Reproductive biology of the starry smooth-hound shark

*Mustelus asterias*: geographic variation and implications for sustainable exploitation

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Examination of the reproductive biology of *Mustelus asterias* in the north-east Atlantic Ocean highlighted apparent geographical variation in maturity, fecundity and ovarian cycle between Atlantic and Mediterranean populations. The stretch total length (LST) and age at 50% maturity for Atlantic males and females were estimated at 78 cm LST and 4–5 years and 87 cm LST and 6 years, respectively. Size at maturity of females was considerably smaller than in Mediterranean specimens (96 cm LST). Ovarian fecundity ranged from eight to 27 oocytes and uterine fecundity from six to 18 embryos. The gestation period was c. 12 months, followed by a resting period of c. 12 months, resulting in a biennial cycle. Females stored sperm in the oviducal gland and, unlike Mediterranean specimens, no uterine compartments were observed in Atlantic specimens. This study reveals the existence of strong, possibly adaptive, divergence in life-history traits in an elasmobranch, whose northern populations may be more susceptible to overexploitation than previously believed.

Key words: elasmobranch; gestation; sperm storage; uterine compartments.

INTRODUCTION

The K-selected life-history strategies of elasmobranchs and their high position in trophic food webs make them more susceptible to fishing pressure than most teleosts (Stevens et al., 2000). Limited biological information for most species has made it difficult to determine their specific vulnerability to exploitation and has subsequently hampered the implementation of conservation and management measures (Frisk et al., 2001). The problem is further exacerbated, as some species are known to exhibit latitudinal or geographic variability of key life-history traits, which may respond rapidly to exploitation (Kuparinen & Merila, 2007), often with undesirable effects on populations, fisheries and ecosystems (Myers et al., 2007). Therefore, the accumulation of life-history data should be a priority for biologists, fisheries scientists and resource managers (Cope, 2006).

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The starry smooth-hound *Mustelus asterias* Cloquet is a relatively small demersal shark whose distribution is limited to continental shelf waters in the north-east Atlantic Ocean, Mediterranean Sea and south to Mauritania (Compagno, 1984). Little is known of its reproductive biology in the north-east Atlantic Ocean due in part to confusion concerning the discrimination of this species from the common smooth-hound *Mustelus mustelus* (L.) whose range partially overlaps with that of *M. asterias*. The recent development of a molecular genetic identification technique has allowed for the reliable identification and discrimination of north-east Atlantic *Mustelus* species (Farrell et al., 2009), enabling more detailed study of the genus.

Members of the *Mustelus* genus are all viviparous; however, they can be separated into two putative clades based on molecular phylogenetic analyses and their respective forms of viviparity (Lopez et al., 2006). Unspotted *Mustelus* species, such as *M. mustelus*, develop a placental connection with the mother through the interaction of the yolk sac, egg envelope and uterine wall (Smale & Compagno, 1997), whereas white-spotted species, such as *M. asterias*, have no physical connection to the mother and rely on the yolk sac during early stages of development. Once this resource is depleted, additional nutrients and water are absorbed from uterine secretions (Capapé, 1983), known as minimal histotrophy (Hamlett et al., 2005a).

Survey data in the north-east Atlantic Ocean suggest that the abundance of *Mustelus* spp. has increased in recent years in the Bristol Channel, Celtic Sea and North Sea and has remained stable in the Irish Sea (ICES, 2007). Decreases in abundance and landings, however, have been reported in the southern Bay of Biscay and Portuguese waters (Quero, 1998; Correia & Smith, 2003). Mediterranean populations have also been severely depleted and possibly locally extirpated in some areas, after decades of overexploitation (Aldebert, 1997; Jukic-Peladic et al., 2001; Ferretti et al., 2005).

The reproductive biology of *M. asterias* has previously been described in Mediterranean waters (Capapé, 1983); however, there have been no studies conducted in the north-east Atlantic Ocean, despite the known geographic variability of reproductive variables in this genus (Francis & Mace, 1980; Lenanton et al., 1990; Yamaguchi et al., 2000; Walker, 2007). This study presents the most exhaustive investigation to date of the reproductive biology of *M. asterias* in the north-east Atlantic Ocean. The findings were compared with previous data on Mediterranean populations.

