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Morpho-physiological predictors of ovulatory success in captive striped bass (*Morone saxatilis*)

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Abstract

This study evaluates morpho-physiological characters as predictors of ovulatory success in cultured striped bass, *Morone saxatilis*, that could be used by farmers to select females for induced spawning. Diameter, size homogeneity and growth of ovarian follicles; blood plasma levels of testosterone (T), oestradiol-17β (E_2) and vitellogenin (VTG); and in vitro maturation of oocytes, in response to a combination of insulin-like growth factor-I (IGF-I, 100 nM) and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S, 290 nM) were examined for females prior to spawning induction and compared with their subsequent ovulatory response. Fish spawning within 8 days of implantation with pelleted analogue of mammalian gonadotropin-releasing hormone analogue (GnRHa; [D-Ala^6-des-Gly^{10}-NEt]-LHRH) were considered to have given a satisfactory maturational response. The in vitro assay was the most reliable predictor for ovulatory success. All fish whose oocytes completed final oocyte maturation (FOM) in vitro in response to the combination of IGF-I and 20β-S spawned, whereas, 12 out of 13 fish, whose oocytes did not complete FOM in vitro, failed to spawn within 8 days of GnRHa treatment. The in vitro assay was field-tested on commercial farms, and correctly identified all four females that spawned out of the eight females that were given hormone treatment. Among the other measurements, follicle diameter best differentiated between fish that later spawned and those that did not spawn. Plasma T concentrations were greater on average in fish that spawned, but the technical complexity of the assay and overlap in T concentrations between fish that spawned and those that did not limits the value of this measurement to farmers. There was no significant difference in follicle size homogeneity, follicle growth over the 2-week period prior to hormone treatment, or plasma levels

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of E_2 and VTG between fish that spawned and those that did not.

Keywords: *Morone*; IGF-I; Oocyte maturation; Spawning; Broodstock

1. Introduction

Farming of striped bass and its hybrids is one of the fastest growing aquaculture industries in the US (USDA, 1992). Striped bass maintained in captivity rarely complete final oocyte maturation (FOM; see Goetz, 1983; Nagahama et al., 1994) and undergo ovarian atresia after completing oocyte growth unless they are induced to undergo oocyte maturation and spawn by exogenous hormone treatment (Sullivan et al., 1997). Injected human chorionic gonadotropin (hCG) is routinely used to induce the maturation and spawning of female striped bass, but the technique is only effective for fish whose oocytes have already initiated FOM. Fish at this stage of maturation can be identified by visual inspection of biopsied oocytes (Rees and Harrell, 1990). Often, by the time members of a broodstock are identified as having initiated FOM, most of the remaining females have begun to resorb their fully grown oocytes. Hodson and Sullivan (1993) demonstrated that fish not yet showing signs of FOM can be induced to spawn with implanted pellets that slowly release mammalian gonadotropin-releasing hormone analogue (GnRHa) with or without subsequent injections of hCG. Female striped bass at these early stages are selected for spawning based on maximum ovarian follicle diameter. When follicle diameter is the only criterion used to select females for spawning, many fish are treated with hormone too early or too late, resulting in the loss of egg production. Follicles can be examined for signs of atresia (Mylonas et al., 1997b) but this only serves to identify fish that can no longer produce viable eggs. Using maximum follicle size as a method to select candidates for induced spawning is inadequate because there is a great disparity among individual fish as to the size at which their follicles become responsive to hormone treatment, as well as the size at which they begin preovulatory atresia. Furthermore, exposure of the fish to altered photothermal regimes used to shift spawning time can have dramatic effects on maximum follicle diameter (Blythe et al., 1994a,b).

