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Improved Induced-Spawning Protocol for the Spotted Rose Snapper (*Lutjanus guttatus*)

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Abstract

An improved protocol for hormonal induction of spawning in spotted rose snapper (*Lutjanus guttatus*) resulted in increased spawning efficiency in newly-caught wild breeders and wild-caught adults maintained in captivity for more than a year. A controlled-release delivery system (implant) based on an ethylene-vinyl acetate copolymer (EVAc) matrix was loaded with gonadotropin-releasing hormone agonist (GnRHa). The required GnRHa dose was established in two stages. The first stage included meta-analysis of our earlier experiments with wild spawners; the second stage included new experiments with wild and captive breeders. A nomograph was developed to calculate the required GnRHa implant dose, taking into account the origin of the female (wild vs. captive), the initial mean oocyte diameter, and body weight. The effective GnRHa dose was greater in wild than captive females and, in both cases, inversely related to mean oocyte diameter. Using this nomograph, over half the wild females with a mean oocyte diameter of ≥425 μm and over half the captive females with a mean oocyte diameter of ≥350 μm responded to GnRHa implant treatment (producing multiple spawning events in captives), with mean total relative fecundity ranging 80-278 x 10^3 eggs/kg body weight and 51-85% fertilization success. The nomograph can be used to calculate the GnRHa implant dose required to induce spawning in this species under commercial aquaculture conditions.

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Introduction

Snappers (family Lutjanidae) are found throughout the tropical and subtropical regions of the world, mainly inhabiting shallow coral reef waters but also present near rocky bottoms at depths over 90 m. Snappers are an important commercial and recreational fish throughout the world, and their excellent flesh quality warrants high prices and demand. However, high demand has led to over exploitation. World landings exceeded 249,000 tons in 2006, while aquaculture production was less than 4,800 tons with Malaysia (95%) being the main producer (FAO, 2008).

In Mexico, efforts to increase production involve fattening of wild-caught juvenile (50-200 g) spotted rose snapper *Lutjanus guttatus* and American Pacific red snapper ("huachinango") *L. peru*. Research is also being carried out on spawning induction and larvae rearing of the spotted rose snapper. However, newly-caught mature wild spawners are unable to spawn in captivity without hormone treatment (Ibarra et al., 2004). A preliminary spawning induction protocol was developed but needs improvement as spawning success, egg fecundity, and egg quality varied widely among treated fish (Ibarra et al., 2004; Ibarra-Castro and Duncan, 2007).

The spotted rose snapper is a batch spawner of asynchronous ovarian development with a long reproductive season comprising two main spawning periods, March-April and August-November (Arellano-Martinez et al., 2001). Gonadotropin-releasing hormone agonist (GnRHa) is used to induce oocyte maturation and spawning of fish (Zohar, 1996; Zohar and Mylonas, 2001) and has been applied to snappers by means of injection (Emata, 2003) and sustained-release implants (Schipp and Pitney, 1995). GnRHa implants are especially recommended to induce spawning in multiple-batch spawning species (Zohar, 1988; Larsson et al., 1997; Mugnier et al, 2000; Fornies et al., 2001; Marino et al., 2003; Mylonas et al., 2004, 2007; Schipp et al., 2007).

The objective of the present study was to improve the GnRHa implant protocol for spotted rose snapper by studying the relationship between initial oocyte diameter, female body weight, and effective GnRHa implant dose.

Materials and Methods

Study 1 (2003-2004). Induced-spawning trials were carried out at the Regional Center for Fishery Research in Bandera Bay (CRIP-BB) from June 2003 to June 2004. Some of the trials have been published (Ibarra et al., 2004; Ibarra-Castro, 2005; Ibarra-Castro and Duncan, 2007). The trials were carried out with mature wild spawners (228-683 g), recently caught at Sayulita Beach, Nayarit (Mexico). Hormone treatments consisting of GnRHa ([D-Ala^6 Pro^9 NEt]-GnRH) were first administered as liquid injections, then in a sustained-release delivery system (implant) based on an ethylene-vinyl acetate copolymer (EVAc) matrix (Zohar and Mylonas, 2001). A total of 57 females were treated, 18 with injections (of which only five spawned), 36 with implants (two of them without hormone), and another three as untreated controls. Among the GnRHa implant-treated females, 22 individuals were grouped in four trials, two of them without hormone (Ibarra-Castro and Duncan, 2007). In the present study, results of the initial implant trials were meta-analyzed (Table 1).

