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Preliminary Trials of Hormone-Induced Production of Fertilised Eggs in the West Australian Dhufish (*Glaucosoma hebraicum*, Richardson, 1845)

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Abstract

This paper describes the first trials of spawning induction on the WA dhufish. Spawning was achieved using hormone treatments at low dose rates such as:

1) 13 µg LHRH-a·kg^{-1} in cholesterol-based implant,
2) a double treatment with 5 µg LHRH-a·kg^{-1}(by implant + saline injection)
3) 6 µg GnRH-a ·kg^{-1} (0.3 ml Ovaprim·kg^{-1}).

Treatments with hCG (900-1600 IU·kg^{-1}) and with implants of LHRH-a at high dose rates (25, 50, 80, 100, 120 µg·kg^{-1}) induced ovulation but not spawning in which case eggs had to be stripped manually. The timing at which to strip females was critical since the viability of eggs dramatically decreased within a few hours after ovulation.

Sperm production by male dhufish post-capture was achieved in 50% of cases with LHRH-a implants at a dose rate of 30 and 100 µg·kg^{-1}. Males captured at least 2.5 months before the spawning season and held in tanks as sole males with a group of females produced highly active sperm naturally. When several males (up to eight) were present in a group with females, a maximum of two males produced viscous sperm with active spermatozoa. Treatments with hCG or LHRH-a performed immediately after capture failed to retain the viscosity of the sperm and activity of spermatozoa.

Introduction

The rapid depletion of many stocks of marine finfish and the growing demand for high quality seafood products has encouraged the development of marine fish farming in Australia which major limitation is still the reliable production of fertilised eggs and juveniles (Carragher & Pankhurst 1991; Battaglene 1996). The West Australian (WA) dhufish, *Glaucosoma hebraicum* Richardson, 1845

(Glaucosomatidae), one of the most popular fish targeted by recreational anglers in temperate Western Australia, which attains a maximum size of 1.22 m TL and 26 kg (Hutchins and Swainston 1986; Kailola et al. 1993), is currently being studied at the Fremantle Maritime Centre as a potential species for aquaculture. The limited data available suggest that this valuable species may have biological attributes that make it highly attractive for aquaculture. For example, ageing studies indicate that dhufish can attain a mean length of 30 cm TL during its first year of life and 40-46 cm TL after three years (Marr 1980). Moreover, a market survey conducted by Barnetson (1995) described the dhufish as a high yielding species with firm, excellent quality flesh and suggested that an opportunity exists in Australia for its aquaculture.

In most cases, wild marine fish are unable to spawn naturally in captivity and fertilised eggs are obtained through manipulation of broodstock fish (Barnabe 1994) using hormones such as human chorionic gonadotropin (hCG) and luteinizing hormone-releasing hormone analogue (LHRH-a) (Lam 1982; Shelton 1989). Dosages of hCG vary from 100 to as high as 40000 $IU \cdot kg^{-1}$ body weight and the rule of thumb is to use 1000 $IU \cdot kg^{-1}$ (Lam 1982). Dosages of LHRH-a in saline injection for marine fishes vary from 4 to 500 $\mu g \cdot kg^{-1}$ body weight (Glubokov et al. 1991; Mayes et al. 1993). Treatments using the above hormones are effective in many marine species of fish (e.g. sparids) while several freshwater and estuarine species respond better when salmonid gonadotropin analogue (sGnRH-a) is injected with a dopamine antagonist, most commonly known as domperidone (Glubokov et al. 1991; Battaglene, submitted).

Dhufish are serial spawners and spawning occurs between late November and March with a peak in January or February; total fecundity estimates per female range from 0.3 to 2.9 million eggs (Moy 1986; Abordi 1986; Hesp 1997). The size of males and females at first maturity is thought to be around 350-400mm and 250-300mm respectively (Hesp 1997).

