INTRODUCTION

Black Sea Bass

The black sea bass, *Centropristis striata,* is a commercially and recreationally important marine finfish species (family Serranidae) found from the Gulf of Maine to Florida (Waltz et al. 1979; Berlinsky et al. 2000) in water 10 to 120 m deep (Kendall 1977). Juveniles often live in estuaries (Tucker 1984) and begin a seasonal migration as they mature, living inshore among hard substrates and traveling offshore to spawn in the summer months (Kendall 1977). They can survive salinities of 10 - 35 ppt and in water temperatures of 5 – 30 °C. However, based on the observed feeding pattern in black sea bass, water temperatures in the range of 18 – 22 °C are preferred (Berlinsky et al. 2000).

Black sea bass are a desirable catch by commercial and recreational fishermen, therefore they are being affected by overfishing (Waltz et al. 1979; Able et al. 1995; Vaughan et al. 1995; Jackson et al. 2001). Pollution and habitat degradation also play a role in the declining number of black sea bass (Tucker 2003). Annual commercial landings were approximately 9,900 metric tons (mt) in 1952 and have decreased since then so that catch has been between 1,000 and 2,000 mt since the mid 1960's (Kendall 1977; Shepherd and Terceiro 1994). Recreational black sea bass landings in 1982 were 8,100 mt but are usually 1,000-2,000 mt annually (US Department of Commerce 1991; Shepherd and Terceiro 1994). They are hardy fish that adapt quickly to captivity, are tolerant of temporary poor water quality, have a high growth rate, and a high market value (Copeland et al. 2002; Copeland et al. 2003; Tucker 2003). Black sea bass are especially profitable in the restaurant and ethnic live fish trade (Berlinsky et al. 2000). They are estimated to grow three times quicker in captivity than in the wild (Copeland et

al. 2002; Copeland et al. 2003). For these reasons, black sea bass are economically attractive candidates for aquaculture. Reliable methods for controlled spawning and production of high quality eggs and juveniles are needed to accelerate development of hatchery and growout technologies for this new species (Lee and Ostrowski 2001).

Reproductive Biology

Black sea bass are protogynous hermaphrodites, changing sex from female to male at approximately 3 - 4 years of age (Waltz et al. 1979; Tucker 1984; Shepherd and Idoine 1993; Sherwood et al. 1993; Berlinsky et al. 2000; Tucker 2003) or 8 - 35 cm (Shepherd and Idoine 1993). Females usually mature by two years of age (Wenner et al. 1986). The optimal sex ratio has not yet been determined but, it is thought that if there are not enough male black sea bass in a population, females will switch to males before they would if there was an abundance of males (Shepherd and Idoine 1993). Males and females are collectively kept in tanks to inhibit social factors causing sex change. Too few males in a population could cause females prematurely to switch to males, as Quinitio et al. (1997) saw in grouper, *Epinephelus coioides*. Additional females must be added to the broodstock to replace the females that change to males.

The sex of a black sea bass can be difficult to determine although they are a sexually dimorphic species. They are very similar in appearance until the spawning season when females are often light gray in color and plump in the abdominal region; while males are darker gray, developing fluorescent blue, green, and teal colors on their head and body, their fins become filamentous, and they grow an adipose, nuchal hump on their head (Hoff 1970; Kendall 1977; Mercer 1989).

Black sea bass produce two or more clutches of oocytes (i.e., multiple clutch group-synchronous spawners) (Wallace and Selman 1981) and will spawn multiple times during their spawning season (Watanabe et al. 2003). In waters off the coast of the southeastern United States, spawning occurs in the spring on an increasing photoperiod (Watanabe et al. 2003). Their major natural spawning season is from March to May, with a short season from September to October (Wenner et al. 1986; Mercer 1989). Fecundity increases with the age and size of the fish, with numbers of eggs increasing from 17,000 to 1,050,000 in fish with a standard length of 108 mm and 438 mm, respectively (Wenner et al. 1986; Mercer 1989). Black sea bass, like many marine finfish species, do not usually spawn naturally in captivity. Hormone treatment along with environmental control are therefore used to induce spawning (Berlinsky et al. 2000; Watanabe et al. 2003), but more work is required to standardize methods for reliable spawning success.

Stages of Gonad Development

Environmental stimuli trigger synthesis and secretion of gonadotropin (GtH), a hormone produced by the pituitary gland that stimulates formation of sex steroids, which directly modulate spermatogenesis in males and oogenesis in females (Harvey and Hoar 1979; Bromage et al. 2001). Males are considered ripe when milt is emitted with slight abdominal pressure (Harvey and Hoar 1979). Sperm is released from the testes through the vas deferens where spermiation occurs; liquid is added making milt. Sperm is activated by the hypertonic composition of seawater and the dilution of potassium. Sperm is motile for 60-120 seconds (Billard 1995). Fish can have two million to sixty-

five billion cells per milliliter of sperm. Captivity often does not alter spermatogenesis, so sperm is readily available (Harvey and Hoar 1979; Tucker 1984).

Many changes in the size and morphology of oocytes occur during oogenesis (Wallace and Selman 1981). Oocyte growth occurs in four stages: primary oocyte, yolk vesicle stage, yolk globule stage, and final oocyte maturation (Wallace and Selman 1981). The pre-vitellogenic phase is GtH independent, there is cytoplasmic and nuclear growth of primary oocytes and follicle formation. The vitellogenic stages occur over a period of several weeks to months and are GtH dependent. Both endogenous vitellogenesis (yolk vesicle stage) and exogenous vitellogenesis (yolk globule stage) take place at this time. Eggs enlarge with the formation of yolk, the larval food source until they are able to feed independently (Tamaru et al. 1993). Oogenesis often stops after the yolk globule stage in captive fish, and eggs are not released (Harvey and Hoar 1979). They do not reach final oocyte maturation (FOM), the final stage of oocyte development before spawning (Golovanenko 1975). During FOM, oocytes enlarge as their water content increases (hydration) in preparation for fertilization, the germinal vesicle migrates to the animal pole, and after fertilization the germinal vesicle breaks down (Golovanenko 1975; Wallace and Selman 1981). At the end of FOM, eggs hydrate and are ovulated, then released through the oviduct and genital pore at oviposition or spawning (Wallace and Selman 1981).

The breakdown and reabsorption of oocytes (atresia), occurs in the ovary when oocytes are old, do not undergo FOM, the fish is sufficiently stressed or has inadequate nutrition (Wallace and Selman 1981), or there is a significant change in water temperature (Kjørsvik et al. 1990). Overripe or atretic oocytes are discolored, opaque, do

not have a smooth surface, and are asymmetrical (Kjørsvik et al. 1990). Once oocytes have reached this stage they are no longer able to produce viable larvae.

Spawning coincides with the environmental conditions that give the young the highest chance for survival (Bromage et al. 2001). Mimicking the natural environment during the spawning season is important for spawning in captivity. Control of photoperiod, water temperature, and introduction of hormones are often necessary for successful, reliable spawning (Lam 1985).

Environmental cues (zeitgebers) such as photoperiod, temperature, rainfall, food supplies, and pheromones are possible spawning stimuli (Bromage et al. 2001). Photoperiod is believed to be the most important influence on the seasonal maturation, ovulation, and spawning of fish (Carrillo et al. 1991; Carrillo et al. 1993). Light acts directly on the pineal gland where photoreceptor cells synthesize melatonin in daily cycles with light and darkness (Bromage et al. 2001). Melatonin triggers the brain to send neurotransmitters to the hypothalamus where neurohormones, gonadotropin releasing hormone (GnRH) and gonadotropin release inhibiting factor (GRIF), are produced. GnRH and GRIF travel through neurosecretory cells to the pituitary gland. GtH is produced in the proximal pars distalis section of the adenohypophysis in the pituitary gland. GnRH stimulates the release of GtH while GRIF inhibits the release of GtH (Mylonas and Zohar 2001). GtH is carried in the blood stream to the gonads, ovaries or testes, where sex steroids are formed. Sex hormones in turn stimulate maturation and release of gametes, eggs and sperm. It is also believed that fish have an internal clock, endogenous mechanism which controls reproduction (Bromage et al. 2001).

Hormone-Induced Spawning

In captivity, female fish often complete vitellogenesis, but do not reach FOM and spawn (Mylonas et al. 1995; Mylonas and Zohar 2001). Reproductive dysfunctions may be due to the stress of captivity or a missing environmental spawning trigger (Zohar and Mylonas 2001).

To induce spawning, hormones should be administered as soon as oocytes reach the final stages of vitellogenesis and their mean critical oocyte diameter (Harvey and Hoar 1979), the minimum size at which exogenous hormone treatment will result in final oocyte maturation and spawning. This increases chances of spawning success (Rottmann 1991a). Ripe oocytes are clear, round, and smooth in appearance as a result of hormone treatment (Rottmann et al. 1991b).

Stress can cause a negative impact on a fish's spawning capacity (Mylonas and Zohar 2001). Injury, handling, stress, and poor water quality can reduce spawning success and fish mortality (Marte et al. 1987). Fish are more susceptible to illness and stress during their spawning season (Mylonas and Zohar 2001).

Hormone implantation is widely used to induce spawning, since the fish experience less handling stress compared to the handling necessary for hormone injections, and the hormone is released into circulation slowly, prolonging its effects (Mylonas and Zohar 2001). Hormone-induced spawning techniques must be optimized to minimize fish handling and maximize fish response to hormone treatment (Mylonas and Zohar 2001).

Success of hormone-induced spawning depends on the pattern of gonadal development, hormone type, dose, and release rate. Fish may spawn once or multiple times from hormone introduction (Garcia 1990).

Hormone Type

Two types of synthetic neuropeptide hormones are commonly used for induced spawning in fish due to effectiveness, availability, and price (Mylonas and Zohar 2001). Synthetic compounds reduce the chance of disease transmission, are more potent, and less expensive than natural hormones (Mylonas and Zohar 2001; Zohar and Mylonas 2001). Luteinizing hormone-releasing hormone analogue (LHRH-a), des-Gly^{10}, [D-Ala]-LHRH ethylamide (Sigma Chemical Company, St. Louis, MO, USA), is a mammalian GnRH analog. Gonadotropin releasing hormone analogue (GnRH-a), a commercially produced product known as Ovaplant (Syndel Laboratories, Vancouver BC, Canada), is a salmon based GnRH analog. Two forms of GnRH are produced in the brain of a black sea bass, the dominant type is salmon GnRH-like which is present in most teleosts (Sherwood et al. 1993). LHRH-a and GnRH-a have been found to raise the level of gonadotropic hormones in fish (Sherwood et al. 1988) and can be implanted to induce FOM and spawning (Lee et al. 1986). The duration of the hormone's effects varies with the dose and release rate of the hormone (Crim 1988; Mylonas and Zohar 2001). Using the correct dosage, correct mixing technique of the matrix, and proper storage of the hormone matrix are very important to successful hormone-induced spawning (Rottmann et al. 1991c).

Dose

The hormone dosage used for induced spawning in fish is calculated based on body weight (Harvey and Hoar 1979). The concentration of hormone equals the desired dose times the weight of the fish divided by the desired volume (Rottmann et al. 1991c). Differing doses of LHRH-a have been found to be effective for induced spawning of different fish species, such as 100 - 200 µg/kg in barramundi (*Lates calcarifer* (Bloch)) (Garcia 1990), 9.0 - 23.5 µg/kg in barramundi (Harvey et al. 1985), and 19.2 - 26.3 µg/kg in milkfish (Marte et al. 1987). GnRH-a is effective at doses of 1-100 µg/kg (Zohar and Mylonas 2001). Determining the most effective dose is important for maximizing production of viable eggs and will save the culturist time and money (Mylonas and Zohar 2001).

Release Rate

To control rate of hormone administration, hormones are often administered by implantation of a small, cylindrical pellet containing the hormone. Pellets contain hormone in a cholesterol/cellulose matrix (Crim et al. 1988). The matrix in nontoxic, inexpensive, and keeps the water-soluble hormone from dissolving immediately (Sherwood et al. 1988). Cellulose, a natural product, binds the cholesterol with the hormone. The release rate varies with the cholesterol/cellulose ratio in the pellet (Sherwood et al. 1988), from several days to weeks (Crim et al. 1988). Pellets with 0 - 100 % cellulose have been successfully used in fish to stimulate FOM and spawning (Sherwood et al. 1988). Pellets with a higher cholesterol concentration tend to prolong

the release of hormone, causing elevated plasma hormone levels for a longer period of time (Crim et al. 1988). Pellets are manufactured easily in the hatchery and remain effective for four months at room temperature or longer if frozen (Mylonas and Zohar 2001).

Egg Quality

Egg quality is "the egg's potential to produce viable fry" (Kjørsvik et al. 1990). The success of marine fish larviculture has been limited due to the high variability of egg quality (Kjørsvik et al. 1990). Many factors determine egg quality including physical, genetic, and chemical aspects, initial physiological processes in the egg, and broodstock husbandry and nutrition. Egg quality varies between species, fish, and spawns (Navas et al. 1995).

Egg quality is important to reliably produce healthy juveniles. In most cases, eggs of good quality are buoyant and float, due to their hardened egg membrane, while eggs of inferior quality sink (Al-Marzouk et al. 1995). This is a useful method to separate spawned eggs, keeping the high quality eggs and discarding the bad eggs. High quality eggs have high rates of fertilization, hatching, and survival to the first-feeding stage (Al-Marzouk et al. 1995), when the eyes are pigmented, the mouth opened, the oil droplet disappears, and exogenous feeding begins (Al-Marzouk et al. 1995). The better the quality of eggs the more larvae that hatch and reach the first feeding stage.

The appearance of egg shape and color could indicate poor egg quality if eggs are wrinkled, dark, "plasma-bulged", have asymmetrical cleavage, or have more than one oil droplet (Kjørsvik et al. 1990). Egg size and larvae size are directly related, but do not

seem to affect quality (Kjørsvik et al. 1990). An egg batch with a close size range is usually of better quality than one with a large range of egg sizes (Kjørsvik et al. 1990). The survival potential of fertilized eggs and hatched larvae could decrease with poor egg quality (Kjørsvik et al. 1990).

