies are specialised in BFT (70% of their incomes is due to BFT). They have contracts with the major French supermarket chains (Carrefour and Auchan) to secure commercialisation of small fresh tuna[4]. All BFT products now reaching Sète harbour are directed to French supermarket chains. The quick Spanish move to fattening "live tunas" induced some negative impacts on local companies and public investments.
(i) Lack of use of transformation plants : the Stock of BFT frozen in Sète facilities is almost at zero;
(ii) 1/5 Reduction of local manpower capacity : qualified manpower used by the processing companies had to be disbanded. For some of the companies that developed partnership with Spanish fatteners, some French qualified workers are sent to Spain in order to form Spanish professionals and work at the Spanish partner site during the high season.

Impacts of Fattening on Murcia Region

In 2000, the BFT annual turnover of 270 millions euros contributes to more than 2,2 % of the regional GDP. Murcia is the first BFT fattening region in the world and the pioneer region in Mediterranean Sea with 6 farms owned by 4 major fish processing companies (Fuentes, Albaladejo, Mendes, Caladeros del Mediterraneo). While fisheries production is stabilising at 10 million Euros in value, the BFT aquaculture passed from 77 tons in 1996 up to 3660 tons in 2000 (Fig. 1) representing 8 times the value of all regional fisheries (80 millions). This evolution is due to their very rapid adaptation to Japanese market, and development of strong partnerships with Japanese companies.

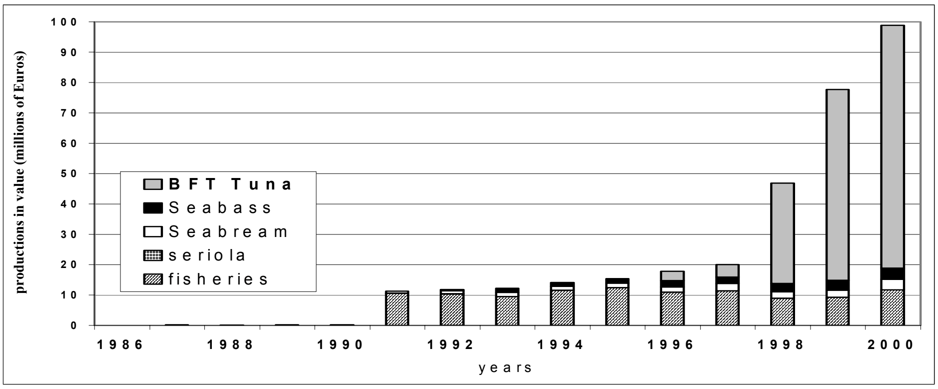

Figure 1: Evolution of Aquaculture/Fisheries production in Murcia region (Services of Fisheries of Murcia, 1999-2000)

Between 1994 and 1999, public investments represented 53 % of the global aquaculture projects (4 millions Euros). With 158 millions Euros of exportations, regional benefits are clear (further detailed study is needed on indirect economical impacts) (CES, 1999 and CES, 2000).

[4] the biggest fresh tuna market for consumption in Europe is the french market

Impact on Murcia commercial exchange

BFT exports are ranked 6th among all other exports from the region and represent now more than 94% of the global marine exports (Fig. 2).

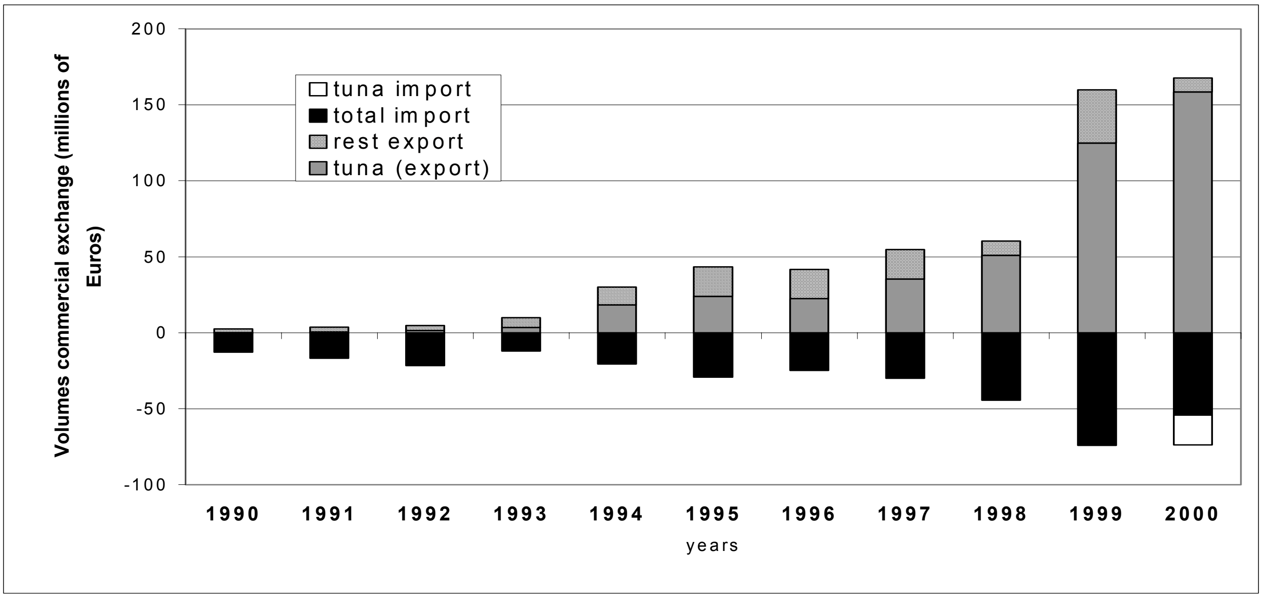

Figure 2: Murcia regional marine products commercial exchange (source: INFO, 2000)

Tuna is the first ranking product exported to Japan with 158 millions Euros in 2000 (94% of all tuna export, 76% of all export to japan) after plastics (12,8%) and wood furniture's (2,3%). Due to BFT fattening (no BFT fishing fleet in the region), Murcia is now the 4th region of Spain for exportation to Japan and the second for tuna exportation. After Asia (Japan) representing 94% of total BFT export, Europe (4%) and USA (2%) are the main exporting regions. France is the country with the highest commercial exchange after Japan (INFO, 2000; INFO 2002).

Murcia fish-processing industry developed various transformation plants, explaining the importation of Tuna from various countries in Europe and Central America. New markets in USA and Europe are also developing in the last years. In 2001, tuna exportations to Europe increased by 50%, to America by 76% and to Asia by 3 %.

BFTexperience valorise strongly Murcia region capacity for exportation and commercial reactivity. Nowadays, Murcia uses Japan experience as a marketing argument (excellence, seriousness) for other countries, especially in Asia. Murcia region is thinking of a marketing campaign in Japan for Murcia agro-products supported by the BFT experience.
In addition, the rapid adaptation of BFT fatteners to Japanese market, it's leading ranking place in the world is a strong example for the younger population in the Murcia region.

Impacts on MURCIA infrastructures (harbours, airports)

Impact on local harbours is important since fatteners are using large amount of supply boats (maintenance, taxes, diesel, etc…), however the present study did not allow this to be studied fully. As an example for 2 farms producing more than 2500 tons in total, they need 40 boats comprising: 5 boats over 30 m length, 14 boats of 15-25 m for feeding supply, 10 small boats for support divers and transfer, 4 tugboats and 8 local fishing boats to tow cages and support the feeding campaign.
After using local Murcia airport, BFT fatteners prefer Alicante airport (no precise data available).
Impact on MURCIA local employment

According to ASETUN, the BFT industry provides 500 places for direct employment. No precise data was available at social or fishery services on indirect employment. However data collection from 2 companies conclude that 70% of the employees are divers working at sea. As an example 2 companies increased from 15 persons to 113 persons in 4 years and employees are young (between

25 and 35 years old). In addition, professional diving with BFT is concerned with security and social regulations especially for BFT transfers after fishing and at BFT killings in the cages.

Interactions of BFT fattening activity with local fisheries

In the beginning, strong conflicts developed because of fisherman's opposition to BFT farms. Although some animosity remains, after 5 years, the relations are better and most of the interactions found between BFT activities and local fisheries are positive.
BFT fattening impacts on local fisheries employment: BFT fattening supports local employment of marine workers. BFT fattening is attractive for young fishermen since the working conditions are better than on the fishing boats (regular hours between 7 AM and 4 PM, regular salary, everyday at home, week-end on land). This has been reported to slightly affect the local fisheries since fisheries have more and more difficulties to find qualified marine workers. However, the decrease in fisheries attractivity is a general difficulty of fisheries to adapt to the evolution of society in Europe. The increase of foreigners (North Africa and Africa) in fishing crews does not seem to be linked specially to BFT fattening. A specific study should conduct to give more precise conclusions.

Reduction of local fishing effort : One of the side effects of BFT fattening is the contracting with small-scale fisheries boats in order to support BFT activities and specially:
(i) feeding BFT cages : for each fattening company, 2-6 fishing boats (with 4-8 crew members) are contracted each year for a period of 5 month between July and December. From fisheries statistics, an average estimation of 3-7% of total catches are not purchased per year ;
(ii) towing cages from fishing grounds to Murcia feeding cages : during Balearic season, high demand on tugboats (BFT, transports, harbours, etc…), increased the price of tugboats renting. Therefore, during this season, fatteners contracted with fishing trawlers (2-6 in total). It can represent an average of 1 – 3 or 4% of annual regional trawler catches that could be saved (to be studied in details).

New incomes for local fishermen (new pelagic fish) : "Alacha" gilt sardine (*Sardinela spp.*), local small pelagic fish were, for years, thrown away at sea by pelagic seine fishers as a by-catch product and is nowadays the 1st major product of landings in the region. While the total fish landing volumes are decreasing since 1995 from 8000 to 5000 tons, gilt sardine passed from less than 5% in 1993 to almost 35% of the landings in 1999 (Fig. 3) mainly because of BFT activity. In addition, gilt sardine prices doubled in 5 years. Mackerel, and Kingfishes are also part of BFT meal when local prices are down. Feeding is one of the highest expense factors and producers are seeking prices below 1 euro/kg (gilt sardine is bought at about 0,5 euros/kg).

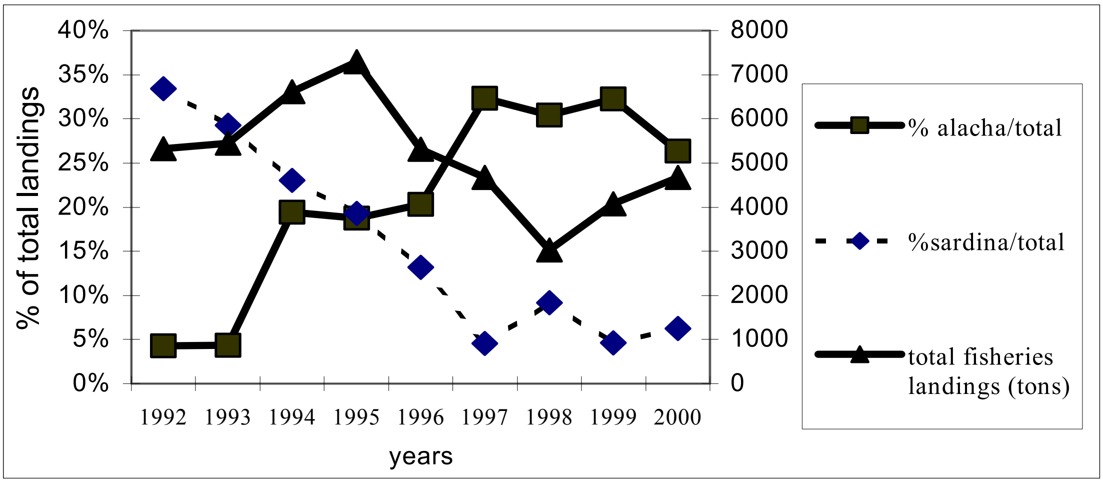

Figure 3 : Valorisation of local gilt sardine (Allacha) small pelagic fish from BFT industry (Fisheries department of Murcia Region 1999-2000; CES 2000)

The supply of BFT production with regional small pelagic fish is not the priority of fatteners because of the small volumes involved. The regional BFT fattening industry approximately requires between 35 000 to 50 000 tons of small pelagic fish/year (estimations) and small pelagic fisheries represent a small part of this needs (2000 tons). An increase of gilt sardine (Sardinela) volumes is however due also to specific fishing campaigns and small pelagic stock management becomes a priority in addition to BFT stock management. Demand and prices on high quality small pelagic could increase (competition of fish meal for aquaculture, risks of metals concentrations in pelagic fish from the North and Baltic Sea).

Some fishermen and environmentalists criticised BFT farming assuming that diminution of pilchard (sardina) captures in the MURCIA region is linked with the development of BFT farms (BFT in cages would scare small pelagic etc…). According to landing statistics, it seems not to be true : the increase of other small pelagic fish (Gilt Sardine and Kingfish) was observed during the last 10 years, and the decrease of pilchard captures occurred since 1990 (much before the first BFT farms (1994-1995)).

Interaction with longliners : Tugboats, with cages of 25-35 m depth, cross from time to time longlines and destroyed them. Some conflicts are presently under discussion (Spanish and Maltese longliners). Some of longliners seem to plant their longlines on purpose in front of the tugboats in order to receive compensation. To solve the problems of expenses, BFT companies prefer to rent special boats in order to guide the tugboats during their long trip from fishing grounds to Murcia (15-50 days),

Interaction with fishing auction hall and local fisheries : BFT fattening, is a fishing-linked activity and not completely an aquaculture one. For these reasons, fishermen are asking that BFT fatteners to use the fishing auction halls and pay taxes, while fatteners refuse because auction halls are not providing to them any service compared to fishermen (weight, collective selling, ice, etc…). For local social peace BFT companies are sometimes making small donations to fishermen organisations.
Some small-scale fisheries confirm that some of them are now often putting their nets close to the cages since the concentration of fauna is higher close to the cages.

Impacts on BFT Spanish fisheries: BFT fatteners interactions with French fleet had impacts of developing new partnerships with Spanish tuna boats for fishing campaigns.

Impacts of BFT fattening on the environment

Here are presented some of the conclusions of discussions with regional officers, farmers and university specialists.

BFT fattening advantages: BFT needs very high quality water (O_2, currents, far from freshwater sources). Thus densities higher than 4kg/m^3 are not possible zoo-technically, the average practice is 2 Kg/m^3. As a comparison, the average density for Seabream-Seabass is 20-25 kg/m3 in cages. The pattern measurements over 4 years does not show an impact on the water column and small differences between control and under cages data for granulometry of sediments and organic matter content. If Anoxia happens, it is partial and recovery of sediments is observed often after the feeding period.

Benthic fauna and algal communities: There is an increase in fish populations under the cages (*Mugilidaes, Salpa salpa, Anguila* spp., etc…) attracted by the feeding and the lack of fisheries around this area. Diversity of fauna of the sediments (polychaetes and annelids) seems to change. Some data shows even an increase of polychaete and annelid diversity compared to controls. Thus, more independent studies have to be done.
The effects of cages on *Posidonia* (Sea grass meadows) and other sensitive populations (Maerl) are clear if the cages are too close (lack of photosynthesis, degradation). Murcia region now imposes a law to place the cages at a minimum of 300 m from *Posidonia* beds.

Thus the main environmental priorities for BFT aquaculture or fattening installations are : (i)study the currents and place the cage regarding to current patterns ; (ii) place the cages at secure distance from sensitive species like *Posidonia* and Maerl (300 m) ; (iii) keep the farms as far as possible from tourism activities and the coast to avoid complaints (visual pollution, fear, interaction with activities) ; (iv) receive a political regional support for specific zones and ICZM approach.

Interactions of BFT fattening and tourism

With more than 700 000 tourists/year (1% of Spain tourism) Murcia tourism is undergoing strong development.

Sport diving on BFT cages : one trial was conducted in Murcia. The experience stopped when, during a feeding watch, a BFT hit a diver with it's fin by accident. Even if interesting economically for the diving company, it became too dangerous for the BFT Company to risk its image for such little activity. Lessons could be taken into account in order to plan a better system with risks controls.

Sportfishing : BFT cages attract fish and sport fishermen, who profit from the ease of catch. They are often fishing close to the cages. It is a problem for professional divers that can hurt themselves with hooks or lines caught in the cage nets. No extension of these interactions are possible, control is difficult.

Competition for space: Future place of Aquaculture in Coastal zone development ? Critics of tourism on BFT cages are more a psychological fear of intensive farms systems (pollution) and visual pollution than knowledge on the real impacts. The regional council has conducted a study in 2000 in order to establish the suitable zones for aquaculture concessions (Services of fisheries, 2000). A SIG cross-mapping has been done according to the possible sites and restricted area presented by each of the offices concerned by coastal zone occupation: Tourism office, Environmental office, Fisheries offices, Marine Military office. In conclusion, little space is available for aquaculture and further political coastal management decisions affect private visibility. A regional coastal zone management plan is therefore needed urgently.

Tourism (actual priority of economical development for the region) interactions and conflicts between BFT fattening will be stronger and stronger in the future even if tourism is also moving from "mass production " to higher quality, cultural and environmentally sustainable tourism.

Increasing Risks for the BFT Production Channels

The main questions arising as expressed by the producers, regional officers and analysed during the study are detailed below. The context of low visibility at middle or long terms reduces the margins of this actual profitable sector and will affect the sustainability of some companies.

Production and investments costs are increasing (boats, tunas, trashfish, insurance,)

For fishermen, as consequence of modernisation and concentration of the fleets, the increase in boat prices impose plan strategies (like major industries) of up to 10-15 years (loans, new investments, etc…). For fatteners, the increase in the prices also affects their profitability (primary BFT products limited by quotas (+ 30% in 4 years), pelagic fish for feeding (+25% in 2001), insurances) since the Japanese market is slowing down.

Insecurity on access to Mediterranean resources

The countries from the south Mediterranean sea (Tunisia, Algeria, Libya) will soon have access to BFT quotas for political reasons between North and South. This competition is strongly feared by French fishermen even more nowadays when Spanish fatteners develop southern-partnerships in order to secure their production and market. In addition, EU regulations and the difficult ICCAT negotiations on Atlantic stocks will also affect private sector visibility.

Insecurity on BFT markets capacity related to international competition

In France, prices for BFT are around 1,5 to 3 euros/kg. BFT industry is completely dependent on the most interesting market of Japan where TORO flesh quality (all fattened BFT in cages are concerned) reaches often 40-57 euros/kg (Canadian Ambassy, 2001). But The Japanese market is changing.

Crisis of Japanese economy/unique market for BFT : Focussing on a unique market (Japan) is becoming risky for fishermen and fatteners. A drop in the Yen in 2000 affected benefits and investments of farmers (- 20% of the precedent year, according to farmers). Statistics show a continuous drop of consumer prices for more than 10 years. Japanese consumers start changing their consuming habits (choosing less expensive products).

Competition on BFT providing resources to main markets : increase of fattening supply by other Mediterranean countries (Malta, Lybya, Tunisia, Italy, Croatia) is observed. In addition, competition increase from other fattening areas in the world (Australia, Mexico, etc…) or from new potential ones (South Africa, Philippines, Pacific Islands, Central America) will probably affect market prices. Their competitiveness is based on better manpower costs than European countries but also on their geographical location allowing savings on feeding costs (trashfish access at better costs) or transportation costs (access to USA and Japanese Market).

Competition on "sushi-sashimi" primary sources / change mentalities : the competition is already starting with other tuna species (big eye, yellowfin) (3-6 euros/kg at Japan import), less expensive than BFT (20-40 euros/kg) or from salmon that becomes a cheap basic product for sushi-sashimi consumers. Japanese youth, are more and more influenced by "supermarket culture", and might be less exacting on sushi-sashimi products as far as quality and freshness are guaranteed.

Perspectives of new markets for Tuna: is "outside of Japan markets" economically sustainable ? The BFT fattening industry is looking to develop it's market outside of the sushi-sashimi niche, on other consumers markets. In the context of a meat crisis, developing a new mass product from the sea, with a high quality product assimilated as " the steak of the sea" presents interesting potentials.
The question remains if future aquaculture production will be economically sustainable while supplying a European market with half price fresh tuna. Nowadays, BFT primary sources in Europe for fresh tuna steak is around 1,5-3 euros/kg which seems incompatible with estimations of 17-18 euros/kg production costs for BFT fatteners. Further technico-economical simulations and potential markets studies are needed to implement decision for research and industry.

Insecurity on environmental regulation and social pressure

Access to new sites and renewing concessions : fishermen interested in diversification of their activity will face European regulations presenting marine aquaculture as a polluting activity. There will be a need for rigorous "impacts studies" and "public studies" before installation or exploitation permits are granted. In addition lack of political concern for coastal zone management of BFT installations, new potential sites or renewing old sites is not supporting the dynamics of the local private sector.

Immediate Adaptative Strategy

Faced by this conjectural insecurity, private stakeholders of the sector are developing adaptive strategies

Fatteners are exploring three directions in order to maintain their competitiveness:
(I) Concentration of BFT industry, integration of fisheries : the aim is to stabilise their activity and integrate the production part in order to secure BFT primary product supply and their markets ;
(ii) International development: The fattening industry tendency is to develop European scale companies and exchanges in order to share costs, risks, export capacities and investments and look for less regulated countries. Recent partnerships have been developed with other fatteners or fishermen in Malta, Croatia, Tunisia, Algeria, Libya ;
(iii) Market niche: in order to be less dependant on Japanese market, to reduce risks, some of BFT fatteners are looking for new commercial opportunities in Asia and USA. Some are also developing direct valorisation at the production site and new commercialisation's channels.

Fishermen are scared by southern Mediterranean fisheries developments, feeling they are late in the aquaculture development. They fear they will loose their independence at the same time some of them are interested in sharing the risk and added value of the fishing products with successful and strong companies. They have a choice on exchanging shares between their companies and BFT fatteners companies or developing diversification and leadership in a fattening project since they have access to the primary resources.

It is assumed that 3 possible prospective directions exist for BFT future activities :
(i) Integrative regional politic strategies? The alternatives for aquaculture depends strongly on regional and European politics and on strengthening annual productive traditional activities (fisheries and aquaculture) in ICZM programs (integrated coastal zone management programs). These permanent activities would play an important role not only in terms of employment and economical impact but also in terms of culture and identity of a region.
(ii) offshore technical developments? The reality of pressure of the public opinion and competition with tourism might impose rapid changes of production areas and move to 5-16 Km offshore. However,

apart from technical aspects, the main problems of offshore techniques are economical ones. A platform installation leads to very expensive costs. In addition, offshore cages do not allow monitoring, feeding, harvesting during heavy seas (winter), thus being too dependent on weather and which affects their economic performance, and the capacity to provide the market all the year round posing a question for their sustainability.
(iii) Southern countries production? If no possible development of aquaculture is realistic in Europe, moving production to southern regions where environmental, tourism or public pressure is less important present most advantages for private companies even if presenting a loss for the regional economy. These strategies are already being developed by some of BFT fatteners.

Breaks for Research Programme

Fishermen are frightened of Aquaculture development of BFT in terms of impacts on their fishing activity (diminution of prices, competition in the same markets, etc…) as has happened with seabream-seabass fisheries (the situation is different because of low prices of BFT). They do not want to support a development that they will neither be able to control nor receive the benefits since they it will be used by larger companies that may even not be linked with fisheries. For developing aquaculture research, they expect strong partnerships in order to be the first informed and able to adapt their private strategies accordingly.

Fatteners are the most interested in research programmes. They expect European protection on European scientific data and priority access to the knowledge.

Regions expect market studies and local impacts of BFT studies before huge investments would be launched in research developments. If they are assured scientific interest, they are suspicious and interested in knowing the feedback of public investments in BFT existing activities in their region.

Consumer Organisation Opinion

The main concern is quality and traceability of the products : (i) there is a lack of communication on aquaculture products (origin, etc…), traceability, and quality and there is strong need for information on aquaculture nutrition process (components) in order to educate populations ; (ii) there are fears of mercury and heavy metals concentrated in large pelagic fishes and specially in BFT ; (iii) Aquaculture, by controlling the feeding sources would be able probably to valorise itself ; (iv) there are also concerns on animal welfare.
The second priority, without direct impacts on prices, are the environmental and ethical aspects. The public is more and more aware and sensitive to sustainable production. An aquaculture that would consume millions of tons of other fishes will probably not be well accepted in the future.

Immediate Needs and Priorities: Secure Development of European Private Sector Capacity

All the priorities below have been define through a participation process. We present a synthesis of the common priorities of research for fishermen and fatteners supported by regional fisheries officers. Fishermen are specifically asking support for diversification and studies on simulations for installations for new fattening activities.

Stocks management research at Mediterranean level

BFT stock management research: all stakeholders are asking for the strengthening of research studies on BFT Mediterranean stocks and discussion at the Mediterranean level (CGPM) and specially on :
(i) The use of genetics to distinguish if possible between two existing Atlantic stocks ;
(ii) To measure the impact of reproductive behaviour and spawning in the cages on BFT stocks;
(iii) To improve data collection from fisheries;
(iv) To provide correcting factors for stock estimations of biomass going from fishing to fattening cages
(v) To identify the origin and future of small BFT huge schools that have been observed this year.

Small pelagic stock management research is required in order to determine pelagic fish stock health and its impacts on BFT and/or large pelagics in the Mediterranean or produce data to explain the decrease of small pelagics captures (environmentalist and fisheries concerns).

Secure information on market and production perspectives

This has a high priority since private sector needs to get a precise idea either on sashimi-sushi market (short term projections) or on fresh Tuna steak new markets (perspective on products issued from complete BFT aquaculture). They identify the following needs for information and research on :
(i) the elasticity of Japanese market on sushi-sashimi, the state of the art of world markets for BFT, the trends of consumer profiles in Europe, USA, Asia in the context of world competition ;
(ii) the future perspectives of BFT new products (filets, cooked meals, steaks, sub-products, etc…) ;
(iii) the economical feasibility and cost effectiveness of complete BFT biological cycle production, the market perspectives for small BFT. Is it economically viable to invest in the complete mastership of the biological lifecycle of BFT for aquaculture or not ?
(iv) Installation costs simulations (immediate need): BFT fishermen ask for costs simulation for fattening installations (minimum size, volumes, and investments). This would allow them to identify their participation in a partnership as well as the types of partnerships needed.

Define potential sites for aquaculture of Tuna in the coastal zone area

BFT requires specific conditions (current, O_2) thus special studies have to be done specifically on: (i) mapping (SIG) presenting the potential sites, constraints and interactions; (ii) summary of coastal zone management and aquaculture installation procedures in each country; summary and information on environmental and coastal zone laws; (iii) They expect both political and inter-sectorial concentration in order to better valorise their activity in coastal zone management (ICZM process).

Technical and economic feasibility of off-shore aquaculture

Technical and even more economical simulations programs are expected to give some answers to cost effectiveness, costs of production and constraints of exploitation in Offshore cage installations.