Study 2 (2005-2007). Subsequent studies with newly-caught wild fish and wild-caught females reared in captivity for one year (referred to as "captive") were carried out in 2005-2007 (Table 2). Adult spotted rose snapper were captured from Sayulita Beach, Nayarit (Mexico), using a modification of the method reported by Ibarra-Castro and Duncan (2007). In brief, scuba fishermen caught fish from coastal reefs with a beach seine. Fish were immediately segregated by sex on board the small fishing vessel. Males were identified by the easy release of milt upon light abdominal pressure, and females by the lack of sperm and the appearance of a genital pore. Males were placed in two 90-l ice boxes and females in a 500-l high density polyethylene (HDPE) tank. Both groups were placed in continuously aerated (by battery-operated blower) ambient sea water (35 ppt, 28°C) and transported to the CRIP-BB laboratory.

Upon arrival at CRIP-BB, each fish was anesthetized with 2-phenoxyethanol (250 ppm; Sigma Chemical Co., St Louis, Mo, USA), and its body weight and total length were measured.

Table 1. Induced spawning of wild female spotted rose snapper using ethylene-vinyl acetate copolymer (EVAc) implants loaded with GnRHa (the initial study).

Trial	*Date*	*Weight (g)*	*Oocyte diameter (µm)*	*GnRHa Dose (µg/kg)*	*Spawn*	*Eggs/kg*	*Floating eggs (% of total)*	*Fertilization success (%)*
P	17 Jun 03	324	412	232	Yes	65,600	58	nd
P	17 Jun 03	322	375	233	Yes	66,000	58	nd
P	17 Jun 03	275	400	273	Yes	77,200	58	nd
P	25 Jun 03	234	460	321	Yes	66,000	78	nd
P	13 Nov 03	364	376	69	No	0	0	0
P	2 Dec 03	228	433	110	No	0	0	0
P	2 Dec 03	265	405	283	Yes	nd	nd	nd
G1	4 May 04	396	506	63	Yes	52,400	nd	nd
G1	4 May 04	367	466	136	No	0	0	0
G1	4 May 04	354	470	212	No	0	0	0
G1	4 May 04	372	359	269	No	0	0	0
H1*	11 May 04	683	496	110	Yes	59,300	97	nd
H1*	11 May 04	382	432	196	No	0	0	0
H1*	11 May 04	471	440	159	No	0	0	0
H1*	11 May 04	475	447	158	Yes	1,600	25	nd
H1*	11 May 04	558	486	134	Yes	22,900	25	nd
H1*	11 May 04	667	457	112	Yes	41,700	46	nd
	11 May 04	495	510	152	Yes	23,900	nd	nd
H2*	26 May 04	290	485	259	Yes	92,800	67	55
H2*	26 May 04	350	473	214	Yes	156,900	41	0
H2*	26 May 04	290	486	259	Yes	288,700	70	63
H2*	26 May 04	290	476	259	Yes	118,100	95	84
H2*	26 May 04	280	441	268	Yes	116,100	94	86
H2*	26 May 04	340	445	221	Yes	85,500	94	80
G2*	31 May 04	360	483	69	Yes	13,900	20	27
G2*	31 May 04	305	493	164	Yes	78,600	38	47
G2*	31 May 04	354	490	212	Yes	68,300	39	50
G2*	31 May 04	314	492	319	No	0	0	0
G3*	6 Jun 04	314	490	80	Yes	76,600	46	38
G3*	6 Jun 04	529	511	95	Yes	55,900	57	0
G3*	6 Jun 04	589	496	127	Yes	65,900	100	93
G3*	6 Jun 04	425	513	235	Yes	43,500	60	40
	6 Jun 04	378	467	132	Yes	30,000	nd	nd
	6 Jun 04	587	495	85	Yes	15,800	nd	nd

* partially published in Ibarra-Castro and Duncan (2007)
P = preliminary
G = dose-dependent trial
H = density trial
nd = no data

Table 2. Induced spawning trials of wild and captive spotted rose snapper using ethylene-vinyl acetate copolymer (EVAc) implants loaded with GnRHa.

