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Progress in controlled breeding of Nassau grouper (*Epinephelus striatus*) broodstock by hormone induction

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Abstract

Human chorionic gonadotropin (HCG), luteinizing hormone-releasing hormone analogue (LHRH-a), and carp pituitary homogenate (CPH), used alone or in various combinations, were tested as spawning agents in captive *E. striatus* broodstock. Fifty hormone-induced strip-spawning trials were attempted using a two-injection sequence in which a priming dose (PD) was followed 24 h later by a resolving dose (RD). Hormone treatment strategies tested included, HCG alone (PD = 1000 IU kg^{-1} bw, RD = 500 IU kg^{-1} bw), LHRH-a alone (PD = 50–100 μg kg^{-1} bw, RD = 100–200 μg kg^{-1} bw), HCG (500 IU kg^{-1} bw) in combination with LHRH-a (50–100 μg kg^{-1} bw) as priming or resolving doses, and CPH (PD = 10 mg kg^{-1} bw) in combination with LHRH-a (RD = 50–100 μg kg^{-1} bw). As an alternative to hormone injection, intramuscular implantation of a cholesterol pellet containing LHRH-a (200–250 μg) was tested in two females.

Females with mean oocyte diameters ranging from 482 to 561 μm were suitable for hormone-induced spawning. Oocyte diameter increased to 524–708 μm within 24 h of the priming dose and to 852–945 μm within 9–16.4 h following the resolving dose. Average diameter of spawned (water hardened) eggs ranged from 879–978 μm.

Although fertilization rate (0–94.7%) varied widely among trials, successful spawnings (fertilization rate ≥50%) were obtained in all of the hormone strategies tested. Use of different hormones in combination showed no advantage over a single-hormone strategy. As HCG appeared to cause an immune response, LHRH-a is recommended for repeated application. Implantation of LHRH-a produced variable results, inducing ovulation in one female, but apparently inducing sex reversal in the other.

Keywords: *Epinephelus striatus*; Induced spawning; Reproduction; Hormone

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1. Introduction

Owing to a high market value and demand as a food fish, and the seasonal and unstable supply of fish from the capture fishery, there is a growing interest in aquaculture of grouper (Family Serranidae) in many areas of the world. In southeast Asia, where grouper are grown commercially in floating cages or earthen ponds, artificial propagation is still not economically feasible due to a lack of standardized methods for controlled breeding and larval rearing. Farmers depend entirely on wild-caught fingerlings for seedstock, which are in short and fluctuating supply (Tookwinas, 1990; Lim, 1993).

The Nassau grouper (*Epinephelus striatus*) is an important commercial species in many areas of the Caribbean and tropical western Atlantic where overfishing and environmental degradation are depleting natural populations (Sadovy, 1989; Sadovy, 1990; Sadovy, 1992). Research programs have recently been initiated to study the reproductive biology and early life history of *E. striatus* as a basis for fishery management (Colin, 1992; Powell and Tucker, 1992; Grover, 1993; Shenker et al., 1993; Tucker et al., 1993) and to develop methods for controlled breeding and larval rearing (Tucker et al., 1991; Head et al., 1994; Watanabe et al., 1994) to support commercial cultivation or stock enhancement (Roberts et al., 1994) programs.

Fish pituitary extracts, mammalian gonadotropins and superactive analogues of mammalian luteinizing hormone-releasing hormone (LHRH-a) have been widely used to induce ovulation and spawning in finfish (see reviews by Lam, 1982; Donaldson and Hunter, 1983; Shelton, 1989). Tucker et al. (1991) reported successful strip-spawning of *E. striatus* adults injected with human chorionic gonadotropin (HCG) within 3–7 h of capture from a spawning aggregation. Recently, Nassau grouper broodstock have been maintained in captivity at the Caribbean Marine Research Center's (CMRC) hatchery facility in the Bahamas, where individual females have been induced to spawn by HCG injection as many as three times over a 5 month season (Head et al., 1994). Information on the efficacies of different hormones on spawning in grouper is lacking. In this study, HCG, LHRH-a, and carp pituitary homogenate (CPH) are evaluated as spawning agents in *E. striatus*.

2. Materials and methods

This study was conducted at the Caribbean Marine Research Center (CMRC) on Lee Stocking Island (Exuma Cays), Bahamas (23° 45′ N, 76° 10′ W). Adult Nassau grouper (*E. striatus*) ranging from approximately 3.2 to 12.0 kg in weight (55–85 cm total length) were trapped at depths of 20–30 m off Lee Stocking Island from September to December 1991, 1992 and 1993. Fish were maintained under ambient photoperiod and temperature in 15 m^3 flow-through seawater (36–38 g l^{-1}) tanks supplied with aeration. Fish were fed daily a commercially-prepared (Zeigler Bros., PA) striped bass grower containing 38% protein or frozen squid and fish. Prepared and non-living diets were fed on alternate days.