**MATERIALS AND METHODS**

**SAMPLING**

A total of 231 *M. asterias*, comprising 113 males and 118 females, were collected between October 2006 and February 2009 from the Irish Sea, Bristol Channel, Celtic Sea and west of Ireland (Fig. 1). There is no commercial fishery targeting *M. asterias* in Ireland; therefore, specimen collection was necessarily opportunistic and samples were not available for each month of the year. Specimens included the discards of commercial trawlers, gillnetters and fisheries research vessels. Sex was recorded and stretch total length (*L*<sub>ST</sub>) was measured (to the cm below) in a straight line from the tip of the snout to the tip of the upper caudal lobe when in a stretched position; males 35–104 cm *L*<sub>ST</sub> and females 43–112 cm *L*<sub>ST</sub> were recorded. Total body mass (*M*<sub>T</sub>) was measured in kg. Each specimen was dissected with a ventral incision from the cloaca to the pectoral girdle in order to expose the body cavity. During the course of the study 118 *M. asterias*, caught by hook and line in the Irish Sea
REPRODUCTIVE BIOLOGY OF *MUSTELUS ASTERIAS*

Fig. 1. Distribution of *Mustelus asterias* samples (◯) collected from the north-east Atlantic Ocean for reproductive analyses. The location of tagging (▲) and recapture (■) of the one returned tag is also shown. Roman numerals indicate ICES assessment areas. High abundance of juveniles has been reported in areas IVc, VIIId and VIIIf.

(Fig. 1), were tagged in the first dorsal fin with individually numbered white plastic tags (Rototag, Dalton; www.dalton.ie) and released. Due to the potential for misidentification, all specimens in this study were genetically identified using the method illustrated in Farrell et al. (2009).

**DATA COLLECTION AND ANALYSES**

**Males**

The calcification of claspers and their length in relation to the pelvic fins, the size and appearance of the testes and the coiling of the sperm ducts were assessed to determine
Table I. *Mustelus asterias* maturity scale used in the current study

<table>
<thead>
<tr>
<th>Stage</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>A Immature (juvenile)</td>
<td>Claspers undeveloped and flexible. Shorter than pelvic fins. Testes small and whitish (&lt;1 g, width = 3–5·1 mm, length = 56·8–76·7 mm), sperm ducts straight.</td>
</tr>
<tr>
<td>B Maturing (adolescent)</td>
<td>Claspers longer than pelvic fins. Tips of claspers becoming structured but still soft and flexible. Testes enlarging (1–6 g, width = 2–12·3 mm, length 49·3–103·6 mm), sperm ducts beginning to coil.</td>
</tr>
<tr>
<td>C Mature (adult)</td>
<td>Claspers structured and calcified. Testes enlarged (6–26 g, width 9·3–20·1 mm, length = 79·3–155·1 mm), well rounded and often reddish in colour. Sperm ducts tightly coiled and filled with sperm.</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>A Immature (juvenile)</td>
<td>Ovary small (&lt;1 g) and difficult to differentiate from epigonal organ, internal structure gelatinous or granulated. Uteri narrow and thread like. Oviducal glands very small and flattened (width = 2–5 mm).</td>
</tr>
<tr>
<td>B Maturing (adolescent)</td>
<td>Ovary becoming larger (1–4 g). Oocytes are white and becoming differentiated to small sizes. Oviducal glands widening and developing (width = 4·3–17·8 mm). Uteri as per stage A, although may widen posteriorly.</td>
</tr>
<tr>
<td>C Mature (adult)</td>
<td>Ovary large, well developed (5–34 g). Oocytes obviously enlarged, yellow and can be easily counted and measured. Uteri well developed and vascularized. Oviducal glands large and well developed (width = 16·1–28·8 mm).</td>
</tr>
<tr>
<td>D Mature (developing)</td>
<td>Uteri filled with yolk balls. Each ball is contained within a separate sac-like egg envelope.</td>
</tr>
<tr>
<td>E Mature (differentiating)</td>
<td>Uteri well filled with yolk balls with developing embryos attached. Embryos small (c. 80 mm stretch total length, ( L_{ST} )) and unpigmented.</td>
</tr>
<tr>
<td>F Mature (expecting)</td>
<td>Embryos are fully formed (&gt;200 mm ( L_{ST} )), pigmented and can be sexed easily. Yolk sacs are used up although yolk stalk is still attached.</td>
</tr>
<tr>
<td>G Mature (post-natal)</td>
<td>Ovary at resting stage, similar to stages A and B. Uteri empty but still widened considerably over their entire length.</td>
</tr>
</tbody>
</table>

