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ORIGINAL ARTICLE

Life history of *Lineus viridis* (Müller, 1774) (Heteronemertea, Nemertea)

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Abstract *Lineus viridis* is a common nemertean species of North-Atlantic intertidal sand flats. Its mating behaviour is peculiar insofar as this species is reported to be polyandric. However, detailed information on this topic is lacking. In order to get more data on the reproduction, oogenesis and life history of this species, a population in the Wadden Sea on the Isle of Sylt (North Sea) was studied between 2005 and 2011. We conducted regular surveys, during which we sampled, measured and recorded the sex status of 25–100 individuals at each sampling event; at least three individuals were fixed for histological studies at each sampling date. In addition, animals were kept in the laboratory for 3 years to complement field data on sexual identity. *Lineus viridis* was found to reproduce annually in several successive year; the females are significantly larger than the males. Oogenesis starts in spring, shortly after the preceding reproductive period, and continues until the end of December. Spermiogenesis starts in late autumn and also ends late in December. During mating, several males are generally found crawling on a single female, which forms a cocoon that encloses both the female and the associated males. Fertilization is internal. While females discharge all of their eggs during a single mating event and lose more than 40% of their wet weight, males only empty a few of their gonads, and are thus able to fertilize more than one female. Our study clearly shows that *Lineus viridis* is a perennial, iteroparous species with a pronounced sexual size dimorphism. During this long-term study, no evidence for sequential hermaphroditism has been found. The observed polyandric mating system in this species raises further questions regarding mate and sperm competition that deserve additional research.

Keywords Oogenesis · Spermiogenesis · Mating · Reproduction · Lophotrochozoa

Communicated by Martin Thiel.

The authors dedicate this publication to Pam Roe who, with her comprehensive works on *Paranemertes peregrina*, inspired this study.

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Introduction

Nemerteans (ribbon worms) are top predators feeding on various prey organisms (crustaceans, annelids and mollusks) (Thiel and Kruse 2001 and references therein) and are common in marine habitats (Gibson 1972, 1995). While the role of nemertean worms as a predators and prey organisms has been studied in a moderate number of species (Nordhausen 1988; Thiel and Reise 1993; Thiel et al. 1995, 2001; Thiel 1998; McDermott 2001; Thiel and Kruse 2001; Caplins and Turbeville 2010), studies of the life cycle of this taxon, comprising some 1275 species (Kajihara et al. 2008), have been done on a broader scale for only a handful of species (for reference Thiel and Junoy 2006). These mainly include studies on species of the genus *Carcinonemertes* Coe, 1902, which are egg predators in commercially exploited crustaceans (e.g. Roe 1979a, 1986; Wickham 1980; Kuris 1993; Santos et al. 2006). Of the free-living nemerteans, data on aspects of the life cycle are available for *Paranemertes peregrina* Coe, 1901 (Roe 1970, 1976, 1979b), *Amphiporus lactifloreus* (Johnston,

1828) (Thiel and Dernedde, 1996), *Tetrastemma fozensis* Gibson & Junoy, 1991 (Thiel and Zubillaga 1998), and *Lineus ruber* (Müller, 1774) (Gontcharoff 1951, 1960; Bierne 1970; Vernet and Bierne 1993).

Lineus viridis (Müller, 1774) is among the most common species of the Northern Hemisphere (Gibson 1995). In the past, *L. viridis* was routinely confused with its sibling species, *Lineus ruber* (Müller, 1774), and it was not until fairly recently that they have been recognized as two, separate species (Gontcharoff 1951; Rogers et al. 1993, 1995; see Friedrich (1979) and Gibson (1995) for an evaluation of older accounts). Due to the unique larval type exhibited by both species, their development has garnered much attention (Desor 1848; Bürger 1895; Nusbaum and Oxner 1913; Gontcharoff 1951, 1960; Schmidt 1964). While there is a sound data base on the reproductive cycle of *L. ruber* (Bierne 1983 and references therein; Vernet and Bierne 1993), findings on mating and reproduction in *L. viridis* are comparably scarce and partly contradictory. In particular, the sexual size dimorphism, the lifespan and the exact mode of fertilization are not entirely known (Gontcharoff 1951, 1960; Riser 1974; Cantell 1975; Bartolomaeus 1984; reviewed by Thiel and Junoy 2006). Internal fertilization simply has never been convincingly demonstrated (see Thiel and Junoy 2006), and contradictory reports have been given on the sexual dimorphism of *L. viridis*. Cantell (1975: in Fig. 3) implies that both sexes are of the same size, whereas Gontcharoff (1951), Riser (1974) and Bartolomaeus (1984) revealed a pronounced sexual size dimorphism. The degree of sexual size difference, however, has not been quantified. While Riser (1974) assumes that this species dies a few weeks after spawning, semelparity has not been reported by any other author. Furthermore, incidental observations made during data collection for a previous study on *L. viridis* do not suggest semelparity. Instead, studies of the reproductive system in this species revealed that the testes are dedifferentiated after sperm release (von Döhren and Bartolomaeus 2006). This, together with assumed iteroparity, led to the hypothesis that *L. viridis* might be a sequential protandric hermaphrodite that changes its sex during ontogeny depending on size or age (von Döhren and Bartolomaeus 2006).

