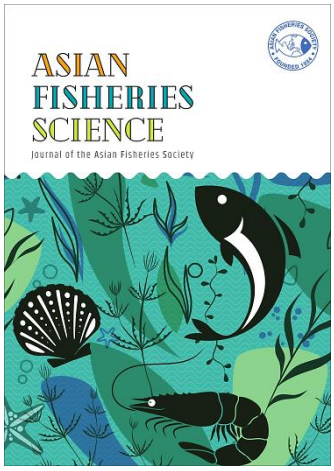

Induced Spawning of Giant Trevally, *Caranx ignobilis* (Forsskål, 1775) using Human Chorionic Gonadotropin (hCG) and Luteinising Hormone-releasing Hormone Analogue (LHRHa)

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Abstract

The giant trevally, *Caranx ignobilis* (Forsskål, 1775) was induced spawned at the National Fisheries Research and Development Institute, Taal, Batangas, Philippines to assess the possibility of captive breeding. *Caranx ignobilis* captive broodstock, six to eight-year-old (4.83 kg mean wt) were injected with: (a) 1,000 IU human chorionic gonadotrophin (hCG).kg^{-1}; (b) 100 µg luteinising hormone-releasing hormone analogue (LHRHa).kg^{-1}; (c) 5 mg carp pituitary extract (CPE).kg^{-1}; and (d) No injection (Control). Ripe female fish was given two sequence intramuscular injections at 0600 to 0700 h and 1800 to 1900 h while male fish was given half dose injection at 1800 to 1900 h. One breeding set (1 female: 2 males) were released to spawn spontaneously in each of the four units of 40-ton circular tanks, done in five replications. Successful spawning was achieved in LHRHa- and hCG-injected fish at 28–30 g.L^{-1} salinity and 27.6–29.25 °C in March to April of 2011 and 2012. Spawning occurred 24–52 h after 2nd injection and eggs hatched in 11–17 h. Fertilisation rate was significantly higher ($P < 0.05$) in LHRHa treatment (60.88 %) than hCG treatment (30.53 %). Mean spawned eggs, hatching rate and mean larvae produced were not statistically different ($P > 0.05$) between hCG (223,068 eggs.kg^{-1} breeder, 43.06 % and 41,547 larvae.kg^{-1} breeder, respectively) and LHRHa treatment (176,524 eggs.kg^{-1} breeder, 71.07 % and 56,040 larvae.kg^{-1} breeder, respectively). This is the first recorded captive breeding of *C. ignobilis* in the Philippines and an initial step towards developing the seed production technology for this new aquaculture species.

Keywords: carangid species, captive breeding, hormone induction, seed production

Introduction

The giant trevally, *Caranx ignobilis* (Forsskål, 1775) of the Family Carangidae, is a highly prized food fish and game fish widely distributed throughout the tropical and sub-tropical regions of the Indian and Pacific Oceans (Smith-Vaniz, 1999). One of the most delicious food fishes in many parts of the world, the giant trevally is widely sought by commercial fishermen and professional game fish anglers because of its high market price and wide consumer preference (Honebrink, 2000). Due to its commercial importance and suitability for culture in captivity, *C. ignobilis* has been identified as a potential new species for aquaculture in several countries (AQUACOP et al., 1989; Liao, 2000; Abdussamad et al., 2008; Alaira and Rebancos, 2014). In the Philippines, *C. ignobilis* which is locally known as "maliputo", is considered as a highly prized food fish and most popular indigenous migratory fish in Lake Taal, Philippines (Herre, 1958; Mercene, 1997; Mutia et al., 2004). Its development for aquaculture has always been one of the priority programs of the government (CNIFDP 2006) through its "maliputo" (*C. ignobilis*) development program.

Production of seedstock is a continuing bottleneck for many marine fish species, and a major challenge in marine finfish hatchery technology is related to the complex life cycles of marine fish compared to freshwater fish (Watanabe et al., 2019). One of the limiting factors in the development of *C. ignobilis* as an aquaculture species is the lack of a seed production technology for at present, wild sourced seedstock has been used for culture. Although Taiwan is reported as

having hatchery-bred *C. ignobilis* produced by the private industry for its aquaculture (Liao, 2000), published literature on captive spawning of this species is lacking. Hence, research on the spawning of *C. ignobilis* is important in the development of a hatchery technology that will support the need for quality fingerlings for aquaculture.

Several carangid species closely related to *C. ignobilis* have been bred successfully either through natural spawning or administration of synthetic hormones. Successful spawning was observed in *Caranx melampygus* Cuvier, 1833 (Moriwake et al., 2001); *Trachinotus carolinus* (Linnaeus, 1766) (Hoff et al., 1972; Weirich and Riley, 2007); *Trachinotus blochii* (Lacepède, 1801) (Gopakumar et al., 2012); *Seriola quinqueradiata* Teminck & Schlegel, 1845 (Nagakura et al., 2003; Hamada and Mushiake, 2006); *Seriola dumerili* Risso, 1810 (Diaz and Garcia, 2001; Jerez et al., 2006; Fernandez-Palacios et al., 2013); *Seriola rivoliana* Valenciennes, 1833 (Roo et al., 2012; Fernandez-Palacios et al., 2015); *Seriola lalandi* Valenciennes, 1833 (Moran et al., 2007; Stuart and Drawbridge, 2012); and *Trachurus japonicus* Temminck & Schegel, 1844 (Nyuji et al., 2013). Taking a cue from the successful development of the hatchery technology of these carangid species, the present study aims to breed captive *C. ignobilis* using commercially available induced spawning agents. The objective of the study is to artificially spawn *C. ignobilis* in captivity as an initial step towards the development of the seed production technology and its promotion as a new species for aquaculture. The successful breeding and artificial propagation of the species will also be useful in stock enhancement.

