Reproduction of *Amphiporus lactifloreus* (Hoplonemertini) on tidal flats: implications for studies on the population biology of nemertines

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ABSTRACT: The reproductive period and life-history parameters were investigated for the hoplonemertine *Amphiporus lactifloreus* found on the tidal flats of the island of Sylt in the northern Wadden Sea. Every six weeks 20 individuals were collected and then histologically examined to determine the development stage of their reproductive organs. *A. lactifloreus* reproduces in the late autumn, its peak reproduction being in the second half of November. Individuals from all size classes > 20 mm body length produced gametes. Individuals of the cohorts that reproduced in the late autumn of 1992 persisted and grew until July 1993, indicating that *A. lactifloreus* is an iteroparous species. The length of relaxed individuals was significantly correlated with their length under anaesthetized conditions, but the regression changed significantly after the reproductive period. Length under ‘relaxed’ conditions was significantly correlated with weight (wet weight, dry weight, and ash-free dry weight); these relationships did not vary significantly before or after the reproductive period. Our results show that important life-history data of intertidal nemertines can be obtained without time-consuming histological studies. On the basis of these findings, recommendations for future studies on the population biology of intertidal nemertines are given. Regular length measurements of nemertines under ‘relaxed’ conditions are proposed as a useful tool for tracking the growth and survival of annual cohorts of intertidal nemertines.

INTRODUCTION

Nemertines are important endobenthic predators in estuarine ecosystems (Roe, 1970, 1976, 1993; Reise, 1985; McDermott, 1984, 1993; Ambrose, 1991). They may have a significant predation effect on their prey populations (Roe, 1976; Nordhausen, 1988; Rowell & Woo, 1990), or they may provoke prey movements within tidal flats (Thiel & Reise, 1993). To understand the role of nemertines in benthic assemblages, a basic knowledge of their population biology is crucial. However, there are only scattered records of the occurrence of intertidal nemertines, and apart from the studies by Roe (1970, 1976, 1993) detailed studies on the population biology of intertidal nemertines do

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not exist. Our present knowledge of interannual variations in nemertine abundance is very limited. When looking for information on recruitment processes and the factors that influence them, one must fall back on reports of an observational nature (e.g. Coe, 1943; Riser, 1974). In the case of most nemertine species it is unknown whether they reproduce more than once during their lifetime. Apart from a few historical reports on individuals surviving for several years in the laboratory (McIntosh, 1873/74), nothing is known about their life-span (Gibson, 1972). This elucidates the need to foster our knowledge of basic life-history information of nemertines.

As a step in this direction, we investigated the reproductive biology of *Amphiporus lactifloreus*. This hoplonemertine is a common endobenthic predator in many coastal areas of Europe (Gibson, 1972, 1982), and it can frequently be found on the tidal flats of the Nord-Sylter Wattenmeer (Reise, 1985; Thiel & Reise, 1993; Albrecht & Reise, 1994). In order to reveal basic information on the life-history of this species and to provide methods to improve studies of the population biology of this and other intertidal nemertine species, we addressed the following questions:

When is the reproductive period of *A. lactifloreus*? – The available data on the reproductive period of this nemertine species in Europe vary considerably (Fig. 1).

Do all individuals of the population participate in a reproduction event? – For population studies it is of prime importance to know when a species achieves maturity.

Is *A. lactifloreus* semelparous or iteroparous? – Coe (1943) mentions that large nemertine species probably reproduce in several successive years (iteroparous), whereas smaller species probably die after their first reproduction. Riser (1974) recorded that individuals of *Lineus viridis* die as a consequence of spawning, and that after the reproductive period only very small individuals can be found on tidal flats.

Furthermore, in order to facilitate future studies on the population biology of *A. lactifloreus* (and other nemertines), we examined whether length is a measure potentially indicative of different age classes.

