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Volitional Spawning of Black Sea Bass *Centropristis striata* Induced with Pelleted Luteinizing Hormone Releasing Hormone-Analogue

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Abstract

The black sea bass is a high-value marine serranid and is a prime candidate for intensive cultivation. Reliable methods for controlled spawning are needed to accelerate the development of hatchery technologies that result in mass production of healthy juveniles. During 1998–2001, spawning studies were conducted at The University of North Carolina at Wilmington (UNCW) and at the South Carolina Department of Natural Resources (SCDNR), Charleston, using pelleted luteinizing hormone releasing hormone analogue (LHRH-a).

From April through July 2001, 28 vitellogenic-stage females, with mean oocyte diameters (MOD) ranging from 277–448 μm, were implanted with a 95% cholesterol-5% cellulose pellet containing LHRH-a (~50 μg/kg body wt) at UNCW. In 10 individual spawning trials, females with MOD of 305–448 μm and maximum oocyte diameter ≥ 475 μm spawned volitionally beginning 2–3 d post-implantation (PI) and continued spawning over an average of 1.9 d (range = 1–4 d). Individual females released a mean total of 149,000 eggs (117,000 eggs/kg) with a mean buoyancy rate of 40.5% (floaters). Fertilization and hatching rates were 98% and 27.2% of floaters, respectively, yielding 14,600 yolksac larvae/female (12,600 yolksac larvae/kg body wt), and overall egg viability averaged 8.9%. In eight group spawning trials (2–3 females/group), average performance of females, including fecundity (103,800 eggs/female; 105,500 eggs/kg body wt), buoyancy rate (42.5%), fertilization and hatching rates (97.7% and 24.3% of floaters), numbers of yolksac larvae produced (10,900 yolksac larvae/

female; 10,100 yolksac larvae/kg body wt), and overall egg viability (10.6%) was comparable to what was seen in individual spawning trials.

From 1998–2000, a total of 58 vitellogenic stage (≥ 70% of oocytes ≥ 500 μm) females were implanted with pelleted LHRH-a (~50 μg/kg body wt) in nine group spawning trials (2–19 females/group) at SCDNR. Volitional spawning typically began 18–42 h PI and recurred every 1–3 d for an average duration of 9 d. Female groups released a mean of 560,000 eggs (84,000/female; 132,000/kg body wt) over the spawning period, with mean buoyancy rate of 25.7% floaters. Fertilization and hatching rates were 17.7% and 11.6 % of floaters, respectively, yielding 4,300 yolksac larvae/female (4,600 yolksac larvae/kg body wt). Overall egg viability was 2.9%.

Captive wild-caught black sea bass were induced to undergo repetitive volitional spawning by implantation of pelleted-LHRH-a, consistent with a multiple clutch group synchronous pattern of ovarian development. Group spawning appears to be a practical way to compensate for variable fecundity and egg viability of individual females. Research is needed to identify optimum hormone treatments and eligibility requirements.

The black sea bass *Centropristis striata* is a high-value marine finfish (family Serranidae) that supports important commercial and recreational fisheries in continental shelf waters from Maine to central Florida, with greatest abundance in the mid-Atlantic region (Steimle and Figley 1966). Black sea bass are currently overexploited in the Middle Atlantic Bight, where the population has declined since the 1950s (Shepherd and Terceiro 1994; NMFS 2002). Commercial landings of black sea bass in the Atlantic and Gulf states peaked at 9,978 mt in 1952, but have fluctuated between 1,301–2,708 mt since the 1970s. In 2000, commercial landings (1,540 mt) were valued at $5.7 million (NMFS 2002). Recreational landings peaked at 6,367 mt in 1986, but have since fluctuated between 1,711–2,673 mt and fell to 759 mt in 1998 (NMFS 2002). Catch quotas, minimum size limits, and gear restrictions are becoming more restrictive.

Black sea bass are protogynous hermaphrodites, beginning life as females and later transforming to males between about 18–25 cm total length and 2–5 yr old (Shepherd and Idoine 1993). They grow in length to 60 cm and over 3.2 kg and reach sexual maturity between 1 and 4 yr of age (Mercer 1978). Black sea bass spawn on the continental shelf (Kendall 1972; Cowen et al. 1993) at depths of around 18–46 m (Able et al. 1995). Along the southeastern U.S. coast, spawning occurs from January through June, peaking from March to May, while in the mid-Atlantic, spawning occurs from June through October, peaking around July (Waltz et al. 1979; Wenner et al. 1986; Dery and Mayo 1988; Vaughan et al. 1995).

There is a small but growing body of published data on culture techniques for black sea bass. Southern sea bass have been induced to spawn in captivity using human chorionic gonadotropin (HCG) and larvae reared through early development stages (Hoff 1970; Roberts et al. 1976; Harpster et al. 1977). Tucker (1984) successfully induced spawning of captive black sea bass with HCG and reared a few larvae through metamorphosis. Berlinsky et al. (2000) studied basic culture requirements for laboratory-spawned black sea bass larvae and juveniles. Copeland et al. (2002) raised wild subadults in recirculating tanks from an average of 270 g to 838 g in 201 d, several times faster than growth observed in natural populations. These workers observed no impairment of growth at biomass densities of up to 27.2 kg/m^3 during ongrowing in recirculating tanks, suggesting that much higher densities may be feasible (Copeland et al. 2002). While the available data suggest the black sea bass to be a prime candidate for intensive cultivation, reliable methods for controlled spawning are needed to accelerate the development of hatchery technologies that result in mass production of healthy juveniles.

