

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 (L_{ST}) and age at 50% maturity for Atlantic males and females were estimated at 78 cm L_{ST} and 4–5 years and 87 cm L_{ST} and 6 years, respectively. Size at maturity of females was considerably smaller than in Mediterranean specimens (96 cm L_{ST}). 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. © 2010 The Authors

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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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1 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.

2 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).

3 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).

4 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.

5 MATERIALS AND METHODS

6 SAMPLING

7 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_{ST}) 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_{ST} and females 43–112 cm L_{ST} were recorded. Total body mass (M_T) 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

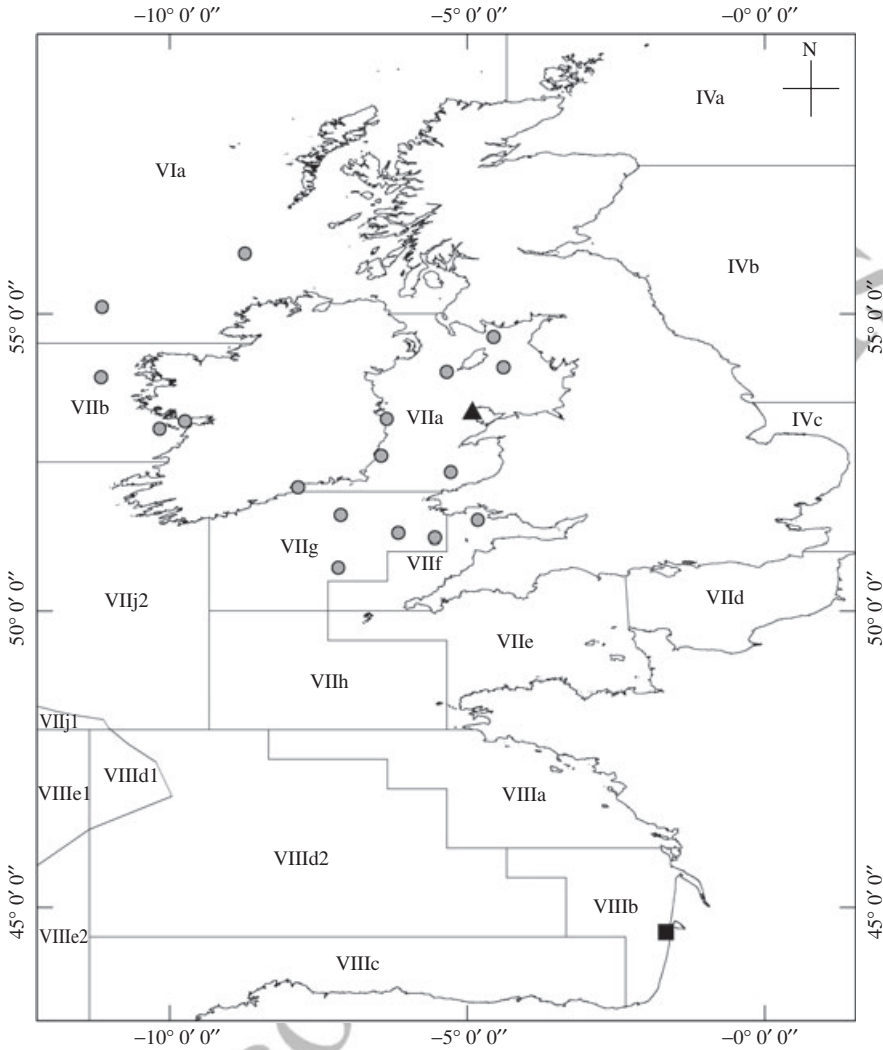


FIG. 1. Distribution of *Mustelus asterias* samples (●) collected from the north-east Atlantic Ocean for reproductive analyses. The location of tagging (▲) and recapture (■) of the one returned tag is also shown. Roman numerals indicate ICES assessment areas. High abundance of juveniles has been reported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

(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

| Stage | Condition |
|----------------------------|--|
| Male | |
| A Immature (juvenile) | Claspers undeveloped and flexible. Shorter than pelvic fins. Testes small and whitish (<1 g, width = 3–5.1 mm, length = 56.8–76.7 mm), sperm ducts straight. |
| B Maturing (adolescent) | 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. |
| C Mature (adult) | 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. |
| Female | |
| A Immature (juvenile) | Ovary small (<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). |
| B Maturing (adolescent) | 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. |
| C Mature (adult) | 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). |
| D Mature (developing) | Uteri filled with yolk balls. Each ball is contained within a separate sac-like egg envelope. |
| E Mature (differentiating) | Uteri well filled with yolk balls with developing embryos attached. Embryos small (<i>c.</i> 80 mm stretch total length, L_{ST}) and unpigmented. |
| F Mature (expecting) | Embryos are fully formed (>200 mm L_{ST}), pigmented and can be sexed easily. Yolk sacs are used up although yolk stalk is still attached. |
| G Mature (post-natal) | Ovary at resting stage, similar to stages A and B. Uteri empty but still widened considerably over their entire length. |