Dhufish breeding in captivity has been successful and the larval development together with a brief summary of the spawning induction of broodstock fish has been described in Pironet & Neira (1998). The present paper examines in detail the effect of specific doses of hCG, LHRH-a and GnRH-a (Ovaprim) on the induction of ovulation and spawning in dhufish. A minor priority was given to the experimental design since the primary aims of this study were the production of fertilised eggs, larvae, and juveniles and the development of a breeding protocol for commercial aquaculture.

Methods

Broodstock capture and management

A total of 135 dhufish were captured between 1995 and 1997 using hand-held lines off Ledge Point (31° 7' S; 115° 22' E) in depths of <25 m. Upon capture, fish were placed directly into a 250 L container filled with sea water, the swim bladder punctured with a sterile 16 gauge hypodermic needle to remove excess gas, and transported to the Fremantle Maritime Centre (Western

Australia). Captured fish were allowed to settle in enclosed tanks (10 to 50 m^3 in capacity) with continuously flowing sand-filtered seawater under natural photoperiod. They were fed ad libitum with a high quality diet consisting of frozen feeds such as squids, prawns, mulies and other fishes; vitamins were regularly added to the diet. Fish biomass ranged between 0.5 and 3.0 kg per m^3. During the first year the ratio of male to female was ≈ 1:1. During the second year, fish distribution in tanks was either one male with two to three females or two males, one being markedly larger, with two to four females.

Water temperature during the spawning season varied between 21 and 27°C and the salinity was 35‰.

Hormone treatments

METHOD

Upon capture, both male and female broodstock fish were anaesthetised using 2-phenoxyethanol at 300 ml/m^3 concentration for intraperitoneal tagging with a passive transponder and weighing.

During the spawning season, testes samples were obtained by manual stripping. Ovarian samples were removed using a sterile cannula, and the diameter of the largest oocytes measured to the nearest 10 µm under a dissecting microscope.

Hormones in liquid form were injected using a sterile 18-gauge hypodermic needle, while implants were inserted using a 2 mm diameter sterile needle. The following hormones were used:

1) hCG (Chorulon)

2) LHRH analogue (des-Gly10, [D-Ala6]-luteinizing hormone-releasing hormone Ethylamide, Sigma) either in dissolved (LHRH-a *sal*) or pelleted form (LHRH-a *imp*)

3) GnRH-a (Ovaprim, Syndel Laboratories).

LHRH-a solutions were prepared by dissolving the hormone in a saline solution at 1 ppt concentration (Pankhurst, N.W., pers.comm.), frozen at –20°C and thawed just before use. LHRH-a implants were prepared with 95% cholesterol and 5% copha oil (Lee et al. 1986) and stored at –20°C until use. Ovaprim contained 20 $\mu g \cdot ml^{-1}$ (D-Arg6, Pro8-NEt) sGnRH and 10$mg \cdot ml^{-1}$ domperidone.

Initial dosages of hCG and LHRH-a used were 1000 $IU \cdot kg^{-1}$ and 100 $\mu g \cdot kg^{-1}$ respectively (Pankhurst, N.W., pers. comm.). Hormone dose rates ranged as follows: 900-1600 $IU \cdot kg^{-1}$ hCG, 5-120 $\mu g \cdot kg^{-1}$ LHRH-a *imp*, 5-100 $\mu g \cdot kg^{-1}$ LHRHa sal, 6 $\mu g \cdot kg^{-1}$ GnRHA.

HORMONE TREATMENTS PERFORMED IMMEDIATELY AFTER CAPTURE

Male treatments : The majority of males (7 out of 11) caught during the first spawning season (1995-1996) were treated within half an hour after capture with ±1000 IU $hCG \cdot kg^{-1}$ (four cases), ±100 µg LHRH-a $sal \cdot kg^{-1}$ (two cases) or 100 µg LHRH-a $imp \cdot kg^{-1}$ (one case). During the second year (1996/97), broodstock collection was completed before the beginning of the spawning season; hormone treatment was performed at the time of capture.