Materials and Methods

Broodstock development

The study used sexually mature, six to eight-year-old *C. ignobilis* captive broodstock, which were selected from the pool of breeders maintained at the National Fisheries Research and Development Institute (NFRDI) in Taal, Batangas, Philippines. The *C. ignobilis* breeders were developed by on-growing fingerlings (mean total length, 5 cm) collected from the wild from Balayan Bay, Taal, Batangas, Philippines and reared to sexual maturity in earthen ponds of NFRDI (1,294 and 1,308 m^{-2} area) at a density of 1 fish per 5–8 m^{-2}. From fingerling size to sub-adult size, the fish were fed chopped trash fish mixed with vitamins at 5 % body weight, which was rationed twice daily at 8 AM and 3 PM. From three years and onwards, fish were fed chopped trash fish and squid mixed with squid oil, egg yolk and vitamins at 3 to 5 % of the total body weight given twice daily. Good water quality in ponds was maintained through weekly water change at 40–50 % exchange rate and removal of benthic aquatic weeds once or twice a week. Water parameters in broodstock ponds were 27.7 to 32.2 °C, 27.3 to 35.1 ppt salinity, 4.1 to 9.0 ppm dissolved oxygen and pH of 7.12 to 8.8, which were within the desirable range for *C. ignobilis* (von Westernhagen, 1974; Graham and Castellanos, 2005).

Selection of ripe breeders for spawning

Ripe breeders used in the spawning experiments during the months of March to April were selected through gonadal biopsy cannulation. The breeders were collected from the pond using soft, fine-meshed seine net, preventing physical injury and scratches on the fish skin. Each fish was sedated in a polyethylene tank (80 cm × 55 cm × 30 cm depth) filled with 120 L water with 200 ppm tricaine methanesulfonate anaesthetic (MS-222; Argent Chemical Laboratories, Redmond, Washington). After 2–3 min in the water containing the anaesthesia, the fish is hauled out of the water and gonad samples were obtained by biopsy, through the insertion of a plastic cannula (1 mm inner diameter, 3 mm outer diameter, 30 cm length; one end slightly cut in 30° angle pointed end) into 3–8 cm deep of the urogenital opening of the fish. Gonad samples were aspirated by mouth via the cannula and blown into a petri dish. Oocyte samples were added with 5–10 drops of clearing agent Serra's fluid and viewed under the microscope (Nikon Eclipse E100) at 100× magnification. Oocyte diameter was measured using an eyepiece micrometre. Germinal vesicle migration stage of at least 100 oocytes were recorded. Female broodstock with more than 50 % of the oocytes at 0.4 to 0.55 mm diameter and at least 60 % of the oocyte samples at the germinal vesicle migration (GVM) stage or nucleus migrating toward the periphery of the cell, were considered as ripe and ready for spawning. Meanwhile, cannulated milt was checked for sperm motility under the microscope. Milky viscous white milt has high sperm motility and males with this milt were selected for spawning. There was no notable physical difference between males and females but there was a distinct difference in the urogenital opening during the spawning season, with the female exhibiting protruding, concentric genitalia while the male having mildly depressed, slit-like opening.

After cannulation biopsy, each fish was placed in a broodfish carrier or mini-hammock made of canvass cloth and immediately weighed, measured for body length and depth and individually tagged with coloured yarn loosely tied at the caudal peduncle. Fish was placed in 200 L oblong plastic tank (80 cm × 55 cm × 30 cm depth) filled with pond water and transferred to a designated conditioning net enclosure (5 m × 10 m) in the pond at a density of not more than 10 fish per conditioning area. Females were confined in separate conditioning areas from the males. Fish was hauled from the oblong tank and revived from sedation by submerging the fish in the pond water while still being held by hands and vigorously splashing pond water into the gills to remove residual anaesthetics. The fish recovered

from sedation in about 2–5 min. Selected ripe female and male broodstock were allowed to rest in the conditioning net enclosure in the pond for one to two days prior to hormone injection. Conditioning area was provided with vigorous aeration using 2 HP ring blower and fountain-type continuous water agitation from ½ HP submersible pump. Fish were fed once in the morning after the cannulation biopsy and during the rest period but were not fed in the afternoon prior to hormone injection.

Induced spawning

For this study, 20 ripe female broodstock (mean weight, 4.83 kg) and 40 males (mean weight, 4.76 kg) were selected to compare the effect on spawning of *C. ignobilis* of the three types of hormones: (1) 1,000 IU.kg^{-1} human chorionic gonadotropin (hCG, Argent Chemical Laboratories, Redmond, Wa.); (2) 100 µg.kg^{-1} luteinising hormone releasing- hormone analogue (LHRHa, Argent Chemical Laboratories, Redmond, Wa.); (3) 5 mg.kg^{-1} carp pituitary extract (CPE, Argent Chemical Laboratories, Redmond, Wa.); and (4) No injection as the Control. Five replicate runs were conducted for this study, with each run composed of four breeding sets (1 female and 2 males per set), representing the four treatments being compared. In each replicate runs, female broodstock had similar stage of gonadal development with no significant difference ($P > 0.05$) in oocyte diameter and maturity stage. The study was conducted in the months of March to April of 2011 and 2012 at the National Fisheries Research and Development Institute-National Fisheries Biological Center in Taal, Batangas, Philippines.