Research on improvement of grow-out techniques

(i) Transportation of live fish procedures;
(ii) The harvesting and killing procedures in relation to fish welfare and the killing systems ;
(iii) Nutrition : flesh quality, trashfish and artificial feed quality and traceability ;
(iv) Pathology : anticipation of problems of pathology (stress, food-handling) ;

Environmental research

Private sector expects research in order to determine the proportional impact of BFT fattening compared with other polluting sources such as tourism, agriculture, industry and other aquaculture species. Local fisheries and regional officers ask for independent research on the local impact of BFT fattening. This would support objective information for all parties and producers could these arguments for communication campaigns.

Image and communication valorisation tools (crisis anticipation)

Fatteners that already suffered from environmentalists and public attacks know that there is collective interest for developing communication tools to face crises like the future possible effects of : (i) environmental impact of BFT on small pelagic fishes, on coastal areas ; (ii) worm, parasites and flesh quality ; (iii) mercury, heavy metals concentrations in large pelagic fishes (toxicity, etc…)

Regional and administrative officers priorities regarding research programmes:

(i) precise the socio-economical impact of BFT industry: The objective is to get a better European and regional picture of socio-economics impacts of BFT on the regions (side effects, inductive employment, impacts on local fisheries and tourism, impacts on public finance, impacts on environment, regional image, etc*…),*
(ii) Socio-economic studies on diversification of tourism: simulations (risks, economic benefits, constraints, legislation, etc…)
(iii) Divers work security conditions harmonisation: There is specific need for studies on new problems: in terms of security, minimum salary, insurance, retirement, legislation, etc…

Conclusions: Specificity of BFT DOTT Research Programs in the Mediterranean

The world global economic situation is defined by rapid changes and the difficulty in planning long term provisional models. Companies will have adapt rapidly and react to market changes while world trade development will impose the concentration of companies into large scale development, to the detriment of small companies.
In addition, environmental and ethical concerns impose regulations and constraints. The increasing importance of these concerns will lead producers to adapt their production programs and facilities in terms of traceability, quality, environmental measures and animal welfare. BFT specific activity (fishermen and fatteners), will need support since we are probably passing from a very profitable pioneer activity to a period where risks and lack of visibility are increasing.
If all stakeholders agree at the moment on international competition and the rapid development of BFT fattening for Japanese sushi-sashimi market, no clear market perspectives are present for ” the steak of the sea” for BFT aquaculture. Studies have to be done on socio-economical sustainability “from larvae production” (see Japanese BFT producers economical constraints while starting from 500 g fishes); in order to answer more precisely the following question :

“ is BFT research on closing the life cycle a long term or a short term story?”

In this context, and according to the above remarks, BFT research programs in the Mediterranean and Europe have to integrate some specific characteristics of the private sector: (i) a private sector in centralising all the biological material for research; (ii) a private sector with fisheries and aquaculture strongly integrated; (iii) a fishery sector with financial potential for diversification strategies.
In this respect, EU concerted research will have to integrate these specificity’s and specially:
(i) Enhance the EU companies competitiveness while sustaining their diversification of production;
(ii) Recognise the rising interaction of fishery/fatteners and their leadership as private partners;
(iii) Develop strong interaction between research, fisheries and fatteners;
(iv) Develop short term as well as long term research to secure investments in a rapidly changing and international competitive environment.

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Australian southern Bluefin Tuna farming and research activity – national report

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SUMMARY – An overview is provided showing how Australian research on the biology and culture of tuna has grown in scope and intensity in step with the successful development of the farming of Southern Bluefin Tuna in South Australia. The nature, progress and future directions are summarised for research on tuna feeds, product quality, environmental management, health and hatchery propagation. Some of the practical problems are discussed, and the approach to coordination of research and industry development is briefly outlined.

Key words: tuna, aquaculture, research, industry development

RESUMÉ

Une critique est fournie qui montre comment les recherches australiènnes sur la biologie et la culture du thon ont augmenteés en portée et en intensité à la mesure du success du développement de l'aquaculture du thon 'Southern Bluefin' en Australie du Sud. La nature, le progrès et les futures directions sont resumés au sujet de la nourriture du thon, de la qualité du produit, de la gestion écologique, de la santé et de la propagation d'éclosion. Quelques-uns des problèmes pratiques sont discutés, et l'approche a la coordination brièvement esquissée.

Mots clés: le thon, l'aquaculture, la recherche, le développement industrièl

Research and development relating to the aquaculture of Southern Bluefin Tuna (SBT), *Thunnus maccoyii,* has naturally evolved in parallel with the emergence of the industry through several phases.

Feasibility Phase

The Australian tuna fishermen and their Japanese colleagues initiated feasibility studies (1989-90), in the face of the challenge of quota limits on the wild fishery. The Tuna Boat Owners Association of Australia (TBOAA) and experts from the Japan Tuna Federation and Japan Sea Farming Association considered possible sites and concluded that wild tuna could in principle be caught and transported to potential farming sites in South and Western Australia.

A three-year project (1991-93) was then undertaken by the TBOAA, the Japanese Overseas Fisheries Cooperation Foundation and the South Australian government, with seed funding from the Fisheries Research and Development Corporation. This project comprised mainly empirical trials of capture, transport and growth in sea-cages, but even at this stage some large fish were set aside for possible future spawning studies, and preliminary environmental models were set up and baseline data collected.

Industry Development

A critical breakthrough came in 1993, when a leading tuna fishing company successfully demonstrated that large numbers of tuna could be encircled by a purse seine net, swum into a hexagonal towing cage, slowly towed back to Port Lincoln, and then swum into grow-out cages. Methods of delivering the feed (baitfish, mainly pilchards) and harvesting fish developed progressively from 1991-3, and the weight gain as a proportion of feed used rapidly increased. Commercial development of the tuna farming industry advanced steadily from 1993 to 1997, with some 18 companies actively involved on their own account, and also beginning to commission and contribute as an industry to research on key issues, and state and national research agencies also accordingly started to take up projects on health, environment and nutrition.

Environmental research identified marked impacts on benthic communities, below and near cages. Very limited baseline data was available on the relevant waterways, but nevertheless rough estimates of carrying capacity were made.

The industry established a program of monitoring, sampling and diagnosis to detect health issues and identify possible pathogens. Little is known about tuna pathogens generally, and so far the incidence of disease problems has been low and has been managed by progressively improved husbandry (particularly water quality, management of nets, and methods of handling and transferring fish). The major health incident so far (in 1996) related to unusual environmental conditions, and the risk of a recurrence has been reduced by moving the cages to deeper water.

Strategic R&D

The most pressing research goal of the industry was to develop manufactured feeds to replace the use of baitfish. The Cooperative Research Centre for Aquaculture (CRC-A) was set up in early 1994, and its program included a small-scale project on this topic as well as studies on the quality of product from the tuna farms. In 1997, the evident economic potential of tuna farming and the range of research activities prompted the Fisheries Research and Development Corporation to establish its Subprogram on SBT Aquaculture, with strong CRC-A involvement, providing a focus for developing research strategy and greatly increasing the resources available, particularly for nutrition and feed development and product quality.

Improved feeds, more specifically the use of manufactured pelleted feeds rather than baitfish, are expected to offer many advantages: higher production efficiency, consistent high product quality, minimal nutrient loss to environment and reduced quarantine risks. There is an ongoing progression in feed research from the quality and efficient use of baitfish, through the use of mashes and moist pellets towards fully manufactured drier feeds. Useful feeds obviously must be attractive to the fish, easily ingested and fully retained by the fish, and efficiently assimilated. They must yield fast growth and the desired characteristics in the final flesh product.

Research methods used have included analysis of tuna flesh and baitfish composition, study of the behavioural and physiological characteristics of tuna and of the anatomy, ultrastructure, and enzymology of the tuna gut, and measurement of ingredient digestibilities. A range of methods have been used to assess the suitability of experimental feeds, including surrogate species (such as salmon), comparative growth trials on a small-scale “research farm”, and biochemical correlates of growth. Archival tags have been used to study the feeding performance of individual fish and to modify feeding strategies accordingly.

The work to date shows that pelleted feeds are capable under research conditions of providing as good growth and condition as do baitfish, but also that rapid weaning onto pellets is crucial to achieve a high survival rate, and to avoid an uneconomic growth lag. By 2000, the feasibility of manufactured feeds was clearly apparent, and Pivot Aquaculture (now Skretting Australia) and the Stehr Group have undertaken commercial-scale trials.

Research on product quality has followed three strands: modification of husbandry and harvest practices to ensure and improve flesh quality; work in parallel with feed development to ensure effects of new diets on quality are satisfactory; and the development of new tools and methods for measurement of quality. Work on tools for quality assessment has addressed the measurement of rigor as predictor of shelf life, the establishment of non-destructive measurements (eg of carcase fat) and minimally destructive sampling techniques, objective colour measurement systems and the use of trained sensory panels.

Amongst the findings of this work are the demonstration that pelleted feeds can yield good colour and fat content, but there are many factors involved here; the fat composition of tuna not surprisingly reflects the diet composition; and inclusion of vitamins in diets may enhance product shelf-life. The study of harvest stress has proved technically very complex in tuna: harvest stress certainly influences the energy status of fish flesh but has not yet been correlated with quality and shelf-life. Methods of achieving rested harvest have been explored but are yet to show an advantage for product quality in tuna.

This period of research highlights some major issues in dealing with Southern Bluefin tuna, as a consequence of its size, value and behaviour. Much use has been made of a “research farm”, using small replicated sea-cages managed by research staff. This system has great value in testing innovative ideas, but is expensive and yields inferior fish performance compared to commercial operations, and this creates a barrier to acceptance of its results by industry. Trials on farms are essential to provide confidence in results, but they present major challenges in implementing scientific methodology, owing to the difficulty in handling the fish without incurring stress-related mortalities, and to the heavy commercial risks. *In vitro* models and surrogate species have also been exploited, but testing their validity as models of SBT is in itself a major challenge, and the models used so far are found to be suitable only for quite specific experimental purposes.

Current and future research

In 2000, a Strategic Research and Development Plan for SBT Aquaculture for 2001-7 was published and a new Cooperative Research Centre for Sustainable Aquaculture of Finfish (Aquafin CRC) was funded by the Australian Government (2001-2008). The Aquafin CRC is the main focus for Australian SBT research and the CRC model has proved a useful mechanism for achieving multi-disciplinary research and industry collaborations, both industry-wide and company-specific. Its program largely implements the Strategic Research and Development Plan, with projects on further diet development, product quality, health and environmental management, and propagation.

Regarding future work on improved manufactured feeds, while much will be achieved by the ongoing efforts of the feed companies, the research agencies also have continuing challenges. A research plant is available locally for small-scale for experimental diet development to enable a wide range of diet modifications to be made, and much effort is being made to develop methods for comparative growth trials which better mirror commercial performance, and because measuring further improvement in feeds (especially feed conversion ratios) is technically difficult in tuna.

Future directions in product quality research will include tracing of fish from cage to market, correlation of price with objective quality parameters, examination of fat accretion and muscle development through grow-out cycle, and further efforts to assess and modify husbandry stresses to achieve the highest quality and price.

Environmental management in aquaculture is a rapidly evolving subject, not least for tuna farming, with Increasing community expectations and regulatory standards, which affect access by industry to farming sites. Industry also well understands the dependence of economic performance on good environmental conditions, and our scientists aware of an extending horizon of environmental impacts, with a concern for nutrients, phytoplankton, and water quality effects over whole water systems. Environmental management is also moving from a past phase of assessment and restriction towards adaptive management and mitigation. This progress, of benefit to industry and the general community, requires and is receiving close collaboration between industry, researchers and government regulators. Present and future research includes the development of tools for fast, cost-effective acquisition of ecosystem data, characterisation of waste flows, and evaluation of methods of impact mitigation. The new focus on regional sustainability assessment involves development of stakeholder teams, new methodologies and models, and rapid acquisition of vast bodies of data.

Tuna health research is becoming more urgent with our expectation of greater health threats as industry intensifies, and perhaps particularly if and when tuna hatcheries are established. The recently initiated health research includes a risk assessment program, strategic development of our ability to characterise and study viruses (through tuna cell line development), and the design and implementation of early-warning surveillance and response procedures.

The domestication of SBT, in the sense of developing hatchery technologies for this species, is a major goal of Aquafin CRC and at least one company. The Aquafin CRC is looking for international collaborators to specifically complement its existing strengths, and preparing a business framework to attract the essential industry collaborations.

An approach to study the nutritional requirements of the bluefin tuna (*Thunnus thynnus thynnus*, L.)

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SUMMARY - The research required in relation to the nutrition of bluefin tuna (BFT) is great. In previous trials in the domestication of large scombrids and in current capture-fattening schemes, nutritional studies have been few. Therefore, virtually nothing is presently known about the nutritional requirements for these species. Nutritional factors are important in any domestication programme in a variety of areas. Correct nutrition is a vital factor in the production of a successful broodstock with high fecundity and fertility producing large numbers of high quality eggs. Reproductive control itself is affected by nutritional factors such as lipid/energy content which can influence sexual maturation in other fish species. Successful larval rearing of marine fish is highly dependent upon suitable diets, whether live prey species or artificial, and their precise composition particularly in relation to fatty acids is an area that still demands much research for all marine species. Clues to the nutritional requirements of any animal can be obtained by looking at the natural food for that animal, in this instance, the natural prey species of the BFT and also by determining the composition of wild caught animals, both prey and predator. The latter certainly being a source of information in terms of lipids and fatty acid requirements. The few data available from previous trials can also add to the overall view. In this section we will briefly review the literature on what is known about the nutrition of the large scombrids and also, importantly, place the problems of their feeding and nutrition in a global perspective.

Key words: Bluefin tuna; Nutrition; Requirements; Diets

Natural Food

Prey species.

There are many reports on the prey species of adult BFT based on the examination of gut contents. The stomach contents of BFT in the Mediterranean (Ligurian Sea) are dominated by anchovies although mesopelagic fish and crustaceans and ommastrephid cephalopods were also present (Orsi-Relini et al. 1995). Immature BFT in the Bay of Biscay consumed fish (anchovy)> crustaceans (euphausids)>cephalopods by frequency of occurrence, and crustaceans> fish >cephalopods by numerical frequency with anchovy the most important prey species (Ortiz de Zarate and Cort 1986). The food spectrum for the tuna changes during ontogeny. Thus, BFT larvae in the northern Pacific feed generally on zooplankton, mainly copepod nauplii, calanoids, cyclopoids, cladocerans and corycaeids (Uotani et al. 1981, 1990; Young and Davis 1990).

Body composition of wild tuna.

The body composition of wild BFT may give some indication of possible dietary requirements, at least in respect of lipid content and fatty acid composition. Clearly, there can be great variation in carcass fat levels reflecting condition factors that are almost certainly related to season. In contrast, the protein composition was observed to be less variable. The very strong inverse relationship between body fat and water in BFT indicated that the fish obtain energy for their migrations from muscle lipid reserves (Clay 1988). The fact that flesh lipid levels can vary so widely has important consequences for farming. Clearly the level of fat in the flesh will be highly dependent upon dietary fat levels but seasonal factors affecting the metabolism of lipids in the fish may also be important. The potential benefits of high fat diets such as rapid growth may have to be balanced with potential deleterious effects such as reduced product quality and consumer acceptance.

The polyunsaturated fatty acid (PUFA) composition of most marine fish are dominated by the n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Sargent et al. 1989). However, the fatty acid compositions of tuna species appear unique in that they are characterised by relatively high levels of the DHA and, especially, a very high DHA:EPA ratio (Sawada et al. 1993). In general, the DHA:EPA ratio of the lipid of northern hemisphere marine fish seldom exceeds 2. In the southern hemisphere, the lipids of marine fish generally show higher EPA levels and consequently even lower DHA:EPA ratios (Ackman 1980). In contrast, Pacific BFT showed flesh DHA levels of between 25% and 36% with DHA:EPA ratios of 3.4 – 5.8, whereas the stomach contents showed a DHA:EPA ratio of just 3.2 (Ishihara and Saito 1996). Muscle phospholipids of Atlantic BFT displayed DHA:EPA ratios of up to 7.4 (Medina et al. 1995). Therefore, the relatively high level of DHA and the high DHA:EPA ratio in tuna appears to be an essential characteristic of tuna species that would probably be required to be reproduced in farmed fish to preserve the qualities that the consumer would expect.

Definition of basic nutritional requirements of the BFT

Energy

Energy is not a nutrient itself, but is present in the chemical bonds that hold the molecules in the nutrients together. The amount of energy in the various nutrients that make up a feed is of great importance as well as the capacity of different species to utilize the energy contained in the different nutrients. Fish, like most animals, eat to satisfy energy needs. Nutrients should be balanced so that the fish will have enough of the essential nutrients for optimum growth when energy needs are satisfied. All energy acquired through the ingestion of food is ultimately lost as wastes in faeces or by excretion, used in metabolic processes or deposited as new body tissues (growth or energy gain, maturation). Bioenergetics is concerned with the study of rates of energy intake and transformation within the organism, providing the physiological framework for the study of the relationships between feeding rates and growth rates or maturation of fish subjected to different environmental conditions. In consequence, the energetic requirements of BFT for maintenance, growth and maturation, and its capacity to utilize the energy contained in the food must be primarily considered. Some studies have been done with skipjack and YFT (Kitchell et al 1978) and SBT (Davis 1997), but very little is known about the bioenergetics of BFT. BFT is a pelagic fish having a swim bladder but without respiratory pump, retention of metabolic heat and a respiratory rate, possibly, independent of ambient temperature and the allometric effect of weight. So, the study of the energy budget at different levels is crucial.

Protein

Dietary protein serves two main purposes in fish diets; firstly, as a source of amino acids required for the synthesis of new proteins both for growth and reproduction and also as replacements for existing protein in the process of turnover; and secondly, any protein in excess to the above requirements will be utilized for energy. An optimal dietary protein to energy balance, the amino acid composition and digestibility of the dietary protein(s), and the amount of the non-protein energy sources in the diet influence the optimal dietary protein level for fish, as well as other animals. Dietary protein requirements can be broken down into three main categories, gross protein requirements and qualitative and quantitative amino acid requirements. Nothing is known about any of these requirements for BFT but some general assumptions can be made based upon existing knowledge of other species.

The estimated gross protein requirements of fish can vary from around 30% up to about 55% of the diet. The lower protein requirements are usually associated with warm freshwater species that can also utilize carbohydrate to a greater extent than most other fish. As marine fish and also top predators, the large scombrid species are likely to be nearer to the high end of this range and it is probably safe to assume that their gross protein requirement is unlikely to be below 40%. Obviously stage of development, and size of fish are likely to affect the gross protein requirement as, generally speaking, it decreases with both age and size (Wilson 1989). Water temperature is another factor that could affect the protein requirement.

Determining the qualitative requirements for amino acids is a tedious and time-consuming process but it has been carried out for many species. The same 10 amino acids (namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) that have been shown to be essential for most animals have also been found to be required by all the finfish studied to date (Wilson 1989). Therefore, it is most likely that the same amino acids will also be required by BFT. The quantitative requirements for all these amino acids have been determined in several species and so can be estimated for BFT using the values obtained for other carnivorous marine fish such as gilthead sea bream or salmon. However, with diets utilizing fishmeal as the sole protein source these requirements should be fully met and so particular attention to the essential amino acids may only be required if diets utilizing other sources of proteins are being used.

Lipids and fatty acids

Fish species that migrate have a higher average fat content and greater variation in fat content than those, which do not, indicating that fat is used as an energy source during migration. The large percentage of body weight that is muscle tissue allows a large amount of energy to be stored in tuna if depot fats are laid down in both red and white muscle. This is a significant source of energy that may be used for movements between areas of food abundance in a habitat where such areas can be widely separated. As with protein above, dietary lipids also serve two main purposes in fish diets; firstly, as a source of fatty acids required for the synthesis of new lipid for growth and reproduction and also for turnover of existing lipid; and secondly, excess lipid is a major energy source. Again lipid requirements can be broken down into three main categories, gross lipid requirement, and qualitative and quantitative essential fatty acid (EFA) requirements. Virtually nothing is known about any of these requirements for BFT but again some general assumptions can be made based on existing knowledge of other species.

Certainly a major consideration in the diet of BFT will be the gross lipid level of the diet. Research will be required to identify the ideal lipid levels at different life stages of the fish, particularly in relation to season and final grow out to ensure optimal growth without compromising quality. Therefore, the dietary protein sparing effect of lipids, which allows protein to be used optimally for growth without depositing excess lipid in the flesh, has to be examined in BFT. It has been concluded that, in general, fish diets containing between 10 to 20% lipid gave optimal protein utilization and growth rates while minimising undesirable alterations in carcass composition (Cowey and Sargent 1979). Considering that the lipid content of the fish used in previous grow out trials with BFT and southern bluefin tuna (see below) will generally have been in this range, this may be a good starting point with the BFT. However, in a market which will probably include export to Japan for the very high value products, sushi and sashimi, and where flesh quality is paramount and so highly prized (high quality fish can realise 4 times the price for lower quality fish), fat content of the diet and feeding regimes will be an area of nutrition requiring close attention.

However, another vital area in lipid nutrition is the provision of sufficient amounts of the correct essential fatty acids (EFA). The EFA requirement of fish varies both in qualitative and quantitative terms. In freshwater fish, including salmonids such as trout and salmon, the EFA requirements can be met by the shorter chain PUFA, α-linolenic (LNA; 18:3n-3) and/or linoleic (LA; 18:2n-6) acids. LNA and LA are converted to HUFA through a series of alternating desaturations and chain elongations mediated by microsomal fatty acid desaturation and elongation systems (Sargent et al., 1989). Freshwater fish, including salmonids, possess both the $\Delta 6$ and $\Delta 5$ fatty acid desaturases required for the production of EPA and DHA from LNA, and arachidonic acid (AA; 20:4n-6) from LA. In contrast, all marine fish studied to date have only very limited ability to produce the biologically active HUFA from LNA and LA and so have an absolute dietary requirement for the preformed HUFA. The relatively high level of DHA in tuna and the high DHA:EPA ratio may have consequences regarding the formulation of artificial diets as the DHA:EPA ratio seldom exceeds 2 in most commercially available fish oils (Ackman 1980). For instance, marine fish generally may have a limited capacity for the conversion of EPA to DHA (Sargent et al. 1993,1995). Therefore, the lipid biochemistry underpinning the high level of DHA and the high DHA:EPA ratio in tuna is unclear, but it has generally been assumed that tuna must selectively accumulate and retain DHA in their tissues (Saito et al. 1996; Ishihara and Saito 1996). Metabolic studies in addition to nutritional trials are required to fully elucidate this area. In the first instance it appears that it would be advisable that the oil used in experimental formulated diets be a high quality marine fish oil containing high total n-3HUFA with as high a DHA:EPA ratio as possible.

Carbohydrate

The capacity of most fish to effectively utilize dietary carbohydrate for energy is limited, particularly in the case of marine fish (Cowey 1988). Indeed the digestive physiology of BFT will probably mean that dietary carbohydrate cannot be utilized, as it is possible that carbohydrate metabolising enzymes in the gut are absent or, at least, very low, as in southern Bluefins. Therefore, in the first instance it is unlikely that carbohydrate will feature in the diets of BFT in a nutritional capacity.

Vitamins and minerals

It is fairly safe to assume that BFT will require the same range of water-soluble and fat-soluble vitamins that have been identified as being required in the diets of all fish studied to date (Halver 1989). The quantitative requirements for each vitamin varies between species and so theoretically will have to be determined in BFT. However, a generalised vitamin premix for marine fish is an appropriate starting point for initial trials and may be sufficient to satisfy the BFT requirements and prevent any vitamin deficiency symptoms. One possible aspect of a putative experimental diet that may have consequences regarding vitamin requirements for BFT is the high level of n-3HUFA and the high DHA:EPA ratio that may be required. Particular attention should be paid to the level of the vitamins that possess antioxidant functions, especially vitamin E (tocopherol) and vitamin C (ascorbate). The possibility that intact phospholipids, particularly phosphatidylcholine and phosphatidylinositol, in addition to the vitamins choline and inositol, may also have a growth promoting effect in larval diets as they appear to have in other marine fish species is an aspect that will require study at some point.

BFT broodstock nutrition

There are a number of aspects of fish reproduction which may be affected by nutritional status: the time to first maturity, the number of eggs produced (fecundity), egg size and egg quality as measured by chemical composition, hatchability and larval survivorship. Energy is partitioned by fish between each of the various physiological processes involved in maintenance, growth and reproduction. The maintenance requirements of fish are met first and then excess energy is divided between growth and reproduction. The relative partitioning of energy between growth and reproduction varies both between species and between strains of individual species.

There are very few studies of the particular reproductive effort and nutrient requirements of aquatic animals for gonadal development and reproduction success, but those that have been undertaken indicate great species variability. Most work has concentrated on essential fatty acid requirements and fat soluble vitamin requirements, and it has been generally assumed that the amino acid requirements of broodstock are similar to those for optimal growth. There must be an optimal protein level for BFT reproductive success, and dietary protein will have to be carefully evaluated for the effects on reproduction if their use for broodstock is to be undertaken with confidence. In general, there is a considerable need for further research in this area, and particularly for BFT.

BFT larval nutrition

The analysis of the variations of energy and nutrient contents during embryogenesis and yolk-sac larvae period can give a very useful information of the nutritional requirements during the early larval stages of BFT (Takii et al 1997). Aspects such as the environmental effects, feeding behaviour, digestive capacity during ontogeny, consumption and assimilation rates and nutritional requirements from first feeding larvae to metamorphosis needs to be investigated for reliable aquaculture of BFT.

Current feeds

Whole fish

Although there have been a number of attempts at BFT culture including both complete aquaculture and grow out/fattening programmes, there are few nutritional data pertaining to those studies (Buchanan 1977; Vincent 1981; Aiken 1984; Okamoto et al. 1984; Belle 1994; Doumenge 1996). The southern bluefin tuna has also been the subject of grow out/fattening trials in South Australia over the last 8-9 years (Jeffriess 1993; Carter et al. 1998; Lee 1998). The necessity to formulate artificial diets has generally been avoided in the above programmes by the use of whole fish derived from local or other commercially available fisheries including trash fisheries. In one of the earliest trials at Kinki University in Japan, wild-caught BFT showed very good growth rates, averaging 20 kg/year, on a diet consisting of fishes including mackerel, anchovy and sand eel (Harada et al. 1971). Subsequent trials elsewhere have used ground trash fish (Vincent 1981) and a mixed diet of local mackerel, herring and butterfish for BFT, and locally caught and frozen pilchards/sardines with southern Bluefins (Fitz-Gerald and Bremner 1998).

