MARINE FISH BROODSTOCK NUTRITION - THE NEED FOR BETTER EGG QUALITY AND SEED FOR INTENSIVE OFFSHORE AQUACULTURE

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TECHNICAL BULLETIN
USSEC SEA 06/2021
AQ2021-01

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Introduction

Aquaculture remains one of the fastest growing food production sectors with a total world production of 66.6 million tonnes in 2012 (FAO, 2014). Over sixty percent of this comes from fresh water bodies that in turn constitute only around 3% of the world's water resources. Marine water in oceans and seas constitute around 97%, indicating that the marine water environment is somewhat under-utilised.

The utilisation of the vast marine environment is required for an increased aquaculture production; however this potential has been limited due to many reasons, primarily various environmental risks posed by aquaculture, mainly due to poorly managed, large scale aquaculture installations that polluted bays. Moreover, some escapes of farmed stock threatened wild fish and spread diseases in some countries, increasing the negative image of aquaculture worldwide. Besides all this negative publicity for the sector, feeding fish on baitfish or wet diets and dry pellets containing fish meal and fish oil also threatens to further deplete world fish populations.

In efforts to neutralise these negative factors, environmental and regulatory frameworks for aquaculture are being developed by many countries worldwide and this creates a pathway for sustainable growth in the marine aquaculture sector. Coastlines and near shore sites for aquaculture are limited due to very high competition for space from many different stakeholders, namely the maritime sector and tourism. This indicates that there is an essential need for the sector to grow in extreme offshore marine waters, which will require specific highly developed structures that will persevere in very exposed sites. It is envisaged that technological developments and advances for offshore aquaculture structures (Holmyard, 2016) will contribute and give rise to a growth in marine aquaculture during the next decade. This will entail a significant increase in the requirements for good quality fingerlings as hatcheries strive to produce more high quality seed to satisfy this increasing demand for growth. Further, marine aquaculture is vital to relieving the pressure on wild fish stocks and creating jobs in coastal areas.

It is widely accepted that effective seed production requires special husbandry and particular nutritional requirements of broodstock fish which significantly affect fecundity, survival, egg size and egg and larval quality (Bromage, 1998). The importance of broodstock nutrition has been highlighted with a number of specific nutrients that have been identified to affect egg quality and fecundity (Watanabe and Vassallo Agius, 2003; Izquierdo et al., 2001). Most studies on broodstock nutrition for marine fish were carried out by Takeshi Watanabe and his scientific research team in Tokyo, Japan, who showed how nutrients affect the spawning performance of red sea bream *Pagrus major* with a number of experiments that initiated interest in this field during the 1980's. (Watanabe and Kiron, 1995). Following this work, hatcheries started to include some vitamins or additives to wet diets for broodstock maturation and improved egg quality.

At the start of this "broodstock nutrition era", specific nutrients for maturation and egg quality were still being investigated and the on-growing dry pellet diets that were in use at the time were not yet providing sufficient micronutrients for an adequate spawning performance. Thus most researchers or hatcheries preferred to keep their broodstock on wet diets and include additives prior to feeding. The red sea bream studies were followed by work on Japanese yellowtail *Seriola quinueradiata*, the gilthead sea bream *Sparus aurata* and striped jack *Pseudocaranx dentex* in the 1990's and early 2000's, that ascertained the importance of specific nutrients that should be included in broodstock diets for marine fish.

Since the turn of the millennium feed companies started producing broodstock dry pellet diets that included specific ingredients based on these studies for improved egg production and quality. Even though broodstock diet formulations are greatly improving, many hatcheries still prefer to use traditional "wet diets" with additives or a mix of the wet diets with the broodstock dry pellets. Arguments in favour of the use of wet diets range from the higher expense incurred to purchase broodstock diets, to lack of palatability especially for wild caught broodstock that prefer to feed on wet diets. On the other hand, the advantages of using dry pellets outweigh the disadvantages, especially when considering that dry pellets are sterile and do not pose a risk of introducing disease into the aquaculture system. Ease of storage, improved environmental sustainability, handling and the possibility of including any ingredient that can be beneficial for egg production and quality far outweigh the disadvantages and in the long term this will be more cost effective. In fact, in commercial production, the amount of formulated dry feed required for Asian grouper to grow 1kg costs $2.00 (Feed Conversion Ratio (FCR) 1.67), whereas if wet diets are used, the FCR would escalate to 6 and the cost per kg of fish produced will be $2.10 (Yang Sim et al., 2005).