Prior to hormone injection, individual broodstock was sedated with 200 ppm MS-222. For the female, a two sequence injection was used with the priming dose injection (50 % of the hormone dosage) done in the morning at 0600 to 0700 h followed 12 h later by a resolving dose injection (remaining 50 % of the dosage) in the afternoon at 1800 to 1900 h. Male broodstocks were injected with 50 % of the required hormone dosage in the afternoon at 1800 to 1900 h. The injection was given intramuscularly between the dorsal fin and lateral line using a 10 ml hypodermic syringe. After the final injection, fish was hauled out of pond, transported in 200 L oval-shaped plastic tank (80 cm × 55 cm × 30 cm depth) with 100 L pond water and transferred to the spawning tank located about 20 meters from the pond. Injected female broodstock were randomly distributed and released to 4 units of 40-ton circular spawning tanks (5 m diameter, 2.5 m water depth), each tank with one female. Two broodstock males of the same hormone injection were then released to each tank to give a sex ratio of 1 female: 2 males. One breeding set of uninjected broodstock were also released into a 40-ton spawning tank.

Seawater supply used for *C. ignobilis* spawning, egg hatching and larval rearing was filtered through a series of filter bags (5 µ and 1 µ) into a reservoir, treated with 5 ppm hypochlorite then neutralised by 10 ppm sodium thiosulfate after 12 h and finally chelated by 5 ppm EDTA after six hours.

Spawning and hatching of fertilised eggs

Spawning behaviour was monitored right after stocking into the tanks and every six h thereafter. Spawning took place at 24 to 52 h after second hormone injection. Each spawning tank had egg collecting chambers (1m × 1m × 1m) on two opposite sides, consisting of inverted mosquito net made of 500 µ organza cloth where spawned eggs were collected. At 20 h after the second injection, monitoring of tanks was done every hour for the presence of eggs and to determine spawning time. Upon spawning, flow-through seawater was provided to the spawning tanks and spawned eggs were collected at the egg collecting chambers. Eggs were collected and transferred to a 10 L bucket filled with seawater. Fertilised eggs floated in water while unfertilised eggs sank to the bottom. Unfertilised eggs were siphoned out into a filter screen (20 cm diameter; 500 µ), transferred to glass petri dish and weighed using digital top loading weighing scale. Fertilised eggs were gradually poured into a 20 cm diameter, 500 µ filter screen submerged in basin with treated seawater. The filter screen with the fertilised eggs was immediately soaked in 10 % iodine solution, with the eggs transferred to glass petri dish and weighed and stocked in hatching tanks. Three sub-samples of 2–3 grams fertilised eggs were counted for the estimation of number of eggs per gram. The fertilisation rate was computed based on the number of eggs fertilised in relation to the total number of collected eggs.

To validate the computed fertilisation rate in the spawning tanks and hatching rate in the hatching tanks, three sets of egg samples of about 100 eggs per set were randomly selected from each spawning tank and placed in a circular basin (30 cm diameter, 3 L capacity) filled with filtered seawater. After 6 h, the number of fertilised and unfertilised eggs were counted under the dissecting microscope. Fertilised eggs are transparent eggs that continue to develop until the gastrula stage. Unfertilised eggs, usually opaque, stopped embryonic development at the blastula or earlier stage. Fertilised eggs were returned back to the basin and allowed to hatch. Hatched eggs were counted after all eggs had hatched. Fertilisation and hatching rates were computed and compared with the calculated rates from the actual collection of eggs in spawning tanks and hatched larvae in hatching tanks.

Fertilised eggs collected from the spawning tank were stocked at 20 gram eggs.tank^{-1} in 200 L (70 cm

diameter) circular incubation or hatching tanks (70,000–80,000 eggs.tank^{-1}) with ample aeration for hatching. At 3 h and 10 h after spawning, residual unfertilised eggs were removed from the incubation tanks by temporarily stopping aeration and gently swirling the water to allow settling of unfertilised eggs at the centre bottom. The eggs were then siphoned out using a 0.5 mm diameter rubber hose. Hatched larvae were estimated using the volumetric method, that is, by taking five sub-samples of 100 mL using a beaker and determining the mean number of larvae per ml which was used to estimate the total larvae in the 200 L hatching tank. At 6 h post-hatch, egg shells were siphoned out of the incubation tank and water change of 50 % was done. At 12 h post-hatch, larvae were transferred to small basins with filtered, treated seawater and stocked in 4-ton (2.5 m diameter), circular canvass tanks for mass larval rearing. The different stages of embryonic and larval development were documented in the laboratory using a stereo microscope and digital camera.

Larval rearing

Larvae were transferred from incubation tanks and stocked at 30 larvae.L^{-1} to larval tanks filled with seawater at 50 cm depth for larval rearing. Larvae were reared using the standard protocol for marine finfish hatchery with minor modification. Live natural food *Nannochloropsis* sp., *Brachionus* sp. and *Artemia salina* (Linnaeus, 1758) were fed to larvae over a 26-day rearing period. *Nannochloropsis* sp. was maintained at a density of 1 × 10^5 cells.mL^{-1} from Day 1 to 26. *Brachionus* sp. was fed at 5–10 ind.mL^{-1} at Day 3–5 which was increased to 11–15 ind.mL^{-1} at Days 6 to 10, 15–20 ind.mL^{-1} at Days 11 to 20 and decreased to 11–15 ind.mL^{-1} at Days 21 to 26. *Artemia salina* was fed at 1–3 ind.mL^{-1} at Days 9 to 15 and increased to 5–10 ind.mL^{-1} at Days 16 to 26. A gradual increase in water depth was done from 50 cm at Day 0 until 1.0 m at Day 15. Water change schedule was the same as with the larval rearing feed experiments done in 150 L tanks.

