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Article · June 1994

DOI: 10.33997/j.afs.1994.7.1.005

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Asian Fisheries Science 7(1994):35-40.
Asian Fisheries Society, Manila, Philippines

Chilled Storage of Pikey Bream *(Acanthopagrus berda)* Sperm and Activation in Different Salinities

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Abstract

Spermatozoa from the pikey bream, *Acanthopagrus berda*, were tested for motility in different salinities and short-term chilled storage with four diluents. Motility was most intense in salinities of 25, 30 and 35 $^{o}/_{oo}$, and its duration was longest at $\geq$15 $^{o}/_{oo}$. Dilution of semen (1:4 *v/v*) with blood plasma from the same species, marine teleost Ringer, a glucose solution (51.7 g/l H_20) and an egg yolk/sodium citrate solution each increased chilled storage life. Sperm in the egg yolk diluent maintained good motility for 13 days, whereas the blood plasma, Ringer and glucose media maintained it for 8 days, compared with only 5 days for undiluted semen.

Introduction

The pikey or black bream (*Acanthopagrus berda* Forsskal) is an important angling and table fish which occurs in rivers and estuaries of northern Australia from Shark Bay (26° 07'S, 113° 25'E) in the west to Rockhampton (23° 22'S, 150° 32'E) in the east (Grant 1987). Its world distribution extends from South Africa to India to the western Pacific, including Japan and northern Australia (Fischer and Bianchi 1984). It belongs to the family Sparidae, some members of which are important species for aquaculture (Kitajima and Tsukashima 1983). The economic significance of this group, and the wide distribution and apparent indifference of *A. berda* to turbid waters (Cyrus and Blaber 1987), suggests they have potential for domestication and aquaculture.

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Specific knowledge of optimal sperm activation and storage techniques can facilitate fish breeding programs (Stoss 1983). The motility of sperm is a useful indicator of appropriate salinities for insemination, and the ability to store semen for short periods can add to the reliability of fish production. Reproductive information on *A. berda* is not available but its more temperate congener, *Acanthopagrus australis*, has shown protandrous sex inversion (Pollock 1985), and a short estuarine spawning peak between July and August in southeastern Queensland (Pollock 1982). Oceanic salinity (36 $^{o}/_{oo}$) has recently been shown to produce maximum sperm motility in *A. australis* (salinities in the range 4.5-36 $^{o}/_{oo}$ tested); storage of undiluted semen was more successful at 4°C than at 23°C (Thorogood and Blackshaw 1992). Chao et al. (1986) reported preliminary investigations into the biological properties and liquid preservation of sperm from the black porgy, *Acanthopagrus schlegeli*. They found undiluted semen maintained good motility for up to 10 days when stored at 4°C.

This study of *A. berda* sperm motility in different salinities provides information on appropriate spawning and insemination salinities, and presents an evaluation of four diluents for improved-chilled storage of semen, which may assist in future breeding programs.

Materials and Methods

Broodstock Maintenance and Sperm Collection

Fish were captured in the Trinity Inlet system in Cairns (16° 55'S, 145° 46'E) and held in 4,000-l tanks with aeration and flow-through of seawater (35 $^{o}/_{oo}$, 26-27.5°C) during the months of August and September. Male fish ranged in total length (TL) from 139 to 225 mm (60-220 g), and females were 205-357 mm long (210-890 g). Female fish were injected with human chorionic gonadotropin (1,000-2,000 IU kg^{-1} body weight), but no ripe oocytes were obtained for fertility testing. Immediately prior to starting each experiment, semen was manually expressed from the genital opening by slight abdominal pressure. Semen was collected in syringes that had been pre-cooled on ice. Before stripping, the area around the genital opening was dried and the bladder was cleared with abdominal pressure, so that contamination of semen with water or urine was minimized.

Activation in Different Salinities

Sperm motility was tested in eight salinities (diluted seawater) from 0 to 35$^{o}/_{oo}$ at 21-25°C. Four semen samples from different fish were tested twice, and two pooled samples from 33 and 4 fish were tested 6 times.

Intensity and duration of motility were assessed according to a method modified after Hogan and Nicholson (1987). Semen adhering to the tip of a probe was mixed with two drops of media in the cavity of a slide. A coverslip was quickly placed over the cavity and motility was observed under 400x magnification. Motility intensity was rated within 10 seconds of activation and ratings were assigned as:

5 Most active sample observed for this species; sperm creating swirling currents obscuring the movement of individual sperm across the field of view
4 Very active sample; all sperm visibly progressing rapidly across the field of view
3 Less energetic head and tail movement; most with forward motion
2 Slow head and tail movement; some individuals progressing slowly
1 Head movement only; no progressive motion
0 No activity

Duration of motility was measured as the time from activation to >99% cessation of movement. Analysis of variance was used to test the significance of treatment effects for motility duration.

