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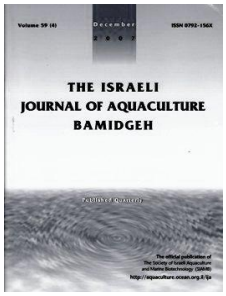
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il



Sperm Characteristics of Wild European Flounder (*Platichthys flesus luscus*)

Temel Şahin[1]*, Erdinç Güneş[2], İlhan Aydın[3], İlker Zeki Kurtoğlu[1]

[1] *Faculty of Fisheries, Rize University, Rize, Turkey*

[2] *Ministry of Food Agriculture and Animal Husbandry, Ankara, Turkey*

[3] *Central Fisheries Research Institute, Trabzon, Turkey*

(Received 1.2.12, Accepted 28.3.12)

Key words: flounder, *Platichthys flesus luscus*, motility, sperm density

Abstract

The spermatologic characteristics of European flounder (*Platichthys flesus luscus*) were determined. Flounder were collected during the spawning season and sperm of eight males was extracted by abdominal massage. Body weight and total length of the flounder were measured, volume, motility, duration of motility, spermatocrit, density, and pH of the sperm were determined, and correlations between the spermatologic characteristics and weight and length were investigated. Mean values were 0.7±0.16 ml for sperm volume, 87.5±3.66% for motility, 22.0±1.49 min for duration of motility, 94.0±1.22% for spermatocrit, 2.7±0.16 × 109/ml for density, and 6.9±0.05 for pH. Body length and sperm volume had positive correlations with body weight (p<0.01), but the correlation between length and sperm volume was negative (p<0.01). Likewise, the correlations between spermatocrit and total length, sperm volume, and density were negative (p<0.05).

* Corresponding author. Tel.: +90-464-2233385 (1427), fax: +90-464-2234118, e-mail: t_sahin@mynet.com

Introduction

Controlled breeding in captivity is an integral component of finfish aquaculture. Even though the quality of both gametes affects fertilization success and larvae survival, fish farming has focused more on the quality of eggs and larvae than sperm. To ensure that high quality semen is available when required and that fertilization is optimal, adequate knowledge of semen properties is necessary.

Spermiation and sperm quality are commonly evaluated by total expressible milt volume, concentration of spermatozoa in the milt (sperm density), percent spermatozoa that exhibit forward motility, and duration of the motility (Billard et al., 1995). The percent and duration of motility play an important role in the total sperm concentration necessary for fertilization. Motility intensity and duration vary according to species and temperature and may fluctuate during the spermiation period (Billard, 1986).

The European flounder, *Platichthys flesus luscus*, is a teleost of the Pleuronectidae family that inhabits coastal and brackish waters. As an important commercial flatfish, it is being considered for aquaculture in Turkey. Larvae rearing (Şahin, 2000), feeding and adaptation of wild-caught juveniles to aquaculture conditions (Ergun and Yalcin, 2006), reproductive characteristics and egg development (Şahin et al., 2008), and spermatological characteristics of hatchery-reared flounder (Aydın et al., 2011) have been studied. The objectives of this study were to characterize sperm from wild-caught European flounder and examine the relationships between body weight and length, and sperm characteristics.

Materials and Methods

Broodstock and semen collection. Wild European flounder (*Platichthys flesus luscus*) adults (96.0±13.19 g, 21.0±0.80 cm total length) were sampled with a trawl net at a depth of 5-70 m off Trabzon, Turkey, during December (the spawning season for flounder in the southeastern Black Sea) by the research vessel of the Central Fisheries Research Institute (CFRI), and transferred to the hatchery. Eight males were stripped during the period coinciding with peak spawning activity (Güneş et al., 2011). The genital pore was rinsed with fresh water and quickly dried. Sperm was collected by gentle abdominal massage from the anterior portion of the testis towards the genital papilla and a sterile graduated silicone tube (1.5 mm inner diameter) and syringe were used to collect sperm from the urogenital hole to prevent contamination by urine, mucus, or blood. Each male was stripped once, the expressed milt was collected individually, and the milt volume was measured. The samples were stored in plastic plates, separately for each fish, and held on dry ice (4°C) until measurement of sperm properties within 1 day after stripping.