Female treatment : Only two females were caught during the spawning season (1995-1996) and one of them was treated within one hour after capture, with 100µg LHRH-a *imp*·kg^{-1}.

HORMONE TREATMENTS PERFORMED AT LEAST TWO DAYS AFTER CAPTURE

Male treatments : Eight males captured within two years of study were treated at least two days after capture with LHRH-a implants at 100 µg·kg^{-1} (five cases) or 30 µg·kg^{-1} (three cases) dose rate.

Female treatments : The majority of female dhufish were captured several weeks to months before the beginning of the spawning seasons. Broodstock tanks were successively assessed at approximately two weeks intervals during the spawning seasons : females from a particular tank were biopsied and treated simultaneously but not necessarily with the same type or dose of hormone. Reproductive hormones were injected intraperitoneally in females with maximum oocyte diameter ≥400 µm. Upon observation of a markedly increased swelling in the female abdomen or eggs released in the tank, fish were anaesthetised and the stage of maturation identified by biopsy or eggs were stripped manually. Anesthesia and egg stripping were repeated at approximately six-hour intervals until assessment of the optimum stripping time.

Stripping and artificial fertilisation

SPERM COLLECTION

Sperm (approximately 0.2 ml per male) was collected using a plastic syringe under anesthesia by exerting pressure along the abdomen. The sperm samples were described as viscous (white, dense) or liquid (cloudy, mixed with urine). The percentage of motile cells just after activation was estimated following Levandusky & Cloud (1988), using seawater as an activating solution. The total duration of forward motion was ascertained following Linhart et al. (1993). Sperm samples showing low or no motility were rejected.

EGG COLLECTION AND FERTILISATION

Females were anaesthetised and eggs were manually stripped by applying a light massage along the abdomen. Eggs were collected in a plastic cylinder and the total volume was recorded. Initially, females were stripped before males; the addition of seawater and fertilisation were performed up to 15 minutes after stripping the eggs, until 17-03-96. After that date, sperms were collected prior to eggs. Straight after egg collection, batches of eggs from different females were mixed and transferred into one litre of seawater (sterilised beforehand using filtration to 1 µm ultraviolet radiation). Active sperm from at least two males was immediately added to the suspension of eggs and gently mixed. After approximately five minutes, the floating fraction of eggs was collected and transferred into an incubator. The rate of fertilisation was assessed within six hours after fertilisation

as the percentage of eggs present in the incubator which were undergoing cell division.

Natural maturation and spawning

To favor natural maturation and spawning, dhufish were mainly captured well before the spawning season and settled in defined groups at low density (± 0.5 $kg \cdot m^{-3}$ in one tank out of five the first year and in six tanks out of eight the second year); incoming seawater produced a slight circular current and at least half of the surface of the tank was shaded from the light. Human interference was minimal.

During the second year (1996-1997), fish distribution in tanks was strictly established as detailed above (ratio of male:female < 1) in order to favor natural maturation and spermiation of males.

Egg collectors (500 µm mesh size) were permanently placed at the outlet of each broodstock tank. Buoyant eggs, produced by natural or hormone-induced spawning, were evacuated together with the surface water of the tank into an egg collector, from which they were harvested by scoop net and transferred into an egg incubator. The rate of fertilisation was evaluated as above.

Results

Hormone treatments performed immediately after capture

MALE TREATMENTS

Each of the seven hormone treatments performed within half an hour after capture during the spawning season failed to retain the viscosity of the sperm and activity of spermatozoa : the percentage of motile cells and duration of forward motion, indicative of sperm quality, decreased to <1% and <1 minute respectively within three or four days.

FEMALE TREATMENT

The female which was treated within one hour after capture with 100µg LHRH-a $imp \cdot kg^{-1}$ ovulated. Eggs stripped during four consecutive days were mostly buoyant but could not be fertilised possibly because of the poor quality of sperm available (Table 2).