The ability for fish to mature is affected by the availability of adequate amounts of food (Bromage et al. 2001). Broodstock diets should be based on the nutritional value of the feed to provide the essential nutrients needed to survive and grow (Kjørsvik et al. 1990; Almansa et al. 1999; Kjørsvik et al. 2003; Watanabe and Vassallo-Agius 2003). Live as well as pelleted feed are necessary to provide their essential nutrients. The exact nutrient requirements of marine fish larvae are difficult to determine and differ between species (Fyhn and Serigstad 1987). Mediterranean sea bass (*Dicentrarchus labrax)* broodstock fed local trash fish have been found to have eggs of higher quality than those fed a diet of fish meal with fish and vegetable oils (Bell et al. 1997). Vegetable oils do not contain the amount of essential fatty acids that fish oils do. Red sea bream obtain twice the amount of *n*-3 HUFA from krill than from corn oil (Kjørsvik et al. 1990). The *n*-3 HUFA level is important to marine fish larval survival, growth, and development (Ako et al. 1991; Navas et al. 1997; Rodríguez et al. 1998; Lee and Ostrowski 2001; Furuita et al. 2002). Malnutrition decreases maternal fecundity, egg viability (Fraser et al. 1988), and egg quality (Kjørsvik et al. 1990; Cerda et al. 1991). Understanding the biochemical correlates of egg quality will improve larval survival and broodstock husbandry practices, yielding more viable fry (Kjørsvik et al. 1990; Kjørsvik et al. 2003).

Nutrient requirements vary depending on the gonadal stage of the broodstock (Navas et al. 1995). Broodstock diet before and during gonadogenesis affects

reproductive performance, egg and larval quality, viability of larvae (Fraser et al. 1988; Bell et al. 1997; Ogata et al. 2003), immune function, and disease resistance (Lall 2000) by supplying the nutrients needed for larval survival and development. Lipid stores in fish are believed to be a reliable marker of nutritional status (Bromage et al. 2001). Proper lipid nutrition in necessary to prevent fin erosion and skin infections in aquaculture raised fish (Lall 2000). Marine fish eggs are high in polyunsaturated fatty acids (PUFA) (Tocher et al. 1985; Lall 2000), which are important for development and responses from the immune system (Lall 2000). PUFA is essential for metabolic energy, and the formation and function of membranes (Bell et al. 1986; Sargent 1995; Sargent et al. 1999), and embryonic development (Navas et al. 1997). Required essential fatty acids (EFA), eicosapentaenoic acid (EPA), DHA, and ARA, are received from their diet (Navas et al. 1997; Rodríguez et al. 1998; Sargent 1995; Sargent et al. 1999; Lall 2000; Ogata et al. 2003). Marine fish with an elevated amount of EPA, which competes with ARA in the eicosanoid biosynthetic pathway, causing eicosanoid deficiency, which may harm the immune system and increase susceptibility to disease or death (Lall 2000; Copeman et al. 2002). Eicosanoids are communication molecules that regulate ions and induce oocyte release (Lall 2000; Copeman et al. 2002). A deficiency of docosahexaenoic acid (DHA) could cause vision impairment in fish (Bell et al. 1997; Furuita et al. 1998). The correct amount and ratio of DHA/ EPA and ARA / EPA is important for proper vision, neural development and functioning in larvae (Bell et al. 1997; Navas et al. 1997; Furuita et al. 1998; Copeman et al. 2002; Watanabe and Vassallo-Agius 2003). The DHA/ EPA ratio in their diet should be ~ 2:1 (Sargent 1995; Sargent et al. 1999). The ratio of *n*-6/ *n*-3 fatty acids (10/1 to 15/1) (Robin 1995), (5/1 –

10/1) (Sargent 1995), or (~5/1) (Sargent et al. 1999) is important for the success and correct time of ovulation and spawning.

Lipids can be extracted and isolated from egg samples in preparation for a FAME reaction and GC-MS (gas chromatography - mass spectrometry) and GC-FID (gas chromatography - flame ionization detection) analysis to compare saturated (SFA), mono-unsaturated (MUFA), PUFA, *n*-3, *n*-6 fatty acids, and individual fatty acids. The FAME reaction converts the polar carboxylic acids in fatty acid samples into a more volatile, relatively nonpolar, methyl ester that is separated better by GC (Furuita et al. 2002). The FAMEs in an egg sample are separated and identified by GC-MS by their retention times and their mass spectra. Fatty acids are identified by comparing their retention times to those of standards (Furuita et al. 2002).

This thesis reports the results of induced volitional spawning trials conducted from 2002-2003 at The University of North Carolina at Wilmington (UNCW) evaluating the efficacy of luteinizing hormone releasing hormone analogue (LHRH-a), administered in slow and fast release (cholesterol-cellulose matrix) implants (Sherwood et al. 1988; Lee et al. 1986; Watanabe et al. 2003), and gonadotropin releasing hormone analogue (GnRH-a) slow release implants as spawning agents in black sea bass. Eggs produced under different hormone treatments were also analyzed for fatty acid content to investigate biochemical determinants of egg quality.

Objectives

I) To determine the effectiveness of LHRH-a and GnRH-a as induced spawning agents in black sea bass.

A) Does spawning success vary with dose of LHRH-a?

Null: LHRH-a dose does not affect spawning success.

B) Is spawning success affected by the type of hormone administered?

Null: The type of hormone administered (LHRH-a vs. GnRH-a) does not affect spawning success.

II) To determine the fatty acid profiles of volitionally spawned eggs from wild-caught captive black sea bass under hormone-induced FOM and ovulation.

A) Does the fatty acid composition vary in eggs of different quality measured by hatching success?

Null: Fatty acid content does not vary in eggs of different qualities.

METHODS

Experimental Animals

This study was conducted at the University of North Carolina at Wilmington, Center for Marine Science (UNCW-CMS) Aquaculture Facility, Wrightsville Beach, North Carolina, USA, from March 2002 through September 2003. Adult black sea bass (mean wt. = 0.927 kg; range = 0.320-1.93 kg) were captured by a commercial fisherman in traps set at a depth of 8-40 m off Carolina Beach in November 2000 (Copeland et al. 2003; Watanabe et al. 2003). The fish grew to a mean of 1.82 kg (range = 0.606 - 3.74 kg) between the 2002 and 2003 spawning seasons.

Experimental System

The black sea bass were held outdoors in six covered round tanks (diam.= 1.83 m, depth = 0.81 m, volume = 2,134 L). Fiberglass tanks were insulated and were supplied with recirculating seawater (32 - 35 g/L) pumped from Banks Channel, Wrightsville Beach. The recirculating system (Fig. 1) consisted of an external bead filter to remove solids, a protein skimmer to remove dissolved organic material, and an ultraviolet sterilizer to kill pathogens. Conical, fiberglass, light-proof covers with automatic, timer-controlled fluorescent light fixtures maintained the desired photoperiod in the tanks and a light intensity of ~ 280 lux at the water surface. The daily water exchange in the system was approximately 25 %. Water temperature, dissolved oxygen, pH, and salinity were monitored daily. Ammonia, nitrate, and nitrite concentrations were measured weekly to determine water quality.

A retractable cover on each tank was left slightly ajar to allow for a normal light regime until spawning commenced when the photoperiod was maintained under constant spring conditions (13L: 11D). The temperature was held at 19 °C with the use of a heat pump to prolong spawning (Berlinsky et al. 2000). Suspending the spring light and temperature pattern lengthened the spawning season in black sea bass (Watanabe et al. unpublished data). The salinity was held between 34 – 35 ppt.

Two tanks were used for holding broodstock and four tanks for spawning experiments (Fig. 1). Cylindrocone egg collectors were located next to the four spawning tanks to receive tank effluent and collect spawned eggs (Fig. 1). Each collector had a 250 μm mesh standpipe, to retain eggs, and sufficient aeration to keep eggs suspended

(Harvey and Hoar 1979). The six tanks were kept at the same light, temperature, and salinity conditions.

The number of broodfish held in the holding tanks varied during the spawning season and ranged from 20 - 95. Females selected for spawning experiments were removed from the holding tanks and were placed in spawning tanks. After spawning, females were placed into another system. The broodstock were fed daily to satiation (until fish stop feeding) on a diet of dry (7 mm) floating commercially prepared pellets (50 % protein, 12 % lipid) (Melick, Catawissa, PA), Atlantic silversides (*Menidia menidia*), smelt, squid, and krill. Different types of feed were alternated daily to vary available nutrients. Fish were observed during feeding for changes in appetite or behavior or signs of illness. Uneaten feed and wastes were siphoned out of the tanks on a daily basis.

Hormone-Induced Spawning

The black sea bass were measured for total body weight, standard body length, and total body length; and the fish were individually tagged and sexed before the experiment began. Fish were anesthetized with tricaine methanesulfonate (100 ppm, MS-222) (Harvey and Hoar 1979; Copeland et al. 2003), before they were measured and tagged.

Fish were checked for milt and, if none was expressed, they were examined for the presence of oocytes. In mature males, milt was easily expressed when the ventral side of the fish was gently massaged from anterior to posterior (Harvey and Hoar 1979). In mature females, gonadal biopsy was used to determine stage of oocyte development

(Harvey et al. 1985). Oocytes were sampled through a polyethylene catheter (1.52 mm o.d., 0.86 mm i.d.) inserted through the genital opening while gentle suction was applied (Rottmann et al. 1991b). These methods of sex differentiation are only successful when the gonads are mature during spawning season. Handling of the black sea bass was gentle and kept to a minimum to reduce the likelihood of stress-triggered resorption of oocytes (Rottmann 1991a).

During spawning experiments, five males were placed into the spawning tank with each female after hormone treatment to encourage volitional spawning (Lee et al. 1988). Running males produced copious amounts of highly motile sperm were chosen for spawning experiments. Motility was determined by placing a drop of sperm of a glass slide, activating with a drop of seawater, and mobility was measured on a global scale of 0-5 (0 = no movement and 5 = very vigorous movement). Females with a mean oocyte diameter of ≥ 0.40 mm (Tucker 1984) were selected for spawning experiments, oocytes were measured to the nearest 0.05 mm with a compound microscope fitted with an ocular micrometer.

Experimental Design

Pellets containing LHRH-a (Sigma Chemical Co., St. Louis, MO) were produced by mixing a prescribed ratio of cellulose, cholesterol, and hormone together creating the desired dosage and hormone release rate (Sherwood et al. 1988). Slow release pellets consisted of 95 % cholesterol and 5 % cellulose, while fast-release pellets had 80 % cholesterol and 20 % cellulose. Slow release pellets raise hormone levels in fish plasma for a period of eight weeks, whereas fast release pellets raise hormone plasma levels for a

period of eight days (Zohar and Mylonas 2001). Twenty percent of the hormone in a slow release pellet is released during the first 24 hours in vitro versus 90 % in fast release implants (Mylonas and Zohar 2001).

The mixture was combined by slowly stirring the cellulose and cholesterol with just enough solvent, 50 % ethanol / 50 % tap water, to make it a liquid. The mixture was dried in a 30 °C oven until no moisture was left, for approximately an hour. When completely dry, the matrix was pressed into pellets. Each pellet was weighed and stored in a separate labeled plastic test tube and kept in the freezer until used.

To compare the effect of LHRH-a dose and release rate on spawning success, female black sea bass were treated at doses of 100 µg/kg body wt. slow (L-100-slow), 50 µg/kg slow (L-50-slow), 50 µg/kg fast (L-50-fast), and 5 µg/kg slow (L-5-slow) with a 0 µg/kg placebo control. The control contained the 95 % cholesterol, 5 % cellulose matrix without the hormone. Different sized pellets were used to account for the difference in fish body weight. The GnRH-a slow release pellets (G-slow) (Ovaplant) (Syndel Laboratories Ltd., Vancouver, BC, Canada) come in a cartridge at only one size. Ovaplant, slow release pellets containing greater than 95 % cholesterol and were purchased as 75 µg and 150 µg pellets. The dose the fish received varied from 49 - 114 µg/kg.

To compare hormone type, response to L-50-slow and L-100-slow were compared to females treated with GnRH-a. Trials were alternated between the types of hormone, and doses, or control, to minimize seasonal influences on spawning success. Fish were anesthetized and pellets were inserted through a small scalpel incision made in the fish's dorsal musculature.

Individual vs. Group Spawning

Group spawning, when females were spawned together, was also examined to compensate for the low fecundity of individual fish. Groups consisted of 6 - 10 males, and 2 - 6 females implanted with pelleted LHRH-a concurrently and held in a common spawning tank. Individual spawning was compared to group spawning using a dose of L-50-slow.

Egg Collection

After hormone treatment, broodstock spawned volitionally, without the need for strip spawning. Egg collectors were monitored daily and spawned eggs were siphoned into a 500 μm mesh bag and transportation into the lab for data collection (Appendix A). Eggs were transferred into a 15 L hatching cone with filtered seawater to allow the floating eggs to separate from the sinking eggs.

In 2002, the eggs separated in the hatching cone into "floaters", good eggs, and "sinkers", inferior quality eggs, but the 2003 spawning season proved different, with eggs rarely floating, and "sinkers" often fertilized and of good quality. In both cases, eggs were drained from the hatching cone into graduated cylinders to volumetrically quantify the eggs (Al-Marzouk et al. 1995). To estimate numbers of eggs volumetrically (Tamaru et al. 1993), the number of eggs were counted in three 1 mL samples of eggs giving a mean and standard error of 1,345 ± 41.6 eggs/ mL. Following a spawn, a sample of at least one hundred eggs was examined under a dissecting scope to determine the fertilization rate (fertilized eggs/ number of eggs sampled) and the predominant stage of

embryonic development (Fig. 2). A sample of eggs, approximately 1 mL, was stored with 5 % formalin and seawater in a plastic test tube for diameter measurements.

To determine egg fatty acid composition, 1 - 2 mL of eggs were placed on a 23 μm mesh sieve and blotted with paper towels to remove excess water, then placed in an airtight glass vial with gaseous nitrogen, and frozen for fatty acid analysis (Al-Marzouk et al. 1995). Eggs were stored under nitrogen gas at a temperature of –80 °C to prevent the oxidation and deterioration of the eggs before they were analyzed.

Egg Quality

To monitor egg quality, eggs were stocked at approximately 179 eggs/ L (~ 2 mL) into two 15-L incubation tanks. Incubators were supplied with flow through, UV-treated seawater, and diffused aeration. Eggs were incubated under a 24 L photoperiod and a light intensity of 40 - 60 lux and a temperature of 19 °C.

To determine spawning success under different induced spawning treatments, a number of parameters were monitored. These included fertilization success, number of oil droplets, mean egg diameter, hatching success, number of yolksac larvae, survival to first-feeding, and number of larvae surviving to the first feeding stage.