Ovarian maturity of broodstock was assessed monthly during the pre-spawning season and at approximately 3 week intervals in-season by in-vivo monitoring (Shehadeh et al., 1973): A polyethylene cannula (1.52 mm o.d., 0.86 mm i.d.) was inserted into the oviduct of an anaesthetized (0.3 g l^{-1} 2-phenoxyethanol) female, and a sample of gonadal tissue

Table 1
Hormones dose schedules used for induced spawning experiments with the Nassau grouper *Epinephelus striatus*. For hormone injection, the resolving dose was given 24 hours following the first priming dose. For LHRH-a pellet implant, a single implant was made. Doses are expressed per unit of body weight, except for LHRH-a implant which indicates quantity contained per pellet

Method of administration	Hormone	Priming dose	Resolving dose
Injection			
	HCG	1000 IU kg^{-1}	500 IU kg^{-1}
	LHRH-a	50–100 $\mu g\ kg^{-1}$	100–200 $\mu g\ kg^{-1}$
	HCG + LHRH-a	500 IU HCG kg^{-1}	10–100 μg LHRH-a kg^{-1}
	LHRH-a + HCG	50 μg LHRH-a kg^{-1}	500 IU HCG kg^{-1}
	CPH + LHRH-a	10 mg CPH kg^{-1}	100 μg LHRH-a kg^{-1}
Implant			
	LHRH-a	200–250 μg	none

was removed by oral suction as the cannula was withdrawn. The 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 (Shehadeh et al., 1973). In males, maturity was assessed by the presence or absence of milt when gentle pressure was applied to the abdomen. Fish were weighed and measured (total length) after sampling.

Hormone-induced spawning was attempted in females having an average vitellogenic oocyte diameter of at least 450 μm. To induce spawning, a series of two injections were used; a first (priming) dose was followed 24 h later by a second (resolving) dose. Hormones (or hormone combinations) tested (Table 1) included, HCG (Sigma Chemical Co., St. Louis, MO), LHRH-a (des-Gly^{10},[D-Ala^{6}]-LH-RH Ethylamide) (Sigma Chemical Co., St. Louis, MO, or Oriental Scientific Instruments and Export Corp., Shanghai), HCG in combination with LHRH-a, and acetone-dried carp pituitary homogenate (CPH) (Stoller Fisheries, IA) in combination with LHRH-a. In some trials, a third injection of HCG was given 24 h after the second injection. HCG and LHRH-a were dissolved with sterile 0.9% saline prior to use and injected intramuscularly into an anaesthetized (0.3 g l^{-1} 2-phenoxyethanol) female near the dorsal fin. Carp pituitary was homogenized in 0.9% saline before injection.

As an alternative to the two injection protocol, intramuscular implantation of a single cholesterol pellet containing 200–250 μg LHRH-a was tested in two females (Table 1). These pellets were prepared and administered as described by Lee et al. (1985).

Hormone preparations were administered between 19:00 and 21:00 h or between 06:00 and 09:00 h. Each female was transferred along with two to three running ripe males to a separate 15 m^3 tank supplied with flow-through seawater and aeration during the treatment period. To monitor oocyte development, in-vivo sampling was performed at 0 and 24 h post-injection, and mean oocyte diameter and morphology were recorded.

Time of strip-spawning was determined using various morphological criteria, including fullness of the belly, protrusion of the urogenital region, and change from a normal color pattern to a 'bicolor' phase (Colin, 1992; Tucker et al., 1993; Head et al., 1994). Fertilization was achieved by the 'wet method': milt from two to three males was stripped into a 20 l

bucket containing 10 l of seawater. Within a period of 2–3 min, eggs were stripped into a dry 8 l bucket, then added to the milt-seawater mixture, stirred, and maintained for 15 min with gentle mixing to allow water-hardening. Eggs were rinsed to remove excess milt and debris before transfer to incubators. Unfertilized and water-hardened eggs were sampled for diameter measurements.

Eggs were incubated in 530–668 l tanks containing seawater (36–38 $g\,l^{-1}$) and supplied with aeration. Total numbers of eggs spawned were estimated volumetrically. In some spawnings, aeration was suspended approximately 30 min post-fertilization, and buoyant eggs were skimmed and transferred to a separate incubator.

Fertilization and hatching successes were expressed as percentages of total eggs and of buoyant eggs.

3. Results

3.1. Hormones and spawning

During the 1992–1993 and 1993–1994 spawning seasons (late November to early May), 50 induced spawning trials were conducted with captive *E. striatus* females. Mean oocyte diameters prior to injection ranged from 457 to 561 μm (Tables 2–4). Average water temperature during these trials varied from 22.0 to 27.7°C (Tables 2–4).