This paper reports the results of induced

spawning trials conducted from 1998–2001 at The University of North Carolina at Wilmington (UNCW) and at the South Carolina Department of Natural Resources (SCDNR), Charleston, evaluating the efficacy of luteinizing hormone releasing hormone analogue (LHRH-a), administered in sustained release (cholesterol pellet-implant) form (Crim 1985; Lee et al. 1986; Sherwood et al. 1988), as a spawning agent in black sea bass.

Materials and Methods

These studies were conducted at the UNCW Center for Marine Science in Wrightsville Beach, North Carolina, USA, and at the SCDNR in Charleston, South Carolina, USA. At UNCW, black sea bass (mean wt. = 318 g; range = 135–620 g) were collected during September 1999 by commercial fishermen using traps in 8–30 m of water off southeastern North Carolina. Fish were transported to the UNCW aquaculture facility, where they were held at a density of 10 fish per tank in 12 outdoor, circular fiberglass tanks (dia. = 1.85 m; depth = 1 m; vol. = 2.6 m^3) supported by a water recirculating system and heat pump for temperature control. Broodtanks were supplied with recirculating seawater at 34 ppt salinity. Each broodtank was provided with a conical fiberlass cover, and fish were maintained at 34 ppt under an ambient photoperiod and a temperature range of 12–27 C for approximately 20 mo.

Beginning in April 2001, females were examined for stage of sexual development. Fish were anesthetized (100-ppm tricaine methane sulfonate), individually tagged and weighed, and their gonads biopsied using a 1.57-mm o.d. × 1.14-mm i.d. polyethylene cannula (Shehadeh et al. 1973). Ovarian samples were fixed in a solution of 10% formalin in seawater. Using a compound microscope fitted with an ocular micrometer, the diameters of at least 100 oocytes were measured to the nearest 50 μm, from which a mean and standard deviation were calculated. General stage of oocyte development (i.e., pre-vitellogenic, cortical vesicle, vitellogenic, and atretic) was determined from microscopic appearance (Kuo et al. 1974; Wallace and Selman 1981). Males were identified by the presence of running milt when pressure was applied to the gonadal area. Fish were fed to satiation twice daily (approximately 0900 and 1500 h) commercially-prepared diets containing 44–54% protein and 9–15% lipid.

Luteinizing hormone releasing hormone [D-Ala^6 Des-Gly^{10}]-LH-RH Ethylamide) (Sigma Chemical Co., Missouri, USA) (LHRH-a) pellets were implanted between 0900 and 1200 h into females with mean vitellogenic oocyte diameters (MOD) ranging from 277–448 μm. Anesthetized females were implanted intramuscularly with a single 95% cholesterol-5% cellulose pellet (2 × 8 mm) (Sherwood et al. 1988) containing LHRH-a at a nominal dose rate of 50 (range = 43.1–61.1) μg/kg body wt. Females were treated individually (individual spawning trials), where the implanted female was paired with 4–5 males in a spawning tank, or in groups (group spawning trials), where 2–3 females were implanted concurrently and paired with 4–5 males in the same spawning tank. Broodstock were held at 19–21 C during induced spawning experiments.

An egg collector, consisting of a 500-L cylindrocone with 250-μm mesh standpipe screen, plumbed to receive the brood tank effluent, was checked daily for spawned eggs. Spawned eggs were transferred to a separatory funnel in 35-ppt seawater, where buoyant, viable eggs (floaters) were separated from non-buoyant, non-viable eggs (sinkers). The numbers of eggs in each fraction and total numbers of eggs (fecundity) were quantified using volumetric methods. Fertilization rate of buoyant eggs was determined as the percentage undergoing embryonic development. Buoyant eggs were transferred to a 15-L incubator (250–450 eggs/L) supplied with diffused aeration and flow-through seawater at 19 C and survival monitored through hatching 2–3 d post-fer-

tilization. Fertilization and hatching rates were expressed as percentages of buoyant eggs (% floaters) and of total (floaters + sinkers) eggs (% overall).

Temperature, salinity, and dissolved oxygen were monitored daily, while pH, total ammonia-nitrogen, nitrite, and nitrate were monitored weekly during these studies. Average daily values (and ranges) were as follows: salinity, 33.5 (32–37) g/L; dissolved oxygen, 6.8 (5.1–8.6) mg/L; pH, 7.9 (7.6–8.3); total ammonia-nitrogen, 0.12 (0.0–0.4) mg/L; nitrite-nitrogen, 0.02 (0–0.11) mg/L; nitrate-nitrogen, 9.6 (1.7–34.6) mg/L.