Hormone treatments performed at least two days after capture

MALE TREATMENTS

During the first spawning season (1995-1996), sperm available for fertilisation was mainly of poor quality and consisted of a low activity, low spermatozoic density mixed sperm-urine fluid.

Some of the treatments with LHRH-a implants performed at least two days after capture were effective on males which were not immediately treated with hormone after capture. Sperm was either produced or its viscosity maintained in two out of five fish after the insertion of an implant of LHRH-a at a dose rate of 100 $\mu g \cdot kg^{-1}$. Sperm was produced in two out of three fish which were administered 30 µg LHRH-a *imp*·kg^{-1}. The quality of sperm produced was high, similar to that obtained by natural maturation, and consisted of a white-colored viscous fluid with highly active spermatozoa (Table 1).

Table 1. Male dhufish: sperm condition (liquid, with a low percentage of active spermatozoa, or viscous, white-couloured, consisting of a high density fluid with a high percentage of active spermatozoa) before and after treatment with LHRH-a implants, performed at least two days after capture from 1995-1997.

Dose rate of LHRH-a *imp*	Number of fish treated	Treatment immediately after capture	Sperm Condition			Time capture-treatment
			Before treatment	1-5 days post-treatment	6-10 days post-treatment	
		none	no sperm	liquid	viscous	> 10 days
		none	viscous	no data	viscous	2 days
100 $\mu g.kg^{-1}$	5	none	no sperm	no sperm	no sperm	> 10 days
		1000 IU hCG·kg^{-1}	no sperm	no sperm	no sperm	6 days
		none	no sperm	no sperm	no sperm	> 10 days
30 $\mu g.kg^{-1}$	3	none	liquid	liquid[1]	viscous	>10 days
		none	no sperm	no sperm	viscous	> 10 days
		none	no sperm	no sperm	liquid	> 10 days

[1]liquid sperm with a high percentage of active spermatozoa.

Table 2. Female dhufish: treatments with hCG and LHRH-a in implants; eggs produced by stripping.

Hormone Type	Dose rate (kg^{-1})	Oocyte diameter (µm)	Time treatment to 1st stripping[1] (h)	Number of ovulations	Number of successful[2] strippings	Total[3] eggs stripped (kg·$fish^{-1}$)	Mean buoyant eggs[4]	Mean fertilisation rate
hCG	1600IU[6]	500	120	1	0	no data	/	/
	1200IU[5]	600	45	4	2	66300	45%	6%
	1000IU	700	≥48	2	0	0	0%	/
	1000IU[5]	600	48	2	0	3000	100%	/
	900IU	600	96	4	1	20200	37%	1%
LHRH-a	120µg[5]	580	116	1	1	1600	94%	0%
	100µg	500	116	5	5	96700	99%	61%
	100µg	400	/	0	0	0	0%	/
	100µg[6]	±600	46	4	4	121400	88%	0%
	100µg	680	43	3	1	21400	9%	0%
	100µg	680	70	4	2	124300	8%	30%
	80µg	460	140	5	4	157000	68%	75%
	50µg	500	74	4	3	72400	71%	no data
	50µg[5]	640	69	4	0	3300	0%	/
	50µg	600	116	>4	4	56400	38%	no data
	25µg[5]	600	79	3	2	118500	40%	no data
	25µg	600	79	3	2	64000	56%	no data
	13µg	620	72	3	2	94100	5%	20%

[1]1st stripping: eggs were mature but not necessarily buoyant.
[2]Which included a fraction of buoyant eggs.
[3]Total eggs stripped (including sinking and buoyant eggs) during five consecutive days.
[4]Fraction of stripped eggs which were buoyant; mean over five days.
[5]Fish died during the experiment.
[6]Female treated within one hour after capture, oocyte diameter estimated onboard the fishing boat.

FEMALE TREATMENTS

Hormone treatments (hCG, LHRH-a, Ovaprim) were effective in inducing ovulation in 95% of treated females (19/20) (Tables 2, 3).