Except for siphoning dead larvae, no water change and scrubbing of tank were done at Days 0 to 7 and Days 18 to 22, during the critical period when larval mortalities were observed. Salinity and temperature of rearing water were maintained at 28–30 ppt and 27–28 °C, respectively. When the temperature dropped below 27 °C, 100 W heaters were provided to maintain the larval tank water temperature at 28 °C.

Statistical analysis

Analysis of variance (ANOVA) was used to analyse data on reproductive parameters and to determine significant differences among treatment means. Duncan's multiple range test (DMRT) was used to determine specific treatment mean differences. Statistical significance was compared at the 5 % probability level. All computations and analysis were carried out using SPSS version 20.

Results

Results of the reproductive parameters are presented in Table 1. All *C. ignobilis* breeders injected with 100 µ LHRHa.kg^{-1} breeder (n = 5) and 1,000 IU hCG.kg^{-1} breeder (n = 5) spawned successfully. Only one fish injected with 100 mg CPE.kg^{-1} breeder spawned but the eggs were not fertilised while four other CPE-treated fish did not release eggs. Uninjected Control fish (n = 5) did not spawn. Egg production per kg of female was not statistically different between treatments (P > 0.05). The hCG treatment, however, resulted to a relatively higher weight of eggs spawned per kg of female (26.85 g to 102.95 g; mean value of 58.27 ± 12.69 g eggs.kg^{-1} female) compared to LHRHa treatment (18.85 to 51.68 g eggs.kg^{-1} female; mean value of 50.44 ± 10.81 g eggs.kg^{-1} female). Each gram of spawned eggs consisted of 3,580 to 4,680 eggs. Thus, the estimated number of spawned eggs.kg^{-1} female in hCG-treated female ranged from 109,798 to 360,313 eggs.kg^{-1} female with mean value of 223,068 ± 40,049 eggs.kg^{-1} female and for LHRHa-female ranging from 65,958 to 299,777 eggs.kg^{-1} female with mean value of 176,524 ± 37,260 eggs.kg^{-1} female. The hCG-treatment also yielded a relatively higher egg production per individual female (483,110 to 1,729,500 eggs.female^{-1}; mean value of 1,007,914 ± 206,460 eggs.female^{-1}) than LHRHa-treatment (336,385 to 1,738,709 eggs.female^{-1}; mean value of 958,404 ± 226,960 eggs.female^{-1}).

Fertilisation rate was significantly higher (P < 0.05) in LHRHa-treated fish at 55.97 ± 2.93 %, (min-max: 47.99 to 63.5 %) than hCG-injected fish with 30.53 ± 9.62 % (min-max: 10.32–62.83 %). There was no significant difference in the hatching rate (P > 0.05) in LHRHa and hCG injections with 60.07 ± 9.11 % (min-max: 30–82 %) and 43.06 ± 5.89 % (min-max: 30–65.3 %), respectively. The higher fertilisation and hatching rates in LHRHa treatment resulted to relatively higher number of mean hatched larvae per kg female at 56,040 ± 12,302 larvae.kg^{-1} female (range: 27,751–82,441 larvae.kg^{-1} female) than hCG treatment with 41,547 ± 26,830 larvae.kg^{-1} female (range: 6,983–147,829 larvae.kg^{-1} female). The mean hatched larvae per kg female, however, was not significantly different between treatments (P > 0.05).

After the release of the broodstock in the spawning tank, they were observed to be circling together in the tanks in normal swimming behaviour. At about 8–12 h before spawning, more active swimming was noted at faster speed, diving to the bottom and going upwards to the water column. Upon nearing the spawning time, broodstock were observed to dive to the tank bottom and return to the water column at the time when spawned eggs were seen at the water surface. All three broodstocks always swam together but it was difficult to observe whether spawning pairs dive to the bottom to spawn due to the depth of the tank. The female, however, were chased by both males with at

Table 1. Spawning and hatching period, weight and number of spawned eggs, hatched larvae, fertilisation and hatching rate of *Caranx ignobilis* injected with different hormones.

Treatment	N	H spawning (min-max)	H hatched (min-max)	Weight spawned eggs.kg^{-1} (min-max)	Number spawned eggs.kg^{-1}♀ (min-max)**	Number spawned eggs.♀breeder^{-1} (min-max)	Hatched larvae.♀breeder^{-1} (min-max)	Fertilisation rate (%) (min-max)	Hatching rate (%) (min-max)
Human chorionic gonadotropin (1,000 IU.kg^{-1})	5	29.6 ± 2.26^{a} (24-36.5)	12.4 ± 0.4^{a} (11-13)	58.27 ± 12.69^{a} (26.85-102.95)	223,068 ± 40,049^{a} (109,798-360,313)	1,007,914 ± 206,460^{a} (483,110-1,729,500)	41,547 ± 26,830^{a} (6,993-147,829)	30.53 ± 9.62^{b} (10.32-62.83)	43.06 ± 5.89^{a} (30.0-65.3)
Luteinizing hormone-releasing hormone analogue (100 µg.kg^{-1})	5	39.25 ± 5.04^{a} (25-52)	13.7 ± 1.04^{a} (11-17)	50.64 ± 10.81^{a} (18.85-51.68)	176,524 ± 37,260^{a} (65,958-299,777)	958,404 ± 226,960^{a} (336,385-1,738,709)	56,040 ± 12,302^{a} (27,751-82,441)	55.97 ± 2.93^{a} (47.99-63.5)	60.07 ± 9.11^{a} (30.0-82.0)
Carp pituitary extract (5 mg.kg^{-1})	5	***							
Uninjected control	5	Did not spawn							

*In a column, means followed by a common letter are not significantly different at 5 % level by Duncan's multiple range test (DMRT); ± indicates standard error of the mean (SEM).
** 1 gram spawned eggs is 3,580 to 4,680 eggs.
*** Only one (1) CPE-injected breeder spawned but released eggs were not fertilised.

least one going in close contact with the female particularly upon nearing spawning.

