Supplemental Effect of Astaxanthin in Broodstock Diets on the Quality of Yellowtail Eggs

Visuthi Verakunpiriya,*¹ Keiichi Mushiake,*² Kazutoshi Kawano,*²
and Takeshi Watanabe*¹

*¹Laboratory of Fish Nutrition, Department of Aquatic Biosciences,
Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan

*²Komame Station of Japan Sea-Farming Association, Komame,
Otsuki, Hata, Kochi 788-03, Japan

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Different levels of pure astaxanthin (0, 20, 30, and 40 ppm) were supplemented in soft-dry pellets (SDP), in order to clarify the effect of this carotenoid on the quality of eggs and spawning performance of yellowtail. Three-year-old fish of average body weight 6.1 kg were separately fed one of each test diet. The feeding experiment was carried out in cages for about 5 months prior to spawning. Seven males and 7 females from each test diet group were transferred to 65 m³ indoor spawning tanks to permit them to spawn spontaneously, while those which remained in the cages were artificially inseminated. Fish of both spawning investigations were induced to maturity by HCG injection (600 IU/kg-fish).

When the brood fish were allowed to spawn spontaneously, those fed SDP supplemented with 30 ppm astaxanthin gave superior results for total production, quality of eggs and the final number of normal larvae. These observations were identical to those from the artificially inseminated group. Thus, the supplementation of astaxanthin in SDP was found to be effective for broodstock yellowtail and seemed to be optimal at around 30 ppm (30 mg/kg-diet); beyond which the quality of eggs diminished.

Key words: astaxanthin, egg quality, yellowtail, broodstock diets

Our preliminary work¹⁾ on "Nutrition and Reproductive Performance of Yellowtail" indicated that soft-dry pellets (SDP) could be employed as diets for the broodstock. On the basis of egg quality¹⁾ and chemical analysis,²⁾ SDP proved superior to both fresh or frozen raw fish and moist pellets generally used for broodstock rearing. Krill meal, which is usually supplemented in the practical SDP to enhance skin pigmentation in yellowtail,³⁾ was postulated to be the effective constituent, specifically the astaxanthin in the meal, as demonstrated in red sea bream broodstock diets.⁴⁾ Results obtained from broodstock fed SDP containing 10% krill meal in the preliminary study¹⁾ and those from fish fed SDP without supplementation of krill meal in a later experiment⁵⁾ were comparable in spawning performance, egg quality, and carotenoid content in eggs. This was because the latter SDP was found to contain carotenoids almost at an identical level as the former SDP. The same experiment⁵⁾ revealed that it was not advisable to include high amounts of krill meal because it seemed to impair the spawning performance and the quality of the eggs, probably due to the excess amount of astaxanthin or components other than carotenoids contained in krill meal.*³

With this background, the present study examined the effect of astaxanthin, the predominant carotenoid component in krill meal,⁶⁾ on the spawning performance and quality of yellowtail eggs. A synthetic carotenoid was supplemented instead of krill meal in the practical SDP and

fed to the three-year-old yellowtail prior to their spawning. The factors relating to spawning were observed and the results are discussed in this paper.

Materials and Methods

Experimental Diets

Table 1 shows the composition of the basal SDP used for all the test diets in this experiment, including the scheme of carotenoid supplementation. Basically, the formulation of the SDP was almost the same as in the previous experiment,^{1,5)} and all the test diets were prepared as soft-dry pellets⁷⁾ by Sakamoto Fish Feed Co., Ltd., using a twin screw extruder (Buhler Co., Ltd.). The basic SDP was formulated to contain about 46% crude protein, in which fish meal was the main protein source in combination with defatted soybean meal, and about 24% crude lipid mainly derived from feed oil (pollack liver oil).

To examine the effect of pure carotenoids on the spawning performance and quality of eggs of yellowtail, astaxanthin which was hypothesized to be the effective component in krill meal was supplemented in that basal SDP at the levels of 0, 20, 30, and 40 ppm (0, 20, 30, and 40 mg astaxanthin per kg-diet, respectively) and are designated as 0-Astx, 20-Astx, 30-Astx, and 40-Astx, respectively. The synthetic carotenoid, Carophyll Pink® (Nippon Roche), which contained 8% astaxanthin, was employed. The ana-

*³ Gyorui Youshoku Kankyo Jikaosen Boushi Gijitsu Kaihatsu Kenkyu (Haigou Shiryō) Nendo Honkokusho: Kouchi-ken Suisan Shikenjou 1981, pp. 1-38 (in Japanese).

