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Maturity index validation in the white-spotted skate *Bathyraja albomaculata*, from the Falkland Islands

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The reproductive systems of 39 male and 54 female Bathyraja albomaculata *were assessed microscopically and compared with macroscopic maturity index values assigned by scientific observers. Inconsistencies were limited to 'developing' maturity stages in both sexes, but such discrepancies were attributed to different factors depending on sex. Furthermore, while spermatozoa were observed in the oviducal gland of females, they do not appear to accommodate long-term sperm storage in this species.*

Keywords: elasmobranchs, Rajidae, fishery, reproduction, histology

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INTRODUCTION

Maturity indices based on macroscopic assessments of the reproductive system are commonly employed in fisheries research, as a standardized approach to identifying the reproductive status of individual fish, and consequently, fish populations (Cailliet *et al.*, 1986; King, 1995). This approach has proved successful with many teleost species (Gillanders *et al.*, 1999; Bobko & Berkeley, 2004; Bromley, 2000; Hannah *et al.*, 2002; Morato *et al.*, 2003), whose gonads can change appearance quite dramatically between immaturity and maturity, and during the reproductive cycle. However, although maturity indices are commonly employed in the monitoring of elasmobranch species as well (e.g. Walmsley-Hart *et al.*, 1999; Kyne & Bennett, 2002; Henderson *et al.*, 2006; Coelho & Erzini, 2007), their suitability is not without question. For example, many species utilize the oviducal gland as a seminal receptacle (Metten, 1939; Pratt, 1993), practising delayed implantation; so empty uteri do not necessarily indicate an absence of sexual activity. Conversely, the presence of early embryos or egg-cases in the uteri is not necessarily indicative of a recent mating event. Furthermore, many maturity indices include a 'maturing' class—a division primarily based on the 'developing' appearance of the reproductive tract—which is somewhat open to interpretation. Inaccuracies in this regard can obviously have adverse effects on resultant population models (Gerritsen & McGrath, 2006).

The white spotted skate *Bathyraja albomaculata* (Norman) is one of the primary species taken in a directed rajid fishery around the Falkland Islands (Malvinas), as well as being taken as by-catch in mixed demersal and *Loligo gahi* fisheries (Agnew *et al.*, 1999). Biological data are collected from the skates by on-board Falkland Islands Fishery Department (FIFD) scientific observers, utilizing the maturity scale presented in Table 1. This information, together with catch data, is used to manage the fishery.

The present study was undertaken to assess the value of this maturity scale (i.e. if the scale allows for immature and reproductively active individuals to be clearly separated), and to investigate the possibility of sperm storage by females of this species. The focus of attention in the female reproductive system was, therefore, the oviducal gland. The terminology relating to this structure follows that of Hamlett *et al.* (1999), while terminology relating to the structure of the male reproductive system (testes and genital ducts), and the processes that occur therein, follows that of Hamlett (1999).

MATERIALS AND METHODS

Testes, genital ducts, and oviducal glands were collected from specimens of *B. albomaculata* by scientific observers aboard demersal trawlers operating within the Falkland Islands Interim Conservation Zone (FICZ), and fixed in 10% neutral buffered formal saline. The disc width (DW) of each specimen was measured to the nearest cm by the scientific observer, who also assigned the specimen a maturity stage (Table 1). After a period of at least one month, the tissue samples were dehydrated through a graded series of alcohols, cleared in chloroform, and infiltrated/embedded in paraffin wax at 56°C. Sections were cut at 5 μm thickness on a rotary microtome, and stained with haematoxylin and eosin following dewaxing and rehydration.

RESULTS

Testes from a total of 39 male specimens, ranging in DW from 26–67 cm (mean 42.4 ± 8.2 SD), were examined (Table 2).

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Table 1. Falkland Islands Fisheries Department maturity scales for rajids.