Fourteen trials used HCG as both priming and resolving injections (Table 2). Successful spawnings (fertilization rate $\geq$50%) were obtained when eggs were stripped 36.3–42 h after the first injection, or within 12.3–18 h of the second injection. Females 1 and 3 (F1 and F3) received a third injection, resulting in partial or no responses, respectively (Table 2). Three females (F1, F3 and F5) that were spawned during the 1991–1992 season with HCG, did not respond to this hormone the following season (1992–1993) (Table 2). All three individuals were spawned later that season with LHRH-a (Table 3).

Twenty eight trials were conducted using LHRH-a as both priming and resolving injections (Table 3). Successful spawnings were obtained when eggs were stripped 33.1–40.4 h after the first injection, or within 9.1–16.4 h of the second injection.

Two females (F3 and F11) with initial mean oocyte diameters of 513 and 494 μm, respectively, showed only partial responses to LHRH-a injection in November 1993 (Table 3). However, both were spawned successfully in January and February 1994 using LHRH-a alone (Table 3) or in combination with HCG (Table 4), suggesting seasonal variation in sensitivity to hormone treatment. In two other females (F13 and F5), egg stripping was prevented by a blockage of the oviduct (Table 3).

Two females (F5 and F2) with initial mean oocyte diameters of 504 and 475 μm, respectively, were each implanted with a LHRH-a cholesterol pellet (Table 3). In F5, strip-spawning at 45.4 h post-implant produced 95 000 eggs, with a fertilization rate of 8.0%. In F2, no response to LHRH implant was observed after 48 h, and no oocytes could be obtained by cannulation from this female by 18 days post-implant, or during subsequent bi-weekly sampling. By 98 days post-implant, however, this individual released milt with abdominal pressure, indicating that sex reversal had occurred. In contrast, the first female (F5) did not undergo sex-reversal and was successfully spawned the next season (Table 3).

Table 2

Summarized data on induced-spawning of *E. striatus* with HCG. A two injection sequence was used in which a priming dose (1000 IU kg^{-1}) was followed 24 h later by a resolving dose (500 IU kg^{-1})

Date	I.D.	Body wt.(kg)	Initial oocyte diam. (μm)	Oocyte diam. 24 h p.i.[a] (μm)	Time of stripping (h p.i.)[a]	Unfertilized egg diam.[b] (μm)	Spawned egg diam.[c] (μm)	Fecundity ($\times 10^6$)	Fertilization rate (% overall)	Floaters (%)	Fertilization rate (% floaters)	Hatching rate (% overall)	Hatching rate (% floaters)	Bicolor before spawn	Avg. temp (°C)
11/21/92	1	8.34	470	449	81[d]	810	*	0.434	0	*	*	*	*	*	26.4
12/6/92	2	6.02	512	549	42	*	910	2.17	92.3	*	96.1	*	81.7	no	25.7
1/10/93	3	4.6	533	534	no spawn[d]	*	*	*	*	*	*	*	*	no	24.7
1/12/93	4	5.22	527	571	41.8	882	907	1.782	11.7	*	*	*	0	yes	24.7
1/12/93	5	4.06	502	520	no spawn	*	*	*	*	*	*	*	*	no	24.7
1/16/93	6	7.43	503	588	39.1	*	880	4.3	89.3	*	97.7	*	81.4	*	24.4
1/16/93	1	8.34	502	498	no spawn	*	*	*	*	*	*	*	*	no	24.4
2/1/93	7	3.52	513	569	42.4	*	940	0.665	86.7	most	*	*	52.4	yes	23.2
2/1/93	3	5.22	518	532	no spawn	*	*	*	*	*	*	*	*	no	23.2
2/18/93	8	6.87	505	524	38	*	950	0.981	94.7	95	95.7	86.3	90.9	yes	23.4
11/30/93	9	5.56	482	548	36.3	933	942	0.765	68.5	63.6	82	51	80.2	yes	26.8
3/7/94	10	5.68	489	526	38.9	891	879	0.333	91.7	100	96	66.9	66.9	yes	24.8
3/13/94	11	12	515	587	41.2	892	908	4.75	85.7	97.6	95.7	73	74.9	yes	24.4
3/13/94	6	7.5	*	*	40.8	839	855	2.593	12.3	0	0	0	0	yes	24.4

[a]h p.i., hours post-first injection.[b]Unfertilized eggs sampled during stripping.[c]Fertilized eggs sampled after water hardening.[d]A third injection (resolving dose) was administered at 48 h p.i. * Not applicable or no data available.