Improved predictors of ovulatory success need to be developed for female striped bass that have not reached stages of oocyte maturation that can be visually identified. The current study evaluates the potential of several morpho-physiological characters to serve as indicators that the fish is ready for hormone treatment. These include homogeneity in diameter of ovarian follicles, rate of follicle growth over a 2-week period, and circulating levels of sex steroid hormones (testosterone, T; and oestradiol-17β, E_2) and the yolk protein precursor vitellogenin (VTG). Homogeneity of follicle size was evaluated because there appears to be a greater heterogeneity in captive striped bass compared with wild fish of the same stock (W. King V, personal observation). Rapid oocyte growth just prior to FOM has been reported to occur in several *Morone* species including striped bass (see Sullivan et al., 1997 for review). Females ready for induced spawning may be fish whose follicles are in the rapid growth stage near the end of

vitellogenesis. The optimal time for GnRHa treatment in milkfish is within the 5-day period just following the end of oocyte growth (Lee et al., 1986). In routine spawning activities, we have also noted a high degree of variability in follicle growth rate among captive fish beginning with similar size follicles. For these reasons, we evaluated whether the rate of follicle growth over a 2-week period could be used to identify fish competent to complete FOM and ovulate in response to GnRHa treatment.

The reproductive cycle of female striped bass has been characterized with regard to the changes in concentrations of steroid hormones, gonadotropin II and VTG in the blood, relative to the histological stages of oocyte development (Berlinsky and Specker, 1991; Woods and Sullivan, 1993; Blythe et al., 1994b; King et al., 1994a; Mylonas et al., 1997a,b, 1998). Blood levels of T and E_2 increase during early stages of FOM. Blood levels of VTG have not been previously examined during striped bass FOM but might be expected to decrease during the postvitellogenic period of oocyte maturation. Blood concentrations of these molecules were measured in the present study to determine if they could serve as predictors of ovulatory success. Plasma T concentration can serve to predict ovulatory success in response to GnRHa for coho salmon (Fitzpatrick et al., 1987).

Ovulatory success of female sturgeon (*Acipenser transmontanus* Richardson) can be predicted based on the in vitro response of follicles from biopsy samples to a steroid that induces FOM, progesterone. Females ready for induced spawning are fish whose oocytes completed FOM in vitro in response to the hormone (Lutes et al., 1987). This test cannot be used with captive striped bass because oocyte development in most individuals is arrested at a stage when oocytes cannot respond yet to the maturation-inducing steroid (MIS; King et al., 1994b). The MIS in striped bass is 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S; King et al., 1997). Gonadotropin (hCG) can act on follicles of striped bass to induce FOM in vitro before the follicles become responsive to the MIS. Even so, follicles of most captive female striped bass do not complete FOM in vitro in response to hCG unless the donor females are injected with gonadotropin prior to biopsy (King et al., 1994b). Potential spawners cannot be injected with gonadotropin for the purpose of this kind of test because such treatment renders the fish unspawnable due to premature activation of FOM in their immature oocytes.

Recent studies by Kagawa et al. (1994) have shown that insulin-like growth factor-I (IGF-I) can induce FOM in oocytes of red seabream (*Pagrus major*) in vitro. We have recently shown that IGF-I can induce FOM in vitro in striped bass follicles that are not yet responsive to gonadotropin or the MIS (Weber et al., 1997). In the present study, an evaluation was made of the use of this in vitro response to IGF-I as a bioassay to predict ovulatory success of female striped bass induced to mature with GnRHa implants.

2. Materials and methods

2.1. Experimental animals

Animals used in the study were striped bass that had been held in captivity at Pamlico Aquaculture Field Laboratory (35°15′ north latitude), North Carolina State University

for a minimum of 2 years as described by Hodson and Sullivan (1993). The fish were derived from wild stocks from the Roanoke River and Santee-Cooper reservoir. The female striped bass ranged from 3.4 to 10.4 kg body weight. All fish were maintained in outdoor 6-m diameter × 1.2-m deep circular tanks supplied with aeration and flow-through 18°C well water (0 ppt salinity, 300 ppm, hardness, 300 ppm alkalinity) or water pumped from a nearby creek (7–15 ppt). Water temperatures ranged between 7°C and 25°C annually. Fish were fed a 38% protein commercial hybrid striped bass production diet (Southern States, VA) twice daily for the duration of the experiment. They were individually identified using passive integrated transponders (PIT, Destron/IDI, Boulder, CO) implanted subdermally.