Fertilization success was determined as the percentage of eggs undergoing embryonic development, at the time of collection. A sample of eggs was preserved in 5 % formalin in seawater. Mean egg diameter was measured before formalin was added, immediately after formalin was added, one day later, and a month and a half later to determine egg size changes during preservation. To measure egg diameters, a minimum

of one hundred eggs from each sample were placed in a grooved microscope slide and measured under a compound microscope with an ocular micrometer.

The number of oil droplet(s) per egg were counted in at least 100 eggs per spawn. The number of eggs with more than one oil droplet (i.e., multiple oil droplets) were recorded as a percentage.

Larvae usually hatched 2 - 3 days post-fertilization (pf) at 19 °C (Fig. 2). The number of larvae present were estimated using volumetric methods. Eggs were quantified in a graduated cylinder and then multiplied by the number of eggs/ mL. Hatching success was expressed as the percentage of hatched larvae to the total number of eggs incubated. The larvae were incubated for an additional 2 - 3 days until they reached the first feeding stage, approximately 4 - 5 days pf at 19 °C (Fig. 2), when the eyes are pigmented, the mouth opened, oil droplet disappears, and exogenous feeding begins (Al-Marzouk et al. 1995). Survival to first feeding was expressed as a percentage of first feeding larvae to the total number of eggs incubated.

Fatty Acid Analysis

To compare fatty acid composition in eggs of low and high quality, eggs produced from one hormone treatment (L-50-slow) were used. Egg batches with < 20 % hatching success were designated as "low quality", while those with > 20 % hatching success were designated as "high quality". Three egg samples (1 - 2 mL) from low quality and high quality spawns were obtained and each had three replicates analyzed for fatty acid content (Appendix C). Triplicate egg samples from each spawn were placed into separate

pre-weighed glass vials to determine wet weight. A solution of 1:1 chloroform: methanol (2 mL) and nitrogen gas were added to each vial before storing at –25 °C until extraction.

Lipid was extracted from the egg sample using a handheld homogenizer and an ultrasonicator with a solution of 1:1 chloroform: methanol. The extract was filtered through a glass filter funnel into a round bottom flask to remove cell debris. The sample was concentrated and then 1:1 chloroform: methanol was added. This solution was filtered through a Kimwipe plugged pipet into a pre-weighed round bottom flask. Solvent was evaporated, and the sample was weighed to obtain the percent lipid. The sample was re-dissolved 1:1 chloroform: methanol (1 mL) and transferred to a conical vial, with stirring magnet, for the fatty acid methyl ester (FAME) reaction.

FAME Reaction

A FAME reaction was done to prepare the sample for GC-MS analysis (Appendix C). The FAME reaction consisted of adding 1 mL of 0.5 M NaOH/ MeOH to the sample and heating for an hour, then adding 1.5 mL of boron trifluoride-methanol (BF_3) complex and heating for 30 min. The mixture was allowed to cool before saturated aqueous NaCl, and hexane were added. The organic layer was separated from the aqueous layer and was removed with a glass pipet. The aqueous layer was re-extracted with hexane and then with 20 % ether/ hexane. The organic layers were filtered through a silica filled pipet into a round bottom flask. The solution was concentrated and transferred to a GC vial with chloroform. Samples were refrigerated in airtight GC vials, under nitrogen, to prevent oxidation and deterioration. The fatty acid composition of the egg samples were

analyzed using GC-MS (column DB1, HP GC 5890 MS 5971) and GC-FID (HP GC FID 5890).

Individual FAMEs were first identified by GC-MS by comparison of retention times and mass spectra to standards, GLC-84 (Nu-Chek Prep). FAMEs from all samples were then identified and quantified using GC-FID. The GC-FID consisted of an auto-sampler, 2 μL injection volume, helium carrier gas, and flame ionization detector (FID). The temperature profile consisted of an initial temperature of 210 °C, which was held for 14 min, followed by a 10 °C/ min ramp to 260 °C, which was held for 5 min, resulting in a total analysis time of 24 min. Standards were routinely analyzed by GC-FID to determine the retention times of individual fatty acids.

Statistics

Data on spawning performance and egg and larval quality parameters were expressed statistically as treatment means and analyzed using an ANOVA. Levene's test was done to check the variences for homogeneity. The Tukey multiple comparison test was applied to compare means. Significance level was set at $P < 0.05$. Counted data were log or square root transformed and percent data were arcsine transformed in order to meet the assumptions of ANOVA. Parameters that did not meet the assumptions of ANOVA were compared non-parametrically by the Kruskal-Wallis test. Lipid regression analysis was done for lipid composition, DHA, EPA, ARA, *n*-3, *n*-6, DHA/ EPA, ARA/ EPA, *n*-6/ *n*-3, and *n*-3/ *n*-6 on percentage of fertilized eggs and hatching success.

RESULTS

Hormone-Induced Spawning

The number of hormone-induced spawning trials for each treatment ranged from *N* = 21 for L-50-slow to *N* = 6 in L-50-fast and G-slow (Table 1). A total of 17 control females were implanted with placebo implants containing no hormone (Table 1).

Volitional spawning was observed in all treatments approximately two days (range = 2 – 6 d) post-implantation. Total number of spawns was greatest in L-50-slow (60), and L-100-slow (50), intermediate in L-5-slow, G-slow (20), and L-50-fast (19), and lowest in the control (8) (Table 1).

The percentage of females that spawned following hormone treatment was highest in L-50-fast and L-100-slow (100 %), and L-50-slow (95.0 %), intermediate in L-5-slow and G-slow (83.3 %), and lowest in the control (41.2 %) (Table 1).

The total number of spawns per female was greatest in L-100-slow (5.56), intermediate in L-50-slow (2.86), L-50-fast (3.17), and G-slow (3.33), and lowest in L-5-slow (1.67), and the control (0.471) (Table 1). All hormone treatments produced a significantly ($P < 0.05$) greater number of spawns per female than the control (Table 1). The total number of spawns per female was significantly ($P < 0.05$) greater in L-100-slow than in L-5-slow and the control (Table 1).

L-100-slow was the only treatment associated with mortalities. Two of the nine females (22 %) implanted with this treatment became egg-bound and died during the spawning period (Table 1).

Egg Production

The mean number of eggs spawned per female (x 10^3) was greatest in L-100-slow (584), intermediate in L-50-slow (197), L-50-fast (223), and G-slow (221), and lowest in L-5-slow (72.0), and in the control (10.2) (Fig. 3, Table 2). The number of eggs spawned per female was significantly ($P < 0.05$) higher in L-50-slow, L-50-fast, L-100-slow, and G-slow than the control (Fig. 3, Table 2). L-100-slow was significantly ($P < 0.05$) higher than in the control, L-5-slow, L-50-slow, and G-slow (Fig. 3, Table 2).

The mean number of eggs spawned per kg female body wt (x 10^3) followed a similar pattern and was greatest in L-100-slow (382), intermediate in L-50-slow (121), L-50-fast (160), and G-slow (170), and lowest in L-5-slow (85.3) and the control (9.40) (Fig. 4, Table 2). Numbers of eggs spawned per kg female body wt was significantly ($P < 0.05$) greater in L-50-slow, L-50-fast, L-100-slow, and G-slow than in the control (Fig. 4, Table 2). The number of eggs spawned per kg female body wt was significantly ($P < 0.05$) greater in L-100-slow than in the control, L-5-slow, and L-50-slow (Fig. 4, Table 2). The L-100-slow treatment produced the most eggs per female and per kg female body wt.

Fertilization Success

Mean fertilization rates were greatest in L-5-slow (41.6 %), intermediate in L-50-slow (38.0 %), L-50-fast (31.9 %), and L-100-slow (37.7 %), and lowest in G-slow (28.0 %), and the control (22.5 %) (Fig. 5, Table 3). However, there were no significant ($P > 0.05$) differences among treatments. Except for the control and L-5-slow treatment, most

Table 2. No. of eggs produced per female and per kg body wt (x10^3) under different hormone treatments. Letter indicates hormone (L = LHRH-a, G = GnRH-a), number indicates dose (µg/kg), and word indicates release rate (slow or fast). Values represent means ± standard error (N = 6 - 21), while numbers in parentheses denote ranges. Mean values in a column followed by a different letter are significantly ($P < 0.05$) different.

Treatment	N	No. eggs produced (x 10^3) per female	per kg body wt
Control	17	10.2 ± 4.22 c (0-65.9)	9.40 ± 4.35 c (0-72.4)
L-5-slow	12	72.0 ± 38.5 bc (0-476)	85.3 ± 48.9 bc (0-605)
L-50-slow	21	197 ± 49.0 b (0-861)	121 ± 27.0 bc (0-420)
L-50-fast	6	223 ± 57.5 ab (17.5-356)	160 ± 45.9 ab (13.0-306)
L-100-slow	9	584 ± 152 a (69.9-1360)	382 ± 81.9 a (42.0-744)
G-slow	6	221 ± 100 b (0-570)	170 ± 79.4 ab (0-427)

Table 3. Egg viability under different hormone treatments, including fertilization success (%), hatching success (%), and survival to first-feeding (%). Data are expressed as a percentage of total eggs spawned under different hormone treatments. Letter indicates hormone (L = LHRH-a, G = GnRH-a), number indicates dose (µg/kg), and word indicates release rate (slow or fast). Values represent means ± standard error (*N* = 6 - 21), while numbers in parentheses denote ranges. Mean values in a column followed by a different letter are significantly ($P < 0.05$) different.

Treatment	N	Fertilized (%)	Hatch (%)	First-feeding (%)
Control	17	22.5 ± 11.4 (0-76.3)	19.6 ± 11.2 (0-53.0)	4.48 ± 2.13 ab (0-9.05)
L-5-slow	12	41.6 ± 7.20 (4.75-73.7)	21.3 ± 4.12 (3.40-38.4)	7.50 ± 2.17 a (0.972-19.9)
L-50-slow	21	38.0 ± 5.17 (0-100)	15.6 ± 2.33 (0-40.2)	4.20 ± 1.05 ab (0-21.0)
L-50-fast	6	31.9 ± 9.45 (0-100)	31.5 ± 10.3 (0.040-87.4)	3.62 ± 1.77 ab (0.009-15.1)
L-100-slow	9	37.7 ± 5.17 (0-100)	11.9 ± 2.00 (0.737-26.7)	2.63 ± 0.684 ab (0-9.38)
G-slow	6	28.0 ± 7.59 (0-100)	14.7 ± 5.79 (0-48.1)	0.456 ± 0.260 b (0-1.90)

treatments showed a high variability in fertilization success, which ranged from 0 – 100 % (Table 3).

Egg Morphology and Development

Black sea bass egg and larval development at 19 °C are described in Fig. 2 and Table 4. It took approximately 51.2 h (2 d 3 h) for the eggs to hatch, and 129 h (5 d 10 h) for the larvae to reach the first-feeding stage from fertilization.

The percentage of eggs with multiple oil droplets was greatest in the control (21.3), L-50-fast (23.1), and G-slow (25.9), intermediate in L-5-slow (17.9) and L-50-slow (14.6), and lowest in L-100-slow (3.90) (Table 5, Fig. 6). No significant ($P > 0.05$) differences in the percentages of eggs with multiple oil droplets were observed (Table 5, Fig. 6).

Storage and preservation of egg samples in a solution of 5 % formalin in 35 ppt seawater did not affect the size of the eggs. There was no significant ($P > 0.05$) difference in mean egg diameter (mm) for samples held in seawater on day 0 (0.916), compared to those preserved in seawater containing 5 % formalin on day 0 (0.921), day 1 (0.915), and day 46 (0.918) (Fig. 7).

Pre-treatment mean oocyte diameters (mm) were very similar among treatments ranging from 0.450 – 0.488, with no significant ($P < 0.05$) differences (Table 6, Fig. 8). The mean egg diameters (mm) of spawned eggs were very similar in the control (0.967), L-5-slow (0.981), L-50-slow (0.919), L-50-fast (0.929), L-100-slow (0.932), and G-slow (0.933) (Table 6, Fig. 9). Mean spawned egg diameters were significantly ($P < 0.05$)

Table 4. Embryonic development of black sea bass, *Centropristis striata,* at 19 ºC.

Time (h) [a]	(d + h.a.h.)[b]	Stage	Description
0 h 55'		2 cells	first cleavage
1 h 18'		4 cells	second cleavage
1 h 30'		8 cells	third cleavage
1 h 51'		16 cells	fourth cleavage
3 h 21'		32 cells	fifth cleavage
3 h 51'		64 cells	sixth cleavage
5 h 21'		128 cells	seventh cleavage
6 h 56'		blastula	
11 h 6'		gastrula	
14 h 6'		neurula	
19 h 6'		early embryo	Embryonic formation. Organogenesis. Eye visible.
27.1 h		late embryo	C-shaped embryo. Heart beat. Embryonic movement.
52.1 h		hatch	
68.3 h	0 + 16.2	larvae	
76.3 h	1 + 0.200	larvae	
100.6 h	2 + 0.500	larvae	
129.9 h	3 + 5.8	first-feeding larvae	Dark eye pigmentation. Gut and anus visible.

[a] Post-fertilization

[b] Days + hours after hatching

Table 5. Percentage of black sea bass eggs with multiple oil droplets following hormone induced spawning. Data are expressed as a percentage of total eggs spawned. Letter indicates hormone (L = LHRH-a, G = GnRH-a), number indicates dose (µg/kg), and word indicates release rate (slow or fast). Values represent means ± standard error (*N* = 6 - 21), while numbers in parentheses denote ranges. No significant ($P > 0.05$) difference among treatments were observed.

Treatment	Multiple oil droplet (%)
Control	21.3 ± 7.73 (0-44.8)
L-5-slow	17.9 ± 8.28 (0-69.6)
L-50-slow	14.6 ± 4.13 (0-100)
L-50-fast	23.1 ± 6.81 (0-90.0)
L-100-slow	3.90 ± 1.27 (0-38.0)
G-slow	25.9 ± 8.45 (0-100)

Table 6. Pre-treatment mean oocyte and mean spawned egg diameter (mm) for black sea bass females under different hormone treatments. Letter indicates hormone (L = LHRH-a, G = GnRH-a), number indicates dose (µg/kg), and word indicates release rate (slow or fast). Values represent means ± standard error (N = 6-21), while numbers in parentheses denote ranges. Mean values in a column followed by a different letter are significantly ($P < 0.05$) different.