	Date of experiment						
	Wild				*Captive*		
	Jun 2003- Jun 2004	*Jun 2005*	*Nov 2005*	*Jun 2006*	*Jun-Jul 2005*	*Aug-Sep 2006*	*Aug 2007*
Wt range (g)	228-683	364-646	284-892	264-634	620-1050	594-1292	1040-1748
Avg wt (g±SEM)	392±20	461±18	446±84	368±28	801±42	920±48	1395±70
No. females	36	5	7	12	11	25	10
Spawned females	26	5	0	0	10	19	10
Spawns/female	1	1	0	0	1-8	1-2	1-4
Avg spawns/female	1	1	0	0	4.2	1.1	2.3
Initial oocyte diameter (µm)	360-510	410-490	360-420	380-430	380-420	350-410	380-430
Avg initial oocyte diameter (µm±SEM)	460±7	440±13	400±8	400±4	400±4	380±3	400±5
GnRHa dose (µg/kg)	63-321	155-275	172-417	197-379	136-273	133-383	209-288
Mean GnRHa dose (µg/kg±SEM)	183±13	237±11	316±28	294±16	211±13	241±11	251±8
Relative batch fecundity (x 10^3/kg)	1.6-288.7	31.4-406.6	0	0	5.7-165.3	24.1-202.5	2.4-329.4
Avg relative batch fecundity (x 10^3/kg±SEM)	71.3±11.5	189.2±72.9	0	0	66.2±8.9	80.6±9.7	79.8±19.2
Relative total fecundity (x 10^3/kg)	1.6-28.7	31.4-406.6	0	0	7.5-497.1	24.0-202.5	10.1-537.5
Avg relative total fecundity (x 10^3/kg±SEM)	71.3±11.5	189.2±72.9	0	0	277.9±65.7	80.6±9.7	199.4±55.2
Fertilization success (%±SEM)	51±9	85±26	0	0	51±5	63±17	0

Female gonad stage was assessed by biopsy; briefly, oocytes were taken from the ovary, approximately 2-3 cm into the genital pore, with a flexible polyethylene catheter (1.0 mm internal diameter, 1.75 mm external). Oocytes were placed in a Sedgwick Rafter counting cell filled with a preservative solution of 1% formalin in 0.9% NaCl. Using a compound microscope, late vitellogenic oocytes were identified by the opacity of the cytoplasm resulting from yolk globule accumulation (Shehadeh et al., 1973a). The diameters of 50 late vitellogenic oocytes were measured using an ocular micrometer. A few drops of Serra solution (6:3:1 v/v ethanol/formalin/acetic acid) were used to clear the cytoplasm and identify evidence of oocyte maturation (e.g., migration of the germinal vesicle, lipid droplet, yolk-globule coalescence) and hydration. In males, the presence and fluidity of milt and the motility of sperm were examined under a compound microscope after activation with a drop of sea water.

The captive fish experiments were conducted at the Centro de Investigacion en Alimentacion y Desarrollo (CIAD) using three groups of broodstock captured a year earlier. After capture, the fish were kept 3-5 days at CRIP-BB and then transported to CIAD for 7 h in 500-l or 1000-l tanks containing cooled (22-24°C) sea water (33-35 ppt), continuously aerated by a battery-operated blower and pure oxygen. Transportation density was 30-60 kg/m^3 and fish were lightly sedated using 25 ppm 2-phenoxyethanol. On arrival at CIAD, the fish were stocked for recovery in 7-m^3 or 25-m^3 fiberglass tanks with vinyl liners and fed fresh oily fish and squid for several weeks. Broodstock caught in June 2004 were stocked into two 18-m^3 fiberglass tanks in equal numbers in August. Broodstock tanks received a constant flow (6-8 volumes/day) of ambient sea water (22-33°C, 33-35 ppt) and strong aeration, and were covered by a shade cloth (70%). Fish were fed a laboratory-made moist diet (50% commercial dry diet and oily fish flesh, 50% frozen squid), six days a week at 3% of their body weight daily and were left undisturbed for seven months. Starting in March, as the spawning season was approaching, fish were fed oily fish, squid, and shrimp at equal proportions fortified with 0.3% vitamin C, 0.1% vitamin E, and 6% menhaden oil. After transition to artificial food, fish caught in 2005 and 2006 were fed a semi-moist diet (50% Fish Breed-M, INVE Aquaculture Inc., 25% frozen squid, 25% oily fish), supplied *ad libitum* once every morning, 6 days a week.