In order to elucidate the life history of this nemertean species and address these inconsistencies, a long-term study was conducted. Regular collections of specimens on the Isle of Sylt in the Wadden Sea (NW Europe) over a period of 2 years were complemented by observations of animals kept in the laboratory during the same time. Additional data on the animal's size and sex ratio were recorded in subsequent years. The data obtained are used to provide a sound understanding of the life cycle of one of the most common intertidal nemerteans in the Northern Hemisphere.

Materials and methods

Species and collecting site

Specimens of *Lineus viridis* (Müller, 1774), (Heteronemertea, Nemertea) were collected in the Odde Watt on the Isle of Sylt (55°2′ N, 8°26′ E) in the muddy to sandy, mid to upper intertidal zone from September 2004 to February 2011. The species was identified according to Gibson (1982) and Gontcharoff (1951). Sexes were distinguished based on the position of the gonopores (males, ventral; females, dorsal) or the contents of their gonads.

Seasonal sampling and measurements

In order to collect data on seasonal population structure, all animals found along a 500-m transect parallel to the Lister Haken were collected during nocturnal low tide every 3–4 months for 2 years starting in May 2005. During the mating period in February, animals were collected from under stones in the area specified above. The animals were relaxed in 7% $MgCl_2$ and individually placed in a Petri-dish without artificially stretching the body. The body length was measured using a scale paper placed underneath the Petri-dish. After recording the body length data, the animals were slightly compressed with a glass slide under a dissecting microscope using transmitted light to record sex and developmental status of the gonads. The wet weight of females and males was measured after having placed the animals on a Kim Wipe™ tissue to remove as much water as possible. Grouped according to the status of their reproductive system, size differences were tested for normal distribution (Shapiro-Wilkes test) and subsequently for significance employing ANOVA for normally distributed data or *H* test for non-normally distributed data.

Histological studies

Three to ten individuals of each survey were fixed in Bouin's fluid (after Dubosq-Brasil) for histological studies to confirm the observed stage of maturity. The samples were dehydrated in an ethanol series and butanol and embedded in paraplast. Sections of 5 µm thickness were Azan stained. The sections were examined with an OLYMPUS BX61 light microscope equipped with colour digital camera (Colour view, SIS) for documentation. The observations are based on a minimum of 20 gonads per individual and sampling date.

Laboratory culturing

Two groups of *Lineus viridis* were kept separately in the laboratory under standard conditions (15°C, weekly water

change with artificial sea water, 12 h:12 h light regime, feeding with *Nereis diversicolor* (Müller, 1776) adding about twice the number of *L. viridis* at 2-week intervals). Each tank measured 20 cm × 20 cm × 5 cm (width x length x height) and was filled with 1,000 ml of artificial sea water. The salinity of the artificial sea water was 34 ± 1‰. Larger pebbles partly covered by water provided shelter for the animals. One tank contained 12 female individuals, collected in May 2005; the other contained 13 male individuals collected in February 2006. The males were taken from mating groups after reproduction. The males were kept alive for 2 years, and the females were kept alive for 3 years. Both groups were allowed to reproduce during each reproductive season in the laboratory, one female with 2–3 males were placed in a Petri-dish (14 cm in diameter) and kept there until the egg string was observed. Males were set up for subsequent matings up to three times until all females had spawned. In 2008, five males and two females were kept from reproduction in order to study the fate of the gametes in unmated specimen. All adult animals were still alive at the termination of laboratory culturing in 2008.

Mating observations

In February 2006, 2008, 2009 and 2011, male and female specimen were collected in the field, brought to the laboratory and measured for size differences between sexes as described above. In 2006 and 2011, each of the females was placed in a Petri-dish (14 cm in diameter) and generally 1–4 males were added. The Petri-dishes were filled with 100 ml sea water and kept at 5–7°C in the dark. 6–10 days later, the dishes were exposed to light and room temperature (20°C). The females immediately started envelope formation. In 2006, we continuously observed the mating process in 5 cases from envelope formation onward until the female had left the mating cocoon. Two mating events were recorded on video (supplementary material). In order to assess the degree of filling of the male gonads, males that had left the mating envelope were slightly compressed with a glass slide and examined with a dissecting microscope with transmitted illumination in 2006 and 2011. In order to obtain information regarding the decrease in body length, females and the males were measured again as described above after reproduction in 2009 and 2011. In both years, mating groups (25 in 2009 and 12 in 2011) were isolated during collection to get deeper insight into the male–female relation. In 2011, the wet weight of 20 females before and after spawning was measured after having placed the animals on a Kim Wipe™ tissue to remove as much water as possible. In order to test for a possible correlation between female size and number of males participating in a mating group, a correlation coefficient between female body length and number of associated males was calculated for the subsamples (Pearson's correlation coefficient).