Maturity stage	Condition
	Female
I: Juvenile	Ovaries leaf-like, thin. No obvious eggs or eggs present in leaf-like opaque zone within ovary with gelatinous or granular appearance (grains of sugar). Nidamental glands* undeveloped, present as opaque bulge in oviduct. Oviducts small and thread-like
II: Adolescent, maturing	Ovary/ovaries containing small eggs. Nidamental glands small and developing. Usually cream in colour with obvious white ends. Oviducts small and thread-like
III: Adult, developing	Ovary/ovaries containing developing eggs some of which are very large. Nidamental glands fully developed, uniformly white. Oviduct walls thickened
IV: Adult, mature	Ovary/ovaries containing large egg/eggs. Large eggs present in Fallopian tubes, or already passing through to nidamental gland. Nidamental glands fully developed. Egg cases, if present, only partially extruded. Oviducts developed, walls thick and venous
V: Adult, laying	Ovary/ovaries containing large egg/eggs. Nidamental glands fully developed, uniformly white. Fully formed egg cases present. Oviducts developed, walls thick and venous or may appear stretched (following extrusion)
VI: Adult, resting	Ovaries containing a variety of eggs in different stages of development. No extremely large eggs present. Nidamental glands fully developed. No eggs present in Fallopian tubes or oviducts. No egg cases present. Oviducts venous and stretched
	Male
I: Juvenile	Claspers less then length of the pelvic fins. Testis undeveloped, thread or leaf-like. Spermatophoric area evident as leaf-like zone within the testis. Sperm duct undeveloped
II: Adolescent, maturing	Claspers longer than tips of pelvic fins. Clasper tips (glans) already structured but skeleton still flexible. Testis swollen with developing spermatophoric spheres. Sperm ducts with obvious structure and of uniform size throughout. Kidneys slightly obscured by developing sperm duct
III: Adult, mature	Claspers rigid and much longer than the pelvic fins, gans structures fully formed. Testis swollen with developing spermatophoric spheres. Sperm ducts with obvious structure separated into two distinct structures and filled with flowing sperm. Sperm dust obscuring at least half of the kidneys
IV: Adult, running	Same as III, except that sperm are present in the glans

*, nidamental gland = oviducal gland.

The testes of all mature individuals (i.e. stages III and IV) contained fully developed spermatocysts, in which spermatozoa formed tight bundles. This was also the case in 12 (i.e. 60%) of the stage II specimens. Of the remaining stage II specimens, two individuals contained spermatozoa in late spermatogenesis, the spermatozoa associating in linear arrays rather than forming tight bundles, while the remaining four specimens had not progressed beyond secondary spermatocytes. Late spermatogenesis was also observed in a 26 cm DW stage I individual, but the remaining three displayed nothing more advanced than secondary spermatocytes.

In all specimens which displayed fully mature spermatocysts, the genital ducts were fully developed and the epididymis, ductus deferens, and seminal vesicle all contained spermatozoa. The condition of the spermatozoa varied with location along the duct system, being present as individual non-aggregated spermatozoa in the lumen of the epididymis, as loosely associated aggregations in the lumen of the ductus deferens and as closely associated aggregations (spermatozeugmata) within the lumen of the seminal vesicle. Moderate amounts of individual and loosely bundled spermatozoa were also observed in the lumen of the seminal vesicle, but it is unclear if this is simply due to mechanical disruption of spermatozeugmata during tissue fixation and processing. The genital ducts of specimens which did not contain fully mature spermatocysts were all devoid of spermatozoa.

Table 2. The numbers of male and female *Bathyraja albomculata* from each maturity stage examined during the present study.

Season	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI
Male	4	20	14	1	n/a	n/a
Female	3	7	12	13	11	8

n/a, not applicable.

Oviducal glands from a total of 54 females, ranging from 23–58 cm DW (mean 42.9 ± 8.9 SD) were processed (Table 2), and their microanatomy and sperm content assessed. As in many elasmobranch species, the opposing secretory faces of the oviducal gland displayed discernible zonal variations in coloration and structure, without magnification. After histological examination it was found that these zones corresponded to the club, papillary, baffle and terminal zones (Hamlett *et al.*, 1999).

Glands from females assigned maturity stages III, IV, V and VI, were all structurally mature, displaying fully differentiated zones, whereas those from all other individuals were poorly developed and the secretory face consisted of an undifferentiated epithelium. The latter were also lacking the secretory tubules which are characteristic of the mature glands.

Spermatozoa were primarily observed in the baffle zone of the gland, within the transverse grooves, spinneret regions, secretory ducts and proximal gland tubules (Figure 1). In all of these areas the spermatozoa were present both as isolated individuals and as non-aggregated collections of individual sperm, which did not appear to be aligned in any way. Spermatozoa were also occasionally observed in the deeper reaches of the tubules, but the very small bore in this region made their detection very difficult and their orientation impossible to determine. Spermatozoa were recorded in females of maturities III through to VI, although a moderate proportion (22.3%) did not contain any sperm.