Treatment	Oocyte	Egg
Control	0.454 ± 0.00885 (0.339-0.503)	0.967 ± 0.00649 ab (0.951-0.982)
L-5-slow	0.480 ± 0.0107 (0.418-0.523)	0.981 ± 0.00820 a (0.933-1.02)
L-50-slow	0.450 ± 0.00917 (0.338-0.526)	0.919 ± 0.00679 c (0.836-0.990)
L-50-fast	0.473 ± 0.0189 (0.422-0.559)	0.929 ± 0.00989 bc (0.864-1.00)
L-100-slow	0.488 ± 0.0128 (0.451-0.569)	0.932 ± 0.00757 bc (0.859-0.996)
G-slow	0.470 ± 0.0183 (0.421-0.545)	0.933 ± 0.00994 bc (0.865-0.990)

greater in L-5-slow than in the other hormone treatments (Table 6, Fig. 9). L-50-slow was significantly ($P < 0.05$) lower than in the control (Table 6, Fig. 9).

Hatching Success

Hatching success (%) was greatest in L-50-fast (31.5), intermediate in the control (19.6) and L-5-slow (21.3), and lowest in L-50-slow (15.6), L-100-slow (11.9), and G-slow (14.7) (Fig. 10, Table 3). There were no significant ($P > 0.05$) differences among treatments.

Yolksac Stage

The mean number of yolksac stage larvae produced per female (x 10^3) was highest in L-50-fast (35.5), and G-slow (34.3), intermediate in L-50-slow (14.0) and L-100-slow (19.4), and lowest in L-5-slow (5.53) and the control (0.664) (Table 7, Fig. 11). There were no significant ($P > 0.05$) differences among treatments.

The results were similar when expressed as number of yolksac larvae produced per kg female body wt (x 10^3); greatest in L-50-fast (23.4) and G-slow (28.0), intermediate in L-5-slow (5.82), L-50-slow (8.76), and L-100-slow (12.7), and lowest in the control (0.068) (Table 7, Fig. 12). There were no significant ($P > 0.05$) differences among treatments.

Survival to First-Feeding

Survival to first-feeding (%) was highest in L-5-slow (7.50), intermediate in the control (4.48), L-50-slow (4.20), L-50-fast (3.62), and L-100-slow (2.63), and lowest in

Table 7. No. of yolksac larvae produced per female and per kg body wt (x10^3) under different hormone treatments. Letter indicates hormone (L = LHRH-a, G = GnRH-a), number indicates dose (µg/kg), and word indicates release rate (slow or fast). Values represent means ± standard error for trials (*N* = 6 - 21), while numbers in parentheses denote ranges. No significant (P > 0.05) differences among treatments were observed.

Treatment	N	No. yolksac larvae produced (x 10^3) per female	per kg body wt
Control	17	0.664 ± 0.392 (0-6.42)	0.680 ± 0.411 (0-6.79)
L-5-slow	12	5.53 ± 2.76 (0-26.9)	5.82 ± 2.87 (0-28.4)
L-50-slow	21	14.0 ± 5.75 (0-111)	8.76 ± 0.336 (0-63.1)
L-50-fast	6	35.5 ± 24.8 (0-149)	23.4 ± 16.7 (0-101)
L-100-slow	9	19.4 ± 9.56 (0-80.0)	12.7 ± 6.49 (0-58.2)
G-slow	6	34.3 ± 22.9 (0-136)	28.0 ± 19.4 (0-117)

G-slow (0.456) (Fig. 13, Table 3). There were no significant ($P < 0.05$) differences among treatments.

First-Feeding Stage

The mean numbers of first-feeding stage larvae produced per female (x 10^3) were highest in L-50-fast (4.16), and L-100-slow (4.42), intermediate in L-5-slow (2.09), and L-50-slow (2.06), and lowest in G-slow (0.711) and the control (0.151) (Fig. 14, Table 8). There were no significant ($P < 0.05$) differences among treatments.

The numbers of first-feeding stage larvae produced per kg female body wt (x 10^3) showed a similar trend (Fig. 15, Table 8); highest in L-50-fast (2.78), and L-100-slow (2.88), intermediate in L-5-slow (2.23), and L-50-slow (1.34), and lowest in G-slow (0.515) and the control (0.143) (Table 8). There were no significant ($P < 0.05$) differences among treatments. The L-100-slow treatment produced the most first-feeding larvae per female and per kg female body wt, with L-50-fast close behind.

Individual vs. Group Spawning

For females implanted with L-50-slow, there were 21 individual spawning trials and 10 group trials, with a mean of 4 (range = 2 - 6) females per group (Table 9). During group spawnings, there was an average of 646 eggs per group (x 10^3). Number of eggs per spawn (x 10^3) was significantly ($P < 0.05$) greater in the group spawns (108) than in the individual spawns (66.7) (Table 9). Number of eggs per female (x 10^3) (197 vs 208), eggs per kg body wt (x 10^3) (121 vs 159), and fertilization success (%) (38.0 vs 41.0)

Table 8. No. of first-feeding larvae produced per female and per kg body wt ($x10^3$) under different hormone treatments. Letter indicates hormone (L = LHRH-a, G = GnRH-a), number indicates dose (µg/kg), and word indicates release rate (slow or fast). Values represent means ± standard error (*N* = 6 - 21), while numbers in parentheses denote ranges. No significant (P > 0.05) difference among treatments were observed.

Treatment	N	No. first-feeding larvae produced ($x 10^3$) per female	per kg body wt
Control	17	0.151 ± 0.0882 (0-1.10)	0.143 ± 0.0833 (0-1.16)
L-5-slow	12	2.09 ± 1.05 (0-10.0)	2.23 ± 1.09 (0-10.6)
L-50-slow	21	2.06 ± 0.718 (0-11.1)	1.34 ± 0.451 (0-6.08)
L-50-fast	6	4.16 ± 3.33 (0-20.4)	2.78 ± 2.25 (0-13.8)
L-100-slow	9	4.42 ± 2.86 (0-24.7)	2.88 ± 2.01 (0-18.0)
G-slow	6	0.711 ± 0.646 (0-3.93)	0.515 ± 0.465 (0-2.83)

Table 9. Individual (L-50-slow) versus group (L-50-slow group) spawning parameters. Letter indicates hormone (L = LHRH-a), number indicates dose (µg/kg), and word indicates release rate (slow or fast). Values represent means ± standard error (*N* = 10, 21), while numbers in parentheses denote ranges. Mean values in a row followed by a different letter are significantly ($P < 0.05$) different.

	Individual	Group
No. of trials	21	10
No. of females per trial	1	4.00 (2.00-6.00)
Eggs per spawn (x 10^3)	66.7 ± 13.8 b (0-679)	108 ± 14.6 a (2.69-504)
Eggs per female (x 10^3)	197 ± 49.0 (0-861)	208 ± 61.9 (67.3-568)
Eggs per group (x 10^3)		646 ± 150 (64.6-1710)
Eggs per kg body wt (x 10^3)	121 ± 27.0 (0-420)	159 ± 46.1 (66.7-175)
Fertilization (%)	38.0 ± 5.17 (0-100)	41.4 ± 4.68 (0-100)
Hatch (%)	15.6 ± 2.33 b (0-40.2)	18.7 ± 1.93 a (0.070-44.9
Yolksac larvae per female (x 10^3)	14.0 ± 5.75 b (0-111)	37.2 ± 12.5 a (1.55-99.7)
Yolksac larvae per group (x 10^3)		108 ± 32.5 (0-299)
Yolksac larvae per kg body wt (x 10^3)	8.76 ± 0.336 (0-63.1)	23.2 ± 8.99 (0-81.3)
First-feeding (%)	4.20 ± 1.05 (0-21.0)	5.45 ± 1.01 (0-28.3)
First-feeding larvae per female (x 10^3)	2.06 ± 0.718 b (0-11.1)	8.56 ± 2.94 a (0.620-24.4)
First-feeding larvae per group (x 10^3)		23.6 ± 7.29 (0-73.2)
First-feeding larvae per kg body wt (x 10^3)	1.34 ± 0.451 (0-6.08)	5.44 ± 2.27 (0-20.5)
Oocyte diameter (mm)	0.450 ± 0.00917 (0.338-0.526)	0.462 ± 0.00605 (0.354-0.527)
Egg diameter (mm)	0.919 ± 0.00679 (0.836-0.990)	0.947 ± 0.00503 (0.892-1.01)
Multiple oil droplet (%)	14.6 ± 4.13 (0-100)	19.0 ± 4.09 (0-100)

were not significantly ($P > 0.05$) different between individual and group spawns, respectively (Table 9).

There was an average of 108 yolksac larvae produced per group (x 10^3). The hatching success (%) (18.7 vs 15.6) and number of yolksac larvae per female (x 10^3) (37.2 vs 14.0) were significantly ($P < 0.05$) greater in the group spawns than in the individual spawns, respectively (Table 9). The number of yolksac larvae per kg body wt (x 10^3) (8.76 vs 23.2) was not significantly ($P < 0.05$) different between individual and group spawns, respectively (Table 9).

Survival to the first-feeding stage (%) (4.20 vs 5.45) and number of first-feeding larvae per kg body wt (x 10^3) (1.34 vs 5.44) were not significantly ($P > 0.05$) different between the individual and group spawnings, respectively (Table 9). The number of first-feeding stage larvae per female (x 10^3) (8.56 vs 2.06) was significantly ($P < 0.05$) greater in the group spawns than in the individual spawns, respectively (Table 9). There was an average of 23.6 first-feeding larvae per group (x 10^3). Group spawning yielded proportionally higher numbers of eggs and viable larvae than individual spawning.

The MOD (mm) (0.450 vs 0.462), spawned egg diameter (mm) (0.919 vs 0.947), and the percentage of eggs with multiple oil droplets (%) (14.6 vs 19.0) were not significantly ($P < 0.05$) different between the group and the individual spawns, respectively (Table 9).

Egg Fatty Acid Composition

The fertilization rates (%) of the low (38.7) and high (55.1) quality egg samples were not significantly ($P > 0.05$) different (Table 10). The hatching success (%) in the

Table 10. Percentage of lipid, fertilization success, hatching success, and survival to first-feeding of low and high quality black sea bass eggs produced following spawning induced by the L-50-slow treatment (see text for explanation of egg quality criteria). Column titles refer to egg quality (low or high). Values represent means ± standard error (N = 3), while numbers in parentheses denote ranges. Mean values in a row followed by a different letter are significantly ($P < 0.05$) different.

Parameter	Low	High
Lipid (%)	4.48 ± 0.217 b (3.47-5.48)	6.19 ± 0.391 a (5.08-7.36)
Fertilization (%)	38.7 ± 11.5 (16.2-54.0)	55.1 ± 23.6 (19.8-100)
Hatching success (%)	7.83 ± 3.85 b (1.40-14.7)	28.8 ± 5.82 a (21.0-40.2)
First-feeding success (%)	1.38 ± 1.08 (0-3.5)	8.58 ± 2.69 (4.56-13.7)

high quality eggs (28.8) was significantly ($P < 0.05$) greater than in the low quality eggs (7.83) (Table 10). The percentage of larvae reaching the first-feeding stage in the low (1.38) and high (8.58) quality egg samples were not significantly ($P > 0.05$) different (Table 10).

Egg lipid content (6.19 vs 4.48 %) was significantly ($P < 0.05$) greater in the high than in the low quality eggs, respectively (Table 10, Fig. 16). The GC-MS chromatogram for FAMEs produced from an egg sample showed the various fatty acids in the sample (Fig. 17). Fourteen peaks were examined and compared in L-50-slow treatment between high and low quality eggs (Fig. 18).

The fatty acids are defined numerically for example 18:1Δ-9 (18 = length of fatty acid carbon chain, :1 = no. of double bonds, Δ-9 = position of double bonds from carboxylic end). The SFAs measured in the low and high quality eggs, respectively, were 14:0 (1.98, 2.01), 15:0 (0.881, 0.720), 16:0 (17.7, 24.3), 17:0 (2.54, 1.57), 18:0 (7.94, 9.77), and 19:0 (1.51, 1.03) (Table 11, Fig. 18). The major SFAs, 16:0 and 18:0, were significantly ($P < 0.05$) greater in high than in low quality eggs. The sum of SFAs (37.1 vs 31.0) was significantly ($P < 0.05$) greater in high than in low quality eggs, respectively (Table 11, Fig. 19).

The MUFAs measured in high and low quality eggs, respectively, were 16:1 (6.62, 7.88), 18:1Δ-9 (13.7, 15.2), and 18:1Δ-11 (3.22, 5.34) (Table 11, Fig. 18). The major MUFAs included 16:1, 18:1Δ-9 and 18:1Δ-11. The 16:1 and 18:1Δ-11 fatty acids were significantly ($P < 0.05$) greater in the high than in the low quality eggs. The sum of MUFAs (28.4 vs 23.6) was significantly ($P < 0.05$) greater in the high than in the low quality eggs, respectively (Table 11, Fig. 19).

Table 11. Fatty acid composition, determined by GC-FID, of low and high quality black sea bass eggs produced following spawning induced by the L-50-slow treatment (see text for explanation of egg quality criteria). Column titles refer to egg quality (low or high). Values represent means ± standard error (*N* = 3), while numbers in parentheses denote ranges. Mean values in a row followed by a different letter are significantly (P < 0.05) different.