Results

Gonad maturation

In females of *Lineus viridis,* the gonads again contained developing eggs shortly after the preceding reproductive season. Onset of oogenesis occurred along with an increase in the size of the nucleolus in certain cells of the germinal epithelium lining the ovary (Fig. 1b). Initially, these cells were attached to the periovarian extracellular matrix (*ecm*) as well as to adjacent ovarian lining cells, which may also differentiate into oocytes. During further development, the nucleus increased in size to form a hypertrophied structure. Vitellogenesis subsequently enlarged the oocyte (May–October), which bulged into the lumen of the ovary, but remained attached to the periovarian extracellular matrix by a stalk-like process. There were no specialized vitellocytes or yolk cells observed.

About 7–8 months after the onset of oogenesis, i.e. from November to December, the first eggs detached from the germinal epithelium, became spherical and rested in the centre of the ovary (Fig. 1c). When females were slightly squeezed with a glass slide, large oocytes were visible in each gonad. Females that were fertile for the first time in their life started to form gonoducts about a month before the first eggs detached from the gonad wall. The gonoducts were formed by epidermal invaginations. In females that had reproduced the year before, gonopores and gonoducts persisted.

In February, up to 15 eggs were found in a single gonad (Fig. 1d). Towards the anterior and posterior end of the body, the number of eggs per gonad decreased, and sometimes only a single egg was found in terminally situated gonads. At this time, vitellogenesis seemed to cease. Developing oocytes were still adhering to the ovarian epithelium. In both first-year females as well as in older females, the gonad was sealed by a small tissue layer between the gonoduct and gonad (Fig. 1c). This layer disintegrated shortly before mating, so that the eggs could be released. Oocyte development was already observed at the next sampling date after the reproductive season. In larger females, we always found small, immature oocytes immediately after spawning.

Sperm masses inside the testes were observed in males collected from early December to April from 2005 to 2007. From May to mid November, there were no signs of male reproductive organs (gonads, gonoduct or gonopores) in