2.2. Experimental design and sampling protocol for Experiment 1

Fish were moved from multiple tanks into a single tank on February 26, and then divided between the two tanks. Twenty of the fish were moved to another outdoor tank as described above, and 20 were moved to an indoor tank. The indoor tank was 2.2-m diameter × 0.84-m deep circular tank equipped with a recirculating water system and lighting that allowed for photothermal regulation. The water temperature was maintained at 10 ± 1°C throughout the study. The photoperiod matched ambient duration. The purpose of the indoor facility was to extend the spawning season by holding broodstock at low temperature to prevent preovulatory atresia, in order to allow more fish to be used in spawning trials. This procedure, by common usage referred to as ''cold banking'' is routinely used by hybrid striped bass fingerling producers to extend the spawning season of female white bass (Smith et al., 1996; Kohler, 1997; Mike Freeze, Keo Fish Farms; and Lee Brothers, Carolina Fisheries, personal communication).

Females were examined 2 weeks before induced spawning was initiated and then examined a second time when they were implanted subcutaneously with two pellets containing [D-Ala^6-des-Gly^{10}-NEt]-LHRH (GnRHa; Sigma, St. Louis, MO) to induce spawning, as described by Hodson and Sullivan (1993). Each of the two implants contained 150 μg GnRHa. The matrix of one GnRHa pellet was 80% cholesterol and 20% cellulose (fast hormone release), and that of the other was 95% cholesterol and 5% cellulose (slow hormone release). Groups of two to five fish from either tank were selected randomly for each spawning trial. Fish from the outdoor tank were first examined on March 25, and April 1, 8, 15 and 22. Fish from the indoor tank were first examined on April 1, 15 and 29, and May 13 and 27. At examination, they were anesthetized in 15 ppm quinaldine sulfate (Aurum Aquaculture, Bothell, WA) and biopsied by inserting a plastic tube through the genital pore to obtain ovarian samples for measurement of follicle diameters and to determine if their oocytes had initiated atresia (King et al., 1994a). First, the diameter of 10 of the largest follicles in the biopsy sample was measured to the nearest 25 μm using a dissecting stereomicroscope fitted with an ocular micrometer to obtain an estimate of maximum diameter. Next, a random sample of 50 vitellogenic stage follicles was measured to obtain a measurement of size homogeneity. The fish were biopsied again 2 weeks later, at which time their follicle diameters were again measured and follicle samples were used for the in vitro bioassay. Blood plasma samples were collected by caudal puncture, processed as described

previously (Tao et al., 1993), and stored at −80°C until being subjected to radioimmunoassay (RIA) for the measurement of steroid hormones or enzyme-linked immunosorbent assay (ELISA) for the measurement of VTG. Steroid hormones in the blood plasma were measured using RIAs for E_2 and T as described by Woods and Sullivan (1993). VTG was measured using an ELISA described by Heppell et al. (1999). All samples were measured in a single assay for each steroid and VTG.

2.3. Spawning

Individual females from the outdoor tank were placed directly into circular, 1.8-m diameter × 0.9-m deep, indoor tanks along with two spermiating males for tank spawning (Smith and Whitehurst, 1990). The water temperature of the outdoor and spawning tanks was ~18°C. Females from the indoor holding tank maintained at ~10°C were first removed to a separate tank filled with 12°C water. The water temperature was increased to 14°C overnight, and then to 18°C over the next 24 h. After acclimation to 18°C, the fish were transferred to individual spawning tanks as described. Females were given up to 7 days to show signs of oocyte maturation and up to 8 days for tank spawning to take place. Spawning trials were terminated after 8 days because holding the fish for longer periods under these conditions is stressful to the females and can disrupt normal maturation (Hodson and Sullivan, 1993; Woods and Sullivan, 1993). The fish were biopsied periodically and injected with hCG to induce spawning upon initiation of FOM (Hodson and Sullivan, 1993).