Maturity, following a maturity scale (Table I) (Stehmann, 2002). Male clasper length \( (L_C) \) was measured to the nearest 0·1 cm along the inner margin from the apex of the cloaca to the clasper tip. The rate of growth of \( L_C \) in proportion to \( L_{ST} \) and maturity stage was described using locally weighted scatter-plot smoothing (LOESS). Testes length and width were measured to the nearest 0·1 mm and weighed to the nearest 0·1 g, before fixation in 10% buffered formalin for histological analyses. Once fixed, a 4 mm thick transverse section was taken from the middle of each testis, dehydrated through a series of
alcohol and solvent solutions and infiltrated with paraffin on an automatic tissue processor (VIP, Tissue-Tek; www.sakura-americas.com). A rotary microtome (HM 325, Thermo Fisher Scientific Inc.; www.thermofisher.com) was used to cut 4 μm thick sections, which were stained with haematoxylin and eosin, cover-slipped with a mounting medium and viewed under an Olympus BX60 light microscope (www.olympusmicro.com). The proportion of the testis section occupied by each stage of spermatogenesis (Maruska et al., 1996; Conrath & Musick, 2002) was measured in a straight line from the germinal zone across the section. The mean proportion of the testis occupied by each stage per month, for which samples were available, was calculated to determine if there was a seasonal pattern in spermatogenesis and thus a probable period of mating activity for males.

Females

The female reproductive tract was observed to assess maturity (Table I) (Stehmann, 2002). Females were considered mature when the largest oocyte was >3 mm in diameter (Walker, 2007). The condition and number of enlarged oocytes (>3 mm diameter) per single (right) ovary of each specimen were recorded and the maximum oocyte diameter (DMO) was measured to the nearest 1 mm. The condition and contents, if any, of the uteri were recorded and measured to determine fecundity, embryo development and timing of the reproductive cycle. The width of each oviducal gland was measured to the nearest 0.1 mm. The oviducal glands of 33 specimens (9 immature and 24 mature) were excised and fixed in 10% buffered formalin for histological analyses. Once fixed, the oviducal glands were sagittally sectioned, processed and stained following the same protocol as the testes. Sections were viewed under a light microscope to determine if female M. asterias store sperm.

The liver mass (ML) of each dissected specimen was measured to the nearest 0.1 g. The hepato-somatic index (IH) was calculated as a measure of condition and plotted against LST for each maturity stage: 

\[ IH = 100 \cdot \frac{M_L}{M_T} \]

The reproductive tract of males and females was dissected out; gonads were carefully separated from the epigonal organ and weighed (MG) to the nearest 0.01 g. The gonado-somatic index (IG) was calculated and plotted against LST for each maturity stage: 

\[ IG = 100 \cdot \frac{M_G}{M_T} \]

All statistical analyses were performed with Microsoft Excel using the data analysis and solver add-ins. The LST at which 50% of males and females were sexually mature was calculated by logistic regression of binomial maturity data (immature = 0, mature = 1) using the following equation (Conrath & Musick, 2002): 

\[ Y = \left(1 + e^{-(a + bX)}\right)^{-1}, \] 

where Y is the proportion of mature individuals, X is LST and a and b are coefficients estimated by fitting a logistic curve to the data. The corresponding age at maturity for males and females was calculated from the age and growth estimates in Farrell et al. (2010).

RESULTS

MALE MATURITY

The smallest mature male was 72 cm LST, while the largest immature male was 85 cm LST. The LST and age at 50% maturity for males were estimated to be 78 cm and 4–5 years (Fig. 2). The upper and lower 95% CI were 80 and 76 cm LST, respectively. The plot of LC and LST showed three distinct phases of clasper growth, relating to three stages of male maturity (Fig. 3). Juveniles (stage A, n = 40, 35–72 cm LST) had very short flexible claspers. Adolescents (stage B, n = 29, 60–85 cm LST) had rapidly elongating claspers. Adults (stage C, n = 44, 72–104 cm LST) had long calcified claspers, the growth of which had slowed considerably. Both testes develop concomitantly in M. asterias, and there was no significant difference between the mass of left and right testes (paired t-test, d.f. = 142, P > 0.05),
Fig. 2. Maturity ogives for stretch total length ($L_{ST}$) for male (●) and female (○) Mustelus asterias in the northeast Atlantic Ocean. The $L_{ST}$ at 50% maturity was 78 and 87 cm for males and females, respectively.

therefore the mean testes mass was calculated for each specimen. Testes of juvenile $M$. asterias were threadlike, undeveloped and difficult to distinguish from the epigonal organ. The $I_G$ showed a rapid increase in testes growth at c. 70 cm $L_{ST}$, which coincides with the adolescent maturity stage and the elongation of the claspers [Figs 3 and 4(a)]. The $I_H$ also increased with $L_{ST}$, though not as significantly as the $I_G$ [Fig. 5(a)].