Fish were selected for the induced spawning trials according to the following criteria: females with late vitellogenic oocytes that had mean diameters of ≥400 μm for wild fish and ≥350 μm for captive fish, males with flowing milt and ≥80% motile spermatozoa. A passive integrated transponder tag (PIT tag, Biomark, Idaho, USA) was implanted in the dorsal muscle. Fish were treated with GnRHa implants, as in the initial trials (Zohar and Mylonas, 2001). In the first five females treated in June 2005 the GnRHa implant was applied intramuscularly; thereafter, implants were applied intraperitoneally. Wild females were treated between 13:00 and 14:00 (3-4 h after capture) and separately placed into 600-l or 1400-l fiberglass tanks with flow-through sea water at a daily exchange rate of 300% and continuous aeration. Captive females were treated between 10:00 and 12:00 and separately placed into 3-m^3 fiberglass tanks, with flow-through sea water at a daily exchange rate of 400% and continuous aeration. Two spermiating males were placed with each female. In June 2005 the males received an intraperitoneal injection (500 IU) of veterinary grade human chorionic gonadotropin (Chorulon, Reg. SAGAR Q-0273-066, Agropecuaria Dasa S.A. de C.V., Mexico). Males in later trials were treated with a GnRHa implant of 25 μg for small fish (0.6-0.8 kg) or 50 μg for large fish (≥1 kg). Fish were not fed during the experiments and were left undisturbed. Each spawning tank was equipped with a 20-l overflow egg collector made of 500-μm mesh, hanging inside a 100-l tank.

The egg collectors were checked for the presence of eggs every hour, starting at 18:00 of day 1 after hormone treatment. Spawning occurred 32-40 h after treatment in wild females and 30-42 h after treatment in captives. When spawning was detected, the time was recorded and fertilization success was estimated using a sample of ~120 eggs (2-16 cell stage) from each collector, examined under a compound microscope. The next morning (12-14 h after spawning),

fertilization success was estimated again from another sample from each collector. The difference between the two fertilization estimates was insignificant (p = 0.095), thus the second estimate is presented in the results. All eggs were harvested and the number of floating (which were ~90% fertilized) and sinking (dead) eggs was estimated volumetrically with a graduated cylinder, based on an earlier estimation of 2,000 eggs/ml (Ibarra-Castro, 2005). Fertilization success was calculated as the number of fertilized eggs/total number of spawned eggs. Clean collecting devices were replaced the following afternoon, before the next spawning.

Results

Study 1 (2003-2004). Results from these experiments were used to develop a preliminary spawning protocol: i.e., a dose of 240-280 µg of GnRHa/kg body weight for females with late vitellogenic oocytes of a mean diameter of 440–500 µm. Using this protocol, there was considerable variation in egg fecundity (Ibarra-Castro and Duncan, 2007). Most of the variation could be explained by species variability and the small number of females with heterogenous stages of sexual development. The lack of standardization of female reproductive development meant that the hormone dose was not determined by oocyte diameter or body weight.

Examination of the results from the initial studies showed a significant negative correlation (p = 0.0036, non-parametric Spearman Rank test) between mean initial oocyte diameter and GnRHa dose (Fig. 1). Spawning success was 86% in females with a mean oocyte diameter >400 µm and treated with ≥200 µg GnRHa/kg body weight (group A), 92% in females with a mean oocyte diameter >460 µm and treated with <200 µg/kg (group B), and only 40% in females with a mean oocyte diameter <460 µm and treated with <200 µg/kg (group C). For group A the mean effective GnRHa implant dose was ~ 250 µg/kg and for group B ~125 µg/kg. Thus, the required implant dose for females could be calculated according to mean oocyte diameter and body weight.