Fatty acid	Low	High
14:0	1.98 ± 0.152	2.01 ± 0.149
15:0	0.881 ± 0.113	0.720 ± 0.0740
16:1	6.62 ± 0.187 b	7.88 ± 0.262 a
16:0	17.7 ±0.606 b	24.3 ± 0.905 a
17:0	2.54 ± 0.360	1.57 ± 0.170
18:2*n*-9,12	3.06 ± 0.345	3.23 ± 0.289
18:1*n*-9	13.7 ± 0.183	15.2 ± 0.567
18:1*n*-11	3.22 ± 0.0848 b	5.34 ± 0.492 a
18:0	7.94 ± 0.317 b	9.77 ± 0.312 a
19:0	1.51 ± 0.228	1.03 ± na[a]
20:5*n*-3	8.99 ± 0.683	7.87 ± 0.430
20:4*n*-6	1.68 ± 0.0472	1.42 ± 0.0941
22:6*n*-3	25.4 ± 0.276 a	17.3 ± 1.40 b
22:5	3.60 ± 0.267	3.47 ± 0.385
∑Saturates	31.0 ± 0.732 b	37.1 ± 0.906 a
	(28.3-34.0)	(31.9-41.2)
∑Monoenes	23.6 ± 0.353 b	28.4 ± 0.800 a
	(22.1-24.9)	(23.9-29.8)
∑Polyunsaturates	42.7 ± 0.547 a	33.0 ± 1.69 b
	(40.3-44.7)	(25.4-44.1)
∑*n*-6	7.96 ± 0.378 b	9.68 ± 0.461 a
	(6.39-9.12)	(7.69-11.3)
∑*n*-3	38.0 ± 0.303 a	28.7 ± 1.66 b
	(36.7-39.3)	(25.6-31.4)
∑*n*-3 HUFA	34.4 ± 0.484 a	25.2 ± 1.66 b
	(32.5-36.7)	(18.6-35.7)
n-6/*n*-3	0.209 ± 0.00917 b	0.351 ± 0.0318 a
	(0.171-0.246)	(0.194-0.495)
n-3/n-6	4.86 ± 0.228 a	3.08 ± 0.333 b
	(4.07-5.86)	(2.02-5.16)
22:6*n*-3/20:5*n*-3	2.97 ± 0.242	2.22 ± 0.155
	(2.10-3.76)	(1.58-2.96)
20:4*n*-6/20:5*n*-3	0.198 ± 0.0191	0.146 ± 0.032
	(0.0322-0.277)	(0.136-0.310)

[a] not applicable

The PUFAs measured in low and high quality eggs, respectively, were 18:2Δ-9,12 (3.06, 3.23), 20:5*n*-3 (*n*-3 = position of double bonds from methyl end) (8.99, 7.87), 20:4*n*-6 (1.68, 1.42), 22:6*n*-3 (25.4, 17.3), and 22:5*n*-3 (3.60, 3.47) (Table 11, Fig. 18). The major PUFAs included 18:2Δ-9,12, 20:4*n*-6, and 22:6*n*-3. The sum of PUFAs (42.7 vs 33.3) was significantly ($P < 0.05$) greater in the low than in the high quality eggs, respectively (Table 11, Fig. 19).

DHA (25.4 vs 17.3) was significantly ($P < 0.05$) greater in the low than in the high quality eggs, respectively (Table 11, Fig. 20). ARA (1.68 vs 1.41) was not significantly ($P < 0.05$) different in low and high quality eggs, respectively (Table 11, Fig. 20). The EPA values (8.99 vs 7.87) were not significantly ($P < 0.05$) different in low and high quality eggs, respectively (Table 11, Fig. 20). The ratio of DHA/ EPA (2.97 vs 2.22) was not significantly ($P< 0.05$) different in low and high quality eggs, respectively (Table 10, Fig. 21). The ratio of ARA/ EPA (0.198 vs 0.146) was not significantly ($P < 0.05$) different in the low and high quality eggs, respectively (Table 11, Fig. 21).

The sum of *n*-3 (38.0 vs 28.7) was significantly ($P < 0.05$) greater in the low than in the high quality eggs, respectively (Table 11, Fig. 22). The sum of *n*-6 (9.68 vs 7.96) was significantly ($P < 0.05$) greater in the high than in the low quality eggs, respectively (Table 11, Fig. 22). The ratio of *n*-6/ *n*-3 fatty acids (0.351 vs 0.209) was significantly ($P < 0.05$) greater in the high than in the low quality eggs, respectively (Table 11, Fig. 21). The ratio of *n*-3/ *n*-6 fatty acids (4.86 vs 3.08) was significantly ($P < 0.05$) greater in the low than in the high quality eggs, respectively (Table 11, Fig. 21).

The linear regression of lipid composition on percentage of fertilized eggs ($r^2 = 0.153$) appeared to show a significant ($0.05 < P < 0.10$) relationship (Table 12, Fig. 23).

The linear regression of *n*-6 on percentage of fertilized eggs ($r^2 = 0.125$) appeared to show a significant ($0.05 < P < 0.10$) relationship (Table 12, Fig. 24). The linear regression of ARA/ EPA on percentage of fertilized eggs ($r^2 = 0.129$) appeared to have a significant ($0.05 < P < 0.10$) relationship (Table 13, Fig. 25). There were no significant ($P > 0.10$) relationships between the other fatty acid individual components or ratios to fertilization success (%) or hatching success (%).

DISCUSSION

Hormone-Induced Ovulation

A single LHRH-a or GnRH-a pellet implant successfully stimulated final oocyte maturation and ovulation in female black sea bass *Centropristis striata* as previously reported by Watanabe et al. (2003). Similar results have been obtained with a number of finfish species (Mylonas and Zohar 2001), including summer flounder *Paralichthys dentatus* (Watanabe et al. 1998), sea bass *Lates calcarifer*, rabbitfish *Siganus guttatus* (Harvey et al. 1985; Almendras et al. 1988), milkfish *Chanos chanos* (Lee et al. 1986), Nassau grouper *Epinephelus striatus,* and squaretail coraltrout *Plectropomus areolatus* (Tucker 2003).

The fertilization rate (%) in the L-50-slow treatment in this study (38.0), which was based on all spawned eggs, appeared to be less than that reported by Watanabe et al. (2003) with a dose of ~50 slow µg/kg (98.0), which were based on floating eggs only. The eggs in this study did not reliably separate into “floaters” and “sinkers”, so the percentage of fertilized eggs was based on overall eggs rather than “floaters”. So in reality fertilization success in both studies were comparable.

Good quality eggs have the highest hatching rates (Navas et al. 1997). Hatching success (%) in this study (15.6) also appeared to be lower than in the previous study (Watanabe et al. 2003) (24.3-27.2). However, the values in this study were based on all spawned eggs instead of only floating eggs (Watanabe et al. 2003), so in reality hatching success in both studies were comparable. The overall egg viability (i.e., fecundity x hatching rate) (%) in the previous study (8.9) (Watanabe et al. 2003) was lower than seen in the L-50-slow treatment (15.6) in this study. In the earlier study (Watanabe et al. 2003), yolksac production (x 10^3) (14.6 yolksac larvae / female, 12.6 yolksac larvae / kg body wt) was similar to the L-50-slow treatment (14.0 yolksac larvae / female, 8.76 yolksac larvae / kg body wt) in the present study.

Volitional Spawning

Once implanted, black sea bass spawned volitionally, consistent with an earlier study by Watanabe et al. (2003). It was not necessary to strip spawn black sea bass to produce viable eggs, as practiced by other workers (Berlinsky et al. 2000; Howell et al. 2003). Strip-spawning causes more handling stress and potential damage to the fish and egg quality (Kjørsvik et al. 2003). Successful volitional spawning of black sea bass in this study was most likely due to careful maintenance of high water quality and control of temperature, photoperiod, and salinity to simulate natural environmental conditions, as well as fish diet, and proper male: female ratio (Zohar and Mylonas 2001).

Seasonality of Spawning

In this study, hormone induced spawning of black sea bass was successfully achieved from March through August, much longer than the natural spawning season, which occurs from March to April in North Carolina waters (Mercer 1989; Waltz et al. 1979; Wenner et al. 1986; Vaughan et al.1995; Watanabe et al. 2003). Once black sea bass spawning commenced in March, the photoperiod was held at 13L: 11D and temperature at 19 ºC, so that fish would continue to spawn. Hence, the spawning season was successfully lengthened by photo-thermal control.

Hormone Dose

The LHRH-a hormone doses used in this study, which ranged from 5 - 100 µg/kg, were similar to the doses successfully used for this hormone in prior studies, which varied from 9.0 - 23.5 µg/kg body wt in sea bass *Lates calcarifer* (Harvey et al. 1985), 20 – 200 µg/kg body wt. in red drum (Thomas and Arnold 1993), ~50 µg/kg body wt. in black sea bass (Watanabe et al. 2003), and 100 µg/kg body wt. in summer flounder (Watanabe et al. 1998). Regardless of dose implanted, the latency periods, or time from implantation to spawning (2 - 6 dpi), were similar to what was reported in previous studies for induced spawning of black sea bass (2.3 - 3 dpi, Watanabe et al. 2003; 2 - 8 dpi, Tucker 1984), southern sea bass (2 - 3 dpi) (Hoff 1970), and summer flounder (2.5 - 5.5 dpi) (Watanabe et al. 1998).

The percentage of females responding to LHRH-a, however, increased with higher hormone dosage from 41.2 % in the control to 100 % in L-100-slow. In a previous study, black sea bass implanted with ~50 µg/kg LHRH-a spawned an average of

two times (Watanabe et al. 2003), which was less than the number of spawnings observed following implantation with L-50-slow (2.86) in this study. The number of spawns per female also increased with higher hormone dosage from 0.471 in the control to 5.56 in L-100-slow, which could be due to more oocytes being hydrated and released at once for higher hormone doses (Thomas and Arnold 1993), or to a longer period that circulating GtH levels are maintained at sufficient levels to trigger final oocyte maturation and ovulation.

The number of spawns and eggs spawned per female and per kg female body wt were significantly influenced by hormone dose, generally increasing with higher hormone dosages. All of the doses tested, except L-5-slow, produced a significantly greater number of eggs per female than in the control. High hormone doses appeared to ripen and release more oocytes over a longer period of time than low doses.

Although higher doses stimulated greater egg production, egg quality appeared to improve at lower doses. While statistical differences could not be determined, the L-5-slow treatment had the greatest fertilization success (41.6) and survival to the first-feeding stage (7.50), and the second highest hatching success (21.3). The higher doses caused a larger number of eggs to be ovulated and spawned, but with apparently lower fertilization and hatching success and survival to first-feeding, possibly due to premature oocyte ripening and ovulation.

Although the higher doses increased egg production, the L-100-slow treatment appeared to be too high for black sea bass, since two of the nine females (22 %) implanted with that dose became egg-bound and died during the spawning period, suggesting hormone over-stimulation. This was the only dose associated with

mortalities. Similar results were observed in orangemouth corvina injected with 500 IU of human chorionic gonadotropin, in which a larger number of oocytes than normal hydrated at one time causing substantial swelling of the ovary and death (Thomas and Arnold 1993).

Release Rate

The time until onset of spawning following hormone treatment (latency period) in black sea bass was similar in fish treated with L-50-fast (2.67 d) and L-50-slow (2.53 d), indicating that the hormone release rate did not have an effect on latency period. The release rate of hormone pellets determines the amount of time and the concentration of hormone in the circulation of the recipient fish, which has been shown to alter the duration of spawning in serially spawning species (Mylonas and Zohar 2001). In this study, the fast and slow release pellets did not affect the number of spawns per female, which was similar in fish implanted with L-50-fast (3.17 d) and L-50-slow (2.86 d). In contrast, barramundi spawned once with GnRH-a fast-release implants but multiple times with slow-release pellets (Almendras et al. 1988; Mylonas and Zohar 2001). The hormone implant release rate did not appear to affect the latency period and number of spawns per female in black sea bass, which suggests that slower release did not cause recruitment of additional clutches of oocytes compared to fast release pellets. Alternatively, fish implanted with the slow release pellets may have continued to spawn past the time of collection which was set at 10 dpi for experimental and practical reasons. No significant differences between L-50-fast and L-50-slow treatments were observed in

terms of number of spawns, spawned egg diameters, egg production, hatching success, or in egg quality as measured by fertilization success.

Hormone Type

The GnRH-a hormone pellets used in this study were applied at doses ranging from 49 - 114 µg/kg, similar to the LHRH-a doses used in this study. Assuming that these hormones are equally effective, the results from G-slow implants were, therefore, expected to be similar to the results in the L-50-slow and L-100-slow treatments. However, the percentage of females spawning was lower in G-slow (83.3) than in L-50-slow (95.0) and L-100-slow (100). The number of spawns per female was not affected by hormone type, being similar in G-slow (3.33), L-50-slow (2.86), and L-100-slow (5.56).

Hormone type did not affect egg production or hatching success in black sea bass. The number of eggs produced per female and per kg body wt. (x 10^3) were similar in G-slow (221, 170), L-50-slow (197, 121) and L-100-slow (584, 382).

Hatching success (%) was similar in G-slow (14.7), L-50-slow (15.6), and L-100-slow (11.9). There appeared to be a greater number of yolksac larvae produced per female and per kg body wt. (x 10^3) in response to G-slow (34.3, 28.0) than L-50-slow (14.0, 8.76) and L-100-slow (19.4, 12.7). The percentage of eggs with multiple oil droplets appeared to be greater in G-slow (25.9) than in L-50-slow (14.6) and in L-100-slow (3.90). The spawned egg size (mm) was similar in G-slow (0.933), L-50-slow (0.919), and L-100-slow (0.932).

To sum up, the number of spawns per female, egg production, egg size, and hatching success were not affected by hormone type. Compared to LHRH-a, the GnRH-a hormone type appeared to decrease in the percentage of females spawning and fertilization success, while it appeared to increase the occurrence of multiple oil droplets in spawned eggs and yolksac larval production. GnRH-a is similar to one of the endogenous hormones found in the brain of black sea bass (Sherwood et al. 1993) and resulted in higher yolksac larval production, while LHRH-a produced higher survival to first-feeding. Due to wide variability in results among treatments, more studies are required to determine the best hormone type for hormone induced spawning in black sea bass.

Mean Oocyte Diameter

Females with a range of MOD (0.338 - 0.488 mm) successfully spawned following implantation with a hormone pellet, and there were no significant differences among treatments in pre-treatment MOD. The MOD range was similar to those used in other studies using pelleted LHRH-a implants in black sea bass (0.305 - 0.448 mm) (Watanabe et al. 2003), in *Lates calcarifer* (≥ 0.46) (Harvey et al. 1985), and summer flounder (0.258 - 0.456) (Watanabe et al. 1998), or black sea bass injected with HCG (≥ 0.400 mm) (Tucker 1984). More research is needed to determine the optimal MOD for black sea bass hormone implantation.

Spawning in the Control

In this study, spawning occurred in the control females, but at a much lower frequency than in hormone treated fish. This is an uncommon occurrence, since spawning in control fish is not often reported for marine finfish species. For example, no spawning occurred in untreated control *Lates calcarifer* and *Siganus guttatus* females (Harvey et al. 1985). Spawning in control fish may have been stimulated by the placebo implant. The placebo contained cholesterol, which is a precursor to steroid hormones and could have been metabolized by the female, inducing spawning (Mylonas and Zohar 2001). Alternatively, spawning could have occurred naturally, since natural spawning was observed on several occasions in holding tanks, containing fish that had not been implanted. However, natural spawning was infrequent and unpredictable in this study and therefore not reliable enough for practical purposes. Since there was some natural spawning in control fish, this mode of spawning may become more reliable with domestication and improved broodstock husbandry.