**FEMALE MATURITY**

The smallest mature female was 83 cm $L_{ST}$, while the largest immature female was 91 cm $L_{ST}$. The estimated $L_{ST}$ and age at 50% maturity for females were estimated to be 87 cm and 6 years (Fig. 2). The upper and lower 95% CI were 88 and 84 cm $L_{ST}$, respectively. The plot of oviducal gland width and $L_{ST}$ showed three phases of development (Fig. 6). Juveniles (stage A, $n = 62$, 43–84 cm $L_{ST}$) had small flattened, undeveloped oviducal glands with an average width of 0.37 cm. Adolescents (stage B, $n = 22$, 69–91 cm $L_{ST}$) showed a marked increase in oviducal gland width, average 1.08 cm, and development. Adult (stages C, D, E and F, $n = 19$, 3, 1 and 11, respectively, 83–112 cm $L_{ST}$) had large swollen oviducal glands with an average width of 2.09 cm. The $I_G$ increased concomitantly with oviducal gland width for stage B and some stage C females, indicating the onset of maturity [Fig. 4(b)]. Some stage C females, however, have a very low $I_G$, which is similar to that of stages E and F females. The $I_H$ increased with $L_{ST}$ for all maturity stages except stage F females, which displayed a marked decline in the $I_H$, consistent with a substantial postovulation maternal investment in the embryos [Fig. 5(b)].
REPRODUCTIVE BIOLOGY OF Mustelus asterias

Fig. 3. The relationship between clasper length ($L_C$), maturity and stretch total length ($L_{ST}$) for male Mustelus asterias in the north-east Atlantic Ocean stages: A (●), B (○) and C (■). A loess curve is fitted to the data; $r^2 = 0.93$.

FECUNDITY

Ovarian fecundity ranged from eight to 27 yellow, spherical oocytes and uterine fecundity from six to 18 embryos. The maximum was observed in a 111 cm $L_{ST}$ female, which had nine embryos in each uterus. There was no significant difference between the contents of left and right uteri (paired $t$-test, d.f. = 13, $P > 0.05$) or between the numbers of male and female embryos (1:1.04) in each litter (paired $t$-test, d.f. = 10, $P > 0.05$). The relationship between uterine fecundity ($F_U$) and $L_{ST}$ was curvilinear and described by the equation: $F_U = 0.00004L_{ST}^{2.6395}$ ($r^2 = 0.39$). Pregnant females were, however, occasionally observed to abort and expel embryos when caught, therefore values of uterine fecundity may be underestimates.

EMBRYO DEVELOPMENT AND GESTATION

Stage D females with uterine eggs were observed in April and June. Uterine eggs were oval, yellow, measured 28–43 mm in length and weighed 3.8–7.5 g each. Each was contained within a brown transparent egg envelope, secreted by the oviducal gland. One stage E female, with developing embryos (8 cm $L_{ST}$) still attached to a large yolk sac, was observed in January. Small stage F embryos with
Fig. 4. The gonado-somatic index ($I_G$) in relation to stretch total length ($L_{ST}$) for (a) male and (b) female *Mustelus asterias* in the north-east Atlantic Ocean. The variation with maturity stage is also shown [stages A (●), B (○), C (■), D (□), E (◆) and F (+)].

both unfixed pigmentation, *i.e.* pigmentation that is not fully developed and can be rubbed off easily (21 cm $L_{ST}$, 23.7 g), and fixed pigmentation (22.5 cm $L_{ST}$, 28 g) and no yolk remaining in the yolk sac were also observed in January. In April, the fully developed embryos had fixed pigmentation and an average $L_{ST}$ of 25 cm and mass of 34 g. In June, stage F embryos had an average $L_{ST}$ of 27 cm and mass of 54 g and in July an average $L_{ST}$ of 31 cm and mass of 86 g. Neonate
Fig. 5. Hepato-somatic index ($I_H$) in relation to for stretch total length ($L_{ST}$) of (a) male and (b) female Mustelus asterias in the north-east Atlantic Ocean. The variation with maturity stage is also shown (stages A (●), B (○), C (■), D (□), E (◆) and F (+)).

*M. asterias* with obvious umbilical scars were caught as early as late April and May. The smallest free-swimming *M. asterias* observed during the study was 28 cm $L_{ST}$ (May) while the largest in utero embryo was 32 cm $L_{ST}$ (July); therefore, the average length at birth was calculated to be 30 cm $L_{ST}$. No stage G, post-natal females were observed; specimens in this category may have been confused with...
stage C individuals, although none were observed with obviously enlarged flaccid post-parturition uteri. The shortest and longest periods between observation of recently ovulated uterine eggs and full-term (fully developed embryos of the birth length) embryos were 11 and 16 months, respectively. Given this and the fact that some degree of asynchronism of embryo development was observed, as illustrated by the occurrence of both stages E and F females in January, it is likely that gestation lasts c. 12 months.

