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Full Paper

Timing of oocyte recruitment and reproductive performance of female hatchery-reared spotted scat (*Scatophagus argus* Linnaeus, 1766) after artificial insemination

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Abstract: Two-year-old spotted scat (*Scatophagus argus*) broodstock [selected mature females (165.0–244.4 g) and males (55.2–122.4 g)] were reared at 10 fish/m^3 in cement tanks and fed to satiation with a 35% protein artificial diet, twice a day until selection, for five consecutive inseminations. The results showed that the timing for oocyte recruitment was 41.6 ± 6.8 days. Most reproductive performance parameters during the first and second artificial inseminations were not significantly different ($P > 0.05$), except for oocyte diameter before hormone injection, ovulation rate and viable eggs for the recruited females during the second insemination (381.3 ± 27.7 µm, 37.5 ± 45.4% and 54.8 ± 36.5% respectively), which were reduced compared with those from the first insemination (422.8 ± 21.0 µm, 72.9 ± 29.4% and 81.3 ± 12.5% respectively). Histological investigations of ovarian development 1 day after stripping showed several residual hydrated oocytes occupying most of the area of the ovaries. After 15 days, most oocytes reached the pre-vitellogenic stage. Vitellogenic oocytes were obviously present after 30 days, while post-vitellogenic oocytes were predominantly located (45.1%) in mature ovaries, 45 days after stripping.

Keywords: spotted scat, oocyte recruitment, reproductive performance, artificial insemination

INTRODUCTION

The spotted scat, *Scatophagus argus* Linnaeus, 1766, is a tropical finfish distributed widely across coastal areas of the Indo-Pacific region [1]. It is a euryhaline species that can thrive in fresh, brackish and sea water, and is highly tolerant and well-adapted to environmental changes, especially salinity and temperature fluctuations [2]. These properties make scat a potential candidate among finfish species for coastal aquaculture [3]. The spotted scat is one of the most highly valued food

Maejo Int. J. Sci. Technol. **2019**, *13*(02), 148-160

fish in countries such as Philippines [4] as well as a popular ornamental, aquarium fish [5] . However, most of these fish are currently caught in the wild, and the quantity of caught fish has been inadequate to meet market demands [4] . Currently, there have been no reports on the mass seed production of this species.

Researchers from many countries such as Philippines [6] , Taiwan [7] , China [8] and Vietnam [9] have attempted to study the reproductive biology of spotted scat and breed them. However, most of them have applied artificial insemination during seed production because this fish has not been reported to spawn in captivity. Generally, after ovulation, the ovaries of the broodstock enter a recovery phase to steadily regenerate new oocytes for the next spawning season. Gonadal development is driven by sex steroids, especially oestradiol, which correlate with oocyte growth [10]. The spotted scat is a multiple-spawner [8], exhibiting group-synchronous oocyte development [11] , with at least two batches of oocytes present in the ovaries during each cycle. In wild fish, larger oocytes spawn naturally during the first breeding cycle, whereas smaller ones will develop and spawn during the next breeding cycle [12] . The stages of gonadal development have been studied in several marine fish species [13-15] , although information regarding the timing or periodicity of oocyte recruitment after breeding and the reproductive performance of newly matured oocytes compared with the previous clutch remains scarce. Understanding the reproductive strategies of individual fish species, especially the timing of gametogenesis, is very useful for hatchery production planning. The ability to accurately count the number of available broodfish and to determine the frequency of egg production is an important factor in attaining the highest level of efficiency for hatchery-reared broodstock [16, 17].

The aim of this study is to determine the timing of oocyte recruitment, the oestradiol profile, and the reproductive performance of female hatchery-reared spotted scat broodstock after artificial insemination and to compare these variables between the first brood and subsequent broods. It tries to provide significant data for the development of artificial insemination protocols for this species, which can be applied to mass seed production in the future.

