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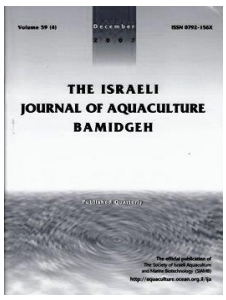

Sperm Properties of Waigieu seaperch (*Psammoperca waigiensis*)

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Key words: Waigieu seaperch , *Psammoperca waigiensis*, seminal plasma, semen properties

Abstract

The aim of this study was to determine the semen properties of Waigieu seaperch (*Psammoperca waigiensis*). The physical properties of the semen were: semen volume 1.28±0.05 ml/fish, sperm density 31.35±0.67x10^9 cells/ml, spermatocrit 87.70±0.80%, and sperm count 40.12±1.68x10^9 cells/fish. The biochemical properties of the seminal plasma were 154.45±0.48 mmol/l sodium, 16.89±0.31 mmol/l potassium, 113.58±0.38 mmol/l chloride, 12.75±0.38 mmol/l calcium, 6.58±0.22 mmol/l magnesium, 1.15±0.08 g/l total protein, 346.37±2.71 mOsm/kg osmolality and 7.85±0.07 pH. Regression analysis showed a significant positive linear relationship between fish length and fish weight, sperm count and semen volume, and sperm density and spermatocrit, supporting the use of spermatocrit as a rapid estimator of sperm density in this species. Sperm motility parameters tested showed sperm motility, sperm velocity and duration of sperm motility as 95.80±0.389%, 144.50±0.96 µm/s and 232.30±2.32 s, respectively. The results from this study provide information on Waigieu seaperch sperm physiology. The values and correlations between the semen properties and the composition of seminal plasma may provide useful information for the formulating a species-specific extender solution for the cryopreservation of Waigieu seaperch sperm.

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Introduction

Controlled breeding in captivity is an integral component of finfish aquaculture. Even though quality of both the sperm and the eggs (the gametes) affects fertilization success and larvae survival, fish farming has focused more on the quality of eggs and larvae than sperm. Adequate knowledge of semen properties is necessary to ensure that high quality semen is available when required and that fertilization is optimal (Şahin et al., 2012). Semen (or milt) is defined as spermatozoa plus seminal plasma, which mediates the chemical function of the ejaculate. Seminal plasma has a unique composition with some components supporting the spermatozoa, while others reflect the functions of the reproductive system and the spermatozoa (Alavi and Cosson, 2006). Studies on semen properties are necessary to understand basic biochemical processes that occur in sperm motility and during fertilization, thereby enabling the evaluation of the reproductive abilities of different fish species and improving methods for short and long-term storage of fish semen ((Alavi and Cosson, 2006; Rurangwa et al., 2004; Alavi and Cosson, 2006).

Waigieu seaperch (*Psammoperca waigiensis)* is a tropical marine fish species of high economic value and has become a cultured species of interest (Shimose and Tachihara, 2006; Pham*,* et al., 2007a, Pham, et al., 2007b; Pham, et al., 2009; Pham, et al., 2010; Pham, et al., 2012). Studies on the reproductive biological characteristics, and artificial seed production, have been published however there has been little research on the physico-biochemical sperm properties of Waigieu seaperch (Nguyen and Luc, 2003; Shimose and Tachihara, 2006; Alavi and Cosson 2005, Alavi and Cosson 2006; Cabrita*,* et al., 2010). Knowledge of the semen properties of fish is necessary for controlled and successful production in aquaculture systems. The objective of the present study is to determine the physical and biochemical properties of Waigieu seaperch sperm at peak spawning season. This information will facilitate the selection of good quality sperm and improve protocols for cryopreservation and artificial propagation methods.

Materials and Methods

The experiments were carried out in the laboratory of the Department of Fisheries Biology, Faculty of Aquaculture, Nha Trang University, Vietnam. Broodstock Waigieu seaperch were collected from the wild. They were separated into two cages and cared for until sexual maturity. Semen was collected during the breeding season by abdominal massage after anesthetization with MS-222 at a concentration of 100 ppm. Semen was collected and stored in 1.5 ml Eppendorf tubes on crushed ice until analysis.