Females treated with hCG at dose rates ranging from 900 to 1600 IU·kg^{-1} ovulated in 100% of cases (n=5) and viable (buoyant and clear) eggs were stripped from two out of five fish. Fertilisation rates were extremely low and a maximum of two consecutive batches of buoyant eggs were obtained per female (Table 2).

Females treated with LHRH-a *imp* at dose rates ranging from 13 to 120 µg·kg^{-1} ovulated in 93% of cases (n=14). One female did not respond to a treatment with 100 µg LHRH-a *imp*·kg^{-1}, but the egg size was only 400 µm in diameter at the time of treatment. At dose rates ranging from 25 to 120 µg LHRH-a *imp*·kg^{-1}, females had to be stripped manually since they did not spawn spontaneously (Table 2).

At a dose rate of 13 µg LHRH-a·kg^{-1} (oocyte diameter at the time of treatment: 620 µm) the treated female spawned spontaneously during three consecutive days. Spawned eggs were highly buoyant and clear but were only fertilised naturally on the first day, at a rate of 0.1%, by males which had been repeatedly anaesthetised and stripped prior to spawning (Table 3).

A dose of 5 µg LHRH-a·kg^{-1} in implant (oocyte diameter : 550 µm) was insufficient to induce ovulation (n=1). A further treatment of the female with 5 µg·kg^{-1} LHRH-a *sal* five days later resulted in a single spawning ± 38 hours after injection. Spawned eggs were buoyant; they were fertilised naturally by the single male present at a rate of 92% (Table 3).

A treatment with 6 µg GnRH-a (0.3 ml Ovaprim)·kg^{-1} at an oocyte diameter of 500 µm resulted in the spawning of a female for three consecutive days. Eggs were fertilised on the first day only (40% fertilisation) (Table 3).

The number of ovulations per female was estimated by stripping and/or by biopsy (Tables 2, 3).

Fertilisation rates of stripped eggs ranged from 0 to 10% and from 3 to 95% with hCG and LHRH-a respectively, many data being unavailable since eggs from different females were mixed prior to fertilisation due to the low quantities of sperm available (Tables 2, 3).

Viable eggs, clear and buoyant, constituted 0 to 99% of total eggs stripped or spawned (Tables 2, 3).

The optimum time of the day to strip hormone-induced females, which corresponded to the time at which a high proportion of eggs stripped were buoyant, had to be assessed after each treatment. This time of day varied as follows: on the first year of study (1996) it was either ± 9h00 or 13h00-14h00 wherein treatments had been performed at 11h00 and 16h00 respectively. During the second year (1997), treatments were performed between 10h30 and 15h00 and the optimum time to strip females was between 23h00 and 2h00 in seven cases out of nine recorded, including cases of eggs released by spawning, and 17h30 - 19h00 in two cases out of nine (Table 4). If the stripping was performed six hours before the optimum time of day, the female belly was flat and no buoyant eggs could be

Table 3. Female dhufish: treatments with LHRH-a (implant and injection) and Ovaprim: eggs produced by spawning.

Hormone treatment (kg^{-1})	Oocyte diameter (µm)	Time treatment to 1st spawning (h)	Number of ovulations	Number of spawnings	Total[1] eggs spawned ($kg \cdot fish^{-1}$)	Mean buoyant eggs	Mean fertilisation rate
13 µg LHRH-a *imp*	620	72	3	3	65800	73%	0%
5 µg LHRH-a *imp*[2]	550	/	0	0	0	/	/
5 µg LHRH-a *sal*[2]	550	±38	1	1	16400	79%	92%
6 µg GnRH-a (Ovaprim)	500	±38	3	3	32400	74%	17%

[1]Total eggs spawned which were harvested from the egg collector (including sinking and buoyant eggs) for three consecutive days.
[2]The same female was administered both treatments at five days interval.