Artificial feeds

The Kinki University experiments included some comparative dietary trials where juvenile BFT were fed four test diets comprising three single species fish diets (sand eel, anchovy and mackerel) and a mixed diet of minced mackerel with a dry commercial diet for yellowtail (Harada et al. 1983). The BFT on both the mackerel diet and the mixture diet showed high survival and had body compositions similar to wild fish. However, whereas the fish on the mackerel diet had the best growth rate, the mixture diet had a relatively poor growth rate. Perhaps surprisingly, the fish on the anchovy diet had the lowest survival, growth rate and fat content (Harada et al. 1983). In more recent studies with the southern bluefin tuna in South Australia, pellets have been trialled. The fish did not accept dry pellets but moist pellets (sausage) containing 40% moisture and based on fishmeal and fish oil were readily accepted. The pellets were 40% protein with lipid levels varying between 12.5% and 7.5%. Growth on the pellets (in combination with bait fish) was similar to that on baitfish (sardines) alone with feed conversions slightly better for pellets (Smart 1995).

Broodstock feeding

Atlantic mackerel (*Scomber scombrus*), Atlantic horse mackerel (*Trachurus trachurus*), and shortfin squid (*Illex coindetti*) have been used as feed for BFT broodstock. The preference and feeding quantity on kind of feed is relating on physiological condition of BFT, especially on maturation (Fushimi et al 1996).

BFT nutrition – The global perspective

As indicated above, the primary goal of nutrition research will be to define the precise nutritional requirements of BFT with the output being the formulation of ideal artificial diets. This will remove the dependence of the prospective tuna industry on whole fish feeds based on local trash fish or individual species fisheries, such as anchovies or pilchards, which can be subject to very great seasonal and environmental variations (Thorpe et al 1997). However, it is very important to note that the projected artificial pellet/sausage diets will still be heavily dependent upon current commercial marine fisheries that provide the global supplies of fish meal and fish oil.

Capture fisheries and aquaculture

Wild fish capture fisheries are finite resources, which, although renewable, are highly vulnerable so that their sustainability is in question. Global capture fisheries have shown little growth over the last 15 years and currently yield less than 100 million tonnes per annum. Over exploitation (fishing) of individual fish species, whether for direct human consumption or reduction to fishmeal and fish oil, has caused the collapse or near collapse of some valuable fisheries. Environmentalists are exerting pressure to further reduce fishing effort and catches by introducing tighter regulatory measures. The realisation that global warming and natural climatic events such as El Niño can profoundly affect major fisheries, especially the anchovy fishery, highlights the inherent vulnerability of global fisheries. Projected stagnation and, perhaps, even declining yield from global fisheries predicts that future demand for fisheries products may exceed supply leading to price increases. Indeed, the cost of fish oil increased substantially from 1997 to 1998 to exceed that of soya oil. Against this background, fish production from aquaculture has increased substantially over the last decade or more (double in the past 15 years). Aquaculture has been the world's fastest growing food production sector for over a decade and it is projected to at least double over the next decade or more (Naylor et al 2000).

Fish meal and fish oil

Erratic global fisheries and rapidly increasing aquaculture as described above have to be considered against the background that fishmeal and fish oil are major feedstocks for cultured marine fish. The dietary requirement of farmed marine fish for high quality protein, rich in essential amino acids, can probably be met by sources other than fishmeal. However, the primary role of marine fish oils in aquaculture is as a dietary source of the HUFA, which together can satisfy the essential fatty acid (EFA) requirements of all fish species. As described above, marine fish in particular have an absolute dietary requirement for the preformed HUFA. Currently there is no feasible, alternative source to fish oil for these nutrients in marine fish feeds. Plant seed (vegetable) oils, rich in LA and LNA may be a partial substitute for HUFA in freshwater and salmonid fish feeds but this is an unknown area with marine fish. The effects of partial substitution of fish oil with plant oils in marine species including tuna would have consequences for growth and health of the fish as well as being potentially undesirable in terms of both consumer acceptance and human nutrition because of dilution of the health promoting effects of fish-derived EPA and DHA. Although this may not be an immediate problem it is certainly one that is projected to have major consequences for aquaculture in the future and it would be highly advisable that the plans for a prospective European tuna farming industry include the necessary steps to address this approaching problem at this early stage.

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Feeding of Bluefin Tuna: experiences in Japan and Spain

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SUMMARY- This paper summarizes the research on feeding the Pacific Bluefin Tuna (PBFT*), Thunnus thynnus orientalis*, carried out at Kinki University (Japan), together work in Spain from 1995 on the Atlantic Bluefin Tuna (ABFT), *Thunnus thynnus thynnus*, at the private company, Tuna Farms of Mediterraneo S.L. (TFM). Results on growth and feeding parameters obtained by the utilisation of several raw fish and moist pellets as food for the on growing of bluefin tuna are presented.

Key words: Feeding, Pacific Bluefin tuna, Atlantic Bluefin tuna, growth.

Introduction

Research on feeding the Pacific Bluefin Tuna (PBFT) carried out at Kinki University (Japan) started in 1974. For sixteen years (1974-1990), several studies were accomplished in order to develop a suitable technology for the growing out of this species. Young PBFT (100-300 g body weight) were caught by trawling. In 1974 mortality rate at stocking was 85%. By removing the right-angled part of the hook used to catch the fish and handling it with care, PBFT injuries were diminished and mortality was reduced up to 15% in 1984. PBFT shown a good adaptability to the rearing conditions in cages, taking in food after 7-10 days after being transferred to the on growing cage. The food used consisted of mackerels (*Scomber japonicus*), horse mackerels (*Trachurus japonicus*), anchovies (*Engraulis japonica*) and cuttlefish (*Sepia esculenta*). In order to maintain suitable water conditions, nets were replaced eleven times along the growing period and new further offshore locations to place the cages were chosen, with good results.

Growth performance of 0-age class fishes (250 g mean body weight, MBW) was 2.6 kg in one year, 74 kg after five years, 145 kg at 15 years-old. The largest PBFT growth rate was 177 kg in weight and 229 cm in length at 16 years old. Spawning in captivity of PBFT occurred in fish over 5 years old.

In order to improve the growth and meat quality of PBFT, several kinds of raw fish were tested as food for the PBFT, most of them based on low cost commercial fish. A summary of the feeding experiments with PBFT carried out by Kinki University for sixteen years were presented in 1990 at the Congress of the Japanese Society of fisheries Science (Harada *et al*. 1990). Previously, some of them were reported in Harada *et al*. (1983).

Further researches in Japan were focused on the comparison of moist pellet and raw fish on the growth and feeding performance of the PBFT. This information was employed and improved since 1995 for the Atlantic Bluefin tuna at the commercial facilities of Tuna Farms of Mediterraneo S.L, TFM (Cartagena, SE Spain).

Material and methods

Experiments in Japan (Kinki University)

In 1981, some experiments were done by the utilization of moist pellet food (M-diet) for feeding PBFT. Four groups of 16 to 22 young PBFT (450 g MBW) were fed with sand eel (*Ammodytes personatus*), anchovy, mackerel and M-diet. The latter consisted of a mixture similar to the one used for yellowtail (*Seriola quinqueradiata*), composed of fishmeal and fish oil, minced mackerel and a vitamin mixture. Fish were stocked in 6 m square cages for 80 days. Temperatures during the experiment ranged between 16.8 and s24.8ºC. Table I show the approximate composition of the four diets, Diet of anchovies and M-diet had the higher fat content, whilst the mackerel one had higher protein content.

Table 1. Approximate composition of experimental diets (%).

Group	Diet	Moisture	Crude Lipid	Crude Protein	Crude Ash
1	Sand eel	77.9	3.0	15.4	2.6
2	Anchovy	73.0	5.4	17.5	4.0
3	Mackerel	72.0	3.0	23.5	1.5
4	M-diet	61.8	4.7	21.8	4.7

Experiments in Spain (TFM S.L.)

Since 1995, feeding knowledge acquired in Japan with the PBFT were put into practise with the ABFT in the growing out facilities from the company TFM, located in "El Gorgel" (Cartagena, Spain). There, polypropylene cages (90 m in diameter) are used over a sea concession of 30-50 m bottom depth. Sea temperatures range from 28ºC in August to 14ºC in February (see Fig. 1). Annual production of TFM increased from 22 tonnes in 1995 to 1800 tonnes in 2000.

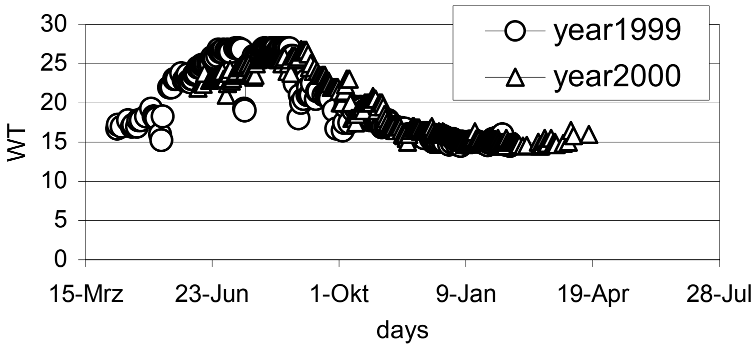

Fig 1. Water temperature in the rearing facilities of TFM in SE Spain 1999-2000.

Regarding the feeding practise, at the beginning food was supplied by hand, but later automatic feeders were used. In order to facilitate the recovering of ABFT after spawning and towing for stocking in cages, and improve its meat quality, some nutritional supplements were included in the diet.

Results and discussion

Experiments in Japan

Results of the experiment made in Japan are shown in Table 2. Survival rate after the experiment was 93.8% in the M-diet group, inferring that the vitamin supplements was the essential. Feeding efficiency in dry matter was 65.4% for the mackerel group.

Table 2. Results on feeding parameters obtained with the four different diets.

Group	Diet	SR*	CM**	FE***
1	Sand eel	81.3	160.5	54.9
2	Anchovy	66.7	63.4	34.3
3	Mackerel	81.8	165.0	64.5
4	M-diet	93.8	105.3	28.0

*SR= survival rate **CM= conversion magnificent ***FE= feed efficiency

Comparing the meat quality of experimental fish from all diets with the one in wild PBFT, an approximate composition analysis was made after the experiment (Table 3). The mackerel and M-diet group had the most similar composition to the wild PBFT ones. Especially, the liver composition of the mackerel group was very close to the wild fish. Although the anchovy feed was higher in crude lipids composition, fat content in this group was quite low. External appearance of PBFT fed with sand eel and mackerel was good, and meat colour of the latter was very similar to wild PBFT.

Table 3. Proximate composition of muscle and liver

Tissues	Group	Moisture	Crude Lipid	Crude Protein	Crude Ash
Dorsal muscle	Sand eel	66.9	7.4	25.1	1.4
	Anchovy	76.4	1.9	20.7	1.5
	Mackerel	70.9	3.8	25.1	1.5
	M-diet	71.0	2.9	25.5	1.4
	Wild	71.3	2.5	25.5	1.7
Liver	Mackerel	67.0	12.1	16.9	1.4
	M-diet	62.2	17.9	14.6	1.3
	Wild	68.9	7.2	28.4	1.4

In conclusion, it was noticed that nutritional supplements can produce a positive influence on growth, survival rate and meat quality. PBFT fed with anchovies resulted in a lower growth and survival rates and inferior meat quality.

Experiments in Spain

Due to the technical improvement of growing ABFT, mortality at towing from the fishing ground to the rearing facilities decreased from 20.8% in 1995 to 3.9% in 2000. After 8 months of rearing, ABFT showed a weight increase of 40-50% in smaller fish and 10-30% in the larger ones. Higher feeding rates (5.2%) of ABFT occurred in summer, 1.5 months after stocking. Thereafter it declines when seawater temperature falls in winter (Fig. 2). From 1995 to 2000, mortality rate of ABFT reared in TFM was reduced from 15.8 to 3.7%. Conversion magnificent (CM) and food efficiency (FE) also decreased from 24.1 to 15.8 and from 5.1 to 4.3 respectively (see Table 4). FE differs when considering the ABFT size, being 5.0% for the larger and 13.8 for the smaller fish.

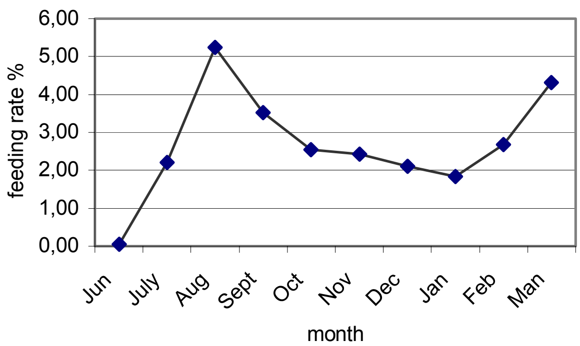

Fig. 2. Feeding rate during the rearing period

Table 4. Yearly results of BFT of mortality and growth at TFM in the period 1995-2000

	1995	1997	1998	1999	2000
Mortality	15.8	38.1	6.1	6.9	3.7
CM*	24.1	8.2	24.7	15.1	15.8
FE**	5.1	2.5	6.2	4.4	4.3

*CM= conversion magnificent
**FE= feed efficiency

It is not possible to compare feed efficiency between ABFT and PBFT, because mass culture of large PBFT doesn't exist. Harada *et al* (1990) reported that FE decreases as the PBFT get older, showing 0.8% in the 14 years old ones. Large ABFT show a FE of 4.3%, which is very efficient.

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Possıbılıtıes for the domestıcatıon of Bluefın Tuna ın the Eastern Medıterranean sea

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SUMMARY - Turkey borders the Black Sea, the Sea of Marmara, the Aegean and the Mediterranean Seas with a total coast line of 8333 km. The Mediterranean coast is 1839 km long. The water temperatures on the Mediterranean coasts of Turkey are good to grow out the bluefin tunas. The monthly minimum mean surface water temperatures in the Western Turkish Mediterranean Coast in Antalya in the coldest month in February was 16.6 ºC. In the hottest month in August the maximum mean surface water temperature was 27.6ºC
.

Key words: Eastern Mediterranean Sea, grow out, bluefin tuna, fishery

In the eastern part of the Turkish Mediterranean Coast in Iskenderun, in the coldest month in February, the monthly minimum mean surface water temperature was 15.6 ºC. In the hottest month in August, the monthly maximum mean surface water temperature was recorded as 29.1ºC.

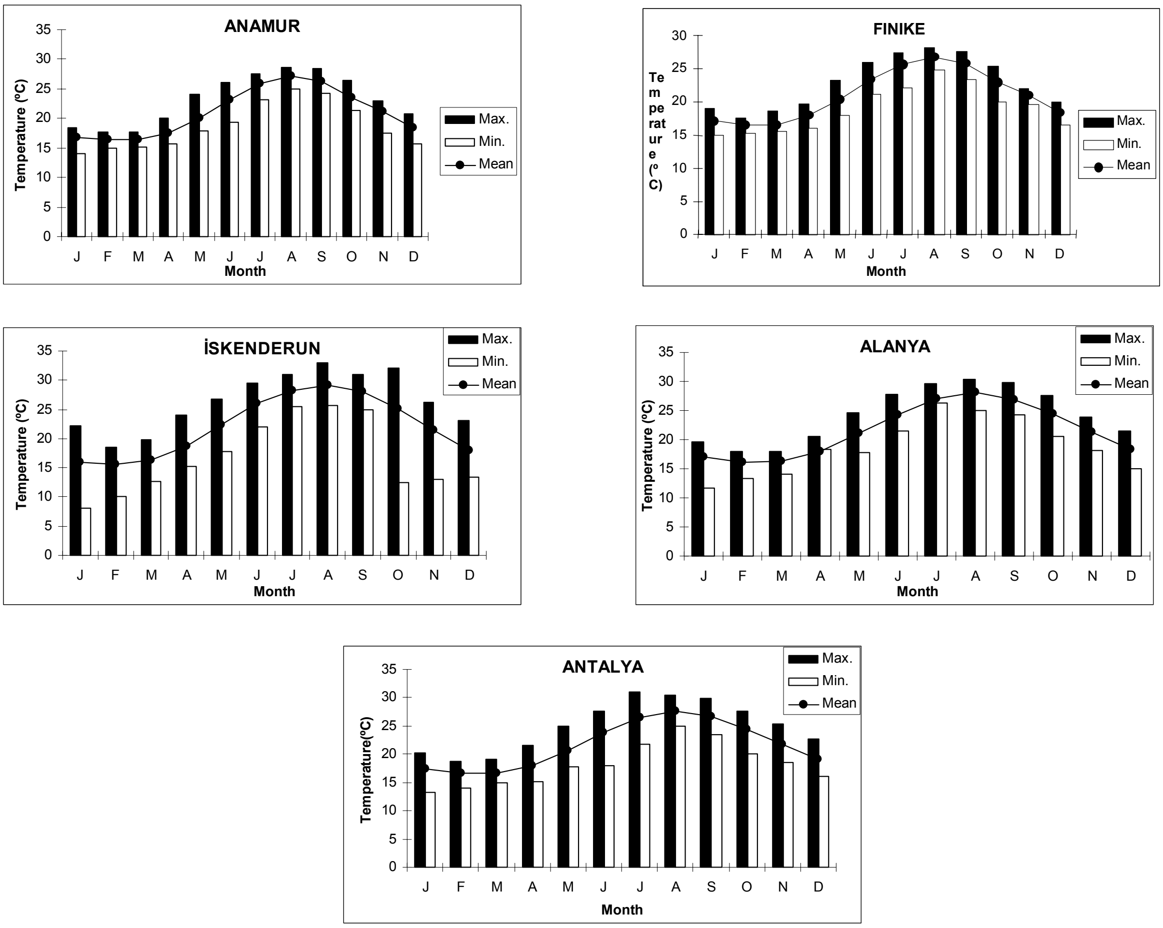

Figure 1: Minimum, maximum and mean sea water temperatures in the Eastern Mediterranean

Up to 1957 bluefin tunas in Turkey were caught in dalians (traps) and by hooks and lines. The most important bluefin tuna dalians were set in the Bosphorus and in the Sea of Marmara. After 1957 (İyigüngör, 1957) bluefin tuna catch was carried out mostly by purse seiners.

Up to 1987 bluefin tunas were caught in the Black Sea and in the Sea of Marmara. Till 1988 bluefin tunas were rarely observed by the fishermen at the entrance of Marmara Sea and the Dardanelles. 1998 and 1999 very small quantities bluefin tunas were caught by purse seiners in the Marmara Sea (Anonymous, 1999). From 2000 on more quantities of bluefin tunas are being caught by purse seiners in the Sea of Marmara.

The catch of bluefin tunas in 1998 of 5889 tons by 62 purse seiners were reduced drastically to about 2000 tons in 2001 by means of 25 purse seiners.

Bluefin tunas are mainly caught in The Northern and in The Southern Aegean Sea and in the Mediterranean Sea in the coastal areas and in the open waters during the winter and in the spring.

For the first time in 2001, the catch period was extended till mid July, in which good catches were made. The weights of the bluefin tunas caught by purse seiners in May to mid July were between 40-350 kg. Good catches were also made in March and April. Few days before Mid July all the bluefin tunas disappeared from the fishing grounds in the Eastern Mediterranean Sea (between Antalya and North Cyprus).

Bluefin tunas in Turkish waters become sexually fully mature at 3-4 years of age by 94.75 – 113.51 cm (Karakulak, 1999). Turkey has a fish production of about 650.000 tons/year. About half of the total catch is comprised by anchovies.

40 miles away from Turkey, on the coasts of North Cyprus exist during the whole year ideal water temperatures for growing out of bluefin tunas. The bluefin tunas in North Cyprus are caught as by catch in the swordfish longlines fishery in small numbers by the artisanal fishery. The weights of these fish are between 10-100 kg. In North Cyprus, a purse seine fishery does not exist. Small and big pelagic fisheries are not developed. There are good chances for developing these fisheries. The monthly minimum mean temperature on the North Cypriot coasts in the coldest month in February was 15.4 ºC. The maximum monthly mean water temperature of the hottest month in August was recorded as 27.9º C.

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Tuna in the international market for seafood

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SUMMARY – Tuna is one of the major products in seafood international trade. World tuna landings are around 3,5 millions tons per year. All tuna fishing countries are involved in international trade. One third of the tuna production is exported as fresh, chilled or frozen fish. The main final markets for tuna are in USA, Japan and European Union, like for most seafood products. The tuna industry is more and more concentrated, with a very limited number of actors at the marketing level. While the market for canned tuna is stabilised in Europe and recessing in the US, the market for fresh or frozen tuna has good perspectives of development.

Key-words: tuna, seafood, international trade

RESUME – Le thon est un des produits majeurs du commerce international des produits de la mer. La production mondiale de thon atteint 3,5 millions de tonnes par an. Tous les pays producteurs sont impliqués dans le commerce international. Un tiers de la production de thon est exportée sous forme de poisson frais ou congelé. Les principaux marchés finaux sont au Japon, aux USA et en Union Européenne, comme pour la plupart des produits de la mer. L'industrie thonière est de plus en plus concentrée, avec un très petit nombre d'acteurs au niveau commercial. Tandis que le marché de la conserve de thon est stable en Europe et en réduction aux USA, le marché du thon frais ou congelé apparaît prometteur.

Mots-clés : thon, produits de la mer, commerce international

World tuna production

Tuna is one of the major products in seafood international trade. It accounts for 4 to 5% of the world fisheries and aquaculture production for human consumption.

World tuna landings are around 3,5 millions tons per year. In spite of an increasing fishing effort throughout the last decade, the landings have been quite stable from 1990 to 1997. Nevertheless, some variations can occur from one year to another and between the different fishing zones. The last three years have been much above the average with a production reaching 4 millions tons (Table 1).

Table 1. World tuna production by countries (1000 metric tons)

	1995	1996	1997	1998	1999
Japan	628	547	630	659	578
Indonesia	261	298	303	395	414
Taiwan	363	382	354	460	405
Spain	285	252	243	234	306
USA	224	206	205	217	217
Philippines	171	171	177	200	204
Ecuador	58	75	113	116	199
Korea	218	196	212	255	182
France	175	165	138	127	154
others	1024	1030	1132	1151	1304
total	**3407**	**3322**	**3507**	**3814**	**3963**

Source: FAO Globefish, 2001

The main fishing zones are the Pacific Ocean (65%), the Indian ocean (22%) and the Atlantic ocean (12%). Eastern Central Pacific and Western Indian are the zones where tuna fisheries have recently expanded most.

The commercial name tuna is used for many species, the most important of which are skipjack (2 millions mt), yellowfin (1,3 millions mt), bigeye (400 000 mt), albacore (252 000 mt) and bluefin (80 000 mt). Skipjack landings have increased by 80% over the last decade (Table 2). Because of its large size, colour, texture and fat content, bluefin is sought-after and commands a higher price than any other tuna. The Mediterranean bluefin tuna fishery accounts for less than 1% of the total world tuna production.

Table 2. World tuna production by species (1000 metric tons)

	1995	1996	1997	1998	1999
skipjack	1655	1584	1613	1884	1976
yellowfin	1115	1083	1213	1252	1258
bigeye	374	370	387	382	400
albacore	191	198	217	230	252
bluefin	72	87	77	66	77
total	**3407**	**3322**	**3507**	**3814**	**3963**

Source: FAO Globefish, 2001

International trade of tuna

Tuna accounts for more than 10% of the world seafood international trade, and ranks second behind shrimp. All tuna fishing countries are involved in international trade. Three main products that are part of the processing chain of tuna are exchanged: whole raw tuna, tuna loins and canned tuna (Josupeit, 2000).

One third of the tuna production is exported as fresh or frozen fish. Within this category, 10% only is fresh and 90% is frozen. The main exporting countries of fresh and frozen tuna are in decreasing value Taiwan, Korea, Spain, France, Japan and Indonesia (Table 3). Indonesia and Taiwan are specialised in fresh tuna exports flown out by air.

Table 3. Exports of fresh and frozen tuna in million US$ (1999)

	value	share
Taiwan	1190	42%
Korea	292	10%
Spain	254	9%
France	121	4%
japan	112	4%
Indonesia	107	4%
others	757	27%
Total	**2832**	

Source: FAO Globefish, 2001

The main importing countries are by far Japan, then Thailand, USA and Spain (Table 4). Except in Japan where the main use of imported tuna is raw sashimi, these imports are mainly directed to the canning industry. Japan is the first importer of fresh tuna (70 000 metric tons per year). Europe is importing more and more loins because they require less manpower than whole frozen tuna in the canning industry.

Table 4. Imports of fresh and frozen tuna in millions US$ (1999)

	value	share
Japan	1307	39%
Thailand	724	22%
USA	237	7%
Italy	125	4%
Singapore	99	3%
Spain	87	3%
others	746	22%
Total	**3325**	

Source: FAO Globefish, 2001

As for canned tuna, the main exporting countries are Thailand, Philippines, Ivory Coast and Spain. In spite of a limited fleet, Thailand has become the first exporting country of canned tuna and plays a major role in production and trade of cans and of loins for other processing countries (Peckham, 1998). Ecuador and Columbia are the main suppliers of loins on the market.

The main markets for tuna

The main final markets for tuna are in the triad, i.e. USA, Japan and European Union, like for most seafood products. Japan is by far the first market with more than 600 000 mt per year, mainly fresh or frozen. The Japanese market depends on imports since almost 300 000 mt are imported each year (Table 5).

Table 5. Imports of tuna by Japan in 1999 (metric tons)

	fresh	frozen
skipjack	167	76 087
albacore	397	1 790
yellowfin	33 405	71 739
bigeye	24 085	108 831
bluefin	9 452	9 301
total	**67 506**	**267 748**

Source: Tanabe, 2000

The first species on the Japanese market are skipjack, then bigeye and yellowfin. Bluefin is the most desired and expensive species, but in limited volumes. Bluefin market share is only 3% in volume and 10% in value.

The sashimi market is the most important in Japan for tuna and amounts to 500 000 metric tons per year. This market is more and more supplied by frozen tuna, especially for supermarket sales. Fresh tuna imports (70 000 metric tons per year) represent only 25% of the total tuna imports in volume. These imports of fresh tuna which are transported by air are composed of yellowfin (50%), bigeye (30%) and bluefin (15%).The import price of yellowfin and of bigeye is around 5 US$/kg while the import price for bluefin is around 20 US$/kg. The price of imported bluefin depends on the origin, with a premium for Spanish farmed bluefin tuna (35 US$/kg).

The American market which used to be focused on canned yellowfin is more and more eager to buy fresh and frozen tuna, as a substitute to beef steak. Thailand is the main supplier of USA for cans, and Ecuador for loins. There is a drop in the can consumption, with a transfer from can to pouch.

The European market revolves around canned tuna, with a consumption assessed to 500 000 t per year. The four main European markets are Italy, Spain, France and United Kingdom. The market for fresh tuna, mainly albacore and bluefin is expanding, based on the catering sector, but still marginal and located along the coast. In France, there are more and more imports of IQF tuna steaks from liners operating in the Central Pacific ocean (French Polynesia and New Caledonia).