Table 1. Composition of the basal soft-dry pellets (SDP) and scheme of the experimental diets

A: Composition of basal SDP (%)	
Fish meal (sand lance)	56.0
Defatted soybean meal	4.0
Wheat flour	10.0
Potato starch	7.0
Vitamin and mineral mixtures	6.2
Feed oil	16.8
B: Scheme of the experimental diets	
SDP	+ 0 ppm Astaxanthin* → 0-Astx
	+ 20 ppm Astaxanthin → 20-Astx
	+ 30 ppm Astaxanthin → 30-Astx
	+ 40 ppm Astaxanthin → 40-Astx

* Carophyll Pink® (Nippon Roche) containing 8% astaxanthin.

Table 2. Proximate composition and carotenoid contents and their composition in the experimental diets

	0-Astx	20-Astx	30-Astx	40-Astx
A: Proximate composition (%)				
Crude protein	43.04	43.81	43.13	43.72
Crude lipid	22.16	22.64	22.51	19.34
Crude ash	8.81	9.06	8.82	8.89
Moisture	7.52	5.51	6.93	8.07
Dry matter basis weight				
Crude protein	46.54	46.36	46.34	47.56
Crude lipid	23.96	23.96	24.19	21.04
VE (µg/g)	295.6	253.1	248.7	273.2
Gross energy (kcal/100 g)* ¹	532.3	547.5	535.7	511.2
B: Carotenoid contents and their composition (mg/kg diet)				
Astaxanthin (free)	0.1	15.1	30.6	37.4
Astaxanthin (esters)	2.3	2.2	2.3	2.2
Xanthophylls* ²	0.1	0.1	0.3	0.6
Astaxanthin content (ppm in diets)	2.4	17.3	32.9	39.6

*¹ As is basis.

*² Zeaxanthin and lutein are included.

lytical data of the experimental diets such as proximate composition and fatty acid composition, which were determined by the methods described previously,²⁾ and carotenoid contents and their composition, which were kindly analyzed by Vitamins & Fine Chemicals Division, Nippon Roche Research Center (Nippon Roche K.K.), are summarized in Tables 2 and 3. On the basis of nutrient requirements of yellowtail, all the test diets contained sufficient amounts of protein (about 46%), lipid (about 24%) and gross energy (about 500 kcal/100 g-diet).⁷⁻⁹⁾ The test diets were similar in fatty acid composition and essential fatty acid (EFA) profiles. They contained around 30–31% each of saturated and mono-unsaturated fatty acids, about 24–25% n-3 highly unsaturated fatty acids (n-3 HUFA), and about 11% each of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). The ratio of DHA to EPA, which has been demonstrated to have an important role during the development of gonads in yellowtail brood fish,²⁾ was kept at the same level of around 0.9 for all the test diets. On the basis of the EFA profile during gonadal development of brood fish, embryogenesis, and that of yolk sac larvae during the endogenous stage, the above composition seems to be adequate for the needs of