MATERIALS AND METHODS

Broodstock Rearing and Conditions

Spotted scat fry, obtained through artificial breeding at the Coastal Aquaculture Research Institute (Songkhla, Thailand), were reared indoors for 2 years at a sex ratio of 1:1 in two 28-m^3 cylindrical cement tanks with a semi-recirculating system and constant aeration. The fish were maintained at 10 fish/m^3 (approximately 1 kg/m^3), with at least 500 fish/tank. Fifty per cent of the water was changed every two weeks. The fish were fed to satiation with a commercially available floating artificial diet (Thai Union Feedmill Co., Samutsakhon, Thailand) containing 35% protein twice a day until selection. The water quality was monitored weekly following the standard procedure detailed by American Public Health Association, American Water Works Association and Water Environment Federation [18]. Rearing conditions were as follows: Salinity, 10–30 ppt; temperature, 26–28°C; pH, 7.5–8.1; dissolved oxygen level, 5.4–6.5 mg/L; ammonia level, 0.05–0.13 mg/L; nitrite level, 0.01–0.02 mg/L.

Broodstock Selection

Broodstock fish were starved for one day prior to selection. Sex differentiation was determined by snout shape (the head profile of females has a relatively constant slope, unlike that of males, which displays an obvious curvature) [4]. Female spotted scat broodstock were selected

using the combined criteria of external morphology (swollen abdomen with abdominal width greater than body width) [19] and ovarian biopsy. Oocyte sampling was conducted by inserting a polyethylene tube (0.5-mm diameter) connected to a 0.1-mL syringe into the approximate central region of one ovary through the ovarian cavity. Hormone injections were then performed to determine the oocyte developmental stage and oocyte diameters were measured using ocular and stage micrometers and a compound microscope with 40× magnification. The diameters of 10 oocytes from each female were measured [16]. Females that achieved a mean oocyte diameter larger than 350 μm and males with expressible milt present after gentle abdominal stripping were identified as mature fish [4] and were selected for artificial breeding. Selected female broodstock, measuring 16.8–18.6 cm in total length and 165.0–244.4 g in weight, was maintained separately in 20-litre floating baskets placed into a 1,000-litre plastic tank, with constant aeration. Male broodstock, measuring 12.1–16.1 cm in total length and 55.2–122.4 g in weight, was maintained in the same tank as the females but allowed to swim freely in the tank.

Artificial Insemination and Reproductive Performance Parameters

Artificial insemination was conducted six times (the final insemination was performed to facilitate histological investigation of oocyte recruitment), at intervals of 1–2 months. After the mature broodstock was selected (a maximum of ten mature females each time), ovulation was induced in all selected females using a single intramuscular hormone injection of a luteinising hormone-releasing hormone analogue (Suprefact, Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany) at 20 μg/kg. The broodfish were examined for signs of ovulation every 30 min.–2 hr, beginning at 32 hr after injection depending on the degree of external abdominal swelling, through the application of gentle abdominal massage. The eggs of each ovulated fish were manually stripped and fertilised immediately with fresh pooled milt collected from 3-4 running males with good sperm motility. The latency time (time from injection to ovulation) of each broodfish was recorded. The ovulation rate (100 × number of ovulating females within 42 hr/number of injected females) for each breeding session was determined. The buoyancy rate (100 × mL of buoyant eggs/total mL of eggs) of the stripped eggs was measured within 20 min. after the eggs were transferred to a 500-mL volumetric flask containing 30 ppt sea water [20]. Oocyte diameters were also determined before hormone injection. The percentage of viable eggs (100 × number of perfectly spherical, translucent eggs without a perivitelline space/total number of sampled eggs) [21] was determined by the microscopic examination of approximately 100 eggs per fish.