The mean time interval from 2nd injection to spawning was shorter and more predictable in hCG-treatment at 29.6 ± 2.26 h (min-max: 24–36.5 h) compared to LHRHa-treatment with variable spawning time at 39.25 ± 5.04 h (min-max: 25–52 h). Majority of the spawnings occurred between 7 PM to 12 midnight (66.67 %) while the rest of the spawnings happened between 1:45 AM to 3:30 AM (16.67 %), 5:30 AM to 10:30 AM (11.11 %) and at 3 PM (5.56 %). Water quality parameters during spawning were 26.3–32.0 °C, 29.0–34.9 ppm salinity, 3.9–10.3 ppm dissolved oxygen and pH 6.7–8.6. Spawned eggs of *C. ignobilis* are pelagic, clear and spherical with single oil globule and mean diameter of 0.8 mm. Actual spawning time was recorded based on collected eggs at the 1–64 cell stage of development. Collection of all the spawned eggs from the collecting chamber took 2–4 h. There was no subsequent spawning after 2–3 days and broodstock were then returned back to broodstock ponds.

Figure 1 shows the embryonic development of *C. ignobilis* documented in the study. Eggs were hatched in 12.4 ± 0.4 h (min-max: 11–13 h) for hCG and 13.7 ± 1.04 h (min-max: 11–17 h) in LHRHa, with no significant difference (P > 0.05) in hatching time between treatments. The first cleavage stage (2-cell division) occurred at 30 min to 1 h followed by 4-cell, 8-cell and 64-cell division in 2–3 h. Morula stage was reached in 5 h and blastula stage at 6 h. Embryonic axis was visible at 7 h with the evolution of the embryo observed at 8 h and segmentation of mid-embryo at 10–12 h. At 13 h, primordial fin formation occurred and embryonic development completed at 11–17 h. Newly hatched larvae were 1.7 mm in mean total length. There was no significant difference (P > 0.05) in the mean readings of water parameters between treatments in spawning tanks during incubation and hatching of eggs which ranged from 28–32.0 °C, 30.0–30.5 ppm salinity, 5.0–6.5 ppm dissolved oxygen and pH range of 7.3–8.6. The larval development of *C. ignobilis* from Days 1–26 is presented in Figure 2. After rearing for 26 days, *C. ignobilis* fry reached 8.1 mm mean total length at a survival rate of 0.4–4.47 %. The critical period for larval rearing Day 1–7 and Day 19–22 when gradual mortalities were observed. Water parameter readings during larval rearing were 26.3–29.8 °C, 27.9–31.6 ppt salinity, 4.9–6.3 ppm dissolved oxygen and pH 6.8–8.5.

Discussion

Reproduction in captivity, either through natural or induced spawning, is essential prior to the introduction and promotion of new fish species for aquaculture. In the present study, *C. ignobilis*, a potential new species for aquaculture in the Philippines, have shown a positive response and enabled spawning in captivity after hormonal injection with LHRHa and hCG. This is the first successful attempt to breed *C. ignobilis* in the country and an initial step towards the development of seed production technology for this species. The two hormones were used in several carangid species. hCG