Table 4. Period of time since treatment and time of day at which the first batch of buoyant eggs were obtained following treatments with hCG, LHRH-a (implant and injection) and Ovaprim.

Date, Time		Eggs stripped or spawned	Number of buoyant eggs in 1st batch $\cdot kg^{-1}$	Fertilisation rate	Type of treatment
Hormone treatment	1st batch of buoyant eggs obtained				
12March96, 16h00	14March96, 13h15	stripped	15400	7%	1200 IU hCG$\cdot kg^{-1}$
12March96, 16h00	17March96, 12h00	stripped	14000	3%	100µgLHRH-a *imp*$\cdot kg^{-1}$
21March96, 11h00	23March96, 9h20	stripped	58800	0%	100µgLHRH-a *imp*$\cdot kg^{-1}$
29March96, 16h00	3April96, 12h00	stripped	1600	0%	120µgLHRH-a *imp*$\cdot kg^{-1}$
10January97, 14h30	16January97, 18h50	stripped	20800	85%	80µgLHRH-a *imp*$\cdot kg^{-1}$
14January97, 15h	18January97, 2h00	stripped	17100	30%	100µgLHRH-a *imp*$\cdot kg^{-1}$
10February97, 14h30	16February97, 2h30	stripped	6100	no data	50µgLHRH-a *imp*$\cdot kg^{-1}$
21February97, 10h30	25February97, 0h15	stripped	25100	no data	50µgLHRH-a *imp*$\cdot kg^{-1}$
21February97, 10h30	25February97, 0h00	stripped	27200	60%	25µgLHRH-a *imp*$\cdot kg^{-1}$
21February97, 10h30	24February97, 17h30	stripped	7300	no data	25µgLHRH-a *imp*$\cdot kg^{-1}$
21February97, 10h30	25February97, 0h00	stripped	3900	20%	13µgLHRH-a *imp*$\cdot kg^{-1}$
12March97, 10h40	14March97, ± 0h30	spawned	12900	92%	5µgLHRH-a *sal*$\cdot kg^{-1}$*
18March97, 10h20	20March97, ± 0h30	spawned	16700	40%	6µgGnRH-a$\cdot kg^{-1}$

*Fish treated beforehand with 5 µg$\cdot kg^{-1}$ of LHRH-a implant on 7-03-97 at 12h00.

extracted, while with eight to ten hours delay the majority of stripped eggs tended to sink.

Natural maturation and spawning in broodstock tanks

NATURAL MATURATION, SPERMIATION AND FERTILISATION

Natural maturation and spermiation, were recorded in only four cases (22% success rate, n=18) during the first spawning season (1995-1996) and were observed at least six months after capture. Firstly, one out of four males produced sperms naturally in a tank holding four females. Secondly one small male (2.6 kg) also spermiated in a tank holding three females and one other male, large (8 kg) but unhealthy and which did not produce any sperm. Finally, on two occasions a male with no sperm, transferred into a tank holding induced females, produced sperm of high quality within 18 days of its transfer.

In 1996-1997, the majority of males (67%, n=21) matured naturally 2.5 to 16 months after capture, whether or not they had been treated with hormone

at capture. Natural maturation was best achieved among males kept for a minimum of 2.5 months with no other male (100% success rate, n=3) or with another male smaller in size and three to four females (75% success rate, n=4). When two males were present in a broodstock tank, the small size males matured in 50% of cases (n=4). In tanks (n=7) holding more than two males (up to eight) with a variable number of females, only one or two of the males, not necessarily the largest, matured naturally while sperm produced by the other males present was liquid or absent (100% of cases).

Natural fertilisation (of eggs spawned by hormone-treated females) occurred on three occasions.

NATURAL OVULATION AND SPAWNING

The majority of captive females (20 out of 22) underwent gonadal development to a maximum oocyte diameter of 680 μm but neither ovulated nor spawned naturally. Two females ovulated naturally in a 40 m^3 concrete tank in December 1995 after being held for four months at a density of 0.5 $kg \cdot m^{-3}$ and one of them spawned spontaneously for three consecutive days.