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

1 alcohol and solvent solutions and infiltrated with paraffin on an automatic tissue processor
2 (VIP, Tissue-Tek; www.sakura-americas.com). A rotary microtome (HM 325, Thermo Fischer
3 Scientific Inc.; www.thermofisher.com) was used to cut 4 μm thick sections, which were
4 stained with haematoxylin and eosin, cover-slipped with a mounting medium and viewed
5 under an Olympus BX60 light microscope (www.olympusmicro.com). The proportion of the
6 testis section occupied by each stage of spermatogenesis (Maruska *et al.*, 1996; Conrath &
7 Musick, 2002) was measured in a straight line from the germinal zone across the section.
8 The mean proportion of the testis occupied by each stage per month, for which samples were
9 available, was calculated to determine if there was a seasonal pattern in spermatogenesis and
10 thus a probable period of mating activity for males.

11 Females

12 The female reproductive tract was observed to assess maturity (Table I) (Stehmann, 2002).
13 Females were considered mature when the largest oocyte was >3 mm in diameter (Walker,
14 2007). The condition and number of enlarged oocytes (>3 mm diameter) per single (right)
15 ovary of each specimen were recorded and the maximum oocyte diameter (D_{MO}) was mea-
16 sured to the nearest 1 mm. The condition and contents, if any, of the uteri were recorded
17 and measured to determine fecundity, embryo development and timing of the reproductive
18 cycle. The width of each oviducal gland was measured to the nearest 0.1 mm. The oviducal
19 glands of 33 specimens (9 immature and 24 mature) were excised and fixed in 10% buffered
20 formalin for histological analyses. Once fixed, the oviducal glands were sagittally sectioned,
21 processed and stained following the same protocol as the testes. Sections were viewed under
22 a light microscope to determine if female *M. asterias* store sperm.

23 The liver mass (M_L) of each dissected specimen was measured to the nearest 0.1 g. The
24 hepato-somatic index (I_H) was calculated as a measure of condition and plotted against L_{ST}
25 for each maturity stage: $I_H = 100 M_L M_T^{-1}$.

26 The reproductive tract of males and females was dissected out; gonads were carefully sep-
27 arated from the epigonal organ and weighed (M_G) to the nearest 0.01 g. The gonado-somatic
28 index (I_G) was calculated and plotted against L_{ST} for each maturity stage:
29 $I_G = 100 M_G M_T^{-1}$.

30 All statistical analyses were performed with Microsoft Excel using the data analysis and
31 solver add-ins. The L_{ST} at which 50% of males and females were sexually mature was
32 calculated by logistic regression of binomial maturity data (immature = 0, mature = 1) using
33 the following equation (Conrath & Musick, 2002): $Y = (1 + e^{-(a+bX)})^{-1}$, where Y is the
34 proportion of mature individuals, X is L_{ST} and a and b are coefficients estimated by fitting
35 a logistic curve to the data. The corresponding age at maturity for males and females was
36 calculated from the age and growth estimates in Farrell *et al.* (2010).

37 RESULTS

38 MALE MATURITY

39 The smallest mature male was 72 cm L_{ST} , while the largest immature male was
40 85 cm L_{ST} . The L_{ST} and age at 50% maturity for males were estimated to be 78 cm
41 and 4–5 years (Fig. 2). The upper and lower 95% CI were 80 and 76 cm L_{ST} , respec-
42 tively. The plot of L_C and L_{ST} showed three distinct phases of clasper growth, relat-
43 ing to three stages of male maturity (Fig. 3). Juveniles (stage A, $n = 40$, 35–72 cm
44 L_{ST}) had very short flexible claspers. Adolescents (stage B, $n = 29$, 60–85 cm
45 L_{ST}) had rapidly elongating claspers. Adults (stage C, $n = 44$, 72–104 cm L_{ST})
46 had long calcified claspers, the growth of which had slowed considerably. Both
47 testes develop concomitantly in *M. asterias*, and there was no significant differ-
48 ence between the mass of left and right testes (paired t -test, d.f. = 142, $P > 0.05$),