The broodstock nutrition work carried out on dry pellets for yellowtail and striped jack in Japan showed that the effective ingredients are species specific, and even their levels of inclusion can alter the results (Watanabe and Vassallo Agius, 2003). In fact, in yellowtail, commercial soft-dry pellets produced for broodstock resulted in a much improved spawning performance when compared to the wet diet (Verakunpiriya, et al., 1996). On the other hand, for striped jack, a broodstock dry pellet that matched the already excellent wet diet in spawning performance results was developed after a number of experiments, showing that it can take a number of years until a broodstock dry diet is developed (Vassallo-Agius, 2001), after which, the diet can consistently be refined. During the past decade, marine broodstock nutrition work was carried out in Europe on two very highly valued species, Greater amberjack *Seriola dumerili* (Scabini, et al., 2011) and Atlantic Blue-fin tuna *Thunnus thynnus* (Selfdott Final Report, 2012), where paprika and squid were used to give excellent spawning results and consolidate the pioneering work set up before and at the turn of the Millennium. Moreover, these results highlight a very important aspect that every species needs to have its broodstock requirements identified for a superior spawning performance with excellent egg quality. Furthermore, when the right ingredients are available, the spawning performance will be improved, resulting in greater numbers of superior quality eggs and larvae.

This bulletin analyses the work carried out on marine broodstock nutrition over the years and identifies the specific ingredients that can be considered by feed manufacturers for inclusion in broodstock dry diets of any marine species. The levels of inclusion of the ingredients in the diets should be fine-tuned according to species and the feed companies should work closely with farmers so that further species specific improvements are obtained. Indeed, due to the species specificity of the ingredients required for a broodstock diet, broodstock feed manufacturers have a much closer working relationship with their customers in order to maximise gains for all concerned (Hutchinson, P. 2010).

It should be emphasized that the production of large numbers of good quality larvae is essential for the development of marine aquaculture, so if a high quality superior wet diet exists, this should be used in parallel so as to maintain quality egg production as feed companies develop quality species specific dry broodstock diets.

Studies on Different Species Over the Years

Pioneering Studies in Japan: Red Sea Bream

The first series of experiments that were carried out on red sea bream broodstock in Japan laid down the foundations for broodstock nutrition work on marine species and revealed the importance of pre-spawning nutritional regimes on egg quality, specifically the effect of broodstock diets on egg quality (Watanabe et al., 1984a), where cuttlefish meal produced a markedly higher number of eggs with higher egg quality. A lower protein level diet produced very low quality eggs whereas phosphorous and essential fatty acid deficient diets produced eggs that did not hatch (Watanabe et al., 1984a). In another experiment, Antarctic krill *Euphasia superba* was fed to the broodstock just before spawning and produced superior spawning results, showing that the diet has a rapid effect on spawning of marine fish species with short vitellogenic periods (Watanabe et al., 1984b). As a matter of fact, egg chemical components showed similar profiles to the broodstock diets indicating that certain nutrients are incorporated into the eggs produced (Watanabe et al., 1984c).

The effective components in frozen krill and cuttlefish meal for improved egg quality were identified as the lipid fraction of krill meal and the non-lipid fraction of cuttlefish meal, which constitutes a better protein source due to its favourable amino acid composition and higher cholesterol and phospholipid content (Watanabe et al., 1991a). The effective components in krill lipids were also identified as phosphatidylcholine in the polar lipid fraction and astaxanthin in the non-polar lipid fraction. Moreover, vitamin E supplemented at 200mg / 100g diet improved the percentages of buoyant eggs, hatching rates and normal larvae produced in red sea bream (Watanabe et al., 1991a). It was concluded that it was the egg quality rather than the gonad maturation or fecundity that was improved by the phosphatidylcholine, astaxanthin and vitamin E in the broodstock diets (Watanabe et al., 1991b). A summary of the various effective ingredients in diets and their effects on fecundity and egg quality for red sea bream are shown in Table 1.

Japanese Yellowtail

Prior to the knowledge gained through broodstock nutrition studies on red sea bream during the 1980's, Japanese yellowtail broodstock were fed on wet diets consisting of chopped Pacific mackerel *Scomber japonicus*, jack mackerel *Trachurus japonicus* or sardines *Sardinops melanostica*. However, with a number of improvements in broodstock management techniques (Mushiake, 1994), an improved broodstock diet was essential. Building on the previous work on red sea bream, and considering that yellowtail did not accept dry feed diets; moist pellets were used as a broodstock diet with improved results (Mushiake et al., 1993).

Better spawning results were required for this species and a breakthrough was achieved when the extruded or soft-dry pellets (SDP) were developed for commercial yellowtail production (Watanabe et al., 1991c). These SDP were easily acceptable to yellowtail, they had a better feed conversion and reduced waste loading during commercial production (Watanabe et al., 1991c). They were tested on wild-caught broodstock and their suitability for egg production and quality was proven when a superior spawning performance in terms of total eggs produced and egg quality was achieved (Watanabe et al., 1996). These results were achieved even though a commercial SDP was used as the broodstock diet, indicating that even though no special ingredients were supplemented; the quality of the commercial dry feed was superior to the wet diet and the moist pellet (Watanabe et al., 1996). With this basis, and considering the previous results on red sea bream studies, the benefits of including krill meal in the commercial SDP to formulate a specific broodstock diet for yellowtail were considered.