Discussion

Introduction

This study describes the first trials of spawning induction on the WA dhufish. Fertilised eggs, thence juveniles, were produced by hormone treatment of females and either artificial or natural fertilisation. Natural maturation of males was readily achieved when held under particular stocking conditions and in some cases sperm was produced by hormone treatment with implants of LHRH-a. In most cases, females required hormone treatment to undergo the final stages of maturation and viable eggs were successfully produced using a range of hormones including hCG, LHRH-a implants, LHRH-a injection and Ovaprim.

Sperm production

Sperm production in captive marine fish usually occurs spontaneously without hormonal treatment at a ratio of males: females ≥ 1 (Smith 1986; Garcia 1989; Zohar et al 1989; Barnabe 1994) while in the present study, when several male dhufish were present with females (ratio male:female ³ 1) only one or two of the males produced sperm naturally. On the contrary, natural maturation and spermiation were readily achieved when male dhufish were held for several months as sole males with at least two females (ratio males: females <1).

If sperm was required from males, which were not spermiating during the spawning season, a treatment with 30 μg LHRH-a·kg^{-1} in a cholesterol-based implant should be attempted.

Dhufish sperm was particulary dense and its total volume per male by manual stripping (1-2 ml) was small compared to that usually obtained with

other cultured marine species such as pink snapper (*Pagrus auratus*) or atlantic cod (*Gadus morhua*) (Jenkins, G.I., unpubl. data; Suquet et al. 1994).

In this study, natural fertilisation of eggs released spontaneously by a female dhufish under hormone treatment was achieved on several occasions. However, repeated anesthesia and stripping of males seemed to affect their ability to perform natural fertilisation.

Viable eggs production

EGGS PRODUCED BY SPAWNING

Spawning of female dhufish was successfully achieved in this study under hormone treatments at low dose rates (≤ 13 µgLHRH-a· *imp* or *sal* kg^{-1}). At higher dose rates of LHRH-a *imp* (25-120 $\mu g \cdot kg^{-1}$), female dhufish ovulated but failed to spawn. Similar results were obtained with sea bream (*Sparus aurata*) which had to be stripped manually when treated with high doses of hCG (≥ 800 $IU \cdot kg^{-1}$) while at a lower dose rate (100-200 IU $hCG \cdot kg^{-1}$), or with 5-20 µg LHRH-a $saline \cdot kg^{-1}$, spawned naturally at daily interval (Gordin & Zohar 1978; Zohar & Gordin 1979; Zohar et al. 1989). Most sea bream females treated only spawned for one to six days and then stopped while the rest of the vitellogenic oocytes underwent rapid atresia (Zohar et al. 1989). Similarly in the present study, dhufish females spawned daily for a short period of one to three days, after which time the oocyte diameter regressed to 600 µm or less.

Hormonal induction of marine species with LHRH-a combined with a dopamine inhibitor (e.g. Ovaprim) is poorly documented to date and several authors recommend not to use such treatments with marine fishes (Bromage & Roberts 1995). However, Battaglene & Allan (1994) showed that treatments with Ovaprim increased the number of ovulations per female in pink snapper compared with treatments with hCG but did not induce females to spawn. In the present study, ovulation and spawning were both successfully achieved after one treatment with Ovaprim (0.3 $ml \cdot kg^{-1}$) but further attempts are required to assess the potential of this hormone with dhufish.

Further hormone treatments with low doses of LHRH-a saline (5 $\mu g \cdot kg^{-1}$) and Ovaprim (6 µg $GnRH\text{-}a \cdot kg^{-1}$) failed to induce spawning of female dhufish in April 1997. However, at that time of year in 1997, wild dhufish at the latitude of the Fremantle Maritime Centre were no longer spawning. The lack of response to treatments may have been caused by the commencement of gonadal atresia in treated fish.