Survival to First-Feeding

Survival to first-feeding was relatively low among treatments (0.456 - 7.50 %). The trends displayed through first-feeding, with the most first-feeding larvae produced in the L-5-slow, L-50-fast, and L-100-slow treatments, were different from those seen through hatching, with the most yolksac larvae produced in the L-50-fast, and G-slow treatments. These differences may not necessarily be related to hormone treatment, because the low survival to first feeding could be related to broodstock diet, sub-optimal incubation temperature (Watanabe et al. 2003), or excessive turbulence in egg incubators

(A. Mangino, personal communication). Poor water quality was not a likely cause because the incubators were supplied with flow-through seawater, greatly reducing the risk of deteriorating water quality. Eggs were incubated at a temperature of 19 ºC which was the spawning temperature. In the wild, eggs and larvae are pelagic (Mercer 1989; Tucker 2003). Surface water in the ocean tends to be warmer, from solar radiation, than at depth. Pelagic eggs may be distributed in surface waters where temperatures are warmer than deeper waters where spawning occurs. This suggests that incubation of eggs and larvae at a higher temperature may improve survival. This is supported by recent studies which have shown that survival of eggs and larvae are optimized at > 22 ºC (Berlinsky et al. 2000) and 22 - 25 ºC in black sea bass (Copeland et al. 2003). Black sea bass larvae are small and fragile. The amount of turbulence, due to aeration in the incubators, may have been excessive, causing stress, exhaustion, damage, and/or death to the larvae (Tucker 2003). Research is needed to improve the survival of larvae through the first-feeding stage.

Egg Size

Storage and preservation of spawned egg samples in 5 % formalin in seawater did not affect the mean spawned egg diameter over a period of 46 d. In contrast, a 10 % formalin solution with hematoxylin and eosin stain was found to decrease the diameter of ovarian follicles by 22 % in black sea bass (Howell et al. 2003).

The spawned egg diameter (mm) in this study (0.836 – 1.02) was similar to what was previously reported in black sea bass (0.9 – 1.0) (Mercer 1989). In a previous study, the mean spawned egg diameter (mm) in barramundi was significantly larger in fish

treated with 9.0 - 23.5 μg/kg LHRH-a (0.509) than in the control fish (0.497) (Harvey et al. 1985). In this study, egg diameter (mm) of the L-5-slow treatment (0.981) while not significantly different from the control (0.967) was significantly larger than in the higher doses (0.919 – 0.933). This could suggest an acceleration of the final maturation process with increasing dose, leading to a subnormal water uptake, yielding a smaller diameter egg. This is consistent with higher egg quality at lower doses.

Eggs with Multiple Oil Droplets

The distribution of lipid droplets in an egg is thought to be an early marker of egg quality (Kjørsvik et al. 1990). Spawned eggs with more than one oil droplet are thought to be of a lower quality than eggs with a single oil droplet (Kjørsvik et al. 1990). However, in this study the percentage of eggs with multiple oil droplets (3.90 - 25.9) were not significantly different among treatments and did not appear to be correlated with egg quality as measured by fertilization, hatching success, or survival to first-feeding.

Individual vs. Group Spawning

Although individual female black sea bass showed relatively low fecundity, group spawnings, where 3 or more females were implanted concurrently, was a practical method for increasing egg and larval production. In this study, individual spawning performance, including eggs per female and per kg body wt, fertilization success, hatching success, first-feeding success, oocyte diameter, and percentage of eggs with multiple oil droplets were comparable to those seen in group spawnings. The number of eggs (x 10^3) (646 vs 197), yolksac larvae (37.2 vs 14.0) and the number of first-feeding

larvae (8.56 vs 2.06) were at least proportionally higher in group than in individual spawns. This is similar to what was observed in a previous study using ~50 μg/kg LHRH-a (Watanabe et al. 2003), where group spawnings produced proportionally higher fecundity to individual spawnings. These results suggest a possibly synergistic effect of group spawning compared to individual spawning.

Fatty Acids

The effect of lipid composition (%) on egg quality varies among fish species. In turbot and European sea bass, a higher lipid content caused a lower hatching and egg viability (Devauchelle et al. 1982). In Japanese flounder, egg lipid content (25.5 - 32.1 %) had no effect on egg viability (Furuita et al. 2002). In this study, however, the percentage of total lipids was significantly greater in the high (6.19 %) than in the low (4.48 %) quality eggs. These results suggest that lipid content is positively correlated to egg quality in black sea bass (Fig. 22). Lipids are important to the metabolism and survival of eggs and larvae (Sargent 1995) and higher levels are a good indicator of proper egg nutrition (Bromage et al. 2001). The greater percent lipid composition in high quality eggs could be the reason good eggs float while poor quality eggs sink.

The sum of SFAs in black sea bass eggs (28.3 – 41.2 %) was similar to Japanese flounder (33.6 – 35.7 %) (Furuita et al. 2002), but greater than in yellowtail flounder (22.1) (Copeman et al. 2002). The sum of MUFAs in black sea bass eggs (22.1 – 29.8 %) was greater than in Japanese flounder (14.3 – 14.9 %) (Furuita et al. 2002), but lower than in yellowtail flounder (42.1) (Copeman et al. 2002). The sum of PUFAs in black sea

bass eggs (25.4 – 44.7 %) was similar to yellowtail flounder (35.8) (Copeman et al. 2002).

In this study, the sum of SFAs was significantly greater in the high (37.1 %) than in the low (31.0 %) quality black sea bass eggs. The sum of MUFAs was significantly greater in the high (28.4 %) than in the low (23.6 %) quality black sea bass eggs. SFAs and MUFAs are important metabolic energy sources during embryonic and early larval development (Sargent 1995). The poor quality eggs appear to be deficient in SFAs and MUFAs which are necessary to complete their growth and development.

The sum of PUFAs was significantly greater in the low (42.7 %) than in the high (33.0 %) quality black sea bass eggs. The PUFA levels, which are important to the development and responses from the immune system (Lall 2000), metabolic energy, formation and function of membranes (Bell et al. 1986; Sargent 1995; Sargent et al. 1999), and embryonic development (Navas et al. 1997), appear to be nutritionally sufficient in the high quality and in excess in the low quality eggs.

The sum of *n*-6 fatty acids in black sea bass eggs (6.39 – 11.3 %) was similar to Japanese flounder (4.9 –8.1 %) (Furuita et al. 2002). The sum of *n*-3 fatty acids was similar in black sea bass eggs (25.6 – 39.3 %) to Japanese flounder (31.4 – 32.8 %) (Furuita et al. 2002). In this study, the *n*-6 fatty acids were greater in the high (9.68) than in the low (7.96) quality eggs.

The *n*-3 fatty acids were greater in the low (38.0) than in the high (28.7) quality eggs. Similar to this study, a greater value of *n*-3 fatty acids caused decreased survival in European sea bass, Atlantic salmon and Channel catfish (Cerdá et al 1995; Lall 2000), suggesting that excessive *n*-3 values were detrimental to egg quality in these species.

However, the opposite was seen in European sea bass, which had greater egg viability and hatching success in broodstock fed high levels of *n*-3 fatty acids (Navas et al. 1997). In this study, the high value of *n*-3 decreased the ratio of *n*-6/ *n*-3 fatty acids in black sea bass eggs, which could be detrimental to egg quality and survival (Robin 1995; Sargent 1995).

Based on fecundity, percentage of buoyant eggs, hatching rates, and percentage of normal larvae, deficiencies or excessive amounts of *n*-3 HUFA were found to negatively affect egg quality in European sea bass, gilthead seabream, and Japanese flounder (Navas et al. 1997; Rodríguez et al. 1998; Furuita et al. 2002). The results of previous studies have been combined to give an optimal, non-species specific, percentage (20 %) of *n*-3 HUFA to the total fatty acids (Furuita et al. 2002). Fish fed higher levels of *n*-3 HUFA had lower quality eggs, and the *n*-3 HUFA levels were above the optimal level in turbot (22-26) (Lavens et al. 1999) and in Japanese flounder (~20 - 25) (Furuita et al. 2002). The *n*-3 HUFA levels (18.6 – 36.7 %) in this study were similar to red sea bream (18.5 – 29.6 %) (Watanabe and Vassollo-Agius 2003). In this study, the *n*-3 HUFA level (%) was greater in the low (34.4) than in the high quality eggs (25.2). These results suggest that percentage *n*-3 HUFA was above optimal in the low quality black sea bass eggs, negatively affecting the growth, and egg quality, and could be a reason for poor survival to first-feeding.

The ratio of *n*-6/ *n*-3 fatty acids in black sea bass eggs (1/5.85 – 1/2.02) was similar to Japanese flounder (1/5 – 1/3.33) (Furuita et al. 2002). The *n*-6/ *n*-3 fatty acid ratio in black sea bass eggs appeared to be greater in the high (0.351) than in the low (0.209) quality eggs but, much lower than the optimal ratio (5/1 – 10/1) (Sargent 1995;

Sargent et al. 1999). This is a nutritional concern making it harder for larvae to react to stressors (Sargent 1995). The low *n*-6/ *n*-3 fatty acid ratio could be related to broodstock diet and could be responsible for poor survival to the first-feeding stage. Black sea bass are omnivorous, therefore, the *n*-6 deficiency could be the result of a diet too high in fish and may be improved by a diet including more plant matter.

DHA values in black sea bass (11.4 – 26.7 %) were similar to previous studies in Japanese flounder (22.5 – 23.3 %) (Furuita et al. 2002), milkfish (23.5), coral trout (23.2) (Ogata et al. 2003), and mangrove red snapper (18.0) (Ogata et al. 2003). High dietary levels of DHA improved survival in the eggs of Japanese flounder (Furuita et al. 1998), mahimahi, moi, milkfish (Ako et al. 1991), and yellowtail flounder (Furuita et al. 1996; Copeman et al. 2002), but appeared to lower egg quality in black sea bass. The DHA value was greater in the low (25.4) than in the high (17.3) quality eggs, suggesting that excessive dietary DHA may reduce egg quality. In this study, the DHA levels, which are important to development of neural tissues like the brain and retina (Rainuzzo et al. 1997), appeared to be nutritionally sufficient in high quality and excessive in low quality eggs. This may be related to inadequate SFA and MUFA reserves in these eggs.

The EPA values in this study (5.59 – 11.8 %) were similar to Japanese flounder (5.5 – 7.1 %) (Furuita et al. 2002), and yellowtail flounder (6.7 %) (Copeman et al. 2002), but greater than in milkfish (4.5 %), coral trout (3.5 %), and mangrove red snapper (4.3 %) (Ogata et al. 2003).

The ARA values in this study (1.06 – 1.90 %) were similar to a previous study in Japanese flounder (1.0 – 1.7 %) (Furuita et al. 2002), but lower than in yellowtail flounder (2.8 %) (Copeman et al. 2002), milkfish (5.6 %), mangrove red snapper (2.3 %),

and coral trout (4.7 %) (Ogata et al. 2003). Higher levels of ARA were directly correlated with egg quality, hatching rate, and lower mortality in Japanese flounder (Furuita et al. 1998), turbot (Furuita et al. 2002; Lavens et al. 1999; Robin 1995), and European sea bass (Bruce et al. 1999; Furuita et al. 2002). In contrast, no apparent relationship between ARA levels and egg quality was seen in yellowtail flounder (Copeman et al. 2002) and black sea bass, in this study. The data suggests that either ARA was too low and/or that EPA was too high in black sea bass eggs.

The ARA content decreased with higher *n*-3 HUFA values in turbot and Japanese flounder (Furuita et al. 2002). In contrast, ARA content in black sea bass eggs increased with increasing *n*-3 HUFA levels. The ARA values in this study, were similar in the low (1.68) and high (1.42) quality eggs. These low ARA values decreased the ratio of ARA/ EPA fatty acids in black sea bass eggs, which could be detrimental to proper vision, and neural development and functioning in the eggs of sea bass *Dicentrarchus labrax* (0.05 – 8.6) (Bell et al. 1997), European sea bass (0.08 – 0.15) (Navas et al. 1997), and in the larvae of yellowtail flounder (0.169 – 1.67) (Copeman et al. 2002). This imbalance could be corrected by a broodstock diet supplemented with a higher proportion of ARA, by using feed high in corn, soy, canola, or sunflower oils, which may improve larval visual and neural development, and increase the ARA/ EPA ratio.

In this study, the ratios of DHA/ EPA (1.58 – 3.76 %) were similar to Japanese flounder (3.3 - 4.0 %) (Furuita et al. 2002), and mangrove red snapper (4.17 %) (Ogata et al. 2003), but higher than in yellowtail flounder (1.1 %) (Copeman et al. 2002). The DHA/ EPA ratio in yellowtail flounder (5.6 vs 1.1) was directly related to survival rate (~22 vs ~5 %) (Copeman et al. 2002). In red drum the DHA/ EPA ratio (0.66-3.78) (Lee

and Ostrowski 2001) was directly related to stress resistance, with higher ratios producing greater resistance. However, in this study, DHA/ EPA ratios in low (2.97) and high (2.22) quality eggs were very similar and met the ratio of 2:1 recommended as nutritionally optimal by previous workers (Sargent 1995; Sargent et al. 1999). This optimal ratio of DHA/ EPA allows for normal larval growth, survival, and quality (Rainuzzo et al. 1997).

The ratio of ARA/ EPA in black sea bass (0.032 – 0.310 %) was similar to Atlantic salmon (0.13 %) and European sea bass (0.36 %) (Ogata et al. 2003), but was lower than in yellowtail flounder (0.417 %) (Copeman et al. 2002), and in mangrove red snapper (0.53 %) (Ogata et al. 2003). The ARA/ EPA ratios in this study, were similar in low (0.198) and in high (0.146) quality eggs and much lower than the ARA/ EPA ratio of 1:1 recommended as nutritionally optimal for marine finfish by previous workers (Sargent et al. 1999). This low ARA/ EPA ratio in black sea bass seems to be low due to a low level of ARA and an elevated level of EPA, which could be detrimental to proper vision, and neural development and functioning in larvae (Bell et al. 1997; Navas et al. 1997; Furuita et al. 1998; Copeman et al. 2002).