**MATERIAL AND METHODS**

The study was conducted on the tidal flats of the Nord-Sylter Wattenmeer. The tidal range is about 1.8 m (Reise, 1985). In this area, *Amphiporus lactifloreus* can reach densities of up to 250 individuals / m² (Thiel & Reise, 1993). Mussel clumps, which are an important micro-habitat of this nemertine, are very abundant in the study area (Reise et al., 1989; Albrecht, 1991).
Examination of the reproductive stage of *Amphiporus lactifloreus*

We collected *A. lactifloreus* from the tidal flats of the island of Sylt in the northern Wadden Sea between August 1992 and July 1993 every 6–8 weeks. On each sampling date we collected 20–24 individuals which were separated from mussel clumps in the field. We measured their length by allowing them to crawl along a ruler (described below in 'Biometrics of *A. lactifloreus*'). Nemertines were grouped into 7 different length classes according to the measurements under 'relaxed' conditions (≤20 mm; 21–30 mm; 31–40 mm; 41–50 mm; 51–60 mm; 61–70 mm; > 70 mm). Before fixation, the nemertines were anaesthetized with 7.5 % MgCl₂-solution. Specimens were fixed in Bouin's fluid for 24 h and then transferred to 70 % alcohol. Animals were embedded in 54 °C paraffin wax for sectioning.

In order to examine the reproductive stage of the gonads, we prepared sections (6–8 μm) from the middle part of every animal's body. The sections were stained using the Mallory-trichrome-method. We examined 50 sections of each individual under the microscope, determined its sex when possible, and classified the developmental stage of the gonads in every section according to the description presented in Table 1. The latest stage of development that was found to be in majority after 50 sections of an individual were examined was used to classify its reproductive stage.

Biometrics of *Amphiporus lactifloreus*

In order to examine the general biometrics of *Amphiporus lactifloreus* and to test whether relationships between different, easily measurable parameters remained consistent, we collected nemertines in the autumn of 1991 (October) and in the spring of 1992 (March). We determined their natural length by allowing them to crawl along a ruler and reading the length when they were fully relaxed (= 'relaxed' nemertines; see also Gibson & Young, 1976). As it proved to be a time-consuming task to get the nemertines to crawl along the ruler and to be more or less relaxed, we anaesthetized the nemertines in a 7.5 % MgCl₂-solution. After anaesthetizing the nemertines for about 10 minutes in the MgCl₂-solution we again measured their length (= 'anaesthetized' nemertines) to establish a

<table>
<thead>
<tr>
<th>Stage</th>
<th>Term</th>
<th>Short description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>undeveloped</td>
<td>No gonads visible in the section</td>
</tr>
<tr>
<td>1</td>
<td>resting</td>
<td>Gonads visible but still adhering close to the body wall; oocytes and spermatogonia visible</td>
</tr>
<tr>
<td>2</td>
<td>maturing</td>
<td>Gonads visibly protruding into the body lumen; oocytes increasing in size, spermatocytes increasing in number</td>
</tr>
<tr>
<td>3</td>
<td>mature</td>
<td>Gonads filling most of the body lumen; oocytes and sperm densely packed in the gonads, gonopores opening</td>
</tr>
<tr>
<td>4</td>
<td>post-reproductive</td>
<td>Gonads empty, only very few gametes remaining in the gonad lumen; gonopores still opened</td>
</tr>
</tbody>
</table>

Table 1: Classification of the reproductive stages of the hoplonemertine *Amphiporus lactifloreus* as recognizable in histological sections (6–8 μm thick)
relationship between living and anaesthetized individuals. We then determined the wet weight, dry weight (after drying them for 3 days at 80°C), ash weight (muffle furnace for 4 hours at 510°C), and ash-free-dry-weight (afdw = dry weight – ash weight).

RESULTS

Reproductive stages and reproductive period of Amphiporus lactifloreus

*Amphiporus lactifloreus* is dioecious. Females and males can be distinguished in the field only during the reproductive period. The reproductive products of mature individuals can be seen through the body wall. Mature eggs have a greenish-white appearance, and the observer in the field can easily recognize between 4 to 10 mature eggs in one ovary (Fig. 2). Male gonads containing sperm appear white. The mature male gonads are heavily folded, and in the field the borders between individual testes cannot be distinguished. Outside the reproductive period, males and females can only be distinguished by histological examination.

Animals collected between April and July sometimes had a bright orange or dark grey coloration, but the histological examination of these individuals showed that they did not contain mature eggs or sperm. During the rest of the year the animals appeared greyish-white.

In the histological sections, the gonads of nemertines that were in reproductive stage 1 (Table 1) were pressed close to the body wall. The intestinal lumen and the intestinal tissues occupied most of the body volume (Figs 3a, 4a).