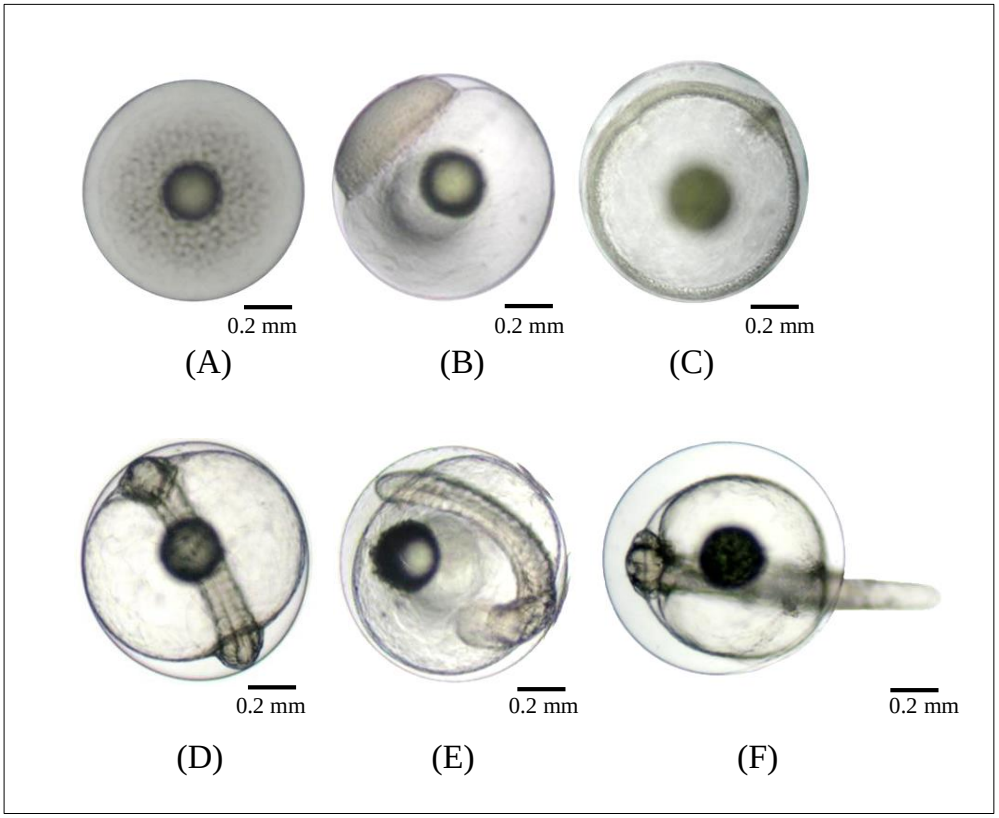

Fig. 1. Giant trevally, "maliputo" (*Caranx ignobilis*) embryonic development at: (A) 1 h, 1st cell division; (B) 3 h, morula stage; (C) 5 h, gastrula stage ; (D) 7 h, evolution of the embryo; (E) 12 h, embryo bends inside egg and primordial fin is formed and; (F) 13 h, hatching of larvae. Viewed under compound microscope, Olympus CH20, at 10× magnification. Scale bar = 0.2mm.

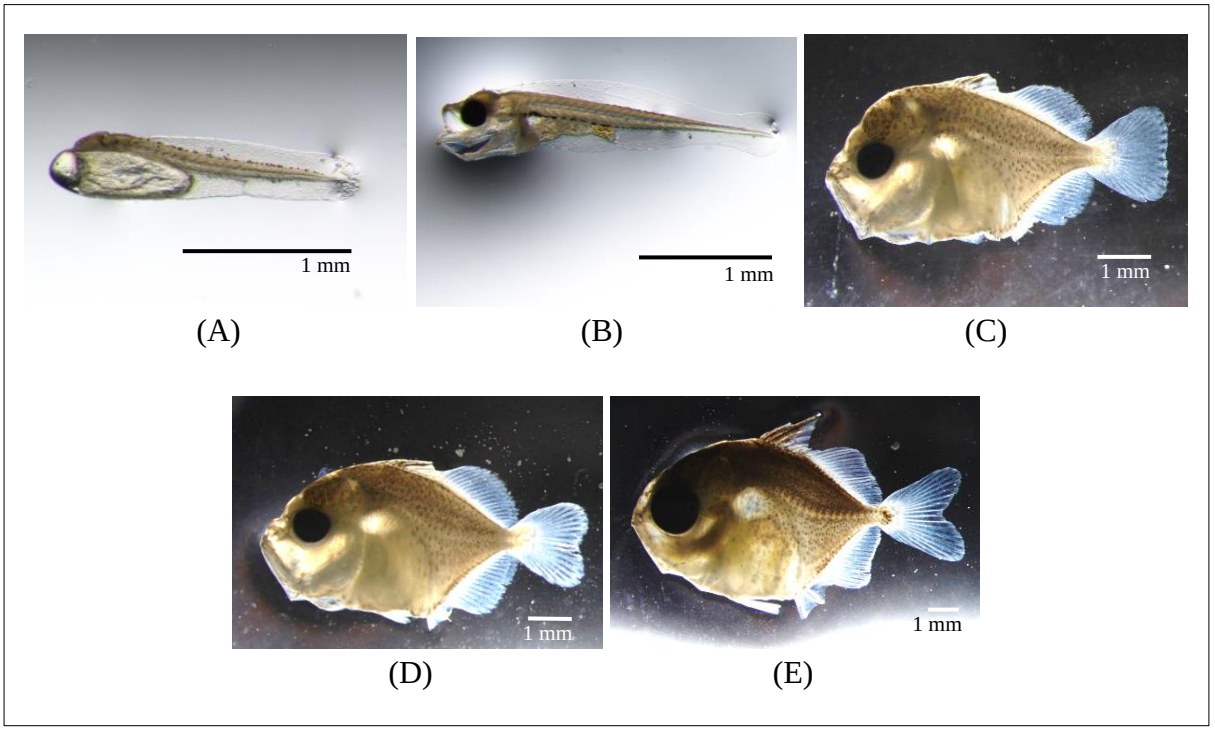

Fig. 2. Larval development of giant trevally, "maliputo" (*Caranx ignobilis*) at: (A) yolk-sac stage Day 0 (1.7 mm); (B) pre-flexion stage at occurring at Day 3–12 (3.1 mm, Day 7); (C) flexion stage occurring at Day 13–15 (4.3 mm, Day 14); (D) organs are fully developed Day 18 (5.0 mm); and (E) fry at full metamorphosis occurring at Day 26-onwards (8.1 mm, Day 26), viewed under stereo microscope, Nikon SMZ800N. Scale bar = 1 mm.