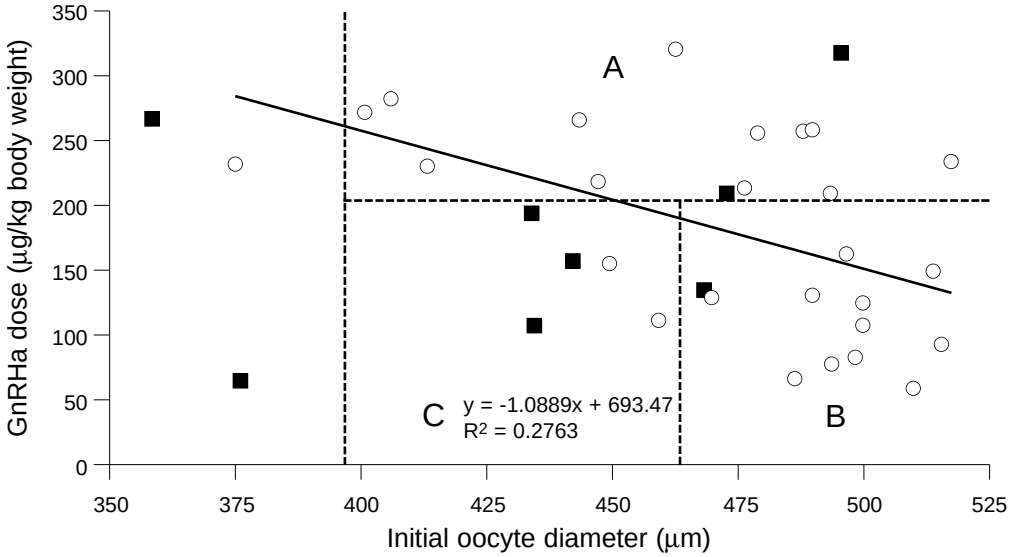

Fig. 1. Study 1 (2003-2004): Relationship between GnRHa dose and mean initial oocyte diameter in spotted rose snapper females that spawned (○) and did not spawn (■). Correlation is significantly negative (p = 0.0036). Percent successful spawning (production of viable eggs) in areas A, B, and C was 86%, 92%, and 40%, respectively.

Study 2 (2005-2007). No wild or captive females showed evidence of oocyte maturation at the time of GnRHa treatment. The total relative fecundity (mean±SEM) of all fish was 209,000±120,000 eggs/kg and the mean fertilization success was 84±26%. The best-spawning females produced a total relative fecundity of 200,000-400,000 eggs/kg with >90% fertilization success. The 19 wild females treated in the November 2005 and June 2006 trials failed to spawn, demonstrating that the mean oocyte diameter must be at least 425 µm for GnRHa implantation to induce spawning. In contrast, most of the captive females with mean oocyte diameters of 350-430 µm spawned, producing a mean total relative fecundity of 162,000±70,000 eggs/kg, with a mean fertilization success of 51±35%. Of the spawned captive females, 80% had multiple spawns on two to eight consecutive nights, whereas a single spawn was obtained from wild fish.

There was a significant negative correlation (p = 0.0011, non-parametric Spearman Rank test) between the required GnRHa dose and mean initial oocyte diameter (Fig. 2, regression not shown). In wild females, spawning success was >50% when oocyte diameters were 425-449 µm and 100% when 500-524 µm, while most females with initial oocyte diameters <425 µm did not spawn (Fig. 3). In captive females, spawning was >50% in females with mean oocyte diameters of 350-374 µm.

To develop a nomograph for determining the required GnRHa dose based on initial oocyte diameter, the mean doses required for ≥50% spawning success were estimated for females grouped according to mean initial oocyte diameter (Fig. 3) and superimposed on the original data (Fig. 2). Two regression lines represent the relationship between effective GnRHa dose and mean initial oocyte diameter, one for captive females (Fig. 2, left) and one for wild females (Fig. 2, right). The regression lines significantly differ (comparing two slopes and two elevations,