Table 3
Summarized data on induced-spawning of *E. striatus* using LHRH-a. A two injection sequence was used in which a priming dose (50 $\mu g\ kg^{-1}$) was followed 24 h later by a resolving dose (100 $\mu g\ kg^{-1}$). Two females received a single LHRH-a pellet implant (200–250 μg)

Date	I.D.	Body wt. (kg)	Initial oocyte diam. (μm)	Oocyte diam. 24 h p.i.[a] (μm)	Time of stripping (h p.i.)[a]	Unfertilized egg diam.[b] (μm)	Spawned egg diam.[c] (μm)	Fecundity ($\times 10^6$)	Fertilization rate (% overall)	Floaters (%)	Fertilization rate (% floaters)	Hatching rate (% overall)	Hatching rate (% floaters)	Bicolor before spawn	Avg. temp (°C)
2/18/93	6	7.09	511	598	34.5	*	*	0.998	85	most	*	46.9	*	yes	23.4
2/18/93	3	5.22	530	549	37.5	*	*	1.13	71.3	*	*	3.1	*	yes	23.4
3/15/93	12	4.71	526	559	39.4	*	905	1.52	73	*	*	49.4	*	*	22.0
3/15/93	1	8.34	525	574	40.4	*	922	0.968	90.3	79.2	93.7	84	*	yes	22.0
4/2/93	3	4.77	519	650	34.1	*	907	2.1	1.1	*	*	0	*	yes	25.8
4/2/93	8	7.86	512	746	34.2	*	928	1.38	1.7	*	*	0	*	yes	25.8
4/2/93	6	7.35	508	633	34.0	*	885	3.43	1	*	*	0	*	yes	25.8
4/2/93	5	4.37	531	671	33.9	*	922	1.25	25	*	78.7	6.5	59	yes	25.8
4/20/93	3	4.77	504	628	34.4	*	911	0.81	32	*	*	12.6	39	yes	24.9
4/20/93	5	4.37	500	668	34.3	*	978	0.734	69.7	*	*	29	42	yes	24.9
4/20/93	13	5.79	561	708	33.1	*	952	2.712	88.7	61.6	96.7	*	*	yes	24.9
5/5/93	5	4.20	498	594	34.2	938	*	0.309	68	*	*	12.8	18.8	yes	25.5
5/5/93	1	8.00	513	589	35.8	*	*	*	*	*	*	*	*	yes	25.5
5/5/93	8	7.89	514	631	33.9	928	*	2.73	63.7	*	*	27.8	45.4	*	25.5
11/27/93	3	5.56	513	569	no spawn	*	*	*	*	*	*	*	*	*	27.2
11/27/93	11	12.00	494	532	no spawn	*	*	*	*	*	*	*	*	*	27.7
11/30/93	5	4.54	497	603	33.5	*	*	0.519	47.3	30.6	79	15.3	50	yes	26.8
12/8/93	14	9.53	504	566	34.8	881	902	4.367	85.3	72	91	50.2	69.7	yes	25.8
12/8/93	13	5.68	457	509	no spawn[d]	874	*	*	*	*	*	*	*	yes	25.8
1/8/94	3	5.56	528	685	34.4	880	920	3.352	69.3	8.79	88.7	*	*	yes	25.1
1/8/94	5	4.54	520	630	35.1	945	949	1.471	84.3	55.6	95.3	37.8	68	yes	25.0
1/17/94	14	9.50	530	639	46.6	877	883	3.61	14	9.5	60	1.38	14.5	yes	24.6
1/23/94	8	8.06	526	581	34.2	*	903	2.48	41.7	44.6	88	38.8	87	yes	23.8
2/12/94	3	5.56	512	696	34.7	875	886	1.41	56.7	4.26	88	2.12	49.7	no	25.5
3/4/94	14	9.53	502	550	36.0	822	855	4.282	7.7	4.91	67	0.04	0.82	yes	25.9
3/4/94	5	4.54	548	616	no spawn[d]	*	*	*	*	*	*	*	*	yes	25.9
3/7/94	13	5.68	502	609	34.3	852	891	1.768	83	93	95.7	26	28.0	yes	24.9
3/7/94	3	5.56	488	649	42.4	*	907	1.733	34.7	70.1	19.3	18.4	26.3	no	24.8
2/18/93	5 (implanted)	4.20	504	494	45.4	*	*	0.095	8	*	*	*	*	yes	23.4
3/12/93	2 (implanted)	5.87	475	486	no spawn[e]	*	*	*	*	*	*	*	*	*	23.4

[a] h p.i., hours post-first injection. [b] Unfertilized eggs sampled during stripping. [c] Fertilized eggs sampled after water hardening. [d] Blockage of the oviduct. [e] Sex-reversal observed by 98 days post-implant. * Not applicable or no data available.

