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Broodstock nutrition research on marine finfish in Japan

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Abstract

Broodstock diet formulations are essential for the development of marine fish breeding and propagation. Japanese research has mainly focused on red sea bream (*Pagrus major*), yellowtail (*Seriola quinqueradiata*), the Japanese flounder (*Paralichthys olivaceus*) and striped jack (*Pseudocaranx dentex*), and indicated that the required quantities of essential dietary components may vary according to species. The more recent work carried out on yellowtail and striped jack consolidated the trends already observed in red sea bream. In yellowtail, soft-dry pellets were used and astaxanthin was found to be the determining factor for good egg quality, when supplemented at around 30 mg/kg. Paprika powder supplementation that provided about 30 mg/kg paprika esters further improved the spawning performance of yellowtail in terms of egg production, egg quality and larval survival whereas squid meal inclusion also showed potential as an effective ingredient. Work on the development of dry pellets for striped jack broodstock revealed that egg production and quality were affected by separate nutrients. Even though striped jack eggs do not contain carotenoids, dietary astaxanthin, added at 10 mg/kg to the diet, increased fecundity whereas egg quality was improved through the replacement of half the fish meal with squid meal. The combination of these two ingredients in dry pellets produced a spawning performance that matched the widely used raw fish mix. Fatty acids, especially $n-3$ highly unsaturated fatty acid (HUFA) in fertilized eggs, were derived from dietary lipids in all the species studied.

From the present information, it is evident that optimal levels of astaxanthin or paprika esters that have high oxygen quenching abilities, squid meal and $n-3$ HUFA play an important role in gonadal development of different marine fish. These dietary components should be utilised for the development of wholesome dry pellets for marine broodstock.
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Keywords: Marine broodstock; Highly unsaturated fatty acid; Finfish

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1. Introduction

Broodstock husbandry and spawning techniques are constantly upgraded as Japanese marine hatcheries require a high number of good quality eggs to satisfy the needs for aquaculture and sea ranching. Despite various technological advances, aquaculture in Japan remains comparatively traditional whereas sea ranching has developed into a completely new sector since its establishment in 1962, with the prime function of enhancing fish production from Japanese waters (Matsuoka, 1989).

Government hatcheries of the Japan Sea Farming Association developed sea farming and the improvement and optimisation of various methods for broodstock culture. However, at the start of the 1980s, it soon became evident that broodstock diets were limiting the spawning performance of various high quality marine species. This concurred with studies on rainbow trout *Oncorhynchus mykiss* that indicated positive and negative effects of broodstock diets on spawning (Takeuchi et al., 1981; Springate et al., 1985; Watanabe et al., 1984a). Today, it is widely accepted that effective seed production demands a thorough understanding of the special husbandry and particular nutritional requirements of broodstock fish which significantly affect fecundity, survival, egg size and egg and larval quality (Bromage, 1998).

A number of experiments on marine broodstock nutrition in Japan have been performed on four species of commercial importance for aquaculture and marine stock enhancement; namely red sea bream *Pagrus major*, yellowtail *Seriola quinqueradiata*, the Japanese flounder *Paralichthys olivaceus* and striped jack *Pseudocaranx dentex*. A characteristic of Japanese broodstock research is that the studies were usually coordinated with the production of eggs for sea farming purposes, thus providing access to large-scale experiments that require results with a practical application under natural photoperiod conditions.

Fig. 1 shows the aquaculture production of the abovementioned species over a 10-year period (Annual Report of Fisheries and Aquaculture of Japan, 1999). Red sea bream and yellowtail constituted the major finfish aquaculture production figures in 1998 at 82,000 and 146,000 metric tones, respectively. Aquaculture production of the Japanese flounder almost doubled during this 10-year period and was around 8000 metric tones in 1998. On the other hand, striped jack production reached a peak of 2653 metric tones in 1995 but has marginally dropped recently, probably due to problems with the striped jack nervous necrosis virus (SJNNV). Since the occurrence of SJNNV (Mushiake et al., 1992, 1993a), Mushiake et al. (1994a) developed protocols to prevent the vertical transmission of this virus through the selection of gene-negative fish for spawning. If this method proves to be successful and reliable on a large scale, the upward trend in production that was observed prior to 1995 may be restored in the near future.

Apart from the need to develop broodstock diets, the rapid decline in harvests of low-value fish such as sardines *Sardinops melanostica*, jack mackerel *Trachurus japonicus* and sand lance *Ammodytes personatus* led Japanese farmers to utilise dry pellets. These could effectively substitute traditional raw fish diets and reduce the dependence on fish meal by employing alternative protein sources (Watanabe, 1996). Various advantages of dry pellets over raw fish diets (frozen fish may introduce fish diseases into a culture system) as well as the need for dry diets that promote gonadal maturation and viable egg production

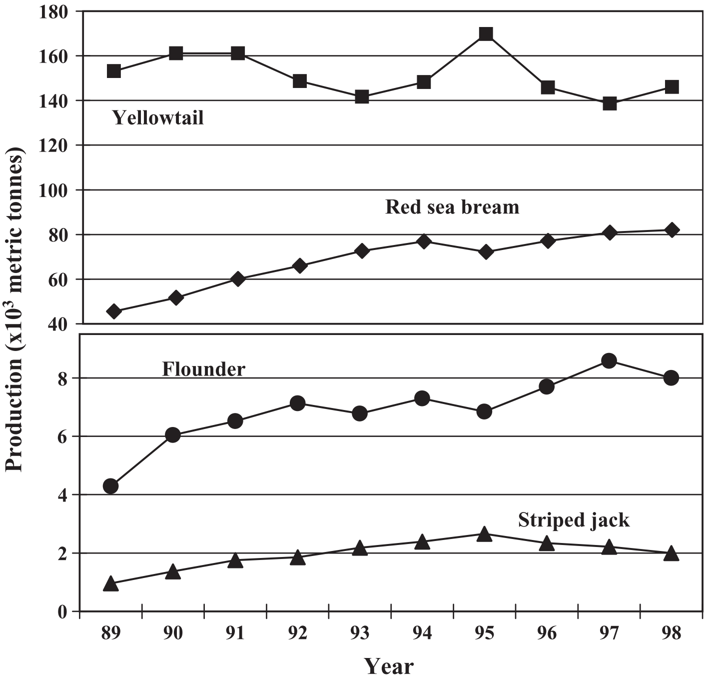

Fig. 1. Recent production trends of main mariculture finfish in Japan.

propelled research towards the formulation and development of quality broodstock feeds for commercially important species.

Research started with a series of experiments on red sea bream in the 1980s that revealed the importance of pre-spawning nutritional regimes in marine fish (Watanabe and Kiron, 1995). This work, along with the development of extruded dry pellets (known as 'soft-dry' pellets because of their high palatability and acceptability, Watanabe et al., 1991a), set the basis for pursuing work on yellowtail (Verakunpiriya, 1996). Moreover, a number of trials on the development of dry pellets for striped jack broodstock were also carried out (Vassallo-Agius, 2001) while other work focused on $n-3$ highly unsaturated fatty acid (HUFA) levels in broodstock diets for the Japanese flounder (Furuita et al., 2000). The present review discusses these studies and focuses on the more recent work on yellowtail and striped jack with the aim of understanding the specific dietary needs of marine broodstock.