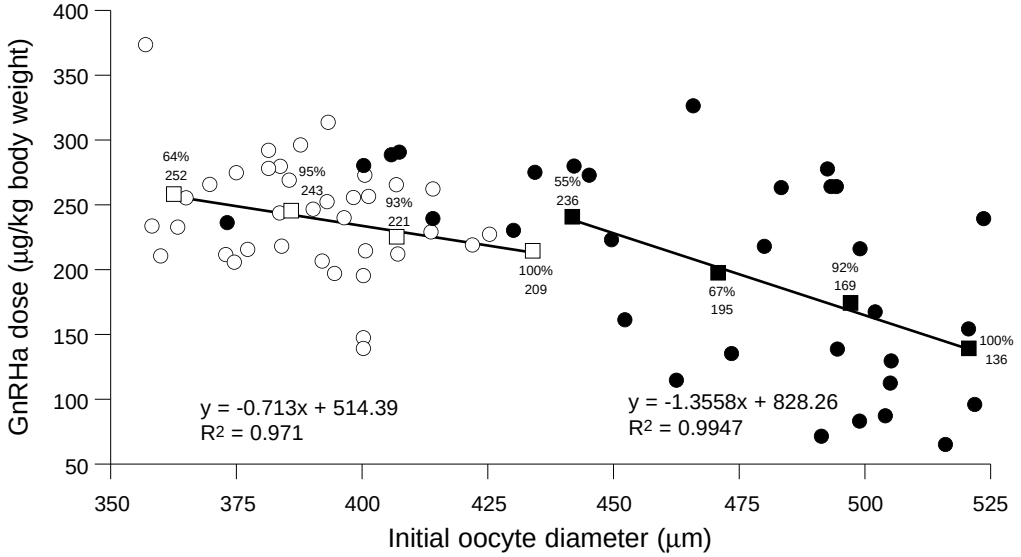

Fig. 2. Study 2 (2005-2007): Relationship between GnRHa dose and mean initial oocyte diameter of captive (○) and wild (●) spotted rose snapper females. Mean GnRHa doses used to induce spawning in females with oocyte diameters within the relevant 25-µm interval are shown for captive (□) and wild (■) females. Mean GnRHa dose and percent of females that spawned in the 25-µm group appear beside the squares.

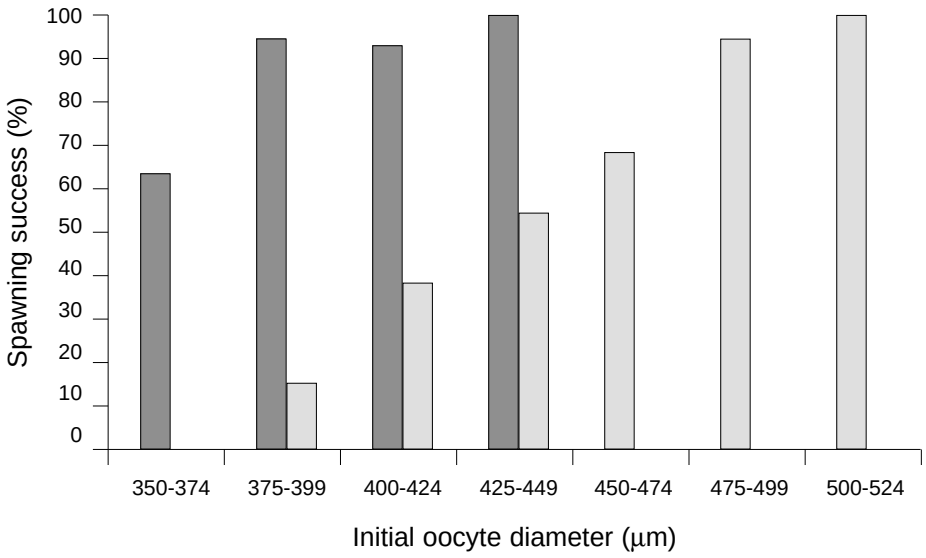

Fig. 3. Spawning success (%) of wild (□) and captive (■) spotted rose snapper females according to mean initial oocyte diameters grouped into 25-μm categories.

$p<0.05$; Zar, 1999). Thus, a nomograph for estimating the GnRHa dose required to induce spawning in at least 50% of females must take the origin of the females (captive or wild) into account, as well as the mean initial oocyte diameter, based on these equations.

