Male characteristics, sperm traits, and reproductive success in winter-spawning Celtic Sea Atlantic herring, *Clupea harengus*

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Abstract The relationship between sperm characteristics and reproductive success was examined in male herring, *Clupea harengus* L. Males were categorised as being first-time or repeat spawners on the basis of their age; they were also grouped according to whether their sperm were immediately active and exhibited forward motion on contact with seawater (FM) or had little or only vibratory motion (VM). Unlike the Pacific herring *C. pallasi* Valenciennes, Atlantic herring sperm is usually motile on contact with seawater. The age, weight and gonadosomatic index (testes mass as a percentage of somatic mass = GSI) were measured and used as characteristics for individual fish. Sperm traits measured were (1) adenosine triphosphate (ATP) concentration, (2) sperm count, (3) duration of sperm motility. Reproductive success for each male was estimated from the fertilisation rate and from the length of larvae at hatching. Fertilisation rates for all fish were generally >80%. The ATP concentration of non-activated spermatozoa was negatively correlated with fertilisation rate. Among repeat spawners, fish with higher GSIs produced larvae that were larger at hatching. VM sperm fertilised eggs at rates equivalent to fertilisation by FM sperm, the larvae produced by VM sperm were significantly smaller at hatching. Larval length tended to increase in parallel with the duration of sperm motility, but the relationship was not significant in these tests. The results did not indicate any age or size pattern to spawning readiness in male herring. Sperm that are not yet ready to be shed are not fully motile on contact with seawater, but are still capable of fertilising eggs that hatch successfully. There is likely to be a progression of males which come into spawning readiness within a spawning shoal; therefore it is possible that paternal influences would result in a progressive decrease in larval size over the spawning period in winter-spawning Celtic Sea herring.

Introduction

The ability of a fish species to sustain a viable population depends upon its reproductive success. In Darwinian terms, this is formally defined as the ability of an individual to produce offspring which themselves reproduce. More informally, an individual’s reproductive success can be assessed indirectly by the quality of its gametes. To date, the majority of work on gamete quality in fish has concentrated on relating egg characterisitics to fertilisation rate and offspring success. For example, egg size has been positively correlated with fertilising capacity, measured as the percentage of eggs fertilised in a batch (Blaxter and Hempel 1963; Buckley et al. 1991), and to larval size (Blaxter and Hempel 1963; Moodie et al. 1989; Marteinsdottir and Able 1992) in several species. However, the influence of sperm traits on the reproductive success of fish has received far less attention.

Sperm traits used to assess a male’s reproductive condition usually include measures of motility (Christen et al. 1987; Aas et al. 1991; Suquet et al. 1992), number of spermatozoa per unit volume (Piironen and Hyvärinen 1983; Aas et al. 1991), and gonadosomatic indices which measure an individual’s relative investment in reproduction (De Vlaming et al. 1982). An additional trait, the adenosine triphosphate (ATP) content of semen, is often measured in conjunction with sperm motility. ATP is the source of energy for sperm movement and can be measured in sperm before activation to assess the potential for motility (Geffen and Frayer...
Herring milt and eggs were obtained from winter-spawning Celtic Sea herring, *Clupea harengus* L., caught off Dunmore East (south-east Ireland) in January 1997. The fish were taken from commercial catches on the last day before the mandated closure of the roe fishery, and thus were assumed to be in spawning condition and part of a spawning shoal (Anonymous 1991, 1997). The maturity ogive for this stock (ICES Management Area: Celtic Sea + Division VIIa and j) indicates that all 3 yr-old fish are reproductively mature (Anonymous 1997).

Eighteen running males were measured, weighed, and their gonads dissected from the body cavity. Each male’s gonads were weighed and subsamples were stored for later analysis. The milt samples were stored refrigerated for no more than 3 h before motility measurements were made. Subsamples of milt used for ATP analysis were frozen in plastic vials at −20 °C.

The GSI was calculated from the testes weight as a percentage of body weight (De Vlaming et al. 1982). The age of adult herring was determined from otolith readings. Both otoliths were dissected from each male, placed in water, and viewed under a dissecting microscope. Ages were determined by counting the number of annual winter rings.

Sperm count was estimated by counting sperm cells on a “improved Neubauer chamber” sperm count, haemocytometer under ×400 magnification (Rosenthal et al. 1988). The samples were diluted (1:1000 v/v) in seawater to bring the spermatozoa to counting concentration (≈100 per chamber). Sperm counts were expressed as the number of spermatozoa ml⁻¹ sperm. Means were calculated from three counts for each fish.

Herring testes are lobular, and the dorsal sperm duct is continuous with the lobular lumen where spermatogenesis and spermiation occur (Nagashama 1983). By the time the testes are judged visually to be ripe, the lobes throughout the testes are packed with spermatozoa (Bowers and Holliday 1961; Blaxter and Holliday 1963). There is no clear progression of ripening from the distant tips of the lobes toward the sperm duct in herring testes and the last stage of spermatogenesis and spermiation probably takes only 24 h (Bowers and Holliday 1961). Therefore, the distribution of ripe spermatozoa within the testes in rather uniform. Herring testes were sliced and pressed lightly onto a microscope slide and then lifted off. The adhering film of milt was activated with a drop of seawater and viewed at ×400 magnification. The duration, or retention, of motility was measured by timing the interval between sperm activation and the cessation of motility. Only forward-moving spermatozoa (Aas et al. 1991) were judged to be fully motile (FM), while those simply vibrating or turning in the two main axes were considered to show only vibrating movement (VM). Of the 18 herring sperm samples, 10 contained only VM spermatozoa. Values of zero were recorded for the duration of motility in these samples. The duration of motility was calculated from the mean of three separate measurements for each fish.

Total ATP concentration of un-activated herring milt was measured using a CPL1 photomultiplier-based luminometer (Celsis, Lumac Ltd., Cambridge, England). This measured the amount of light emitted through a sample of milt after the addition of a bioluminescence reagent (luciferin-luciferase). The ATP concentration of each sample was determined by comparing the test result with a calibration curve measured from serial dilutions of an ATP standard prepared using ATP-depleted water (Celsis.Lumac Ltd). The ATP working-range was 10⁻³ to 10⁻¹ mol l⁻¹; all test results fell between these extremes. The herring milt was collected as the fluid that dripped from the testes when sliced. The milt was blended in a food processor, drawn up through a plastic syringe so that milt volume could be determined, and diluted (1:1 v/v) with filtered (2 μm GF/C glass microfibre followed by 0.2 μm cellulose nitrate membrane-filters) distilled water. Results from ATP analyses were expressed both in absolute concentrations (μmol ATP 1⁻¹ milt) and in standardised units (e.g. nmol ATP per 10⁶ spermatozoa).

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### Materials and methods

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