Semen physical properties analysis. After semen volume was determined, spermatozoa density was measured using a hemocytometer counting chamber. The semen was diluted 1000 times by pipetting 10 µl of semen in 990 µl 0.2% formaldehyde. A droplet of the diluted semen was placed on a hemocytometer slide (depth 0.1 mm) with a coverslip and counted using light microscopy (400X magnification). After allowing sperm sedimentation for 3-5 min, the sperm cells were counted in 16 chamber cells and calculated for sperm density (Le, et al., 2007). Spermatocrit is defined as the ratio of white packed material volume to the total volume of semen multiplied by 100 (Le*,* et al., 2011a). Glass microhematocrit capillary tubes (75 mm length and 1.1-1.2 mm diameter) were filled with semen and one end of each tube was sealed with clay. The capillary tubes were centrifuged at 15,000 rpm for 10 min. Spermatocrit counts were read in a Hawksley micro-hematocrit reader (Hawksley and Sons Ltd., England). Sperm count was calculated as the product of semen volume and sperm density.

Seminal plasma biochemical properties analysis. To analyze biochemical properties in the seminal plasma, semen was separated from the seminal plasma by centrifugation (15,000 rpm for 10 min). Seminal plasma was centrifuged twice to avoid possible contamination with spermatozoa. The supernatants were frozen and stored in the freezer for 3 days until analysis. Biochemical components of seminal plasma were determined using Fuji Dri-Chem 3500 (Fujifilm Co. Ltd., Japan). A pH meter (pH test, Romania) and an osmometer (Advanced Instruments Inc., USA) were used to measure the pH and osmolality of seminal plasma.

Sperm motility parameters analysis. To measure sperm motility parameters (sperm motility, sperm velocity, duration of sperm motility), 1 µl of the diluted sperm was placed on a glass slide (Teflon Printed Glass Slide; 21 wells; diameter of well, 4 mm; Funakoshi Co., Japan) without a cover slide. Sperm motility parameters were observed immediately

at 400X magnification under a microscope (Olympus BX41TF, Tokyo, Japan) setting and recorded with video camera (Nikon D5200, Japan). A sample was observed three times under the microscope and the time until the each sperm stopped moving was recorded. To analyze sperm motility parameters, recorded files were played on video software using computer aided sperm analysis (CASA) software. Duration was calculated after 100% of sperm stopped moving (Le, et al., 2011b; 2011c; 2011d).

Statistical analysis. Means are expressed as means±standard deviation or standard error. Data analyses were carried out using SPSS 16.0 for windows software package. Differences with a probability value of $P<0.05$ were considered significant.

Results

Table 1 shows the following results for W. Seaperch: *the physical properties of semen*: semen volumes (1.28±0.05), density (31.35±0.67x10), spermatocrit (87.70±0.80%) and sperm count (40.12±1.68x10); *the biochemical properties of the seminal plasma*: sodium and chloride were predominant ions, the mean value of osmolality for seminal plasma was 346.37±2.71 mOsm/kg; and *sperm motility parameters*: sperm motility, sperm velocity and duration of sperm motility were 95.80±0.389%, 144.50±0.96 µm/s and 232.30±2.23 s, respectively.

Table 1. Descriptive statistics of sampled fish, physical and biochemical properties of Waigieu seaperch *Psammoperca waigiensis* semen (n=10)