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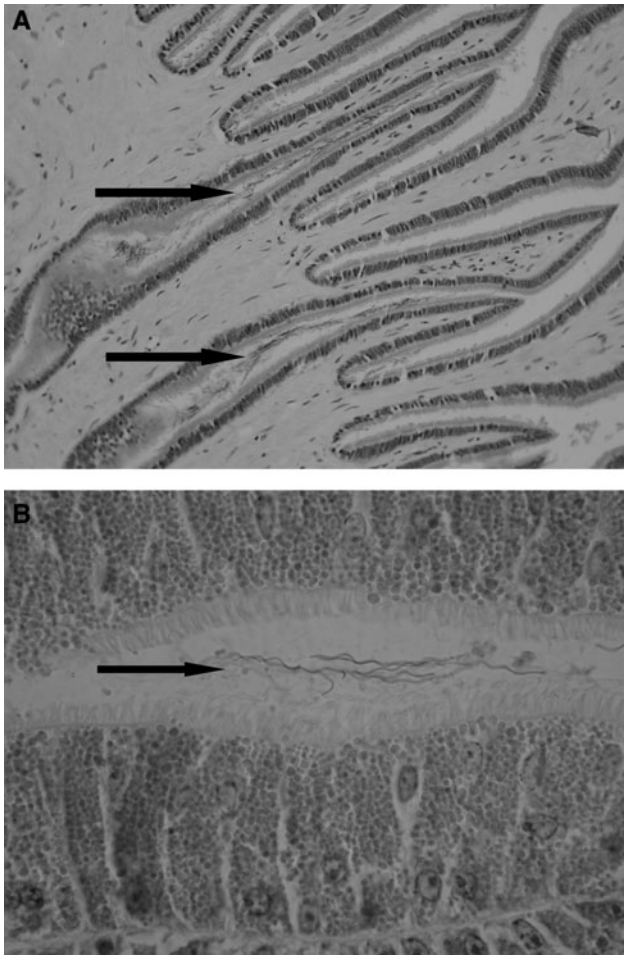

Fig. 1. Light micrographs showing sperm (arrows) within the secretory ducts (20×) (A), and within the proximal gland tubules (100×) (B) of the oviducal gland baffle zone, in *Bathyraja albomaculata* from the Falkland Islands.

DISCUSSION

Histological examination found that oviducal glands assigned maturities III–VI were all structurally mature, while those of stages I and II were not mature, thus confirming the assessment of mature or immature status by scientific observers. However, it is important to note that all stage III females contained spermatozoa and had therefore mated, when in fact these animals would be regarded as being mature but not yet sexually active. This indicates that the differentiation of females into the stage III and stage IV categories serves no real purpose. While the female maturity scale employed by FIFD is less detailed than that used in some other rajid studies (e.g. Walmsley-Hart *et al.*, 1999), there is a general trend towards the simplification of maturity indices (Stehmann, 2002). However, the results of the current study suggest that the FIFD index may benefit from further refinement. Indeed, the female maturity scale suggested by Stehmann (2002) is lacking a stage corresponding to stage III in the FIFD index.

The results from the male aspect of the work yielded similar discrepancies. The testes which had been classed as mature (i.e. stages III and IV) all contained fully developed spermatocysts, and the seminal vesicles contained fully formed spermatozeugmata, thus confirming that they were indeed mature. However, 60% of stage II males also contained fully mature spermatocysts and fully formed spermatozeugmata, when these individuals would be regarded as 'maturing' rather than mature. There are two possible reasons for this: (a) scientific observers find it difficult to distinguish between stage II and stage III males, and are consequently inaccurate in their maturity assessment; or (b) the process of physiological maturation occurs at a faster rate than physical maturation (i.e. maturation of the claspers), and individuals may be capable of producing sperm prior to being capable of copulation. If it was a case of the former, one would expect to find immature individuals among the mature classes, but this did not happen. Furthermore, Foley (1998) found that gonad maturation occurs before physical maturity in four rajid species from the Irish Sea. It therefore seems that male *B. albomaculata* are also, for a short period at least, reproductively precocious. While the sample size on which these results are based is admittedly small, the high occurrence of these precocious individuals is clearly of importance. This condition has also been observed in the bonnethead shark (*Sphyrna tiburo*) (J. Gelsleichter, personal communication), and so is possibly common across elasmobranch taxa. As opposed to precocious teleosts, which can contribute sperm to the reproductive population, the underdeveloped claspers of the precocious elasmobranch presumably preclude it from copulating. It is therefore unclear what advantage this route of development might yield.

The presence of spermatozoa within the tubules of the gland indicates that it acts as a seminal receptacle (Pratt, 1993). However, it is thought that long-term storage of spermatozoa in elasmobranchs is limited to the terminal zone of the oviducal gland (Hamlett *et al.*, 2002, 2005), and it therefore seems unlikely that *B. albomaculata* engages in long-term sperm storage. The sperm observed during the present study were most likely the remnants of a relatively recent insemination.

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