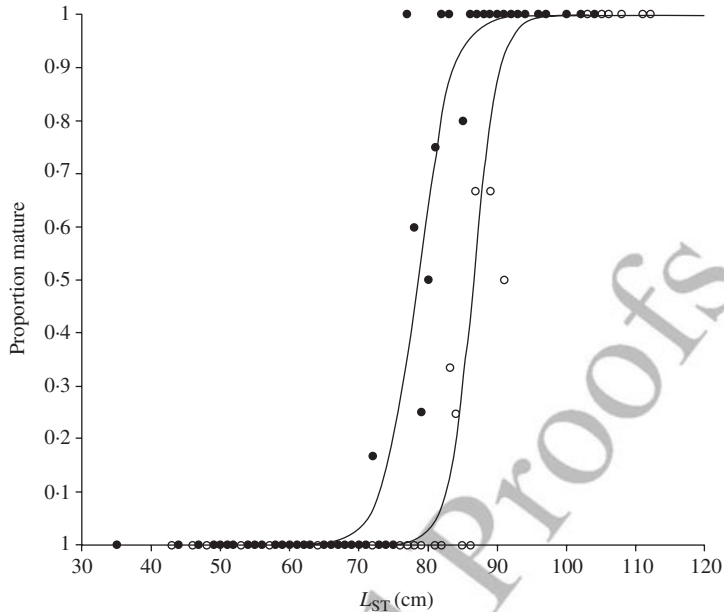


FIG. 2. Maturity ogives for stretch total length (L_{ST}) for male (●) and female (○) *Mustelus asterias* in the north-east Atlantic Ocean. The L_{ST} at 50% maturity was 78 and 87 cm for males and females, respectively.

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)].

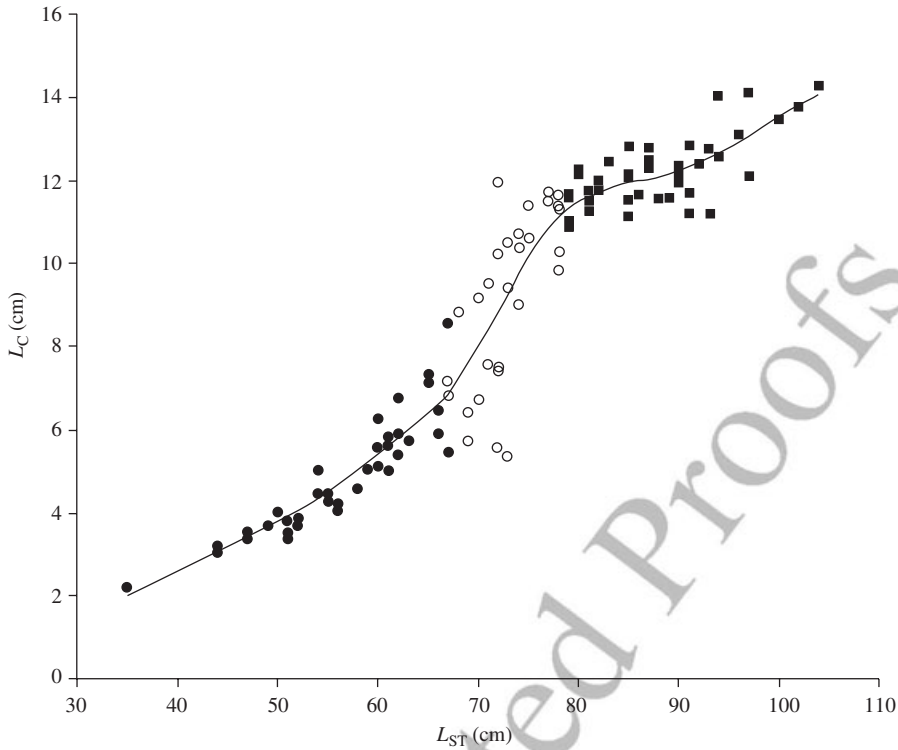


FIG. 3. The relationship between clasper length (L_C), maturity and stretch total length (L_{ST}) for male *Mustelus asterias* in the north-east Atlantic Ocean stages: A (●), B (○) and C (■). A loess curve is fitted to the data; $r^2 = 0.93$.

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 (1:1.016) in females (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