2. Red sea bream

Red sea bream, which symbolises good fortune, has a great commercial importance in Japan. It is known as *madai* and is usually eaten raw as *sashimi* or presented in ceremonies such as weddings, where it is seasoned with salt and grilled. It therefore comes as no surprise that its production has steadily increased (Fig. 1) and is only

Table 1
Selected dietary ingredients/composition and spawning results of red sea bream fed experimental diets (Watanabe et al., 1984b)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredient (%)					
Fish meal[a]	72.0	52.0	72.0	–	–
Casein	–	–	–	51.0	–
Cuttlefish meal[b]	–	–	–	–	57.0
Amino acid mix[c]	–	–	–	4.0	–
Cellulose	1.0	14.0	1.0	10.0	15.0
Cuttlefish oil[d]	4.0	11.0	4.0	–	5.0
Beef tallow	–	–	–	5.0	–
Corn oil	–	–	–	7.0	–
Others[e]	23.0	23.0	23.0	23.0	23.0
Proximate composition (%)					
Crude protein	51.0	35.8	50.1	48.6	45.7
Crude lipid	10.7	15.5	10.7	10.6	11.5
Phosphorus	2.8	2.1	2.2	1.2	1.4
Gross energy (kcal/100 g)	447.0	471.0	466.0	507.0	479.0
Main fatty acids (% area)					
14:0	6.3	5.2	6.3	1.3	2.7
14:1	0.3	0.3	0.3	traces	0.1
15:0	0.3	0.3	0.3	traces	0.6
16:0	18.5	17.6	18.5	17.9	23.9
16:1	7.0	6.1	7.0	1.5	3.0
18:0	3.0	2.9	3.0	9.2	8.0
18:1$n-9$	15.0	15.9	15.0	36.3	10.1
18:2$n-6$	0.9	1.0	0.9	28.2	0.9
18:3$n-3$	0.3	0.6	0.3	0.7	0.3
18:4$n-3$	0.1	0.2	0.1	0.2	0.1
20:1	14.3	12.6	14.3	0.5	6.7
20:3$n-3$ + 20:4$n-6$	1.0	1.3	1.0	–	4.7
20:4$n-3$	0.3	0.5	0.3	–	0.9
20:5$n-3$	8.7	10.2	8.7	–	10.8
22:1	7.8	6.9	7.8	–	2.6
22:5$n-6$	0.1	0.2	0.1	–	0.7
22:5$n-3$	2.1	2.2	2.1	–	1.7
22:6$n-3$	7.4	9.8	7.4	–	16.2
$\Sigma n-3$ HUFA	18.5	22.7	18.5	–	29.6
Spawning results					
Total eggs ($\times 10^3$/female)	1005	727	841	1165	1735
Hatching rate (%)[f]	62.4 ± 20.4	3.8 ± 6.3	6.2 ± 5.2	–	97.6 ± 3.4

Phosphorus was deleted from the mineral mix included in diet 3.
[a] Crude protein: 62.5%, crude lipid: 8.3%, $\Sigma n-3$ HUFA: 9.9%.
[b] Crude protein: 78.9%, crude lipid: 10.5%, $\Sigma n-3$ HUFA: 37.1%.
[c] L-Phe: 0.6%, L-Arg: 1.5%, L-Trp: 0.2%, L-AspNa: 1.0%, L-Val: 0.7%.
[d] Contains 1.0% vitamin E.
[e] α-Starch: 15.0%, mineral mix (Ogino salt mix): 5.0%, vitamin mix: 2.0%, choline chloride: 1.0%.
[f] Rate of normal larvae obtained: mean ± S.D., $n = 7$.

second to that of yellowtail. Artificial breeding was first attempted in 1887 in Hiroshima and the first production of juveniles was reported in Kanagawa in 1962 (Davy, 1991). With the established technology for larval mass-culture (Fukusho, 1989), the need to investigate the effects of broodstock diets became evident and led to a series of pioneering experiments on its pre-spawning nutritional needs (Watanabe and Kiron, 1995).

In an initial investigation (Table 1), four experimental diets were formulated and compared to a control diet (diet 1). These were low in protein (diet 2), low in available phosphorus (diet 3), deficient in essential fatty acids (EFA) (diet 4; based on casein with corn oil and beef tallow plus an amino acid mixture) or containing cuttlefish meal (diet 5) instead of fish meal (Watanabe et al., 1984b). Spawning results in terms of total eggs, buoyant eggs, egg normality and hatching rates showed that the broodstock diet containing cuttlefish meal was superior. On the other hand, the low-protein, available phosphorus-deficient and EFA-deficient diets produced abnormal eggs of low hatchability and high larval deformities (Table 1). Although dietary phosphorus deficiency did not affect the mineral compositions of broodstock tissues and eggs, the low-protein diet produced eggs with decreased protein levels. Egg essential fatty acids (EFA) were also correspondingly greatly affected by the level of these EFA in the diets (Watanabe et al., 1984c). Another study determined the optimum protein level to be around 45% (Watanabe et al., 1984d) and confirmed the superiority of cuttlefish meal over fish meal as a protein source. As shown in Table 2, improved egg quality was also observed when the broodstock were fed frozen raw Antarctic krill *Euphasia superba* or diets containing canthaxanthin, or cuttlefish liver oil just before spawning (Watanabe et al., 1984e). This experiment also indicated the importance of the nutritional quality of the diet, and its rapid effect on marine fish species with short vitellogenic periods. Although the analyses of egg chemical components showed profiles that corresponded to the broodstock diets, there was no particular relation to egg quality (Watanabe et al., 1985a). Further work proved the efficacy of cuttlefish meal and frozen raw krill in broodstock diets for this species (Watanabe et al., 1985b). Watanabe et al. (1991b) even showed that it was the lipid fraction of krill meal and the nonlipid fraction of cuttlefish meal that resulted in the best egg quality.

The superiority of cuttlefish meal as a better protein source for red sea bream broodstock was attributed to its favourable amino acid composition and higher cholesterol and phospholipid content (Watanabe et al., 1991b). On the other hand, the effective components in krill lipids were identified as phosphatidylcholine in the polar lipid fraction and astaxanthin in the nonpolar lipid fraction (Watanabe et al., 1991c). Moreover, vitamin E, supplemented at 200 mg/100 g diet as DL-α-tocopheryl acetate improved egg quality (Watanabe et al., 1985b, 1991b,c) and was easily incorporated into egg lipids (Watanabe et al., 1985a, 1991b).

Watanabe et al. (1991c) concluded that egg quality rather than gonad maturation of red sea bream is aided by phosphatidylcholine, astaxanthin and vitamin E which may be involved as free radical scavengers. These experiments mainly attributed importance to the effective components in cuttlefish meal and krill meal and set a standard for further investigations towards the development of broodstock diets for marine fish.

Table 2
Selected dietary ingredients/composition and spawning results of red sea bream fed experimental diets (Watanabe et al., 1984d,e)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredient (%)						
Fish meal[a]	82.0	34.0	82.0	74.5	frozen	67.0
Cuttlefish meal[b]	–	31.0	–	–		–
α-Starch	7.0	15.0	7.0	9.0		15.0
Mineral mix[c]	5.0	5.0	5.0	4.5	krill	5.0
Vitamin mix	2.0	2.0	2.0	1.8		2.0
Choline chloride	1.0	1.0	1.0	0.9		1.0
Cuttlefish oil[d]	3.0	3.0	3.0	–		–
Oil extracts from krill[e]	–	–	–	9.0		–
Corn oil	–	–	–	–		10.0
β-Carotene[f]	–	–	0.1	–		–
Canthaxanthin[g]	–	–	0.3	–		–
Proximate composition (%)						
Crude protein	52.1	43.6	53.0	50.1	–	45.7
Crude lipid	9.5	9.0	9.9	14.9	–	16.6
Main fatty acids (% area)						
14:0	4.5	4.0	5.2	8.8	–	1.5
16:0	17.0	18.4	18.7	21.3	–	14.1
16:1	6.3	5.5	6.5	8.3	–	2.3
18:0	2.5	3.8	2.9	2.0	–	2.2
18:1$n-9$	16.6	14.8	19.6	20.1	–	30.6
18:2$n-6$	1.4	1.8	1.7	3.0	–	34.0
18:3$n-3$	0.8	0.9	0.8	0.5	–	0.6
18:4$n-3$	1.9	1.4	1.6	1.0	–	1.2
20:1	10.4	9.3	9.9	5.1	–	3.7
20:4$n-6$	0.9	1.8	1.0	0.6	–	0.2
20:4$n-3$	0.4	0.4	0.6	0.5	–	0.1
20:5$n-3$	12.3	12.1	10.2	10.4	–	2.7
22:1	6.2	4.8	5.2	4.6	–	1.8
22:5$n-3$	0.8	0.9	0.8	0.4	–	0.2
22:6$n-3$	11.7	13.4	10.1	5.8	–	2.2
24:1	1.3	1.2	1.2	1.5	–	0.5
$\Sigma n-3$ HUFA	25.2	26.8	21.7	17.1	–	5.2
Spawning results						
Total eggs ($\times 10^3$/female)	1495	1216	1204	901	2021	583
Hatching rate (%)[h]	21.1	52.8	39.1	41.4	68.1	1.2

[a] Crude protein: 66.9%, crude lipid: 8.8%, $\Sigma n-3$ HUFA: 24.1%.
[b] Crude protein: 73.6%, crude lipid: 13.6%, $\Sigma n-3$ HUFA: 33.6%.
[c] Ogino salt mixture.
[d] Contains 1% vitamin E.
[e] Contains 108 mg/100 g total carotenoids; 91% astaxanthin esters.
[f] Oil containing 30% β-carotene.
[g] 10% purity.
[h] Rate of normal larvae obtained: mean ± S.D., $n=7$ (for diet 6, $n=2$).