The following breeding performance variables were also investigated: fertilisation rate (100 × number of eggs at the 4-8 cell-stage/total number of eggs), determined under a binocular microscope 45-60 min. after incubation; hatching rate (100 × number of larvae/total number of eggs), determined after one day of incubation at 26–28 °C; and absolute fecundity (number of eggs obtained from stripping per fish), determined by counting a subsample (1 mL of eggs collected with a 1-mL syringe) in triplicate, counting the eggs, and multiplying the number of eggs in 1 mL by the total volume of ovulated eggs. The relative fecundity was calculated as the number of eggs obtained from each stripping per g body weight. Larval quality was monitored based on the following parameters: 1) total length of day-0 larvae (6–9 hr post hatch), measured under a binocular microscope using an ocular and stage micrometer (n = 20); 2) larval abnormality rate (abnormal larvae were characterised by vertebral deformities), determined under a binocular microscope (100 × number of abnormal larvae/total number of larvae) (n = 100); and 3) survival rate of day-3 larvae (100 × number of surviving larvae/initial number of larvae) [20], based on the average number of

surviving larvae 3 days after hatching, which was calculated from three replicates, reared at a density of 15 larvae/L in 2-litre plastic containers without aeration or feeding.

After ovulation and oocyte stripping, all broodfish were tagged with passive-integrated transponder tags inserted into the dorsal musculature for broodstock identification before being moved back to the broodstock tank, where they were maintained separately in a 1-m^3 floating net cage under the same conditions as for the initial maturation. The tagged ovulating females were reared under the same conditions as those used during the first insemination until oocyte recruitment. Non-ovulating females during the first induction were discarded, whereas ovulating brooders were checked for oocyte recruitment every 2 weeks, using the same criteria used for broodfish selection. When each fish reached the recruitment gravid stage, the second artificial insemination was conducted and all data were recorded using the same methods and variables as those used for the first artificial insemination. The timing of oocyte recruitment between the first and second maturation in this study was recorded as the period between the two inseminations. The experiment was ended when all females had been induced for breeding twice, with the exception of those broodfish that were injected only once due to death or observed oocyte recruitment failure (abdominal width did not increase within one month).

Sex Steroid Hormone Levels

After insemination, the female broodfish were divided into four groups, based on the period after egg stripping: 1, 15, 30 and 45 days. Blood samples were taken from the caudal vein of four stripped females in each group after insemination and centrifuged at 6,000 rpm for 5 min. at 4°C (Beckman, Avanti^{TM} 30 Centrifuge). The serum was separated and stored at -20°C for the determination of sex steroid hormone concentration (17β-oestradiol: E2) by electrochemiluminescence immunoassay, using an Elecsys Estradiol III kit (Roche Diagnostics, Germany) with an immunoassay analyser (Modular Analytics E170) according to the manufacturer's instructions. The sensitivity of the assay was 5.0 pg/mL.

Ovarian Histology

After the ovulated eggs were stripped for the last time, ten fish were maintained in the broodstock tank. Two fish were randomly sampled and sacrificed for ovary collection at days 1, 15, 30 and 45 after egg stripping. Small fractions of the central regions of the ovaries were removed from the body cavity, placed in 10% buffered formalin, dehydrated with gradient alcohol and cleared with xylene before being embedded in paraffin. Sections were cut at 3–5-µm thickness and stained with haematoxylin and eosin. Histological investigation divided the maturation of the ovaries into six stages, depending on diameter [22], combined with morphological features of the dominant oocytes from hatchery-reared spotted scat [23] (Table 1). Subsequently, the diameter of ten largest oocytes (residual hydrated oocytes were not included) and the percentage of oocytes observed in each stage for each sample from a permanent slide were measured with an ocular micrometer, using a 40× light microscope. The development of the oocytes was observed and photographs were taken using a digital camera. The following four stages of spotted scat oocyte development were determined, as suggested by Shao et al. [24] and Zhang et al. [25]: pre-vitellogenic oocyte (O1), vitellogenic oocyte (O2), post-vitellogenic oocyte (O3) and hydrated oocyte (HO).