Table 1. Effective ingredients used for red sea bream broodstock nutrition studies and their effects on fecundity and egg quality

Ingredient	(%)	Fecundity	Egg Quality
Raw Krill (frozen)	100	+	+
Cuttlefish Meal	57	+	+
Cuttlefish Meal Oil	4	+	-
Defatted Cuttle Fish Meal	55	-	+
Krill Oil	5	-	+
Krill Oil	2.5	no effect	+
Krill Polar Lipids	2.5	-	+
Krill Non-Polar Lipids	2.5	-	+
Defatted Krill Meal	64	-	-
Vitamin E	200 mg/100g	+	+

Negative effects (-) or Positive effects (+) are shown with respect to the control diets for each experiment.

Krill meal was added to the SDP as 20% or 30% of the diet and was compared to the commercial SDP that was used as the control. Results did not show any improvements in spawning quality; on the contrary, there was deterioration in spawning quality (Verakunpiriya et al., 1997a). This was unexpected following the previous work on red sea bream where the frozen krill had produced excellent results and it was postulated that chemical components other than the carotenoids derived from the krill might have impaired the physiological abilities of the broodstock (Verakunpiriya et al., 1997a). Alternatively, it may have been a mere "overdose" as during the previous experiment, a 10% krill meal inclusion that was present in the commercial SDP gave very good spawning results (Watanabe et al., 1996).

In another attempt to improve the SDP for yellowtail broodstock, different levels of pure astaxanthin were supplemented in the commercial SDP, this time fine-tuning the astaxanthin contents in the diets. Pure astaxanthin was selected since it was this single carotenoid that was identified as the effective component in the non-polar lipid fraction of the krill lipids (Watanabe and Kiron, 1995). Four diets were produced for this experiment, the control was a commercial SDP with no added astaxanthin, and this was compared to three SDP diets with 20ppm, 30ppm and 40ppm astaxanthin (Verakunpiriya et al., 1997b). The results showed a very clear trend, where the optimal astaxanthin supplemental level of 30ppm produced superior results (Verakunpiriya et al., 1997b), while at 20ppm and at 40ppm supplementation; the results were inferior. This again clearly indicated that below and above certain levels of supplementation, the beneficial ingredients can become detrimental to the spawning performance. Moreover, the yellow egg coloration that indicated the presence of zeaxanthin and lutein in the eggs, converted from astaxanthin in the diets was more pronounced in the eggs from the 30ppm astaxanthin diet, indicating that carotenoids were directly incorporated into the eggs with a positive effect on egg quality (Verakunpiriya et al., 1997b).

The detrimental effect on spawning of the inclusion of excess quantities of ingredients in broodstock diets on the spawning performance was demonstrated when excess carotenoids were included in another SDP experimental diet for yellowtail broodstock. *Spirulina* was supplemented as a source of zeaxanthin which was the most abundant yellowtail egg carotenoid; converted from the astaxanthin in the previous experiment. However, with the intention of supplementing 30ppm zeaxanthin, the presence of other carotenoids such as β-carotene and lutein in the *Spirulina* was overlooked and the total carotenoids in the diet were circa 200ppm (Watanabe and Vassallo-Agius 2003). This resulted in a very poor spawning performance and verified that an overdose of carotenoids is detrimental to the spawning performance. These results were in line with the postulation by Watanabe and Vassallo Agius (2003); that an overdose of carotenoids could have been the reason behind the detrimental effect of krill carotenoids in the 20% and 30% supplementations in previous work.

In further efforts to improve the spawning performance of yellowtail, other ingredients were experimented upon in yellowtail broodstock diets; namely paprika powder and squid meal. Paprika powder contains the red carotenoids, capsanthin and capsorubin that have a better radical scavenging ability than astaxanthin (Hirayama, et al., 1994). Paprika was already proven effective for the improvement of nauplii quality when included in the broodstock maturation diet of the Pacific white-leg shrimp *Litopenaeus vannamei* (Wyban et al., 1997). It was supplemented as 2% of the SDP diet so that 30ppm paprika esters were supplemented, and this diet was compared to a commercial SDP and to another SDP diet containing 30ppm astaxanthin. The SDP containing paprika produced better egg quantity and quality (Vassallo-Agius et al., 2001a) and this superiority was confirmed when another experiment comparing the same paprika SDP diet to the control astaxanthin SDP diet and another paprika SDP containing squid meal (Vassallo-Agius et al., 2002). Both diets containing the paprika carotenoids produced superior egg quality as calculated by the hatching rate of normal larvae from total eggs produced.