Three full-term (stage F) female specimens also carried undeveloped eggs or underdeveloped and deformed embryos in their uteri. One case of twins was observed in a 112 cm LST female. The two male embryos were together in the same egg envelope and both were underdeveloped compared to the other embryos, 6 cm shorter and 30 g lighter than the average embryo size. Unlike some other Mustelus species, no uterine compartments were observed (Yamaguchi et al., 2000; Walker, 2007); however, the egg envelope persisted throughout the pregnancy and was full of clear liquid once the embryo was at stage F development.

**Sperm Storage**

The morphology of the oviducal gland of *M. asterias* [Fig. 7(a)] appeared identical to that of *Mustelus antarcticus* Günther and *Mustelus canis* (Mitchill) (Hamlett et al., 1998; Storrie et al., 2008). Thirteen of the 33 sectioned oviducal glands did
not contain any sperm, while 20 contained clearly visible sperm bundles [Fig. 7(b)]. Of these, one was from a stage B immature female and sperm was only present in the terminal zone [Fig. 7(a)]. Two stage C mature specimens had sperm in the terminal zone, baffle zone and in the lumen of the oviducal gland. The remaining 17 specimens, comprising seven stage C, three stage D, one stage E and six stage F individuals, all had sperm bundles in the terminal zone. These specimens were collected over a number of months including January, February, April, June and October; therefore, sperm storage occurs throughout the year.

**REPRODUCTIVE CYCLE**

Histological analyses of the testes of mature males (Fig. 8) showed their structure to be similar to those of *M. canis* (Conrath & Musick, 2002). Due to the small
sample size \((n = 44)\), no significant differences in the proportion of sperm stages throughout the year were found. Trends suggest however, that the highest proportion of evacuated spermatocysts (stage 7) and lowest proportion of spermatocysts with mature sperm (stage 6) were observed in June (Fig. 9). Mature males caught in October also often had large amounts of semen in their seminal vesicles and two stage C females caught in October had sperm in the terminal zone, baffle zone and in the lumen of the oviducal gland.
Fig. 9. The mean proportion of the testes of mature male *Mustelus asterias* \((n = 44)\) occupied by each stage of spermatogenesis \([1, 2, 3, 4, 5, 6, 7]\) from January to December.

Non-pregnant, mature females had an average \(D_{MO}\) of 6 mm in April \((n = 5)\), 10 mm in October \((n = 7)\), 14 mm in November \((n = 2)\) and 15 mm in February \((n = 4)\). The widest \(D_{MO}\), 23 mm, was observed in a stage D female captured in April, which had ovulated two eggs into each uterus, suggesting that ovulation occurs around this time of year after a c. 12 month period of oocyte development. Pregnant females with full-term embryos (stage F) had immature oocytes in the ovaries (<5 mm). This suggests that females do not become pregnant soon after parturition, but probably have a period of rest, during which oocytes develop to ovulation size. Considering the c. 12 month gestation period and this post-parturition rest period, the reproductive cycle is most probably biennial.

**TAGGING**

One tagged *M. asterias* has been recaptured to date. A female specimen was tagged in May 2008 off Holyhead, Wales, and was recaptured by a commercial fishing vessel near Arcachon in the Bay of Biscay in March 2009 (Fig. 1). After 257 days at liberty, the minimum distance travelled was 1109 km with a minimum distance travelled per day of 4.3 km (Fig. 1). Although preliminary, the result of the tagging may have implications for fisheries management.

**DISCUSSION**

*Mustelus asterias* is currently undergoing an apparent upward shift in abundance in the north-east Atlantic Ocean (ICES, 2007). Commercial landings of this species are, however, concurrently increasing (FAO, 2000), and there are no management
measures in place despite the known vulnerability of European Mustelus species to exploitation (Aldebert, 1997; Quero, 1998; Jukic-Peladic et al., 2001; Correia & Smith, 2003; Ferretti et al., 2005). The current study provides vital reproductive biological traits of M. asterias in the north-east Atlantic Ocean and reveals geographic variation and potential local adaptation in the region.

MATURITY AND FECUNDITY

Male M. asterias mature earlier and at a smaller $L_{ST}$ than females, which is common among members of the Mustelus genus (Walker, 2007). The estimated $L_{ST}$ at 50% maturity for male M. asterias (78 cm) is similar to the 75 cm estimated by Capapé (1983) for Mediterranean males. Interestingly though, the $L_{ST}$ at 50% maturity for females (87 cm) is considerably shorter than the 96 cm estimated by Capapé (1983). The corresponding ages at maturity for male and female M. asterias, 4 to 5 and 6 years, respectively (Farrell et al., 2010), are more than twice those previously estimated (Francis, 1981).

