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Hormone-Induced Ovulation of Sand Whiting *Sillago ciliata*

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Abstract

Sand whiting *Sillago ciliata* were captured by seine netting ocean beaches. Mature females were given single intraperitoneal injections of 300 IU•kg^{-1} human chorionic gonadotropin (hCG) or 0.5 ml•kg^{-1} Ovaprim (a synthetic GnRH analog with domperidone). One female and a spermiating male were then placed in 70-l or 1,000-l tanks. Both hCG and Ovaprim induced ovulation but not spawning in 38% and 75% of females, respectively (n=8). Females injected with distilled water (n=4) failed to ovulate. Fish were stripped, and the eggs fertilized 32-48 h after injection; mean fertilization was 85.6±15.4% (n=3, mean±SD) and 89.5±12.0% (n=6) in females injected with hCG or Ovaprim, respectively. Tank size had no apparent effect on the number of females that ovulated. A separate experiment was conducted to determine if natu-rally ovulating females could be successfully collected from the wild and stripped. The mean fertilization in naturally ovulating females was 71.0±17.1% (n=4), lower than for Ovaprim-in-jected mature fish colected at the same time (89.5±12.0%, n=2), but higher than in hCG-in-jected fish (32.7±5.5%, n=3). The fertility of eggs from females injected with hCG was higher in the first experiment. Reasons for the different response to hCG between experiments were un-clear, but the results suggest that Ovaprim is a more reliable hormone that hCG for inducing ovulation in sand whiting.

Introduction

The whitings or sand smelt of the genus *Sillago* (Family Sillaginidae) are commercially and recreationally important estuarine and near shore species of the Indo-west Pacific region (McKay 1985; Paxton et al. 1989). Members of the genus are frequently used in early life stage experiments because their biology and ecology in the wild is well understood, and they are relatively small, daily spawners, and commonly available in mature condition. For example, *Sillago japonica* and *S. sihama* have been used in Japan to study the effects of temperature and photoperiod on spawning, temperature and salinity on egg

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development, and the effects of steroids on germinal vesicle breakdown (Lee and Hirano 1985; Oozeki and Hirano 1985; Matsuyama et al. 1990; Furukawa et al. 1991). The supply of eggs usually comes from spawning fish either caught in the wild or held in captivity (Lee and Hirano 1985; Matsuyama et al. 1990). Alternatively, broodstock can be induced to ovulate and spawn with hormone injections (Lee et al. 1981; Oozeki and Hirano 1985).

The sand whiting, *S. ciliata* Cuvier, is common in northern New South Wales (NSW) and southern Queensland. It is a highly fecund, multiple spawner with a prolonged spawning season extending from September to March (Morton 1985; Burchmore et al. 1988; Young 1991). Mature sand whiting have been successfully induced to ovulate using human chorionic gonadotropin (hCG) and the larvae reared intensively (Young 1991; Battaglene et al. 1994).

Reliable production of good quality fertilized eggs and larvae is required if sand whiting is to become a useful model experimental animal, and if the suggested aquaculture potential of the species is to be assessed (Treadwell et al. 1992). Young (1991) successfully stripped captive sand whiting injected with 100-2,700 IU•kg^{-1} hCG. The lowest reliable dose that induced ovulation was 300 IU•kg^{-1} hCG, and higher doses did not improve the success of fertilization. The retention of ovulated eggs decreased their viability and there was great variability between females with respect to the fertility of their eggs (Young 1991).

The objectives of the currect study were to determine if (1) wild-caught sand whiting ovulate; (2) non-ovulating fish can be induced to ovulate using hormones, and (3) eggs produced from naturally ovulating and hormone-induced fish can be successfully fertilized.

Materials and Methods

General

Two experiments were conducted with wild-caught sand whiting. Mature fish were seine netted from an ocean beach at Boat Harbour, NSW, Australia (32° 47'50^{s} S 152° 6'80^{s} E) during summer (February 1992 and January 1993). Fish were transported to the Port Stephens Research Centre hatchery in a 750-l tank within 1 h of capture and transferred to a 4,000-l tank with flow-through salt water. In the first experiment, fish were left for 24 h before being anesthetized with p-aminobenzoate (50 mg•l^{-1}) between 1400 and 1600 h. In the second experiment, fish were anesthetized within 8 h of capture, between 2000 and 2200 h. Males were examined for expressible milt, and only spermiating males with very active sperm were used. A sample of oocytes was obtained from females by inserting a fine bore (1 mm) silicone tube into the oviduct and aspirating tissue by mouth. The diameter of the 10 largest oocytes was measured (± 30 μm) for each female. Fish were considered to have started final oocyte maturation if hydrated oocytes (cleared yolk) were detected.