With reference to squid meal inclusion in SDP for yellowtail, this was included as a replacement of 50% of the fish meal component in the diet; however the spawning quality from fish fed the diet containing squid meal was very similar to that of the diet containing only fish meal as a protein source (Vassallo-Agius et al., 2002). Squid meal is a prime valid ingredient for marine broodstock nutrition, however this result showed that more species specific work is required to identify the level at which this ingredient could benefit the spawning performance of yellowtail. Ingredients and their effects on the spawning performance of yellowtail as described above are summarised in Table 2.

Table 2. Effective ingredients used for yellowtail broodstock nutrition studies and their effects on fecundity and egg quality

Ingredient	Inclusion in SDP	Fecundity	Egg Quality
Krill Meal*	20%	-	-
Krill Meal*	30%	-	-
Pure Astaxanthin	30ppm	+	+
Pure Astaxanthin*	40ppm	-	-
Spirulina*	200ppm	-	-
Paprika	2%	+	+
Squid Meal	50%	no effect	no effect

Negative effects (-) or Positive effects (+) are shown with respect to the control diets for each experiment.
*Negative effects probably due to over supplementation.

Gilthead Sea Bream

Following the pioneering work on red sea bream and in line with work on yellowtail in Japan, a number of experiments were carried out on the broodstock nutrition of gilthead sea bream. In fact, gilthead sea bream showed that an increase in dietary *n-3* highly unsaturated fatty acids (n-3 HUFA) was found to increase fecundity (Fernández-Palacios et al., 1995). This increase was observed up to levels of 1.6% *n-3* HUFA in the diet, as higher levels of dietary *n-3* HUFA reduced the total amount of eggs produced even though there was an increase in egg *n-3* HUFA concentration (Fernández-Palacios et al., 1995). This once again indicated that nutrient levels included in marine broodstock diets can have negative effects if their optimum inclusion level is not respected.

A positive effect on the reproductive performance of gilthead sea bream was observed to be due to the protein component of cuttlefish and squid together with the optimal concentration of HUFA (Izquierdo et al., 2001). Moreover, as previously observed in red sea bream, vitamin E was shown to affect fecundity in gilthead sea bream (Izquierdo and Fernández-Palacios, 1997) with an increase in dietary α-tocopherol levels up to 125mg/kg resulting in an improved fecundity (Izquierdo et al., 2001). Recently, the dietary increase of an oleoresin preparation from paprika as a source of carotenoids improved the reproductive performance of gilthead sea bream (Scabini et al, 2011).

Striped Jack

During the 1980's and up to the 1990's there were various developments in wet diets used for striped jack in Japan, culminating in an very high quality diet made of jack mackerel, squid *Todarodes pacificus* and shrimp *Atypopenaeus sp.* in the ratio of 2:2:1, with added feed oil, vitamin E oil and cuttlefish oil (Watanabe and Vassallo Agius, 2003). This wet diet incorporated all the known ingredients beneficial to red sea bream as identified in previous work, and thus produced a very high fecundity and egg quality for juvenile production.

With the trend to shift to dry pellets for various advantages as already highlighted above, experiments started to examine the effective ingredients for dry diets in the mid to late 1990's. In parallel with yellowtail and gilthead sea bream studies, the effects of soft-dry pellets were examined, but contrary to the immediate success that was observed for yellowtail, the spawning performance of striped jack was significantly inferior when compared to the results obtained with the wet diet, and the total eggs produced by the commercial SDP were only one third of those produced by the wet diet (Watanabe et al., 1998). Moreover, no carotenoids were detected in the eggs obtained from both diets, indicating that in contrast to yellowtail in previous studies, there was no transfer of dietary carotenoids from broodstock into the eggs produced (Vassallo-Agius et al., 1998). Furthermore, a 2% supplementation of *Spirulina* in the SDP, which was already proven to be effective in striped jack diets for improved colour, texture, taste (Liao et al., 1990) and pigmentation (Okada et al., 1991) did not produce encouraging results (Vassallo-Agius et al., 1999).

In a separate study, it was also noted that striped jack broodstock fed on steam dry pellets showed better feeding activity and improved egg production (Watanabe and Vassallo Agius, 2003), most probably due to the lower lipid content (15% lipid content on a dry basis when compared to over 20% in SDP), which prevented excessive abdominal lipid accumulation that interfered with spawning. For these reasons, it was decided to shift to steam dry pellets for the next experiments and improvements in the spawning performance and egg quality were pursued by experimenting with astaxanthin, squid meal or squid meal and krill meal. Astaxanthin was included separately in a first experiment as pure astaxanthin, supplemented at 10ppm in the dry diet – a level that matched the carotenoid levels in the wet diet on a dry basis. This improved fecundity when compared to a commercial dry pellet, but the results were slightly lower than those for the control high quality wet diet (Vassallo-Agius et al., 2001b). In another experiment, the inclusion of squid meal as a replacement of 50% of the fish meal in the diet improved egg quality when compared to both the dry diet and the control wet diet (Vassallo-Agius, 2001c). In the same experiment, a diet containing squid meal, fish meal and krill meal in equal proportions as the protein sources (1:1:1) gave egg quality similar to the control wet diet but these were lower than the dry pellet that contained squid meal and fish meal without krill meal (Vassallo-Agius, 2001c).