Table 4
Summarized data on induced-spawning of *E. striatus* with HCG or CPH in combination with LHRH-a. A two injection sequence was used in which a priming dose was followed 24 h later by a resolving dose

Date	I.D.	Body wt. (kg)	Initial oocyte diam. (μm)	Primary hormone-dose[a]	Resolving hormone-dose[a]	Oocyte diam. 24 h p.i.[b] (μm)	Time of stripping (h p.i.)[b]	Fertilized egg diam. (μm)	Spawned egg diam. (μm)	Fecundity ($\times 10^6$)	Fertilization rate (% overall)	Floaters (%)	Fertilization rate (% floaters)	Hatching rate (% overall)	Hatching rate (% floaters)	Bicolor before spawn	Avg. temp (°C)
3/15/93	15	6.16	514	L-50	H-500	615	36.5	*	939	2.69	79.7	*	*	63	*	intermit.	22.0
1/23/94	10	5.68	511	H-500	L-100	539	37	933	*	0.096	90	95	99	93	98	yes	23.8
2/12/94	11	12.03	554	H-500	L-100	549	36.8	899	906	3.34	83.3	17.7	93.7	*	*	yes	25.5
2/12/94	13	5.68	490	L-50	H-500	585	36.3	882	901	2.304	22	26.9	92	20.5	76.1	yes	25.5
3/4/94	7	3.63	543	L-50	H-500	616	39.8	821	*	1.335	0	0	0	0	0	yes	25.9
3/4/94	8	8.06	515	H-500	L-10	545	36.1	847	864	2.18	30.9	22	90	21.3	96.7	yes	27.2
1/17/94	6	7.5	535	C-10	L-100	639	34.3	850	857	1.33	12.3	0	*	*	*	yes	24.6
1/23/94	13	5.67	537	C-10	L-100	601	35.3	878	877	2.907	50	9.88	83.3	5.08	51.4	yes	23.8

[a]L, LHRH-a; H, HCG; C, CPH (dose expressed as mg kg^{-1} for LHRH-a, IU kg^{-1} for HCG and mg kg^{-1} for CPH). [b]h p.i., hours post-first injection. * not applicable or no data available.

Six trials were conducted using HCG in combination with LHRH-a as priming or resolving injections, while two trials were made using CPH as a priming injection and LHRH-a as a resolving injection (Table 4). Successful spawnings were obtained when females were stripped 35.3–37 h after the first injection, or within 11.3–13 h of the second injection.

3.2. *Oocyte size frequency distribution and morphology*

Mean oocyte diameter of females prior to successful hormone-induced spawning ranged from 482–561 μm (Tables 2–4). The oocyte diameter frequency distribution was typically unimodal (Fig. 1). In F11 for example, which was spawned by HCG injection with a fertilization rate of 85.7% (Table 2), the initial mean oocyte diameter was 515 μm (Fig. 1a), with 85.7% of the oocytes ranging from 500–600 μm and 11.1% ranging from 200–500 μm.

In spawnings resulting in moderate ($\geq 50\%$) to high ($\geq 75\%$) fertilization rates, mean oocyte diameter showed a distinct increase from 482–561 μm to 524–708 μm within 24 h of the first injection (Tables 2–4, Fig. 1b), and the frequency distribution of oocyte diameters remained unimodal (Fig. 1b). This increase in mean diameter was accompanied by a coalescence of lipid droplets in the cytoplasm, visualized by a central clearing of the oocytes. In successful spawnings, mean diameter of unfertilized eggs at time of stripping ranged from 852 to 945 μm (Tables 2–4, Fig. 1c), while diameters of fertilized eggs following water hardening ranged from 879–978 μm (Fig. 1d).

3.3. *Fecundity and frequency of spawning*

Individual females released from 0.095 to 4.75×10^6 eggs when stripped (Tables 2–4), with the numbers of eggs generally increasing with female body weight. This relationship could be described by a highly significant ($P < 0.001$) linear regression (Fig. 2).

Some females were strip-spawned more than once during a season. One individual (F5), was strip-spawned four times from 18 February, to 5 May, 1993 at intervals of 43, 18 and 15 days, respectively, producing a total of 2.39×10^6 eggs (Table 3). Both female and male brooders were resistant to handling during induced spawning; only one female died following stripping, due a blockage that caused a rupture of the ovary.

3.4. *Egg buoyancy, fertilization and hatching*

During incubation of eggs in full-strength seawater (36–38 g l^{-1}), the percentage of eggs that remained buoyant when aeration was suspended varied among spawnings, generally increasing with higher fertilization rate. This relationship could be described by the linear regression, $y = 0.8774x - 4.9702$ ($r^2 = 0.59$, $n = 24$, $P < 0.01$), where y is the buoyancy rate (%) and x is the fertilization rate (%).

Hatching rate (range = 0–94%) varied widely among spawnings, generally increasing with higher fertilization rate (Tables 2–4). This trend could be described by the linear regression, $y = 0.6862x - 6.7482$ ($n = 32$, $r^2 = 0.6405$, $P < 0.001$), where y is the hatching rate (%) and x is the fertilization rate (%).