Gonads of females in stage 1 contained previtellogenic oocytes (Fig. 3b). The nucleus appears translucent, and chromosomes are visible (Fig. 3b). Up to 15 of these previtellogenic oocytes can be found in one section of an ovary. Gonopores can be recognized in the sections, but their opening to the outside is very narrow. During the vitellogenic phase, the oocytes increase in size, the nucleus is no longer translucent in sections, and
Reproduction of *Amphiporus lactifloreus*

![Cross sections of female *Amphiporus lactifloreus*.](image)

*Fig. 3.* Cross sections of female *Amphiporus lactifloreus*. a: overview; b: ovary in stage 1; c: ovary in stage 2; d: ovary with mature eggs. G gonads; g gonopores; I intestine; L lateral nerve cord; M longitudinal muscles; R rhynchocoel. Scale bar = 0.1 mm in a–d

the ovaries protrude into the body (stage 2 – Fig. 3c). After maturation, ovaries containing oocytes almost completely occupy the body volume (stage 3 – Fig. 3d). Up to 5 mature oocytes can be seen in one section of an ovary. Mature oocytes are covered by a thin, hard layer, which is difficult to section and often causes artifacts in the sections (Fig. 3d).

Gonads of males in stage 1 contain spermatogonia (Fig. 4a). During spermatogenesis the number of spermatocytes rapidly increases, and subsequently the gonads extend into
Fig. 4. Cross sections of male *Amphiporus lactifloreus*. a: testes in stage 1 (resting); b: testes in stage 2; c: testes with mature sperm – stage 3; d: empty testes immediately after the reproductive period – stage 4. G gonads; g gonopores; I intestine; L lateral nerve cord. Scale bar = 0.1 mm in a–d
Reproduction of *Amphiporus lactifloreus*

![Reproduction stages diagram](image)

**Fig. 5. Numbers of *Amphiporus lactifloreus* from the Nord-Sylter Wattenmeer in the respective reproductive stages during the 8 sampling periods.** Stage 0 – undeveloped; stage 1 – resting; stage 2 – maturing; stage 3 – mature; stage 4 – post-reproductive. For further explanation see Table 1

*Amphiporus lactifloreus* has a clearly confined reproductive period in the Nord-Sylter Wattenmeer (Fig. 5). Maturation of the gonads does not begin until August. In October 1992 almost all of the nemertines that were collected were in stage 2, and the ovaries and testes were maturing (Fig. 5). At the end of November (27th November 1992) more than half of the nemertines that were collected contained fully mature gametes, and 7 of 24 nemertines had already released their reproductive products. By the end of January (31st January 1993), all of the nemertines had spawned, and the gonads were again in stage 1.

**Biometrics of *Amphiporus lactifloreus***

Nemertines were found in all length classes. Both sexes were represented in all length classes more or less equally (Fig. 6), except in the class ≤20 mm, in which only...
Fig. 6. Numbers of *Amphiporus lactifloreus* in each of the 7 size classes during the 8 sampling periods.
Reproduction of *Amphiporus lactifloreus*

Fig. 7. Biometric relationship for *Amphiporus lactifloreus* in October 1991 (n = 78 individuals) and March 1992 (n = 40 individuals). A: Linear regression of the length of anaesthetized individuals and the length of 'relaxed' individuals. B: Linear regression of wet weight and 'relaxed' length of different sizes of *A. lactifloreus*. C: Linear regression of dry weight and 'relaxed' length. D: Linear regression of ash-free dry weight and 'relaxed' length. Linear regressions between 'relaxed' length and weight data were only calculated for nemertines longer than the 20 mm 'relaxed' length (October: n = 77 and March: n = 40)
one male was found. Male *Amphiporus lactifloreus* were in the majority before the reproductive period, whereas females dominated after the reproductive period (Fig. 6).

In January 1993, the numbers in the different length classes were almost the same as in November 1992. Thus it can be concluded that the majority of nemertines survived the reproductive period. Between August 1992 and January 1993, the distribution of nemertines in different length classes indicates a cohort with a modal size between 40 and 60 mm (Fig. 6). Beginning in March 1993, this cohort seemed to grow further, and in July 1993 most of the nemertines were longer than 60 mm.