2.4. Experimental design and sampling protocol for Experiment 2

The in vitro bioassay was field-tested using ovarian biopsy samples from striped bass at two commercial fish farms. Fish were biopsied and ovarian follicles were incubated in medium without hormone (control), medium containing IGF-I (100 nM) together with 20β-S (290 nM), and medium containing hCG (25 IU/ml; Sigma). Fish were implanted with GnRHa pellets and induced to spawn as described for Experiment 1, except that they were manually stripped of their eggs following ovulation. The tests were conducted using four fish from each farm, with follicle diameters greater than 850 μm. The ovarian follicle samples were incubated at ambient temperature at the farms for 3–4 days before being examined to enumerate maturing oocytes.

2.5. In vitro bioassay

In vitro incubations of ovarian follicles and subsequent evaluation of oocyte maturational stages followed our routine procedures previously described by King et al. (1994b). Briefly, biopsy samples were incubated in Cortland's balanced salt solution (chemicals purchased from Fisher Scientific, Pittsburgh, PA) buffered with 15 mM HEPES (Sigma) and supplemented with a combination of IGF-I (100 nM; GroPep, Adelaide, Australia) together with 20β-S (290 nM; Steraloids, Wilton, NH). Penicillin and streptomycin (100 units and 0.1 mg ml^{-1}, respectively; Sigma) and 0.1% bovine serum albumin (Sigma) were also added to the medium. Biopsy samples were twice

rinsed with sterile Cortland's balanced salt solution before groups of follicles weighing approximately 0.1 g were placed into wells of a Falcon 24-well culture plate (Becton Dickinson, Franklin Lakes, NJ) with 1 ml of incubation medium containing the hormones. Up to three wells were used per fish depending on the size of the biopsy sample obtained. The follicles were incubated up to 4 days at 22°C. On day 4, the follicles were cleared with an oocyte clearing solution of ethanol:formalin:acetic acid (6:3:1 v/v; Pankhurst, 1985; chemicals purchased from Fisher Scientific), and were examined using a dissecting microscope for signs of maturation including germinal vesicle migration (GVM) and germinal vesicle breakdown (GVBD). GVM was used as an indication that oocyte maturation had been initiated, and GVBD was used as an indication that FOM had occurred.

2.6. Statistical analysis

Data were subjected to two-way ANOVA to test for significant differences in means for fish that did and did not spawn within the designated time period, and for the effects of being maintained outdoors versus indoors (Zar, 1974). The a priori level of statistical significance chosen for all tests was $P \leq 0.05$ and the statistical analysis was performed using the statistical software SUPERANOVA (Abacus Concepts, Berkeley, CA). Significant differences were not observed for the effects of being maintained outdoors versus indoors so the combined data are reported.

3. Results

3.1. Experiment 1

3.1.1. Study animals

Data from 29 of the 40 fish are presented for the morphometric and blood-borne characters. Five of the 11 fish not included in these analyses died during the study. The remaining six fish were excluded because they either had many atretic oocytes when examined just prior to spawning trials, were mature enough to be eligible for hCG-induced spawning when they were first examined, or had follicles smaller than 700 μm in diameter at the time for hormone implantation. Over several years, it has never been proven that it is possible to induce females with oocytes ≤ 700 μm in diameter to ovulate with GnRHa implants, in spite of repeated trials (C.V. Sullivan and R.G. Hodson, unpublished). Nine of the remaining 29 fish were not represented in the data for the in vitro studies. Three fish were not represented because, due to logistical problems, in vitro incubations were not conducted for the final group of fish spawned in this study. Three fish were not included because they were eligible for hCG-induced spawning at the time when they were to be implanted with hormones. Atretic oocytes were already present in one additional fish at the time of hormone implantation. Severe microbial contamination was observed in cultures from two additional fish, resulting in death of nearly all the follicles. Data from these two fish were not included in the in vitro analysis.

3.1.2. In vitro bioassay

All seven fish whose oocytes completed GVBD in vitro in response to a combination of IGF-I and 20β-S, ovulated in vivo within 8 days in response to GnRHa implants and hCG injections (Table 1). Only one of 13 fish whose oocytes did not respond to the in vitro treatment ovulated within 8 days (on day 7). Fish whose oocytes showed only early signs of maturation in vitro did not ovulate in vivo within 8 days. Three fish that did not respond to in vitro hormone treatment were allowed 20 days to ovulate. Two of these fish ovulated, one at 12 days and one at 16 days following GnRHa administration.