These experiments clearly showed that the egg production or fecundity and the egg quality were improved by different ingredients; astaxanthin in the former and squid meal in the latter. Therefore, in the next experiment, both significant improvements were put together when a dry pellet with squid meal (fish meal: squid meal, 1:1) and astaxanthin supplementation (10ppm) was developed. As expected, these beneficial ingredients worked and the total eggs produced and the egg quality matched those from eggs produced by the wet diet (Vassallo-Agius et al., 2001d). All ingredients and their effects on the spawning performance of striped jack during these studies are summarised in Table 3.

Greater Amberjack

Although the greater amberjack has a very high commercial value and is of great interest to aquaculture due to its fast growing capabilities, the production of sufficient amounts of juveniles in Europe has been a bottle-neck due to a lack of high quality spawns. Without the availability of a dry pellet broodstock diets for amberjack, broodstock management improvements with high quality wet broodstock diets showed a marked increase in the numbers of eggs produced. Amberjack broodstock reared in Malta (average BW 20.7±2.3kg) were fed on wet diets that consisted of mackerel *Scomber scombrus* and squid *Lolito vulgaris* (1:1) supplemented with 2% paprika powder. These fish produced between 2.3 and 4.6 million eggs / female per spawning season during three subsequent spawning seasons between

Table 3. Effective ingredients used for stiped jack broodstock nutrition studies and their effects on fecundity and egg quality

Ingredient	Inclusion in Dry Diet	Fecundity	Egg Quality
Spirulina	2%	-	-
Pure Astaxanthin	10ppm	+	-
Fish Meal (FM) and Squid Meal (SM)	1:1	-	+
FM, SM and Krill Meal	1:1:1	-	no effect
FM, SM and Pure Astaxanthin[1]	1:1; (10ppm)	+	+

Negative effects (-) or Positive effects (+) are shown with respect to the control diets for each experiment.
[1] 10ppm included to match astaxanthin content in wet diet.

2010 and 2012, which relates to 111,000 to 222,000 eggs kg $^{-1}$female BW season^{-1}, with average fertilization rates of 91.2%, 87.5% and 90.5% (Vassallo-Agius, unpublished data).In comparison to another study on captive amberjack broodstock one female produced around 55,000 eggs kg $^{-1}$female BW season^{-1} with fertilization rates around 13% (Mylonas et al., 2004), whereas 25kg amberjack broodstock fed on a wet diet based on mackerel, the fecundity was around 114,490 eggs kg $^{-1}$female BW season^{-1} with an average fertilization rate of 61.7% (Jerez et al., 2006).

These results indicate that, in agreement with previous work on a number of marine species, squid as a protein source and paprika supplementation increased fecundity and egg quality in the greater amberjack, producing up to 222,000 eggs kg $^{-1}$female BW season^{-1}. This indicates that squid and paprika supplementation improved spawning maturation, fecundity and egg quality however it will be very interesting to identify the individual effect of both nutritional components separately on fecundity or egg quality. It must be noted that the maturation of amberjack is very delicate and without the correct broodstock management practice, results can be compromised. Further work needs to be done to identify the different levels of the individual ingredients and their effect on fecundity and egg quality. These improvements and the excellent adaptability of this species to offshore aquaculture augurs well for the future of greater amberjack aquaculture through higher numbers of good quality juveniles.

Atlantic Blue-Fin Tuna

Until recently, one of the major bottlenecks in the development of techniques for the production of Atlantic blue-fin tuna juveniles has been the collection of high quality eggs. Similar to egg collection of Pacific blue-fin tuna *Thunnnus orientalis* in Japan, Atlantic blue-fin tuna large scale egg collection was first achieved in offshore cages in Italy (DeMetrio et al, 2010), followed by Spain, Malta and Croatia. In the first years in the mid 2000's, diets mainly consisting of Pacific mackerel *Scomber japonicus* and herring *Clupea harengus* at a ratio of 9:1 were fed to broodstock in Italy (DeMetrio et al., 2010) while mackerel *Scomber scombrus* and Spanish mackerel *Scomber japonicus* were fed to broodstock in Spain (Selfdott Final Report, 2012). Some actual work on broodstock nutrition was carried out during the Selfdott Project, a European Union Fifth Framework Programme funded project, where two groups of Atlantic blue-fin tuna were fed different diets and their maturation was directly assessed (Selfdott Final Report, 2012).