The differences in $L_{ST}$ at maturity for male M. asterias in the north-east Atlantic Ocean and Mediterranean Sea appear negligible; however, for females the differences may suggest a degree of geographic variation. It must be noted, however, that Capapé (1983) did not provide a detailed description of the methods employed for measuring the length of specimens and as such the differences may reflect some degree of methodological bias. If it is assumed that the differences are valid, then a plausible explanation is required. Yamaguchi et al. (2000) found that Mustelus manazo Bleeker populations in higher water temperature matured earlier and at a smaller $L_{ST}$ than populations living in lower water temperature. The findings of the present study may delineate the opposite pattern in M. asterias, as the north-east Atlantic Ocean has significantly lower water temperatures than the Mediterranean Sea, yet females here reached maturity at a smaller size than in the warmer Mediterranean waters (Capapé, 1983). One possible explanation is that the growth rate in the Mediterranean Sea is higher, although no published age and growth studies are available to confirm this. Limited food availability in warmer waters may have also slowed down the rate of maturity as demonstrated in the bonnethead shark Sphyrna tiburo (L.) (Parsons, 1993). More notably, the observed differences may result from fisheries-induced evolution, plastic response or distortion of the size-frequency compositions of one or more cohorts in the population by size-selective fishing. Walker (2007) found that the length at maturity of M. antarcticus increased with rising length-selective fishing mortality from gillnets and subsequently decreased with falling fishing mortality. The level of fishing mortality of M. asterias has historically been high in the Mediterranean Sea and low in the north-east Atlantic Ocean and thus length-selective fishing methods may have had an important role in shaping the reproductive characteristics in these regions.

The maximum uterine fecundity of 18 embryos should be viewed with caution as this was observed in a 111 cm $L_{ST}$ specimen. The maximum observed $L_{ST}$ of a reliably identified M. asterias in the north-east Atlantic Ocean is 133 cm $L_{ST}$ (Farrell et al., 2010). Uterine fecundity increases with $L_{ST}$, therefore, females of this size would be capable of carrying larger litters than those observed. The relationship between litter size and maternal $L_{ST}$ has also been described for other aplacental Mustelus species; curvilinear for M. antarcticus (Lenanton et al., 1990; Walker,
2007) and Mustelus lenticulatus Phillipps (Francis & Mace, 1980) and linear for M. manazo (Yamaguchi et al., 1997). Capapé (1983) observed a maximum fecundity of 35 embryos in a 125 cm total length, \( L_T \), specimen, which is much greater than the fecundity observed in the present study. This may also be explained by lower water temperature in the north-east Atlantic Ocean than the Mediterranean Sea as litter size in M. manazo decreases with increasing latitude and possibly decreasing water temperature (Yamaguchi et al., 2000), a phenomenon also seen in M. antarcticus (Walker, 2007).

The marked decline in the \( I_H \) for stage F females, carrying full-term embryos, indicates a substantial postovulation maternal investment in the embryos [Figs 4(b) and 5(b)]. This suggests that M. asterias is a matrotrophic species rather than lecithotrophic (Hamlett et al., 2005a; Musick & Ellis, 2005). The mechanisms of such matrotrophy in the white-spotted Mustelus species appear to be mucoid histotrophy, which has been confirmed in M. antarcticus (Storrie et al., 2009).

### GESTATION PERIOD AND BIENNIAL REPRODUCTIVE CYCLE

The processes of ovulation and parturition within a population do not necessarily occur instantaneously and individuals within that population will probably display a small degree of asynchrony, which complicates the definition of ‘period of gestation’ (Walker, 2007). In this study, the shortest period between observation of uterine eggs and of full-term embryos and free-swimming neonates was 11 months, while the longest period was 16 months. Considering the small degree of asynchrony in embryo development from specimens in January (both stages E and F were observed), the gestation period for the population was considered to be c. 12 months. This is consistent with observations from other aplacental and placental Mustelus species (Francis & Mace, 1980; Capapé, 1983; Lenanton et al., 1990; Smale & Compagno, 1997; Yamaguchi et al., 1997; Chiaramonte & Pettovello, 2000; Conrath & Musick, 2002; Oddone et al., 2005; Walker, 2007; Saidi et al., 2008, 2009).