3. Yellowtail

Yellowtail is very popular and its production is the highest among cultured finfish species in Japan (Fig. 1). This species is often referred to as a progressive fish since its Japanese name changes with size. Fingerlings, known as *mojako*, are caught from the wild and cultured to *hamachi* and *buri* that have a body length of 20–30 cm or over 65 cm, respectively. Yellowtail is widely consumed as *sashimi* but it may also be cooked or seasoned with salt and grilled.

Yellowtail farming first started in 1928 but it was not until the 1960s and 1970s that a major increase in its production was observed (Davy, 1991). With strict regulations being imposed by the government on *mojako* catches (Davy, 1991) and the improvement of management techniques for broodstock yellowtail (Mushiake, 1994; Mushiake et al., 1993b, 1994b), a series of studies on the broodstock nutritional requirements of this species were performed (Verakunpiriya, 1996). These studies made use of ‘soft-dry’ pellets (SDP) that were prepared by thoroughly mixing and processing the ingredients with a twin screw extruder (Watanabe et al., 1991a). Initially, the suitability of these pellets for egg production in this species was examined (Watanabe et al., 1996).

Prior to the start of these studies, yellowtail broodstock were fed on raw fish diets (chopped Pacific mackerel *Scomber japonicus*, jack mackerel or sardines) or moist pellets. The first trial was designed to compare the SDP with either a raw fish diet (Pacific/jack mackerel) or freshly prepared moist pellets (Table 3; Watanabe et al., 1996). Results were very encouraging because the spawning performance showed an improved egg production and quality for the SDP-fed group during the first day of spawning, when the best quality eggs are obtained from yellowtail. From a very high β-carotene content in the moist pellets, only a small amount was transferred to the eggs with no apparent effect on egg quality. On the other hand, astaxanthin in SDP (derived from the 10% krill meal; see Table 3, footnote d) possibly improved egg quality by increasing zeaxanthin and lutein levels in the eggs (Watanabe et al., 1996; Verakunpiriya et al., 1996). The fatty acid profile and vitamin E content in eggs and milt were related to their availability in the broodstock diets. Indeed, EFA of eggs produced by the SDP group contained the highest levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Verakunpiriya et al., 1996).

These results were pursued in a subsequent study that utilised SDP and different supplemental levels of krill meal in the diets as shown in Table 4 (Verakunpiriya et al., 1997a). This was based on the assumption that in yellowtail, as previously observed in red sea bream (Watanabe et al., 1991a,b), the improved egg quality was due to the 10% krill meal incorporated in SDP which led to superior palatability and colour enhancement. Krill meal was supplemented at 0%, 20% and 30% in SDP that were fed to the fish for about 5 months before spawning. Egg production and quality were not according to expectations and the 0% level produced the best spawning performance. This study showed that even though frozen krill meal improved spawning in red sea bream, a supplemental level above 10% in SDP deteriorated spawning. In their conclusions, Verakunpiriya et al. (1997a) postulated that chemical components other than carotenoids derived from krill meal might have impaired the physiological abilities of the broodstock.

Table 3
Selected dietary ingredients/composition and spawning results[a] of yellowtail fed experimental diets (Watanabe et al., 1996; Verakunpiriya et al., 1996)

	FF[b]	MP[c]	SDP[d]
Proximate composition (% d.b.)			
Crude protein	72.3	56.8	50.5
Crude lipid	18.2	16.0	23.9
Carotenoid composition (mg/kg diet d.b.)			
Astaxanthin	–	–	7.4
β-Carotene	0.3	867.3	3.6
Zeaxanthin	–	–	3.6
Lutein	–	–	4.9
Tunaxanthin	trace	–	–
Others	0.1	trace	6.4
Main fatty acids (% area)			
16:0	20.8	20.1	13.8
16:1	3.3	5	4.6
18:0	3.8	3.8	2.1
18:1	10.4	21.3	10.9
18:2$n-6$	1.6	17.6	2.1
18:3$n-3$	1.1	0.6	0.8
20:4$n-6$	0.5	0.6	0.4
20:5$n-3$	6.6	7.5	7.1
22:5$n-3$	1.1	0.6	1.3
22:6$n-3$	13.1	6.3	6.7
$\Sigma n-3$ HUFA	24.6	15.1	23.0
Spawning results			
Mean female body weight (kg)[e]	4.3 ± 0.3	3.8 ± 0.6	4.3 ± 0.3
Total eggs (× 10^3/kg-female)	3.0	28.2	36.3
Hatching rate (%)[f]	0.0	76.4	87.8

[a] Results from first day of spawning.
[b] Frozen horse mackerel or jack mackerel + 1% vitamin premix (Pfizer Chemical).
[c] Oregon type moist pellet; freshly prepared; raw fish: formulated mash (1:1).
[d] Soft-dry pellets containing local sardine meal: 55.0%, krill meal (Maruha, Japan): 10%, wheat flour: 12.0%, wheat gluten: 3.0%, potato starch: 3.0%, vitamin mix: 2.0%, mineral mix: 2.0%, pollack liver oil: 13.0%.
[e] Mean ± S.D., $n=5$.
[f] Rate of normal larvae obtained from total eggs.

The next experiment was based on the supplementation of pure astaxanthin in SDP for yellowtail broodstock and four diets were formulated to contain 0, 20, 30 and 40 mg/kg astaxanthin, respectively (Table 5; Verakunpiriya et al., 1997b). Consistent with the results obtained in red sea bream (Watanabe et al., 1991a,b), astaxanthin was beneficial to yellowtail broodstock and the spawning performance improved with an increase in dietary astaxanthin up to 30 mg/kg. At 40 mg/kg supplementation, egg quality diminished and the authors concluded that the optimal supplemental level was around 30 mg/kg for yellowtail broodstock (Verakunpiriya et al., 1997b). It would be interesting to see whether experiments using krill meal supplements between 0% and 20% in SDP would produce similar results in this species. On the other hand, it should be noted that krill meal contains diesterified astaxanthin, which has no radical

Table 4
Selected dietary ingredients/composition and spawning results[a] of yellowtail fed experimental diets containing krill meal (Verakunpiriya et al., 1997a)

	0% KM	20% KM	30% KM
Ingredient (%)			
Local sardine meal	67.0	47.0	37.0
Krill meal[b]	0.0	20.0	30.0
Others[c]	33.0	33.0	33.0
Proximate composition (% d.b.)			
Crude protein	55.2	53.3	51.9
Crude lipid	23.0	25.9	25.0
Carotenoid composition (mg/kg diet d.b.)			
Astaxanthin	1.0	14.2	23.3
β-Carotene	2.0	0.7	0.9
Canthaxanthin	1.3	0.4	0.8
Lutein	1.1	0.1	2.2
Others	0.9	6.2	8.1
Main fatty acids (% area)			
16:0	19.3	19.1	18.6
16:1	6.6	6.7	6.3
18:0	3.4	3.2	3.1
18:1	16.5	16.6	17.2
$18:2n-6$	2.7	2.2	2.8
$18:3n-3$	0.9	0.9	0.9
$20:4n-6$	1.0	0.9	0.9
$20:5n-3$	11.0	11.4	11.4
$22:5n-3$	1.5	1.5	1.4
$22:6n-3$	10.2	9.9	9.7
$\Sigma n-3$ HUFA	23.4	23.6	23.3
Spawning results			
Mean female body weight (kg)[d]	7.7 ± 0.8	7.9 ± 0.9	8.1 ± 0.6
Total eggs ($\times 10^3$/kg-female)	79	35	30
Hatching rate (%)[e]	73.0	42.8	14.6

[a] Results from first day of spawning.
[b] Produced by Maruha.
[c] Wheat flour: 5.5%, potato starch: 5.5%, vitamin mix: 2.0%, mineral mix: 2.2%, pollack liver oil: 17.8%.
[d] Mean ± S.D., $n=5$.
[e] Rate of normal larvae obtained from total eggs.

scavenging ability, whereas pure astaxanthin in the free form has a potent radical scavenging ability (Miki, 1991).