Maejo Int. J. Sci. Technol. **2019**, *13*(02), 148-160

Table 1. Maturity stages of spotted scat ovaries, according to oocyte size and development

Ovary stage	Oocyte size (μm)[I]	Most advanced group of oocytes[II]
Immature	≤20	pre-vitellogenic oocyte: chromatin nucleolus
Developing	21–100	pre-vitellogenic oocyte: perinucleolus
Maturing	101–300	vitellogenic oocyte: cortical alveoli
Mature	301–600	post-vitellogenic oocytes
Spawning	601–750	full of hydrated oocytes
Spent	<750	small number of hydrated oocytes

[I] modified from Barry and Fast [4] and Gandhi et al. [22]
[II] Ruensirikul et al. [23]

Statistical Analysis

Reproductive parameters and timing of oocyte recruitment are expressed as mean ± standard deviation (SD). Statistical differences for all variables between the first and second artificial inseminations were determined using paired Student's *t*-tests, with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Timing for Oocyte Recruitment

The mean oocyte recruitment timing measured for a total of 19 female spotted scat broodstock after oocyte stripping ranged from 35.0 ± 15.0 to 51.9 ± 28.2 days (average 41.6 ± 6.8 days) (Figure 1) across five artificial insemination sessions. The shortest and longest periods for the development of a new batch of oocytes in the ovaries were 21 days and 60 days respectively.

Data regarding the timing of oocyte recruitment in hatchery-reared fish after spawning or after being stripped for artificial breeding are limited. Morehead et al. [10] found that the timing of egg production in captive striped trumpeter (*Latris lineata*) was 22–37 days, which was slightly shorter than the period observed in this study. Hatchery-raised meagre (*Argyrosomus regius*) broodstock were found to have similar egg production timing as that of the spotted scat, as vitellogenesis in this species was completed within 2 months [16], whereas in some species such as the bighead carp (*Aristichthys nobilis*), longer than 2 months (71–107 days) was required for oocyte recruitment [26]. The annual reproductive period for fish depends on the reproductive strategy of each species. In species with group-synchronous spawning the reproductive cycle has been found to be shorter, whereas it appears to be longer in asynchronous spawning fish. In addition, germ cell renewal has been observed to be more intense during the annual reproductive cycle occurring during the regenerative phase and development phase but become sharply reduced during the spawning phase [27]. The regenerative phase is the recovery phase when new oocytes are generated for the next spawning or for the next stripping, when artificial insemination is applied.

The oocyte recruitment periods observed for the female broodstock in this study fitted well with the seed production cycle of this species, the timing for the development from day-1 larvae into juveniles being 40–45 days [7]. This timing enables efficient utilisation of hatchery facilities, especially for small-scale hatcheries which may plan to sell or move juveniles that are produced after the first insemination before inducing ovulation for the next breeding cycle.