Parameters	*n*	*Range*	*Minimum*	*Maximum*	*Mean*		*Std. Dev.*
	Statistic	*Statistic*	*Statistic*	*Statistic*	*Statistic*	*Standard Error*	*Statistic*
Fish weight (g)	10	329.00	421.00	750.00	580.60	35.12	111.02
Fish length (cm)	10	3.20	25.30	28.50	26.82	0.35	1.10
Semen volume (ml/fish)	10	0.40	1.10	1.50	1.28	0.05	0.15
Concentration (cells*10^9/ml)	10	5.70	28.70	34.40	31.35	0.67	2.12
Spermatocrit (%)	10	8.00	84.00	92.00	87.70	0.80	2.54
Sperm count (cells*10^9/fish)	10	15.93	32.23	48.16	40.12	1.68	5.31
Sodium (mmol/l)	10	4.90	151.50	156.40	154.45	0.48	1.53
Potassium (mmol/l)	10	2.70	15.70	18.40	16.89	0.31	0.98
Chloride (mmol/l)	10	3.90	111.90	115.80	113.58	0.38	1.19
Magnesium (mmol/l)	10	2.40	5.70	8.10	6.58	0.22	0.70
Calcium (mmol/l)	10	3.70	11.20	14.90	12.75	0.38	1.19
Total protein (g/l)	10	0.70	0.80	1.50	1.15	0.08	0.25
Osmolality (mOsm/kg)	10	26.50	334.80	361.30	346.37	2.71	8.57
pH	10	0.60	7.60	8.20	7.85	0.08	0.24
Salinity (ppt)	10	5.00	27.00	32.00	29.50	0.50	1.58
Sperm motility (%)	10	4.00	94.00	98.00	95.80	0.39	1.23
Duration sperm motility(s)	10	18.00	225.00	243.00	232.30	2.23	7.04
Sperm velocity (µm/s)	10	10.00	140.00	150.00	144.50	0.96	3.03

Correlations between fish weight/length, spermatological properties and biochemical properties are presented in Table 2.

Table 2. Correlation between spermatological properties and seminal plasma composition of Waigieu seaperch *Psammoperca waigiensis* semen (n=10)

	FW	FL	SV	SD	ST	SC	Na^+	K^+	Cl^-	Mg^{2+}	Ca^{2+}	TP	Osm	pH
Fish weight (FW)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fish length (FL)	.978*	-	-	-	-	-	-	-	-	-	-	-	-	-
Semen volume (SV)	-.334	-.312	-	-	-	-	-	-	-	-	-	-	-	-
Sperm density (SD)	-.164	-.061	-.036	-	-	-	-	-	-	-	-	-	-	-
Spermatocrit (ST)	-.214	-.156	.041	.834*	-	-	-	-	-	-	-	-	-	-
Sperm count (SC)	-.381	-.306	.847*	.499	.480	-	-	-	-	-	-	-	-	-
Sodium (Na^+)	.089	-.024	-.266	.243	.556*	-.102	-	-	-	-	-	-	-	-
Potassium (K^+)	.562*	.595*	-.156	-.324	-.400	-.327	-.193	-	-	-	-	-	-	-
Chloride (Cl^-)	-.046	-.020	-.426	.308	.291	-.198	.158	-.395	-	-	-	-	-	-
Magnesium (Mg^{2+})	.335	.317	-.400	.023	-.072	-.347	.014	.283	-.513	-	-	-	-	-
Calcium (Ca^{2+})	-.105	.001	.108	.368	.194	.327	-.275	-.515	.391	-.351	-	-	-	-
Total protein (TP)	.011	-.057	-.122	.129	-.098	-.018	.055	-.562	.246	-.026	.432	-	-	-
Osmolality (Osm)	.477	.433	-.420	-.505	-.540	-.637	-.232	.297	.323	-.007	-.180	.211	-	-
pH	-.489	-.469	-.350	.414	.249	-.081	.130	-.219	-.146	.480	-.042	.162	-.412	-
Salinity (Sa)	.367	.440	.333	.184	.318	.394	-.044	-.054	.224	-.320	.441	-.243	-.111	-.697

*Correlation is significant at $P<0.05$.

Significant polynomial correlations were observed between fish length and fish weight (r=0.978, $P<0.05$), sperm count and sperm volume (r=0.847, $P<0.05$), and sperm density and spermatocrit (r=0.834, $P<0.05$).

Discussion

The semen volume of Waigieu seaperch was lower than that of black porgy and European perch, but higher than that of yellow croaker (Chang, et al., 1995; Alavi, et al., 2007; Le, et al., 2011a). The present study showed that the Waigieu seaperch produces semen with a very low density compared to other teleosts such as yellow perch but higher than European perch and yellow croaker (Ciereszko and Dabrowski, 1993; Alavi, et al., 2007; Le, et al., 2011a). The spermatocrit of Waigieu seaperch, was higher than yellow perch, but lower than yellow croaker (Ciereszko and Dabrowski 1993; Le, et al., 2011a). Observational differences in semen properties support the assumption that semen production in Waigieu seaperch may be related to various factors, including age and weight of males, rearing conditions, nutrition, breeding seasonality, methods of spawning induction and spawning behavior of broodfish, sampling period, and method (Le et al., 2011a; Şahin et al., 2012).