Chilled Storage with Diluents

Aliquots of semen (0.2 ml) were taken from a pooled collection from 28 fish and stored undiluted or diluted (1:4 *v/v*) with four storage media. These diluents were pre-cooled on ice in 2-ml plastic vials before adding semen, and vials were capped during storage. The diluents tested were:

1. centifuged blood plasma from *A. berda* (400 rpm, 15 minutes);
2. marine teleost Ringer (7.25 g NaCl, 0.38 g KCl, 0.24 g $CaCl_2 \cdot 2H_2O$, 0.27 g $MgSO_4 \cdot 7H_2O$, 0.01 g $NaHCO_3$, 0.41 g $NaH_2PO_4 \cdot 2H_2O$, 1.01 g glucose, and H_2O to 1 liter);
3. D^+ glucose (51.7 g/l H_2O) solution (10 ml) adjusted to pH 7.4 with PO_4 buffer (0.1 ml); and,
4. sodium citrate solution (3.1 g $Na_3C_6H_5O_7 \cdot 2H_2O$ and 90 ml H_2O) buffered to pH 7.4 with 5% citric acid and made up to 100 ml with water, then added to an equal volume of freshly separated chicken egg yolk from a fresh egg (after Hogan et al. 1987).

Antibiotics were added to all storage vials at a rate of 500 IU penicillin (Crystapen^R Benzyl penicillin sodium BP) and 500 mg streptomycin (Strepolin^R Streptomycin sulphate Injection BP) per ml of the final volume in each vial (after Stoss and Holtz 1983).

Storage treatments were replicated 6 times and were stored in a refrigerator set to 5°C (3.5-9°C). The intensity of motility of sperm in each vial was tested daily in a salinity of 25 $‰$ until motility had ceased in all vials.

Results

No sperm activated in salinities of 0 and 5 $‰$. Table 1 shows the mean motility intensity and duration in *A. berda* sperm following activation in salinities $\geq$ 10 $‰$. Motility was more intense in salinities $\geq$ 20 $‰$ than in 10 and 15 $‰$. Duration of motility was shorter in 10 $‰$ than in salinities $\geq$ 15 $‰$. No differ-

Table 1. Mean spermatozoal motility intensity (0-5) and duration (minutes) in *A. berda* following activation in different salinities.

Salinity (‰)	Motility intensity	Motility duration
10	1.3 ± 0.53	17.4 ± 9.40[b]
15	2.4 ± 0.22	75.8 ± 42.18[a]
20	3.1 ± 0.09	84.6 ± 9.03[a]
25	3.6 ± 0.19	104.1 ± 8.61[a]
30	3.6 ± 0.23	83.3 ± 14.04[a]
35	3.7 + 0.20	93.7 ± 16.79[a]

[a-b] Corresponding letters with duration means indicate no significant difference. Otherwise, differences are significant ($P < 0.05$). Standard errors are provided.

ences ($P>0.05$) in motility duration were found in salinities between 15 and 35‰.

Storage life of semen is shown in Fig. 1. Immediately after mixing semen with diluents (day 0), motility intensity was lower from the Ringer and glucose media than from the other treatments. Sperm stored in the egg yolk medium had higher initial motility following activation than sperm from other media. Motility intensity declined most rapidly during storage in undiluted semen and was less than 3 after 5 days. The egg yolk diluent maintained motility intensity above 3 for 13 days, whereas the blood plasma, Ringer and glucose media maintained it above 3 for only 8 days.