Perhaps the most significant finding of the current study is the occurrence of small to medium-sized oocytes in the ovary of full-term (stage F) females and the low \( I_G \) and \( I_H \) [Figs 4(b) and 5(b)]. This indicates that females do not become pregnant soon after parturition, but have a resting period during which oocytes develop to ovulation size. Trends in oocyte development suggest that this process takes c. 12 months. This scenario contrasts with Mediterranean M. asterias, where full-term pregnant females also had fully enlarged oocytes, meaning that females are capable of becoming pregnant very soon after parturition, with a full female reproductive cycle lasting c. 1 year (Capapé, 1983). This apparent geographic variation in the duration of the reproductive cycle has also been observed in at least two other species of ‘white-spotted’ aplacental Mustelus species, M. manazo and M. antarcticus (Yamaguchi et al., 2000; Walker, 2007), with biennial ovarian cycles in populations from colder waters and annual cycles in populations from warmer waters. Until the current study, these were the only two elasmobranch species reported to have ovarian cycles with different periods in separate regions (Walker, 2007). From a management perspective, this is highly important as the productivity of the cold water populations is effectively half that of the warm water populations, which may be further reduced with the lower fecundity observed in colder waters.

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MATING, SPERM STORAGE AND PARTURITION

Insufficient sample size precluded the determination of a seasonal pattern in spermatogenesis. Trends, however, suggest that testes of males in June had the highest proportion of evacuated spermatocysts (stage 7) and lowest proportion of stage 6 spermatocysts with mature sperm [Figs 8 and 9]. Caution should be applied to the interpretation of this result although it suggests that mature sperm were present in the reproductive system at this stage and may be released from the testes at this time and stored until required. Many species of male elasmobranchs have been shown to be able to store sperm in their epididymis or seminal vesicles (Pratt & Tanaka, 1994). Mature males caught in October also often had large amounts of semen in their seminal vesicles and two mature females captured in October had free sperm in the baffle zone and lumen of the oviducal gland in addition to the sperm bundles in the terminal zone. This suggests that mating may occur at this time of year. These females, however, had a mean maximum oocyte diameter of 9.9 mm, which means it is unlikely that this sperm was used immediately to fertilize eggs and more likely it was destined for storage in the oviducal gland.

Females appear to be able to store sperm for extended periods of time, as even those with full-term embryos had sperm bundles in the terminal zone of the oviducal gland [Fig. 7(a), (b)]. During pregnancy, the uteri are effectively sealed by the uterine sphincter and filled with embryos encased in egg envelopes which should prevent the movement of sperm through the uteri into the oviducal gland (Storrie et al., 2008). Therefore, female M. asterias must store sperm for at least the length of gestation which is c. 12 months. The observation of sperm in the oviducal gland of one adolescent female is interesting as it means that M. asterias mate and store sperm before reaching sexual maturity. This has also been observed in M. antarcticus (Storrie et al., 2008), where sperm was found in the isthmus, uterus and uterine sphincter throughout the reproductive cycle which means that copulation occurs year round in M. antarcticus. In the current study, only the oviducal gland was tested for sperm and it is possible that sperm may have been present in other areas of the female reproductive tract.

Parturition in M. asterias occurs from April to July and neonates have been observed in shallow waters off sandy beaches on the south-east coast of Ireland (E. D. Farrell, pers. obs.). No published information exists on the location of parturition and nursery areas of M. asterias; however, neonates and juveniles are also periodically abundant in shallow areas of the English Channel, southern North Sea and Thames Estuary and Bristol Channel (Fig. 1) (Ellis et al., 2004). In the Irish Sea, large females (>90 cm L_T) many showing obviously distended abdomens, are seasonally abundant in May off Holyhead, Wales (E. D. Farrell, pers. obs.). These aggregations only remain in this area for c. 1 month after which they disappear. The one tag returned to date revealed that at least one of these females travelled south from Holyhead to the southern Bay of Biscay (Fig. 1). The gestational stage of this specimen was unknown; however, given the L_ST of 97 cm upon recapture, it was probably mature. Seasonal migrations of Mustelus species are not unusual (Bigelow & Schroeder, 1948; King, 1984; Oddone et al., 2005) and female M. lenticulatus and M. antarcticus are known to make considerably further migrations than males, with mature females travelling further than immature females (Francis, 1988). The purpose of such a migration to the Bay of Biscay is unknown and given that only one tag has been returned to date it is difficult to draw meaningful conclusions; however, it
may be related to seasonal bottom temperature fluctuations in the north-east Atlantic Ocean and food resources. The \( I_H \) of stage F females with full-term embryos was very low as they had utilized their lipid store [Fig. 5(b)]. They could therefore experience nutritional stress as seen in \( M. \) lenticulatus (King, 1984) and may have to migrate further to find optimal habitat and resources.