At SCDNR, black sea bass (mean wt. = 732 g; range = 312–1,121 g) were collected off South Carolina and Virginia during 1998–2000. Fish were transported to the SCDNR's Charleston facility, where they were held at densities of 50–130 fish per tank in two indoor, circular fiberglass tanks (diam. = 3.7 m; depth = 1.1 m; vol. = 12.9 m^3). Broodtanks were supplied with recirculating seawater at 33–35 ppt salinity. Illumination was supplied to the broodtanks from four 32-W fluorescent lamps. Fish were conditioned using photoperiod (10.5–14.5 h light) and temperature (12–27 C) regimes typical of their spawning grounds. Broodstock were fed a diet of cut fish, squid, and shrimp for 3–6 mo before spawning experiments were initiated.

At SCDNR, induced spawning experiments using LHRH-a pellets were conducted from 1998 to 2000 using procedures similar to those described above. Females (mean length and weight = 354 mm and 732 g) were treated at a nominal dose rate of 50 (range = 46–84) μg/kg. Important differences in methodology were as follows: Females in which at least 70% of the sampled oocytes were ≥ 500 μm were selected for hormone treatment. Females were treated in group spawning trials, where 2–19 females were implanted concurrently and paired with 8–20 running males (mean length and weight = 395 mm and 893 g) in the same spawning tank. Brooders were held at 15–18.5 C during induced spawning experiments.

Mean numbers of eggs per female (and per kg body wt) were calculated for each day of spawning, for the duration of a spawning trial, and over all spawning trials. Mean percentages (unweighted for spawn size) of floaters, fertilization and hatching rates of floaters, overall egg viability (% floaters × % hatching success), numbers of yolksac larvae per female (and per kg body wt) for individual and group spawning trials were calculated for each female or female group, as well as over all individual or group spawning trials.

Results

At UNCW, a total of 28 vitellogenic-stage females with initial MOD of 277–448 μm were implanted with pelleted LHRH-a from April through August 2001. Of these 28 females, 11 were treated in individual spawning trials (Table 1), while 17 were treated in eight group spawning trials, consisting of 2–3 females per group (Table 2).

Oocyte diameter-frequency distributions of a representative female black sea bass during the 2001 reproductive season is shown in Fig. 1. At the onset of the reproductive season (29 March), a unimodal oocyte diameter frequency distribution (Fig. 1a) was observed, with an overall mean of 299 μm (range = 225–375 μm), consisting primarily of yolk vesicle and early to mid-vitellogenic stage oocytes. On 25 April, when this female was implanted with pelleted LHRH-a, size and frequency of late vitellogenic stage oocytes (425–525 μm) were increased, and MOD was 369 μm (range = 225–525 μm) (Fig. 1b). Volitional spawning (i.e., spontaneous release of ovulated eggs) began on 28 April (d 3 post-implantation = d3 PI) and continued for three consecutive days. Diameter of fertilized eggs following water hardening was 931 ± 14.3 (mean ± SE) and ranged from 850–1,005 μm, and eggs were typically translucent and contained a single oil droplet. By 2 May (d7 PI), MOD was 431 μm

TABLE 1. *Data on induced volitional spawning of black sea bass with pelleted LHRH-a at UNCW. LHRH-a dose range = 47.7–61.1 μg/kg body weight. Data represent individual females (individual spawning trials).*

Date of implant (2001)	Fish ID	Body wt. (kg)	Initial MOD[a] (mm)	Time of spawn (d PI)[b]	Eggs/ spawn ($\times 10^3$)	Eggs/ female ($\times 10^3$)	Eggs/kg body wt. ($\times 10^3$)	Floaters (% overall)	Fertilization (% floaters)	Hatch (% floaters)	Egg viability (% overall)
4/17	1	1.21	433	2	114.7	114.7	94.8	14.3	95.0	18.7	2.7
5/10	2	1.23	nd[c]	2	232.7			68.3	96.7	29.8	20.4
				3	165.5			13.9	100	25.5	3.5
				5	27.9			41.2	100	20.2	8.3
				6	39.3	465.4	378.4	25.0	97.4	9.1	2.3
5/10	3	nd	412	3	45.9			10.7	98.2	33.8	3.6
				4	98.3	144.2	nd	30.0	87.9	16.9	5.1
5/15	4	1.78	441	2	180.3	180.3	101.6	54.5	95.8	4.28	2.3
5/15	5	1.17	nd	2	98.3			16.7	100	13.5	2.2
				3	27.9	126.2	107.8	100.0	100	5.48	5.5
6/1	6	1.50	nd	2	45.9			35.7	95.0	55.9	20.0
				3	44.3	90.2	60.1	25.9	99.3	50.4	13.1
6/19	7	1.78	305	1	29.5			55.6	100	32.4	18.0
				2	41.0	70.5	39.6	40.0	100	61.4	24.6
6/25	8	2.08	277	ns[d]							
7/3	9	1.78	402	4	37.7	37.7	21.2	21.7	100	17.8	3.9
7/14	10	0.855	448	3	38.8			30	100	8.1	0.9
				4	28.8			39	99.0	83	9.3
				6	42.8	110.4	129.1	81.5	97.0	17.7	14.4
7/27	11	1.5	423	2	154.5			65.3	100	13.5	8.8

[a] MOD = mean oocyte diameter.
[b] d PI = days post implantation. For some fish, spawning on more than 1 d PI was observed.
[c] nd = no data.
[d] ns = no spawn.