Since the most abundant yellowtail egg carotenoid was zeaxanthin, converted from dietary astaxanthin (Verakunpiriya, 1996), it was presumed that the direct supplementation of zeaxanthin into SDP would be effective for improved yellowtail spawning. Pure zeaxanthin is not commercially available. However, *Spirulina* contains between 15% and 35% zeaxanthin (Miki et al., 1986) as well as various other yellow carotenoids. In a preliminary study, the inclusion of *Spirulina* sufficient to supplement 30 mg/kg zeaxanthin in SDP introduced, as well, high quantities of other carotenoids such as β-carotene and lutein. Indeed, the total carotenoid content of the *Spirulina* supplemented diet was as high

Table 5
Selected dietary ingredients/composition and spawning results[a] of yellowtail fed experimental diets containing astaxanthin (Verakunpiriya et al., 1997b)

	0 Astx	20 Astx	30 Astx	40 Astx
Basal SDP ingredients (%)[b]	100.0	100.0	100.0	100.0
Supplementation				
Astaxanthin[c] (mg/kg diet)	0.0	20.0	30.0	40.0
Proximate composition (% d.b.)				
Crude protein	46.5	46.4	46.3	47.6
Crude lipid	24.0	24.0	24.2	21.0
Carotenoid composition (mg/kg diet d.b.)				
Astaxanthin (free)	0.1	15.1	30.6	37.4
Astaxanthin (esters)	2.3	2.2	2.3	2.2
Astaxanthin (total)	2.4	17.3	32.9	39.6
Xanthophylls[d]	0.1	0.1	0.3	0.6
Main fatty acids (% area)				
16:0	18.5	18.7	18.9	18.6
$16{:}1n-7$	6.6	6.7	6.8	6.5
18:0	3.9	4.0	3.9	3.8
18:1	15.7	16.1	16.0	15.9
$18{:}2n-6$	2.7	2.8	2.9	3.6
$18{:}3n-3$	0.9	0.9	0.9	1.0
$20{:}4n-6$	1.2	1.2	1.2	1.1
$20{:}5n-3$	11.6	11.3	11.3	11.2
$22{:}5n-3$	2.0	1.9	1.8	1.9
$22{:}6n-3$	10.7	10.3	10.1	10.9
$\Sigma n-3$ HUFA	25.1	24.4	23.9	24.6
Spawning results				
Mean female body weight (kg)[e]	8.0 ± 0.8	8.3 ± 0.5	8.0 ± 0.4	8.1 ± 1.4
Total eggs ($\times 10^3$/kg-female)	87	38	116	53
Hatching rate (%)[f]	48.1	51.6	64.3	29.8

[a] Results from first day of spawning.
[b] Fish meal (sand lance): 56.0%, defatted soybean meal: 4.0%, wheat flour: 10.0%, potato starch: 7.0%, vitamins and minerals: 6.2%, feed oil: 16.8%.
[c] Carophyll Pink (Roche Vitamins KK, Japan) containing 8% astaxanthin.
[d] Includes lutein and zeaxanthin.
[e] Mean ± S.D., $n=5$.
[f] Rate of normal larvae obtained from total eggs.

as 200 mg/kg, half of which was β-carotene. These very high carotenoid contents or components in *Spirulina* may have interfered with spawning and resulted in a poorer spawning performance (Vassallo-Agius and Watanabe, unpublished data).

Paprika was used as an ingredient for the next experiment on broodstock yellowtail because it was effective for the improvement of nauplii quality in Pacific white-leg shrimp *Litopenaeus vannamei* (Wyban et al., 1997), the pigmentation of fancy red carp *Cyprinus carpio* (Morimoto et al., 1989) and the pigmentation of goldfish *Carassius auratus* (Tsushima et al., 1998). Vassallo-Agius et al. (2001a) formulated three SDP diets: the first with no additional carotenoids (SDP), the second with 30 mg/kg astaxanthin (a-SDP) and the third with 2% paprika powder (p-SDP). Paprika powder

Table 6
Selected dietary ingredients/composition and spawning results[a] of yellowtail fed experimental diets containing astaxanthin or paprika (Vassallo-Agius et al., 2001a)

	SDP	a-SDP	p-SDP
Ingredient (%)			
Fish meal[b]	50.0	50.0	49.0
Fish oil[c]	22.5	22.5	22.0
Wheat flour	12.0	12.0	11.5
Paprika[d]	–	–	2.0
Others[e]	15.5	15.5	15.5
Supplementation			
Astaxanthin[f] (mg/kg diet)	–	30.0	–
Proximate composition (% d.b.)			
Crude protein	44.4	43.8	44.2
Crude lipid	29.1	29.5	28.4
Carotenoid composition (mg/kg d.b.)			
Astaxanthin[g]	–	22.4	1.3
Other red xanthophylls	–	–	24.1
Yellow xanthophylls[h]	22.0	16.8	17.5
Main fatty acids (% area)			
16:0	17.2	17.4	16.7
$16:1n-7$	6.2	6.3	6.0
18:0	2.8	2.8	2.8
18:1	12.9	12.8	13.2
$18:2n-6$	7.4	7.8	8.4
$18:3n-3$	1.6	1.7	1.8
$20:4n-6$	0.4	0.3	0.3
$20:5n-3$	8.4	8.0	8.3
$22:5n-3$	0.7	0.6	0.7
$22:6n-3$	9.7	9.0	9.4
$\Sigma n-3$ HUFA	19.4	18.5	19.2
Trial 1 spawning results			
Mean female body weight (kg)[i]	8.5 ± 1.7	7.0 ± 0.7	8.2 ± 0.8
Total eggs ($\times 10^3$/kg-female)	51	61	66
Hatching rate (%)[j]	59.4	63.9	75.2
Trial 2 spawning results			
Mean female body weight (kg)[i]	7.8 ± 1.6	7.1 ± 1.2	7.6 ± 0.7
Total eggs ($\times 10^3$/kg-female)	90	94	124
Hatching rate (%)[j]	37.2	52.9	61.8

[a] Results from first day of spawning.
[b] Jack mackerel, product of Chile.
[c] Sandlance oil (Esbjerg Fiskeindustri, Denmark).
[d] Contained 1.74 g/kg xanthophylls (Bayer, Japan).
[e] Soybean meal: 4.0%, corn gluten meal: 3.0%, potato starch: 5.0, vitamins and minerals (Ueno Pharmaceuticals, Japan): 3.5%.
[f] Carophyll Pink (Roche Vitamins KK) containing 8% astaxanthin.
[g] Free and esterified forms.
[h] Includes β-carotene, lutein and zeaxanthin.
[i] Mean ± S.D., $n=5$.
[j] Rate of normal larvae obtained from total eggs.

was added at 2% in order to supplement 30 mg/kg paprika esters and provide a similar carotenoid content to the a-SDP. Even though the a-SDP diet was once again confirmed to be superior to the SDP diet, p-SDP produced the best spawning performance in terms of egg production and quality (Table 6; Vassallo-Agius et al., 2001a). The superiority of paprika esters to astaxanthin for yellowtail broodstock was confirmed in a subsequent experiment (Vassallo-Agius et al., 2002) that compared the spawning performance of fish fed a-SDP, p-SDP or ps-SDP (containing 30 mg/kg paprika esters along with squid meal in replacement of 50% of fish meal in SDP). The best spawning results were obtained from fish fed ps-SDP, although these were only marginally better than those of

Table 7
Selected dietary ingredients/composition and spawning results[a] of yellowtail fed experimental diets containing astaxanthin, paprika or paprika and squid meal (Vassallo-Agius et al., 2002)

	a-SDP	p-SDP	ps-SDP
Ingredient (%)			
Fish meal[b]	50.0	49.0	28.0
Squid meal[c]	–	–	28.0
Soybean meal[d]	4.0	4.0	–
Corn gluten meal	3.0	3.0	–
Wheat flour	12.0	11.5	11.5
Fish oil[e]	22.5	22.0	22.0
Paprika[f]	–	2.0	2.0
Others[g]	8.5	8.5	8.5
Supplementation			
Astaxanthin[h] (mg/kg diet)	30.0	–	–
Proximate composition (% d.b.)			
Crude protein	47.1	46.5	48.4
Crude lipid	27.4	27.6	28.7
$n-3$ HUFA	5.9	5.8	6.4
Carotenoid composition (mg/kg diet d.b.)			
Astaxanthin[i]	23.7	1.1	1.1
Other red xanthophylls	–	10.9	10.5
Yellow xanthophylls[j]	12.9	26.1	24.3
Spawning results			
Mean female body weight (kg)[k]	9.3 ± 2.3	9.6 ± 1.4	8.9 ± 1.2
Total eggs ($\times 10^3$/kg-female)	107	89	89
Hatching rate (%)[l]	40.2	68.9	70.1