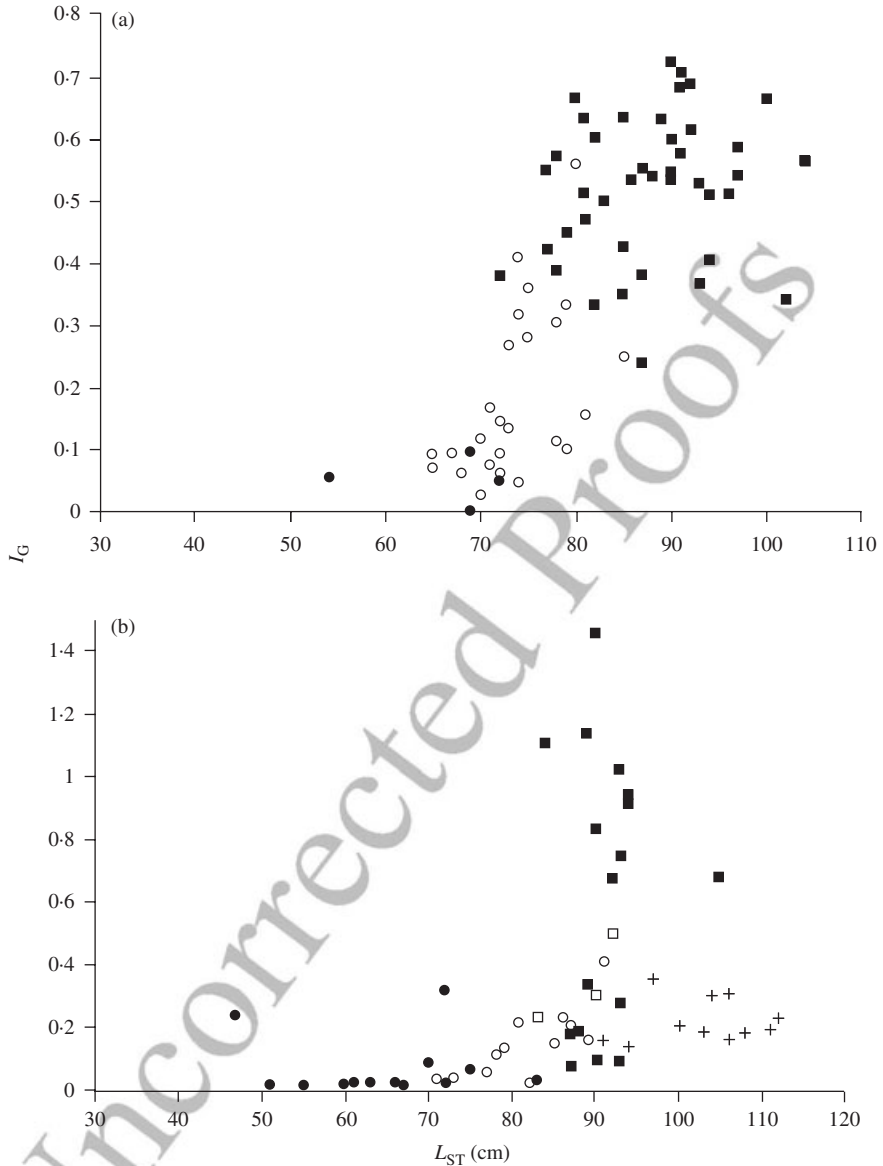


FIG. 4. The gonado-somatic index (I_G) in relation to stretch total length (L_{ST}) for (a) male and (b) female *Mustelus asterias* in the north-east Atlantic Ocean. The variation with maturity stage is also shown [stages A (●), B (○), C (■), D (□), E (◆) and F (+)].

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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