CONCLUSIONS

The results support the practical application of LHRH-a and GnRH-a pellet implants as volitional spawning devices in black sea bass. LHRH-a doses of 5 - 100 µg/kg and GnRH-a doses of 49 - 114 µg/kg were used successfully to induce spawning but, there was a wide variability in fertilization, hatching success, and survival to first-feeding. Percentage of fertilized eggs was generally high among treatments therefore,

was not a good indicator of egg quality in black sea bass. The hatching success of black sea bass eggs appeared to be a better parameter of quality than survival to first-feeding. The survival to first-feeding could be a more reliable parameter of egg quality if more information on the factors that influence survival of yolksac larvae can be obtained to improve black sea bass larviculture, decreasing the high mortality between hatching and first-feeding. Effects of hormone treatments were most clearly seen in egg production and the number of yolksac larvae produced. The dose of L-100-slow resulted in a 100 % female response as well as the highest number of spawns per female, greatest number of eggs and first-feeding stage larvae produced per female, and per kg female body weight than any other dose. Unfortunately, L-100-slow was the only dose associated with mortalities. Two of the nine females, or 22 % of the fish implanted with L-100-slow, became egg-bound and died during the spawning period, suggesting hormone over-stimulation.

There were many similarities and differences between black sea bass egg fatty acids and those of other species. In this study, high quality eggs had a greater percent lipid composition, hatching success, SFAs, MUFAs, *n*-6 fatty acids, and *n*-6/ *n*-3 ratio and appeared to have higher fertilization, and survival to first-feeding. Low quality eggs had greater percent PUFAs, *n*-3 fatty acids, *n*-3 HUFAs, DHA, and *n*-3/ *n*-6 ratio. Hatching success, which reflects both fertilization success and survival of embryos through hatching, appeared to be a good parameter of low and high quality spawns. More research is needed on eggs from different hormone dosages and from broodstock fed different diets to determine how the amounts of different fatty acids relate to egg quality.

Future Directions for Research

Slow and fast release rate at dose rates between 50 and 100 µg/kg pellets merit evaluation. A dose of L-75 µg/kg, for example, will hopefully induce a high spawning frequency and production of eggs and first-feeding stage larvae, without risking overstimulation and mortality.

Studies should examine incubation temperatures and aeration turbulence. The survival to first-feeding larval stage would most likely be increased at a higher temperature and optimal turbulence.

Research on ethylene vinyl acetate implants (EVAcs) and microspheres is necessary to see what effect mode of GnRH-a administration will have on spawning performance, or fecundity and egg quality. EVAcs have a rubbery, non-degradable shell made of a copolymer of ethylene and vinyl acetate monomers, which is filled with a sponge-like matrix and GnRH-a (Mylonas et al. 1995; Mylonas and Zohar 2001). Microspheres are tiny spheres that are injected into broodstock in solution. Their biodegradable shell holds a porous matrix containing GnRH-a (Yang et al. 2000; Yang et al. 2001). EVAcs and microspheres release hormone over a longer period of time, two to eight weeks, and at a steadier rate than slow release pellets and may produce more consistent results than the LHRH-a implants which release 20 % - 90 % of the hormone within the first 24 hours in vitro. Forniés et al. (2001) found that GnRH-a in three different modes of administration altered the spawning of European sea bass females, *Dicentrarchus labrax*. Injected GnRH-a caused one day of spawning while EVAc and

microspheres triggered up to four days of spawning (Forniés et al. 2001; Mylonas et al. 1995).

A problem with spawning black sea bass is that they are protogynous hermaphrodites. A hatchery needs to bring in female broodstock to replace the females that have changed to males. Research on suppression of sex reversal by treating females with female hormones to prevent the switch to males could give the hatchery more large spawning females (Tucker 2003).

LITERATURE CITED

Able, K.W., M.P. Fahay, and G.R. Shepherd. 1995. Early life history of black sea bass, *Centropristis striata,* in the mid-Atlantic Bight and a New Jersey estuary. Fishery Bulletin 93:429-445.

Ako, H., S. Kraul, and C. Tamaru. 1991. Pattern of fatty acid loss in several warmwater fish species during early development. Pages 23-25 *in* P. Lavens, P. Sorgeloos, E. Jaspers, and F. Ollevier, editors. LARVI '91 – Fish & crustacean larviculture symposium. European Aquaculture Society, Special Publication 15, Gent, Belgium.

Almansa, E., M.J. Pérez, J.R. Cejas, P. Badía, J.E. Villamandos, and A. Lorenzo. 1999. Influence of broodstock gilthead seabream (*Sparus aurata* L.) dietary fatty acids on egg quality and egg fatty acid composition throughout the spawning season. Aquaculture 170(3-4):323-336.

Al-Marzouk, A., K.P. Lone, and S.K. Teng. 1995. Egg and larval size and fatty acid composition of eggs of sobaity, *Sparidentex hasta* (Teleost: Sparidae) under different temperature and photoperiod regimes. Pakistan J.Zool. 26(3):207-214.

Almendras, J.M., C. Duenas, J. Nacario, N.M. Sherwood, and L.W. Crim. 1988. Sustained hormone release. III. Use of gonadotropin releasing hormone analogues to induce multiple spawnings in sea bass, *Lates calcarifer.* Aquaculture 74(1-2):97-111.

Bell, M.V. R.J. Henderson, and J.R. Sargent. 1986. Minireview: The role of polyunsaturated fatty acids in fish. Comp. Biochem. Physiol. 83B(4):711-719.

Bell, J.G., B.M. Farndale, M.P. Bruce, J.M. Navas, and M. Carillo. 1997. Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax).* Aquaculture 149(1-2):107-119.

Berlinsky, D., M. Watson, G. Nardi, and T.M. Bradley. 2000. Investigations of selected parameters for growth of larval and juvenile black sea bass *Centropristis striata* L. Journal of the World Aquaculture Society 31(3):426-435.

Billard, R., J. Cosson, L.W. Crim, and M. Suquet. 1995. Sperm physiology and quality. Pages 25-52 *in* Bromage, N.R. and R.J. Roberts, editors. Broodstock management and egg and larval quality. Blackwell Science, Oxford, England.

Bromage, N., M. Porter, and C. Randall. 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197:63-98.

Carrillo, M., N. Bromage, S. Zanuy, R. Serrano, and J. Ramos. 1991. Egg quality and fecundity in the sea bass (*Dicentrarchus labrax)* and the effects of photoperiodically-induced advances and delays of spawing time. Pages 259-261 *in* Proceedings of the fourth international symposium on the reproductive physiology of fish, Norwich, UK.

Carrillo, M., S. Zanuy, F. Prat, R. Serrano, and N. Bromage. 1993. Environmental and hormonal control of reproduction in sea bass. Recent advances in aquaculture 4:43-54.

Cerdá, J.L., M. Carrillo, S. Zanuy, J. Ramos, and R. Serrano. 1991. Effects on fecundity and egg quality of three different diets supplied during two reproductive cycles to broodstock sea bass (*Dicentrarchus labrax* L.). Page 270 *in* Scott, A.P., J.P. Sumpter, D.E. Kime, and M.S. Rolfe, editors. Proceedings of the fourth international symposium on the reproductive physiology of fish, 7-12 July 1991, Norwich, UK.

Cerdá, J.L., M. Carrillo, S. Zanuy, J. Ramos, and R. Serrano. 1995. Short and long-term dietary effects on female sea bass (*Dicentrarchus labrax*): seasonal changes in plasma profiles of lipids in relation to reproduction. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology and Toxicology 111(1):83-91.

Copeland, K.A., W.O. Watanabe, and P.M. Carroll. 2002. Growth and feed utilization of captive wild subadult black sea bass *Centropristis striata* fed practical diets in a recirculation system. Journal of the World Aquaculture Society 33(2):97-109.

Copeland, K.A., W.O. Watanabe, P.M. Carroll, K.S. Wheatley, and T.M. Losordo. 2003. Growth and feed utilization of captive wild black sea bass *Centropristis striata* at 4 different densities in a recirculating tank system. Journal of the World Aquaculture Society 34(3):300-307.

Copeman, L.A., C.C. Parrish, J.A. Brown, and M. Harel. 2002. Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition, and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment. Aquaculture 210:285-304.

Crim, L.W., N.M. Sherwood, and C.E. Wilson. 1988. Sustained hormone release. II. Effectiveness of LHRH analog (LHRHa) administration by either single time injection or cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. Aquaculture 74:87-95.

Devauchelle, N., G. Brichon, F. Lamour, and G. Stephan. 1982. Biochemical composition of ovules and fecund eggs of sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*). Pages 155-157 *in* Richter, C.J.J., and Goos, H.J.Th., editors. Proceedings of International Symposium on Reproductive Physiology in Fish.

Forniés, M.A., E. Mañanós, M. Carrillo, A. Rocha, S. Laureau, C.C. Mylonas, Y. Zohar, and S. Zanuy. 2001. Spawning induction of individual European sea bass females (*Dicentrarchus labrax)* using different GnRH-a delivery systems. Aquaculture 202:221-234.

Fraser, A.J., J.C. Gamble, and J.R. Sargent. 1988. Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua)*. Marine Biology 99:307-313.

Furuita, H., T. Takeuchi, T. Watanabe, H. Fujimoto, S. Sekiya, and K. Imaizumi. 1996. Requirements of larval yellowtail for eicosapentaenoic acid, docosahexaenoic acid and *n*-3 highly unsaturated fatty acids. Fish. Sci. 62:372-379.

Furuita, H., T. Takeuchi, and K. Uematsu. 1998. Effects of eicosapentaenoic and docosahexaenoic acids on growth, survival and brain development of larval Japanese flounder (*Paralichthys olivaceus*). Aquaculture 161:269-279.

Furuita, H., H. Tanaka, T. Yamamoto, N. Suzuki, and T. Takeuchi. 2002. Effects of high levels of *n*-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus*. Aquaculture 210:323-333.

Fyhn, H.J., and B. Serigstad. 1987. Free amino acids as energy substrate in developing eggs and larvae of the cod *Gadus morhua*. Marine biology 96:335-341.

Garcia, L.M.B. 1990. Advancement of sexual maturation and spawning of sea bass, *Lates calcarifer* (Bloch), using pelleted luteinizing hormone-releasing hormone analogue and 17a-methyltestosterone. Aquaculture 86:333-345.

Golovanenko, L.F., T.F. Shuvatova, and A.L. Arakelova. 1975. Qualitative evaluation of the sexual products of the bream (*Abramis brama*) from biochemical and histological indicators. Journal of Ichthyology 15:499-502.

Harvey, B.J., and W.S. Hoar. 1979. The theory and practice of induced breeding in fish. IDRCTS21e. Ottawa, Canada.

Harvey, B., J. Nacario, L.W. Crim, J.V. Juario, and C.L. Marte. 1985. Induced spawning of sea bass, *Lates calcarifer*, and rabbitfish, *Siganus guttatus*, after implantation of pelleted LHRH analogue. Aquaculture 47:53-59.

Hoff, F.H. Jr. 1970. Artificial spawning of black sea bass, *Centropristis striata melanus* Ginsburg, aided by chorionic gonadotrophic hormones. Special Scientific Report No. 25. Florida Department of Natural Resources Marine Research Laboratory, St. Petersburg, Florida, USA 1-17.

Howell, R.A., D.L. Berlinsky, and T.M. Bradley. 2003. The effects of photoperiod manipulation on the reproduction of black sea bass, *Centropristis striata*. Aquaculture 218:651-669.

Jackson, J.B.C., M.X. Kirby, W.H. Berger, K.A. Bjornal, L.W. Botsford, B.J. Bourque, R.H. Bradbury, R. Cooke, J. Erlandson, J.A. Estes, T.P. Hughes, S. Kidwell, C.B. Lange, H.S. Lenihan, J.M. Pandolfi, C.H. Peterson, R.S. Steneck, M.J.Tegner, and R.R. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629-638.

Kendall, A.W. 1977. Biological and fisheries data on black sea bass, *Centropristes striatus* (Linnaeus). Sandy Hook Laboratory, NMFS, NOAA. Technical series report(7):1-14.

Kjørsvik, E., A. Mangor-Jensen, and I. Holmefjord. 1990. Egg quality in fishes. Advances in marine biology 26:71-113.

Kjørsvik, E., K. Hoehne-Reitan, and K.I. Reitan. 2003. Egg and larval quality criteria as predictive measures for juvenile production in turbot (*Scophthalmus maximus* L.). Aquaculture 227:9-20.

Lall, S.P. 2000. Nutrition and health of fish. In: L.E. Cruz-Suárez, D. Ricque-Marie, M. Tapia-Salazar, M.A. Olvera-Nova, R. Civera-Cerecedo (Eds.). Avances en Nutrición Acuícola:19-22.

Lam, T.J. 1985. Induced spawning in fish. Pages 14-56 *in* C.S. Lee and I.C. Liao, editors. Reproduction and culture of milkfish. Oceanic Institute, HI.

Lavens, P., E. Leregue, H. Jaunet, A. Brunel, Ph. Dhert, and P. Sorgeloos. 1999. Effect of dietary essential fatty acids and vitamins on egg quality in turbot broodstocks. Aquaculture International 7:225-240.

Lee, C.S., C.S. Tamaru, J.E. Banno, C.D. Kelley, A. Bocek, and J.A. Wyban. 1986. Induced maturation and spawning of milkfish, *Chanos chanos* Forsskal, by hormone implantation. Aquaculture 52:199-205.

Lee, C.S., C.S. Tamaru, and C.D. Kelley. 1988. The cost effectiveness of CPH, HCG and LHRH-a on the induced spawning of grey mullet, *Mugil cephalus*. Aquaculture 73:341-347.

Lee, C.S., and A.C. Ostrowski. 2001. Current status of marine finfish larviculture in the United States. Aquaculute 200:89-109.

Marte, C.L., N.M. Sherwood, L.M. Crim, and B. Harvey. 1987. Induced spawning of maturing milkfish (*Chanos chanos* Forsskal) with gonadotropin-releasing hormone (GnRH) analogues administered in various ways. Aquaculture 60:311-321.

Mercer L.P. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic) -black sea bass. U.S. Fish and Wildlife Service Biological Report 82(11.99):1-16.

Mylonas, C.C., Y. Tabata, R. Langer, and Y. Zohar. 1995. Preparation and evaluation of polyanhydride microspheres containing gonadotropin-releasing hormone (GnRH), for inducing ovulation and spermiation in fish. Journal of Controlled Release 35:23-34.

Mylonas, C.C., and Y. Zohar. 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. Reviews in Fish Biology and Fisheries 10:463-491.

Navas, M., M. Thrush, J. Ramos, M. Bruce, M. Carrillo, S. Zanuy, and N. Bromage. 1995. The effect of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass (*Dicentrarchus labrax*). Pages 108-110 *in* Goetz, F.W., and P. Thomas, editors. Proceedings of the fifth international symposium on the reproduction of fish, Austin, TX.