Both in November 1991 and March 1992, there was a strong correlation between the length of the nemertines measured when they were crawling along the ruler under 'relaxed' conditions and their length measured after being anaesthetized (Fig. 7a). A significant relationship existed between the relaxed length and the weight-data (ww, dw and afdw) of *A. lactifloreus* (Fig. 7b, c, d). No significant differences for any of the examined 'relaxed' length-weight relationships were found between the two sampling dates of October 1991 and March 1992 (t-test; p > 0.05). However, the relationship between the length of 'relaxed' and anaesthetized nemertines changed significantly after the reproductive period (t-test; p < 0.02). The relationship between anaesthetized length and wet weight also changed significantly from October 1991 to March 1992 (t-test; p < 0.001), whereas the other length-weight relationships (dw, afdw) under anaesthetized conditions did not change in this time period (p > 0.05).

**DISCUSSION**

**Reproductive period of *Amphiporus lactifloreus***

The hoplonemertine *Amphiporus lactifloreus* in the Wadden Sea reproduces during the late autumn. This is in clear contrast to most other reports of the reproductive period of this species (McIntosh, 1873–74; Bürger, 1897–1907; Franzen, 1956, 1983; Gibson, 1972). Bierre & Rué (1979), who also examined histological sections, found a reproductive period in *A. lactifloreus* on the French Channel coast that is similar to that observed by us in the Wadden Sea. In April and June of 1993 we found individuals that matched the colour description given by Gibson (1972) for *A. lactifloreus* during their presumptive reproductive period, but none of our individuals revealed maturing or mature gonads during the histological examination (Fig 5). Individuals collected in November 1992 contained mature gonads. They could clearly be recognized as mature individuals in the field with the naked eye or with the aid of a hand lens. Interestingly, in none of the above-mentioned reports is a description of mature individuals given that matches our observations. This leads us to suspect that in most cases the reported reproductive period of *A. lactifloreus* was not accurately identified. Mature individuals can easily be recognized in the field, providing an easy tool for re-examining the reproductive period of *A. lactifloreus* along the European coasts.

Most other nemertines from temperate regions of the northern hemisphere, for which reproductive data are available, reproduce during the spring and summer months (Fig. 8). The majority of nemertines are benthic predators, so it can be assumed that developing juveniles will find abundant prey resources during the summer and early autumn. Very few nemertines other than *A. lactifloreus* reproduce during the autumn (Friedrich,
Fig. 8. Reproductive periods of 53 nemertine species from the temperate regions of the northern hemisphere. Horizontal lines separate the different species from each other. Information from various geographical areas or different authors may be given for particular species (see also Fig. 1). Where several reports are available for one species, the thickness of the horizontal columns is decreased.

1979). No unifying character can be identified among these different groups of nemertines which reproduce in autumn.

During the reproductive period and following it, high numbers of *A. lactifloreus* could be observed at the sediment surface during nocturnal low tides (Thiel, 1992; Thiel et al., 1995). Throughout our observations and sample-collecting trips we found no indication of how *A. lactifloreus* reproduction might function. Some authors mention that *A. lactifloreus* deposits fertilized eggs in a cocoon-like mass on the bottom where they undergo direct development (Thorson, 1950, 1957), whereas other authors report that the gametes are freely shed into the water column (Bierne, 1983). Intensive field observations
during night-time low tides in November are a promising project that may shed more light on how reproduction functions in *A. lactifloreus*.

**Life-history of Amphiporus lactifloreus**

The hoplonemertine *Amphiporus lactifloreus* is a very common intertidal nemertine along European coasts (Gibson, 1982). It feeds preferentially on amphipods of the genus *Gammarus* (Jennings & Gibson, 1969; McDermott & Roe, 1985). *A. lactifloreus* can be found in aggregations comprising several intermingled individuals within mussel clumps. Densities may be as high as 250 individuals/m² within these micro-habitats (Thiel & Reise, 1993). The average food requirement of an adult individual *A. lactifloreus* was estimated from laboratory experiments to be about 0.15 amphipods/day (Thiel, 1992), a value which corresponds with values provided by McDermott (1984, 1993) for another hoplonemertine feeding on amphipods of a size similar to those preyed upon by *A. lactifloreus*.