[a] Results from first day of spawning.
[b] Jack mackerel, product of Chile.
[c] Product of Paudal Shokuzai, Japan.
[d] Crude protein: 45.0%, crude lipid: 1.0% (defatted).
[e] Sandlance oil (Esbjerg Fiskeindustri).
[f] Contained 1.74 g/kg xanthophylls (Bayer).
[g] Potato starch: 5.0%, vitamins and minerals (Ueno Pharmaceuticals): 3.5%.
[h] Carophyll Pink (Roche Vitamins KK) containing 8% astaxanthin.
[i] Free and esterified forms.
[j] Includes β-carotene, lutein and zeaxanthin.
[k] Mean ± S.D., $n=5$.
[l] Rate of normal larvae obtained from total eggs.

p-SDP, both these dietary treatments were markedly better than a-SDP (Table 7; Vassallo-Agius et al., 2002). These studies confirmed the importance of carotenoids for sexual maturation in yellowtail as well as the superiority of paprika esters when compared to pure astaxanthin. However, more work on squid meal incorporation levels needs to be done to define any advantages that may be obtained through its inclusion in SDP for broodstock yellowtail.

Table 8

Selected dietary ingredients/composition and spawning results of Japanese flounder fed experimental diets containing different $n-3$ HUFA levels (Furuita et al., 2000)

	Diet 1	Diet 2	Diet 3
Ingredient (%)			
Defatted fish meal[1]	65.0	65.0	65.0
Wheat flour	15.2	15.2	15.2
Vitamins and minerals	5.0	5.0	5.0
Pollock visceral oil	–	2.0	10.0
Palm olein	10.0	8.0	–
Others[2]	4.8	4.8	4.8
Proximate composition (% d.b.)			
Crude protein	56.1	56.3	55.8
Crude lipid	17.7	18.7	17.3
$n-3$ HUFA	0.4	0.8	2.1
Main fatty acids (% area)			
16:0	34.4	29.5	17.7
16:1$n-7$	0.6	3.1	9.3
18:0	3.1	3.1	2.1
18:1	33.8	31.9	18.1
18:2$n-6$	19.9	19.2	14.2
18:3$n-3$	1.3	1.4	2.0
20:4$n-6$	0.1	0.1	0.2
20:5$n-3$	1.1	2.2	7.3
22:5$n-3$	0.2	0.4	0.5
22:6$n-3$	1.1	1.7	4.0
$\Sigma n-3$ HUFA	2.4	4.3	12.2
Spawning results			
Mean female body weight (kg)[3]	1.7	1.6	1.9
Total eggs ($\times 10^3$/kg-female/spawn)[4]	43	48	51
Hatching rate (%)[5]	76.8 ± 4.6^b	53.1 ± 4.7^a	89.2 ± 2.6^b
Normal larvae (%)[5]	62.6 ± 4.5^a	71.2 ± 4.4^a	76.9 ± 5.6^{ab}
Survival (%)[5]	67.8 ± 5.8^a	76.9 ± 5.6^{ab}	94.1 ± 2.6^b

Values within a row with different superscript letters are significantly different ($P<0.01$).

[1] White fish meal: crude protein 78.5%, crude lipid 3.2%, $n-3$ HUFA 0.7% (d.b.).

[2] Soybean lecithin: 3.0%, choline chloride: 0.1%, carophyll pink (containing 8% synthesized astaxanthin; Nippon Roche): 0.1%, Hospitan C (Ascorbate-2-phosphate Mg salt; Showa Denko): 0.5%, vitamin E: 0.1%, attractant (inosine-5-monophosphate disodium: 400 mg/100 g diet, L-alanine: 100 mg/100 g diet, glycine: 200 mg/100 g diet, betaine–HCl: 300 mg/100g diet): 1.0%.

[3] Mean value ($n=2$).

[4] $n=133$ for diet 1, $n=73$ for diet 2 and $n=65$ for diet 3.

[5] Results represent mean ± SE. $n=50$ for diet 1, $n=51$ for diet 2 and $n=40$ for diet 3.

4. Japanese flounder

The Japanese flounder or *hirame* is another very highly rated marine fish in Japan. It is usually eaten fresh as *sashimi* and its popularity is such that it very often fetches market prices similar to those of red sea bream. The marine stock enhancement programs for this species are on a similar scale to those of red sea bream and release over 20 million juveniles each year (Furusawa, 1997).

Recently, Furuita et al. (2000) investigated the effects of $n-3$ HUFA levels on the spawning performance of the Japanese flounder. They suggested that higher dietary arachidonic acid levels (0.2% of total fatty acids) may have positive effects on spawning whereas larval normality and survival are improved if the diet contained between 1.5% and 2% $n-3$ HUFA (Table 8). Apart from this work on $n-3$ HUFA levels in flounder broodstock diets, no other studies have been carried out on the effects of carotenoids, krill meal or squid meal on the spawning performance of this species. More studies are required to identify the effective components that will improve the overall spawning performance of this species.

5. Striped jack

Striped jack or *shimaaji* is selected by most as the best fish for *sashimi* and is a much sought-after delicacy in Japan, making it the most expensive fish among the Carangids. The culture of wild-caught juveniles started during the early 1960s while fertilized eggs and hatched larvae were first obtained in 1973 (Harada et al., 1984a,b). Egg and larval development and the effects of different water temperatures on hatching were examined (Murai et al., 1987; Kawabe et al., 1991) followed later by Mushiake (1994) who performed a series of studies to determine the optimal spawning techniques and broodstock management.

Various diets ranging from raw fish diets to moist pellet diets have been used for striped jack broodstock prior to, and during the early 1990s. At the start of broodstock rearing and egg production, several kinds of minced and chopped fish were fed (Harada et al., 1984a) throughout the year. Some moist pellets that were mainly made of minced sardines were also used but these diets caused intestinal lipid accumulation that hindered spawning (Mushiake, 1994). Mushiake (1994) designated discrete broodstock feeding periods, defining them as pre-spawning (November to January), spawning (February to May) and post-spawning (June to October) periods as summarised in Table 9. The fish were fed a raw fish mix (RF) made of jack mackerel, squid *Todarodes pacificus* and shrimp *Atypopenaeus* sp. that was mixed at a ratio of 2:2:1 during the pre-spawning and spawning periods. The RF diet fed during the spawning period contained added cuttlefish oil and feed oil in order to fortify the dietary HUFA. During the post-spawning period, the fish were fed moist pellets made of a commercial mash and a minced raw fish mix (jack mackerel, squid and shrimp at 2:1:1). At present, a standard RF (Mushiake, 1994) is used all year round at Japan Sea Farming Association centres. It consists of jack mackerel, squid and shrimp that are mixed at a ratio of 2:2:1 and supplemented with 1% cuttlefish liver oil and 1%

Table 9
Pre-spawning, spawning and post-spawning diets for striped jack broodstock during the late 1980s and early 1990s (Mushiake, 1994)

Pre-spawning period	Spawning period	Post-spawning period
November–January RF[a] + 1.5% feed oil + 3% vitamin E oil	February–May RF + 3.0% feed oil + 1.5% vitamin E oil + 3.0% cuttlefish oil	June–October MP[b]

[a] Jack mackerel, squid and shrimp mixed at a ratio of 2:2:1, respectively.
[b] Commercial mash and RF mixed at a ratio of 1:1.

vitamin mix prior to feeding. The frozen jack mackerel and squid are chopped to a suitable size and thawed before being fed whereas the frozen shrimps are thawed and fed directly.