The control diet was mackerel alone while the experimental diet was made of a 1:1 mix of squid and mackerel that was supplemented with 2% commercial paprika powder added for nutritional enhancement due to its high content of carotenoids - capsorbin and capsanthin. Following a three month period prior to spawning, the experimental diet was shown to result in a superior growth, higher Gonado Somatic Index values (GSI), as well as significantly higher condition factors for both males and females. Fish fed the experimental diet showed a more advanced gonadal development, with females having oocytes of greater diameter than those fed on the control diet. Moreover, the improved broodstock diet was associated with a lower occurrence of apoptosis of vitellogenic oocytes and a higher vitellogenin gene expression (Selfdott Final Report, 2012).

Furthermore, fertilized eggs collected from Atlantic blue-fin tuna were pigmented light or dark yellow after being fed on squid that contained a pink / red internal coloration, indicating that carotenoids present within the squid were converted and transferred into the eggs (Vassallo-Agius, unpublished data).

Other species of blue-fin tuna showed an improved spawning performance when their broodstock were fed on quality diets. Following on from Japanese practice on Pacific blue-fin tuna, yellow-fin tuna *Thunnus albacores*, were fed on mackerel and squid with vitamin C and E supplements prior to spawning and high quality eggs produced (Hutapea et al., 2009). Moreover, soft pellet formulas including fish, crustaceans and squid in similar percentages to those found in wild caught mature fish together with appropriate additives were proposed for Southern blue-fin tuna *Thunnus* maccoyii (Young P.C., 2001). Identical to other marine fish, these studies confirmed the importance of including squid and antioxidants in broodstock diets for a superior spawning quality in blue-fin tuna species. Nevertheless, more work needs to be done on the specific ingredients, their levels of inclusion in the diet and their effects on spawning.

Current Status and the Importance to Replace Wet Fish Diets with Dry Pellets for Broodstock

The shift from traditional wet diets to dry pellet diets used for aquaculture is significant and of great importance for the sustainable and increased development of global aquaculture that is foreseen in the coming years. The production of dry feeds leads the way for sustainability as the required ingredients, as well as alternative protein sources can be included in the diet that will significantly reduce environmental pollution and losses. More importantly, wet diets hold a risk of bacterial pathogen transfer to the cultured fish, as shown through controlled tests in Indonesia where bacterial concentrations in trash fish were significantly higher (ten to 100 times higher) than those in dry diets over a three day period (Hasan, R., 2009). Other advantages of dry diets include easier handling and storage in addition to the fact that the species specific needs can be fine-tuned according to the size and stage of growth of the cultured species.

Along with the importance to shift to dry feeds for commercial production, broodstock dry feeds should also be produced though joint efforts between feed mills and the hatcheries. In a situation where no broodstock dry diet is readily available, it must be understood that gonadal maturation requires dietary protein and energy levels that may not be adequate in normal grow-out diets. For this reason it is essential that feed companies work hand in hand with the hatchery towards developing broodstock sized dry pellet diets. A good starter point would be to utilise formulations for younger fish and produce larger sized dry pellets; in view of the fact that younger fish usually require more protein and energy levels in their diets, equivalent to the parent fish. Squid as a protein source, the inclusion of antioxidants and other ingredients that may be beneficial should be carried out step by step so that positive steps forward can be identified towards the production of a high quality broodstock dry diet.

Effective Ingredients for Marine Fish Broodstock Diets

Various effective ingredients have been identified for marine fish broodstock diets that require the right ingredients at the right supplemental levels for effective results. Studies towards the formulation of wholesome broodstock diets should be carried in a methodical manner whereby optimal ingredient levels can be determined. The cost of the ingredients, their availability and the advantages they offer should also be considered.

Squid

The main ingredient that has shown positive results in all species is squid meal that is usually included as a replacement for part of the fish meal in dry diets. The efficacy of squid has been attributed to its superior protein quality, higher phospholipid and cholesterol content (Watanabe et al., 1991a) or its better apparent protein digestibility coefficients (Fernández-Palacios et al., 1997). It has been shown to improve both fecundity and egg quality in most fish species however its percentage inclusion needs to be defined more precisely.

Squid meal was included in dry diets for red sea bream (Watanabe et al., 1991a), gilthead sea bream (Fernández-Palacios et al., 1995) and yellowtail (Vassallo-Agius et al., 2002) with very good results. It is recommended for inclusion in all marine fish broodstock dry diets; however its level of inclusion needs to be investigated for an optimal effect on the spawning performance, as well as for economic reasons due to its higher price. Although no specific investigations have concluded the proportions of fish and squid fed in wet diets, it has been included at a 1:1 ratio with baitfish such as mackerel for blue-fin tuna (Selfdott Final Report, 2012), for the dusky grouper *Epinephelus marginatus* (Pierre et al., 2008) and amberjacks in the Mediterranean (Vassallo-Agius, unpublished data). Basically, squid or squid meal is used in all fish marine broodstock diets across the world (Izquierdo et al., 2001; Watanabe and Vassallo-Agius, 2003; Fotedar and Minh Sang, 2011; Young P.C., 2001).