The nemertine *A. lactifloreus* has the capacity to reproduce several times in subsequent reproductive periods, as can be concluded from our data. To our knowledge the present study is the first to show that a relatively small nemertine species is iteroparous. We conclude that in the field *A. lactifloreus* can reach an age of 2 years, because some individuals of the cohort that reproduced in November 1992 persisted in the field until July 1993 (see Figs 5 and 6).

The explanations for *A. lactifloreus* reproducing in autumn remain speculative. Gammarid amphipods in the Nord-Sylter Wattenmeer and along the northern European coasts reach their highest densities in spring and autumn (personal obs.). The reproductive period of *A. lactifloreus* immediately follows the period when gammarids reached peak numbers in autumn. This leads to the assumption that food availability may be a factor influencing the timing of reproduction in *A. lactifloreus*.

An alternative hypothesis would be the lack of potential predators of *A. lactifloreus* in its micro-habitat during autumn and winter months. *A. lactifloreus* preferentially inhabits mussel clumps on tidal flats and lives in large numbers under small rocks on rocky shores. The nemertine shares this habitat with many other invertebrates. Despite the widespread notion that nemertines have few or no enemies, shore crabs prey upon small nemertines (Gibson, pers. comm.; Thiel, 1992). Juvenile shore crabs (*Carcinus maenas*), which inhabit mussel clumps in large numbers, leave this habitat during the late fall (Thiel & Dernedde, 1994). Thus, during the winter, small juveniles of *A. lactifloreus* would have a relatively predator-free habitat in mussel clumps.

**Biometrics of Amphiporus lactifloreus**

Measurements of length proved to be a relatively useful tool for investigating the population biology of the nemertine *A. lactifloreus*. Despite the fact that the size and shape of nemertines are highly variable (Gibson, 1972, 1982; Riser, 1974), their length is a good and consistent measure of their condition under both the ‘relaxed’ and anaesthetized condition, which is also true for the heteronemertine *Lineus viridis* (Thiel, unpubl. data). In the nemertine *Nipponemertes pulcher*, body length is a consistent measure for the number of eyes (Berg, 1972), and Heine et al. (1991) report the body length of
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*Parborlasia corrugatus* to be correlated with wet weight. The study of Roe (1976) shows that length measurements of 'relaxed' nemertines are a good tool for tracking annual cohorts over various time periods. Gibson (1983) and Roe (1976) demonstrated that 'body bulk' (length* maximum width) is a good measure for wet weight. Figure 5 shows that with the help of the length measurements of 'relaxed' individuals, cohorts from different years can also be followed in the nemertine *A. lactifloreus*. As length measurements of anaesthetized individuals do not seem to be as consistent over time as those of 'relaxed' nemertines, we propose the latter measure for future studies on their population biology.

Future studies on the population biology of intertidal nemertines

Ready access and the relatively high abundances of nemertines found on the tidal flats invite studies on the population biology of intertidal species. Regular length measurements of nemertines promise to provide important information on the growth and survival of annual cohorts of nemertines. Furthermore, they will reveal whether the nemertine under investigation is an annual species. Optical examinations of living specimens often allow a determination to be made whether individuals have mature gonads, and when combined with data from length measurements, these data can indicate whether it is an iteroparous or semelparous nemertine, and whether all members of the population produce gametes.

The most difficult problem in future studies on the population biology of intertidal nemertines is to determine the recruitment process and the factors influencing its success. Future studies should first focus on those species that produce cocoons, as in these species the small juveniles hatch directly from the cocoon, e.g. in *Lineus viridis* (Bartolomaeus, 1984). Not surprisingly, *L. viridis* is one of the very few nemertines for which the juvenile habitat and development are known, but no data on juvenile growth and mortality are available at present (Cantell, 1989). Prime factors to be investigated with respect to the recruitment success of nemertines should be the food of small juveniles, their susceptibility to potential predators, and the reproductive potential of the adult population.

Our study shows that important life-history data of intertidal nemertines can be relatively easily obtained without time-consuming histological investigations. These data provide the basic information necessary for a better understanding of interannual variations in nemertine abundance and may subsequently help to understand the fluctuations in the abundance of their prey species.

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