Maejo Int. J. Sci. Technol. **2019**, *13*(02), 148-160

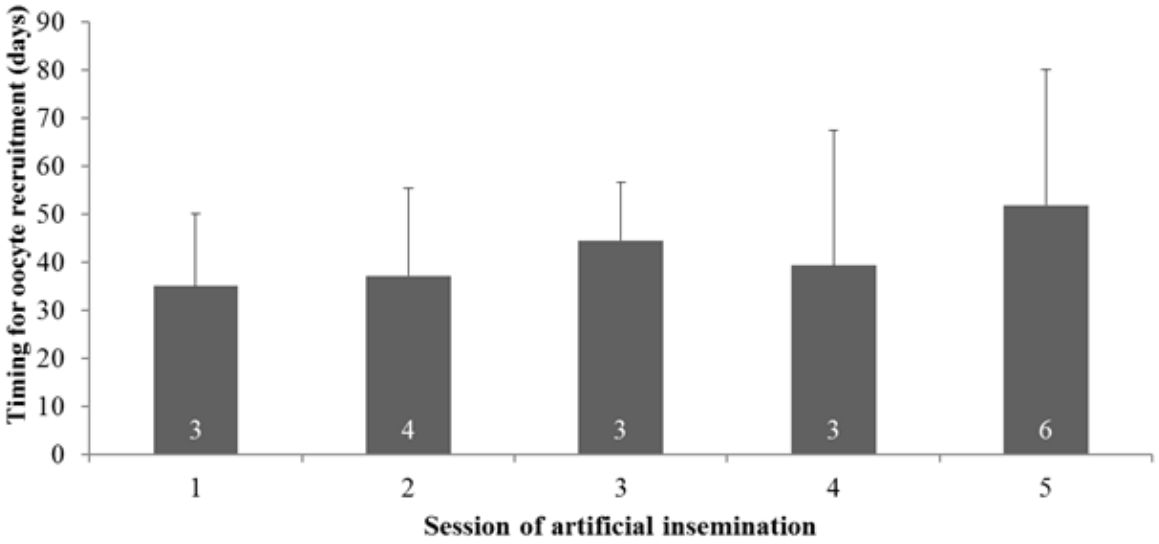

Figure 1. Timing of oocyte recruitment for hatchery-reared spotted scat (*Scatophagus argus*) during five artificial insemination sessions (number inside each bar indicates number of fish that undergo oocyte recruitment for each cycle.)

Reproductive Performance

The reproductive performance variables are presented in Table 2. There were no significant differences between the first and second artificial inseminations (after oocyte recruitment) for almost all of the examined parameters ($P > 0.05$), except for oocyte diameter before hormone injection, ovulation rate and per cent viable eggs from recruited females after ovulation (381.3 ± 27.7 µm, 37.5 ± 45.4% , and 54.8 ± 36.5% respectively), which were reduced in the second insemination compared with the first (422.8 ± 21.0 µm, 72.9 ± 29.4% and 81.3 ± 12.5% respectively). The reproductive performance variables after ovulation that did not differ between the first and second inseminations were latency time (38.6 ± 2.1 hr and 38.3 ± 1.0 hr respectively), buoyancy rate (65.0 ± 15.4% and 64.1 ± 64.6% respectively), and oocyte diameter of viable eggs (622.4 ± 20.3 µm and 624.4 ± 40.2 µm respectively). The respective breeding performance parameters measured after the first and second artificial inseminations was: fertilisation rate, 50.7 ± 24.4% and 48.2 ± 36.5%; hatching rate, 63.0 ± 19.4% and 67.1 ± 36.7%; absolute fecundity, 197.2 ± 75.7 × 10^3 eggs/fish and 177.5 ± 27.0 × 10^3 eggs/fish; and relative fecundity, 348.1 ± 133.4 eggs/g fish and 426.7 ± 313.3 eggs/g fish. The respective larval qualities from the first and second artificial inseminations were assessed: length of day-0 larvae, 1.88 ± 0.1 mm and 1.83 ± 0.1 mm; larval abnormality rate, 3.3 ± 4.3% and 2.5 ± 2.9%; survival rate to day 3, 69.3 ± 15.1% and 62.6 ± 41.4%.

Some of the reproductive performance parameters between the two consecutive inseminations were insignificantly different. Thus, these results indicated that the quantity and quality of the gonadally-recruited broodfish did not decline when the same broodfish were re-inseminated, which would be beneficial for seed production planning in this species. Although the spotted scat is not currently a domesticated species, and the broodstock remain unable to spawn naturally while in captivity, they are able to continually produce reproductive cells under captive conditions. These results provide evidence that the spotted scat can adapt well to an artificial environment, which is similar to the findings of previous research [3].

Maejo Int. J. Sci. Technol. **2019**, *13*(02), 148-160

Table 2. Reproductive performance variables (mean ± SD) of hatchery-reared spotted scat after first artificial insemination and when reared until oocyte recruitment (second insemination)

Reproductive performance parameter	First insemination	Insemination after oocyte recruitment
Before injection	*(n = 32)*	*(n = 32)*
Oocyte diameter (μm)	422.8 ± 21.0	381.3 ± 27.7*
After ovulation	*(n = 12)*	*(n = 12)*
Latency time (hr)	38.6 ± 2.1	38.3 ± 1.0
Ovulation rate (%)	72.9 ± 29.4	37.5 ± 45.4*
Buoyancy rate (%)	65.0 ± 15.4	64.1 ± 64.6
Oocyte diameter (μm)	622.4 ± 20.3	624.4 ± 40.2
Viable eggs (%)	81.3 ± 12.5	54.8 ± 36.5*
Breeding performance		
Fertilisation rate (%)	50.7 ± 24.4	48.2 ± 36.5
Hatching rate (%)	63.0 ± 19.4	67.1 ± 36.7
Absolute fecundity (× 10^3 eggs/fish)	197.2 ± 75.7	177.5 ± 27.0
Relative fecundity (eggs/g fish)	348.1 ± 133.4	426.7 ± 313.3
Larval quality		
Length of larvae on day 0 (mm)	1.88 ± 0.1	1.83 ± 0.1
Larval abnormality rate (%)	3.3 ± 4.3	2.5 ± 2.9
Survival rate to day 3 (%)	69.3 ± 15.1	62.6 ± 41.4