3.1.3. Morphometric criteria

Follicle diameter was greater for fish that ovulated compared with fish that failed to ovulate within 8 days. This was true when 50 randomly selected vitellogenic follicles were measured (spawned: 950 ± 23 μm; no spawn: 778 ± 19 μm; $P \leq 0.001$), and when only 10 of the largest follicles were measured (spawned: 1058 ± 29 μm; no spawn: 881 ± 20 μm; $P \leq 0.001$; Fig. 1). Despite this significant difference, there was much overlap in mean follicle diameter between individual fish that did ovulate versus those that did not. Seven of 12 of the fish that spawned had smaller follicle diameters, based on measuring 10 of the largest follicles, than a fish that did not ovulate; and seven of the 17 fish that did not spawn had follicle diameters larger than that of a fish that did

Table 1
Comparison of the response of ovarian biopsy samples from striped bass incubated in a combination of IGF-I (100 nM) plus 20β-S (290 nM), to the in vivo response of the same fish to GnRHa implants
The following abbreviations were used to denote observed response of ovarian fragments incubated with the hormone combination: GVBD, germinal vesicle breakdown; GVM, initiation of germinal vesicle migration and coalescing of oil droplets; N, no maturation observed.

Date implanted	In vitro response	Days until spawned
4/8	N	–
4/8	N	–
4/8	N	–
4/8	N	–
4/15	N	–
4/15	N	–
4/29	N	–
5/13	N	–
5/13	N	7
5/13	N	12
5/13	N	16
4/15	GVM	–
4/15	GVM	–
4/8	GVBD	3
4/15	GVBD	5
4/15	GVBD	8
4/29	GVBD	2
5/13	GVBD	3
5/13	GVBD	4
5/13	GVBD	5

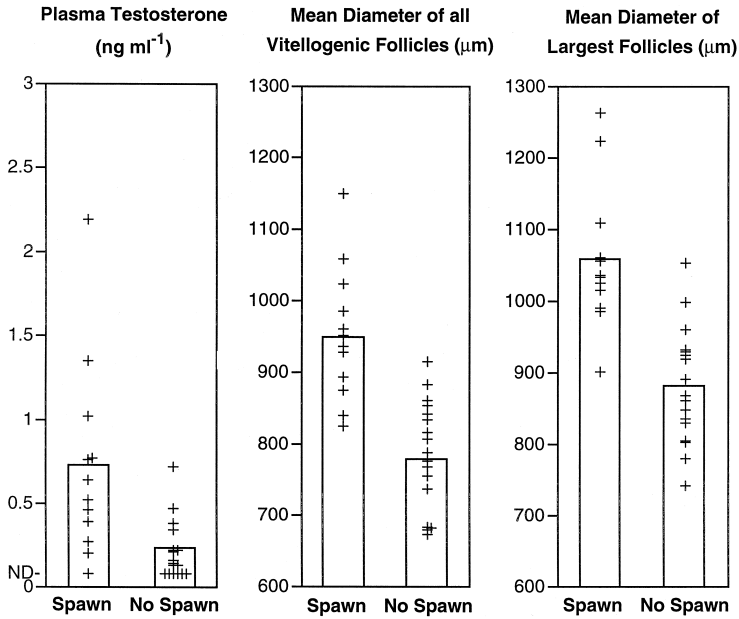

Fig. 1. Mean plasma concentrations of T and diameters of ovarian follicles biopsied from striped bass that spawned ($n = 12$) or did not spawn ($n = 17$) within 8 days of being implanted with GnRHa pellets. Blood was drawn the day the implant was administered. Follicle diameter measurements include diameters of 50 vitellogenic follicles selected at random or 10 of the largest vitellogenic follicles, from biopsy samples taken the day the implants were administered. Crosses (+) indicate values for individual fish. ND denotes detectable limits of the T assay ($P \leq 0.005$, ANOVA). Mean ± SEM values are shown in the text.