Table 3. Distribution of fatty acids in the experimental diets

Fatty acid (% area)	0-Astx	20-Astx	30-Astx	40-Astx
14:0	6.41	6.42	6.63	6.32
14:1	0.24	0.24	0.25	0.17
15:0	0.59	0.58	0.60	0.57
16:0	18.50	18.67	18.90	18.63
16:1n-7	6.62	6.65	6.80	6.47
17:0	0.46	0.47	0.46	0.44
16:3n-6	0.97	0.94	0.97	0.91
16:3n-3	0.19	0.20	0.19	0.19
16:4n-1	0.86	0.83	0.90	0.87
18:0	3.94	3.95	3.88	3.84
18:1n-9	12.57	12.80	12.76	12.70
18:1n-7	2.97	3.09	3.06	2.97
18:1n-5	0.18	0.18	0.19	0.18
18:2n-6	2.73	2.80	2.87	3.58
18:3n-6	0.12	0.09	0.11	0.12
18:3n-3	0.87	0.87	0.88	0.95
18:4n-3	1.83	1.81	1.88	1.88
18:4n-1	0.15	0.14	0.15	0.14
20:0	0.32	0.32	0.30	0.30
20:1n-11	1.29	1.38	1.32	1.26
20:1n-9	2.62	2.70	2.64	2.71
20:1n-7	0.29	0.31	0.29	0.33
20:2n-9	0.10	0.10	0.10	0.10
20:2n-6	0.21	0.21	0.20	0.20
20:3n-6	0.10	0.10	0.09	0.09
20:4n-6	1.23	1.16	1.16	1.13
20:3n-3	0.09	0.17	0.09	0.09
20:4n-3	0.66	0.65	0.62	0.64
20:5n-3	11.64	11.32	11.28	11.19
22:0	0.09	tr	tr	tr
22:1n-(13+11)	3.28	3.50	3.29	3.46
22:1n-9	0.40	0.43	0.40	0.41
22:1n-7	0.09	tr	tr	tr
22:4n-9	0.43	0.41	0.40	0.40
22:4n-6	tr* ¹	tr	tr	tr
22:5n-6	0.35	0.30	0.31	0.30
22:4n-3	ND* ²	ND	ND	ND
22:5n-3	2.01	1.94	1.83	1.85
22:6n-3	10.68	10.31	10.12	10.85
ΣSaturate	30.31	30.41	30.77	30.10
ΣMonoene	30.55	31.28	31.00	30.66
Σn-3HUFA	25.08	24.39	23.94	24.62
DHA/EPA	0.92	0.91	0.90	0.97

*¹ Trace amount (<0.005).

*² Not detected.

the different developmental stages.^{2,10,11)}

The content of astaxanthin in each test diet was proportional to the amount of the supplemented synthetic astaxanthin; the 0-Astx, 20-Astx, 30-Astx, and 40-Astx diets contained 2.4, 17.3, 32.9, and 39.6 ppm, respectively. The composition of carotenoids in the SDP supplemented with different levels of synthetic astaxanthin was similar, free astaxanthin being the main component (87.3–94.4%), followed by astaxanthin esters (5.6–12.7%), and trace amounts of xanthophylls. On the other hand, the main component in the SDP without supplementation of synthetic astaxanthin (0-Astx) was esterified astaxanthin (95.8%) whilst the others such as free astaxanthin and xanthophylls were only present in traces. The little amount of astaxanthin in the unsupplemented SDP may have been

directly derived from protein and/or lipid sources (fish meal and feed oil).

Feeding of Yellowtail Broodstock

Three-year-old yellowtail, (*Seriola quinqueradiata* TEMMINCK & SCHLEGEL) having a body weight of 6.1 ± 0.9 kg and fork length of 69.1 ± 2.9 cm (condition factor: 18.5) were used. Forty fish each (approximately 20 males and 20 females) were separately stocked into $5 \times 10 \times 5$ m floating net cages that were installed at the inner sea of the Komame bay, close to the Komame Station (Kochi Prefecture) of Japan Sea-Farming Association (JASFA). Each of the four broodstock groups was offered the designated test diets at a ration of about 2.5% of their body weight, once every other day for about 5 months prior to spawning. The water temperature during the feeding period (November 1995–April 1996) fluctuated between 21.7 and 9.8°C (average: $17.5 \pm 1.8^\circ\text{C}$).

Investigation of Spawning and Evaluation of Egg Quality

Assessment of spawning was made after completing the feeding experiment. Healthy fish selected from each experimental lot were induced hormonally and either allowed to spawn spontaneously in 65 m^3 indoor tanks or inseminated artificially at the cage site.

Spontaneous spawning: Seven males and an equal number of females having an average body weight of about 8 kg were selected from each test diet group to induce maturity and stimulate spawning. The fish were injected with HCG (human chorionic gonadotropin) at a dosage of 600 IU/kg-fish,¹²⁾ and they were immediately transferred and stocked separately into the 65 m^3 indoor spawning concrete tanks on land. Spawning conditions such as water temperature, water exchange and light intensity were controlled as described elsewhere.^{1,12–14)}

Eggs produced by female brood fish of each test diet group were collected at 09:00 h and then at 17:00 h to enumerate the daily rate, until no further eggs were laid. The degree of success of spawning was determined on the basis of the number of eggs produced, the rate of buoyant and fertilized eggs at morula stage, diameter of eggs and oil globules etc. Buoyant eggs, which are generally assumed to be useful for the evaluation of egg quality, were then stocked into hatching nets in order to assess the rate of hatching and to observe the nature of the hatched larvae. Survival activity index, which indicates the response of the larvae under starvation;¹³⁾ was also determined. Under commercial mariculture operations, the larvae that can reach the stage of hatchery reared juveniles, ready for release, are termed “normal larvae”. In the present experiment, the calculation was carried out for the normal larvae obtained, based on total egg output using the formula described in the footnote of Table 5.