(Fig. 1c), and a multi-modal diameter-frequency distribution was evident, with an advanced mode consisting of hydrated eggs in the final maturational stages (625–825 μm dia.), as well as a smaller mode of younger vitellogenic oocytes (225–525 μm dia.) (Fig. 1c).

Summarized data for the 11 individual spawning trials are presented in Table 1. Mean female body wt was 1.49 kg (range = 0.86–2.08 kg). Females with MOD ranging from 305–448 μm and maximum oocyte diameters ≥ 475 μm spawned volitionally as a result of LHRH-a pellet implantation. Only one female, with a MOD diameter of 277 μm (maximum oocyte diameter = 425 μm), did not respond to hormone treatment.

Volitional spawning typically began on d2 PI (range = d1–3 PI). Individual females spawned on an average of 1.9 d (range = 1–4 d), and females released an average of 78,630 eggs on each day of spawning for a total fecundity of 148,700 eggs (116,600 eggs/kg body wt) for the duration of the spawning period, but range values varied widely (27,900–232,700 eggs/spawn; 37,700–465,000 total fecundity; 21,200–378,400 eggs/kg body wt) (Table 1). The mean percentage of floaters was 40.5% (range = 10.7–100%), while mean fertilization rate was 98.0% (range = 87.9–100%) of floaters. Mean hatching rate was 27.2% (range = 4.3–83.0%) of floaters, for a mean egg viability of 8.9% (range = 0.9–24.6%) overall.

Summarized data for the eight group spawning trials at UNCW are presented in Table 2. A total of 17 females were tested in eight group spawning trials, consisting of 2–3 females per group (Table 2). For group spawning trials, volitional spawning typi-

TABLE 2. *Data on induced volitional spawning of black sea bass with pelleted LHRH-a at UNCW. LHRH-a dose range = 47.2–57.7 μg/kg body weight. Data represent groups of 2–3 females (group spawning trials).*

Date of implant (2001)	Fish ID/ body wt. (kg)	Avg. group body wt. (kg)	Total female body wt. (kg)	Initial MOD[a] (μm)	Time of spawn (d PI)[b]	Eggs/ group[c] (×10³)	Total eggs/ group[d] (×10³)	Total eggs/ female (×10³)	Total eggs/ kg body wt.	Floaters (% overall)	Fert. (% floaters)	Hatch (% floaters)	Egg viability (% overall)
4/25	1/0.91			369	3	34.4				4.8	100	8.7	0.4
4/25	2/2.09	1.50	3.00	414	4	59.0				44.4	100	5.0	2.2
					5	28.7	122.1	61.1	40.7	31.4	93.8	42.1	13.2
4/25	3/1.08			475	9	47.5				100	98.0	7.6	7.6
4/25	4/1.31	1.20	2.39	464	10	60.6	108.2	54.1	45.3	24.3	100	0.5	0.1
5/4	5/1.26			354	2	31.1				31.6	92.6	41.1	13.0
5/4	6/1.14	1.20	2.40	395	4	59.0				16.7	100	12.9	2.2
					5	70.5				41.9	94.6	32.9	13.8
					6	34.4	195.0	97.5	81.3	52.4	100	2.5	1.3
5/4	7/0.92			389	3	172.1	172.1	86.0	91.5	52.4	99.5	16.7	8.8
	8/0.96	0.94	1.88	306									
5/18	9/0.96				2	41.0				20.0	100	14.6	2.9
5/18	10/1.44	1.20	2.39	462	7	22.9	63.9	32.0	26.7	14.3	98.0	16.5	2.4
5/25	11/1.18			366	2	37.7				56.5	96.0	58.2	
5/25	12/1.00	1.09	2.18	368	3	29.5				33.3	96.8	27.4	
					5	36.1				31.8	91.0	41.8	
					6	80.3				75.5	100	22.1	
					7	19.7	203.2	101.6	93.2	100	99.4	28.8	
6/5	13/1.33			486									
6/5	14/0.74			nd	1	80.3				40.8	95.1	24.0	
6/5	15/1.44	1.17	3.50	447	2	101.6	181.9	60.6	52.0	35.5	93.7	36.1	
6/14	16/1.0			372	2	295.0				22.2	100	30.3	
6/14	17/1.1	1.05	2.1	409	3	85.2				23.1	100	21.7	
					4	155.7				52.6	99.5	42.1	
					5	139.3	675.3	337.6	321.6	70.6	100	24.9	

[a] MOD = mean oocyte diameter.
[b] d PI = days post-implantation.
[c] Represents eggs per group per day.
[d] Represents eggs per group over the duration of the spawning period.

cally began on d2 PI (range = d2–9 PI). Female groups released eggs on an average of 2.9 d (range = 1–5 d). Total duration of the spawning period for these groups averaged 3.6 d (range = 1–6 d). Female groups released an average of 74,900 (range = 19,700–295,000) eggs on each day of spawning, for a total fecundity of 215,200 eggs (103,800 eggs/female, 105,500 eggs/kg female body wt) for the duration of the spawning period (Table 2). The percentage of floaters averaged 42.4% (range = 4.8–100%) overall, and mean fertilization rate was 97.7% (range = 91–100%) of floaters. Mean hatching rate was 24.3% (range = 0.5–58.2%) floaters for a mean egg viability of 10.6% (range = 0.1–32.9%) overall.