spawn. Regarding the homogeneity of follicle size as indicated by standard deviation, and follicle growth, there were no significant differences between fish that ovulated versus those that failed to ovulate within 8 days. Standard deviations of follicle diameters when 50 randomly selected vitellogenic follicles were measured were 94 ± 9 μm for fish that spawned and 80 ± 5 μm for fish that failed to spawn ($P \geq 0.16$). Changes in follicle diameter over a 2-week period when 50 randomly selected vitellogenic follicles were measured were 49 ± 10 and 26 ± 12 μm for fish that spawned and fish that did not spawn, respectively ($P \geq 0.24$). The changes in diameter were 73 ± 11 μm for fish that spawned and 39 ± 12 μm for fish that failed to spawn when only 10 of the largest follicles were measured ($P \leq 0.09$). Follicle diameter decreased in six fish based on the measurements of 10 of the largest follicles, and all of these fish failed to ovulate. Three of these were among the final group of fish to be implanted with GnRHa.

3.1.4. Plasma steroid and VTG concentrations

Plasma T concentration was greater in fish that ovulated than for fish that did not ovulate within 8 days of hormone treatment (spawned: 0.72 ± 0.17 ng ml^{-1}; no spawn: 0.21 ± 0.4 ng ml^{-1}; $P \leq 0.0032$; Fig. 1). Despite the significant difference in means,

there was much overlap of individual values for this comparison. There were no significant differences between fish that ovulated and those that failed to ovulate within 8 days in plasma levels of E_2 (spawned: 1.10 ± 0.20 ng ml^{-1}; no spawn: 0.66 ± 0.17 ng ml^{-1}; $P \geq 0.08$) or VTG (spawned: 1.25 ± 1.15 mg ml^{-1}; no spawn: 1.11 ± 0.25 mg ml^{-1}; $P \geq 0.60$).

3.2. Experiment 2

FOM was induced in vivo by GnRHa implants in two of four fish at each of the two commercial farms. Only oocytes from biopsy samples of the fish that initiated FOM in vivo completed GVBD in vitro in response to the IGF-I/20β-S combination (Fig. 2). Maturation was not induced in vitro in biopsy samples from any fish that did not initiate FOM in vivo. In vitro exposure to hCG induced maturation in oocytes from only one fish. Early GVM and partial coalescence of oil droplets in the ooplasm was induced in 5% of the oocytes from the biopsy sample from this fish and no oocytes completed GVBD.