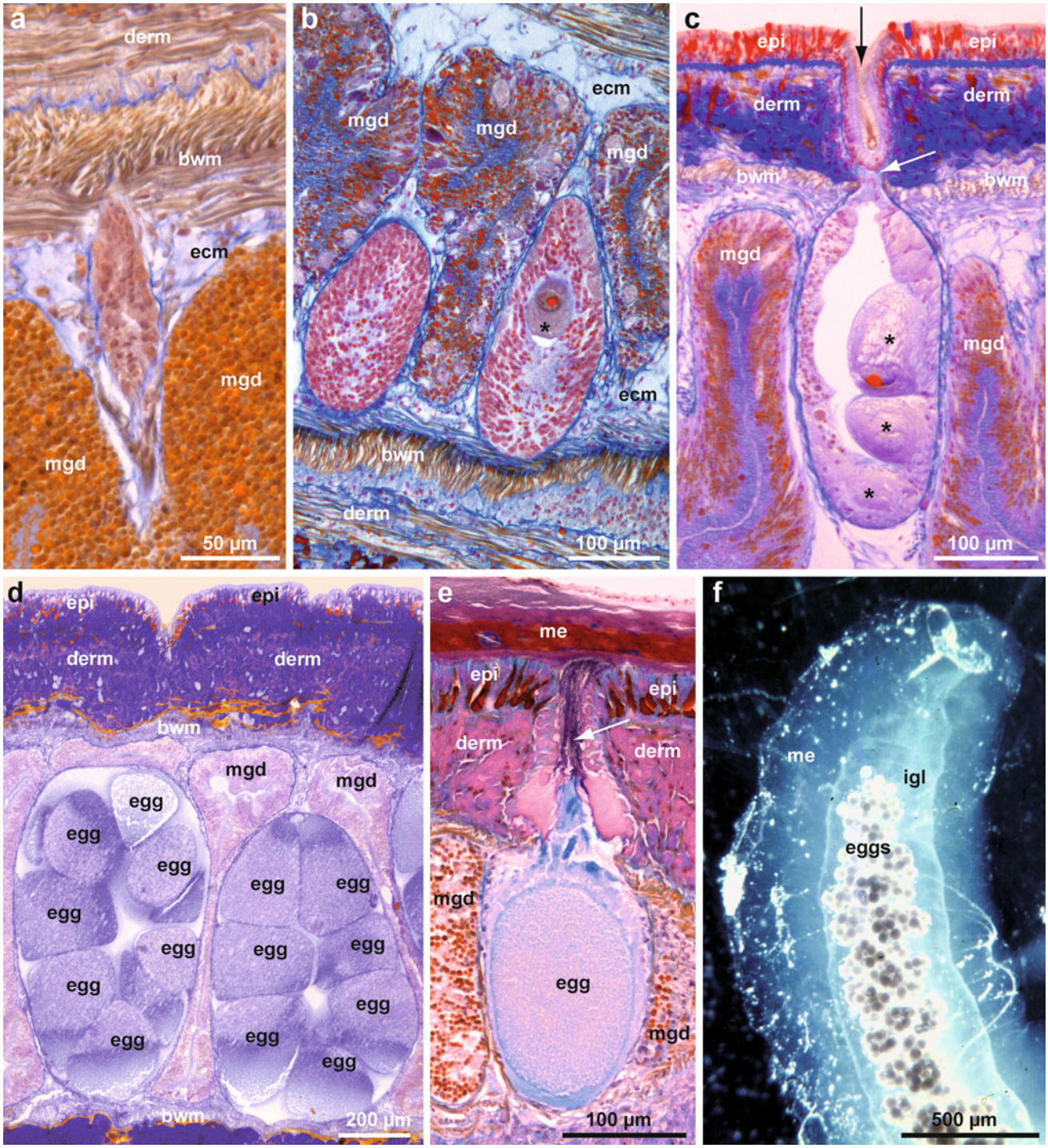

Fig. 1 *Lineus viridis*. Bouin fixation, paraffin section 5 µm thickness, Azan staining. Animals collected in the "Odde-Watt", List/Sylt. **a** Infertile specimen, gonad located between two adjacent midgut diverticula (*mgd*), Mai 2005. **b** Gonad status in April 2005, reserve material is starting to be accumulated in a few eggs (*asterisk*). Animal were fixed 3 weeks after reproduction. **c** Gonad status end of July 2005. Eggs (*asterisks*) increased in size. It should be noted that the tissue closure between gonad and gonoduct (*white arrow*), gonopore is marked by a *black arrow*. **d** Gonad status in February 2006. More than ten large eggs are tightly packed inside the gonad. Due to the size, nuclei are not shown in this section. **e** Female fixed during mating envelope (*me*) production. Note spermatozoa inside the gonoduct (*white arrow*). **f** Mating cocoon consisting of the former mating envelope and an inner gelatinous layer (*igl*). *bwm* body wall muscles, *ecm* extracellular matrix, *derm* dermis, *epi* epidermis

any specimen of *L. viridis*. Thus, during this time, males could not be identified.

Seasonal population structure

In May 2005, 6 weeks after spawning, two groups of females could be distinguished: females lacking gonopores but having gonads showing early stages of oogenesis, and females exhibiting both gonopores and developing gonads (Fig. 1b). While the latter measured on average 77 ± 23 mm ($N = 59$), females without gonopores were 51 ± 16 mm long ($N = 20$). The difference in length between both groups of females was significant ($P < 0.05$). There were also infertile animals that showed no sign of gametogenesis (Fig. 1a), measuring 32 ± 17 mm ($N = 11$) in length (Fig. 2). The body length of both groups of females was significantly different from that of the infertile animals ($P < 0.01$). Ten weeks later, at the end of July 2005, the mean size of the animals had significantly increased in all three groups ($P < 0.05$) (Fig. 2). Females with gonopores were significantly longer than females without gonopores ($P < 0.01$), and these were significantly longer than infertile animals ($P < 0.05$).

In October 2005, only females with gonopores were found. The mean size of the females was 89 ± 20 mm ($N = 45$); no infertile specimens were recorded (Fig. 2). Late in December 2005, males were found associated with females. Males and females with fully differentiated gonads and gonopores were observed. Males ($N = 9$) were significantly ($P < 0.01$) smaller than the females ($N = 13$). Metric data collected at the onset of the mating period in February 2006 showed that both sexes continued to differ significantly ($P < 0.01$) in their total body length (Fig. 2). Males were less than half as long as the females (47.9%).

Early in July 2006, we found similar results to those of the year before. Infertile animals measured 34 ± 16 mm ($N = 30$), females without gonopores were 74 ± 17 mm ($N = 22$) and females with gonopores were 96 ± 22 mm ($N = 23$) in length (Fig. 2). Size differences between the groups were highly significant ($P < 0.01$). When compared with the observations made in May 2005, neither infertile animals nor any of the female groups differed significantly in length between years (Fig. 2). At the end of November 2006, we recorded data from females with gonopores that had nearly completed oogenesis. They did not differ significantly from the group of females recorded late in October 2005. In November 2006, only one male measuring 72 mm was found in the surveyed transect.

Mating observations

Shortly before the first mating cocoons of *Lineus viridis* were observed in the field, males and females were often found under stones, forming mating groups that consisted