At SCDNR, a total of 58 vitellogenic stage females (≥ 70% oocyte diameters ≥ 500 μm) were implanted with pelleted LHRH-a from 1998–2001 in nine group spawning trials consisting of 2–19 females per group (Table 3). Volitional spawning was observed in all spawning groups. For the nine group spawning trials, female biomass averaged 6.12 kg (range = 3.11–13.3 kg). Volitional spawning was observed in all groups and typically began 18–42 h (0.8–1.8 d) PI and recurred every 1–3 d, sometimes up to d32 PI. Female groups re-

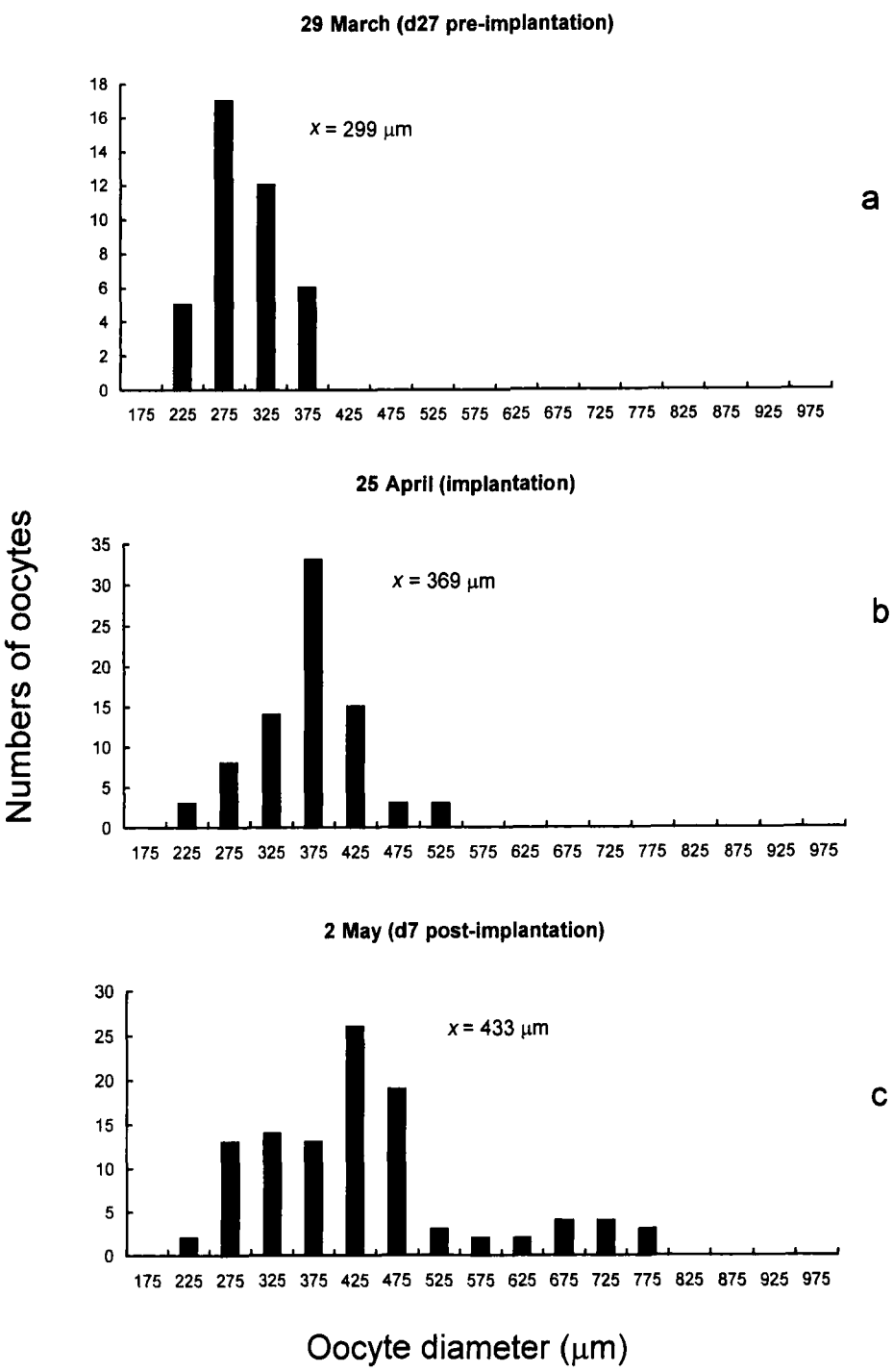

FIGURE 1. Oocyte diameter-frequency distribution of a representative female black sea bass at various stages of LHRH-a induced spawning: (a) 27 d pre-implantation, (b) implantation, and (c) 7 d post-implantation. Spawning occurred on 28 April.