* Significant differences ($P < 0.05$)
n = number of broodfish (data from fish that did not experience a second breeding were discarded.)

However, in the present study some parameters were significantly different between the first and second inseminations, such as egg size before the hormone injection, the ovulation rate, and the percentage of viable eggs, which were inferior during the second insemination compared with the first. These results may be due to accumulated stress of broodfish after the first stripping because they were maintained in a small floating net cage inside the broodstock tank after stripping for more convenient monitoring of further maturation. Fish stress clearly affects reproductive capability, especially gamete quality as judged by decreased egg sizes or low hatching rates [28, 29]. Furthermore, captive stress can cause oocyte developmental dysfunctions. Imanaga et al. [15] found that 60% of captive females failed to undergo vitellogenesis.

Moreover, in the present study the subsequent effect of decreased egg size before hormone inducement may cause inconsistent ovulation rates and egg viability among the recruited females. Thus, the average ovulation rate of recruited broodfish during the second insemination period was almost half of that during the first insemination period. Spedicato et al. [30] showed that one indicator of the success of hormone-induced spawning in dusky grouper was the initial oocyte diameter (vitellogenic oocyte), which should be larger than 420 μm. This was very similar to the average oocyte diameter of 363 μm found for spotted scat broodfish in an earlier study in which only 10% of the broodfish were successfully artificially bred [23]. However, Mylonas et al. [31] found that in multi-batch group-synchronous fish the egg size may decline with subsequent spawning events. Therefore, under optimal environmental and nutritional conditions, spotted scat broodstock reared in captivity can provide adequate eggs and have the potential for sustainable seed production.

Maejo Int. J. Sci. Technol. **2019**, *13*(02), 148-160

Sex Steroid Hormone Levels and Oocyte Sizes

One day after stripping, the average serum E2 level of brooders was 55.1 ± 19.5 pg/mL and it peaked significantly 15 days after stripping (657.5 ± 201.9 pg/mL). After that, the E2 level gradually decreased to 355.9 ± 147.5 pg/mL and 192.7 ± 47.3 pg/mL, 30 and 45 days after breeding respectively. However, the E2 levels of the female fish 30 days after stripping were not significantly different from the peak value at 15 days after stripping ($P > 0.05$) (Figure 2a). The oocyte diameter of the female fish at 1 day after stripping (46.1 ± 7.9 µm) and 15 days after stripping (45.7 ± 11.1 µm) did not change. Thereafter, the diameter increased gradually from 30 days after stripping (104.4 ± 18.2 µm) to 45 days after stripping (319.8 ± 76.5 µm), which was significantly larger ($P < 0.05$) than at 1, 15 and 30 days after egg stripping (Figure 2b).

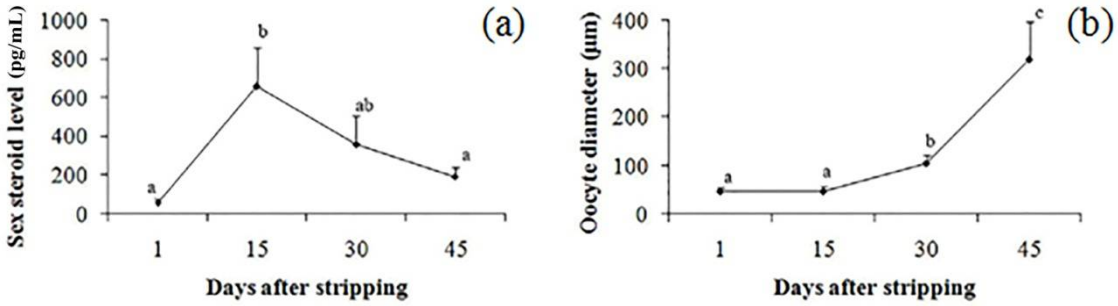

Figure 2. Serum sex steroid (17β-oestradiol) level (a) and oocyte diameter (b) of hatchery-reared spotted scat (*Scatophagus argus*) after stripping. Error bars represent SD (n = 4 for sex steroid, n = 10 for oocyte diameter). Bars with the same letters are not significantly different ($P > 0.05$).