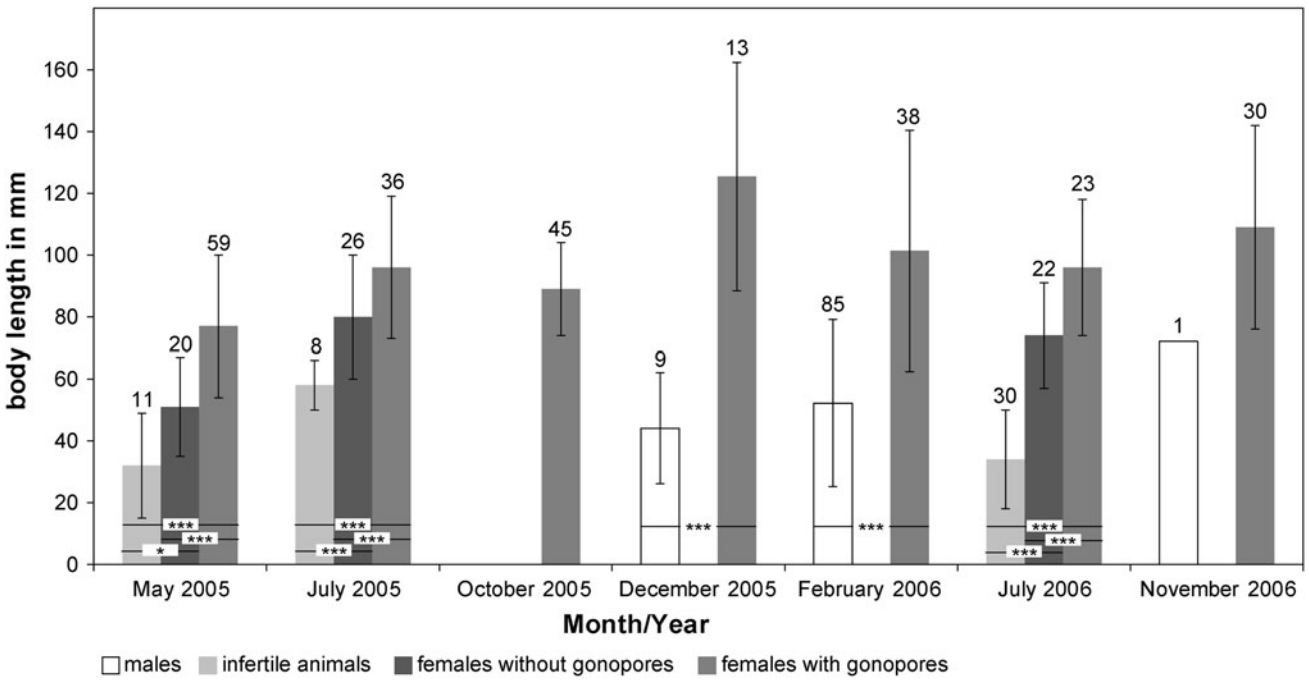

Fig. 2 Seasonal population structure in *Lineus viridis* (Nemertea) recorded between May 2005 and November 2006 in the "Odde Watt", List/Sylt. Males and females differ significantly in size. Asterisks marks the level of significance (*$P < 0.05$, ***$P < 0.001$). Numbers above branches indicate sample size (N)

of a few females and several males. The global ratio between females and males was always male-biased, being 1:2.3 ($n = 123$) in 2006 and in 1:2.8 ($n = 42$) in 2008, 1:3.1 ($n = 196$) in 2009 and 1:1.5 ($n = 113$) in 2011. In the 2009 and 2011 surveys, we recorded the sex ratio of females to males per mating group. The median was 2 males per female (lower quartile = 1.5, upper quartile = 4) in 2009 and 1.5 males per females (lower quartile 1, upper quartile 2.3) in 2011. The number of males per female ranged between 1 and 14 in 2009, and 1 and 8 in 2011. In the mating groups, we found no correlation between female body length and number of males ($r = 0.15$ in 2009 and $r = 0.09$ in 2011). In the mating groups, the males were always smaller than the females, reaching on average 36.3% of the females' length ($n = 123$) in 2009 and 51% of the females length ($n = 68$) in 2011.

Mating in *L. viridis* consists of four subsequent phases. The duration of these phases was measured in 5 mating events. During phase 1, the female forms a mucous mating envelope enclosing itself and one or several males, and the males release their sperm while crawling on the female's dorsum. This phase takes about 103 ± 40 min and ends when the last male has left the mating envelope. In the following phase 2, females start to form a second gelatinous layer that exceeds the first layer in width. In females fixed during this phase, sperm cells are observed in the gonoduct (Fig. 1e). The female then starts to discharge eggs. All eggs from one gonad are contained in one pear-shaped capsule. The egg capsules are embedded in two rows in the second gelatinous layer (Bartolomaeus 1984, Fig. 2). When all egg capsules are deposited in the gelatinous layer, phase 2 ends. This phase takes about 32 ± 19 min. The number of eggs per capsules is highly dependent on the size of the female. In mating cocoons, as in the gonads, the terminally located capsules contain fewer eggs than the capsules located in the middle of the mating cocoon (Fig. 1f). Finally, in the final phase 3, the female exits the mating cocoon, which is then left behind. Phase 3 takes 72 ± 51 min. In females that had left the mating cocoon, all gonads were completely devoid of fertile oocytes. In males that had left the gelatinous mass, some gonads still contained sperm. This observation was studied in more detail in 2006 and 2011.

In 2006, the 14 largest out of 80 males were selected and checked for gonad status. Only one had completely emptied all of its gonads, while in eight males the gonads appeared to be full, and in five males only a fraction of the gonads had been emptied. Out of 80 males, 35 males that had successfully reproduced were placed in Petri-dishes that contained an unmated female ($n = 9$). They were observed to immediately form mating groups and later successfully reproduced. This test was repeated in 2011 with three females and 12 males that already released part of their gametes and yielded the same result, successful reproduction.