In a first study towards the development of dry pellets for the broodstock of this species, Watanabe et al. (1998) compared a commercial SDP to the raw fish mix (RF) (Table 10). Results showed that although striped jack were able to spawn after being fed on SDP, egg quality and production were inferior, suggesting SDP needed to be improved in order to allow the proper gonadal maturation of this species. The dietary ingredients, proximate composition, carotenoid composition, main fatty acid composition and spawning parameters of this experiment may be compared for both SDP and RF in Table 10. The total lipids, fatty acid profiles and lipid classes of fertilized eggs produced by this species were related to their availability in the diets, and indicated the importance of pre-spawning nutritional regimes in striped jack (Vassallo-Agius et al., 1998). Although a correlation existed between dietary and egg lipids, striped jack eggs were colourless and contained no carotenoids (Vassallo-Agius et al., 1998) even though the diets contained carotenoids. This was in contrast with eggs of red sea bream and yellowtail, which were pigmented as they contained carotenoids that were incorporated or transferred from the diets (Miki et al., 1984; Verakunpiriya et al., 1996).

The effectiveness of *Spirulina* in striped jack diets for improved colour, texture and ventral muscle taste (Liao et al., 1990), as well as improved pigmentation (Okada et al., 1991), growth and flesh quality (Watanabe et al., 1993) indicated that this species was capable of utilising *Spirulina*, which might possibly have a positive effect on spawning. Further, it is known that *Spirulina* is a source of zeaxanthin (Miki et al., 1986), the main carotenoid converted from dietary astaxanthin in yellowtail eggs (Verakunpiriya et al., 1996). However, a 2% *Spirulina* supplementation in SDP did not produce encouraging results in spawning when compared to RF (Vassallo-Agius et al., 1999). Again, no carotenoids were detected in the fertilized eggs produced by both groups (Vassallo-Agius et al., 1999). In a related study, another group of striped jack broodstock fed on steam dry pellets (15% lipid content on a dry basis; d.b.) showed better feeding activity and improved spawning performance when compared to fish fed on extruded dry pellets (Japan Sea Farming Association, unpublished data). This suggested that the higher lipid content in the extruded pellets (22.3–24.1% d.b.) caused excessive abdominal lipid accumulation that interfered with spawning. Therefore, steam dry pellets were used in the following experiments on this species.

Table 10
Selected dietary ingredients/composition and spawning results of striped jack fed commercial SDP or a raw fish mix (Watanabe et al., 1998; Vassallo-Agius et al., 1998)

	SDP[a]	RF[b]
Proximate composition (% d.b.)		
Crude protein	48.0	71.8
Crude lipid	24.1	14.9
Crude ash	11.1	11.0
$n-3$ HUFA	5.0	3.6
Carotenoid composition (mg/kg diet d.b.)		
Astaxanthin[c]	0.6	7.7
Lutein	4.7	–
Others	traces	–
Main fatty acids (% area)		
16:0	12.6	19.5
16:1$n-7$	3.8	3.6
18:0	2.6	5.3
18:1	12.7	12.7
18:2$n-6$	2.3	8.9
20:4$n-6$	0.5	1.5
20:5$n-3$	7.7	6.4
22:5$n-3$	1.0	0.9
22:6$n-3$	11.6	16.3
$\Sigma n-3$ HUFA	20.9	24.0
Spawning results		
Mean female body weight (kg)[d]	5.4 ± 1.2	5.1 ± 0.5
Total eggs ($\times 10^3$/kg-female)	571	1553
Hatching rate (%)[e]	45.9	60.3

[a] Commercial soft-dry pellets consisting of: fish meal: 56.0%, defatted soybean meal: 4.0%, wheat flour: 10.0%, potato starch: 7.0%, vitamins and minerals: 6.2%, fish oil: 16.8%.

[b] Raw fish mix made of jack mackerel, squid and shrimp mixed at a ratio of 2:2:1, respectively; supplemented with vitamin E oil, fish oil and squid oil at 1%.

[c] Free and esterified forms.

[d] Mean ± S.D., $n=5$ for SDP, $n=4$ for RF.

[e] Rate of normal larvae obtained from total eggs.

The next two experiments were planned to shed light upon the effectiveness of astaxanthin, squid meal or krill meal on the spawning performance of striped jack. In the first study, three diets were used; the RF as a control, steam dry pellets (DP) and DP supplemented with 10 mg/kg pure astaxanthin (a-DP) (Table 11; Vassallo-Agius et al., 2001b). The astaxanthin supplementation was 10 mg/kg in order to match the astaxanthin content of RF that was 7.7 and 6.4 mg/kg (d.b.) in the previous experiments (Watanabe et al., 1998; Vassallo-Agius et al., 1999). The results showed that the 10 mg/kg astaxanthin significantly improved fecundity when compared to fish fed the non-supplemented DP (Vassallo-Agius et al., 2001b). The DP diet produced eggs of equal fertilization and hatching rates to those produced by the RF group, but fecundity was extremely low, rendering the diet inappropriate for large-scale egg production. It was therefore postulated that since dietary carotenoids were not transferred to the eggs, the

Table 11
Selected dietary ingredients/composition and spawning results of striped jack fed steam dry pellets, steam dry pellets containing astaxanthin or a raw fish mix (Vassallo-Agius et al., 2001b)

	DP	a-DP	RF[a]
Basal steam-dry pellet ingredients (%)[b]	100.0	100.0	–
Supplementation			
Astaxanthin[c] (mg/kg diet)	–	10.0	–
Crude protein	49.0	49.8	66.0
Crude lipid	17.9	17.0	17.2
Crude ash	9.5	9.5	9.6
$n-3$ HUFA	3.6	3.3	4.5
Carotenoid composition (mg/kg diet d.b.)			
Astaxanthin[d]	1.5	7.7	21.9
Yellow xanthophylls[e]	17.0	16.3	3.7
Main fatty acids (% area)			
16:0	16.8	17.8	19.1
16:1$n-7$	5.3	5.5	7.7
18:0	2.8	2.9	5.2
18:1	13.7	13.1	13.2
18:2$n-6$	12.9	12.5	0.7
20:4$n-6$	0.3	0.3	1.2
20:5$n-3$	7.7	7.3	8.7
22:5$n-3$	0.6	0.6	1.4
22:6$n-3$	9.2	8.9	15.6
$\Sigma n-3$ HUFA	18.2	17.4	26.3
Spawning results			
Mean female body weight (kg)[f]	3.3 ± 0.2	4.0 ± 0.7	3.5 ± 0.6
Total eggs ($\times 10^3$/kg-female)	308	832	1044
Hatching rate (%)[g]	63.5	55.3	63.5

[a] Raw fish mix made of jack mackerel, squid and shrimp mixed at a ratio of 2:2:1, respectively; supplemented with feed oil, and vitamin mix at 1%.

[b] Steam-dry pellets consisting of: Fish meal (Jack mackerel, produced in Chile): 45.0%, defatted soybean meal (crude protein 45%, crude lipid 1%): 10.0%, corn gluten meal (crude protein 60%): 9.0%, wheat flour: 23.5%, vitamins and minerals: 3.0%, fish oil: 9.5%.

[c] Carophyll Pink (Roche Vitamins KK) containing 8% astaxanthin.

[d] Free and esterified forms.

[e] Includes β-carotene, lutein and zeaxanthin.

[f] Mean ± S.D., $n=5$ for SDP, $n=4$ for RF.

[g] Rate of normal larvae obtained from total eggs.

astaxanthin present in the diet improved the broodstock health condition due to its various physiological functions resulting in enhanced total egg production (Vassallo-Agius et al., 2001b).