The E2 level results confirmed that the spotted scat experiences group-synchronous ovary development (successive clutches of germ cells that will mature and be spawned are recruited from a population of vitellogenic oocytes) because the highest level of E2 was observed in the post-ovulating broodfish, which is similar to the results reported by Utoh et al. [32] who studied the Japanese conger, *Conger myriaster*. However, these results contrast with those of other studies, in which the E2 level typically peaked during the vitellogenesis phase [33, 34]. In the present study the E2 level peaked 15 days after stripping (Figure 2a), during which time the diameter of the recruited oocytes had not yet increased and remained similar to that observed 1 day after stripping. However, the development of oocytes for the next period was observed after the initial E2 level enhancement. Utoh et al. [32] suggested that the early peak in the E2 level was likely the result of secretion from the remaining vitellogenic oocytes, although oocytes in this stage were rarely observed in the present study. Most of the remaining oocytes during this period were perinucleolar and residual hydrated oocytes. However, hatchery-reared broodfish easily undergo reproductive dysfunction that can affect their hormonal profiles [15]. Thus, the captive scat broodstock used in this study might have suffered captive stress, which might have affected the E2 profile. This possibility is supported by the oocyte size not exceeding 400 µm in diameter even when mature, unlike the mature oocyte size observed in wild scat (430-460 µm) [22].

Ovarian Histology

Histological investigation of ovaries collected 1 day after stripping showed that the spent ovaries (post-stripping) contained several residual hydrated oocytes (6.8% by number), which were

Maejo Int. J. Sci. Technol. **2019**, *13*(02), 148-160

sparsely scattered within the ovaries. In addition, most of the hydrated oocytes were irregular in shape. Pre-vitellogenic oocytes, primarily perinucleolus oocyte (93.2%), were located near the ovary wall while most of the inner area of the ovaries was empty (Figure 3a and Table 3). Post-vitellogenic oocytes (0.1%) and several post-ovulatory follicles were found in the ovaries during this period in one of two broodfish examined (Figure 3b and Table 3). After 15 days, the ovaries were classified as being in the developing stage; however, oocytes in the pre-vitellogenic stage, which were primarily perinucleolar (99.3%), still occupied most of the ovary area. These oocytes were located inside the well-developed ovarian lamellae. However, some hydrated oocytes were still found (0.7%) in one of the two broodfish observed. During this period, post-ovulatory follicles were completely absent (Figures 3c and d). At 30 days after stripping, the oocytes were more developed and the proportion of pre-vitellogenic oocytes (71.7%) decreased while that of vitellogenic oocytes (25.0%) increased (Figure 3e and Table 3), with some oocytes reaching the post-vitellogenic stage (3.3%). At 45 days after striping, the ovaries were dominated by post-vitellogenic oocytes (45.1%), which occupied most of the area of the ovaries while pre-vitellogenic oocytes (52.9%) and vitellogenic oocytes (2.0%) continued to decrease in number (Figure 3f), showing signs of maturation.