Details on egg collection and evaluation, investigation of egg quality and assessment of hatched larvae were described elsewhere.^{1,12–14)}

Artificial insemination: The three male and three female brood fish selected from each test diet group for artificial insemination were also induced to mature by HCG injection at the same dosage used for the spontaneous spawning method. Normally, the yellowtail brood fish reached maturity within 48 h after the injection of 600 IU-HCG/

kg-fish,¹²⁾ and the stage of maturity was confirmed either by canulation or gentle stripping, before inseminating them. Milt was pooled from all the male brood fish of each test diet group and then preserved in an ice-cooler box (temperature $< 5^\circ\text{C}$). Ovulating eggs of individual female brood fish of each test diet group were obtained by abdominal stripping and they were fertilized separately with spermatozoa from the same group by the dry method as described by Mushiaki *et al.*¹²⁾ The production was assessed as described earlier for the spontaneous spawning.

Results and Discussion

Feed Performance and Status of the Broodstock Yellowtail

It has been established that SDP are acceptable, palatable, and digestible by yellowtail and produce good growth.⁷⁾ The results obtained from our preliminary study¹⁾ also proved that the fish could be cultured with this diet type from the juvenile stage under captivity. They first spawned within 3 years, even when they were relatively small in size (average body weight 4.3 kg, fork length 59.4 cm, and condition factor 20.5) compared to 8 kg spawners used commonly for seed-production operations.^{12–14)} The efficacy of the SDP was further demonstrated in a later study,⁵⁾ when the same broodstock yellowtail spawned for the second year in succession.

Table 4 shows the status of broodstock used for the spontaneous spawning. The body weight of the male brood fish ranged from 7.5–8.4 kg, the fork length 70.4–72.9 cm and the condition factor 20.9–22.2. The corresponding figures for the female fish were 8.0–8.3 kg body weight, 70.8–72.4 cm fork length, and 21.0–22.5 condition factor. On the basis of fish size and their condition factors, it was considered that the inclusion of synthetic astaxanthin, up to 40 ppm, in the SDP fed to yellowtail broodstock for about 5 months did not produce any negative effects on their growth or gonadal development. They grew from about 6.1 kg body weight at the initiation of the feeding experiment to between 7.5 and 8.4 kg just before spawning. The condition factor of the fish in this experiment was high compared to those of wild and cultured yellowtail broodstock of the same age.^{12–14)} Under hormone

Table 4. Status of the broodstock used for spontaneous spawning

	Experimental groups			
	0-Astx	20-Astx	30-Astx	40-Astx
Male fish*¹				
Body weight (kg)	8.4 ± 1.2	7.5 ± 0.6	8.2 ± 1.0	7.6 ± 1.2
Fork length (cm)	72.2 ± 3.5	70.4 ± 2.7	72.9 ± 2.7	71.3 ± 3.6
Condition factor* ²	22.2 ± 1.9	21.5 ± 1.7	21.1 ± 1.4	20.9 ± 0.7
Female fish*¹				
Body weight (kg)	8.0 ± 0.8	8.3 ± 0.5	8.0 ± 0.4	8.1 ± 1.4
Fork length (cm)	70.8 ± 1.4	71.6 ± 2.5	72.4 ± 1.3	72.2 ± 3.5
Condition factor	22.5 ± 1.8	22.5 ± 1.3	21.0 ± 0.8	21.5 ± 2.2

*¹ Mean $\pm$ SD, n = 5.

*² Condition factor = $\frac{\text{BW}}{\text{FL}^3} \times 1000$.

stimulation, they spawned normally during the spawning season (March-May).