Mature females which had not started final oocyte maturation and spermiating males were given a single intraperitoneal injection with either

300 IU•kg^{-1} hCG (Pregnyl®, Organon [Australia] Pty Ltd) or 0.5 ml•kg^{-1} of Ovaprim® ([D-Arg^6, Pro^a - NEt], a synthetic GnRH analog [20 µg•ml] with a dopamine antagonist domperidone [10 µg•ml] (Syndel, Vancouver, Canada) dissolved in distilled water. Fish in control treatments were injected with 1 ml•kg^{-1} distilled water. Fish were randomly allocated to treatments, with one female and male placed into either, 70-l glass aquaria with continuously flowing seawater, or 1,000-l static fiberglass tanks. Tanks and aquaria were kept in the dark and aerated, and maintained at a temperature of 23±1°C.

Experiment 1

The aim of the first experiment was to determine the effect of hCG and Ovaprim on ovulation in mature wild-caught females held in different holding systems. Eight replicate pairs of fish (one male and one female) were treated with hCG, eight with Ovaprim, and four with distilled water as controls. Four pairs of fish from each hormone treatment and the controls were placed in 1,000-l tanks, and four pairs of hCG- and Ovaprim-treated fish in 70-l aquaria. Two different tank sizes were used to increase sample sizes and to determine if ovulation was influenced by the type of holding system used. Fish were anesthetized at approximately 24, 32, 34, 36 and 48 h after injection and examined for signs of ovulation by applying some pressure to the abdomen. If ovulation had occurred, females were stripped.

Experiment 2

The aim of the second experiment was to compare ovulation, percentage fertilization and hatch in hormone-induced females and ovulating wild-caught females. Four ovulating wild-caught females and four spermiating males were stripped, and the eggs fertilized without hormone treatment. Four replicate pairs of fish were also treated with hCG or Ovaprim and placed in 70-l aquaria. Hormone dosages were the same as in the first experiment, and fish were anesthetized after 34 h and stripped. Females were then examined every 30 minutes until 37 h after injection. Fish which failed to ovulate were then checked again 40 h after injection.

Stripped eggs were collected in 200 ml seawater and fertilized by milt obtained from stripped males. Eggs were incubated in 70-l glass aquaria with aeration (100 l air•minutes^{-1}) and continuously flowing seawater at 22±1°C. Fertilization was determined by microscopically examining 100 or more eggs per incubator 2 h after stripping. The number of eggs per stripping was calculated volumetrically by counting five aliquots from each incubator. Eggs hatch in 24 h at 24±1°C (Battaglene et al. 1994), so after 22 h, approximately 200 eggs were sampled from each incubator, the number of live embryos counted, and the percentage hatch estimated. Calculation of hatching was not possible immediately following hatch because whiting larvae are extremely delicate and heterogeneously distributed.

Differences in ovulatioin in hormone-treated females and the effect of holding tanks on ovulation were analyzed using 2x2 contingency tables (Winer

1971). Separate one-way analyses of variance were used to test for differences in female weight and mean oocyte diameter. Treatment means were compared using SNK tests. Homogeneity of variance was evaluated using Cochran's test.

Results and Discussion

In the first experiment, 75% of females injected with Ovaprim, 38% of those treated with hCG and none of the fish in the control treatments ovulated (Table 1). Overall, there was no significant difference in the number of females which ovulated between fish injected with Ovaprim or hCG ($X^2=2.29$, df=1, $P>0.05$). Tank size had no apparent effect on the number of females which ovulated ($X^2=0.25$, df=1, $P>0.05$). Fish injected with Ovaprim were successfully stripped more often than those injected with hCG but subsequent strippings produced few fertilized eggs (Table 1). Fish injected with Ovaprim generally ovulated before those injected with hCG, and percentage fertilization

Table 1. Hormone induction data for wild-caught female sand whiting *Sillago ciliata* injected with distilled water, 300 IU•kg^{-1} hCG or 0.5 ml•kg^{-1} Ovaprim, and incubated in either 1,000-l tanks or 70-l glass aquaria in February 1992 (experiment 1).

Treatment	Weight (g)	Mean oocyte size[1] mean±SD (mm)	Fertilization (%)	Hatch (%)	No. larvae[2]	No. ovulated
Control	297	0.42±0.01	-	-	-	0/4
	266	0.44±0.01	-	-	-	
	200	0.42±0.02	-	-	-	
	230	0.46±0.02	-	-	-	
Ovaprim	300	0.43±0.02	-	-	-	3/4
(1,000-l tank)	252	0.44±0.01	33	10	464	
	215	0.42±0.03	97	96	39,987	
	274	0.44±0.01	98	96	55,193	
		2nd strip	42	10	800	
Ovaprim	248	0.44±0.02	97	94	17,005	3/4
(70-l tank)	229	0.44±0.02	92	83	22,399	
		2nd strip	0	0	0	
	175	0.42±0.01	-	-	-	
	260	0.42±0.03	97	95	24,522	
		2nd strip	59	36	3,802	
hCG	355	0.43±0.02	92	95	266,962	2/4
(1,000-l tank)	287	0.43±0.07	96	98	66,118	
	261	0.73±0.03	-	-	-	
	299	0.45±0.02	-	-	-	
hCG	248	0.45±0.02	68	65	104,486	1/4
(70-l tank)	229	0.43±0.02	-	-	-	
	2005	0.43±0.02	-	-	-	
	218	0.44±0.01	-	-	-	