TABLE 3. *Data on induced volitional spawning of black sea bass with pelleted LHRH-a at SCDNR. LHRH-a dose range = 21–85 μg/kg body weight. Data represent group spawning trials with 2–19 females per group.*

Trial no.	Female	Male	Female body wt.	Male body wt.	Spawning period (d)	No. of spawns	Eggs/ group ($\times 10^3$)	Eggs/ female ($\times 10^3$)	Eggs/kg ($\times 10^3$)	Floaters (%)	Fertilized (%)	Hatch (%)
98-01	3	9	1.12	1.28	10	5	205	68.3	61	27.9	4.1	0
98-02	6	18	0.96	1.07	32	13	990	165.0	177.4	31.6	11.5	1.4
99-01	3	6	na[a]	na	15	2	1	0.3	na	0	0	0
99-02	2	6	na	na	5	4	150	75	na	2.2	1.5	0
99-03	3	6	na	na	20	7	260	86.7	na	5.5	5.1	48.3
99-04	2	6	na	na	22	8	205	102.5	na	32.6	29.4	15.7
00-01	10	20	0.31	0.54	6	5	106	10.6	34	25.2	20.4	15.8
00-02	10	10	0.63	0.89	11	8	1,705	170.5	268.9	72.6	62.2	13.2
00-03	19	8	0.63	0.68	9	9	1,415	74.5	117.8	33.3	25.5	10.4

[a] na—Length and weight data not taken for those fish.

leased eggs on an average of 9.2 d (range = 2–13 d), and total duration of the spawning period for these groups averaged 14 d (range = 5–32 d). Female groups released an average of 163,900 eggs on each day of spawning, for a total fecundity of 560,000 eggs for the duration of the spawning period (84,000 eggs/female; 132,000 eggs/kg body wt). Mean buoyancy rate was 25.7% (range = 0–72.6%) overall, and mean fertilization and hatching rates were 17.7% (range = 0–62.2%) and 11.6% (range = 0–48.3%) of floaters, respectively (Table 3). Mean egg viability was 2.9% (range = 0–10.0%) overall.

Table 4 compares individual and group spawning trial results at UNCW and at SCDNR. Females used at SCDNR (mean = 0.73 kg) were much smaller than those used at UNCW (mean = 1.17–1.49 kg). At UNCW, total fecundity for the duration of the spawning period averaged 149,000 in individual spawning trials and 215,200 in group spawning trials, compared with 560,000 in group spawnings at SCDNR. At UNCW, eggs per kg female body weight averaged 117,000 in individual spawnings and 105,500 in group spawnings, compared to 132,000 per kg at SCDNR. At UNCW, percentage of buoyant eggs (40.9–42.5%) and fertilization rate (97.7–98.0%) were higher than those recorded at SCDNR (17.7 and 25.7%, respectively). Hatching success (% floaters) at UNCW averaged 24.3–27.2, compared to 11.6 at SCDNR. Numbers of yolksac larvae per female at UNCW ranged from 10,900–14,600 (10,100–12,600/kg body wt) compared to an average of 4,300 per female (4,600/kg body wt) at SCDNR.

Discussion

Wild-caught black sea bass adapted readily to captivity and successfully completed gonadal development during their first and second seasons in captivity when held under artificial photothermal conditions simulating natural habitat conditions. At UNCW, females with vitellogenic stage oocytes were first observed in March and April, consistent with the natural spawning period of these fish in southeastern U.S. waters (Mercer 1978; Waltz et al. 1979; Wenner et al. 1986; Dery and Mayo 1988; Vaughan et al. 1995).

In this study, a single application of LHRH-a in sustained-release pellet form was highly effective in stimulating final maturation and ovulation in female black sea bass, in agreement with what has been reported in a number of teleosts, including sea bass *Lates calcarifer,* rabbit fish *Siganus guttatus* (Harvey et al. 1985; Almendras et al. 1988), milkfish *Chanos chanos* (Lee et al. 1986), striped bass *Morone saxatilis*

TABLE 4. *Summarized data on LHRH-a induced individual and group spawning trials at UNCW and SCDNR (1998–2001). For each location, values in left and right columns represent means and ranges, respectively.*