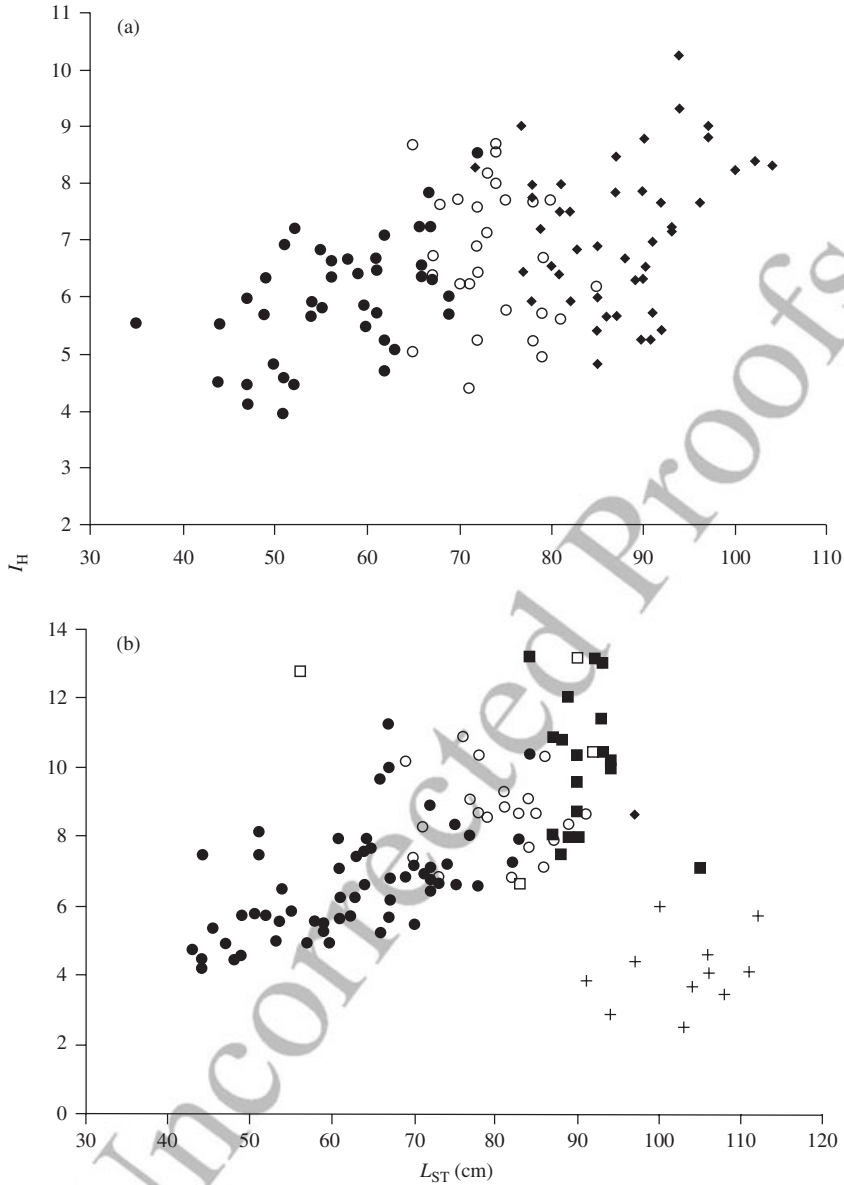


FIG. 5. Hepato-somatic index (I_H) in relation to stretch total length (L_{ST}) of (a) male and (b) female *Mustelus asterias* in the north-east Atlantic Ocean. The variation with maturity stage is also shown [stages A (●), B (○), C (■), D (□), E (◆) and F (+)].

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

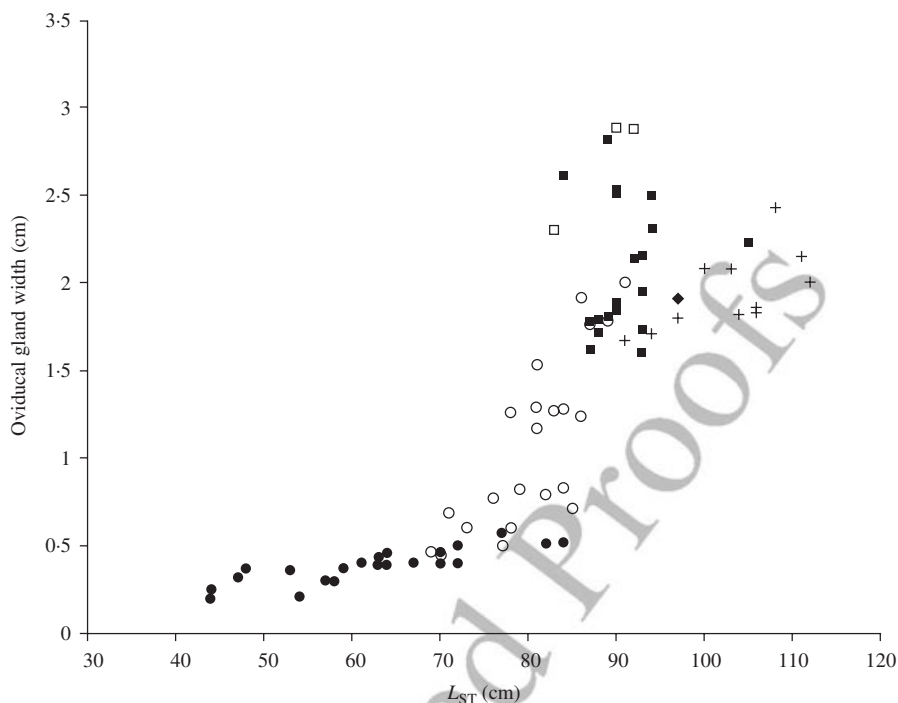


FIG. 6. The relationship between oviducal gland width, maturity and stretch total length (L_{ST}) for female *Mustelus asterias* in the north-east Atlantic Ocean [stages A (●), B (○), C (■), D (□), E (◆) and F (+)].

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 L_{ST} 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