Sperm density and spermatocrit. Sperm density (two replicates) was determined by the hemacytometric method. Semen was diluted 1000 times by pipetting 10 µl semen into 990 µl non-activating medium as described for sea bass (*Dicentrarchus labrax*) sperm (Fauvel et al., 1998). One droplet of diluted semen was placed on a hemocytometer slide (depth 0.1 mm) with a cover slip, the sperm was allowed to settle for 3-5 min, sperm cells were counted in 16 chamber cells using light microscopy (× 400), and spermatozoa density was expressed as $\times 10^9$/ml. To determine spermatocrit, heparinized microhematocrit capillary tubes (75 × 1.1-1.2 mm) were filled with semen, one end was sealed with clay, and the tubes were centrifuged at 10,000 rpm for 10 min. Spermatocrit was defined as the ratio of the volume of the white packed material to the total volume of semen, multiplied by 100 (Rurangwa et al., 2004).

Sperm motility, duration of motility, and pH. Spermatozoa from each male was evaluated immediately after semen was collected. Sperm motility was determined as the percent spermatozoa exhibiting rapid, vigorous, forward movement under a microscope (× 400) after diluting the semen in 100% filtrated sea water (filter and UV filter) at a ratio of 1:100 (1 µl sperm to 99 µl sea water). Duration of motility was determined as the time from activation to complete cessation of activity of the last spermatozoa. Motility was evaluated in three replicates per sample and by a single person, to decrease the degree of variation. pH was measured with indicator papers (Merck 6.4-8).

Statistical analysis. Each sample was evaluated in triplicate and averages of the three replicates were used in subsequent statistical analyses. Data are expressed as means±standard error. Data were analyzed using SPSS 15.0 for Windows software package. Motility data were normalized by arcsine transformation. Pearson correlation analysis was used to estimate spermatologic parameters. Differences with a probability of 0.01 or 0.05 were considered significant.

Results

Sperm quality is summarized in Table 1. Correlations between fish weight, fish length, and spermatologic properties are presented in Table 2.

Table 1. Sperm characteristics of wild European flounder (*Platichthys flesus luscus*), n = 8.

	Mean±SE	*Range*
Total length (cm)	21.0±0.80	18.4-24.1
Body wt (g)	96.0±13.19	58.4-177.0
Volume (ml)	0.7±0.16	0.2-1.3
Motility (%)	87.5±3.66	70.0-100.0
Duration (min)	22±1.49	16-28
Spermatocrit (%)	94.0±1.22	88.0-98.8
Density (× 10^9/ml)	2.7±0.16	2.1-3.2
pH	6.9±0.05	6.6-7.1

Table 2. Correlations between length, weight, and sperm characteristics of wild European flounder (*Platichthys flesus luscus*), * $p<0.05$, ** $p<0.01$.

	Weight	*Length*	*Volume*	*Motility*	*Duration*	*Density*	*Sperm-atocrit*
Length	0.884**	-	-	-	-	-	-
Volume	0.856**	-0.940**	-	-	-	-	-
Motility	-0.309	-0.264	-0.450	-	-	-	-
Duration	-0.303	-0.179	-0.382	0.507	-	-	-
Density	-0.071	0.255	0.180	-0.210	-0.275	-	-
Spermatocrit	-0.385	-0.702*	-0.646*	0.160	0.203	-0.743*	-
pH	-0.097	-0.385	-0.312	0.382	-0.408	-0.398	0.543