Carotenoids: Astaxanthin and Paprika Esters

Carotenoids constitute the group of other supplemental ingredients that have shown beneficial effects repeatedly on marine fish spawning performance. It is known that carotenoids have a number of biological functions in animals (Latscha T, 1991) and their oxygen quenching ability suppresses oxygen free radicals to produce harmless end products.

Firstly, the astaxanthin found in krill meal was effective for red sea bream spawning (Watanabe et al., 1991a), giving rise to a number of experiments on yellowtail where its effect at 30ppm was beneficial to both egg quantity and quality (Verakunpiriya et al., 1997b) and striped jack, where it was found to improve fecundity rather than egg quality (Vassallo-Agius et al., 2001d).

The paprika esters capsanthin and capsorbin were very effective and further improved spawning results in yellowtail (Vassallo-Agius et al, 2002), and produced very good overall results along with squid in blue-fin tuna (Selfdott Final Repot, 2012) and amberjacks (Vassallo-Agius unpublished data). In the absence of dry diet formulation, paprika powder was added at 2% to wet diets consisting of squid and mackerel at a ratio of 1:1 for bluefin tuna and greater amberjacks.

It was suggested that the radical scavenging ability of capsanthin was especially due to its conjugated keto group and the polyene chain that results in a more potent activity (Matsufuji et al., 1998). In agreement with this premise, it was already shown that capsanthin and capsorbin have a better radical scavenging ability than astaxanthin (Hirayama et al, 1994).The incorporation of carotenoids from the broodstock diet into eggs was observed in some species such as the yellowtail (Verakunpiriya et al., 1997b) and Atlantic blue-fin tuna (Selfdott Final Report, 2012), where egg quality markedly improved. On the other hand, amberjack eggs were similar to striped jack eggs that were colourless; indicating that carotenoids were not incorporated into the eggs from the broodstock diet (Vassallo-Agius et al., 1998).

More work needs to be done to determine the supplemental levels in diets and identify the biological pathways of carotenoids in marine broodstock fish. It will also be interesting to investigate the effects of these carotenoids on fish that do not incorporate carotenoids into their eggs but show an overall improved spawning performance through carotenoid supplementation in their diets prior to spawning.

Krill and Spirulina

At the start of broodstock nutrition investigations, krill meal was supplemented to red sea bream diets with positive effects on spawning, even when a 100% krill diet was fed (Watanabe et al., 1984a; 1984b). However, incorporation of krill meal in yellowtail (Verakunpiriya et al., 1997a) and striped jack diets (Vassallo-Agius et al., 2001c) did not give convincingly good results in subsequent years. In view of this, it would be justifiable to investigate krill or krill meal inclusion as a superior protein source or as a source of astaxanthin since it is possible that the negative effect on spawning of the two latter species could have been due to an overdose of the ingredient (Watanabe and Vassallo-Agius, 2003).

Similarly, *Spirulina* added to SDP diets for striped jack (Vassallo-Agius et al., 1999) and yellowtail (Vassallo-Agius unpublished data) did not yield positive results as the quantity of supplemented *Spirulina* for the latter was based on 30ppm zeaxanthin and ignored the fact that there were various other yellow carotenoid such as β-carotene and lutein. This caused an overdose of carotenoids in the diet, as also reported for striped jack (Watanabe and Vassallo-Agius, 2003). It would be beneficial to understand the effect of lower doses of *Spirulina* on the spawning performance of marine fish as this can determine if this readily available source of carotenoids can be utilised for broodstock diets.

Vitamins

Vitamins are compounds that have antioxidant capabilities; however their inclusion in broodstock diets for marine fish has not been studied in great detail. The requirement of vitamin E as an essential dietary component in fish has long been recognised and minimum requirements for many fish species have already been determined (Palace and Werner, 2006).

Vitamin E requirement is highly dependent on the amount of fatty acids in the diet as well as dietary concentrations of selenium, sulphur containing amino acids, other antioxidants, iron, vitamin A and C, and quinines (Palace and Werner, 2006). Vitamins C and E are also potent antioxidants that provide an important protective role for sperm cells during spermatogenesis and until fertilization by reducing the risk of lipid peroxidation (Izquierdo et al, 2001).