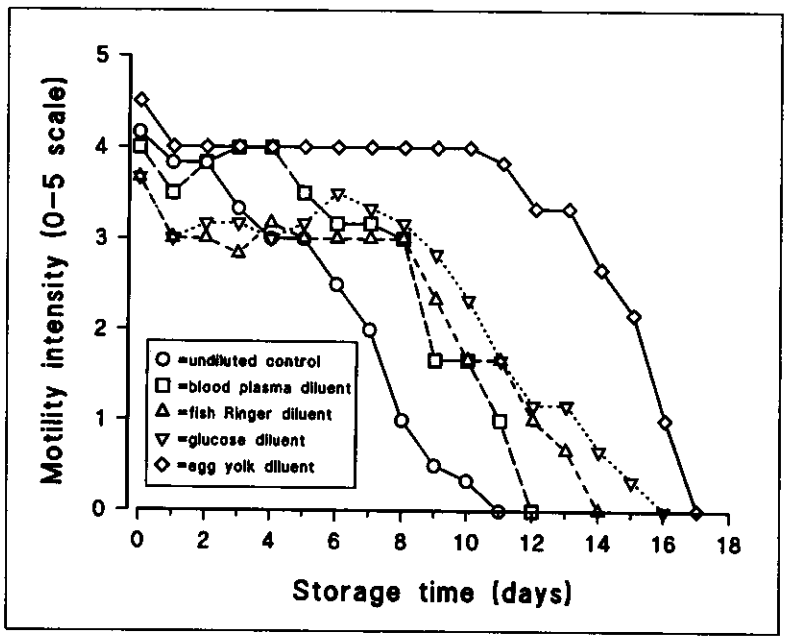

Fig. 1. Daily motility intensity of *A. berda* sperm activated in a seawater solution (25 ‰) following chilled storage (3-9°C) with different diluents. Data are presented as means ± SE (n=6).

Discussion

Sperm motility can be a useful parameter for evaluating viability, as a correlation generally exists between the presence of motility in fish sperm and their capacity to fertilize eggs (Stoss 1983). However, it is worth noting that motility is not always a guarantee of fertilizing capacity (Ginzburg 1972), particularly when sperm are stored *in vitro* for several days. This is due to different parts of the spermatozoon being responsible for locomotion (tail) and fertility (head), thereby making possible differential pathological changes which may be increased with prolonged storage. Forward progression of sperm is presumed necessary for penetration of the micropyle in teleosts (Ginzburg 1972), and accordingly, sperm exhibiting motility ratings lower than a 3 in the present study were assumed to have reduced viability.

Motility ratings for *A. berda* sperm were less than 3 at or below salinities of 15 $^{o}/_{oo}$. This suggests that spawning may be successful in a range of salinities above 15 $^{o}/_{oo}$. In contrast, *A. australis* appears to require oceanic salinity for maximum sperm motility (Thorogood and Blackshaw 1992), such as is characteristic of the open surf bars where this species spawns (Pollock 1982). This broader range of salinities for optimal sperm activation in *A. berda* compared to *A. australis*, may reflect an adaption of *A. berda* to the higher rainfall in northern Australia where this species would more frequently experience marked fluctuations in inshore salinity.

All of the diluents tested enhanced storage time. Egg yolk/sodium citrate was most effective with low motility still evident after 16 days of storage. Chilled storage of undiluted sperm was least successful, with motility falling below 3 after 5 days, and no motility after 11 days. This could be due to a number of factors including adverse dehydration effects in undiluted samples, and higher nutrient availability in diluted samples, even though oviparous fish spermatozoa are thought to show limited glycolytic activity (Mounib 1967). High motility levels were maintained for 10 days at 4°C in undiluted sperm from *A. schlegeli* (Chao et al. 1986), but for fewer than 2 days in undiluted *A. australis* sperm held at 4°C (Thorogood and Blackshaw 1992). *In vivo* ageing of sperm causing within season variation in sperm quality can reduce their keeping qualities (Stoss 1983). This was noted in the work with *A. australis* and *A. schlegeli*, suggesting chilled storage of *A. berda* sperm may also be less successful with semen collected late in the spawning season.

Hara et al. (1982) found blood serum to be a superior storage medium for milkfish (*Chanos chanos*) sperm. For this species, glucose and sodium citrate media were unsatisfactory although both have been used successfully in other fish species (Harvey and Kelley 1984; Chao et al. 1986). Hogan et al. (1987) reported chilled storage of barramundi (*Lates calcarifer*) sperm using the same diluents tested herein for *A. berda*. In this case, the egg yolk diluent was again most effective, maintaining motility at or above 3 for six days, compared with less than two days in undiluted sperm. More recent experiments with barramundi sperm (P.J. Palmer, unpubl. data) suggests that the egg yolk diluent can create water quality problems in incubators with low water exchange, and so effective washing of inseminated eggs before their introduction into hatching systems may be advantageous for this diluent.

Fertility experiments dealing with the insemination of ova are necessary to validate these storage techniques. However, this study provides useful preliminary information for such validation by screening potential diluents, providing a time frame for storage and outlining appropriate insemination salinities. Though crypreservation of sperm cells can offer long-term advantages, this study shows the potential of chilled-storage techniques which require less sophisticated equipment and methods.

Acknowledgements

The authors wish to thank Mr. Les Faithful for his help in acquiring broodstock, and staff from the Northern Fisheries Centre for technical assistance and support during the project. Dr. A.W. Blackshaw of the University of Queensland and staff at the QDPI Southern Fisheries Centre, provided constructive criticism of the manuscript. Thanks also to Dr. R. Brown and participants in the QDPI publishing workshop program for editorial advice.

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Manuscript received 16 October 1992; revised ms received 25 August 1993; accepted 16 February 1994.

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