In the quest for an improved egg quality, the second experiment investigated the effectiveness of fish meal (f), squid meal (s) and krill meal (k) in the DP diet, comparing it to RF (Table 12; Vassallo-Agius et al., 2001c). The fs-DP markedly improved egg quality resulting in fertilization and hatching rates that were better than those obtained by RF but fecundity was low. On the other hand, krill meal, included at 21.5% along with fish meal (22%) and squid meal (22%) in fsk-DP, did not bring about any improvements (Vassallo-

Table 12
Selected dietary ingredients/composition and spawning results of striped jack fed steam dry pellets containing squid meal, steam dry pellets containing squid meal and krill meal or a raw fish mix (Vassallo-Agius et al., 2001c)

	fs-DP	fsk-DP	RF[a]
Ingredients			
Fish meal[b]	32.0	22.0	–
Squid meal[c]	31.0	22.0	–
Krill meal[d]	–	21.5	–
Wheat flour	23.0	21.5	–
Vitamins and minerals	3.0	3.0	–
Fish oil	11.0	10.0	–
Proximate composition (% d.b.)			
Crude protein	51.9	51.4	66.0
Crude lipid	17.7	19.3	17.2
Crude ash	8.3	8.6	9.6
$n-3$ HUFA	3.2	3.5	4.5
Carotenoid composition (mg/kg diet d.b.)			
Astaxanthin[e]	3.5	12.5	21.9
Yellow xanthophylls[f]	5.8	4.6	3.7
Main fatty acids (% area)			
16:0	19.2	19.3	19.1
16:1$n-7$	4.7	5.0	7.7
18:0	3.2	2.9	5.2
18:1	15.4	15.5	13.2
18:2$n-6$	12.1	11.3	0.7
20:4$n-6$	0.4	0.3	1.2
20:5$n-3$	7.5	7.8	8.7
22:5$n-3$	0.6	0.5	1.4
22:6$n-3$	10.4	9.3	15.6
$\Sigma n-3$ HUFA	19.1	18.3	26.3
Spawning results			
Mean female body weight (kg)[g]	3.8 ± 0.3	3.5 ± 0.4	3.5 ± 0.5
Total eggs ($\times 10^3$/kg-female)	539	349	1133
Hatching rate (%)[h]	59.2	49.1	50.0

[a] Raw fish mix made of jack mackerel, squid and shrimp mixed at a ratio of 2:2:1, respectively; supplemented with feed oil, and vitamin mix at 1%.
[b] Jack mackerel, produced in Chile.
[c] Crude protein 75.0%, crude lipid 6.7%, crude ash 5.9%, moisture 11.1% (Paudal Shokuzai).
[d] Crude protein 59.2%, crude lipid 6.1%, crude ash 5.9%, moisture 17.0% (Maruha).
[e] Free and esterified forms.
[f] Includes β-carotene, lutein and zeaxanthin.
[g] Mean ± S.D., $n=5$.
[h] Rate of normal larvae obtained from total eggs.

Agius et al., 2001c). This lack of performance for the DP which contained krill meal may have been due to the inability of striped jack to utilise the available astaxanthin diesters from krill meal, or a suboptimal dietary carotenoid level or other factors that may have interfered with spawning.

These two studies (Vassallo-Agius et al., 2001b,c) led to another trial for the improvement of both fecundity and egg quality by using the as-DP containing both astaxanthin and squid meal and comparing it to RF as shown in Table 13 (Vassallo-Agius et al., 2001d). The combination of these two ingredients produced the predicted results and the spawning performance (egg production, fertilization and hatching rates) was shown equal to that of fish fed RF (Vassallo-Agius et al., 2001d).

Table 13
Selected dietary ingredients/composition and spawning results of striped jack fed steam dry pellets containing squid meal and astaxanthin or a raw fish mix (Vassallo-Agius et al., 2001d)

	as-DP	RF[a]
Ingredients		
Fish meal[b]	32.0	–
Squid meal[c]	31.0	–
Wheat flour	23.0	–
Vitamins and minerals	3.0	–
Fish oil[d]	11.0	–
Supplementation		
Astaxanthin[e] (mg/kg diet)	10.0	–
Proximate composition (% d.b.)		
Crude protein	55.6	72.1
Crude lipid	15.4	17.4
Crude ash	9.0	10.5
$n-3$ HUFA	3.5	4.8
Astaxanthin[f]	8.2	33.4
Yellow xanthophylls[g]	8.4	0.2
Main fatty acids (% area)		
16:0	18.4	21.2
16:1$n-7$	4.8	5.1
18:0	3.3	7.4
18:1	13.2	23.5
18:2$n-6$	7.3	0.9
18:3$n-3$	1.1	0.4
20:4$n-6$	0.5	1.2
20:5$n-3$	8.5	7.6
22:5$n-3$	0.8	1.8
22:6$n-3$	12.8	16.3
$\Sigma n-3$ HUFA	22.7	26.3
Spawning results		
Mean body weight (kg)[h]	3.8 ± 0.6	3.8 ± 0.5
Total eggs ($\times 10^3$/kg-female)	928	873
Hatching rate (%)[i]	73.1	77.9

[a] Raw fish mix made of jack mackerel, squid and shrimp mixed at a ratio of 2:2:1, respectively; supplemented with feed oil, and vitamin mix at 1%.
[b] Jack mackerel, produced in Chile.
[c] Produced by Paudal Shokuzai.
[d] Sandlance oil, produced by Esbjerg Fiskeindustri.
[e] Carophyll Pink (Roche Vitamins KK) containing 8% astaxanthin.
[f] Free and esterified forms.
[g] Includes β-carotene, lutein and zeaxanthin.
[h] Mean ± S.D., $n=14$.
[i] Rate of normal larvae obtained from total eggs.

The sequence of studies hitherto carried out on striped jack underlined the importance of astaxanthin and squid meal in marine broodstock nutrition, confirming guidelines initiated by previous work on red sea bream and yellowtail.

6. Discussion

The demands of marine seed production for a variety of species are constantly increasing as aquaculture producers strive to introduce new species of high market value. Apart from broodstock management and spawning techniques, it is well documented that nutritional factors greatly affect the reproductive performance of fish (Hardy, 1985; Luquet and Watanabe, 1986; Bromage, 1995, 1998). Fertilized eggs are completely dependent on yolk nutrients for survival as carbohydrates, lipids and proteins are consumed prior to hatching (Heming and Buddington, 1988). Newly hatched larvae remain dependent on the depleting yolk reserves until the mouth-opening stage when they start exogenous feeding (Watanabe and Kiron, 1994). Apart from several egg chemical components (Watanabe et al., 1984c; Verakunpiriya et al., 1996; Bell et al., 1997; Vassallo-Agius et al., 1998; Almansa et al., 1999), fecundity as well may be influenced by dietary composition (Bromage, 1998; Vassallo-Agius et al., 2001b; Izquierdo et al., 2001), highlighting the importance of pre-spawning nutritional regimes. An appropriately formulated broodstock diet must satisfy all requirements for high fecundity as well as egg quality for an optimum spawning performance.

6.1. Feeding ration

There have been no studies on the effect of different feeding rations on spawning in marine fish although work on rainbow trout showed that eggs produced by fish fed a half ration were significantly smaller compared to eggs from broodstock fed a complete ration. On the other hand, there were no differences in the proportional biochemical composition of eggs in these treatments (Knox et al., 1988). In the studies reviewed in this paper, red sea bream were fed the experimental diets twice daily whereas yellowtail, striped jack and the Japanese flounder were fed once every other day during the months prior to spawning. Yellowtail do not feed during their short spawning period; however, individuals of the other species all have extended spawning periods during which they were fed once a day. In these studies, feeding times were pre-determined and quantities fed were to near satiation.

6.2. Fecundity

During the first trials on striped jack, fish fed on dry pellets had a markedly lower fecundity than fish fed RF (Watanabe et al., 1998, Vassallo-Agius et al., 1999). This was significantly improved when astaxanthin was supplemented at 10 mg kg^{-1} (Vassallo-Agius et al., 2001b). In yellowtail, nonsupplemented SDP produced only half the total number of eggs produced by a moist pellet diet (Watanabe et al., 1996) but astaxanthin-supplemented SDP improved egg production (Verakunpiriya et al., 1997b). Although astaxanthin facilitated egg production in striped jack (Vassallo-Agius et al., 2001b) and yellowtail (Verakunpiriya et al., 1997b), yellowtail broodstock fed this diet showed that this dietary carotenoid apparently had a greater influence on egg quality rather than fecundity. Studies on red sea bream also showed that fish fed frozen raw krill rich in astaxanthin had a high fecundity, but cuttlefish meal, cuttlefish meal oil and vitamin E also improved egg production (Watanabe et al., 1991b). In other marine fin fish, Bruce et al. (1999) suggested that although the higher arachidonic acid available in tuna orbital oil improved egg viability when included as a lipid source in dry pellets for European sea bass *Dicentrarchus labrax*, there was no effect by this fatty acid on egg fecundity. In gilthead sea bream *Sparus aurata*, Fernández-Palacios et al. (1995) demonstrated that an increase up to 1.6% in dietary $n-3$ HUFA resulted in increased fecundity. Although there is some inconsistency, these studies indicate that certain dietary additives can influence egg fecundity and their optimal levels should be determined. Future work should focus on determining the right balance of these ingredients in order to improve fecundity.