Evolution of the tuna market

The tuna industry is more and more concentrated, with a very limited number of actors at the marketing level. These firms have heavily invested in the production and processing sectors, with consequences on the profitability in case of bad economic context. Nevertheless, the tuna market has been able to cope with the Japanese economic recession thanks to the diversity of its outlets: different products (cans, pouches, fresh steaks, ready meals) and different markets (USA, Japan, Europe).

After two years of extremely low prices, the tuna market stabilised in 2001 at a level considered economically viable by the operators, thanks to voluntary fishing bans (Fig. 1). Prices of canning material are expected to stay stable at the present levels, while the sashimi market depends on the quality of the fish and of the demand in Japan.

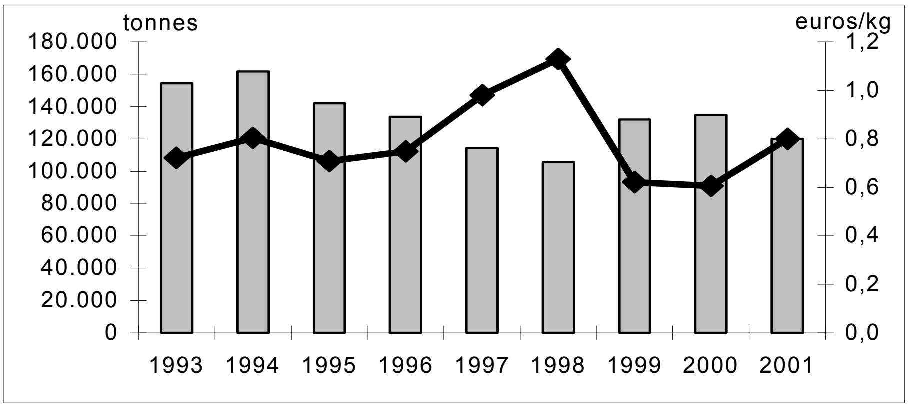

Fig. 1. French frozen tuna landings and price 1993-2001 (source OFIMER)

New products for new markets ?

While the market for canned tuna is stabilised in Europe and recessing in the US, the market for fresh or frozen tuna has good perspectives of development. After a long period of recession, the Japanese sashimi market is improving in volume, although not in price. But the mad cow crisis which has strongly hit the Japanese beef market will likely induce more consumer purchase transfers towards poultry and pork than towards fish on the long term (Katsuyama, 2000). In France, for instance, the positive impact of the mad cow crisis on the market for fresh fish has lasted only six months. That is the reason why the sashimi Japanese market which relies more and more on cheap frozen tuna may not be the only target for farmed bluefin tuna on the long term.

In Europe, the consumption of fresh and frozen fillets of fish has been increasing for the last ten years at a moderate but regular pace, thanks to the development of new species from fisheries like hoki, orange roughy, Nile perch or from aquaculture like tilapia. This market is a wide market of several hundred of thousand tons, but with heavy price constraints since the wholesale price of fresh fillets of fish has to be under 6 to 7 US$/kg.

Indeed, in a situation of saturation of proteins, there is a strong competition between meat and fish products. Therefore, fish has to prove it has a good value for price in terms of sanitary quality, traceability, cooking easiness and nutritional composition. On the European market for fish, a real qualitative effort is required to increase the market share of tuna, as well for fresh products as for processed products. These efforts have to focus on onboard handling and stocking, standardisation, labellisation and marketing.

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Morphological and histological changes of the parenchimatous organs of Bluefin Tuna, *Thynnus thynnus* (linnaeus, 1758)

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SUMMARY – An account is given of pathology examination made on cage reared Tuna. The three main findings were the presence of chronic granulomatous splenitis, acute cholangiohepatitis and pasteurellosis.

Keywords: Post Mortem, spleen,liver, pasteurellosis, splenitis, cholangiohepatitis, Tuna

Introduction and Methods

During last the two years, tuna on-growing has reached 1000 T of harvested fish. As far as health problems progressed during the cage rearing, in the period of June – December, there were no morbidities or mortalities reported.

In December 2001, during processing and packing of harvested tuna, extracted organs were examined for any abnormalities or tissue changes. Post mortem examination included spleen, liver, stomach, intestine, kidney. Time of sampling and examination was one hour after death. Cause of death was brain pithing with a by sharp object. From the moment of death carcasses were conserved on ice. A total of 25 harvested fish were examined.

After gross morphological examination, tissues were fixed in 10% buffered formaldehyde, processed, and stained with haematoxylin-eosin.

Results

The following findings were observed:

Chronic granulomatous splenitis – spleen was enlarged with rugged surface. The colour was lighter than other spleens examined. On the sections, multifocal chronic granulomatous changes were evident, encircled with strong cellular infiltrations. Some solitary short plump rods, measuring 2-3μ were observed on the edges of the granulomas. The granulomas seemed to be not active and process seemed to be in the stadium of resorption. Similar changes were seen in the case of chronic pasteurellosis in adult sparid brood fish.

Acute cholangiohepatitis - liver on gross examination had punctual green spots. On the sections there was evident bile stagnation, cloudy swelling, and picnosis of the hepatic cells. In some areas, haemorrhages followed the inflammatory changes. Cell infiltration followed tissue injury.

During this examination, organs examined were considered more as indication of the health status, rather than the disease of BFT since there was no evidence of morbidity. Till now, noncaseating multifocal granulomatous changes in spleen, in Mediterranean finfish are characteristic changes for Pasteurellosis, caused by bacterium *Photobacterium damsella subsp. piscicida*.

Malta national program towards farming of the Bluefin Tuna present and future

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SUMMARY – The exploitation of Tuna as a farming and fattening facility are outlined for the island of Malta. The present situation and the planning for the future is also discussed.

Keywords: Tuna fattening, Aquaculture Policy, body weight, feeding, cages

In Malta, blue fin tuna are fattened from the beginning of June to January, by which time they are harvested and exported to Japan. The farming of this species around the Maltese islands started in 2000 with one farm producing 330 tonnes. At present there are two farms that will export a total of about 1200 tonnes during 2001. The farms that are currently in operation stock their fish in a total of thirteen 45 and 60 m diameter offshore cages that are anchored 1 km off the Maltese coastline. The cages are HDP floating circular cages. Cages are moored independently, with a minimum depth of 38m and maximum depth of 55m.

The tuna are caught by purse-seine fishing and weigh between 50 and 300 kg. They are fed on Mediterranean frozen fish such as sardines and mackerel. The financial FCR achieved over a period of 180 days is 13. Feed percent varies from 2.21% body weight (BW) to 2.73% at the maximum seawater temperature of 26^{o}C. SGR from 0.17 to 0.21. The average BW for one cage is 116.6 kg at the beginning of ongrowing in June, increasing to 159.4 kg on harvest in December. The minimum and maximum seawater temperatures are 14^{o}C in February and 26^{o}C in September, respectively.

Over the next two years another three companies will start tuna farming around the Maltese islands and export figures are expected to escalate to 2500 tonnes per year. The Malta Centre for Fisheries Sciences (MCFS) is in the process of starting research projects for the development of techniques for broodstock rearing, egg collection and larval development. Moreover, the Laboratory of Fish Diseases within the MCFS is collecting tissue samples from harvested tuna.

A revised National Aquaculture Policy to be adopted in February 2002, will control the issue of licences to aquaculture operations, with sites having a minimum depth of 50m. The licence will also include the registered state of the seabed and obligatory environmental monitoring with yearly revisions of the licenses. Immediate research priorities are the reproduction of BFT and environmental impact studies.

General introduction about topics of interest in BFT research :a fishery and aquaculture integrated challenge

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SUMMARY - A three step procedure is outlined for the devlopment of BFT aquaculture entailing (i) Identifciation of the major challenges (ii) Translation into scientific questions (iii) Structured work packages to answer these questions.

Keywords Sustainability, priority planning, support, workshops

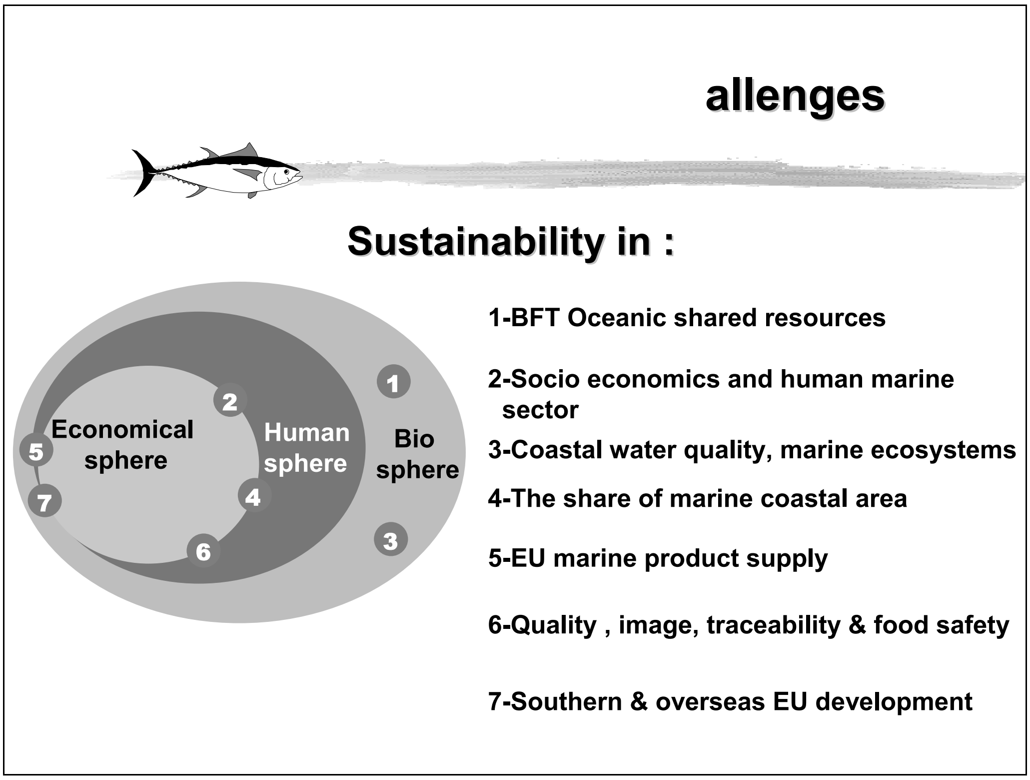
The aim of the research community in the identification of topics of interest in BFT Research is to serve human society for a sustainable development.

In order to fit this aim we propose the following 3-step procedure.

(i) First of all there is a need for a clear identification of the major development challenges for Society : At the regional, national and EU level, at the administrative, political and policy level, from fisherman, aquaculturists, environmentalists and the scientific community.

The scientific community can greatly help Society by determining the state of the science of the BFT and develop links, common shared values and recommendation between these different communities, which was one of the major aims during first days of the tuna conference.

The position and nature of the major development challenges identified, are summarized and shown in the following figure.



(ii) The second step:

These different challenges need now to be translated into scientific questions by scientists.

(iii) The third step:

once translated, these scientific question are structured into research work packages clustered within a scientific programme. These programmes can then be submitted to policy makers for financial support. Together these two new steps constitute the goal of the workshop sessions held in Cartagena.

The research requirement can be dispatched by taking in account three different requirements:

i. Support to Tuna farmers
ii. Answers to Human Society queries
iii. Resolution of bottlenecks for future development

We can now identify our research field in term of priority planning
(i) Immediate support to Tuna farmers: for biotechnological support

- Cage and mooring engineering
- Nutrition
- Flesh quality
- Behaviour
- Pathology
- Food safety
- Market accessibility
- Economic analysis

(ii) Immediate answers to Society: for a secure and sustainable integrated development

- Ecological impact at the local scale
- Ecological impact at the global scale
- Socio economical impact at the local scale
- Socio economical impact at the global scale
- Tuna farming impact at the market

(iii) Medium term resolutions of main potential bottlenecks to future development: for a responsible and precautionary approach

- Sustainability of BFT resources for fattening development purpose
- Sustainability in BFT food supply
- Market sustainability

To achieve these 3 goals will require specific research actions: There is need of immediate long-term research for the medium term development

i. BFT reproduction control in captivity: an ambitious and, at least, ten year objective requiring an immediate major research programme
ii. BFT food supply improvements
iii. Market trend analysis

Taking in account these different identified needs of research we propose the following workshop structure.

i. DOTT-Reproduction
ii. DOTT-Genetics
iii. DOTT-Husbandry (biology)
iv. DOTT-Husbandry (engineering)
v. DOTT-Socio-economics & environmental impacts
vi. DOTT-Larval & juvenile rearing

Engineering tasks for BFT quality assurance

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SUMMARY - Due to the spread of blue fin tuna farms and processing plants, automation (among many other engineering techniques), may help in the development of effective quality assurance methods. This paper seeks to display the technological resources available, as part of an ideal system conceived to achieve this goal from a global standpoint.

Key Words: Engineering resources, Intelligent cages, Onboard , Plant Processing

Motivation

As in many other fields, the needs detected by biologists, scientists and farmers in the BFT farming industry should be covered by the developments offered by the engineers. As in the starting point of any other technological design, it is necessary to know the final scope of development in order to set the capabilities/cost balance.

At this point, it should be said that this ratio will be different for scientific and for industrial purposes due to the difference in pursued results; thus leading to the first restrictions for the design. In scientific plants, information data should have priority over the efficiency of the production; while in industrial farms, is obvious that this balance will be in the opposite direction. In order to cover both worlds, the scientific and the industrial, this paper will consider the design of a integral system that would cover the necessities of both parts.

Our proposal

The improvement of the BFT processing chain should be centered on the development and adoption of specially designed techniques to assure an increase in: Quality assurance, Product tracking and Process automation.

Going further, these three topics should be approached from a common waypoint in order to maximize the pursued benefits in front of the economical effort for the adoption. On the other hand, the final system should integrate all of the proposed techniques within the managing system at production in order to get the desired improvements.

The proposed system will be structured in three stages, covering the whole process from the feeding of the tuna at the cages, the harvesting and preprocessing onboard and the final processing in the plant; each one of these should be set as follow:

Intelligent cages

Quality assurance should start at the feeding stage in the nets, recording the different data such as the kind and amount of feed, conditions of the exploitation environment, fish population and many others. This stage will comprise the design of an "Intelligent Cage" fitted with a transducer network specially conceived for the measurement of several physical and chemical parameters of the farming cage environment.

The sensing network, (Fig. 1), could include information for the measurement of different parameters such as the water temperature, turbidity and speed, asses the fish population conditions (such as biomass estimation through acoustic techniques, presence of other species, etc.), and relay information about the strain forces in the net and the mooring of the cage as in Bugrov, L. (1997). Due to the natural trend to force cage location off-shore, a GPS or Galileo positioning system receiver will help in the detection of the net in case of mooring system failure (Savage, G.H. *et al.* 1997). In order to acquire the data, a solar powered autonomous data logging system would send all the information data through a standard communication channel (RF, GSM, GPRS, UMTS), in response to remote query commands or local alarms, to the host placed either onboard or in plant, and indexed within the exploitation data base.

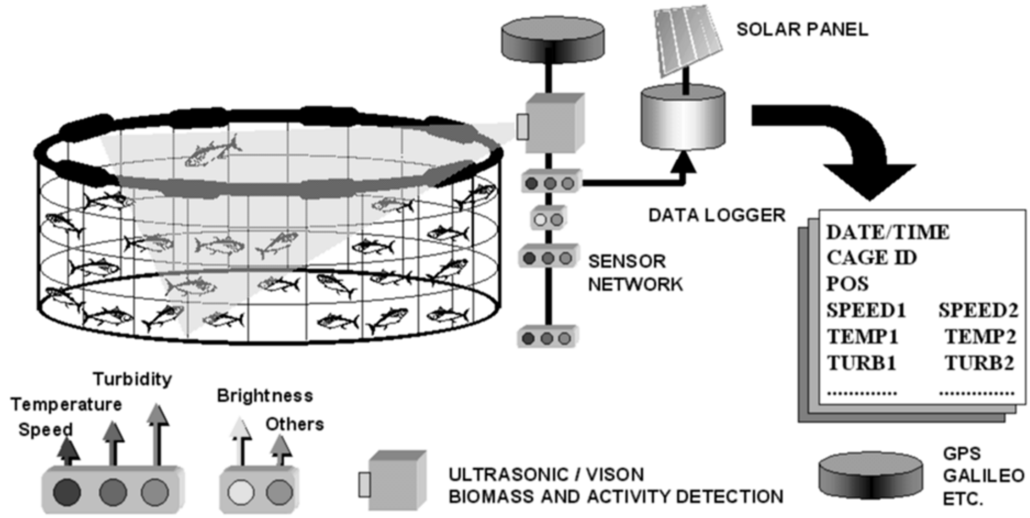

Fig. 1. Intelligent Cage: Sensing System

On the other hand, the cage should be fitted with some additional installations not covered by the existing regulations in order to extend current systems capabilities. In this line, a hydrophone network could offer the possibility of tuna sound recording in order to analyze the differences with the wild behavior, and set feeding strategies such as those used in other inland farms. This point could also be achieved by means of a water sprinkling system designed to alter the water surface as the little fish do in nature in order to induce the feeding (Fig.2).

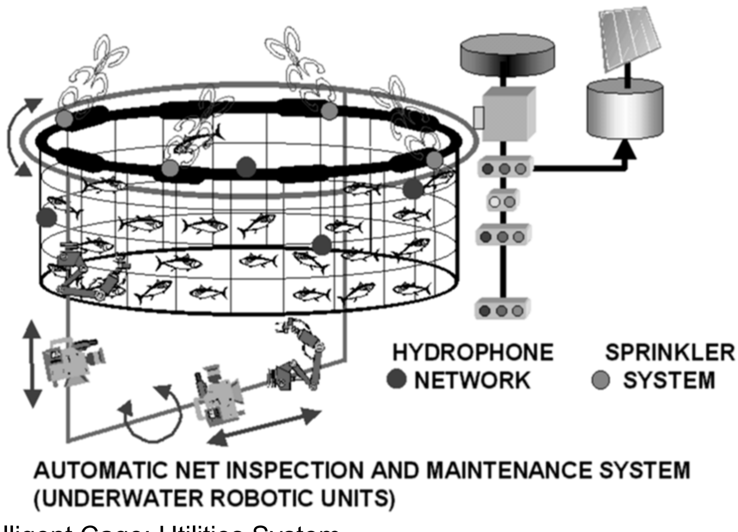

Fig. 2. Intelligent Cage: Utilities System

ONBOARD PREPROCESSING

After the slaughtering onboard, each one of the fishes should be tagged by means of an electronic transponder containing a chip able of recording multiple data such the weight (by means of a motion-compensated cell load at the elevating crane), dimensions (obtained by an artificial vision system), capture details (date, time, ship, fisherman, method, etc.), processing instructions (destination, method). All of these data would be available at the plant as soon as the fishes were captured in real time in order to prepare the resources for the processing work (trucks, people, packaging elements, etc.) as seen in Fig. 3.

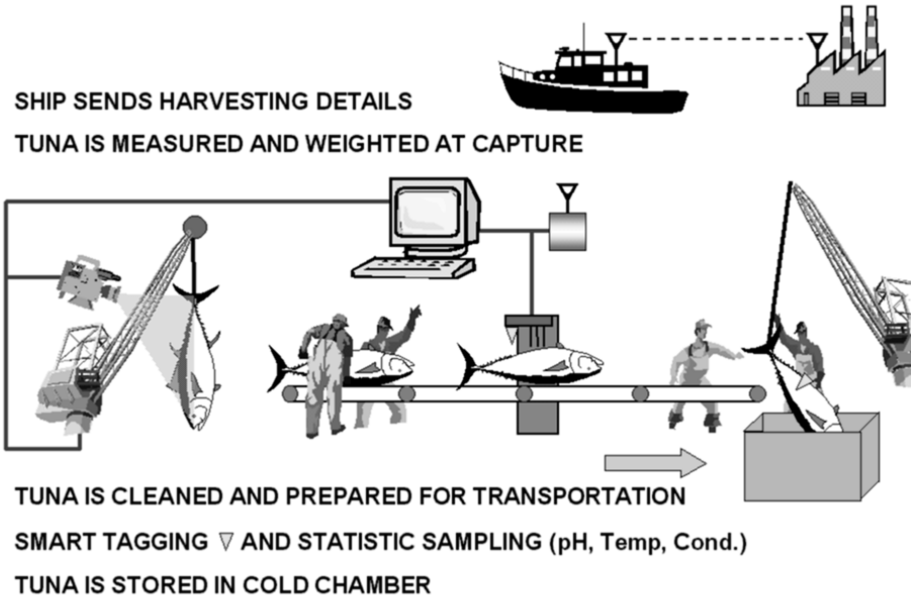

Fig. 3 Onboard Preprocessing

Plant Processing

Once the fishes were unloaded at port, these electronic tags, powered with small data logging capabilities (i-button, touch free cards, etc.), would be able to record the temperature along the transportation to the processing plant, in order to guarantee freezing chain continuity (Fig. 4.)

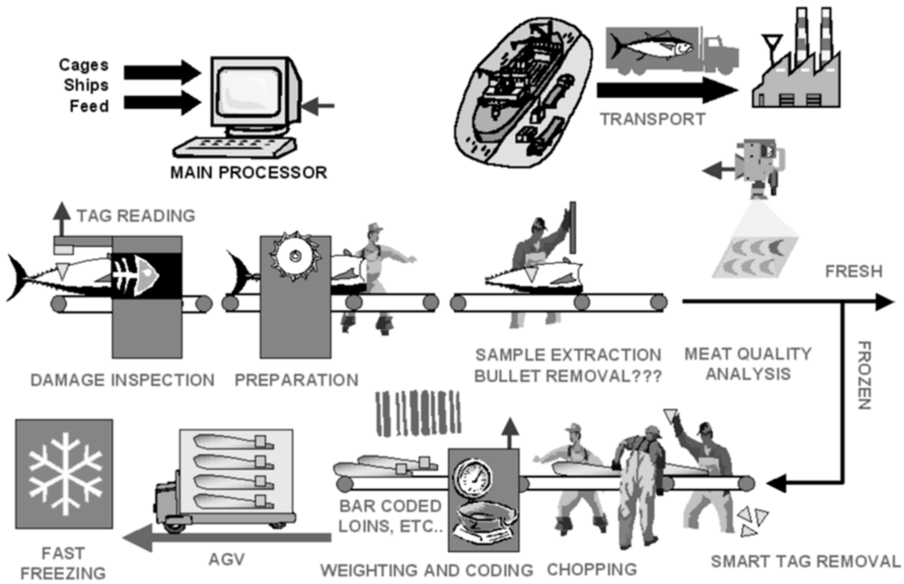

Fig. 4. Plant Processing

These tags should be automatically read once in plant to record the registered information within the processing database, in order to decide the correct processing path for each one of the fishes. Once on the processing chain, the fish would enter in the damage inspection system which would determine the effects of slaughtering (spinal damage, bullet presence, etc.) by means of an artificial vision system based upon an industrial X-rays or echo-graphic tunnel, data that will be used to select the final processing method (whole or in pieces).

In the next stage, the quality inspection, fat content, color and texture of the meat would be evaluated by means of bio-impedance measures and an artificial vision system that would objectively set the quality rank for the final product. At this point it should be important to mention the necessity for establishing quality standards in terms of quantitative indicators such as weight, fat content, pH, color, texture, etc. The packaging island would label each one of the products according to the data registered along the whole process, with a barcode or a smart label that could include a small temperature data logging system for quality assurance over the shipping to the final dealer. Additional improvements could include an autonomous guided vehicle (AGV) for automatic stock piling up at the freezing chambers (-60 ºC) as seen in Figs.5 a and b.

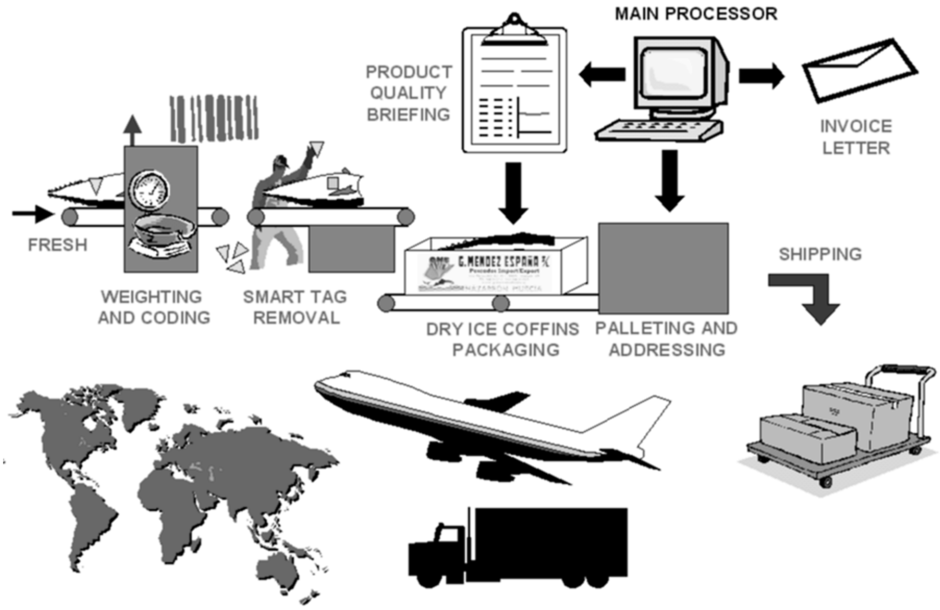

Fig. 5.a Final Product Handling (Fresh Processing)

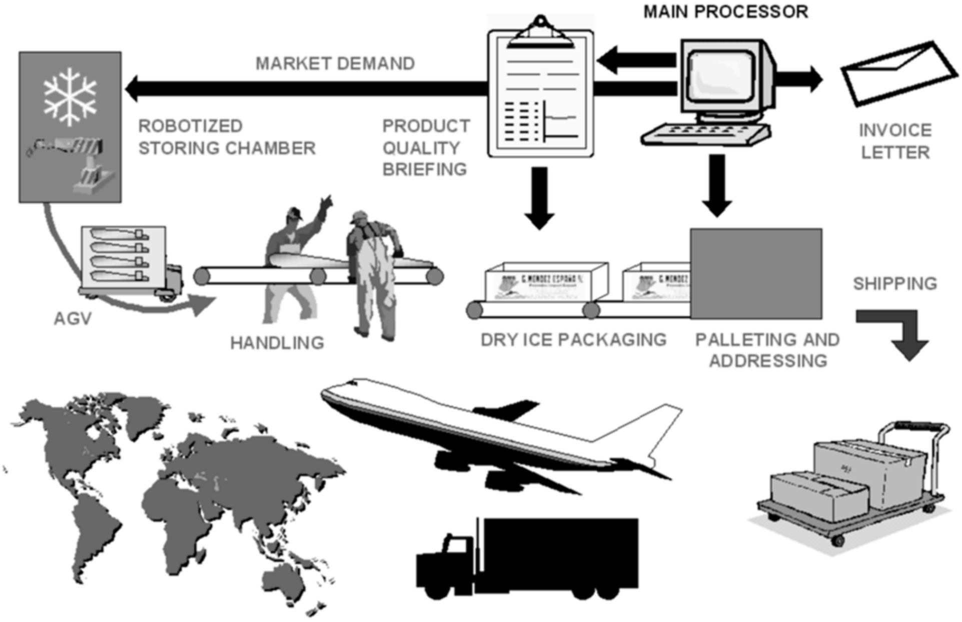

Fig. 5.b Final product Handling (Frozen processing)

Conclusions

This Sensing-Utilities-Communications architecture has proved its validity through the years in many other industrial fields and should be considered in the design of the future farms and processing plants. The introduction of new technologies in the BFT farming business, will decrease the production costs and at the same time open new research lines, favored by the availability of an integral data set of the productive process. The growth of this technology will be guaranteed by availability of existent, widely used, moderate cost standard industrial components. The proposed system, which may be seen in Fig.6, has been conceived as an integral approach to the quality assurance requirements that the emerging business of BFT has set.