Parameter	Individual (UNCW)		Group (UNCW)		Group (SCDNR)	
	Mean	Range	Mean	Range	Mean	Range
No. of trials	10	na[b]	8	na	9	na
No. of females/trial	1	na	2.1	2–3	6.4	2–19
Female body wt. (kg)	1.49	0.86–2.08	1.2	0.7–2.1	0.73	0.31–1.12
Total female biomass (kg)	1.49	0.86–2.08	2.5	1.9–3.5	6.1	3.1–12.0
Initial MOD[a] (μm)	409	305–448	405	354–486	540	nd[c]
Initial spawn (d PI)	2.3	1–3	3	1–9	1.2	0.8–1.8
No. of spawns	1.9	1–4	2.9	1–5	6.8	2–13
Duration (d)	1.9	1–5	3.6	1–6	14	5–32
No. eggs/spawn ($\times 10^3$)	79	27.9–233	74.9	19.7–295	82.5	0.5–213.1
Fecundity (eggs/group) ($\times 10^3$)[d]	na	na	215.2	63.9–202	560	1–170.5
Fecundity (eggs/female) ($\times 10^3$)[d]	149	37.7–465	103.8	32–338	84	0.3–170.5
Fecundity (eggs/kg) ($\times 10^3$)[d]	117	21.2–378	105.5	26.7–322	132	34.0–268.9
Floaters (%)	40.9	10.7–100	42.4	4.8–100	25.7	0.0–72.6
Fertilization (% floaters)	98	87.9–100	97.7	91–100	17.7	0.0–62.2
Hatch (% floaters)	27.2	4.3–83	24.3	0.5–58.2	11.6	0.0–48.3
Egg viability (% overall)	8.9	0.9–24.6	10.6	0.1–32.9	2.9	0.0–10
No. yolksac larvae/group ($\times 10^3$)	na	na	22.6	1.7–82.9	27.8	0.0–172.3
No. yolksac larvae/female ($\times 10^3$)	14.6	1.5–56	10.9	0.9–41.5	4.3	0.0–17.2
No. yolksac larvae/kg ($\times 10^3$)	12.6	0.8–45.9	10.6	0.1–32.9	4.6	0.0–19.5

[a] MOD = mean oocyte diameter.
[b] na = not applicable.
[c] nd = no data.
[d] Total eggs spawned for the duration of the spawning period.

(Hodson and Sullivan 1993), southern flounder *Paralichthys lethostigma* (Berlinsky et al. 1996), and summer flounder *P. dentatus* (Berlinsky et al. 1997; Watanabe et al. 1998). During induced spawning of black sea bass with HCG, a two or three injection sequence on consecutive days was required for successful ovulation, followed by strip spawning and artificial fertilization (Tucker 1984). HCG induced spawnings apparently yielded small numbers of viable eggs, although data on fertilization or hatching successes was not provided. In the present study, a single implantation of pelleted LHRH-a not only induced ovulation, but also promoted repetitive, volitional spawning, yielding significant quantities of viable eggs. Volitional spawning following implantation of pelleted LHRH-a has also been reported in summer flounder (Watanabe et al. 1998) broodstock held for more than a year in captivity. In this study, successful gonadal maturation and volitional spawning of black sea bass at SCDNR was routinely achieved in females held captive for as little as 2 mo.

An optimum initial MOD for induced spawning by LHRH-a pellet implantation was not determined in this study, since vitellogenic stage females with a wide range of initial MOD (305–448 μm) showed a rapid ovulatory response, generally spawning within 2–3 d PI. Tucker (1984) reported that success in using HCG to induce ovulation of black sea bass was attained only in females with mean oocyte diameters of 400 μm or greater. This suggests that, while acute HCG injections may be effective only in females that have fully completed vitellogenesis, a pelleted LHRH-a implant is able to induce maturation and ovulation in fish of lesser maturity, presumably due to

sustained delivery of LHRH-a, which provides greater flexibility in the timing of application (Mylonas and Zohar 2001).

A critical minimum MOD for successful induced spawning was also not determined, since only one female (MOD = 277 μm; maximum oocyte diameter = 425 μm) did not spawn volitionally following LHRH-a pellet implantation. In all females that were successfully spawned, maximum oocyte diameter was ≥ 475 μm. While more studies are required, the available data suggest that critical minimum MOD may be between 277 and 305 μm and, that females with maximum oocyte diameters of at least 475 μm are eligible for hormone induced spawning with pelleted LHRH-a.

During individual spawning trials, females generally spawned twice following implantation of pelleted LHRH-a and sometimes as many as four times. Furthermore, a multi-modal egg diameter-frequency distribution (Fig. 1c) comprising eggs in the vitellogenic and final maturational (ripe) stages was evident in an implanted fish on d7 PI, 3 d after the initial spawning. These observations are consistent with a continuous recruitment of ripe eggs from a pool of smaller, vitellogenic oocytes and with a serial (i.e., multiple clutch, group-synchronous) spawner that releases repetitive clutches of eggs during a spawning season (Wallace and Selman 1981). The results suggest that black sea bass females may potentially be induced to spawn by LHRH-a pellet implant more than once during the spawning season as has been demonstrated in the summer flounder, another multiple clutch group synchronous species (Watanabe et al. 1998).

In individual and group spawning trials at UNCW, the initial spawning was observed on an average of 2.3 to 3 d PI, although this varied from 1 to 9 d PI among trials. This is similar to what was reported during induced spawning of black sea bass (Tucker 1984) and southern sea bass (Hoff 1970) with HCG, where ovulation occurred from 2–8 d and 2–3 d, respectively, after the first injection was administered. While it was anticipated that the time to spawning following LHRH-a pellet implantation (latency period) would depend on stage of gonad development, no clear relationships between latency period and initial MOD or frequency distribution of oocyte diameters were evident. However, latency period may have also been influenced by other factors, including handling, water temperature, or rates of hormone release from individual pellets, which have been reported to vary significantly in cholesterol pellet delivery systems (Carolsfeld et al. 1988).