The relationship between fish length and fish weight (r=0.975, P<0.05), and the relationship between sperm count and sperm volume (r=0.989, P<0.05) in Waigieu seaperch was comparable to that of yellow croaker (Le, et al., 2011a). Additionally, a strong positive relationship was observed between sperm density and spermatocrit. Similar correlations have been reported for many other teleost fish species such as yellow croaker and yellow perch (Le, et al., 2011a; Ciereszko and Dabrowski, 1993). A significant correlation was found between spermatocrit and optical density, but no

significant correlation was found between spermatocrit and sperm density, in turbot (Suquet, et al., 1992). Spermatocrit can be used as a quick and easy technique for estimating sperm density in yellow croaker and significant correlation was found between spermatocrit and sperm density in yellow croaker (Le, et al., 2011a). Using this technique to estimate sperm density, a significant correlation was found between spermatocrit and sperm density in Waigieu seaperch.

Biochemical criteria of importance are the presence or absence of inorganic and organic components in the semen and the osmolality and pH of seminal plasma (Alavi and Cosson, 2006). Sodium, potassium, and chloride ion concentrations that predominate in seminal plasma of Waigieu seaperch, were 169.5, 4.9 and 156.0 mM/l in black porgy, and 148.3, 14.0 and 115.5 mM/l in yellow croaker, respectively (Chang, et al., 1995; Le, et al., 2011a). The calcium and magnesium levels of Waigieu seaperch seminal plasma are higher than in yellow croaker (Le, et al., 2011a). The osmolality value of seminal plasma in Waigieu seaperch was higher than in European perch, 298.1 mOsm/kg, and in yellow croaker, 342.5 mOsm/kg ((Alavi, et al., 2007; Le, et al., 2011a). In addition, the osmolality value of seminal plasma in Waigieu seaperch was lower than that of black porgy (Chang, et al., 1995). It is clear that sperm motility is influenced by hypo- and hyper-osmotic pressure in freshwater and marine fishes, respectively (Alavi and Cosson, 2006; Alavi, et al., 2007). The osmolality in freshwater fishes is generally lower than that of marine fishes (Alavi and Cosson, 2006). The pH value of seminal plasma in the present study was higher than in yellow croaker, and lower than in black porgy (Le, et al., 2011a; Chang, et al., 1995). The pH value in fish seminal plasma may be representative of high spermatozoa viability during the spawning period (Dziewulska*,* et al., 2008). In the case of protein, although the origin and functions of protein in fish seminal plasma is not completely known, part of the proteins present in seminal plasma may originate from disrupted spermatozoa (Le, et al., 2011a).

The duration of sperm motility was more than 3 minutes for Waigieu seaperch whereas in striped bass it was 1.4 min, (Şahin, et al., 2012), and in yellow croaker it was more than 4 min (Le, et al., 2011a). The motility parameters of sperm (sperm motility, velocity and duration of sperm motility) can vary according to spawning season, biochemical properties, or osmolality and pH of the seminal plasma (Butts, et al., 2010; Alavi, et al., 2009). The most reliable indicator of sperm quality is spermatozoa motility and these indicators are used to select good sperm for artificial insemination.

Some of the observed differences between the present study and those published in other literature may be related to a number of factors including spawning periodicity, contamination of semen by urine during stripping, the period of spermatization during the breeding season and endocrine parameters such as steroids during spermatogenesis (Alavi and Cosson, 2006; Hajirezaee, et al., 2011; Le*,* et al., 2011a; Şahin, et al., 2012).

Our data may be useful for estimating the ratio of male:mature females, required in a hatchery. The values and correlations between the semen properties and the composition of seminal plasma may be useful in formulating a species-specific extender solution (diluent) to mimic seminal plasma for cryopreservation of Waigieu seaperch semen and may provide a basis for future evaluation and control of the reproduction in Waigieu seaperch.

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