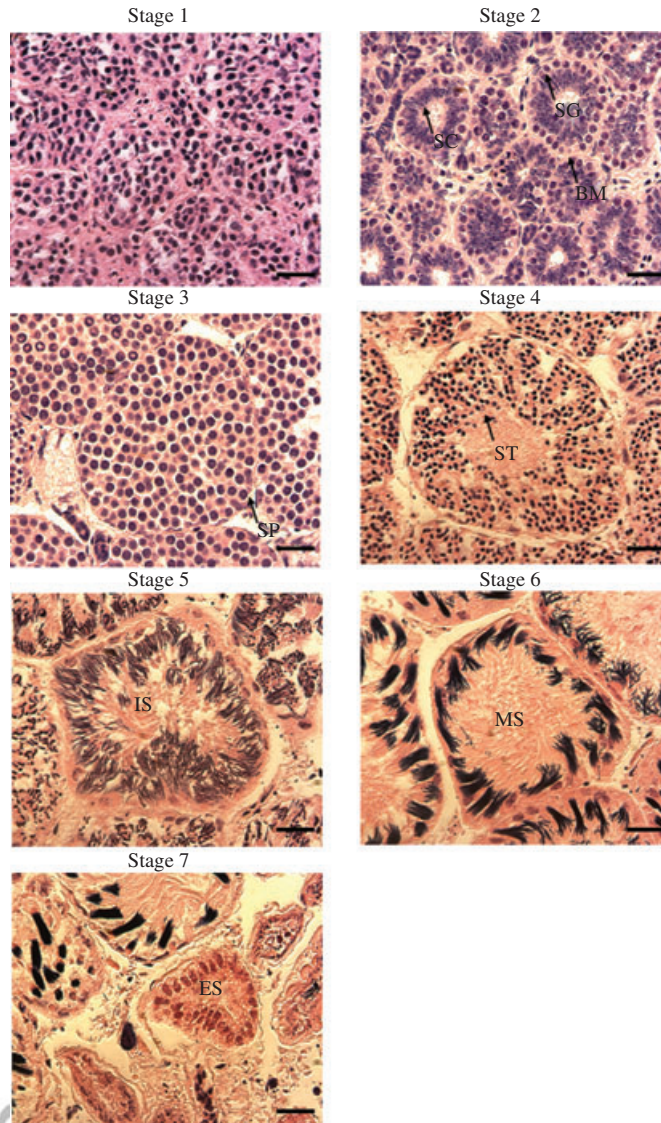


FIG. 7. (a) Sagittally sectioned oviducal gland of a mature *Mustelus asterias*. CZ, club zone; PZ, papillary zone; BZ, baffle zone; TZ, terminal zone; SB, sperm bundle. Scale bar = 2 mm. (b) A sperm storage tubule containing a sperm bundle. Scale bar = 40 μm.

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



37 FIG. 8. Sperm stages of *Mustelus asterias* testes: stages 1 to 7. SG, spermatogonia; SC, sertoli cells;
38 BM, basement membrane; SP, spermatocytes; IS, immature sperm; MS, mature sperm; ES, evacuated
39 spermatocyst; ST, spermatids. Scale bar = 50 μ m.

40
41
42 sample size ($n = 44$), no significant differences in the proportion of sperm stages
43 throughout the year were found. Trends suggest however, that the highest proportion
44 of evacuated spermatocysts (stage 7) and lowest proportion of spermatocysts with
45 mature sperm (stage 6) were observed in June (Fig. 9). Mature males caught in
46 October also often had large amounts of semen in their seminal vesicles and two
47 stage C females caught in October had sperm in the terminal zone, baffle zone and
48 in the lumen of the oviducal gland.

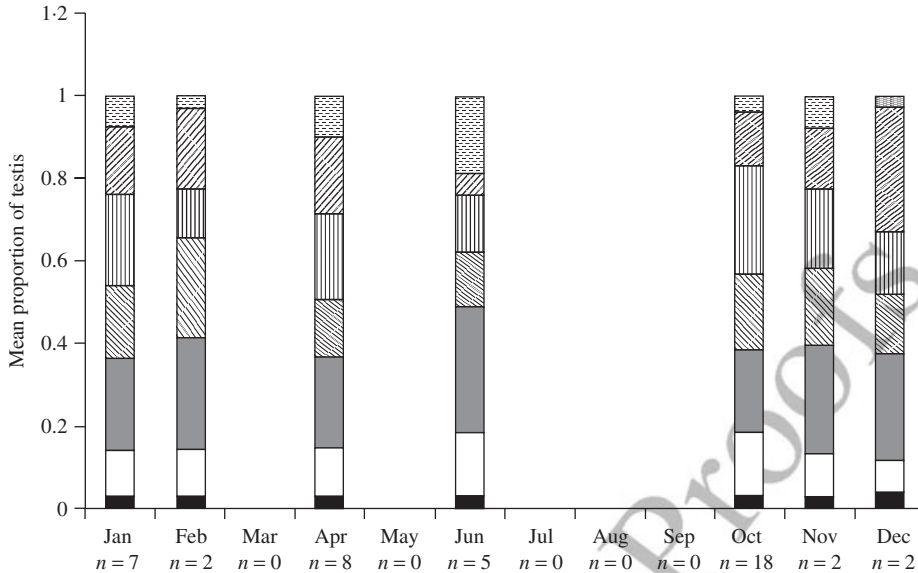


Fig. 9. The mean proportion of the testes of mature male *Mustelus asterias* ($n = 44$) occupied by each stage of spermatogenesis (■, 1; □, 2; ■, 3; ▨, 4; ▩, 5; ▤, 6; ▥, 7) from January to December.

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

1 measures in place despite the known vulnerability of European *Mustelus* species
2 to exploitation (Aldebert, 1997; Quero, 1998; Jukic-Peladic *et al.*, 2001; Correia &
3 Smith, 2003; Ferretti *et al.*, 2005). The current study provides vital reproductive bio-
4 logical traits of *M. asterias* in the north-east Atlantic Ocean and reveals geographic
5 variation and potential local adaptation in the region.

7 MATURITY AND FECUNDITY

8
9 Male *M. asterias* mature earlier and at a smaller L_{ST} than females, which is
10 common among members of the *Mustelus* genus (Walker, 2007). The estimated L_{ST}
11 at 50% maturity for male *M. asterias* (78 cm) is similar to the 75 cm estimated
12 by Capapé (1983) for Mediterranean males. Interestingly though, the L_{ST} at 50%
13 maturity for females (87 cm) is considerably shorter than the 96 cm estimated by
14 Capapé (1983). The corresponding ages at maturity for male and female *M. asterias*,
15 4 to 5 and 6 years, respectively (Farrell *et al.*, 2010), are more than twice those
16 previously estimated (Francis, 1981).