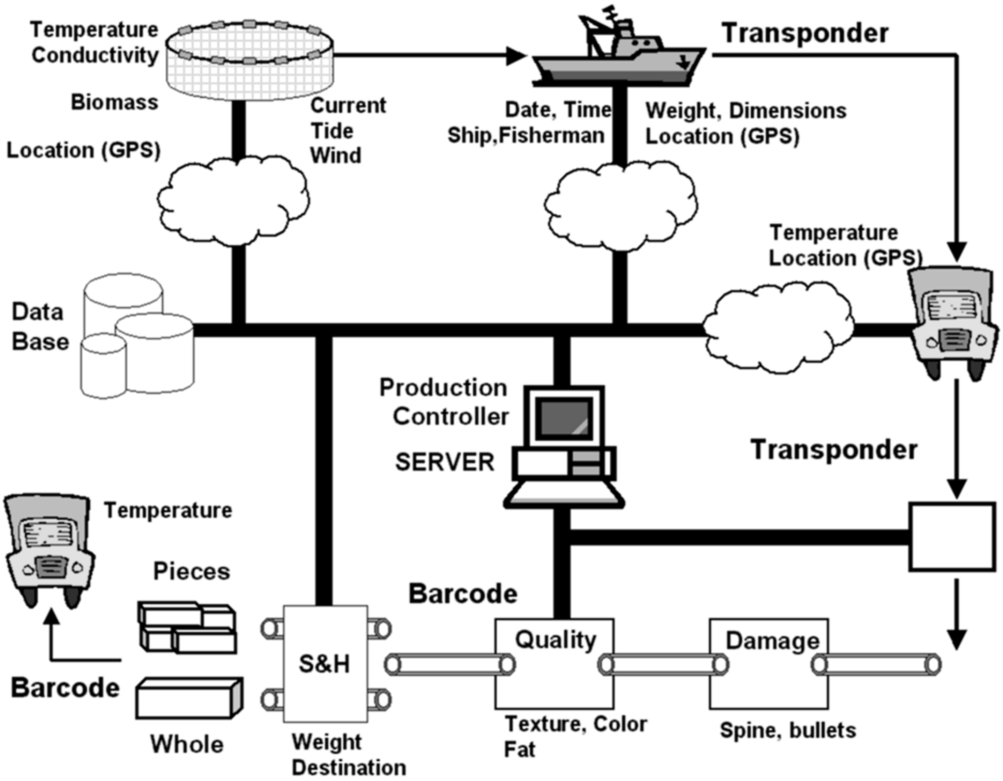

Fig. 6 Proposed System

As a last remark, it should be stated that the present solution comprises only of the technological resources available up to date. The technology is expected to develop as the BFT farming industry spreads worldwide.

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Preliminary study of the key hormones regulating reproduction in the Bluefin Tuna (BFT): the brain gonadotropin- releasing hormones (GnRHs) and the pituitary gonadotropins, FSH and LH

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SUMMARY- As a first step towards understanding the spatio-temporal profiles of the key reproductive hormones in Bluefin Tuna (BFT; *Thunnus thynnus*), the present study was aimed to assess the available immunological tools to detect the gonadotropin (GtH) LH and the gonadotropin-releasing hormone (GnRH) isoforms in tuna species. Our preliminary studies demonstrated that the heterologous ELISA, developed to measure striped bass LH (stbLH), successfully determined LH levels in pituitaries and plasma of bonito (*Sarda sarda*) and yellowfin tuna (*T. albacares*). All displacement curves were parallel to the standard curve, allowing the use of the system for determining the cognate hormone in tuna species. In addition, our specific ELISAs for GnRH isoforms successfully detected the salmon GnRH (sGnRH) and the sea bream GnRH (sbGnRH) in pituitary extracts of bonito and yellowfin tuna. Unfortunately, we could not detect the chicken GnRH-II (cGnRH-II) isoform. However, as all fish species studied to date exhibit cGnRH-II, we assume that our failure to detect this particular isoform in tuna is technical, and that tuna species, like other perciform fish posses the three GnRH isoformes: sbGnRH, sGnRH and cGnRH-II.

Key words: *Sarda sarda*; *Thunnus albacares* ; *Thunnus thynnus*; ELISA; GnRH isoforms; LH.

Introduction

The Bluefin tuna (BFT) is a highly evolved perciform species with exceedingly commercial value; nevertheless, during the last 30 years it has become a threatened species due to over fishing. In order to resolve the contradiction between environmental and fisheries needs, BFT was chosen as a prime candidate for domestication. Yet, it is clear by now that the development of current methods to induce successful reproduction in captive fish has been feasible only through a basic understanding of the species' reproductive endocrinology. Characterizing the pivotal regulators of reproduction, i.e. gonadotropin-releasing hormones (GnRHs) and gonadotropins (GtHs), and monitoring their circulating levels, were found to be indispensable for developing GnRH-based spawning induction therapies. These therapies were already found to be most effective for spawning– induction in many marine finfish of commercial importance (reviewed by Zohar, 1996; Zohar and Mylonas, 2001).

In nature, the Atlantic BFT are managed as separated eastern and western stocks, which parallel their spawning grounds, Mediterranean and Gulf of Mexico, respectively (Block *et al*., 2001). Recently, it was demonstrated that the eastern stock of BFT reaches its sexual maturity at the age of 3 years. Females of the concurrent stock are characterized by asynchronous gonadal development and daily spawning cycles (Abascal *et al*., this issue), similar to the reproductive strategy of the sea bream (Zohar and Gordin, 1979;Gothilf *et al*., 1997).

Two distinct GtHs, FSH and LH, were isolated from the pituitaries of tuna species (Koide *et al*., 1993; Okada *et al*., 1994; Garcia-Hernandez *et al*., 1997), which share the highest sequence identity with the respective hormones of other perciform fish, i.e., striped bass, tilapia and sea bream. In addition, the pituitaries of immature BFT were found to express both gonadotropins, each in a distinct gonadotropic cell type (Kagawa *et al*., 1998; Rodriguez-Gomez *et al*., 2001), resembling the situation found in tilapia (Melamed *et al*., 1998) and sea bream (Elizur *et al*., 2000). However, despite the isolation and characterization of tuna LH and FSH, until now no assays have been developed to monitor these hormones in tunas, and therefore, no information is available concerning their profiles during the reproductive cycle or in response to hormonal manipulation.

Three distinct forms of GnRH, namely: chicken GnRH-II (cGnRH-II), salmon GnRH (sGnRH) and sea bream GnRH (sbGnRH), have been characterized in the brains of all perciform fish studied to date

(Powell *et al*., 1994; Gothilf *et al*., 1995; White *et al*., 1995; Weber *et al*., 1997). Based on the resemblance of tuna reproductive features to that of several well-studied perciform fish, it is expected that the same identity of GnRH forms is conserved in BFT, however no information on this subject is available so far.

In order to expedite the study on the reproduction in BFT, the current study examined whether heterologous assay systems, available in our laboratories, are suitable for monitoring hormone profiles in the tuna.

Materials and methods

Validation of the stbLH ELISA for tuna pituitary and plasma samples

Pituitary and plasma LH content was measured using an ELISA developed to measure stbLH (Mañanós *et al*, 1997). Pituitaries from two male bonito and one male yellow fin tuna were sonicated in 200 µl of double distilled (dd) H_2O. The samples were assayed at five serial dilutions (1/2,500, 1/5,000, 1/10,000, 1/20,000, 1/40,000).

Validation of the GnRH ELISAs for tuna pituitary samples

Pituitaries of two male bonito and one male yellow fin tuna were analyzed for GnRH content using an ELISA, previously developed for the quantification of cGnRH- II, sGnRH, and sbGnRH isoforms (Holland *et al*., 1998). Briefly: pituitaries were sonicated in 200 µl dd H_2O and extracted with an equal volume of 4N acetic acid. After spin down, supernatants were collected, lyophilized and reconstituted in EIA-buffer. The samples were assayed at five serial dilutions (1/4, 1/8, 1/16, 1/32, and 1/64).

Results and discussion

In order to test the possibility of using the stbLH ELISA for LH measurement in tuna species, displacement curves obtained with serial dilution of pituitary and plasma extracts from bonito and yellowfin tuna were compared with the stbLH standard curve (Fig. 1). A clear linearity was obtained in the dilution of the plasma or pituitary of the bonito (A, C and D) and the yellowfin tuna (B). Moreover, these dilution curves exhibited parallelism with the standard stbLH enabling the determination of LH in tuna species.

A similar parallelism was obtained in linearized displacement curves of the standards sGnRH and sbGnRH as compared to serial dilutions of pituitary extracts of bonito (Fig. 2) and yellowfin tuna (data not shown). However, using the same procedure we could not detect cGnRH-II.

Thus far, we have demonstrated the ability of sGnRH and sbGnRH ELISAs to detect the respective hormones in tuna species. Yet, we ascribe our disability to monitor the cGnRH-II isoform to technical problems (which can be solved once additional samples will be obtained) and assume, therefore, that tuna species, like all other perciformes, are characterized by three GnRH forms, namely: sGnRH, sbGnRH and cGnRH-II. Our assumption is based on accumulating data, which indicate that cGnRH-II is synthesized in all studied fish, including the more phylogenetically ancient ones (Carolsfeld *et al*., 2000).

Although our findings are of preliminary nature, we believe that acquisition of immunological tools necessary to analyze these key hormones will be useful in the development of spawning-induction therapies for BFT, and will alleviate many experiments based on trial and error. Therefore, the findings should be considered an important step leading to BFT domestication.

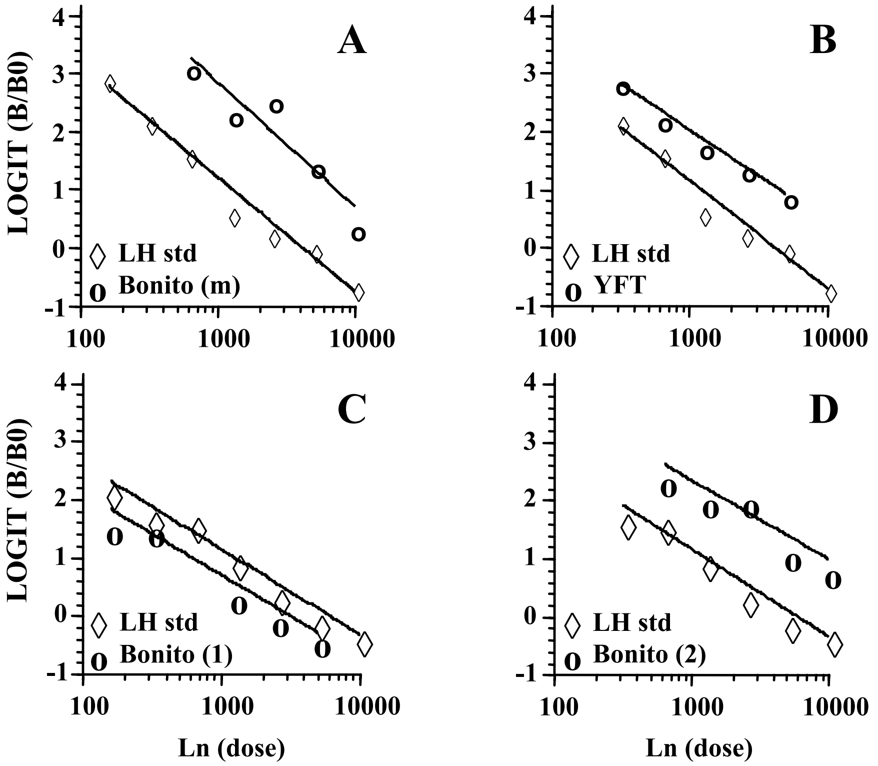

Fig. 1 Displacement curves for standard native stbLH and serial dilutions of plasma (A, B) and pituitary extract (C, D) samples from male bonito (A, C, D) and yellowfin tuna (B). The LOGIT function was utilized to transform standard curve to a linear plot. Each point is the mean of two determinations.

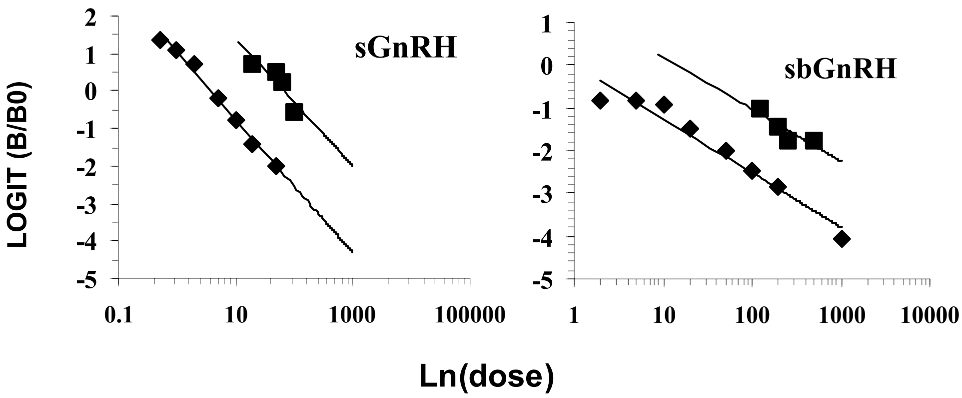

Fig. 2 Displacement curves for standard sGnRH and sbGnRH and serial dilutions of pituitary samples from male bonito. The LOGIT function was utilized to transform standard curve to a linear plot. Each point is the mean of two determinations.

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Testicular cycle of the Mediterranean Bluefin Tuna (*Thunnus thynnus* L.)

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SUMMARY – The results obtained by the histological analysis of Mediterranean bluefin tuna testes collected over a seven-month period (February-August) are reported. Five phases of the testicular cycle were characterised: 1) Quiescence (February-March); 2) Early spermatogenesis (April-early May); 3) Late spermatogenesis (middle May); 4) Spawning (late May-early July); 5) Regression (late July-September).

Key words: Bluefin tuna; Reproductve cycle; Testis; Histology; Mediterranean Sea

Introduction

The knowledge of Mediterranean bluefin tuna (BFT) reproductive cycle is limited to research based on the study of seasonal variations of the gonadosomatic index (IG) (de la Serna & Alot, 1992), and on macroscopic classification of the gonads (Rodríguez-Roda, 1964; 1967). Susca et al. (2001) carried out the first attempt to correlate vitellogenin (VTG) and sex steroid plasma levels with ovarian cycle. Here a histological description of the changes occurring in BFT testis throughout the reproductive cycle is reported.

Materials and methods

Testis samples were obtained from 81 adult (fork length ≥ 120 cm) bluefin tuna caught by professional vessels from February to September in Italian and Spanish seas. The samples were fixed in Bouin's solution, dehydrated in ethanol and embedded in paraffin wax. Sections (5 µm thick) were stained with haematoxylin-eosin.

Results

Bluefin tuna testis (Fig. 1A) is constituted by seminipherous tubules radiating from the longitudinal main sperm duct toward the testicular periphery. Testicular structure is cystic: each cyst contains germinal cells in the same development stage, branched by the cytoplasm of somatic cells (Sertoli cells). The activity of the testes showed seasonal changes allowing the characterisation of five periods during the reproductive cycle: 1) Quiescence (February-March) – Seminipherous tubules showed germinal cysts containing spermatogonia and spermatocysts. Rare spermatidic cysts and few spermatozoa in the lumina were also observed (Fig. 1B). 2) Early spermatogenesis (April-early May) – Testes showed germ cells at all stages of spermatogenesis and there was an increase in the number of spermatocytes and spermatids. Only few spermatozoa were observed in tubule lumina (Fig. 1C). 3) Late spermatogenesis (middle May) – Active spermatogenesis took place in testes. The wall of seminipherous tublues was lined with meiotic and spermatidic cysts. Spermatozoa were more abundant in the lumen of seminipherous tubules, efferent ducts and main sperm duct than in previous stage (Fig. 1D). 4) Spawning (late May-early July) – The lumen of seminipherous tubules, efferents and main sperm duct were filled with spermatozoa. Residual meiotic and spermatidic cysts were still present along the tubule wall (Fig. 1E). 5) Regression (late July-September) – Lumina of seminipherous tubules and efferent ducts were almost devoid of spermatozoa, whereas residual spermatozoa could be observed in efferent ducts and in the main sperm duct (Fig. 1F).

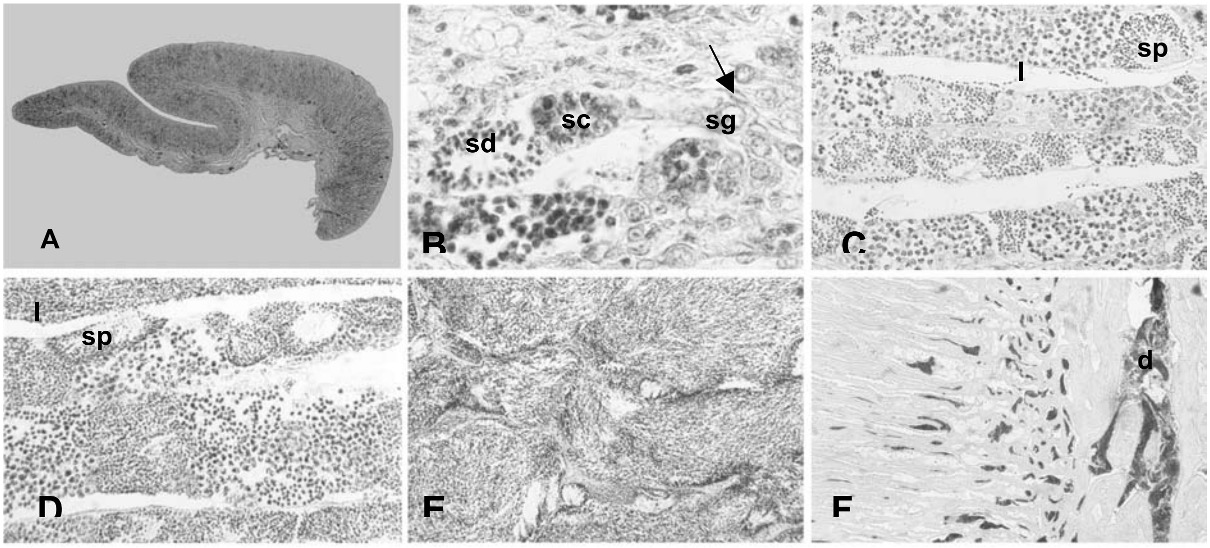

Fig. 1. - A - Photomicrographs of the testis from a BFT specimen caught in March. Magnification, x 9. B - Higher magnification of part of Fig. 1A showing a seminipherous tubule. Magnification, x 500. C - Photomicrographs of the testis from a BFT specimen caught in April. Note the presence of rare spermatozoa in the lumen. Magnification, x 250. D - Photomicrographs of the testis from a BFT specimen caught in May. Note the abundance of sperm cysts and spermatozoa in the lumen of seminipherous tubule. Magnification, x 250. E - Photomicrographs of the testis from a BFT specimen caught in June. Seminipherous tubules are filled with spermatozoa. Magnification, x 123. F - Photomicrographs of the testis from a BFT specimen caught in September showing residual spermatozoa in the efferent ducts and in the main sperm duct. Magnification, x 30. Haematoxylin-Eosin staining. Arrow, Sertoli cell nucleus; d: main sperm duct; l, lumen of seminipherous tubule; sg, spermatogonium; sc, spermatocytic cyst; sd, spermatidic cyst; sp, spermic cysts.

Conclusions

The results obtained in this study show that maturity development of BFT testes starts in early spring with the renewal of spermatogonial mitotic activity. Testes are full mature from late May to the end of July when seminipherous tubules, efferent ducts and main sperm duct are filled with spermatozoa. The quiescent phase starts at the end of July when testes appear to regress, spermatogenetic activity is stopped, and only residual spermatozoa can be observed.

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Results from project COPEMED '*large pelagic* applicable to Bluefin Tuna aquaculture

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SUMMARY:-This paper review the results obtained in the FAO-COPEMED project on "Large pelagic" fish, which may have applicability into the bluefin tuna aquaculture development in the Mediterrean.

Key words: Bluefin, fisheries, catches, length distribution, maturity, aquaculture

Taking into account the development of Bluefin Tuna (BFT) in the Mediterranean Sea. Considering that the Scientific Committee of ICCAT recommended further research in BFT aquaculture. Having in mind that BFT could be a possible way to control fishing effort and to optimise allowed catches (TAC), currently a management tool for BFT fisheries, Project COPEMED '*Large Pelagic* preliminary results could significantly contribute to establishing and developing BFT aquaculture:

A.- From the fishing point of view, preliminary results of Project COPEMED *'Large Pelagic* can contribute with some information about where, when, which fishing gears and the amount (yield) of BFT that can be caught alive for subsequently processing in the aquaculture installations. (Fig. 1)

B.- From the biological point of view, preliminary results from Project COPEMED '*Large Pelagic* would contribute with some information about the biological characteristics of BFT caught for farming:

1. BFT size: length distributions by gear (trap and/ or purse seine) by spatial- temporal strata. (Fig. 2)

BFT vitality condition: condition factor or fattening degree based on length- weight relationships by fishing gear and spatial- temporal strata. (Table I and Fig. 3)

2. Differences in yield: female and male proportion by length class, fishing gear and spatial- temporal strata. (Fig. 4)
3. Demographic composition: growth studies: age- length keys. (Fig. 5)

Recommended fishing periods: Trends in BFT sexual maturity by fishing gear and area by means of Gonadosomatic Index analysis. (Fig. 6)

4. Spawning foresight: length/ Age at first maturity by means of histological analysis of gonads in order to develop reproduction techniques during captivity. (Fig. 7)
5. Guarantee capital investment: trends in abundance (Abundance Indices) by fishing gear and spatial- temporal strata. (Fig. 8)
6. Farming installation location and adaptability: BFT and environment interactions for consecutive physiological stage (genetic and trophic migrations, reproduction, etc. (Fig. 9).
7. Profitability: studies on stock structure and genetic identification. This factor is considered as highly important due to its association with essential biological parameters for farming purposes such as: growth, sexual maturity (and fecundity) as well as acclimatisation to the environmental characteristics of the farming areas. (Fig. 10)

C.- Data on export**:** Finally, Project COPEMED may propitiate the development of a biological sampling plan addressed to obtaining several biometrics relationships as well as conversion factors in order to improve ICCAT's BFT Statistical Document.