Appraisal of Spontaneous Spawning

Entire Spawning Period: The results for the entire period of spontaneous spawning of yellowtail brood fish fed SDP supplemented with the different levels of synthetic astaxanthin for about 5 months prior to their spawning are summarized in Table 5. The injection of HCG at a dosage of 600 IU/kg-fish stimulated the spawning in all the treatments and female brood fish laid their eggs around 48–52 hrs after hormone injection as usual (Fig. 1). Although the average size of female brood fish did not differ among the groups, the 0-Astx, 20-Astx, 30-Astx, and 40-Astx groups produced eggs for 6, 4, 7, and 5 days, respectively. The corresponding figures for total eggs produced throughout the spawning period were 1755×10^3 , 503×10^3 , 1786×10^3 , and 1285×10^3 eggs/female (219×10^3 , 61×10^3 , 224×10^3 , and 158×10^3 eggs/kg-female), respectively. Based on the total eggs produced, the rates of buoyant eggs were 31.4, 51.6, 52.6, and 21.2%; the rates of fertilized eggs were 28.9, 41.2, 47.4, and 15.5%; and the rates of normal eggs having one oil globule were 28.7, 47.6, 49.4, and 19.3%, respectively. The results over the entire spawning period showed that brood fish fed the SDP supplemented with astaxanthin produced a greater number of normal eggs comprising high percentages of buoyant and fertilized eggs compared to the figures usually obtained during seed production operations at the Komame Station (JASFA).^{12–14)}

These values reached an optimum when the supplemental level was 30 ppm astaxanthin. Supplementation of 40 ppm astaxanthin in the SDP seemed to be an over-dose because the quality of eggs deteriorated (Figs. 1 and 2). There was a general decrease in the sizes of eggs and oil globules, as the spawning advanced in all the treatments (Fig. 3); the values obtained from the 30-Astx group were superior. Although, the sizes differed among the groups, the difference was not large, and the values were comparable to those obtained from wild or raw fish fed yellowtail.^{1,12–14)}

Basically, animals are unable to synthesize carotenoids *de novo*. The organisms that are able to do so are certain microorganisms, fungi, algae, and higher plants. Animals, including marine fish, can incorporate or deposit carotenoids in their skin, and flesh, after deriving them from either the food chain or directly from the carotenoid-supplemented diets. The degree of incorporation and/or deposition is presumed to be directly linked to the amount consumed. In this study, the eggs produced by brood fish were pale yellow in color when fed 0-Astx diet, yellow in the case of the 20-Astx group, darker yellow in the 30-Astx group, whilst the intensity of the yellow color for the 40-Astx group was comparable to that of the 20-Astx group. The visible yellow color of yellowtail eggs is known to be characterized by zeaxanthin, which is found to be the main carotenoid component.¹⁵⁾ In addition, zeaxanthin in eggs was available from the bioconversion of astaxanthin via broodstock diets.²⁾ In this experiment, the amount of

Table 5. Cumulative spawning performance of yellowtail brood fish fed the SDP supplemented with different levels of astaxanthin for about 5 months prior to spawning*¹

Experimental factors		Experimental groups			
		0-Astx	20-Astx	30-Astx	40-Astx
Average BW of female	(kg)	8.00	8.26	7.97	8.12
Number of spawners	(male:female)	7:7	7:7	7:7	7:7
Spawning duration	(days)	6	4	7	5
Eggs					
Eggs produced* ²	($\times 10^3$ /female)	1755	503	1786	1285
	($\times 10^3$ /kg-female)	219	61	224	158
Buoyancy rate	(%)	31.4	51.6	52.6	21.2
Fertilization rate among:	(%)	28.9	41.2	47.4	15.5
—Total eggs produced	(%)	91.8	79.8	90.1	73.4
—Buoyant eggs	(%)	28.7	47.6	49.4	19.3
Normal eggs* ³ among:	(%)	91.3	92.4	94.0	91.1
—Total eggs produced	(%)	1110 $\pm$ 43	1131 $\pm$ 46	1154 $\pm$ 37	1106 $\pm$ 53
—Buoyant eggs	(%)	288 $\pm$ 18	296 $\pm$ 19	306 $\pm$ 10	293 $\pm$ 22
Egg diameter* ⁴	(μ m)				
Oil-globule diameter* ⁴	(μ m)				
Larvae					
Rate of hatching among:	(%)	21.2	40.0	45.2	13.7
—Total eggs produced	(%)	67.6	77.6	85.9	64.9
—Buoyant eggs	(%)	73.6	97.2	95.3	88.4
—Fertilized eggs	(%)	373	201	806	177
Larvae obtained	($\times 10^3$ /female)	47	24	101	22
	($\times 10^3$ /kg-female)				
Survival activity index	(range)	10.1–18.0	8.4	12.0–21.9	10.8–17.0
Normal larvae obtained* ⁵	(%)	19.4	36.9	42.6	12.5

*¹ This data was obtained from the entire period of spawning.