17 The differences in L_{ST} at maturity for male *M. asterias* in the north-east Atlantic
18 Ocean and Mediterranean Sea appear negligible; however, for females the differences
19 may suggest a degree of geographic variation. It must be noted, however, that Capapé
20 (1983) did not provide a detailed description of the methods employed for measur-
21 ing the length of specimens and as such the differences may reflect some degree of
22 methodological bias. If it is assumed that the differences are valid, then a plausible
23 explanation is required. Yamaguchi *et al.* (2000) found that *Mustelus manazo* Bleeker
24 populations in higher water temperature matured earlier and at a smaller L_{ST} than
25 populations living in lower water temperature. The findings of the present study may
26 delineate the opposite pattern in *M. asterias*, as the north-east Atlantic Ocean has
27 significantly lower water temperatures than the Mediterranean Sea, yet females here
28 reached maturity at a smaller size than in the warmer Mediterranean waters (Capapé,
29 1983). One possible explanation is that the growth rate in the Mediterranean Sea is
30 higher, although no published age and growth studies are available to confirm this.
31 Limited food availability in warmer waters may have also slowed down the rate
32 of maturity as demonstrated in the bonnethead shark *Sphyrna tiburo* (L.) (Parsons,
33 1993). More notably, the observed differences may result from fisheries-induced
34 evolution, plastic response or distortion of the size-frequency compositions of one
35 or more cohorts in the population by size-selective fishing. Walker (2007) found that
36 the length at maturity of *M. antarcticus* increased with rising length-selective fishing
37 mortality from gillnets and subsequently decreased with falling fishing mortality. The
38 level of fishing mortality of *M. asterias* has historically been high in the Mediter-
39 ranean Sea and low in the north-east Atlantic Ocean and thus length-selective fishing
40 methods may have had an important role in shaping the reproductive characteristics
41 in these regions.

42 The maximum uterine fecundity of 18 embryos should be viewed with caution
43 as this was observed in a 111 cm L_{ST} specimen. The maximum observed L_{ST} of a
44 reliably identified *M. asterias* in the north-east Atlantic Ocean is 133 cm L_{ST} (Far-
45 rell *et al.*, 2010). Uterine fecundity increases with L_{ST} , therefore, females of this
46 size would be capable of carrying larger litters than those observed. The relationship
47 between litter size and maternal L_{ST} has also been described for other aplacen-
48 tal *Mustelus* species; curvilinear for *M. antarcticus* (Lenanton *et al.*, 1990; Walker,

1 2007) and *Mustelus lenticulatus* Phillipps (Francis & Mace, 1980) and linear for
2 *M. manazo* (Yamaguchi *et al.*, 1997). Capapé (1983) observed a maximum fecundity
3 of 35 embryos in a 125 cm total length, L_T , specimen, which is much greater than the
4 fecundity observed in the present study. This may also be explained by lower water
5 temperature in the north-east Atlantic Ocean than the Mediterranean Sea as litter
6 size in *M. manazo* decreases with increasing latitude and possibly decreasing water
7 temperature (Yamaguchi *et al.*, 2000), a phenomenon also seen in *M. antarcticus*
8 (Walker, 2007).

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

15 16 17 GESTATION PERIOD AND BIENNIAL REPRODUCTIVE CYCLE

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

30 Perhaps the most significant finding of the current study is the occurrence of small
31 to medium-sized oocytes in the ovary of full-term (stage F) females and the low I_G
32 and I_H [Figs 4(b) and 5(b)]. This indicates that females do not become pregnant soon
33 after parturition, but have a resting period during which oocytes develop to ovulation
34 size. Trends in oocyte development suggest that this process takes *c.* 12 months. This
35 scenario contrasts with Mediterranean *M. asterias*, where full-term pregnant females
36 also had fully enlarged oocytes, meaning that females are capable of becoming
37 pregnant very soon after parturition, with a full female reproductive cycle lasting
38 *c.* 1 year (Capapé, 1983). This apparent geographic variation in the duration of the
39 reproductive cycle has also been observed in at least two other species of 'white-
40 spotted' aplacental *Mustelus* species, *M. manazo* and *M. antarcticus* (Yamaguchi
41 *et al.*, 2000; Walker, 2007), with biennial ovarian cycles in populations from colder
42 waters and annual cycles in populations from warmer waters. Until the current study,
43 these were the only two elasmobranch species reported to have ovarian cycles with
44 different periods in separate regions (Walker, 2007). From a management perspective,
45 this is highly important as the productivity of the cold water populations is effectively
46 half that of the warm water populations, which may be further reduced with the lower
47 fecundity observed in colder waters.
48