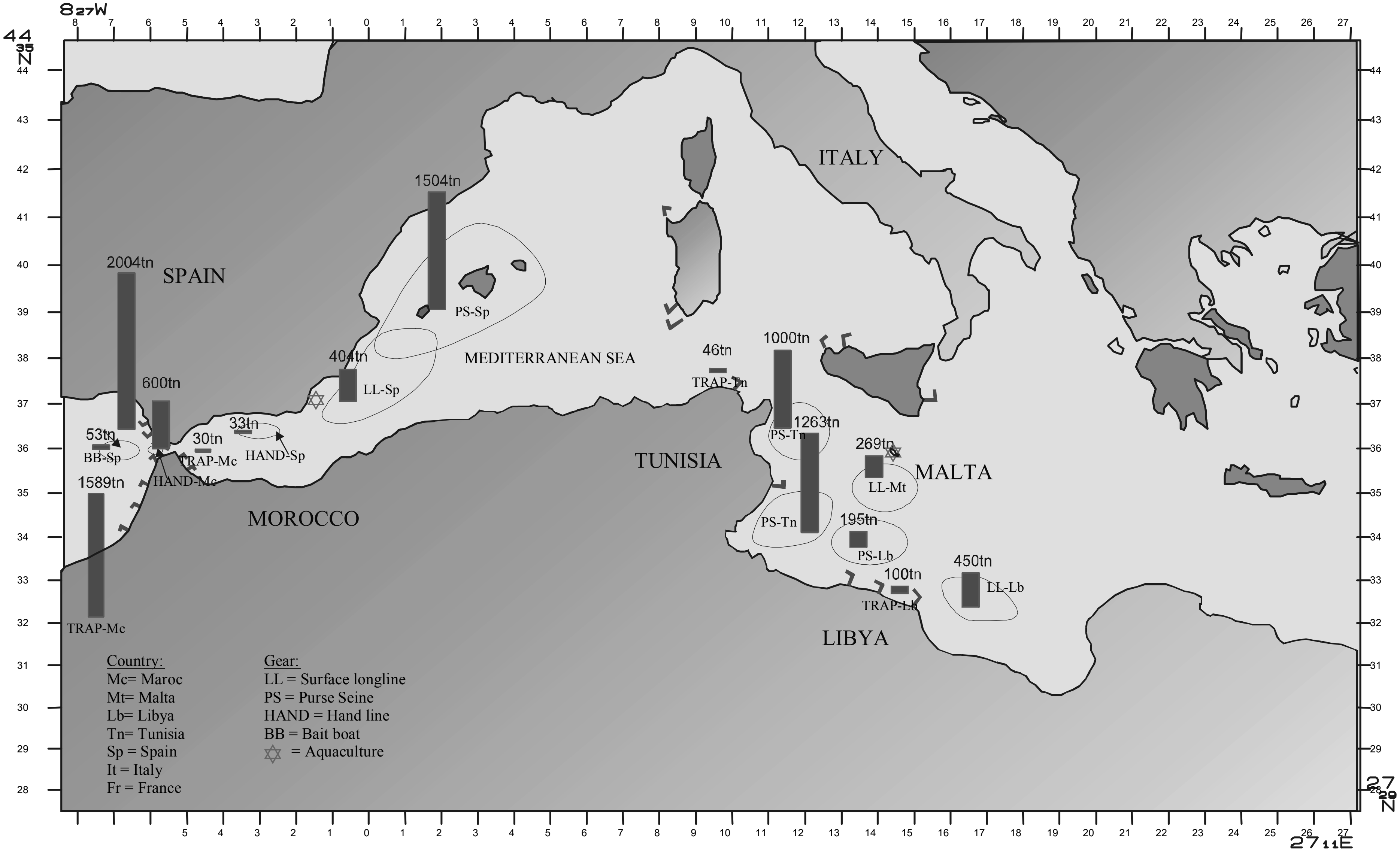


Fig. 1- Bluefin tuna fisheries for participant countries in Project FAO-COPEMED

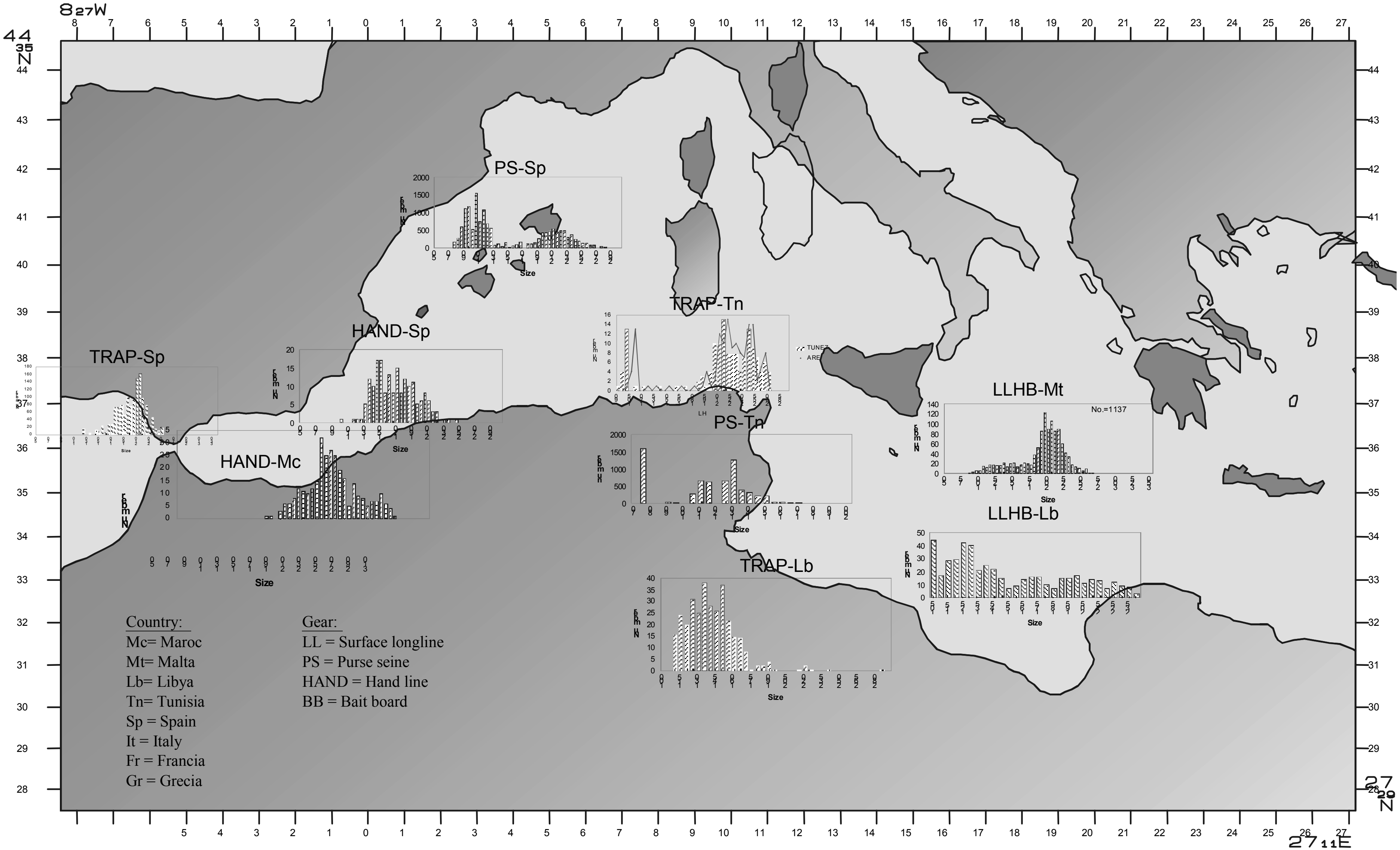
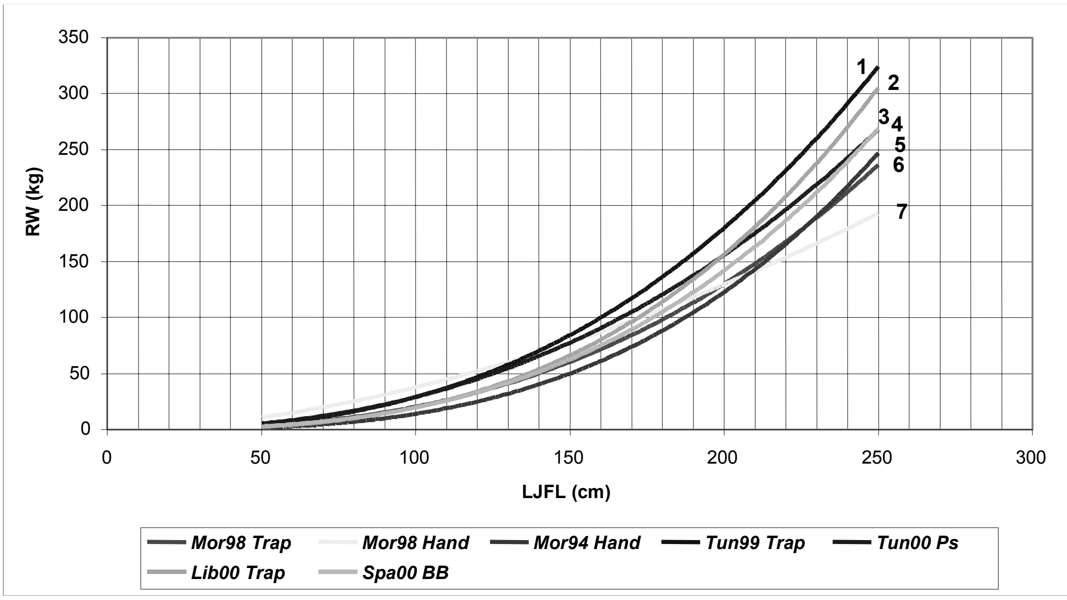


Fig. 2. Length distributions by gear (trap and/ or purse seine) by spatial- temporal strata (bluefin tuna size). Project FAO-COPEMED

Table I. Length-weight regression line by fishing gear and country.

Specie	Regression line*	Fishing gear**	Year	nº fish	Country
BFT	$RW=0.0101LF^{1.7854}$	HAND	1998	65	Morocco
BFT	$RW=9E\text{-}05\ LF^{2.677}$	TRAP	1997	54	Morocco
BFT	$RW=15.3*10^{-5}LF^{2.6381}$	TRAP	1998	118	Tunisia
BFT	$RW=3.74869E\text{-}05*LF^{2.8589}$	BB	2000	503	Spain
BFT	$RW=0.0004\ LF^{2.4295}$	PS	2000	200	Tunisia
BFT	$DW=0.0026\ LF^{2.0775}$	LL	2000	141	Malta
BFT	$RW=2E\text{-}05\ LF^{2.9957}$	TRAP	2000	197	Libya

* RW: weight; LF: fork length.
**HAND: hand line; BB: Bait boat; PS: purse seine; LL: surface longline.



1.- 1.53E-04 * RW ^ 2.6381
2.- 2E-05 * RW ^ 2.9957
3.- 4E-04 * RW ^ 2.4295
4.- 3,74869E-05 * RW ^ 2.8589
5.- 7.6416E-06 * RW ^ 3.1316
6.- 9E-05 * RW ^ 2.677
7.- 0.0101 * RW ^ 1.7854

Fig. 3. Condition factor or fattening degree based on length- weight relationships by fishing gear and spatial-temporal strata (BFT vitality condition). Project FAO-COPEMED

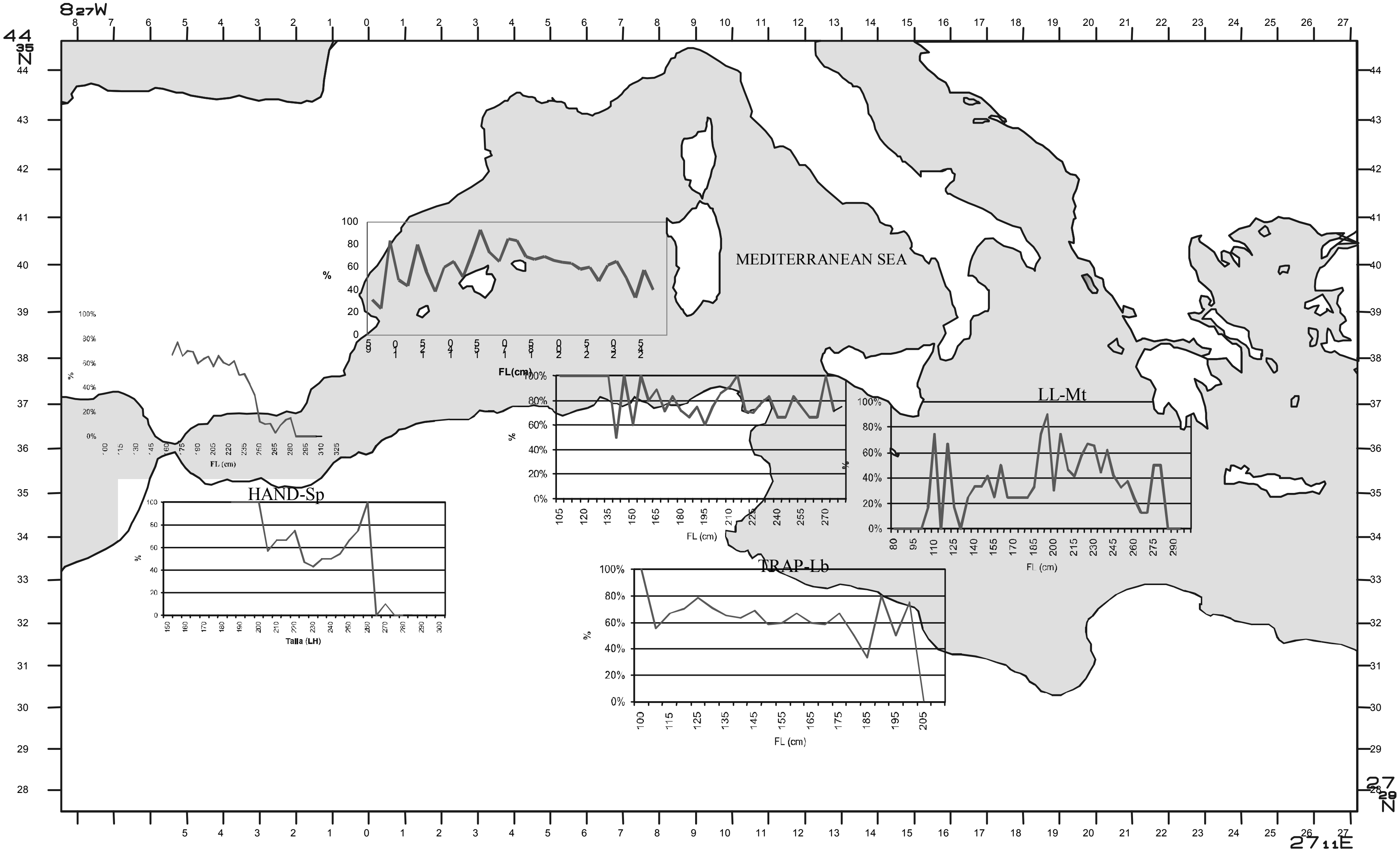
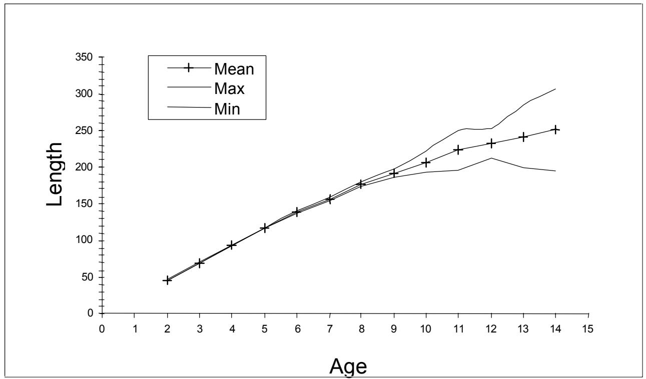


FIG. 4. FEMALE AND MALE PROPORTION BY LENGTH CLASS, FISHING GEAR AND SPATIAL-TEMPORAL STRATA (DIFFERENCES IN YIELD).

PROJECT FAO-COPEMED

	Age											
Length	4	5	6	7	8	9	10	11	12	14	16	Total
110-												
115-	2											2
120-		2										2
125-	1	3										4
130-		1										1
135-		2	3	1								6
140-				1								1
145-			4	2								6
150-			4	3								7
155-			7	2								9
160-			2	7	1	1						11
165-				4	2		1					7
170-				2	4							6
175-					2							2
180-				3	1	1	1					6
185-					1							1
190-					1				1			2
195-					1					1		2
200-					1	2						3
205-						1						1
210-												
215-								1				1
220-						1	1					2
225-												
230-							1					1
235-												
240-										1		1
245-												
250-												
255-												
260-												
265-								1				
270-												
275-												
280-												
285-									1		1	2
Tota	3	8	20	25	14	6	4	2	2	2	1	87



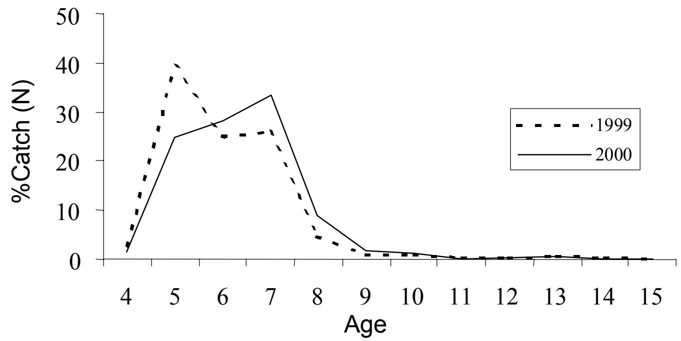


Fig. 5. Growth studies: age- length keys (demographic composition). Project FAO-COPEMED

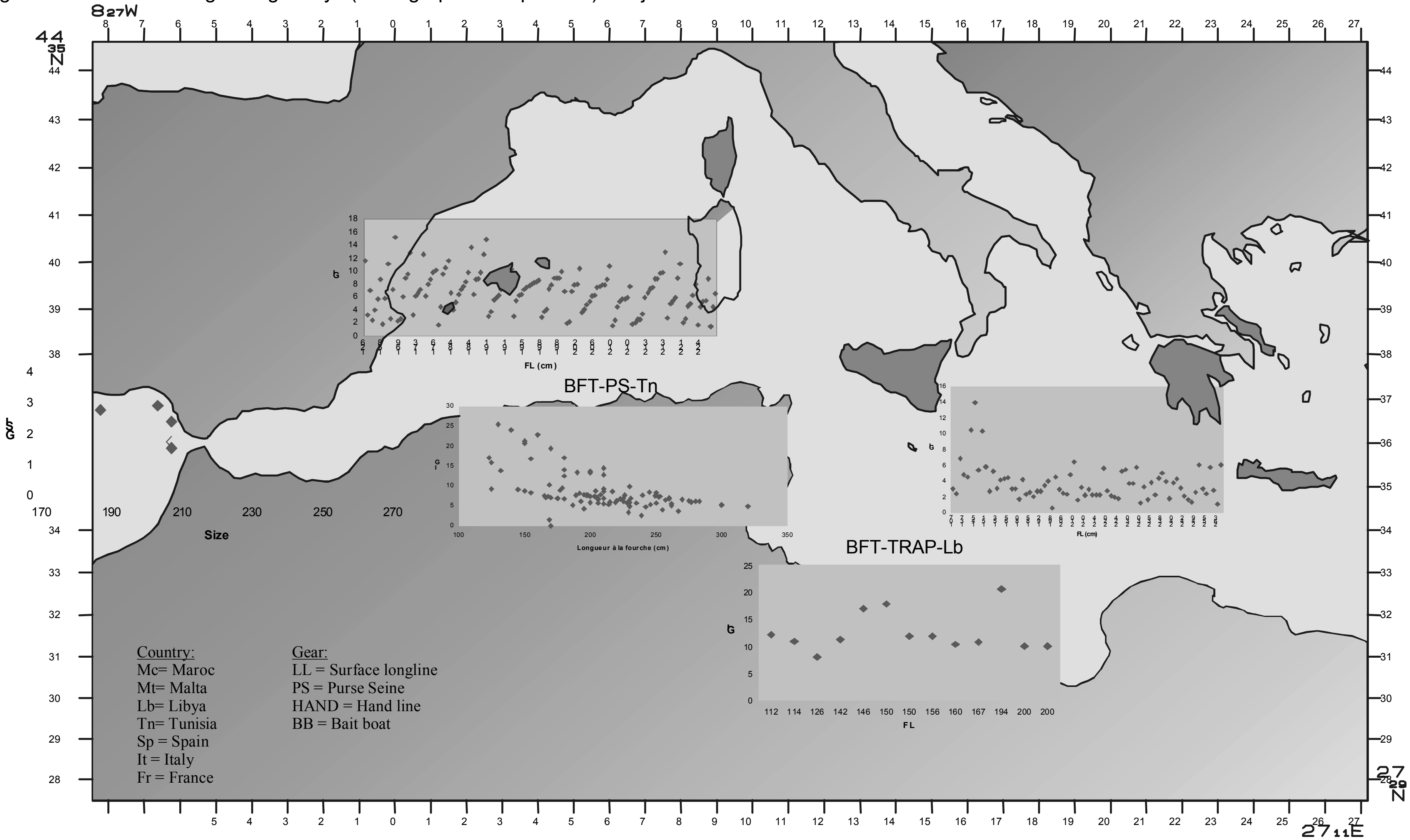


Fig. 6. Trends in Bluefin tuna sexual maturity by fishing gear and area by means of Gonadosomatic Index analyses (recommended fishing period). Project

FAO-COPEMED.

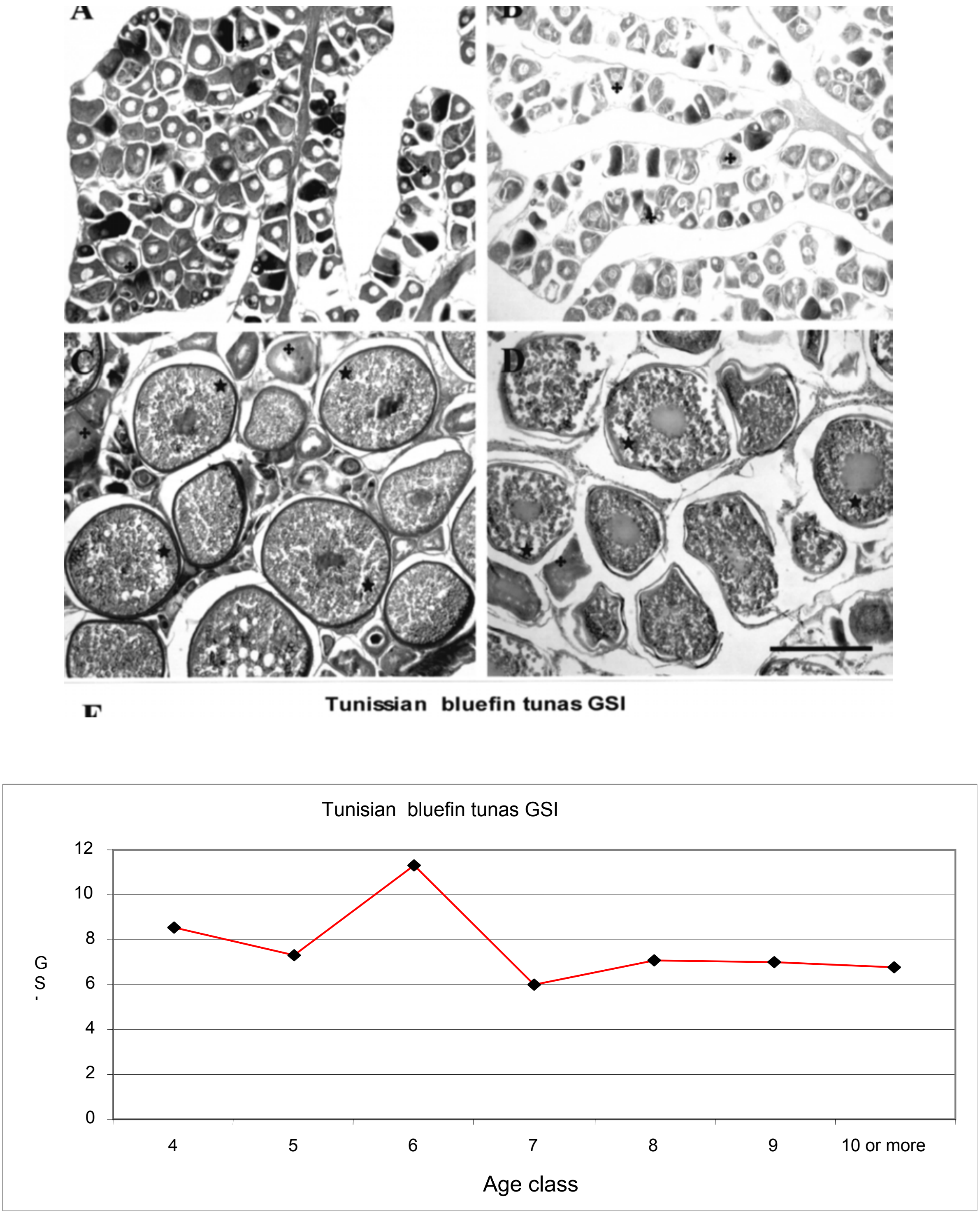
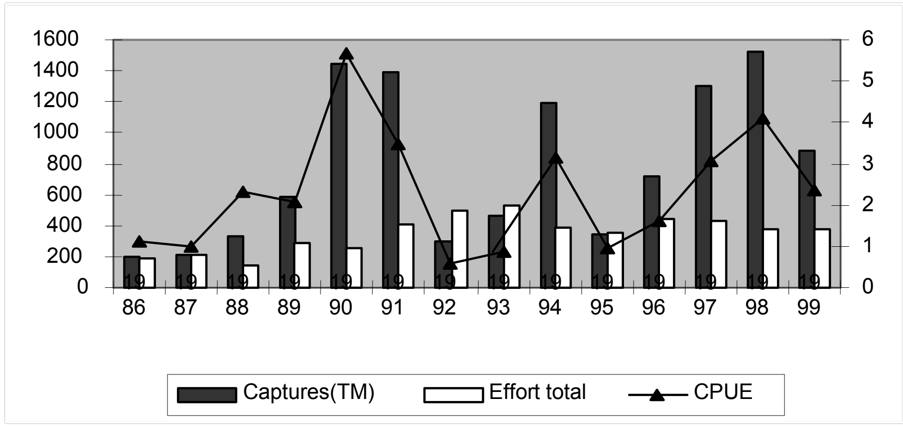
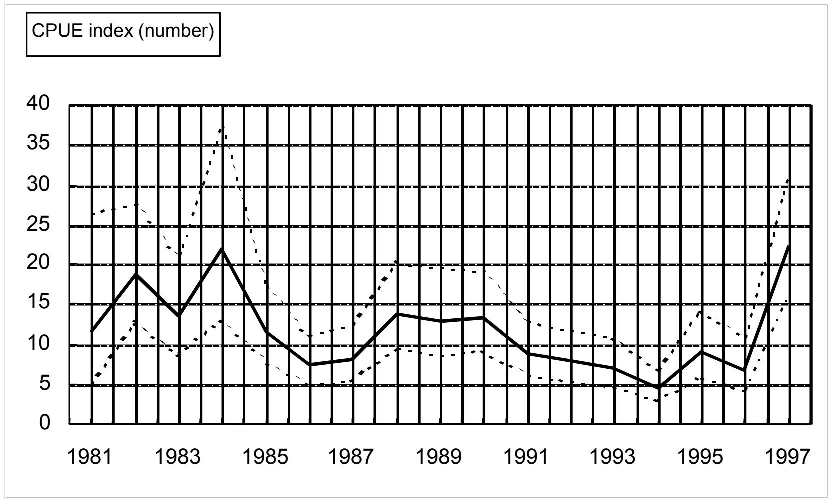


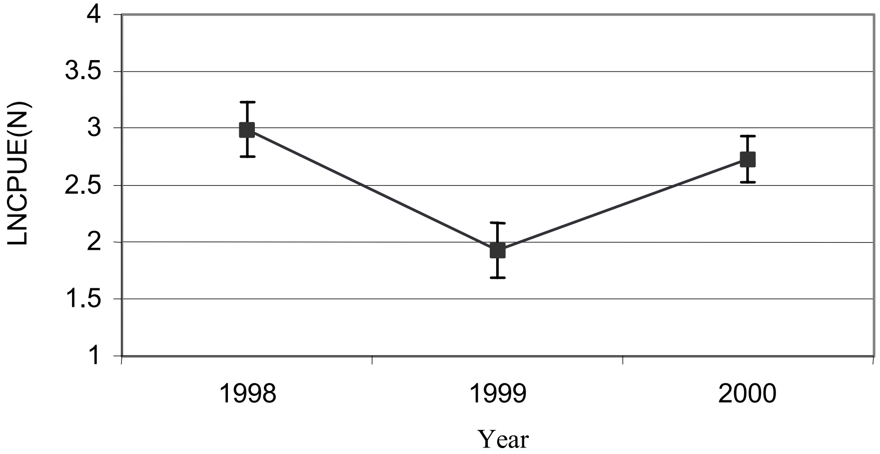
Fig. 7. Maturity stages in the Tunisian Bluefin tunas (bar = 400 mm). A and B: immature three years old ovaries that show a lot of unyolked oocytes (cross). C: A five years old mature ovary that shows maturity signs; yolked oocytes (stars). D: four years old mature ovary with many yolked oocytes(stars) and some unyolked oocytes(cross). E: Gonadosomatic index by age class of the tuna used in this study.