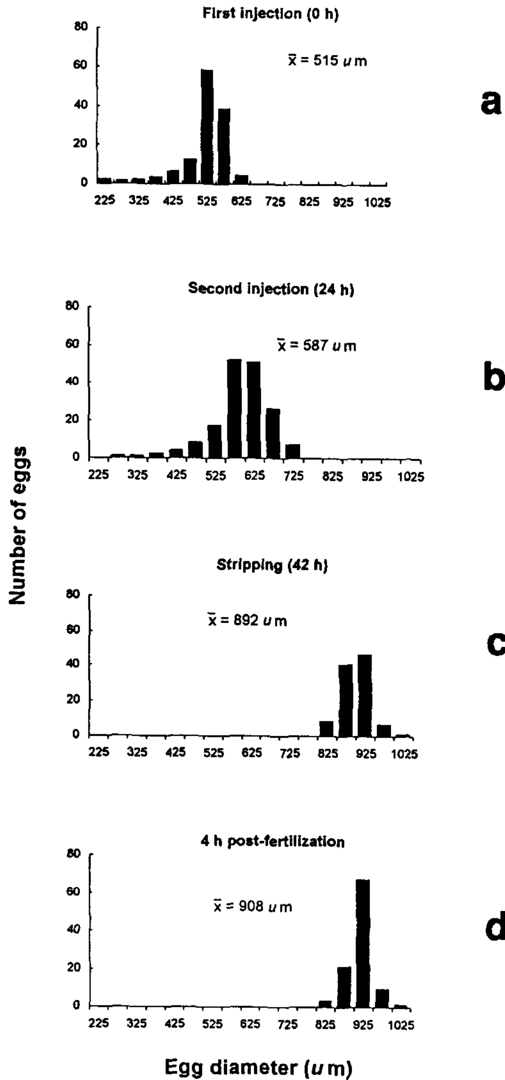

Fig. 1. Oocyte diameter-frequency distribution in *E. striatus* (female 11, Table 3) at various stages of HCG-induced maturation and spawning: (a) first injection, (b) second injection (24 h), (c) unfertilized eggs at time of stripping (42 h) and (d) fertilized eggs at 4 h post-fertilization. Distributions are based on samples of 101–169 eggs.

3.5. Courtship behavior

Prior to spawning, males displayed courtship behavior (following and circling of the female) as described by Colin (1992) for fish under natural conditions. Subsequently, a change from a normal to a distinct 'bicolor' phase (Colin, 1992; Smith, 1972; Tucker et al., 1993; Head et al., 1994) was often observed in both males and females. Small numbers

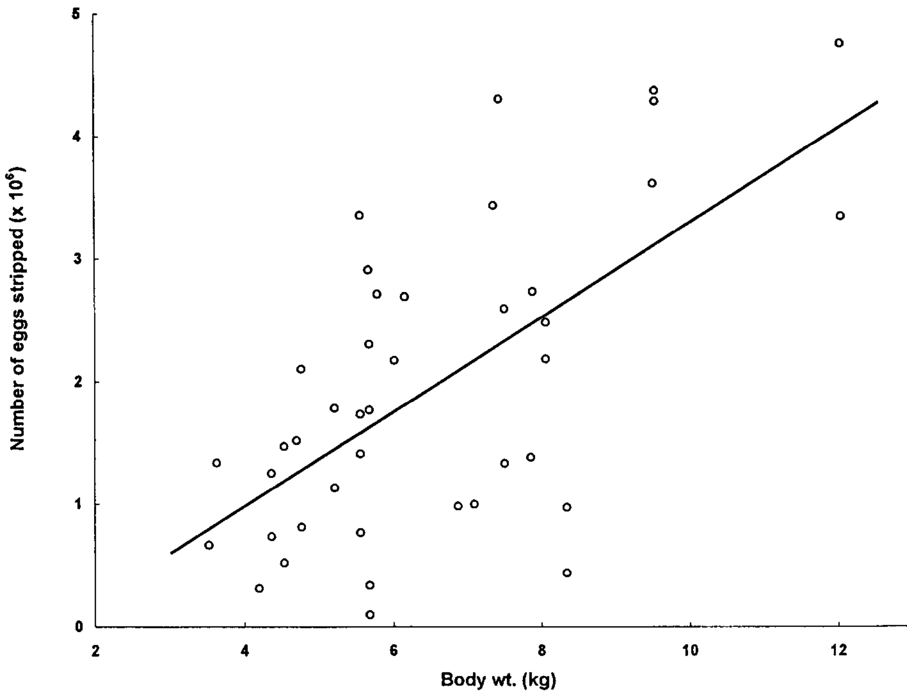

Fig. 2. Relationship between number of eggs released (y) and female body weight (x, kg) in *E. striatus* during hormone-induced strip-spawning: $y = 0.385x - 0.5589$ ($r^2 = 0.4009$, $n = 41$, $P < 0.001$).

of hydrated eggs were sometimes spontaneously released into the spawning tank following hormone induction, but fertilization was not observed during these trials.