Vitamin E supplemented at 200mg / 100g diet improved the percentages of buoyant eggs, hatching rates and normal larvae produced in red sea bream (Watanabe et al., 1991a). In broodstock sea bream fed on several vitamin E and *n-3* HUFA levels and an increase in dietary α-tocopherol levels up to 125mg/kg, an improvement in fecundity was obtained (Izquierdo et al., 2001). Moreover, the addition of vitamin E oil at 1% to wet broodstock diets for striped jack (Watanabe et al., 1998) was included after the work on red sea bream (Watanabe et al., 1991a) but its effect was not directly investigated.

It is essential that vitamins are added or supplemented to any marine fish broodstock diet as they cannot be synthesised (Palace and Werner, 2006), however specific studies on broodstock fish requirements for vitamins are very limiting. It is common practice to add a vitamin premix to wet broodstock diets for many marine fish species, following on from the specific studies carried out on red sea bream (Watanabe et al., 1991a) and gilthead sea bream (Izquierdo et al., 2001). Further work is required to determine the exact quantities of vitamins for marine fish broodstock.

Broodstock Feed Management

We can distinguish between continuous spawners with short vitellogenic cycles including gilthead sea bream and annual spawners with long vitellogenic cycles (Fernández-Palacios et al., 1997). The former are more sensitive to dietary changes and ingredients given on the verge of spawning as these will have an immediate effect on the spawning performance (Watanabe et al., 1984b). In annual spawners, egg quality is determined by mobilisation of nutrients from longer term muscle storage sites (Izquierdo et al., 2001). These factors should play a part in the feed management strategy throughout the year.

The broodstock annual cycle can be divided into three; the pre-spawning period, the spawning period and the post-spawning period (Mushiake, 1994). The length of the pre-spawning, spawning and post-spawning feeding periods may alter according to species, but the quality of the diet should be optimum during the pre-spawning and spawning periods (Muskiake, 1994), with the pre-spawning period usually around 3 months prior to spawning (Watanabe and Vassallo-Agius, 2003). The pre-spawning period can probably be shorter for species that have short vitellogenic cycles; a 30 day period was used for gilthead sea bream (Scabini et al., 2011). During the post-spawning period, a maintenance diet should be fed, and this may consist of commercial pellets or wet diets without any particular need for additives.

Japanese research usually basis fish broodstock feeding on three times per week to near satiation (Watanabe and Vassallo-Agius, 2003), whereas daily feeding was implemented for gilthead sea bream (Scabini et al., 2011) and Atlantic blue-fin tuna broodstock were fed six days a week (Selfdott Final Report, 2012). As in most topics on marine fish broodstock nutrition, further work is needed to identify the different periods, feeding frequency, feeding ratios and diets for the specific species so that broodstock feed management can be optimised for a more efficient quality production.

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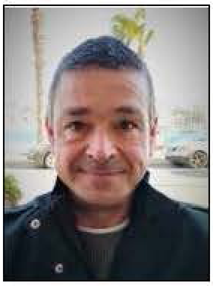

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His involvement in aquaculture spans over the last 34 years, starting as an undergraduate student working in Malta's pilot marine hatchery, then progressing to obtain his MSc and PhD from Tokyo University of Fisheries, specialising in marine finfish broodstock nutrition.

During these years he has contributed to the development of species of commercial interest for aquaculture development like yellowtail and striped jack in Japan and Atlantic blue-fin tuna and the Mediterranean amberjack in Europe.

Along the way he has gained experience in marine hatcheries and spent the last 20 years spearheading the development of new species for aquaculture, to the strategic and development issues in his home country, Malta, as well as the Mediterranean region. His major role is to develop sustainable aquaculture and diversify aquaculture development through private and EU funded research projects.

He is the former Head of Aquaculture Research at the Malta Aquaculture Research Centre where he successfully spearheaded innovative research and development in amberjack and bluefin tuna propagation, along with the National Aquaculture Strategy for Malta. Apart from aquaculture consultancy work, he also teaches aquaculture related topics at first degree level at the University of Malta and at the Malta College of Arts, Science and Technology.

Soy In Aquaculture Program

This technical paper was created through the USSEC Soy In Aquaculture (SIA) program and the USSEC Southeast Asian Regional Program. USSEC works with target audiences in Southeast Asia and globally to show the utility and benefits of using United States soybean products in aquaculture diets.

The SIA program replaces the Managed Aquaculture Marketing and Research Program (the AquaSoy Initiative, funded and supported by the United Soybean Board and American Soybean Association) which was designed to remove the barrier to soybean meal use in diets fed to aquaculture species.

The objective of the SIA is to optimize soy product use in aquaculture diets and to create a preference for U.S. soy products in particular, including but not limited to U.S. soybean meal, soybean oil, soybean lecithin, and "advanced soy proteins" such as fermented soy and soybean protein concentrate.

This paper follows the tradition of USSEC to provide useful technical materials to target audiences in the aquaculture industry.

For more information on soybean use in aquaculture and to view additional technical papers, please visit the Soy-In-Aquaculture website at www.soyaqua.org.

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