**EVOLUTIONARY CONSIDERATIONS AND CONSERVATION**

The geographic variation in reproductive cycle duration, alongside the apparent lack of uterine compartments in \( M. \) asterias in the current study and their presence in Mediterranean specimens (Capapé, 1983), underlines the potential for local adaptation of reproductive variables in this species. It also poses interesting evolutionary questions about the developmental biology of \( M. \) asterias. Uterine compartments, which form as extensions of the uterine mucosa, are found in all placental and some non-placental sharks (Hamlett et al., 2005b; Storrie et al., 2009). They increase the surface area of the uterus for metabolic exchanges and prevent yolk stalks or umbilical cords becoming tangled or abraded (Storrie et al., 2009). The presence or absence of uterine compartments has actually been used as a criterion in the classification of the genus \( Mustelus \) (Whitley, 1945). The present study, however, demonstrates geographic and intraspecific variability in this characteristic. Similar geographic variation has been observed in \( M. \) manazo (Yamaguchi et al., 2000), with uterine compartments persisting throughout pregnancy in southerly regions, opposed to a total lack of uterine compartments in the most northern regions. The uterine compartments in intermediate regions formed but disappeared during gestation. The mechanisms underlying these patterns of variability remain unclarified. Although, given their explicit association with remarkably different geographic locations, it is reasonable to expect that they may be related to environmental gradients, such as temperature and dissolved oxygen levels. Such factors are likely to affect metabolic activity and perhaps embryo development, suggesting that spatially explicit intraspecific variation in reproductive traits may have an adaptive value. While the present data do not allow disentangling of the roles of genomic and phenotypic-level mechanisms for this variation, further investigation of these aspects may add to understanding of the reproductive biology of these species and the evolution of the different forms of viviparity.

The ability of the cold water populations to adapt to increasing water temperatures, which are predicted under the climate change scenario, is also of interest. Will the distribution or phenology of these species be forced to change or has it already started to? Recreational anglers have reported catching \( M. \) asterias in more northerly areas (Scotland) than they were previously considered to inhabit. Survey data also show that \( M. \) asterias is currently undergoing an apparent upward shift in abundance in the north-east Atlantic Ocean (ICES, 2007). This should be viewed with caution as the majority of these surveys are conducted in the fourth quarter of the year and as such give a temporally limited view of the abundance of the species. Perhaps warming water temperatures have delayed the movement of \( M. \) asterias to their winter grounds and as such the abundance is apparently higher than in previous years. While difficult to test and prove, it is worth considering when developing management strategies.
The aforementioned variations in the reproductive variables of *M. asterias* illustrate the complexity of elasmobranch populations and also the current lack of knowledge of the basic biology of many species. Based on their reproduction and other life-history traits (Farrell *et al*., 2010), the north-east Atlantic Ocean and Mediterranean Sea probably represent two different populations of *M. asterias*. In-depth genetic studies and tagging are needed to delineate these populations, uncover any productively isolated sub-populations within these regions, and subsequently develop sound management and conservation measures for this area.

With the introduction of proper management controls, *Mustelus* species have been shown to support sustainable and stable fisheries (Walker, 1998). In the north-east Atlantic Ocean, most *M. asterias* are taken as by-catch in mixed demersal fisheries by a number of countries, using a variety of gears and across a large geographic area. They are generally discarded (ICES, 2007) and the survival rate is unknown. When they are landed, it is usually in a number of mixed categories such as ‘hounds nei (not elsewhere identified)’ and ‘smooth-hounds nei’ or even as ‘dogfish nei’ (ICES, 2007). They may even be landed in mixed boxes with tope *Galeorhinus galeus* (L.) and spurdog *Squalus acanthias* L. (E. D. Farrell, pers. obs.). This makes it impossible to accurately quantify landings and discards and thus difficult to implement management and conservation strategies for the species, as effective conservation of elasmobranch species requires species-specific monitoring of abundance and rates of mortality caused by exploitation (Dulvy *et al*., 2000). Further to this, the present study shows that area-specific management for some species is required, in addition to species-specific monitoring, in order to account for the variability in reproductive variables.

Currently available life-history evidence (Farrell *et al*., 2010; this study) calls for the establishment of a management plan for the north-east Atlantic population of *M. asterias*, although future assessment of the reproductive variables of the species in the North Sea is required, and more thorough stock identification methods should be applied throughout the distribution range of the species. The detailed account of the reproductive biology and cycle, in conjunction with genetic identification methods and age and growth estimates (Farrell *et al*., 2009, 2010), should be used now to devise conservation and management strategies, while the stock is still in an apparently healthy state (ICES, 2007).

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**References**


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Electronic References


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