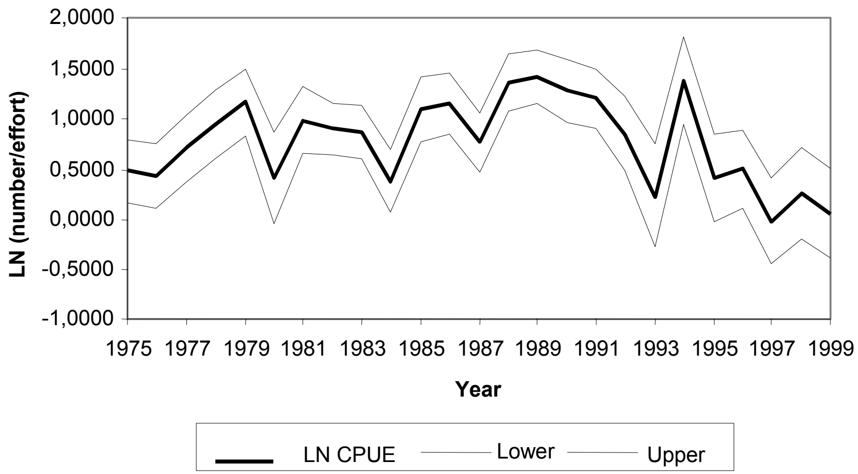
Standardized CPUE index.





Spain

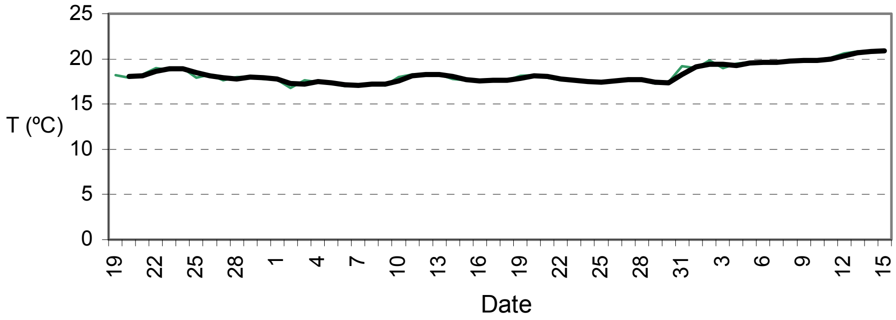




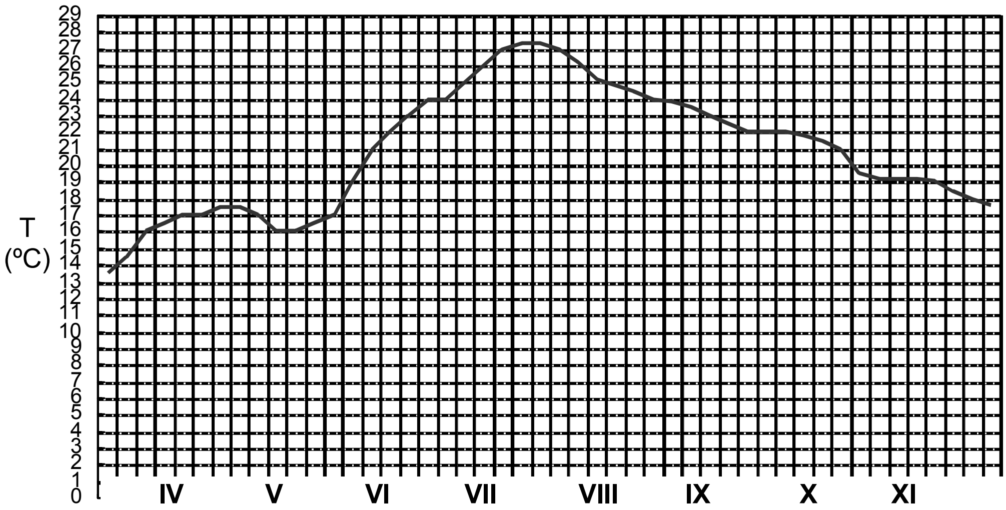
Morocco

Tunisia

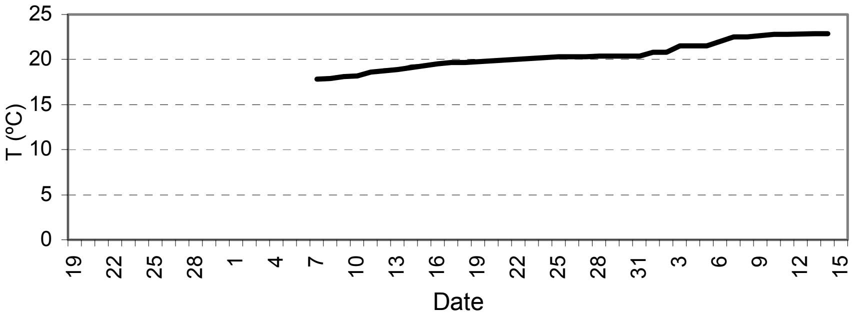
Fig. 8. Trends in abundance (Abundance Indices) by fishing gear and spatial- temporal strata (guarantee capital investment).



TRAP (Atl) – Spain (V-VI)

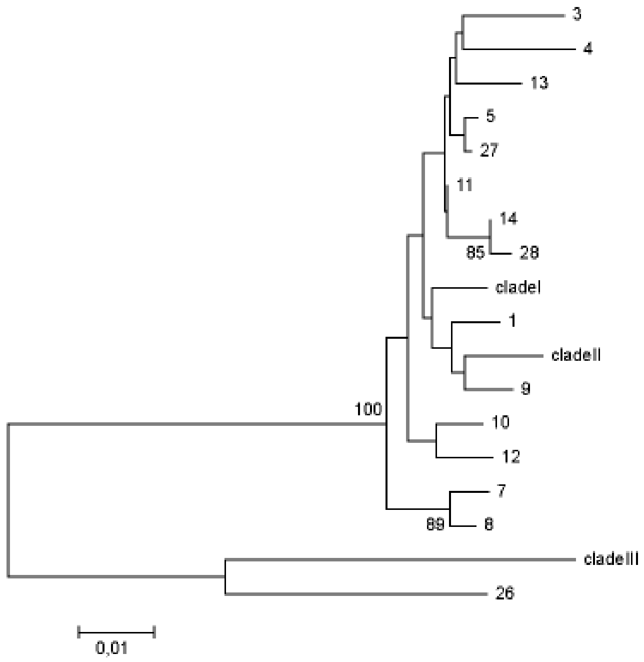


LL – Balearic Islands



LL - Malta (V-VI)

Fig. 9. BFT and environment interactions for consecutive physiological stages (genetic and trophic migrations, reproduction, etc.) (farming installations location and adaptability)



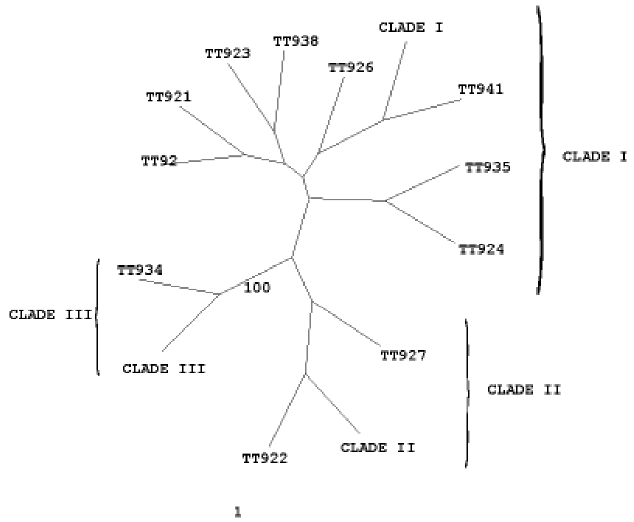


Fig. 10. Phyllogenetic tree based on the Neighbour-joining distances of the different sequences. Branch names shows bootstrap values over 50%.

Nutritional management and development of manufactured feeds for tuna aquaculture

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SUMMARY - At present, all tuna farming is based on wild capture and feed lotting. The impounded tuna are fed on a diet of small pelagic fish such as sardines, mackerel and herring. The manufactured feeds developed thus far have focussed on moist and semi-moist feeds, which produce a soft-textured product, however dry feeds have also been trialed. Farmed tuna are generally fed for 3-6 months before harvest. Feed intake can start at > 10% of body wt per day (bw/d) for raw fish feeds, stabilising to < 5% bw/d nearer to harvest. FCR (dry feed weight) values range from 3-6:1. Cumulative SGR's of 0.5-0.6 have been achieved.

Key words: Manufactured feeds, proximate composition, feed intake, feedback loops, FCR, SGR.

Background

The high value of tuna on the predominantly Japanese market is largely attributed to the red colour and firm but tender texture of the thin pieces of raw flesh served as sushi or sashimi. Northern bluefin tuna (*Thunnus thynnus*) is the most highly regarded and farmed fish average between 4000-6000 ¥/kg depending on size, condition and quality factors. Farmed southern bluefin tuna (T. maccoyii) from Australia are the next most valuable tuna species averaging around 2800-3200 ¥/kg over a season. Other tuna species with aquaculture potential are the warmer water bigeye tuna (*T. obesus*), and yellowfin tuna (*T. albacares*), however these have only recently been trialed in farms in Mexico and the Canary Islands. At present, all tuna farming is based on wild capture and feed lotting and is centred in Australia and the Mediterranean (Fig. 1).

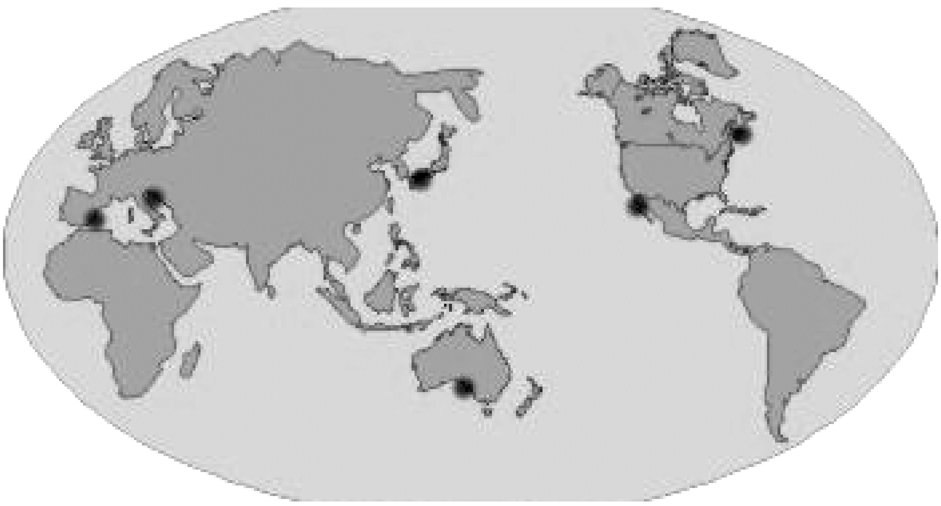

Figure 1. Map of world showing the main tuna farming locations.

Feed development

The impounded wild-caught tuna are fed on a diet of small pelagic fish such as sardines, mackerel and herring. Local sources of these small pelagics are not always available or in sufficient quantities for farmers and therefore importing frozen stocks are often required. Also, they are expensive to use in terms of labour costs and freezer facilities for storage (Fitz-Gerald and Bremner, 1994). There are a number of other feed management issues associated with raw fish diets. For instance, they carry an increased risk of disease transmission (Wee and Tacon, 1992) and thawed fish have associated fragments of blood and scales, etc which are released into the water column leading to poor water quality and pollution (Watanabe, 1991). In addition, improper storage techniques can bring about the destruction of certain vitamins and fats affecting fish health and growth (Roessink, 1989).

In order to combat the problems associated with trash fish feeds and to maximise production yields at the lowest cost, manufactured feeds were developed for the Australian tuna farming industry (Smart, 1996). Manufactured feeds have less bulk to store, are of uniform quality, and allow control over feed formulation (Lovell, 1993). The diets developed thus far have focussed on moist and semi-moist feeds, which produce a soft-textured product, however dry feeds have also been well accepted. Diet formulation development has largely taken place in Australia and has been based on the composition of southern bluefin tuna muscle and natural prey species (Clarke et al., 1997). These analyses indicated a high energy, high protein, diet typical of an opportunistic carnivore was most suitable. Analysis of amino acid profile (Table 1) was used to help formulate early manufactured feeds trialed with tuna (van Barneveld et al., 1997) In addition, a range of experiments were conducted to assess the *in vivo* and in vitro digestibility and transit time of feed ingredients, manufactured diets and pilchards fed to farmed tuna (van Barneveld et al., 1997; Carter et al., 1997; Clarke et al., 1997).

Table 1. Essential amino acid content of red and white muscle in SBT (g/kg, dry matter), dietary amino acid balance (used prior to 1997) and proposed ideal amino acid balance (from van Barneveld, 1997).

Amino acid	Red muscle	White muscle	Dietary balance	Ideal balance
Methionine	11.0	10.9	49	35
Threonine	18.2	17.2	56	56
Valine	20.4	19.2	74	63
Isoleucine	18.7	17.1	61	57
Leucine	29.3	27.6	110	90
Phenylalanine	15.1	14.9	63	47
Lysine	32.5	30.7	100	100
Histidine	30.5	30.3	37	96
Arginine	23.9	22.3	93	73

Performance of farmed tuna

Feed intake

Tuna have sensitive sensory systems, including their visual acuity (Dickson, 1995) and well developed olfactory systems (Atema et al., 1980). The rapid training of a wild tuna to eat manufactured feeds requires a suitable weaning program that makes a transition to the intended feed. Learned behaviour is important in this process along with fish condition (health status) and hunger level.

Farmed tuna are regarded as being in marketable condition after about 3-6 months of feeding and the premiums achieved are generally based on freshness, high condition index, and fat content (Goodrick et al., 1999). The first month of feeding can see feed intake rise above 10%

of body wt per day for raw fish feeds and feeding behaviour is initially very active. These levels stabilise to less than 5% bw/d nearer to harvest (Sylvia et al., 1999).

Growth, FCR and condition factor

The logistics of managing the feeding of such large quantities of sardines pose a significant challenge. The nutritional quality of small pelagics rapidly decreases over time and oxidation is a major concern, especially when uneaten feed is retained for feeding the following day. The raw fish diets are broadcast by means of hand, shovel, and various pumps. Some feeds are added to the water frozen in 10-20kg blocks inside small floating enclosures within the cages and as they thaw, they sink to the tuna below. The use of feedback loops is quite limited with some companies using cameras or divers and this area needs attention to avoid overfeeding. Recorded FCR values range from 3-6:1 (dry weight feeds: wet weight tuna) for pilchards and moist pellets (Smart, 1998). Cumulative SGR's of 0.6 for *T. thynnus*, 0.5 for *T. obesus*, and 0.45 for *T. albacares* have been achieved over a 4 month feeding period (Sylvia et al., 1999), with rapid growth in the first month after capture which is typically at higher water temperatures. The industry has dramatically improved FCR over time and this evolution will continue with the introduction of extruded diets and feedback mechanisms. The condition factor of farmed tuna typically increases from 1.9 to 2.5 over the 3-6 month fattening period. The shape of the tuna is another important factor for the Japanese marketplace.

This summary attempts to give a general global overview of tuna feed management, however it must be recognised that the scientific literature is typically well behind the rapidly evolving finfish aquaculture industry.

Conclusions

There have been some highly successful recent commercial trials with extruded feeds, which produced good fish performance and market acceptance (G. Bayly, pers. comm. Skretting Australia) but these have not been covered in this review. As the tuna industry moves forward with manufactured feeds, the key issue now will be managing the replacement of the raw fish diets with pelleted feeds.
A quantum leap will be achieved once hatchery culture of tuna juveniles is established and this will enable feed development to move more rapidly into the area of drier extruded diets, following a similar evolution to the Atlantic salmon (*Salmo salar*) industry. However, this is likely to be a different product to the current feed-lotted tuna as it may not be economic to on-grow propagated tuna juveniles to the size of wild caught fish. As a result, in the future we may see a farm-reared tuna industry producing fish of 5-15kg for a new 'farmed' market sector that may expand significantly outside Japan.

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The sustainability of the Bluefin Tuna resource: A fishery and aquaculture integrated challenge

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SUMMARY – This short introduction indicates the various directions in which fisheries and aquaculture could harmonise in the future to provide a sustainable fishery for BFT.

Key words: Resources, sustainability, fisheries, aquaculture

Today the preservation of the sea's resources must be approached in terms of three main themes:
(i) First the management of living resources and the quality of the environment,
(ii) Secondly the preservation of the human and socio-economic environment of the world of fishing,
(iii) Finally, meeting consumers' demands by ensuring the development of seafood production.

The resources of Tuna fish, and in particular of BlueFin Tuna in the temperate zones and big eye Tuna in tropical zones, are an integral part of this challenge.

In Europe, the fishermen and the fish merchants themselves have taken the initiative for the progressive change from fishing to aquaculture. This is a remarkable adventure which is now well on the way in the Mediterranean in the Spanish region of Murcia and Cartagena. This shows just how strong is the capacity for innovation, risk-taking and the desire to modernise within these industries.

This evolution from capture to aquaculture with the creation of added economic value is unique in Europe. It creates jobs and maritime activities without entailing a significant increase in captures which could be detrimental to the stock.
It was in Spain, which currently holds the presidency of the EU, hosts and co-organises this meeting and is Europe's leading country in terms of fishing, where this profound change first took place.

As of today, we must ask ourselves questions concerning the long term sustainability of the Tuna fishing and aquaculture activities:
(i) Sustainability of the resource in the Atlantic Ocean and Mediterranean seas to be shared between a rich North and a poorer South,
(ii) Sustainability of the socio-professional tissue of fishing and its related jobs,
(iii) Sustainability of the quality of the coastal environment, which is increasingly attracting new activities: tourism, green tourism, aquaculture, fishing as a leisure activity etc…
(iv) Sustainability in sharing and maintaining a balance between the different uses and users in the coastal environment,
(v) Sustainability of the provision of sea products in Europe,
(vi) Sustainability in terms of the quality, safety, tracability and the image of European productions,
(vii) Finally, sustainability of development at the extreme periphery of the European Union in regions where the great pelagic species are an important source of wealth.

This sustainability can only be obtained through the acquisition of scientific and technical knowledge. Your seminar should lay the foundations for this international research effort aiming at the domestication of the BlueFin Tuna, the objective of which is to secure these different aspects of sustainability both through fishing and aquaculture.

To my mind, this is the real meaning of your research into the domestication of the BlueFin Tuna.

Only a strong and co-ordinated commitment of European research in this field, supported by the European parliament and with the help of the European Commission will enable the identification and implementation of the ways and means to carry out such an ambition.

Naturally the worldwide co-operation with your natural partners in this field, Japan, the United States and Australia, will need to be strengthened.

It is in this spirit of responsibility and sustainability that the parliament supports and will continue to support this work, which will mobilise many different disciplines.

Study of first sexual maturity in female bluefin tuna (*Thunnus thynnus*) from the central Mediterranean Sea

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SUMMARY- This preliminary study indicates that the minimum size at maturity is 110 cm (L_F) in female BFT from the Mediterranean Sea. It is notable that pre-adult specimens (with L_F ranging from 100 to 110 cm) showed signs of gonadal development, characterised by the appearance of oocytes in the lipid stage. This finding occurred contemporaneously to both the appearance of E_2 and Vtg in the plasma. The evidence of the present study needs to be confirmed in a larger fish sample including sampling at the different phases of the reproductive cycle.

Key words: Tuna, sexual maturity, *Thunnus thynnus,* vitellogenin, oocyte diameter, fork length

Introduction

The bluefin tuna BFT (*Thunnus thynnus*) is one of the most important economic fishing resources in the world. This species is subject to an intense fishing effort, which has raised questions concerning the survival of the stock (Sissenwine *et al.*, 1998). In spite of the economic importance of the species, the knowledge of its reproductive biology is limited to research based on: macroscopic classification of the gonads (Rodríguez-Roda, 1964, 1967); seasonal variations of the gonadosomatic index (I_G) (de la Serna & Alot, 1992) and the distribution of eggs and larvae (Piccinetti *et al*., 1977, 1997; Cavallaro *et al*., 1997; Nishida *et al*., 1997). In depth studies, including histological analysis of ovaries, have been carried out in Western Atlantic BFT (Baglin, 1982), whereas histological as well as endocrinological studies are lacking in Eastern Atlantic and Mediterranean BFT stocks.

Since knowledge of reproductive biology is an extremely important tool for determining the correct policy for the management of fish stocks the present study deals with the size at first sexual maturity of female BFT from the Mediterranean Sea, correlating plasma levels of Vitellogenin and 17ß-Estradiol and histological maturity stage of the ovaries.

Specimen Collection

Blood and gonad samples were obtained, during the months of April and May 1998 and 1999 from female BFT with L_F ranging from 90 and 140 cm, caught commercially by long lines in the North Ionian Sea (Gulf of Taranto) and by traditional traps (Tonnare) operating in Carloforte and Portoscuso (Sardinia, Italy). Males were also sampled and used as controls. Soon after the capture fish L_F was measured and blood was collected from the heart with heparinized syringes and cannula (longline fish) or directly in 15 ml tube (trap fish) from a cut, made by fishermen, under the pectoral fin to bleed the fish. The blood (with 1 mM PMSF) was kept on ice after sampling at sea, then centrifuged and plasma collected and stored at -20°C (usually < 4 hrs after capture). Fragments of ovaries were fixed in Bouin‘s solution for histological analysis and immunohistochemical investigations.

Methods

Histological and Immunohistochemical Evaluation of ovary maturity stage

Ovary samples were embedded in paraffin wax. Sections (5 µm thick) were stained with Haematoxylin – Eosin.The immunohistochemical detection of anti BFT-Vtg serum positive cells was performed using the avidin-biotin-peroxidase complex (ABC) procedure. Peroxidase activity was visualised by incubating with Vector DAB Peroxidase Substrate Kit (Vector,

Burlingame, CA). Oocyte diameters were measured on histological slides using Quantimet (Leica, Cambridge, UK) image analyser.
Plasma 17ß-Estradiol (e_2) and Vitellogenin (vtg) Measurement

For the measurement of E_2 200 µl of plasma were extracted two times with Dichloromethane, dissolved again in PBS and measured by ELISA as described by Cuisset *et al.* (1994).The assay for BFT Vtg was established combining several different methods for Vitellogenin ELISA as reported in the literature for other fish species.

Results

The results obtained correlating Vitellogenin (Vtg) and 17ß-Estradiol (E_2) plasma levels and histological analysis are summarised as follows in Figure 1:
Recrudescence Period (April)
a) Fish with L_F ranging from 90 and 100 cm showed almost undetectable (<0.15ng/ml; <0.16 mg/ml) E_2 and Vtg plasma levels. The histological observations revealed immature ovaries with oocytes in perinucleolus stage.
b) Fish with L_F ranging from 100 and 110 cm showed mean E_2 and Vtg plasma levels of 1.04 ng/ml and 0.93 mg/ml respectively. The histological analysis demonstrated the presence of both perinucleolar and lipid stage oocytes.
c) Fish with L_F ranging from 110 and 120 cm showed mean E_2 and Vtg plasma levels of 1.44 ng/ml and 1.74 mg/ml respectively. The ovaries showed no histological difference from the previous size class.
Ripening Period (May)
a) Fish with L_F ranging from 100 and 110 cm showed a slight increase of E_2 and Vtg plasma levels respect to the previous month (2.5 ng/ml and 3.33 mg/ml respectively), but no evolution of ovary maturity stage
b) Fish with L_F ranging from 110 and 120 cm showed a significant increase of E_2 and Vtg plasma levels (12.98 ng/ml and 25.70 mg/ml respectively). The histological analysis demonstrated the presence of oocytes in late vitellogenic stage. The mean oocyte diameter increased significantly, in respect to the previous class (from 55 to 90 µm).
c) Fish with L_F > 120 cm showed an increase of Vtg plasma level and mean oocyte diameter (32.54 mg/ml and 106 µm respectively) in respect to the previous length class and a significant increase of percentage of vitellogenic oocytes (from 5% of the previous class to 11.2%).
The immunohistochemical staining of ovaries with anti BFT-Vtg serum revealed the presence of Vtg-like material in oocyte having a minimum diameter of 220 µm. Immunopositive oocytes were observed only in ovaries of specimens caught in May with L_F ³ 110 cm

Conclusion

Determination of reproductive status is a key requirement for any fisheries management programme. Vitellogenin (Vtg) has been widespread used in aquaculture species as an indicator of sexual maturity (Mañanos *et al*., 1994; Bon *et al.*, 1997; Mosconi *et al.*, 1998).
In wild fish populations, such as the large pelagic bluefin tuna (BFT), the purification of Vtg is particularly difficult due to the problem of obtaining fresh samples from female fish in the correct maturity state. In fact, during sampling the plasma of this fish has often showed high contamination with haemoglobin (our own observation), due to haemolysis from the catch stress, difficulties in blood extraction or the relatively long time between blood sampling and centrifugation.

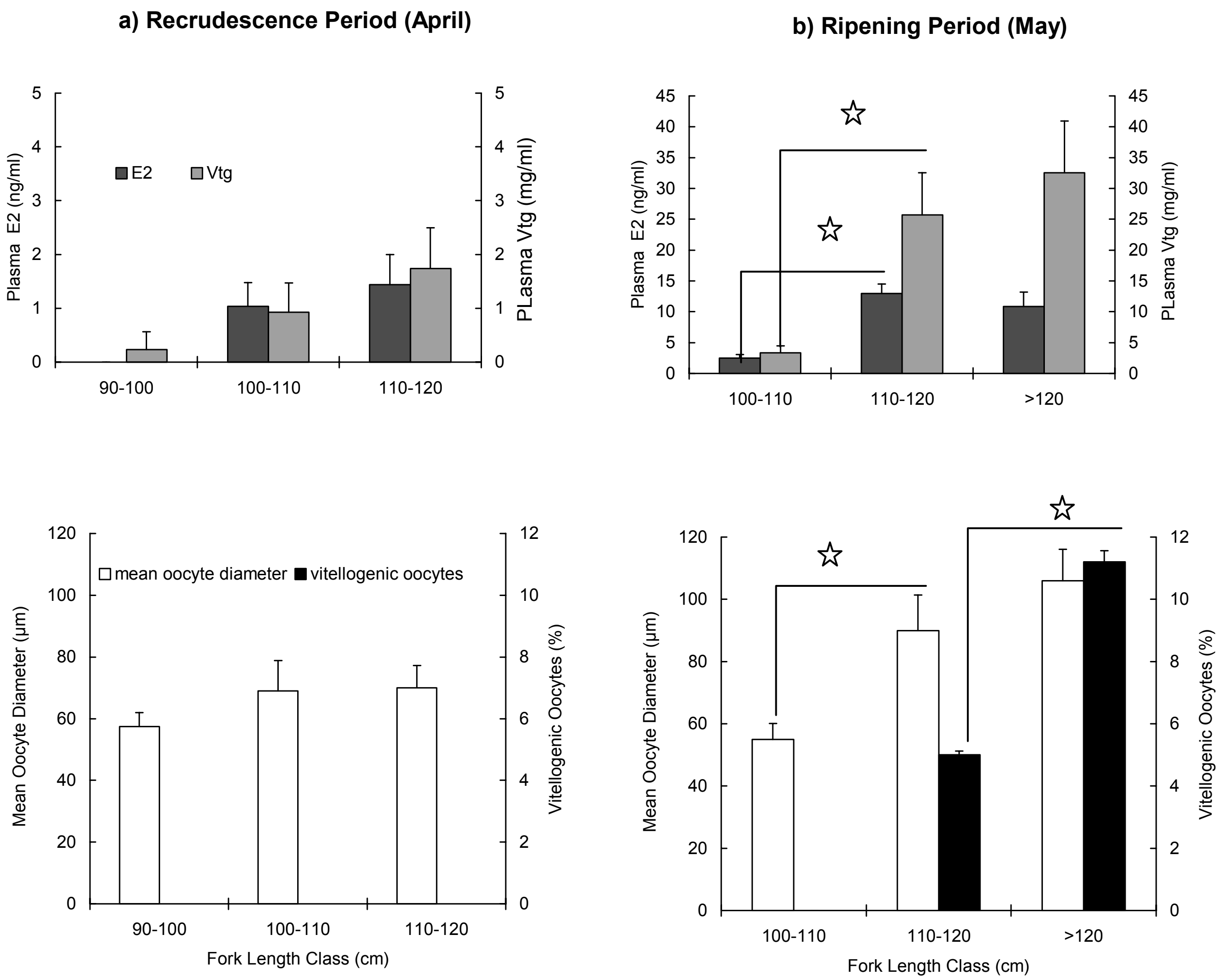

Figure 1. Changes in plasma levels of 17ß-Estradiol (E_2), Vitellogenin (Vtg), mean oocyte diameter and percentage of vitellogenic oocytes in two different periods of the female BFT reproductive cycle: a) recrudescence (April) and b) ripening (May). Stars represent statistical significance ($p<0.05$) assessed by t-Test, N = 7.