Navas, J.M., M. Bruce, M. Thrush, B.M. Farndale, N. Bromage, S. Zanuy, M. Carrillo, J.G. Bell, and J. Ramos. 1997. The impact of seasonal alternation in the lipid composition of broodstock diets on egg quality in the European sea bass. Journal of Fish Biology 51:760-773.

Ogata, H.Y., A.C. Emata, E.S. Garibay, and H. Furuita. 2003. Fatty acid composition of five candidate aquaculture species in Central Philippines. Aquaculture (in press).

Quinitio, G.F., N.B. Caberoy, and D.M. Reyes Jr. 1997. Induction of sex change in female *Epinephelus coioides* by social control. The Israeli Journal of Aquaculture-Bamidgeh 49(2):77-83.

Rainuzzo, J.R., K.I. Reitan, and Y. Olsen. 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155:103-115.

Robin, J.H. 1995. The importance of *n*-6 fatty acids in the culture of marine fish larvae. ICES mar. Sci. Symp. 201:106-111.

Rodríguez, C., J.A. Pérez, P. Badía, M.S. Izquierdo, H. Fernández-Palacios, and A.L. Hernández. 1998. The *n*-3 highly unsaturated fatty acids requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/ EPA ratio in the diet. Aquaculture 169:9-23.

Rottmann, R.W., J.V. Shireman, and F.A. Chapman. 1991a. Capturing, handling, transporting, injecting, and holding brood fish for induced spawning. Southern Regional Aquaculture Center Publication 422.

Rottmann, R.W., J.V. Shireman, and F.A. Chapman. 1991b. Determining sexual maturity of broodstock for induced spawning of fish. Southern Regional Aquaculture Center Publication 423.

Rottmann, R.W., J.V. Shireman, and F.A. Chapman. 1991c. Hormone preparation, dosage calculation, and injection techniques for induced spawning of fish. Southern Regional Aquaculture Center Publication 425.

Sargent, J.R. 1995. Origins and functions of egg lipids: Nutritional implications. Pages 353-372 *in* Bromage, N.R., and R.J. Roberts, editors. Broodstock management and egg and larval quality. Blackwell Science Ltd, Osney Mead, Oxford, England.

Sargent, J., G. Bell, L. McEvoy, D. Tocher, and A. Estevez. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177:191-199.

Shepherd, G.R., and J.S. Idoine. 1993. Length-based analyses of yield and spawning biomass per recruit for black sea bass *Centropristis striata*, a protogynous hermaphrodite. Fishery Bulletin 91:328-337.

Shepherd, G.R., and M. Terceiro. 1994. The summer flounder, scup, and black sea bass fishery of the middle Atlantic bight and southern New England waters. NOAA Technical Report NMFS 122:1-13.

Sherwood, N.M., L.W. Crim, J. Carolsfeld, and S.M. Walters. 1988. Sustained hormone release. I. Characteristics of in vitro release gonadotropin-releasing hormone analogue (GnRH-a) from pellets. Aquaculture 74:75-86.

Sherwood, N.M., H.J. Grier, C. Warby, J. Peute, and R.G. Taylor. 1993. Gonadotropin-releasing hormones, including a novel form, in snook *Centropomus undecimalis*, in comparison with forms in black sea bass *Centropristis striata*. Regulatory peptides 46:523-534.

Tamaru, C.S., W.J. FitzGerald, and V. Sato. 1993. Hatchery manual for the artificial propagation of striped mullet (*Mugil cephalus* L.). Guam Aquaculture Development and Training Center 1-167.

Thomas, P., and C.R. Arnold. 1993. Environmental and hormonal control of reproduction in sciaenid fish. Recent Advances in Aquaculture 4:31-42.

Tucker, J.W. Jr. 1984. Research and development communication: Hormone-induced ovulation of Black sea bass and rearing larvae. Progressive fish-culturist 46:201-203.

Tucker, J.W. Jr. 2003. Grouper culture. World Aquaculture 34(3):32-59.

U.S. Department of Commerce. 1991. Status of fishery resources off the Northern United States for 1991. NOAA Tech. Mem. NMFS 83.

Vaughan, D.S., M.R. Collins, and D.J. Schmidt. 1995. Population characteristics of the black sea bass *Centropristis striata* from the southeastern. U.S. Bulletin of Marine Science 56:250-267.

Wallace, R.A., and K. Selman. 1981. Cellular and dynamics aspects of oocyte growth in teleosts. American Zoologist 21:325-343.

Waltz, W., W.A. Roumillat, and P.K. Ashe. 1979. Distribution, age structure, and sex composition of the black sea bass, *Centropristis striata,* sampled along the southeastern coast of the United States. Marine Resources Research Institute South Carolina Wildlife and Marine Resources Department Charleston, SC 29412. Technical Report 43:1-18.

Watanabe, T., and R.V. Vassallo-Agius. 2003. Broodstock nutrition research on marine finfish in Japan. Aquaculture 227:35-61.

Watanabe, W.O., E.P. Ellis, S.C. Ellis, and M.W. Feeley. 1998. Progress in controlled maturation and spawning of summer flounder *Paralichthys dentatus* broodstock. Journal of the World Aquaculture Society 29(4):393-404.

Watanabe, W.O., T.I.J. Smith, D.L. Berlinsky, C.A. Woolridge, K.R. Stuart, K.A. Copeland, and M.R. Denson. 2003. Volitional spawning of black sea bass *Centropristis striata* induced with pelleted luteinizing hormone releasing hormone-analogue. Journal of the World Aquaculture Society 34(3):319-331.

Wenner, C.A., W.A. Roumillat, and C.W. Waltz. 1986. Contributions to the life history of black sea bass, *Centropristis striata,* off the southeastern United States. Fishery Bulletin 84:723-741.

Yang, Y.Y., T.S. Chung, X.L. Bai, and W.K. Chan. 2000. Effect of preparation conditions on morphology and release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method. Chemical Engineering Science 55:2223-2236.

Yang, Y.Y., T.S. Chung, and N.P. Ng. 2001. Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. Biomaterials 22:231-241.

Zohar, Y., and C.C. Mylonas. 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquaculture 197:99-136.

APPENDIX

Appendix A. Example of black sea bass spawning data collection sheets.

Black Sea Bass Spawning Worksheet

IMPLANT DATA: Tank: A Date: / /03

Time: Tank temp: °C

Tag # Female:

Tag # Male:

Hormone Type: LHRH-a or GnRH-a Dosage: 0,5,50, 100 ug/kg

Release rate: Fast or Slow Mean egg diameter: Before:

Expected implant mass: mg After:

Actual implant mass: mg

SPAWNING DATA: Tank: A Date: / /03

Time: Tank temp: °C

Overall Fecundity Estimate

Volume Floaters: mL

Volume Sinkers: mL

Total Volume Eggs: mL

% Floating eggs: %

Fertility:

# fertilized	# abnormal	# dead	# unfert	Total #

(# fert/Total) *100 = % fertilized: %

Date: / /03 Time:

Predominant Cell-stage: # Oil droplets:

Clear: Yes or No Round: Yes or No Smooth: Yes or No

Comments:

COLLECT: Test tube of eggs for measuring: Yes or No **(In 5% Formalin)**

Fatty Acid sample: Yes or No

STOCKING INFO.: 5 mL / Bucket = eggs

Incubator #: Eggs/mL: Total Eggs:

Temp: °C

Incubator #: Eggs/mL: Total Eggs:

Temp: °C

Incubator #: Eggs/mL: Total Eggs:

Temp: °C

Comments:

Black Sea Bass Hatch and First Feeding

HATCH DATA: Date: / /03 Time:

Incubator #: Total Larva: Mean:

Counts/mL: Larva Larva Larva /mL

mL mL mL

Temp: °C % Hatch:

Comments:

Date: / /03 Time:

Incubator #: Total Larva: Mean:

Counts/mL: Larva Larva Larva /mL

mL mL mL

Temp: °C % Hatch:

Comments:

Date: / /03 Time:

Incubator #: Total Larva: Mean:

Counts/mL: Larva Larva Larva /mL

mL mL mL

Temp: °C % Hatch:

Comments:

FIRST FEEDING DATA: Date: / /03 Time:

Incubator #: Total Larva: Mean:

Counts/mL: Larva Larva Larva /mL

mL mL mL

Temp: °C % Survival:

Comments:

Date: / /03 Time:

Incubator #: Total Larva: Mean:

Counts/mL: Larva Larva Larva /mL

mL mL mL

Temp: °C % Survival:

Comments:

Date: / /03 Time:

Incubator #: Total Larva: Mean:

Counts/mL: Larva Larva Larva /mL

mL mL mL

Temp: °C % Survival:

Comments:

Appendix C. Procedure used to extract lipids from black sea bass egg samples, in triplicate to produce and analyze FAMEs.

1. Label and weigh 3 empty vials.
2. Get egg sample from freezer.
3. Use spatula to put equal amounts of eggs into the 3 pre-weighed vials.
4. Re-weigh vials with eggs.
5. Add enough 1:1 Chloroform/Methanol to fill each vial ¼ of the way.
6. Add Nitrogen gas to vial #2 and #3, put cap on and put in refrigerator so no oxidization occurs.
7. Check to make sure there is at least 0.1 g of sample in each vial.
8. Carefully poor eggs from vial #1 into Pyrex hand held homogenizer. Rinse eggs remaining in vial into homogenizer with 1:1 Chloroform/Methanol. Gently crush eggs with plunger for ~5 min. to mechanically breakdown eggs. Remove plunger and rinse sample from it into homogenizer with 1:1 Chloroform/Methanol.
9. Attach homogenizer to ring stand and lower into ultrasonic machine for about 2 min. to further breakdown eggs.
10. Remove from ring stand. Replace plunger and mechanically crush eggs for an additional 1-2 min. Remove plunger and rinse it into the homogenizer with 1:1 Chloroform/Methanol. Place in ultrasonic machine again.
11. Label 3 small round bottom flasks. Pour sample through glass filter funnel into a round bottom flask to remove solids. Use vacuum tube to help pull liquid through filter.
12. Rinse homogenizer with as little 1:1 Chloroform/Methanol as necessary.

13. Prepare rotovap by adding ice, turn water on low, flip switch to turn vacuum on, clamp sample on with plastic clasp, start rotation, open rotovap vacuum, and lower flask into water.
14. Set rotovap at a speed of 35 for ~40 min, or until sample is dry.
15. While 1st sample is drying start with 2nd and 3rd sample. Get them from refrigerator and start with step #8. Continue to check on progress of 1st sample.
16. When sample is dry slowly turn off rotovap vacuum, stop rotation, remove round bottom flask.
17. Make sure all ports of the vacuum in the hood are closed (horizontal). Flip switch to turn vacuum on. Let dial reach 2. Put glass stopper in flask and attach it to vacuum hose. Turn port on, vertical position. Dry sample for ~5 min.
18. Make 3 filter pipets by stuffing 2 small pieces of Kim wipe gently into pipet towards the tip with the tip of a long pipet.
19. Pre-weigh 3 clean, dry round bottom flasks that have been vacuumed and dried with Kim wipes.
20. Take dry sample from hood vacuum. Leave glass plug there for next sample.
21. Rinse sample with 1mL (about one pipet) of chloroform methanol solution (1:1). Do this 2-3 times moving the sample through the filter pipet into a pre-weighed round bottom flask.

22. Dry the sample using the rotovap, then hood vacuum. Dry flask with Kim wipe, and re-weigh sample. Divide dry fatty acid weight by the wet weight of the eggs.
23. Label 3 conical vials with a stir magnet in each.
24. Rinse dried sample twice with a chloroform methanol solution (1:1 by volume, 1 mL) into one of the conical vials.
25. Clamp conical vial in the hood with nitrogen gas blowing through a needle onto the sample. Let dry until most of sample evaporates (~10 min).
26. Fatty acid methyl esters reaction should be done in the hood. Position hot plate with aluminum vial holder near water supply. Attach 3 condensers to each other with hoses, and to the conical vials with yellow plastic clamps. Water goes in through the bottom and out through the top of each condenser. Place thermometer into the hole on the metal plate and secure it.
27. Start water running through condensers. Set heat and stir speed to a little under 3. Watch thermometer to keep temperature 80-110°C.
28. Add NaOH-MeOH (0.5M, 1mL) to sample in the conical vial. Place vials in aluminum plate on hot plate and place condensers on them. Ensure that a good seal is made. Heat for 30 min.
29. Make 2 silica powder filter pipets. Stuff 2 small pieces of Kim wipe gently into pipet towards the tip with a long pipet. Do not pack too tightly. Add silica powder on top of Kim wipe until pipet is half full. Do Not Breathe In Silica Powder.

30. Add boron trifluoride-methanol complex (1.5mL) by autopipeting 0.75mL twice. Heat for 30 min.

31. Let sample cool. Add 1 mL of saturated NaCl by pipet then add 1mL of hexane by pipet. Put lid on and shake well then let settle.

32. Carefully remove hexane layer (top layer) with a pipet so that the salt layer is not disturbed and run it through the silica filter into a ball flask. Repeat #30 and #31 adding saturated NaCl (1 mL) and hexane (1 mL).

33. Add Ether/Hexane (20%, 1mL). Shake and let settle. Remove Hexane layer and run through silica filter. Put Ether/Hexane (20%, 1mL) through column.

34. Rotovap sample until dry.

35. Add of chloroform (300μm) by micropipeter once or twice to the ball flask. Transfer sample by pipet to a clean, labeled GC vial.

36. Add nitrogen gas to vial and screw lid on (Only until rubber begins to suck inward).

37. Store sample in refrigerator until put through GC-MS.

38. After sample run through GC-MS add nitrogen to vial and use a new cap. Archive sample in refrigerator.

BIOGRAPHICAL SKETCH

Allison Elizabeth White was born on February 16, 1977, in Hingham, Massachusetts. She graduated from the College of Charleston in Charleston, South Carolina in May 1999 with a B.S. degree in Marine Biology. She has worked as a research diver at the National Undersea Research Center on a coral reef assessment and monitoring project in Key Largo, Florida for two years. She has been published in the journal, *Marine Pollution Bulletin*. In 2001 she entered the graduate masters program in Marine Science at the University of North Carolina at Wilmington where she worked as a research assistant under the direction of Dr. Wade O. Watanabe. Ms. White graduated in July 2004, and will begin a wildlife biologist position at the South Carolina Department of Natural Resources at Charleston, SC in August 2004.