EGGS PRODUCED BY STRIPPING

Treatments with high dose rates of hCG (1000 $IU \cdot kg^{-1}$) and LHRH-a (25-120 $\mu g \cdot kg^{-1}$) in implants induced ovulation in the majority of females treated but not spawning. Females reacted to treatments at high dose rates by a massive swelling of their abdomen and in many cases eggs were difficult to extract by stripping. With hCG, fertilisation rates were extremely poor but may have

been affected by a delay of up to 15 minutes between egg stripping and fertilisation, the overripening of ova or the low quality of sperm available. Eggs spontaneously released in the water by females, which had been treated with high doses of hormone, were always overripe and unfertilised.

Implants of LHRH-a induced the ovulation of all but one female dhufish. The latter had a maximum oocyte diameter of 400 µm at the time of treatment. It is difficult to induce ovulation in broodstock which have not attained the critical stage of gonadal development including a certain species-specific oocyte diameter (Lam 1982; Bromage & Roberts 1995) and it is possible that an oocyte diameter of 400 µm may be too small for female dhufish to respond to a treatment with slow-release implants.

Egg quality and fertilisation success are maximum when eggs are stripped a certain time post-ovulation (Bromage et al. 1994). After this time, eggs gradually become overmature over a period which is species-specific (e.g. ≤8 hours in pink snapper, Scott et al. 1993) and can be as short as one hour (Shelton 1989; Bromage & Roberts 1995). Stripping female dhufish at an inappropriate time of day resulted in eggs being overripe or hard to strip and forming clusters, or the abdomen soft and empty. The optimum time of day to strip female dhufish varied from one experiment to another, did not appear to be related to the time of day at which the treatment was performed, and was usually identified as the earliest time when an easy flowing, fluid mass of highly buoyant eggs was obtained after light massage of the female abdomen. Indeed, the time when eggs flow freely given slight pressure on the abdomen of female southern flounder (*Paralichthys lethostigma*) corresponds approximately to the time of ovulation (Berlinsky et al (1996). Moreover, the correlation between egg buoyancy and potential for fertilisation success has been demonstrated for a number of marine species (Carrillo et al. 1989; Watanabe et al. 1995).

A delay of eight to ten hours in stripping female dhufish resulted in a dramatic decrease of the proportion of buoyant eggs extracted.

During the assessment of the correct time to strip, in the present study, many spawns were wasted and 30% of broodstock female died as a result of repeated handling. Loss of fish in these circumstances is a common complaint and is unacceptable when dealing with costly broodstock (Hodson & Sullivan 1993) such as the WA dhufish. Moreover, stress is well known to exert a deleterious effect on the reproductive physiology of females in species such as snapper (Carragher & Pankhurst 1991) and can cause failure to ovulate and atresia besides the loss of broodstock (Billard et al. 1981; Sumpter et al. 1987; Hodson & Sullivan 1993). For these reasons, induced spawning presents obvious advantages over egg collection by manual stripping.

Conclusion

Both male and female dhufish appeared to be particularly sensitive to capture, anaesthesia, handling and hormone treatments. Female dhufish were overwhelmed by the process of induced ovulation at high hormone dose rates

and required human intervention by means of stripping to obtain viable eggs, with a variable degree of success. According to the preliminary results obtained in this study, it seems more appropriate to investigate egg production further with treatments at low hormone dose rates such as 4-6 µg LHRH-a or GnRH-a·kg^{-1}, possibly as a double injection. Indeed, at such low dosage treatments, females seemed to retain some control over the post-ovulatory process, maintain their ability to spawn and even trigger natural fertilisation in some cases.

Favoring natural maturation, spermiation and fertilisation by holding males in suitable environmental conditions (f.i. low fish density) with a small number of females and no other male and by avoiding hormone treatments, anaesthesia and handling seems equally as important to achieve a reliable production of fertilised eggs from dhufish.

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