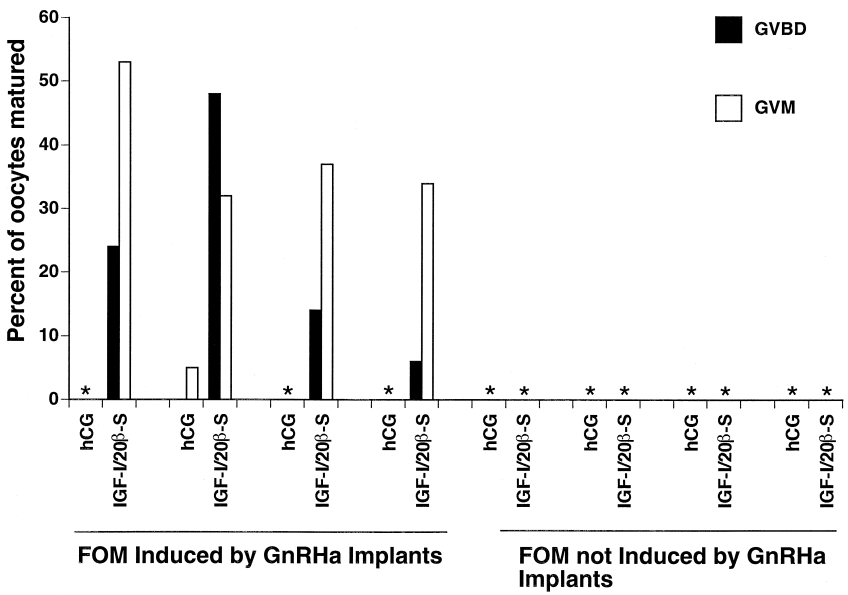

Fig. 2. Farm trials of in vitro assay to predict ovulatory success. Shown is the response of ovarian follicles obtained by biopsy, to hormone treatment in vitro and response of the females from which the biopsies were taken, to GnRHa implants for induced spawning. Ovarian follicles were biopsied from the fish and incubated for 3–4 days in the presence of a combination of IGF-I (100 nM) and 20β-S (290 nM) or hCG (25 IU/ml). Vertical bars indicate the percentage of oocytes completing GVBD (black bars) or GVM (white bars) for individual fish. Asterisks denote cultures in which no oocytes matured. Following biopsy, the females were implanted with GnRHa pellets to induce spawning. Horizontal lines and their legend indicate fish that ovulated and spawned versus those that failed to complete FOM.

4. Discussion

The in vitro response of follicles from biopsy samples to IGF-I and 20β-S served as the most reliable predictor of ovulatory success in our laboratory trials. The in vitro response predicted the in vivo response in 95% of the 20 fish tested. The feasibility of using the in vitro test in a commercial setting was confirmed by our field trials. Four of eight fish ovulated in vivo and biopsy samples from these same four fish were the only samples containing oocytes that completed GVBD in vitro. Microbial contamination was not observed when conducting the in vitro assay in the commercial hatcheries, despite incubating samples out in the open on a bench. Successful execution of the in vitro assay on the farm is not surprising because the farmers routinely conduct most of the procedures required for the assay. They biopsy fish to measure follicle diameters as a means for selecting females for spawning using the same tools and methods we used to collect samples for the in vitro assay. The farmers also monitor follicles for maturation (FOM) as a criterion for decisions about when to spawn the fish. Doing a biopsy on the fish does not prevent the fish from responding to hormone treatment even if the fish are not selected for spawning until days or weeks later (Sullivan et al., 1997). Although signs of microbial contamination were not observed in cultures conducted at the farm, we expect a minimum of training in sterile techniques would be beneficial to the farmers.

There should be no restrictions for using the technique because the IGF-I and 20β-S do not come in contact with the whole animals, eliminating regulatory concerns, and both hormones are commercially available. The addition of 20β-S to the medium is probably not even necessary for the in vitro assay. Subsequent to the initiation of these studies, we have found that IGF-I acts via a steroid-independent pathway in striped bass (Weber et al., 1997).

Potential drawbacks to the in vitro test of maturational competency are the time required for response and potential contamination by microbial agents. The concentration of IGF-I used in the assay was derived from studies on reproductive physiology in which 100-nM IGF-I was the maximum concentration examined (Weber et al., 1997). The time required for response may be decreased with increased concentrations of IGF-I or the use of various IGF-I analogues. The oocytes of most fish that completed FOM in vitro also rapidly completed GVM and oil droplet coalescence in their ooplasm. Although we used GVBD as a marker for maturation in our study, it may be possible to use the completion of oil droplet coalescence or GVM as end points. Studies testing these end points are planned. The anti-fungal agent, amphotericin B (2.5 $\mu g\ ml^{-1}$) is also now routinely added to our culture medium to reduce the incidence of microbial contamination.

The simple procedure of measuring follicle diameter of 10 of the largest follicles appears to serve as a good predictor of ovulatory response based on the separation of values for fish that spawned and those that did not. In our study, 1000 μm would serve as a good demarcating size. However, this size is selected a posteriori, and thus, potential success in discriminating between females competent to respond to GnRHa versus those not competent to respond is artificially inflated. Female striped bass broodstock may become maturationally competent or atretic over a different range of

mean follicle diameters than observed in our study, dependent upon factors such as environmental conditions and genetic stock. As an example, Mylonas et al. (1997b) reported the mean maximum diameter of the oocytes in their broodstock to be only 838 μm, based on measuring the diameter of 10 of the largest oocytes. Thus, a priori determination of a follicle size for selection of spawners would not be expected to result in the same degree of success predicting maturation as our a posteriori results. Another problem with follicle size as an index of maturity for the selection of spawners is not apparent from our data because fish whose oocytes were already atretic when first examined were not included in the study. Eight of the original 40 fish had oocytes that underwent atresia before their mean follicle diameter reached 1000 μm, the size at which they would be expected to mature fully in response to GnRHa. Loss of fish to atresia before they become maturationally competent and can be spawned has previously been identified as a major deficiency to using oocyte diameter as a method for selecting females for spawning (Hodson and Sullivan, 1993). The primary advantage of the in vitro bioassay over using follicle diameter is the ability to identify fish as being ready for induced spawning early enough to avoid losing them to preovulatory atresia.