1 MATING, SPERM STORAGE AND PARTURITION

2 Insufficient sample size precluded the determination of a seasonal pattern in sper-
3 matogenesis. Trends, however, suggest that testes of males in June had the highest
4 proportion of evacuated spermatocysts (stage 7) and lowest proportion of stage 6
5 spermatocysts with mature sperm [Figs 8 and 9]. Caution should be applied to the
6 interpretation of this result although it suggests that mature sperm were present in
7 the reproductive system at this stage and may be released from the testes at this time
8 and stored until required. Many species of male elasmobranchs have been shown
9 to be able to store sperm in their epididymis or seminal vesicles (Pratt & Tanaka,
10 1994). Mature males caught in October also often had large amounts of semen in
11 their seminal vesicles and two mature females captured in October had free sperm
12 in the baffle zone and lumen of the oviducal gland in addition to the sperm bundles
13 in the terminal zone. This suggests that mating may occur at this time of year. These
14 females, however, had a mean maximum oocyte diameter of 9.9 mm, which means
15 it is unlikely that this sperm was used immediately to fertilize eggs and more likely
16 it was destined for storage in the oviducal gland.

17 Females appear to be able to store sperm for extended periods of time, as even
18 those with full-term embryos had sperm bundles in the terminal zone of the oviducal
19 gland [Fig. 7(a), (b)]. During pregnancy, the uteri are effectively sealed by the uterine
20 sphincter and filled with embryos encased in egg envelopes which should prevent
21 the movement of sperm through the uteri into the oviducal gland (Storrie *et al.*,
22 2008). Therefore, female *M. asterias* must store sperm for at least the length of
23 gestation which is *c.* 12 months. The observation of sperm in the oviducal gland
24 of one adolescent female is interesting as it means that *M. asterias* mate and store
25 sperm before reaching sexual maturity. This has also been observed in *M. antarcticus*
26 (Storrie *et al.*, 2008), where sperm was found in the isthmus, uterus and uterine
27 sphincter throughout the reproductive cycle which means that copulation occurs year
28 round in *M. antarcticus*. In the current study, only the oviducal gland was tested for
29 sperm and it is possible that sperm may have been present in other areas of the
30 female reproductive tract.

31 Parturition in *M. asterias* occurs from April to July and neonates have been
32 observed in shallow waters off sandy beaches on the south-east coast of Ireland
33 (E. D. Farrell, pers. obs.). No published information exists on the location of par-
34 turition and nursery areas of *M. asterias*; however, neonates and juveniles are also
35 periodically abundant in shallow areas of the English Channel, southern North Sea
36 and Thames Estuary and Bristol Channel (Fig. 1) (Ellis *et al.*, 2004). In the Irish
37 Sea, large females (>90 cm L_T), many showing obviously distended abdomens, are
38 seasonally abundant in May off Holyhead, Wales (E. D. Farrell, pers. obs.). These
39 aggregations only remain in this area for *c.* 1 month after which they disappear. The
40 one tag returned to date revealed that at least one of these females travelled south
41 from Holyhead to the southern Bay of Biscay (Fig. 1). The gestational stage of this
42 specimen was unknown; however, given the L_{ST} of 97 cm upon recapture, it was
43 probably mature. Seasonal migrations of *Mustelus* species are not unusual (Bigelow
44 & Schroeder, 1948; King, 1984; Oddone *et al.*, 2005) and female *M. lenticulatus* and
45 *M. antarcticus* are known to make considerably further migrations than males, with
46 mature females travelling further than immature females (Francis, 1988). The pur-
47 pose of such a migration to the Bay of Biscay is unknown and given that only one tag
48 has been returned to date it is difficult to draw meaningful conclusions; however, it

1 may be related to seasonal bottom temperature fluctuations in the north-east Atlantic
2 Ocean and food resources. The I_H of stage F females with full-term embryos was
3 very low as they had utilized their lipid store [Fig. 5(b)]. They could therefore expe-
4 rience nutritional stress as seen in *M. lenticulatus* (King, 1984) and may have to
5 migrate further to find optimal habitat and resources.

6 7 8 EVOLUTIONARY CONSIDERATIONS AND CONSERVATION

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

35 The ability of the cold water populations to adapt to increasing water tempera-
36 tures, which are predicted under the climate change scenario, is also of interest. Will
37 the distribution or phenology of these species be forced to change or has it already
38 started to? Recreational anglers have reported catching *M. asterias* in more northerly
39 areas (Scotland) than they were previously considered to inhabit. Survey data also
40 show that *M. asterias* is currently undergoing an apparent upward shift in abundance
41 in the north-east Atlantic Ocean (ICES, 2007). This should be viewed with caution
42 as the majority of these surveys are conducted in the fourth quarter of the year and as
43 such give a temporally limited view of the abundance of the species. Perhaps warm-
44 ing water temperatures have delayed the movement of *M. asterias* to their winter
45 grounds and as such the abundance is apparently higher than in previous years. While
46 difficult to test and prove, it is worth considering when developing management
47 strategies.
48

1 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 reproductively isolated sub-populations within these regions, and subsequently develop sound management and conservation measures for this area.

9 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.

24 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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