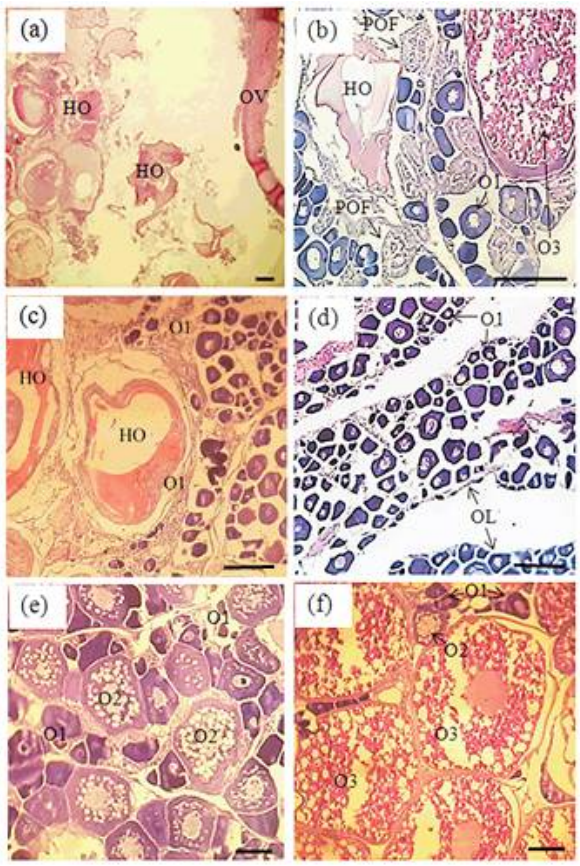

Figure 3. Histological haematoxylin-eosin-stained sections of hatchery-reared spotted scat (*Scatophagus argus*) ovaries: spent ovaries (a and b) 1 day after stripping; developing ovaries (c and d) after 15 days; maturing ovaries (e) after 30 days; mature ovaries (f) after 45 days. HO = hydrated oocytes; OV = ovary wall; O1 = pre-vitellogenic oocytes; O2 = vitellogenic oocytes; O3 = post-vitellogenic oocytes; OL = ovarian lamellae; POF = post-ovulatory follicles. Scale bar = 100 µm.

Maejo Int. J. Sci. Technol. **2019**, *13*(02), 148-160

Table 3. Oocyte size and percentage of oocytes (mean ± SD) at each stage of development in hatchery-reared spotted scat females after egg stripping

Days after stripping	Ovary stage	Oocyte size[I] (μm)	Percentage of oocytes at each stage (%)[II]				POF
			O1	O2	O3	HO	
1	spent	46.1 ± 7.8	93.2 ± 4.8	0.0	0.1 ± 0.04[III]	6.8 ± 4.8[IV]	present[III]
15	developing	45.7 ± 11.1	99.3 ± 1.3[IV]	0.0	0.0	0.7 ± 1.3[III]	not found
30	maturing	104.4 ± 18.2	71.7 ± 9.3	25.0 ± 10.6[IV]	3.3 ± 1.4	0.0	not found
45	mature	319.8 ± 76.5	52.9 ± 11.5	2.0 ± 1.7	45.1 ± 13.3[IV]	0.0	not found

[I] Oocytes of largest size group in ovaries from one broodfish
[II] Average from two broodfish per day after stripping
[III] Found in only one (of two) broodfish
[IV] Oocytes that occupy greatest area in ovaries among two broodfish
O1=pre-vitellogenic oocyte; O2 = vitellogenic oocyte; O3 = post-vitellogenic oocyte; HO = hydrated oocyte; POF= post-ovulatory follicle

The results obtained from ovarian histological analyses clearly showed that the new oocytes were firmly established inside the ovarian lamellae, whereas the formerly mature oocytes degenerated within 15 days after stripping. These findings are consistent with previous reports, suggesting that the ovarian development of spotted scat is group-synchronous [11], and therefore, scat can spawn several times during the spawning season [8].

CONCLUSIONS

The results of this study have shown that the timing of oocyte recruitment in hatchery-reared spotted scat is 41.6 ± 6.8 days. The vitellogenic oocyte diameters, ovulation rates and percentages of viable eggs of oocyte-recruited females are reduced compared with those of matured broodfish harvested the first time (P<0.05), whereas the other reproductive performance parameters between the first and second artificial inseminations are not significantly different. The information obtained from this study may be beneficial for the hatchery management of spotted scat, which is gradually gaining economic importance in South-east Asia.

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Maejo Int. J. Sci. Technol. **2019**, *13*(02), 148-160

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