*² Average values from 7 females which were stocked in each spawning tank.

*³ Eggs with one oil globule.

*⁴ Mean $\pm$ SD, n=25.

*⁵ Buoyancy ratio $\times$ Fertilization ratio (among buoyant eggs) $\times$ Ratio of normal eggs (among buoyant eggs) $\times$ Hatching ratio (among fertilized eggs) $\times$ 100.

astaxanthin supplemented in SDP and subsequently fed to broodstock affected the intensity of pigmentation of the eggs, but peaked at the 30 ppm astaxanthin level. Therefore, it is thought that yellowtail have only limited ability

to utilize this carotenoid directly. However, since this was a qualitative study, it would be better understood if assessed chemically based on the utilization and bioconversion of astaxanthin.

The results of evaluation of eggs in terms of the numbers of larvae obtained, their hatching rate and the rate of normal larvae out of total eggs produced are summarized in Table 5. The rates of hatched larvae were 21.2, 40.0, 45.2, and 13.7%; the number of larvae obtained per female were 373×10^3 , 201×10^3 , 806×10^3 , and 177×10^3 (47×10^3 , 24×10^3 , 101×10^3 , and 22×10^3 larvae/kg-female); the rates of normal larvae obtained were 19.4, 36.9, 42.6, and 12.5%; and the ranges of survival activity index were 10.1–18.0, 8.4 (data from the first day only), 12.0–21.9, and 10.8–17.0, for the 0, 20, 30, and 40-Astx groups, respectively. The results revealed that the final seed production was elevated by feeding broodstock yellowtail with SDP supplemented with astaxanthin. Fish fed 30-Astx diet

Fig. 1. Egg production of yellowtail brood fish fed SDP supplemented with different levels of astaxanthin.

Fig. 2. Quality of eggs obtained during the entire period of spontaneous spawning from yellowtail brood fish fed diets containing different supplemental levels of astaxanthin.

Fig. 3. Mean diameters of eggs and their oil globules produced by yellowtail brood fish at different spawning days, when fed diets containing various supplemental levels of astaxanthin.

produced normal larvae 2.2, 1.2, and 3.4 times that of those obtained from the 0, 20, and 40-Astx groups, respectively. The supplementation levels of astaxanthin seemed to be optimal at around 30 mg per kg-diet, beyond which (40-Astx) returns diminished (Fig. 2). Moreover, the percentage of normal larvae obtained from brood fish fed SDP supplemented with 20 and 30 ppm astaxanthin were higher than those obtained from brood fish fed SDP with or without krill meal supplementation in a previous study.⁵⁾ This indicates the superiority of pure astaxanthin compared to krill meal.⁶⁾

First-day of Spawning: In our previous study,^{1,2,5,16)} we found a general decline in the production and quality of the eggs of yellowtail as the spawning period advanced. Critically examining the spawning process induced by HCG injection and the chemical profile of the eggs, we could classify the spawning period into 3 phases: initial, middle and final; the initial phase, particularly the production on the first-day, was the best since the egg quality dropped during the subsequent days. The supplemental effect of astaxanthin in SDP on the spawning performance of yellowtail broodstock based on the data obtained on the first day of spawning is summarized in Table 6. Eggs produced per female brood fish were 699×10^3 , 318×10^3 , 924×10^3 , and 430×10^3 (87×10^3 , 38×10^3 , 116×10^3 , and 53×10^3 eggs/kg-female) when fed SDP supplemented with 0, 20, 30, and 40 ppm astaxanthin, respectively. Calculated based on the total eggs produced, the corresponding figures for the rates of buoyant eggs were 75.6, 81.0, 82.5, and 54.6%; the fertilization rates were 70.3, 64.8, 77.1, and 39.6%; the rates of normal eggs were 69.1, 66.1, 70.1, and 46.7%; the rates of hatched larvae were 52.6, 63.3, 75.6, and 34.8%; the rates of normal larvae

were 48.1, 51.6, 64.3, and 29.8%; and finally, the survival activity index values indicating larval health were 12.2, 8.4, 21.9, and 15.4, respectively. Thus, the number of normal larvae produced by brood fish fed SDP containing 30 ppm astaxanthin were higher than those obtained from the others, the figures being 1.3, 1.2, and 2.2 times higher than those of the 0, 20, and 40-Astx groups, respectively. Therefore, the spawning performance when evaluated on the basis of the first-day production exhibited more clearly the superiority of the 30-Astx group.