Different aspect of changes in body length and wet weight during reproduction were recorded in 2006, 2009 and 2011. In 2006, the mean body length in females was 114 ± 38 mm before reproduction and 82 ± 27 mm ($n = 28$) after spawning. Thus, spawning led to a reduction in body length by almost one-third. The males that were measured after reproduction had diminished in body length by approximately 24% from 82 ± 18 mm to 62 ± 19 mm ($n = 16$) after reproduction. In February 2009, the average wet weight of females was 220 ± 180 mg ($n = 29$) and in males 35 ± 30 mg ($n = 46$). In February 2011, we recorded the wet weight of the females before and after reproduction. The wet weight of the females decreased from 254 ± 166 mg ($n = 18$) to 132 ± 93 mg ($n = 18$). The mean loss of weight is 48%. When collected in February shortly before mating, females did not feed but resumed hunting and feeding a few hours after having spawned.

Growth and sexual maturity in laboratory cultures

The two groups of males and females of *Lineus viridis* that were kept in the laboratory survived for at least 2–3 years, respectively. In the laboratory cultures, the course of development in the consecutive years was similar. Neither of the sexes increased in body length during the study. The females retained their dorsally situated gonopores at all times. In males, the gonopores were absent from May to September, and it was not until October that the gonopores of the males reappeared. All animals that had reproduced as males in March 2006 developed ventral gonopores and testes. In February 2007, both males and females from the laboratory cultures reproduced successfully with each other. In February 2008, sperm masses were resorbed in the males that had been kept from reproduction. The eggs of females that had been kept away from males during that time were shed in a normal-looking mating cocoon. However, no developing embryos were observed in these mating cocoons.

Discussion

Seasonal population structure and gonad maturation

The seasonal cycle of *Lineus viridis* can be roughly divided into two phases: the resting and the reproductive phase. In females, the resting phase begins after mating, continues until late November and is characterized by gonad maturation. In this phase, the ovaries contain only immature oocytes that grow during the course of the year and reach

maturity after almost 10 months (Fig. 3). During oogenesis, *L. viridis* females increase in diameter and length. Due to the lack of accessory vitellogenic cells, it is assumed that vitellogenesis in oocytes is autosynthetic. This has also been reported in *L. ruber* (Bierne 1983). After spawning, adult females of *L. viridis* release all mature ova in a single spawning event. In doing so, females considerably diminish in length (Fig. 3), since the eggs and mucus given off account for almost half of the total weight of a gravid female. The large amount of yolk contained in the eggs enables the lecithotrophic feeding habits of the Desor-larva, which completes its metamorphosis a relatively long time before hatching (Gontcharoff 1960; Gibson 1972; Friedrich 1979 and references therein). This contrasts with data reported for *L. ruber*, in which some eggs are spawned unfertilized to serve as food for developing larvae (Gontcharoff 1960; Schmidt 1964). Gametogenesis in female *L. viridis* takes at least 10 months until the oocytes are mature. In females that reach sexual maturity for the first time, there are no visible gonopores during oogenesis. In *L. ruber* females, the gonads degenerate after each spawning and are reformed 5–6 months prior to spawning (Bierne 1983). Thus, in *L. ruber* considerably fewer oocytes are supplied with yolk than in *L. viridis*. This is congruent with the differing observations regarding the number of larvae and their mode of development in the two species (see above and Gontcharoff 1960; Schmidt 1964). The gonopores and gonoducts are formed by epidermal invaginations (Bierne 1983 for *Lineus ruber*). Their initial occurrence in *L. viridis* coincides with first gonad maturity. We therefore assume that formation of gonoducts (including gonopores) is induced by gonad maturity. While oviducts are sealed after each spawning, the gonopores, once formed, persist for the rest of the lifetime of the female.

In males, the observed pattern is different. After one mating event, a large portion of the gonads of most males still contains sperm cells. Even if these are not given off in subsequent mating events they will degenerate along with the testes, gonoducts and gonopores (von Döhren and Bartolomaeus 2006). During the summer months, there is no sign of reproductive organs or gonadogenesis in males. Thus, formation of gonads and sperm maturation seems to