4. Discussion

All of the different hormones tested, including HCG (Table 2), LHRH-a (Table 3) and CPH (Table 4), used alone or in various combinations, were effective in stimulating final oocyte maturation (hydration and ovulation) in *E. striatus*, although the fertilization rate varied widely among trials. In addition to *E. striatus* (Tucker et al., 1991; Head et al., 1994), HCG, used alone or in combination with piscine pituitary extracts, has been used to induce spawning in a number of grouper species (see Tucker, 1994 for review), including *E. tauvina* (Chen et al., 1977), *E. malabaricus*, *E. salmoides* (Kungvankij et al., 1986; Tookwinas, 1990), *E. microdon* (Fukuhara, 1989) and *E. fario* (Kuo et al., 1988). Use of LHRH-a to induce spawning in grouper is not well studied and has been reported in only one species (*E. salmoides*) (Kungvankij et al., 1986).

Although results of this and earlier studies (Tucker et al., 1991; Head et al., 1994) suggest HCG, a heterologous gonadotropin of a high molecular weight glycoprotein, to be an effective spawning agent in *E. striatus*, the partial or lack of responses in three females,

spawned with HCG the previous season, indicates that repeated use may cause an immune response (Lam, 1982; Crim et al., 1983; Shelton, 1989). As definitive studies are lacking, and contradictory evidence has been reported in a cyprinid (*Hypopthalmichthys molitrix*) (Van Der Kraak et al., 1989), this possibility requires further study. LHRH-a, a short-chain peptide, was also an effective spawning agent and may be preferable to HCG for repeated application.

Successful spawnings were obtained using HCG in combination with LHRH-a as priming or resolving injections, suggesting that these hormones can be used interchangeably in *E. striatus*. Unlike HCG, which exerts gonadotropic activity, LHRH-a is a hypothalamic hormone which stimulates release of endogenous gonadotropin stored in the pituitary (Lam, 1982; Donaldson and Hunter, 1983; Peter et al., 1988). Preliminary results also indicate that CPH, a crude extract containing gonadotropin as well as other hormones and water-soluble compounds, can also be used as a priming injection in combination with LHRH-a.

Use of LHRH-a as both priming and resolving doses could conceivably cause a depletion of pituitary gonadotropin reserves, which would be prevented by use of HCG and LHRH-a in combination. As no clear advantages to the hormone combination strategy were evident, a single-hormone strategy may be preferable.

Implantation of an LHRH-a cholesterol pellet induced ovulation and spawning in one female, but may have induced sex reversal in another. Hormone implantation (crystal methyltestosterone plus LHRH-a) has been used to induce sex reversal in the brown-marbled grouper *E. fuscoguttatus* (Lim, 1993). However, groupers are known to be protogynous hermaphrodites (Shapiro, 1987), thus sex reversal following implantation may have been fortuitous. Although the cause of the different responses to LHRH-a implant in this study is unclear, the dose rate was slightly higher in the first female, which had a lower body weight (4.2 vs. 5.87 kg). Further studies are required to elucidate the effects of LHRH-a implantation on reproduction in Nassau grouper.

Results of this study indicate that Nassau grouper females with average oocyte diameters ranging from 482 to 561 μm are suitable for hormone-induced spawning. Changes in oocyte size and morphology observed during the pre-spawning period in *E. striatus* are similar to those described for the grey mullet, *Mugil cephalus*. In both species, a distinct increase in mean oocyte diameter during the 24 h after the first injection (Watanabe and Kuo, 1986) is accompanied by a clearing of the oocytes centrally due to the coalescence of lipid droplets and fusion of yolk globules (Kuo et al., 1974). The second injection produces a rapid hydration, an increase in egg diameter over the ensuing 9–18 h (Watanabe and Kuo, 1986), and a transition from an opaque to a transparent condition.

Time of stripping in relation to ovulation is critical to egg quality and fertilization success (Shelton, 1989). In this study, time of stripping following hormone injection was estimated based on subjective morphological criteria as well as on empirical data. Bicoloration, while clearly associated with courtship in this species (Colin, 1992; Smith, 1972; Tucker et al., 1993; Head et al., 1994), was not a reliable criterion by itself, since color change was sometimes absent, intermittent, or at times associated with poor fertilization success (Tables 2–4).

Temperature is known to affect time to ovulation during hormone-induced spawning in fish (Shelton, 1989). In *E. striatus*, time to stripping during HCG-induced spawning is inversely related to temperature (Head et al., unpublished data). Assuming that temperature