Appraisal of Artificial Insemination

To confirm the results obtained from fish that were allowed to spawn spontaneously, the production from artificially inseminated eggs was compared. Milt and eggs were gently stripped 48 hours after the injection of HCG. The maturity of the eggs was confirmed prior to stripping because the ripeness may also be dependent on factors such as water temperature and fish size.¹⁷⁾ Table 7 shows the results obtained on artificial breeding of yellowtail brood fish fed SDP supplemented with astaxanthin at different levels for about 5 months prior to spawning. Three females of each test diet group were employed and the values were expressed as average numbers. Eggs produced per female brood fish were 505×10^3 , 414×10^3 , 598×10^3 , and 522×10^3 (71×10^3 , 48×10^3 , 81×10^3 , and 61×10^3 eggs/kg-female) when fed SDP supplemented with 0, 20, 30, and 40 ppm astaxanthin, respectively. The corresponding figures for the rates of buoyant eggs were 95.7, 94.1, 91.2, and 83.0%; the fertilization rates were 74.9, 67.9, 77.2, and 61.0%; and the rates of normal eggs were 81.9, 83.1, 83.7, and 77.9%, all calculations made in relation to total eggs produced. These values are similar to

Table 6. The first-day spawning performance of yellowtail broodstock fed SDP supplemented with different levels of astaxanthin

Experimental factors		Experimental groups			
		0-Astx	20-Astx	30-Astx	40-Astx
Average BW of female	(kg)	8.00	8.26	7.97	8.12
Number of spawners	(male:female)	7:7	7:7	7:7	7:7
Eggs					
Eggs produced* ¹	($\times 10^3$ /female)	699	318	924	430
	($\times 10^3$ /kg-female)	87	38	116	53
Buoyancy rate	(%)	75.6	81.0	82.5	54.6
Fertilization rate among:	(%)	70.3	64.8	77.1	39.6
—Total eggs produced	(%)	93.0	80.0	93.5	72.5
—Buoyant eggs	(%)	69.1	66.1	70.1	46.7
Normal eggs* ¹ among:	(%)	91.5	81.5	85.0	85.5
—Total eggs produced	(%)	1187 $\pm$ 57	1197 $\pm$ 52	1222 $\pm$ 29	1199 $\pm$ 59
—Buoyant eggs	(%)	310 $\pm$ 14	321 $\pm$ 11	321 $\pm$ 11	330 $\pm$ 10
Egg diameter* ¹	(μ m)				
Oil-globule diameter* ¹	(μ m)				
Larvae					
Rate of hatching among:	(%)	52.6	63.3	75.6	34.8
—Total eggs produced	(%)	69.6	78.1	91.7	63.8
—Buoyant eggs	(%)	74.8	97.6	98.1	88.0
—Fertilized eggs	(%)	368	201	699	150
Larvae obtained	($\times 10^3$ /female)	46	24	88	18
	($\times 10^3$ /kg-female)	12.2	8.4	21.9	15.4
Survival activity index					
Normal larvae obtained* ¹	(%)	48.1	51.6	64.3	29.8

*¹ See the footnote of Table 5.

Table 7. Results of seed production on artificial insemination in yellowtail brood fish fed SDP supplemented with different levels of astaxanthin for about 5 months prior to spawning^{*1}