6.3. Egg quality

Various dietary ingredients such as squid meal, squid oil, carotenoids, vitamin E, frozen krill or krill oil, and tuna orbital oil have all been shown to improve egg quality in a number of marine species. Squid protein and squid lipid sources have improved egg quality in red sea bream (Watanabe et al., 1984d,e, 1985b, 1991b), gilthead sea bream *S. aurata* (Fernández-Palacios et al., 1997) and striped jack (Vassallo-Agius et al., 2001c,d), making it the most effective dietary component. Its efficacy has been ascribed to its superior protein quality, higher phospholipid and cholesterol content (Watanabe et al., 1991b) or its better apparent protein digestibility coefficients (Fernández-Palacios et al., 1997).

Pigmented eggs have shown improved quality when broodstock were fed diets containing carotenoids, primarily red carotenoids like astaxanthin. These compounds play an important role as antioxidants that offer protection from damage caused by free radicals and active oxygen species (Miki et al., 1994; Shimidzu et al., 1998). In the case of red sea bream, the diesterified astaxanthin component from krill meal (Watanabe et al., 1991b,c) was the determining factor for improved egg quality whereas for yellowtail, free astaxanthin supplemented in SDP was the effective component (Verakunpiriya et al., 1997b). On the other hand, over 20% krill meal supplementation in diets of yellowtail (Verakunpiriya et al., 1997a) and striped jack (Vassallo-Agius et al., 2001c) caused a deterioration in egg quality. Investigations into the biological pathways of carotenoid utilization in broodstock marine fish would clarify the effectiveness of krill meal as appropriate levels of inclusion still need to be confirmed for yellowtail and striped jack broodstock. It is possible that red sea bream have a mechanism whereby they can utilize or convert large quantities of diesterified astaxanthin from krill meal. Otherwise, it may have been a mere krill meal 'overdose' in the diets for striped jack or yellowtail that caused its negative effect, especially when considering the fact that 10% krill meal in SDP gave very good spawning results in yellowtail (Watanabe et al., 1996). Moreover, the diesterified form of astaxanthin has no radical scavenging ability, despite the potent ability of the non-esterified form (Miki, 1991).

The importance of carotenoids was highlighted with the improvement of yellowtail spawning when paprika esters were supplemented in SDP (Vassallo-Agius et al., 2001a, 2002). This was probably due to the potent free radical scavenging abilities of paprika esters (Matsufuji et al., 1998) that are capable of replacing astaxanthin, as previously observed in the white-leg shrimp (Wyban et al., 1997). Carotenoids have a number of biological functions in animals (Latscha, 1991; Christiansen, 1996) and their oxygen quenching ability is dependent on the length of the conjugated double bonds and the functional groups (Hirayama et al., 1994). Active oxygen species such as superoxide anions, hydrogen peroxide and singlet oxygen can induce enzyme inactivation, DNA-strand cleavage and membrane lipid peroxidation (Hirayama et al., 1994). Consequently, it is important that carotenoids quench these free radicals to produce harmless end products. Matsufuji et al. (1998) suggested that the radical scavenging ability of capsanthin, which along with capsorbin form the main carotenoids in paprika, was especially due to its conjugated keto-group as well as the polyene chain. These authors speculated that this was the reason why capsorbin, which has two conjugated keto-groups, showed a more potent activity. The superior spawning performance of fish fed SDP supplemented with paprika confirms the fact that capsanthin and capsorbin have a better radical scavenging ability than astaxanthin (Hirayama et al., 1994).

Essential fatty acids, such as $n-3$ and $n-6$ HUFA play a very important role in marine broodstock nutrition, as unlike fresh water fish, marine fish are incapable of elongating the shorter chain fatty acids. Sargent (1995) stressed the importance of the dietary $n-3$:$n-6$ HUFA ratio but pointed out that changes in the absolute amounts of one fatty acid group can alter the relative dietary contribution of the other group. Studies on gilthead sea bream have demonstrated that $n-3$ HUFA are essential for good egg quality, indicating the importance of maintaining the balance between $n-3$ HUFA and other shorter chain fatty acids for a high spawning quality (Fernández-Palacios et al., 1995; Rodríguez et al., 1998; Almansa et al., 1999). Other work on European sea bass showed that tuna orbital oil provides an adequate level of DHA together with a sufficient level of arachidonic acid (AA) with respect to EPA resulting in an improvement in egg viability, survival and hatching (Bruce et al., 1999). Due to the competitive interactions in polyunsaturated fatty acid metabolism, the requirements of DHA, EPA and AA should be investigated in more detail.

Vitamin E and its effectiveness on spawning in marine species was studied in red sea bream when a 200 mg/100 g supplementation improved egg quality to a level similar to that of fish fed frozen raw krill (Watanabe et al., 1991c). Like carotenoids, vitamin E plays a determining role in egg quality due to its free radical scavenging ability (Watanabe et al., 1991c). The subsequent experiments on yellowtail and striped jack did not investigate the optimal vitamin E levels required as the broodstock dry pellets studied contained similar vitamin levels to those required for commercial use. In gilthead sea bream, an increase in the level of α-tocopherol from 22 to 125 mg/kg significantly reduced egg abnormality (Fernández-Palacios et al., 1997) whereas the lowest fertility and larval survival was reported in eggs from broodstock fed the lowest dietary levels of α-tocopherol (Izquierdo et al., 2001).

Watanabe et al. (1984d) carried out a study on the optimal protein requirement of red sea bream and determined it to be around 45%. In another study on European sea bass, reduced levels of dietary protein correlated with lower female body weight and low egg buoyancy and hatchability (Cerdá et al., 1994). In the case of crude lipids, there are no reported studies on the optimum crude lipid requirements of marine broodstock, even though they probably vary from those required for optimal growth as most of the nutrients that affect egg quality are transported to the eggs via lipid micelles during gonad maturation. The studies on egg lipid contents of yellowtail (Verakunpiriya et al., 1996) and striped jack (Vassallo-Agius et al., 1998) showed that a lower lipid diet resulted in decreased egg lipids. However, it is not yet understood whether high or low lipids are better for optimal egg development.

The work on red sea bream showed that a change in diet shortly before spawning could affect egg quality (Watanabe et al., 1984e, 1985b). To date, no experiments have been carried out to determine the feeding period required for a good nutritional status at the start of gonad growth. This period will vary among the different fish species and will probably depend on whether the fish have long or short vitellogenic periods or whether they feed during spawning. Moreover, it should be remembered that the different stages of gonadal development may require different levels of nutrients so it may become important to feed the fish different diets during their sexual maturation. Apart from good gonad growth, a nutritionally adequate diet should allow all females to spawn freely. So, when investigating the efficacy of broodstock diets for egg quality, the total egg production from all individuals in a broodstock population should also be emphasised.

6.4. Sperm quality

Male sexual maturation, sperm production and its quality must also be optimised by the broodstock diets as it can be highly variable and is partly dependent on nutrition (Billard et al., 1995). Data on sperm motility of marine species indicates a variation between 2 and 20 min of motility among the species studied (Billard, 1978; Suquet et al., 1992; Billard et al., 1993). Even fatty acid profiles of semen have shown their dependence on dietary fatty acid profiles in yellowtail (Verakunpiriya et al., 1996) and the European sea bass (Bell et al., 1996) and are likely to play an important role for optimal sperm motility and duration. Vassallo-Agius et al. (2001e) investigated the effects of an $n-3$ HUFA-deficient diet on eggs and sperm of rainbow trout and showed that the spawning quality was lower for crossings that were performed with sperm from males fed an $n-3$ HUFA-deficient diet. This confirmed that the male spawning performance could also be affected by dietary composition.

Finally, it must be remembered that broodstock maturation takes place over extended periods and the fish must be cultivated under optimum conditions with minimum stress throughout this period. Thus there is a need for low stocking densities, good water quality, and appropriately formulated diets that are species specific and contain a selection of ingredients that improve fecundity and egg quality. The available information should be considered and applied to future studies that will lead to the formulation of wholesome broodstock diets for currently cultured species and those with future potential for aquaculture such as the purplish amberjack *Seriola dumerilii* (Kawabe et al., 1996) and bluefin tuna *Thunnus thynnus* (Katabami et al., 1997).

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