The other morpho-physiological parameters examined do not appear to be beneficial for selecting female striped bass for spawning. Follicle size homogeneity was similar between fish that spawned and those that did not spawn. There was a high degree of size heterogeneity for both groups as indicated by the standard deviations for individual fish. Induced spawning of curimbata (*Prochilodus scrofa*) and milkfish (*Chanos chanos*) is less successful for fish with a high degree of follicle size heterogeneity owing to the bi-modal size frequency distributions (Fenerich-Verani et al., 1984; Lee et al., 1986). However, the size frequency distributions for striped bass in the present study all appeared to be unimodal.

All fish showing a decrease in mean diameter of the largest follicles over the 2-week period preceding hormone treatment failed to spawn. However, three of these six fish were from the last group of fish that were implanted with GnRHa on June 10, and one was from the group implanted on May 27. This may suggest that a decrease in maximum follicle diameter better serves as an indicator for the end of spawning season rather than to predict poor ovulatory success.

Ovarian follicles were still growing in the female striped bass within 2 weeks of their becoming competent to respond to GnRHa treatment for induced spawning. Mean follicle growth over the 2-week period prior to hormone treatment for fish that spawned was 49 ± 10 μm. The optimal time for GnRHa treatment in milkfish is within the 5-day period following the end of oocyte growth (Lee et al., 1986). More refined sampling is required to determine whether there is an optimal time during the period of follicle growth for hormone treatment of striped bass, as seen in milkfish. Even if oocyte growth rate can be used to identify an optimal time for GnRHa treatment, repeated biopsy of the fish over very short intervals late in their reproductive cycle would likely cause too much stress to the broodstock to be of practical value to farmers.

In striped bass, T and E_2 levels increase near the end of vitellogenic oocyte growth and early FOM and then decrease concomitant with an increase in 20β-S and 17,20β-dihydroxy-4-pregnen-3-one (a precursor to the MIS, 20β-S) associated with GVBD (Berlinsky and Specker, 1991; Blythe et al., 1994b; King et al., 1994a; Mylonas et al.,

1997a, 1998). Plasma levels of T and E_2 measured in this study are consistent with the patterns and concentrations reported in the prior studies. King et al. (1994a) report that T and E_2 reach maximum levels when completion of oil droplet coalescence and GVM are seen in the oocytes. The wide range of plasma T values among fish that spawned suggests fish are responsive to GnRHa treatment throughout most of the period when T is increasing. Although the mean E_2 concentration of the fish that spawned was 1.10 ng ml^{-1} compared to 0.66 ng ml^{-1} for fish that did not spawn, the difference was not statistically significant. VTG levels in the blood were similar for fish that did and did not spawn. These findings are consistent with oocytes continuing to grow in the fish during the 2-week period preceding the induced spawning trials. The results suggest that, although there is a difference in mean T concentrations for fish that did and did not spawn, there is too much overlap among individuals to make this analysis a valuable tool for farmers. In addition, there are practical difficulties involved in farmers conducting RIAs.

In summary, the in vitro response of follicles to IGF-I plus 20β-S functions as a good predictor for ovulatory success in striped bass. The technique relies mostly on skills the farmers already practice and should therefore be easy to implement on the farm. The use of this technique should result in more efficient use of broodstock because fewer females will be lost to preovulatory atresia or treated with hormones too early for them to successfully complete FOM, ovulate and spawn. The in vitro test for maturational competence may also find application to other species that must be induced to spawn before morphological signs of FOM are evident.

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