Experimental factors		Experimental groups			
		0-Astx	20-Astx	30-Astx	40-Astx
Average BW of female	(kg)	7.27 ± 1.01	8.21 ± 0.83	7.43 ± 1.36	7.63 ± 1.53
<i>Eggs</i>					
Eggs produced	(× 10 ³ /female)	505 ± 227	414 ± 310	598 ± 215	522 ± 538
	(× 10 ³ /kg-female)	71 ± 33	48 ± 35	81 ± 27	61 ± 56
Buoyancy rate	(%)	95.7 ± 0.9	94.1 ± 22.4	91.2 ± 0.7	83.0 ± 4.4
Fertilization rate among:					
—Total eggs produced	(%)	74.9 ± 9.2	67.9 ± 37.2	77.2 ± 4.8	61.0 ± 3.0
—Buoyant eggs	(%)	78.2 ± 10.0	72.2 ± 31.4	84.7 ± 5.9	73.5 ± 4.6
Normal eggs ^{*2} among:					
—Total eggs produced	(%)	81.9 ± 12.8	83.1 ± 33.3	83.7 ± 4.9	77.9 ± 9.0
—Buoyant eggs	(%)	85.5 ± 14.0	88.3 ± 19.3	91.8 ± 4.9	93.8 ± 7.8
Egg diameter ^{*2}	(μm)	1203 ± 41	1189 ± 42	1200 ± 20	1180 ± 31
Oil-globule diameter ^{*2}	(μm)	311 ± 8	308 ± 18	306 ± 8	314 ± 15
<i>Larvae</i>					
Rate of hatching among:					
—Total eggs produced	(%)	48.7 ± 19.4	64.5 ± 40.0	70.6 ± 2.4	29.1 ± 6.3
—Buoyant eggs	(%)	50.8 ± 20.5	68.6 ± 35.9	77.5 ± 2.3	35.1 ± 8.8
—Fertilized eggs	(%)	65.0 ± 18.9	95.0 ± 12.1	91.5 ± 8.0	47.7 ± 9.9
Larvae obtained	(× 10 ³ /female)	246 ± 188	267 ± 288	422 ± 151	152 ± 183
	(× 10 ³ /kg-female)	33 ± 21	31 ± 33	57 ± 20	18 ± 19
Survival activity index		13.9	15.1	17.8	18.3
Normal larvae obtained ^{*2}	(%)	41.6	57.0	64.9	27.3

^{*1} Mean ± SD, n=3.^{*2} See the footnote of Table 5.

those obtained from spontaneous spawning, implying that the production was improved by the inclusion of astaxanthin in the diets up to 30 ppm, beyond which there was a notable reduction.

The above findings are corroborated when the evaluations are based on the larvae produced. The figures obtained from fish fed SDP supplemented with 0, 20, 30, and 40 ppm astaxanthin were 246 × 10³, 267 × 10³, 422 × 10³, and 152 × 10³ larvae/female (33 × 10³, 31 × 10³, 57 × 10³, and 18 × 10³ larvae/kg-female); and when expressed on the basis of total eggs produced the percentages of hatched larvae were 48.7, 64.5, 70.6, and 29.1 and the rates of normal larvae were 41.6, 57.0, 64.9, and 27.3%, respectively. Interestingly, the figures for normal larvae obtained from artificial insemination were almost same as those obtained on the first day of spontaneous spawning in this study. This confirms the validity of the assessment of spawning performance and quality of eggs based on the first-day production in this species.

The observations made in this study have clearly indicated that the spawning performance and quality of eggs of yellowtail in terms of the rate of buoyant and fertilized eggs, number of hatched larvae, and especially the rate of normal larvae obtained, could be improved by the supplementation of pure astaxanthin in the diets for broodstock. The effectiveness of astaxanthin was in good agreement with the research of Watanabe *et al.*¹⁸⁾ on broodstock red sea bream. The optimal level of astaxanthin in the diets for yellowtail broodstock is thus suggested to be 30 mg/kg-diet, almost close to that recommended for red sea bream (20 mg/kg-diet).¹⁹⁾ The slight difference may be species-specific, size-dependent, fecundity-based, spawning rhythm-oriented or utilization-related. Further studies of the chemical aspects could help us understand the

metabolism and functions of this carotenoid.

Generally, researchers in Japan involved in yellowtail seed production, expect one female brood fish to produce about 1 million eggs, 50% of which should ultimately yield normal hatched larvae. Brood fish fed SDP supplemented with 30 ppm astaxanthin for about 5 months prior to their spawning should be able to meet that demand. However, the effect of other carotenoid sources that can be freely converted to zeaxanthin and subsequently incorporated into yellowtail eggs, and that of other nutrients like vitamins E and C, phospholipids, minerals, etc. on the spawning performance and quality of eggs of this species should also be examined to obtain further basic data necessary for the formulation of wholesome broodstock diets.

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