was used to spawn Florida pompano, *T. carolinus* (Hoff et al., 1972; Gopakumar et al., 2012); Japanese amberjack yellowtail, *S. quinqueradiata* (Nagakura et al., 2003; Hamada and Mushiake, 2006); and greater amberjack, *S. dumerili* (Jerez et al., 2006) while LHRHa was employed in the spawning of *S. dumerili* (Diaz and Garcia, 2001). GnRHa injection was also reported to be effective in spawning Almaco jack, *S. rivoliana* (Roo et al., 2012; Fernandez-Palacios et al., 2015); *S. dumerili* (Fernandez-Palacios et al., 2013); and Japanese jack, *T. japonicus* (Nyuji et al., 2013). The pelleted implant of gonadotropin-releasing hormone analogue (GnRHa) has induced spawning in the *T. carolinus* (Weirich and Riley, 2007) and *S. dumerili* (Mylonas et al., 2004). This study showed that CPE was ineffective in induced spawning of *C. ignobilis* despite having oocytes at ripe stage (0.53 mm ± 0.012 oocyte diameter and 63.0 ± 2.0 % of oocyte at GVM stage) which is not significantly different from those of hCG-injected fish (0.46 mm ± 0.024 oocyte diameter and 57.0 ± 4.36 % of oocyte at GVM stage) and LHRHa (0.53 mm ± 0.12 oocyte diameter and 64.0 ± 6.78 % of oocyte at GVM stage). The CPE is widely used as an effective hormone for induced spawning of many freshwater fish and reported to be successful in few marine species such as summer flounder, *Paralichthys dentatus* (Linnaeus, 1766) (Berlinsky et al., 1997), Brazilian flounder *Paralichthys orbignyanus* (Valenciennes, 1839) (Sampaio et al., 2008) and English sole, *Parophrys vetulus* Girard, 1854 (Sanborn and Misitano, 1991). The unsuccessful spawning in CPE treatment in this study may be because carp pituitary is highly impure, and because the number and amount of components in a given pituitary is unknown, their action is unpredictable (Harvey and Carolsfeld, 1993), thus its effectiveness may be limited to some fish species. Among the carangid fishes known in aquaculture, natural spawning in captivity have been reported for *C. melampygus* (Moriwake et al., 2001); *S. lalandi* (Moran et al., 2007; Stuart and Drawbridge, 2012); and *S. dumerili* (Jerez et al., 2006). However, in this study the uninjected Control *C. ignobilis* did not spawn naturally despite having similar ripe oocytes (0.50 ± 0.016 mm diameter and 61.84 ± 2.7 % at GVM stage) as in injected experimental fish. Natural spawning in a confined environment such as the 40-ton spawning tank may not be suitable enough to induce the spontaneous release of eggs. Further studies may be needed on necessary cues to induce natural spawning in captivity of this species. Compared to other carangid fishes, fertilisation and hatching rate of *C. ignobilis* in this study were relatively lower. Fertilisation rate of 30.53 % achieved in *C. ignobilis* using hCG was lower than that of another carangid fish, *S. dumerili* with 70–89.8 % injected with the same hormone (Chen et al., 1998). Similarly, the use of LHRHa in *C. ignobilis* attained relatively lower fertilisation rate of 55.97 % compared to GnRHa injected carangid fish *S. rivoliana* with 92.5 % (Roo et al., 2012). Hatching rate was also relatively lower in *C. ignobilis* compared to reported hatching in *S. dumerili* using hCG with 61–82 % (Chen et al., 1998) and 72.6 % using GnRHa (Roo et al., 2012). Gonadotropin-releasing hormone (GnRH) induces the secretion of the fish's own gonadotropin from the pituitary, thereby overcoming endocrine failure observed in captive broodstocks (Zohar and Mylonas, 2001), thus becoming more effective than hCG which, on the other hand, provides elevated gonadotropins to the recipient fish but may not ensure stimulation of final oocyte maturation and spermiation. Moreover, the molecule of hCG is very different from fish gonadotropin so that high doses must be used for many species and some may not respond at all (Harvey and Carolsfeld, 1993). In the present study, the significantly higher fertilisation rate achieved in LHRHa may indicate that the hormone effectively stimulated final oocyte maturation causing the release of mainly mature oocytes that are available for fertilisation by the milt from the male. In the case of hCG, however, more eggs were released but these include immature eggs, thus resulting to lower fertilisation rate. It is worth noting that in at least one of the LHRHa-treated tanks, the release of milt, indicated by fishy smell and cloudiness of tank water, was observed 14 h earlier than the release of the eggs by the female breeder. Thus, fertilisation rate in LHRHa may still be improved if hormone injection in males is adjusted to proper time for the simultaneous release of milt with the female egg. The effective dosage of different hormones vary among species. In this study, the use of 1,000 IU hCG.kg^{-1} breeder and 100 µg LHRHa.kg^{-1} breeder as dosage for the induced spawning was based from the recommended effective dose by the hormone manufacturer, Argent Chemical Laboratories, Redmond, Washington. This was tested during the preliminary breeding trials conducted at NFRDI that yielded initial successful spawning of the species. This dosage is comparable with those used in other marine species such as *Chanos chanos* (Forsskål, 1775), *Lates calcalifer* (Bloch, 1790), *Siganus canaliculatus* (Park, 1797), *Siganus rivulatus* (Forsskål & Niebuhr, 1775), *Siganus argenteus* (Quoy & Gaimard, 1825), *Siganus guttatus* (Bloch, 1787), *Epinephelus akaara* (Temminck & Schlegel, 1842), *Epinephelus salmoides* (Valenciennes, 1828), *Epinephelus tauvina* (Forsskål, 1775) which spawned successfully in 50 IU to 1,000 IU hCG and 37 to 100 µg LHRHa (Marte, 1989). Other researchers had used 500–5,000 IU hCG.kg^{-1} fish to successfully spawn marine fishes like *Epinephelus striatus* (Bloch, 1792) (Watanabe et al., 1995), *Sparus aurata* Linnaeus, 1758 (Waribi et al., 2017), *Mycteroperca rosacea* (Streets, 1877) (Kiewek-Martinez et al., 2010), *Lutjanus argentimaculatus* (Forsskål, 1775) (Emata, 2003). The use of LHRHa at a dosage of 50–150 µg.kg^{-1} fish had been reported to successfully spawn several marine species such as *Paralabrax maculatofasciatus* (Steindachner, 1868) (Alcantar-Vasquez et al., 2016), *Centropristis striata* Linnaeus, 1758 (Berlinsky et al., 2005), *E. striatus* (Watanabe et al., 1995), *M. rosacea* (Kiewek-Martinez et al., 2010), *E. akaara* (Park et al., 2016) and *L. argentimaculatus* (Emata, 2003). In comparison with the works of other researchers on