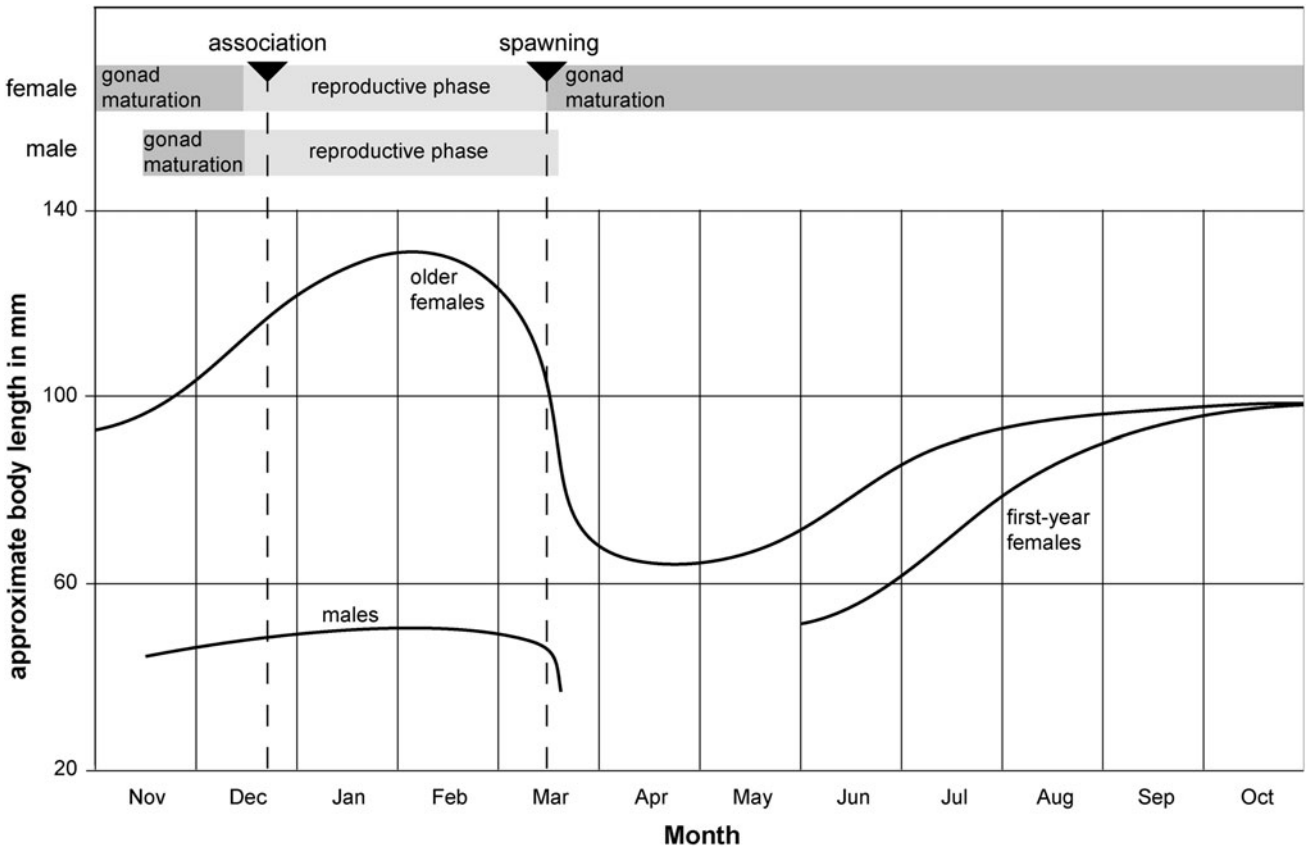

Fig. 3 Schematic representation of the change in size during the seasonal life cycle in both sexes of *Lineus viridis*. It should be noted that while older females are observable throughout the year, males can only be identified during gonad maturation and reproductive phase. First-year females can be discriminated by not having formed gonopores yet. When the gonopores in females appear for the first time they become indistinctive from older females (hence the decrease in mean body length in older females between October and November). Infertile animals not shown

be a rapid process which is initiated and completed within a month (Fig. 3). It is not until the testes are mature that gonopores and gonoducts appear. During the time that males can be identified, they show no significant increase in size (Fig. 3). As in first-year females, gonoduct formation seems to be induced by the maturity of the gonads. In contrast to the females, the male reproductive system is a transient structure that, except for some mitotic cells, largely disappears (von Döhren and Bartolomaeus 2006) and is differentiated de novo each reproductive season. Thus, there are no criteria during the resting phase to tell subadult animals and males apart in the field, whereas first-year and older females can be identified beyond any doubt. Nevertheless, it is suggested that the animals that show no sign of gonads during the resting phase are made up for the most part of male individuals, to a lesser degree of small first-year females that lag behind in their initial gonad formation, and finally some juvenile animals. The proportion of the juveniles in our study, however, is supposed to be negligible because small specimens of *L. viridis*, arguably juveniles, have been found between patches of blue mussels (*Mytilus edulis* Linnaeus, 1758) in the lower intertidal zone (unpublished observation). We therefore assume that this is more likely to be the original habitat of juvenile animals. The recruitment of males and females from the group of juvenile animals, however, needs further, preferably long-term investigation that includes rearing and maintaining of animals in the laboratory. Unfortunately, this has so far never been successful in *L. viridis* (Gontcharoff 1960, own observations).

Reproduction in *Lineus viridis*

During the reproductive phase, adult animals of both sexes have fully mature gonads. This phase can be divided into two sub phases, the non-associative phase, in which animals live solitarily, and the association phase, starting in December, in which one to several males form mating groups with one female. In the non-associative phase, i.e. in October and November, adult females possess mature gonads and gonopores. Females that become fertile for the first time have completed oogenesis and have formed gonopores. Thus, they cannot be discriminated from older females at that time.