[1]Before injection
[2]Number of viable larvae = no. eggs x percentage hatch

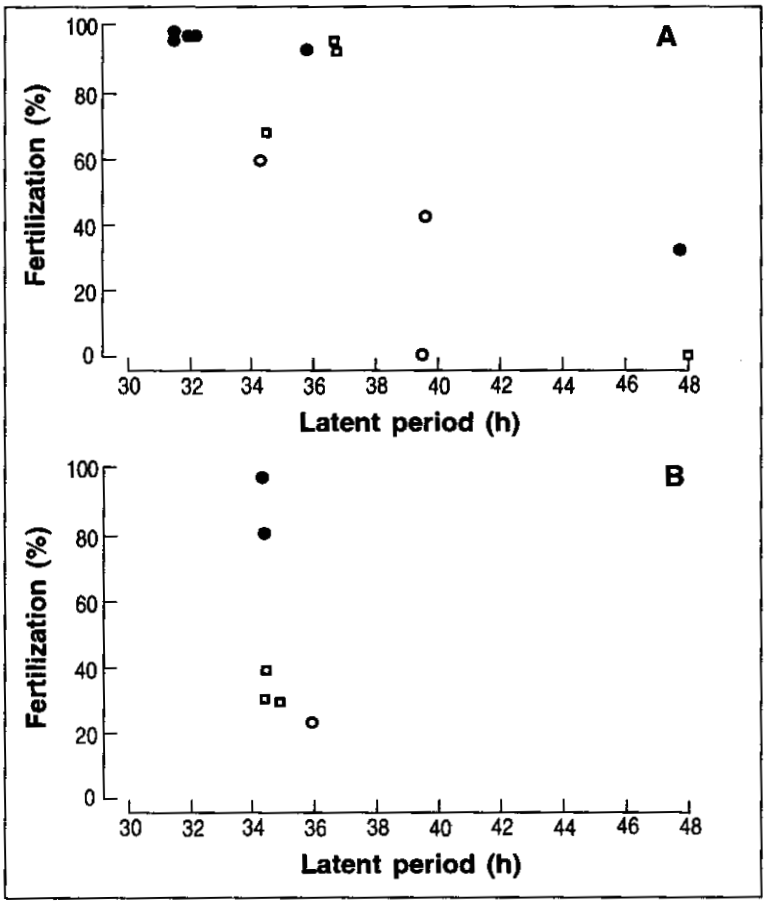

Fig. 1. Latent period and mean fertilization for sand whiting *Sillago ciliata* induced to ovulate with Ovaprim (● = first stripping; ○= second stripping) and human chorionic gonadotropin (□). Figs. A and B give results for experiments 1 and 2, respectively.

and hatch declined after 37 h in both treatments (Fig. 1). Fish injected with Ovaprim may have been initially stripped before they completed final oocyte maturation and ovulation. This could explain why fish injected with Ovaprim could be stripped more than once and why eggs from latter strippings had lower fertility (Table 1).

The fertilization of eggs stripped in experiment 1 was generally above 90% and higher than that achieved by Young (1991). In many species, low fertilization can result from over-ripening of oocytes, as they undergo morphological, biochemical and physiological changes following ovulation (McEvoy 1985; Kjorsvik et al. 1990). The low fertilization experienced following repeat strippings was probably the result of over-ripening.

Highest percentage fertilization in experiment 1 occurred between 32 and 36 h, considerably earlier than the 48 h found by Young (1991). However, it should be noted that Young (1991) used captive fish with initially smaller oocytes, held at 21°C, compared with 23°C in the current study. Fertilized eggs floated and had a diameter of 700±20 μm (mean±SD, n=10) with a single oil droplet, measuring 185±5 μm. Multiple oil droplets were present in some eggs

from poorly fertilized batches. Further egg and larval development was as described by Tosh (1903) and Young (1991).

The failure of some fish to ovulate, and differences in the response to hormones did not appear to be related to variation in size or maturity of the broodstock. The mean weight of females among treatments in experiment 1 was not significantly different ($P>0.05$), and only one female in the hCG (1,000-l tank) treatment had significantly larger oocytes of 0.73 mm (Table 1) ($P<0.01$). The mean oocyte diameter among treatments was also not significantly different ($F=1.05$, $df=19$, $P>0.05$).