related carangid species, female broodstock injected with hCG (Hoff et al., 1972; Nagakura et al., 2003; Hamada and Mushiake, 2006; Jerez et al., 2006; Gopakumar et al., 2012) and LHRHa (Diaz and Garcia, 2001) had spawned using lower hormone dosages. Further studies are therefore needed to determine the most cost-effective dose of these hormones for spawning *C. ignobilis*. Moreover, the use of GnRHa was reported to be effective in the artificial spawning of other carangid species (Mylonas et al., 2004; Weirich and Riley, 2007; Fernandez-Palacios et al., 2013; Nyuji et al., 2013;), thus, future researches may study its effectivity on *C. ignobilis*.

The number of spawned eggs of captive-bred *C. ignobilis* (223,068 eggs.kg^{-1} for hCG; 176,524 eggs.kg^{-1} for LHRHa) was comparable with those reported in other Carangidae fishes that has been bred in captivity. For *T. carolinus*, spawned eggs was 234,000 to 302,000 eggs.female^{-1} (Weirich and Riley, 2007); *S. dumerili* spawned 380,821 eggs.2 females^{-1}.induction^{-1} as reported by Fernandez-Palacios et al. (2013) and 14 M eggs.38 spawns^{-1} reported by Jerez et al. (2006); and 41,690–149,450 eggs in *T. japonicus* (Nyuji et al., 2013). Fecundity of *C. ignobilis* wild population is not available but a closely related species, the blue fin trevally, *C. melampygus* has a higher recorded fecundity of more than 4 million ova in 6.5 kg female (Dale et al., 2011). Egg diameter of spawned eggs of *C. ignobilis* in this study is comparable to those of carangid eggs collected from the wild at 0.6 to 1.6 mm (Tresher, 1984).

The time to ovulate or spawn from the second hormone injection was more predictable in hCG at 24–36 h in contrast to LHRHa with longer range difference of 25–52 h at the same mean readings of temperature and salinity. These results are relatively similar with those of other carangid fish like *T. carolinus* which took 36 h using GnRHa (Weirich and Riley, 2007), 7–28 h with 0.55 IU and 0.275 IU hCG (Hoff et al., 1978), 80 h in hCG and pregnant mare serum (PMS) (Hoff et al., 1972), 30–36 h in 75 µg slow-release pellet GnRHa; in *S. dumerili* which took 34–36 h (Mylonas et al., 2004; Jerez et al., 2006); and in *T. japonicus* by Nyuji et al. (2013) which spawned in 1–2 days.

The results of the present study showed that temperature affects hatching period of spawned eggs, with the higher mean temperature (27.6 °C) in this *C. ignobilis* study taking shorter hatching period (11–17 h) compared to other carangid species. Florida pompano took 30–36 h (Weirich and Riley, 2007) and greater amberjack, *S. dumerili* took 35–48 h to hatch (Jerez et al., 2006) at lower water temperature. In the wild, other carangid species were hatched in 12–24 h (Tresher, 1984), at a relatively similar water temperature with this *C. ignobilis* study.

The criteria for selection of ripe female broodstock used in the present study with more than 50 % of oocyte samples with a diameter of 0.4 to 0.5 mm and at least 60 % at GVM stage, was based on preliminary breeding experiments. Thus the selection of ripe females was an appropriate criterion that gave 100 % successful spawning of *C. ignobilis* injected with hCG and LHRHa. Similar parameters were also used in other marine finfish that had been successfully spawned such as greater amberjack, *S. dumerili* at 400–500 µm (Marino et al., 1995), dusky grouper, *Epinephelus marginatus* (Lowe, 1834) at 325–400 µm (Marino et al., 2003); *T. carolinus* at >500 µm (Weirich and Riley, 2007); Nassau grouper, *E. striatus* at 482–561µm (Watanabe et al., 1995); rabbitfish, *S. guttatus* at >0.45 µm (Ayson, 1991); black sea bass, *Centropistis striata* (Linnaeus, 1758) at 305–500 µm (Watanabe et al., 2003; Berlinsky et al., 2005); and mangrove red snapper, *L. argentimaculatus* at >0.4 mm (Emata, 2003).

Conclusion

This study has shown the possibility of captive breeding of *C. ignobilis* using LHRHa and hCG. However, further research is still needed to address the problems related to mortalities encountered during the critical periods in larval rearing. As the first recorded spawning of *C. ignobilis* in the Philippines, this study provides crucial baseline information on the breeding of the species and an initial step towards the development of its hatchery technology for the country and in other parts of the world. Based on the positive response of *C. ignobilis* to induced spawning with good fertilisation and hatching rates, further studies could improve the fertilisation and hatching rates, thus providing opportunities for hatchery seed production to support the development of a new species for aquaculture.

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