In this study we established an ELISA for the detection of Vtg and validated the assay correlating the plasma levels of Vtg to those of E_2. Furthermore the immunohistochemical study confirmed the specificity of the anti-Vtg serum.

This preliminary study indicates that the minimum size at maturity is 110 cm (L_F) in female BFT from the Mediterranean Sea. This seems to be in agreement with Rodríguez-Roda (1967) who by macroscopic observation of gonadal maturity stage, found 100% mature females of Eastern Atlantic BFT over 120 cm (L_F).

It is notable that pre-adult specimens (with L_F ranging from 100 to 110 cm) showed a slight gonadal development, characterised by the appearance of oocytes in the lipid stage. This finding occurred contemporaneously to both the appearance of E_2 and Vtg in the plasma. The simulation of gonadal development has been reported by Baglin (1982) in sexual immature Western Atlantic BFT.

The findings of the present study need to be confirmed in a larger fish sample including sampling at the different phases of the reproductive cycle.

Acknowledgements

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Current status and future prospective of bluefin tuna (*Thunnus thynnus orientalis*) farming in Mexico and the West Coast of the United States

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SUMMARY - This paper provides a brief summary of the history of tuna farming in Mexico and the United States as well as future prospects for tuna farming in both countries. Production and growth performance data from a Mexican farming operation are discussed for bluefin, bigeye and yellowfin tuna. The domestic and international market situation is also reviewed. Due to the lack of quota regulations, increased national and international interests and the development of a more skilled workforce for these types of operations, both Mexico and the U.S. are uniquely poised to expand and contribute more significantly to worldwide tuna farming production.

Key words: Tuna, husbandry, performance, markets.

World production of farmed northern and southern bluefin tuna is likely to exceed 20,000 metric tonnes (mt) in 2001-2002. Mexican tuna farming operations currently represent 3% of world production. The majority of operations are located on the Pacific side of the Baja peninsula. Tuna farming began in Mexico in 1996 with marginal success and with only 1 or 2 companies in business at any one time. The limited success was largely due to weather events such as El Niño and Hurricane Nora at the time but also due to a general lack of tuna farming experience, which led to high mortalities. However, the development of many innovative techniques for both fishing and farming by Mexican operations in recent years have allowed them to emerge as significant competitors in a relatively young but growing industry.

Mexican farming operations have grown from <50 mt to 600 mt in the last 5 years. As of December 2001, there were 5 permitted tuna farm sites with 4 different owners. Three are for bluefin and are located in Cedros Island, Ensenada and the Coronado Islands, respectively. Two of these sites are for yellowfin and are located in Bahia Magdalena and Islas Marias. The original operators were solely Mexican owned and operated. The currently producing operations have a combination of Mexican, American, Australian and Japanese partners. As of March 2002, 6 new lease sites have been granted for bluefin, which will all be located in the Ensenada area with an allowable production of 400 mt each. The original operating farms currently do not have any production restrictions. Mexico is particularly inviting for tuna farming because of its temperate weather conditions, an abundant supply of locally caught feed, proximity to major international airports in the USA, lack of various regulations and low labor costs.

Fishing bluefin in Mexican waters for farming operations have proven more difficult than in other parts of the world. In Australia, live bait is used to readily and consistently attract southern bluefin tuna schools before they are seined. Atlantic bluefin migrate throughout the Mediterranean during their spawning season. Surface feeding and birds typically locate pre-season fish before being seined. However, the majority of catches in the Mediterranean occur in mid spawning season where the fish are typically congregated at the surface in large schools of very mixed sizes, allowing for larger and more predictable seine catches. In Mexico, many factors such as water depth, fish behavior and unique weather conditions have contributed to inconsistent and unpredictable seasons. The majority of catches occur at night and rely mainly on the presence of bioluminescence to locate bluefin schools. Typical size at capture ranges from 15-45 kilograms, with smaller fish being caught in southern areas and larger fish to the north, and in many instances over the USA border. The catching season typically ranges from July to late August but can extend into November, depending on fishing location. Towing distances can range from 96 - >800 kilometers.

The production cycle typically ranges from 3-8 months, depending on the farm site but also depending on the size of the fish. Smaller fish that are caught in the southern areas are usually held in the cages for longer periods of time. Water temperatures typically range from 18-22ºC. Cage systems are 40-50 meters diameter, 15-20 meters deep, with holding volumes of 18,000m^3- 20,000m^3. Fish densities can range from 2-5 kg/m^3, water currents from <1-2 knots depending on the farm site. Fish are fed both fresh and frozen sardines. Some sites also feed mackerel and squid. Weight gain can range from 30-90% of initial weight, depending on the farm site and the use of various husbandry techniques. Fish are harvested from December-April/May using the Australian method.

There are a variety of concerns particular to Mexico. Although seldom referred to or discussed is the issue of predators, in particular, sea lions. Many areas along the coastline of Mexico and its associated islands support large colonies of sea lions. They are attracted to the farms for both the tuna in the cages but also by excess feed that falls through the cages or that is discarded. Many of the farms do not use predator nets on their cage systems but instead use fences around the perimeter of the cages to prevent the sea lions from hauling out on the cages and from jumping in the cages. Some farms use electric fences around the cage surface perimeter. Although these are effective measures, significant predator effects continue to be a problem. Stress and poor growth performance are common in most of the farms. Fresh or healing wounds from the nails of the flippers or from the mouths of the sea lions are often present on the flanks and underside of the fish in at least 1/3 but sometimes up to ½ of the fish being harvested. Although many fish survive these wounds, their value is significantly decreased in the marketplace. Additionally, many mortalities that occur from these sea lion attacks are not accounted for or go un-noticed. The use of predator nets would greatly improve this situation. There is also a rumor from the Japanese marketplace that the flesh of the Mexican fish has a “bait smell” and is often unattractive to buyers and consumers. It is currently believed that this is caused by feeding the fish only one type of feed. The cause is debatable at this point.

As per usual with other countries farming tuna, the primary market is Japan. However, Mexico as well as the U.S. are beginning to experience the advantages of the rapidly expanding U.S. market for sashimi products. This is of particular importance to Mexico because of the typical small size of their farmed tuna. The smaller tuna simply cannot compete with the larger tunas farmed in other countries. As of February 2002, average prices for Mexican tuna on the Japanese market ranged from 3300 - 3500¥/kg ($25-$27 USD/kg). Larger fish from other countries routinely average at least 4500¥/kg ($34 USD/kg). Many smaller fish go unsold on the auction floor and are typically distributed to other Japanese customers at average prices of 1500-1800¥/kg ($11-$13 USD/kg). When all costs are taken into account it can be predicted that at some point in the future, marketing these small fish in Japan may become cost prohibitive. At this point, only the best quality fish go to Japan. Poor quality and predator- affected fish are sold in the U.S. In the future, it is predicted that a larger percentage of farmed tuna from Mexico as well as the U.S. will be marketed in the U.S. As the market continues to develop and mature in the U.S., higher quality fish will stay in the U.S., ultimately presenting a significant savings on the final price by just eliminating shipping and import taxes. However, because Mexico and the West coast of the U.S. are in close proximity to major international airports and routine flights to Japan, and in order to remain competitive in the global marketplace, a percentage of fish will always target the Japanese market.

Of particular interest is the fact that other, commercially valuable tuna species such as bigeye (*T. obesus*) and yellowfin (*T. albacares*) are common inhabitants of Mexican, southern California and Hawaiian waters. These species are beginning to emerge as a valuable alternative to bluefin in the marketplace. Efforts to farm bigeye are already underway in Spain, Chile and Hawaii. Yellowfin are successfully being farmed in Mexico. During the tuna season of 1998, the farm located in Cedros Island, Mexico had the opportunity to rear bluefin, bigeye and yellowfin in the same cage system. This was the first time that bigeye were ever farmed and provided a unique opportunity to compare the growth performance, behavior, husbandry requirements and post-harvest flesh quality for all three species

Table 1: Growth Performance of Bluefin, Bigeye and Yellowfin Tuna Reared in the Same Cage System-Data from Atunera Nair, Cedros Island, Mexico.

Species	Start Wt. (kg)	Final Wt. (kg)	FCR	SGR (%bw/d)	Cond. Fact.
Bluefin	45	70	7:1	.6	2.2
Bigeye	25-30	45	9:1	.5	2.3
Yellowfin	20-25	35	10:1*	.45	2.1

Production Cycle = 2.5 months   FCR's = Wet Weights   Water Temp = 18-22ºC
*Yellowfin would most likely perform better if in warmer temps.

Table 1 provides a summary of various growth performance characteristics of each species during the study.

There were a variety of differences between all species with regards to behavior, parasite and disease susceptibility, and post harvest flesh quality. The details of these differences will not be discussed in this paper, as they are the subjects of another publication that is currently in progress. However, this study demonstrated that bigeye and yellowfin tuna show promise as alternative species for tuna aquaculture and may serve as surrogate species for hatchery culture of commercially valuable tunas.

Captive bluefin tuna research began on the east coast of the United States in 1989 at the New England Aquarium in Boston, Massachusetts. This research focused on basic biology, physiology, reproductive biology, early life history and the development of many handling and other husbandry techniques for captive tunas. Tuna were reared in closed, filtered seawater systems. Over 7 years of study, collection, transport, handling, anesthesia, blood sampling, ultrasound, pathology, wild larval and juvenile collection techniques and methods were developed for bluefin. This study culminated in the first-ever permitted open ocean sea cage for tuna culture in the U.S. in 1996. The cage was located 24 miles offshore and was primarily focused on demonstrating the feasibility of tuna aquaculture to government, scientists, fishermen and other interested parties. At that time and until this day, tuna aquaculture was too risky for east coast fishermen to undertake. This is primarily because of strict quotas and regulations for bluefin tuna that virtually make it un-economical to farm on the east coast of the U.S.

As of February 2002, there is still no tuna aquaculture in the U.S. Development has been hampered primarily by coastal user conflicts and stringent regulatory limitations. However, interest and initiatives are currently in progress for developing tuna farming operations off the west coast of the United States, particularly in southern California but also off the coast of Hawaii. The advent of several new offshore aquaculture projects in the U.S. coupled with strong governmental initiatives is providing the groundwork necessary to initiate tuna and other aquaculture operations in the U.S. Southern Californian and Mexican waters share many of the same characteristics that lend themselves as prime conditions for tuna farming as well as having the three most valuable tunas as common inhabitants. Due to the lack of quota regulations on the west coast, increased national and international interests and the development of a more skilled workforce for these types of operations, both Mexico and the U.S. are uniquely poised to expand and contribute more significantly to worldwide tuna farming production.

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Ovarian cycle of the Mediterranean Bluefin Tuna (*Thunnus thynnus* L.)

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SUMMARY - The results obtained by the histological analysis of Mediterranean bluefin tuna ovaries collected over a six-month period (March-August) are reported. Five phases of ovarian cycle were characterised: *Recrudescence* (March-early May); *Ripening* (middle May); *Pre-spawning* (late May-June); *Spawning* (late June-early July); *Spent* (late July-August). The ovaries of five specimens caught during the spawning period in the North Ionian Sea and South Adriatic Sea displayed extensive vitellogenic atresia. In females which find themselves in unfavourable environmental condition during the spawning period, follicular atresia could represent a way to re-absorb highly energetic yolk reserve.

Key words: Bluefin tuna; Reproductive Cycle; Ovary; Histology; Mediterranean Sea.

Introduction

The knowledge of Mediterranean bluefin tuna (BFT) reproductive cycle is limited to research based on seasonal changes of the gonadosomatic index (de la Serna and Alot, 1992), and on macroscopic classification of the gonads (Rodríguez-Roda, 1964; 1967). Susca *et al*. (2001) carried out the first attempt to correlate vitellogenin (VTG) and sex steroid plasma levels with ovarian cycle. Here a histological description of the changes occurring in BFT ovary throughout the reproductive cycle is reported.

Materials and methods

Ovary samples were obtained from 101 adult (fork length ≥ 120 cm) bluefin tuna. The samples were collected on board of professional vessels in Italian and Spanish seas. Fragments of the gonads were fixed in Bouin's solution or neutral 10% formaline, dehydrated in ethanol and embedded in paraffin wax. Sections (5 µm thick) were stained with haematoxylin-eosin. To i8dentify vitellogenic oocytes, certain sections were immunostained with rabbit anti BFT vitellogenin serum (abBFT-VTG). The immunohistochemical reaction was visualised by means of the avidin-biotin peroxidase complex (ABC) procedure.

Results

The ovary consists of a thick muscle wall and numerous follicles in different stages of development (asynchronous ovary) embedded in a mass of connective tissue. Each follicle consists of an oocyte rounded by a single layer of follicular cells.
The activity of the ovaries showed seasonal changes allowing the characterisation of the five periods during the reproductive cycle:
1) Recrudescence period (March-early May) – The specimens caught during the recrudescence period showed oocytes at perinucleolus and lipid stage. Perinucleolus stage (diameter 10 -110 µm) was characterised by intense ooplasm basophily and numerous small

nucleoli adjoining the nuclear envelope (Fig. 1). Oocytes at lipid stage (diameter 110-220 µm) exhibited a weak ooplasm basophily and were characterised by small lipid droplets (Fig. 1A). *2) Ripening period* (middle May) – All the specimens analysed showed the presence both of previtellogenic and vitellogenic oocytes. Vitellogenic oocytes (diameter 220-500 µm) were immunopositive with the anti Vtg serum (Fig. 1B). *3) Pre-spawning period* (late May-June) – In the ovaries of the specimens caught in this period, migratory nucleus stage oocytes (diameter ranging from 500 to 600 µm) could be observed, together with the previous stages (Fig. 1C); *4) Spawning period* (late June-early July) – All the females caught in this period showed pre-mature (diameter 600-700 µm) or mature (diameter 700-850 µm) oocytes (Fig. 1D). *5) Spent period* (late July-August) – In this period, only perinucleolus stage oocytes were found. Irregular cell masses containing pigmented inclusions and large lipid droplets, likely residue of the re-absorbing process could be observed (Fig. 1E). The ovaries of five specimens caught during the spawning period in the North Ionian Sea and South Adriatic Sea displayed extensive vitellogenic atresia (Fig. 1F).

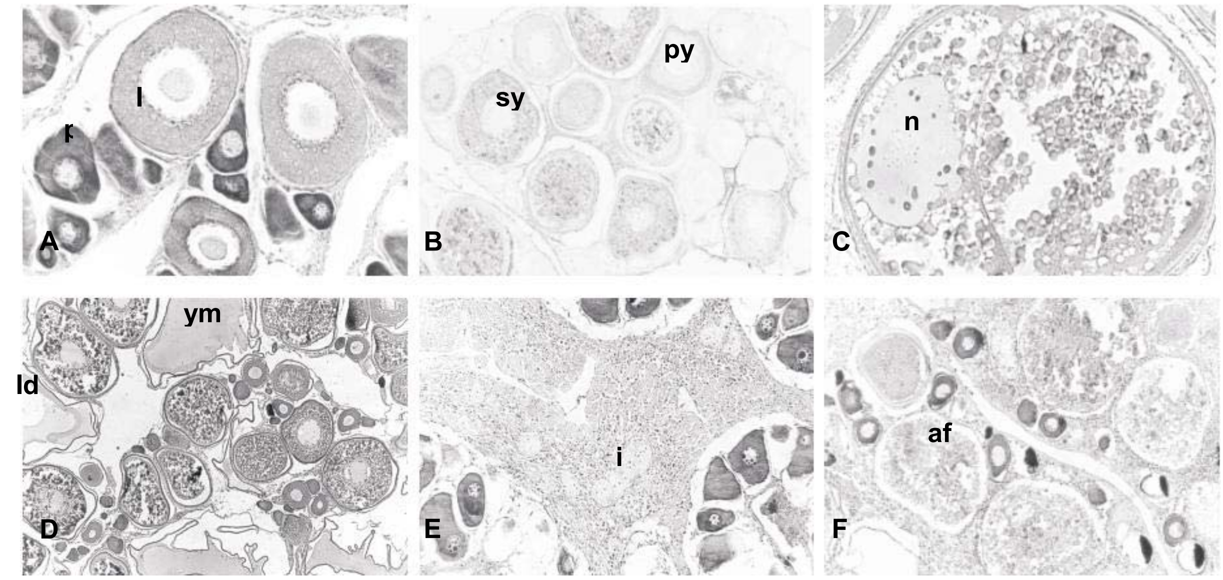

Fig. 1 – A - Photomicrograph of the ovary from a BFT specimen caught in April showing oocytes at perinucleolus and lipid stage. Magnification, x 100. B – Section of the ovary from a BFT specimen caught in May immunostained with abBFT-VTG. Vtg immunoreactive staining was detected in ooplasm of vitellogenic oocytes. Magnification, x 32. C – Section of the ovary from a BFT specimen caught in June showing a migratory nucleus stage oocyte. Magnification, x 88. D – Section of the ovary from a BFT specimen caught at the beginning of July showing mature oocytes. Magnification, x 36. E – Section of the ovary from a BFT specimen caught in August. Only perinucleolus stage oocyte can be observed. Irregular cell masses, likely residue of the re-absorbing process, are present in the connective tissue. Magnification, x 48. F – Photomicrograph of the ovary from a BFT specimen caught in July in the South Adriatic Sea showing extensive atresia. Magnification, x 48. Haematoxylin-Eosin staining. af, atretic follicle; i, irregular cell mass; l, lipid stage; ld, lipid droplet, n, nucleus; p, perinucleolus stage; py, primary yolk stage; sy, secondary yolk stage; ym, yolk mass.

Conclusions

Maturity development of BFT ovaries starts in early spring when the oocytes enter endogenous vitellogenesis. From May throughout June, exogenous vitellogenesis takes place in the ovaries. Vitellogenin uptake starts in oocytes having a minimum diameter of 220 µm, as revealed by immunohistochemical staining with abBFT-VTG. Spawning occurs in late June-early July when pre-mature and mature oocytes were found in the ovaries of specimens caught in the Balearic Sea. In the ovaries of the specimens caught during the spawning period in the North Ionian Sea and South Adriatic Sea no sign of recent spawning was observed and most of unyolked and yolked oocytes were atretic. Therefore, it could be supposed that adult females, which find themselves in unfavourable environmental conditions during the spawning period, are unable to complete the maturation process and re-absorb their developing oocytes.

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Summary of Meeting Conclusions:

The Steering Committee of the DOTT symposium is hoping that new working groups will be formed and emerge as a result of the Conference and the workshops. These groups should put the foundation for a rational and sound program for the domestication of The Blue Fin Tuna. For many of us this meeting was just the first step, with many to follow. The immediate one is the REPRO.DOTT project, which has been approved by the EU Commission and will start its research in 2003.

The closing session of the conference was devoted to drawing a set of resolutions and adopting them by the plenary forum. These resolutions are aimed at increasing the awareness of the public, the different governments and the EU Community and its administration prospects of domestication the BFT and what it involves.

The DOTT conference recognized the serious plight of the BFT stock(s) and made the following general recommendations:

1. **It is believed that understanding all life cycle phases of the BFT will enhance the two facets of this species' interaction with Man;**

    a. **Reducing the pressure on wild populations of the BFT**

    b. **Setting the rational foundation for sustainable aquaculture and the domestication of this species.**

2. **These objectives will only be attained through the joint participation of fishing industry, farmers, management, regulators and scientists.**

3. **The DOTT conference recognizes the positive collaboration between the fishers and farmers, and calls for a stronger integration between the BFT fishing and the farming industries for future mutual benefit of <u>all parties</u>.**

4. **The DOTT conference recognizes that successful and sustainable aquaculture of BFT will only be possible if they are environmentally and socially acceptable.**

5. **Domestication of the BFT is a long, complex and expensive undertaking. Considering the trans-national dimensions of the problems, international strategic Research and Development efforts are called for as well as funding. The industry is seen as an active participant in the drive.**

6. **The Conference calls for the establishment of a European RTD "Virtual" Center for the BFT domestication, of which part will be a land-based facility for holding mature animals (BFT).**

7. **The DOTT Conference plenary session is calling on all relevant governments, under the leadership of the European Community, to support the drive for a sustainable development of the BFT domestication and farming, by creating a liaison committee which will facilitate interactions among the fishing industry, farmers, management, regulators and scientists.**

Domesticating the BFT is not a simple project; it will take at least a decade, may be even longer, to complete all aspects of the domestication process. The only way to succeed in this endeavor is to work together on the European level and on the global level. No one country or nation can do it by itself; it is very complex, very expensive and requires a long time. We have to develop the ways to integrate all parties involved: Fishermen, farmers, scientists (including environmentalists) and governments on all levels, into a working force which will deliver the domestication at the end of the day, and facilitate a durable, rational, environmental friendly and sustainable farming as well as sea-ranching technologies for the benefit of Mankind and the BFT.

It was quite clear from the different presentations regarding the status of the many of the Tunid wild populations that over-fishing may cause irreversible damage to them. The BFT is one of the most looked after species by fishermen due to the high prices it commands in the Japanese sushi/sashimi markets and other markets the around the world. The present practice of fattening wild caught large to giant BFT is pressuring further the wild populations. It was the general understanding that the present “farming” practice of the BFT, will it be in Europe, Australia, Mexico and other places in the world is not a long-term sustainable procedure. On the other hand, it became quite clear that the BFT is a very interesting candidate for marine farming, having the theoretical potential of helping close the gap between demand and supply of finfish in the future. The prospect of taming the BFT into a true-farmed animal incited the minds of the vast majority of the DOTT participants.

A series of of DOTT RTD strategies were defined as follows:

1. **The RTD should be a multidisciplinary processes involving various thematic working teams including genetics** (**analysis of naturally occurring genetic variability - choosing the brood stocks**), **reproduction control, juvenile production, nutrition, behavior and physiology, grow-out and husbandry, engineering aspects, disease control, socio-economics and environmental.**

2. **A BFT virtual RTD Center should be set-up shortly. It should engulf all active teams, which are dealing with the DOTT. One element of the above center must be a physical one that will have land-based facilities in which large, and sexually mature BFT, can be maintained, manipulated and experimented with. All teams could carry some of their research there during different parts of the year.**

3. **The BFT RTD Program should be run in coordination amongst all thematic projects and managed by a board made up by the coordinators of all projects.**

The purpose of this meeting was to inform the participants on the State of Art regarding the BFT fisheries biology, physiology and farming, socio-economic aspects of moving towards mass culture of BFT, integrating the fishing industry into the domestication drive. In addition, some advancement was achieved in the formation of working groups in specific areas of the BFT domestication process, such as: genetics, reproduction control, larval rearing, nutrition, disease control, husbandry, engineering, handling, socio-economics and environmental impacts. It is our hope that these working groups will eventually submit R&D proposals (together with the industry) to the EU. We also share the hope that scientists from around the world will participate in such research and development programs.

Obituaries

Dr Zarko Peric DVM (Belgrade) MVSc(Belgrade)

Dr Peric graduated as Doctor of Veterinary Medicine at the University of Belgrade in Yugoslavia, after which he worked in the Fish Disease Laboratory at the National Veterinary Research Institute of Yugoslavia where he also graduated in the degree of Master of Veterinary Sciences. During the early 1990's, he spent four years at the National Aquaculture Centre in Fort San Lucjan in Malta, where he participated in setting up the pilot marine finfish hatchery, the Fish Disease Laboratory and the pathology service to the local industry. At this time, Dr Peric also took up the position of Fish Health Manager at Malta Mariculture Ltd. In 1996, he moved to Sicily where he was employed as a full time consultant in fish pathology to Acqua Azzurra SpA, and later Production Director. From the year 2000, he was also consultant to Acqua Azzurra SpA, Ittica Mediterranea SpA (Italy) and Stirling Aquaculture, University of Stirling (Scotland). In early 2001 he was appointed Aquaculture Consultant with the Department of Fisheries and Aquaculture of Malta.

Dr Peric has worked extensively on research aimed at solving endemic disease problems in the Mediterranean, improving laboratory diagnostic techniques and diversifying the species used for aquaculture production. At Fort San Lucjan, he was currently working on the immunogenic properties of Atlantic cod PNNV in sea bass, vaccine formulations and testing for Aquaculture Vaccines Ltd. (UK). Zarko was a very hard worker, ever willing to share his considerable knowledge with all his colleagues. Besides his day to day duties, he initiated and pursued various academic activities with articles in various scientific journals, notably the cell culture isolation of Noda virus in vitro as published in the Journal of General Virology in 1996. Zarko was entrusted with the organisation of the 11th International Conference of the European Association of Fish Pathologists that will be held in Malta in September 2003. He was also a member of the steering committee for the European project on the domestication of Bluefin tuna (DOTT-REPRODOTT). More recently, due to Zarko's particular contribution to Noda virus research, he was invited by top international pathologists to join them in undertaking an EU-funded project that would scrutinise the scientific basis for current zoo-sanitary control requirements imposed upon transfer and trade in seed stocks for international aquaculture.

Bart Vlaminck B.Sc. (Ghent), M.Sc. (Stirling)
Bart had 18 years experience in both temperate and tropical freshwater and marine aquaculture and fisheries. He had particular experience of the practical aspects of offshore fish farming, and was managing a marine hatchery and offshore project in Sicily. To be extended

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