In this study, repetitive spawning following implantation with pelleted LHRH-a was characterized by variable numbers of eggs released and fertilization and hatching rates on each day of spawning, with no clear trend in egg viability among primary or secondary spawnings. For example, during individual spawnings at UNCW, one female (No. 2, Table 1) spawned from d2 to d6 PI, with a general trend toward reduced fecundity and viability in secondary spawnings. On the other hand, another female (No. 10, Table 1) spawned from d3 to d6 PI, with a trend toward increased fecundity and viability in secondary spawnings. In both the southern flounder *Paralichthys lethostigma* (Berlinsky et al. 1996) and summer flounder *P. dentatus* (Watanabe et al. 1998), implantation of pelleted LHRH-a produced repetitive spawnings, but with a trend toward diminishing fecundity and egg viability in secondary spawnings. Tucker (1984) noted that in black sea bass, eggs ovulated and stripped later than 94 h (3.9 d) after injection with HCG were of poor viability. In this study, the sustained release LHRH-a delivery system resulted in eggs of similar quality in both primary and secondary spawnings. By promoting repetitive spawnings, sustained release LHRH-a delivery systems enhance reproductive output through increased fecundity, fertilization, and hatching success (Watanabe et al. 1998; Mylonas and Zohar 2001).

Hatching rates of floaters were relatively

low, averaging from 24.3–27.2% at UNCW despite consistently high fertilization rates (97.7–98% of floaters). Considering that incubators received flow through seawater with minimal aeration, physical damage to eggs or deteriorating water quality were unlikely to have impaired hatching success. Sub-optimal incubation temperature (19 C), however, may have influenced these results. Recent studies in our laboratories indicate that survival of embryos and early larvae are optimized at 22–25 C (Berlinsky et al. 2000; Copeland and Watanabe, unpublished data). Relatively little data are available on the interactive effects of environmental factors (e.g., temperature, salinity, and light) on early life stages of black sea bass, and more studies are needed.

Production of viable larvae (i.e., fecundity × hatching rate) is a practical measure of spawning success. During individual spawning trials at UNCW, females produced an average of 14,600 yolksac larvae (12,600/kg body wt) during the spawning period, but range values varied widely from 1,500–56,000/female (800–45,900/kg body wt). The results support the practical application of LHRH-a pellet implants as a spawning device in black sea bass, if predictable rates of fecundity, fertilization, hatching, and larval survival to the first-feeding stage can be achieved. A clearer understanding of the relationships between brooder size, oocyte diameter-frequency distribution, dose, and pattern of LHRH-a administration, environmental conditions (e.g., temperature) during spawning, and the recruitment of ripe oocytes from the vitellogenic pool is needed.

During individual spawning trials at UNCW, fecundity averaged 149,000 per female, while overall egg viability rates averaged 8.9%, with range values varying widely among individual females (Table 4). Group spawning trials were conducted to compensate for relatively low and variable fecundity and egg viability of individual females. In group spawning trials, individual female spawning performance, including fecundity (103,800/female) and overall egg viability (10.5%) were comparable to those obtained in individual spawning trials. Hence, yolksac larval production under group spawnings (10,900 larvae/female; 10,100 larvae/kg body wt) and in individual spawning trials (14,600 larvae/female; 12,600 larvae/kg body wt) were also comparable. Because fish spawned volitionally, without the need to strip-spawn multiple females, group spawning was a practical way to compensate for variable performance of individual females.

Some important differences in broodstock characteristics between research sites (UNCW vs. SCDNR) were evident during the study. Whereas female broodstock at SCDNR were much smaller (0.73 vs. 1.49 kg) than those used at UNCW, their fecundity was considerably higher (132,000 eggs/kg body wt vs. 105,500–117,000 eggs/kg body wt). Despite higher fecundity, egg viability was lower at SCDNR (2.9% vs. 8.9–10.5%), resulting in lower numbers of yolksac larvae per unit weight (4,600 larvae/kg) compared to UNCW (10,100–12,600 larvae/kg). This suggests that smaller females were more fecund per unit body weight, but produced eggs of lower viability. In the European sea bass *Dicentrarchus labrax* (Family Serranidae) larger broodfish have lower relative fecundities (i.e., numbers of eggs produced per unit broodfish weight); however, egg quality is poorer in first-time spawners, so females in their second to fifth spawning seasons are preferred by culturists (Carrillo et al. 1995). Another factor that may have lowered egg viability at SCDNR was temperature, which ranged from 15–18.5 C compared to 19 C at UNCW. Temperatures of 21–22 C or higher may be optimal for embryonic through yolksac stages of black sea bass (Berlinsky et al. 2000; Copeland and Watanabe, unpublished data).

While additional work is needed to improve and standardize these procedures, the results of this study demonstrated that captive wild-caught black sea bass successfully

completed gonadal development over at least two successive seasons and can be induced to undergo repetitive volitional spawning by implantation of sustained-release pelleted LHRH-a. Research is needed to improve predictability of spawning in terms of fecundity and egg viability and to improve egg quality and survival through hatching and the first-feeding larval stages. In specific, optimum frequency distribution of oocyte diameter and GnRH-a type, dose, and release rates for induced spawning must be determined. Since female to male transformation occurs after 2–3 yr of captivity, research is also needed to regulate sex transformation in this protogynous hermaphrodite.

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