The fact that there were no males found in October 2005 may be due to two alternative causes. This might have been a sampling artefact. During this time of the year, sampling was hampered by large numbers of dead blades of dwarf eelgrass (*Zostera noltii* Hornemann, 1832) at the sampling site, which could be confused with small specimens of *Lineus viridis*. It is therefore likely that eelgrass blades along with bad lighting conditions (nocturnal sampling) kept us from finding small males of *L. viridis*. The alternative is that males of *L. viridis* were elsewhere in the intertidal zone or not actively moving during the time of the survey and were therefore not recorded. Cryptic behaviour of males probably also accounts for their low numbers in November 2006. A similar observation has been made by Thiel and Reise (1993) and Thiel et al. (1995). However, judging from the data obtained from the animals maintained in culture, it is very probable that mature males are present during these months.

From January on, females are associated in mating groups with males. From our findings, it is impossible to say whether males are confined to one female or whether they frequently change from one female to another. However, larger females do not seem to be more attractive to males since there is no correlation between female body length and number of males associated with it. While females are still actively moving in December, they are largely immobile in February. During this time, mating groups are found under stones and in patches of Portuguese oysters [*Crassostrea gigas* (Thunberg, 1793)] and blue mussels (*Mytilus edulis*). In culture, generally neither males nor females feed during the height of the mating season. Gametogenesis is completed in both sexes and there are no infertile animals found in the mating groups. At the end of the associative period, mating and spawning take place. Mating includes the production of a mating envelope by the female and fertilization of ova by the males which subsequently leave the mating envelope one after the other. The tissue barrier in the female gonoduct is dissolved so that the sperm cells are able to enter the ovary for fertilization. Spawning comprises the production of the second gelatinous layer and the shedding of fertilized eggs in a pear-shaped egg capsule into the gelatinous layer (Cantell 1975; Bartolomaeus 1984). Finally, the female exits the mating cocoon and leaves it behind. Since, contrary to what has been previously reported for *L. viridis* most likely collected in Roscoff (France) (Bierne 1983), in our study females spawned unfertilized eggs even if they had no male partner, we assume that the maturation and egg release is irreversible and independent of fertilization. Oogenesis thus is an enormous investment which seems only advantageous if fertilization is ensured. Therefore, it is important for females to be found by males. How mating groups are established and maintained is so far not known, but it seems likely that chemical communication is involved in these processes.

Conclusions

Fertilization in *Lineus viridis* has clearly been shown to be internal (Gontcharoff 1951; Bartolomaeus 1984; Fig. 1e). Spawning in this species complies with the definition of

mucus spawning given by Thiel and Junoy (2006). We therefore suggest that fertilization and spawning should be discerned as two separate processes instead of as a single process like the statements of Thiel and Junoy (2006) imply.

After mating, both sexes decreased in size, but the females in particular diminish enormously in size. This has lead to the assumption that *L. viridis* is semelparous, only reproducing once in its lifetime and then dying (Riser 1974). Even though our results confirm this diminishment in size in *L. viridis*, semelparity could be clearly ruled out. Animals that were taken into culture after successfully spawning survived for subsequent reproductive seasons for several years. Thus, it is obvious that *L. viridis* in the North Sea is iteroparous. The interpretation of Riser (1974) is most likely due to a misinterpretation of the loss in body length and mass observed in animals after spawning. Alternatively, *L. viridis* on the coast of New England might show a different life history that involves mating only once in its lifetime.

Degeneration of the testes along with the observation that *L. viridis* survives mating led us to assume earlier that males of *L. viridis* change their sex by dedifferentiation of testes and subsequent formation of female gonads (von Döhren and Bartolomaeus 2006). Such sequential hermaphroditism along with sexual size dimorphism has been reported for the terrestrial hoplonemertean *Argonemertes* (*Geonemertes*) *dendyi* (Dakin, 1915) (Pantin 1969). For *L. viridis*, this hypothesis initially seemed to be supported by the pronounced sexual size dimorphism and the observation of developing ovaries without the presence of gonopores in some animals. This condition was consequently interpreted as an intermediate intersexual state. However, there is an overlap in body lengths of the sexes (observed in February 2006: 2.5% of the males were longer than the mean body length of females, and 4.8% of the females were shorter than the mean body length of males; in total, 3.3% of all sampled animals fell into the overlapping size range). This overlap in size of males and females, with males being larger than some females and vice versa as well as stability of sex observed in cultured animals clearly show that *L. viridis* is gonochoristic.

Due to the polyandrous mating system and internal fertilization present in *L. viridis,* male mate or sperm competition has been suspected to occur in this species (Thiel and Junoy 2006). However, direct mate competition, e.g. preventing other males actively from mating with the female was not observed. The size of the female compared with that of the associated males makes it physically impossible for one male to monopolize all of the female gonopores. Nevertheless, there is some circumstantial evidence that competition for the female is at work in *L. viridis*. Most males only empty some or none of their gonads and seemingly retain sperm for future potential fertilizations. The exact mechanisms of mate competition and whether there is sperm competition involved in the reproduction of *L. viridis*, however, will have to be elucidated in future studies involving detailed surveys of mating groups along with experimental manipulation and assessment of multiple paternities (e.g. paternity tests with microsatellite-DNA).

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