The nomograph consists of three parallel lines, one for mean initial oocyte diameter, one for female body weight, and one for required GnRHa implant dose (Fig. 4). The known variables (oocyte diameter and body weight) are placed on the right and left while the result scale (GnRHa implant dose) is in the center. To determine the required GnRHa implant dose, a line is drawn between the initial mean oocyte diameter and the body weight of the female. The line intersects the center line at the required GnRHa implant dose, the left-hand side for wild females and the right-hand side for captives. Since GnRHa doses are available in increments of 25 μg, when the line intersects the dose scale between two quantities, the higher dose should be used.

Discussion

Meta-analysis of the initial spawning results shows that (a) there is a relationship between female maturity (evaluated by mean oocyte diameter) and GnRHa dose, and (b) spawning success data should be considered individually for each fish. Based on earlier trials with wild spotted rose snapper from the same site using 75 μg GnRHa implants for females ranging 260-700 g with post-vitellogenic oocytes of >430 μm, a narrow range of GnRHa implant doses (240-280 μg/kg) for females with a relatively broad range of oocyte diameters (440-500 μm) was recommended (Ibarra-Castro and Duncan, 2007). However, this recommendation is appropriate for only a small proportion of females (see Fig. 2).

There was considerable variation in quantity and quality of eggs produced by wild females. Most of the variation can be explained by species variability and the small number of females. Limited availability of females meant that the fish used in the experiments were not in a uniform stage of sexual development for all treatments and replicates. In experiments involving standardization of spawning protocol, it is necessary that females have equally developed ovaries for

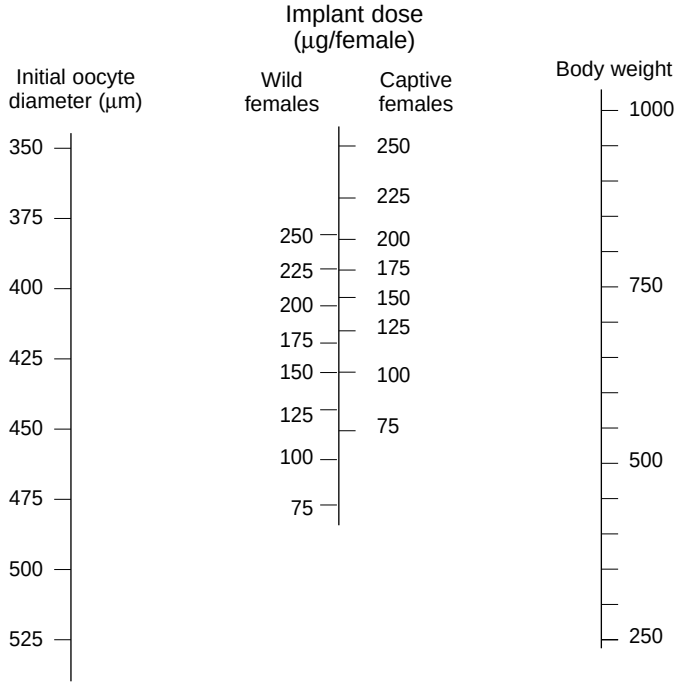

Fig. 4. Nomograph for calculating GnRHa implant dose required to induce oocyte maturation, ovulation, and spawning of wild and captive spotted rose snapper, based on mean initial oocyte diameter and body weight of the female.

all treatments and replicates (Sneed and Clemens, 1960; Fornies et al., 2001). The lack of standardization means that the hormone dose recommended in the initial experiments was not determined by oocyte diameter or body weight.

Fish must have reached a minimum developmental stage of sexual maturity for hormone therapy to succeed (Sneed and Clemens, 1960; Sneed and Dupree, 1961; Kuo and Nash, 1975). Minimum oocyte diameters for successful spawning induction (called threshold oocyte diameter by Colura, 1974), have been reported for many species. For example, the threshold oocyte diatemer for red mangrove snapper (*L. argentimaculatus*) is ≥400 µm (Lim et al., 1985; Emata, 2003), for Asian sea bass (*Lates calcarifer*) >450 µm (Garcia, 1989), for striped mullet (*Mugil cephalus*) ≥600 µm (Kuo et al., 1973; Shehadeh et al., 1973b), and for milkfish (*Chanos chanos*) ≥750 µm (Tamaru et al., 1988). In the present study, the threshold oocyte diameter to obtain ≥50% spawning by GnRHa implants was 425 µm for wild and 350 µm for captive females. This differs from a previous study that recommended a minimum diameter of 440 µm for wild spawners (Ibarra-Castro and Duncan, 2007).