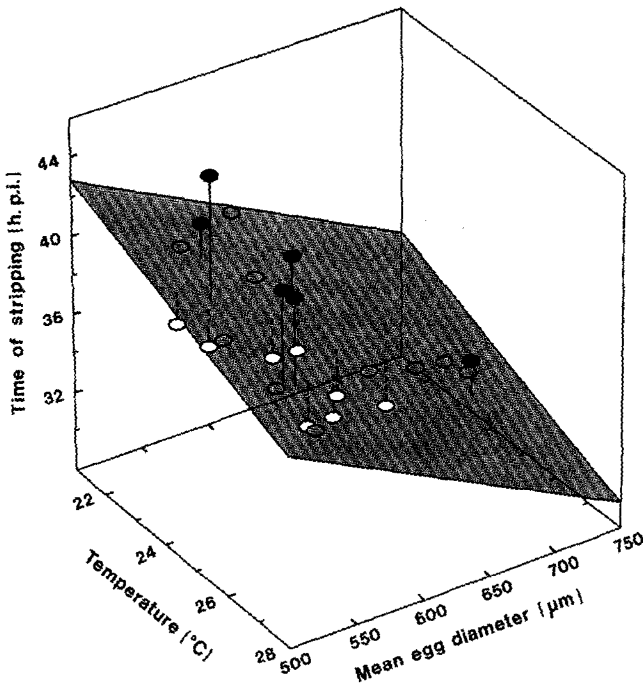

Fig. 3. Optimum time of stripping (y) in *E. striatus* during hormone-induced spawning as a function of mean oocyte diameter at 24 h following the priming hormone injection (x_1) and the average water temperature during the pre-spawning period (x_2). Multiple regression analysis, based on spawnings resulting in ≥50% fertilization rate (plotted symbols), defined this relationship as follows: $y = 68.186 - 0.029x_1 - 0.580x_2$ ($n = 24$, $R^2 = 0.442$, $P < 0.01$). Predicted stripping times are represented by the gridded plane. Symbols lie over (●), under (○) or on (▩) the plane. Vertical lines denote distance from the plane.

affects the rate of oocyte development as assessed by mean size, data for all successful spawnings (Fig. 3) was used to develop a model (Fig. 3). This highly significant ($P < 0.01$) multiple regression predicts the optimal time to stripping based on mean oocyte diameter at 24 h following the first injection and on average water temperature during the pre-spawning period. Larger mean diameters and higher temperatures predict an earlier time of stripping. Oocyte diameter at time of the first injection was not an accurate predictor of stripping time when used in this model.

If dopaminergic inhibition occurs in *E. striatus*, use of LHRH-a plus a dopamine receptor antagonist (e.g. pimozide or domperidone) may potentiate the actions of LHRH-a on gonadotropin secretion (Peter et al., 1988). This method could further improve the rate and predictability of ovulation and time of stripping.

Oocyte samples averaging 482–561 µm in diameter often included early as well as late vitellogenic-stage oocytes. Furthermore, females with post-vitellogenic oocytes were commonly observed with varying percentages of partially hydrated, atretic follicles. This is consistent with observations of this and an earlier study (Head et al., unpublished data) that individual females may be induced to spawn repeatedly within a season at intervals of as little as 15 days.

Successful induced spawnings were obtained from late November through early May, supporting an earlier report (Head et al., 1994) that a 5–6 month spawning season is feasible for captive *E. striatus* broodstock. High numbers ($0.095-4.75\times10^6$ eggs) of eggs released by individual females at each spawning and the capacity for repeated spawning within a season suggests that only a few broodstock would be required for a commercial hatchery if even modest larval survival rates were achieved.

In this study, two females which showed partial responses to LHRH-a in mid-November were spawned with this hormone in January and February. This suggests that oogenesis in these females had not progressed sufficiently by mid-November and that sensitivity to exogenous hormone induction may be poor early in the spawning season (Shelton, 1989).

As reported by Tucker (1994), female Nassau grouper were extremely resistant to handling during induced spawning procedures, which included net capture, anaesthetization, egg sampling, hormone injections and stripping of gametes. When time of stripping was optimum, eggs were rapidly expelled in a fluid stream with minimal pressure, and fertilization success was high. Difficulty in extruding eggs was generally associated with poor fertilization success.

A direct relationship between egg buoyancy and fertilization rate indicates that buoyancy provides an early indication of fertilization success in *E. striatus* and a mechanism for separating fertilized from non-fertilized eggs (Lim, 1993). A clear trend toward higher fertilization and hatching rates from spawnings with higher percentages of buoyant eggs suggests that buoyancy is also related to egg quality.

In this study, courtship behavior and release of hydrated eggs into the spawning tank following hormone induction of females indicates that voluntary spawning is possible. Natural spawning in *E. striatus* has been observed in a public aquarium in Cuba (Guitart Manday and Juarez Fernandez, 1966 in Tucker, 1994) and in a floating sea cage off Grand Cayman Island (Tucker et al., 1993). Other groupers, including *E. tauvina* (Hussain and Higuchi, 1980; Lim, 1993), *E. salmoides*, *E. fasciatus* (see Fukuhara, 1989 for review), and *E. microdon* (AQUACOP, 1990) have also been reported to spawn naturally in tanks. Recently, natural spawning of broodstock *E. striatus* has been observed in a concrete raceway in Florida (J.W. Tucker, personal communication) and in seawater pools in the Bahamas (W.O. Watanabe et al., unpublished data), with high fertilization rates. Natural spawning would preclude handling of broodstock and the need to predict time of stripping, thereby improving egg quality and fertilization success. Hormone induction, however, is an important method to obtain spawning on demand.

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