Discussion

The volume of milt of the European flounder (0.7 ml) was similar to that of yellowtail flounder (<1 ml; Suquet et al., 1994) but lower than that of other Pleuronectiformes such as turbot (1.6 ml; Suquet et al., 1992) and winter flounder (48.6±8.5 ml; Shangguan and Crim, 1999) and higher than that of Brazilian flounder (250±71 µl; Lanes et al., 2010) and Senegalese sole (18.9-32.6 µl; Beirão et al., 2011). The sperm volume was higher in wild European flounder than in cultured (0.2 ml; Aydın et al., 2011). The mean semen density (2.7 x 10^9/ml) was lower than in marbled sole (3.6 x 10^9/ml; Chang and Chang, 2002) and Brazilian flounder (8.9-12.7 x 10^9/ml; Lanes et al., 2010) but within the range of turbot (0.4-4.4 x 10^9/ml; Chereguini et al., 2003), Senegalese sole (1.60-3.38 x 10^9/ml; Beirão et al., 2011), and cultured European flounder (2.8 x 10^9/ml; Aydın et al., 2011). Differences in sperm production can be related to age and weight of males, sampling period, sampling method (Suquet et al., 1994), rearing conditions, nutrition, breeding seasonality, method of spawning induction, spawning behavior (Rurangwa et al., 2004), feeding conditions and regime, environmental factors, or spawning time (Bozkurt et al., 2006).

The relationship between semen volume and fish weight was similar to the relationships in yellow croaker (r = 0.975, $p<0.05$; Le et al., 2011) and cultured European flounder (r = 0.990, $p<0.01$; Aydın et al., 2011). In contrast, the relationship between semen volume and fish weight was insignificant in scaly carp (r = 0.2580, $p>0.05$; Bozkurt, 2006) and brown trout (r = 0.4310, $p>0.05$; Bozkurt et al., 2006). In the present study, spermatocrit negatively correlated with fish length, semen volume, and density, contrary to the strong positive correlation between spermatocrit and sperm density in rainbow trout, whitefish, and yellow perch (Ciereszko and Dabrowski, 1993), Atlantic cod (Rakitin et al., 1999), Atlantic halibut (Tvedt et al., 2001), haddock (Rideout et al., 2004), yamú (Casallas et al., 2007), and yellow croaker (Le et al., 2011). There was a significant relationship between spermatocrit and optical density in turbot, but no significant correlation between spermatocrit and sperm density (Suquet et al., 1992) and a strong negative correlation between spermatocrit and sperm density in cultured flounder (Aydın et al., 2011). The correlations between body weight or length and spermatological parameters generally were negative and insignificant in our study. The relationships in brown trout were similar, possibly indicating that the physical condition of mature fish has no influence on sperm quality (Bozkurt et al., 2006).

The duration of sperm motility for the wild European flounder was long (22±1.49 min), similar to the duration in cultured individuals (25.4±4.20 min; Aydın et al., 2011) but longer than in common carp (1.5 min; Billard et al., 1995), striped bass (1.4 min; Holland et al., 1996), and turbot (1.7 min; Chauvaud et al., 1995). The sperm of many marine and freshwater fish species contain motile spermatozoa >30 min after activation (Toth et al., 1997). Pacific herring sperm were motile 2-3 h after activation (Yanagimachi et al., 1992). Sperm motility in turbot dropped to 50% of its initial value 1 h after activation (Geffen and Frayer, 1993). The duration and motility of sperm can vary according to season (Benau and Terner, 1980), biochemical composition, or osmolality of the seminal plasma (Alavi et al., 2009). The most reliable indicator of sperm quality is spermatozoa motility and this indicator is used to select sperm for insemination. Motility correlates with fertilization capacity in rainbow trout semen, using subjective estimation methods to determine motility (Ciereszko and Dabrowski, 1994). The long motility duration of the flounder spermatozoa may enhance fertilization success.

In conclusion, our results can be used to select high quality mature males for fertilizing eggs in commercial aquaculture operations and provide a basis for future evaluation and control of reproduction in flounder. Further work is needed on wild European flounder sperm and egg quality to fully understand the reproductive potential of this species and to develop broodstock management protocols.

Acknowledgements

The authors thank Central Fisheries Research Institute, Trabzon, Turkey, for the equipment and material support.

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