The present study shows that the effectiveness of hormone treatment in spotted rose snapper increases and the required hormone dose decreases as mean initial oocyte diameter increases, similar to other species (Harvey and Hoar, 1979). There was an inverse linear relationship between oocyte diameter and dose of partially purified salmon gonadotropin required to induce spawning in striped mullet (Kuo et al., 1974), although this was not the case when hCG

was used (Kuo et al., 1973). Recommended GnRHa doses for marine fishes are 40-100 μg/kg (Garcia, 1989, 1992; Schipp and Pitney, 1995; Mugnier et al., 2000; Fornies et al., 2001; Watanabe et al., 2003) although some studies used doses up to 200 μg/kg (Larsson et al., 1997). For the spotted rose snapper, a similar high dose was recommended by Ibarra-Castro and Duncan (2007). In the present study, only females with oocyte diameters <475 μm required such doses, whereas some females with oocyte diameters of ≥475-500 μm required doses as low as 75-100 μg GnRHa/kg.

Stress, through its impact on the hypothalamus-hypophyseal-gonad axis, is an important cause of detrimental effects on reproduction in fish (Schreck et al., 2001; Pottinger, 2008). Environmental and handling stressors are main blocks to fish reproduction, causing gonad regression or even mortality. Limiting stress on broodstock is essential for controlling reproduction in captivity (Billard et al., 1981; Zohar, 1988; Harvey and Carolsfeld, 1993; Bromage, 1995; Patino, 1997; Mylonas and Zohar, 2001). Capture and handling stress is common in wild spawners, particularly sensitive species such as milkfish and mullets (family Mugilidae). In these species, hormone therapy should start immediately upon arrival at the laboratory. According to our results, handling and GnRHa therapy were stressful to wild females, since GnRHa implants did not induce multiple spawning as they did in captive females.

Latency between hormone therapy and spawning was similar to that of *L. argentimaculatus* (Emata, 2003). The average number of eggs produced per kg by wild females with the improved spawning protocol was much higher than in previous experiments with spawners of the same size (Ibarra-Castro and Duncan, 2007; Boza-Abarca et al., 2008). Spawning performance was within the range of best egg production for other snappers (Davis et al., 2000). Captive spawners exhibited higher total fecundity and egg quality than in other reports (Moretti et al., 1999; Schipp et al., 2007).

The nomograph for calculating GnRHa implant dose fulfils the general rules of nomographs, i.e., they are designed to solve specific equations in one step, with a precision limited to practical use (Doerfler, 2008). Nomographs are used for scientific, technical, and human health calculations. As far as we know, only one nomograph concerns spawning in fish and it is used to determine the weight of a pituitary donor and receiver (Makeyeva and Verigin, 1970). The nomograph in the present study consists of three vertical scales, each representing one variable in an equation. The divisions on the middle scale are not uniform, which might have been possible if a curved scale was used, but we chose to use the classic straight-line presentation. Because GnRHa implants contain fixed amounts, when the dose indicated on the nomograph falls between values, it is recommended to use the higher dose. To administer the precise required dose, a controlled-release delivery system such as microspheres could be used (Mylonas et al., 1995). But then, a new nomograph would have to be developed since the release kinetics of GnRHa microspheres differ from those of GnRHa implants, and the dose-responses of wild and captive females with different initial oocyte sizes would have to be determined anew.

In summary, the protocol for inducing spawning in wild and captive spotted rose snapper females using GnRHa implants was improved. The minimum initial oocyte diameter and minimum GnRHa implant dose necessary to achieve >50% successful spawning was determined for wild and captive females. Results were used to develop a nomograph that can be used in commercial hatcheries to quickly estimate the GnHRa dose needed to induce spawning in spotted rose snapper females.

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