In experiment 2, all ovulating wild-caught females were successfully stripped without the use of hormones. Females injected with hCG produced larger numbers of eggs than naturally ovulating females or those injected with Ovaprim. However, hCG-injected females had lower percentage fertilization and hatch (Table 2). The latent period for injected fish was narrower than in experiment 1, varying from 34 to 36 h. One fish injected with hCG died, and two treated with Ovaprim failed to ovulate within 40 h of injection.

The absence of control fish (non-ovulating and not injected) in experiment 2 was due to a shortage of mature females. It is possible that some of the females which could not be stripped at the start of experiment 2 may have ovulated without injection of hormones. However, control females in experiment 1 did not ovulate and we have not observed ovulation in sand whiting during other hatchery trials, unless they can be stripped when captured (Battaglene, unpubl. data 1993).

Table 2. Comparison of eggs produced by sand whiting *Sillago ciliata*, naturally ovulating and from fish injected with 300 IU•kg-1 hCG or 0.5 ml•kg-1 Ovaprim in January 1993 (experiment 2).

Treatment	Weight (g)	Mean oocyte size[1] mean±SD (mm)	Fertilization (%)	Hatch (%)	No. eggs	No. ovulating
Natural ovulation	550	-	85	85	62,570	4/4
	308	-	77	76	51,816	
	296	-	46	46	47,905	
	400	-	76	76	10,754	
Mean±SD			71.0±17.1	70.8±17.0	43,261±2,254	
hCG	400	0.43±0.03	29	12	192,600	3/4
	350	0.42±0.03	-	-	-	
	400	0.43±0.03	39	0	101,677	
	310	0.42±0.02	30	0	111,325[1]	
Mean±SD			32.7±5.5	4.0±6.9	135,201±49,943	
Ovaprim	320	0.42±0.02	98	95	60,526	2/4
	250	0.46±0.03	-	-	-	
	256	0.39±0.03	81	80	68,437	
	221	0.40±0.02	-	-	-	
Mean±SD			89.5±12.0	87.5±10.6	64,526±5,530	

[1]Including 38,000 unfertilized eggs collected from the aquaria

The mean fertilization for hCG-treated fish in experiment 2 was 32.7±5.5%, lower than the 85.6±15.4% in experiment 1. In contrast, the mean percentage fertilization for Ovaprim-treated fish in experiment 2 was 85.8±26.1% and similar to the 89.5±12.0% in experiment 1. Reasons for the different fertilization rates using hCG but not Ovaprim between experiments were unclear but probably relate to the timing of stripping in relation to ovulation.

The 24-h delay in hormone injection in experiment 1 compared with less than 8 h in experiment 2, and the earlier timing of injection in relation to daylight in experiment 1 may also be important, particularly since sand whiting are daily spawners. The time of day when hormones are administered has been suggested to influence the success of ovulation in cyprinids (Peter et al. 1988), gilthead bream, *Sparus auratus* (Zohar 1988) and European sea bass *Dicentrarchus labrax* (Alvarino et al. 1992). It has been postulated that there is a daily change in sensitivity either for the pituitary for LHRHa or for the ovary to gonadotropin in some species (Alvarino et al. 1992).

The closely related Japanese whiting, *Sillago japonica*, are known to have a circadian rhythm with respect to ovulation and production of 17α,20β-dihydroxy-4-pregnen-3-one, a possible maturation-inducing steroid, and spawning occurs between 1800 and 2400 h daily during the summer (Kashiwagi et al. 1987; Matsuyama et al. 1990; Furukawa et al. 1991). The exact time of ovulation and spawning in sand whiting is still unknown (Morton 1985; Burchmore et al. 1988).

Morton (1985) suggested that sand whiting are multiple spawners on the basis of bimodal distributions in oocyte sizes, a protracted spawning season, and lack of synchronization in mature fish. He also estimated fecundity to be highly variable and within the range of egg numbers produced by fish injected with hormones in the current study (Tables 1 and 2).

The results demonstrate that hCG and Ovaprim can induce ovulation in sand whiting. The protocol outlined using Ovaprim gave repeatable results, and maturation time was short and predictable in ovulating females at first stripping, generally resulting in more than 80% fertilized eggs. These features are some of the important criteria needed for assessing the effectiveness of induced ovulation (Peter et al. 1988). The response to hCG was not consistent between experiments, although hCG-injected females generally produced more eggs than those injected with Ovaprim. Ovaprim therefore appears to be a more reliable hormone than hCG for inducing ovulation in sand whiting. However, the results should be interpreted with caution because not all sand whiting responded to hormone treatment and the number of females and the dosages tested were relatively small. Further research is required to determine optimum hormone dosages and if the dopamine antagonist, domperidone, is beneficial.

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