Reproductive biology of several species of recently collected pelagic nemerteans

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Abstract

The reproductive biology and morphology of six polystiliferous and one monostiliferous species of pelagic nemerteans was studied in specimens recently collected off California. Depth distributions for these specimens ranged from 250 m to 3250 m, with most specimens obtained between 700 m and 1750 m. Length of sexually mature individuals ranged from 2 mm for the monostiliferan to 61 mm for a male *Phallonemertes cf. murrayi*. Among *P. cf. murrayi* and *Nectonemertes cf. mirabilis*, which yielded the largest specimens studied, mature males were larger than mature females and mature animals were larger than those in which gonads were not apparent. Females typically outnumbered males, although *N. cf. mirabilis* approached a 1:1 sex ratio. In the polystiliferans studied by light microscopy, accessory ovarian cells appeared to translocate yolk or yolk precursors to oocytes via cytoplasmic bridges, a mechanism typically associated with nurse cells and not previously reported from nemerteans. Mature oocytes 0.5–1 mm in diameter were common, making them very large compared to those of benthic nemerteans. Sperm possessed elongated heads and nuclei. In general, the pelagic nemerteans studied appeared: a) to produce relatively few mature gametes at a time, b) spawn in close proximity to each other, c) undergo iteroparous reproduction, and d) display moderately long-lived life cycles. In addition, data for *P. cf. murrayi* and possibly *N. cf. mirabilis* demonstrates potential seasonal peaks in reproductive activity.

Introduction

Most benthic, shallow-water nemerteans shed hundreds to thousands of oocytes per female, either directly into the sea or into benthic gelatinous cocoons, with external fertilization (Coe, 1905; Riser, 1974; Bierne, 1983; Franzén, 1983). Most also show seasonal periodicity in spawning (Riser, 1974). Both *Paranemertes peregrina* and *Pantinonemertes californiensis*, for example, have one major spawning season per year, although a few reproductively mature animals can be found throughout most of the year (Roe, 1976, 1993). In contrast, the deep sea long has been considered an area of relative constancy over space and time, with most of its fauna expected to show no reproductive seasonality (Gage & Tyler, 1991; Young, 1994). However, recent evidence indicates seasonal reproductive patterns in several, mostly benthic, abyssal invertebrates (Gage & Tyler, 1991; Gage, 1994; Tyler, et al., 1994).

According to Brinkmann (1917a) reproduction in *Nectonemertes cf. mirabilis* was non-seasonal, because he found a full range of reproductive maturity in the 82 specimens obtained form the North Sea during June and July, 1910. Coe (1926) concurred, primarily on the premise that 'surface-conditions do not vary the environment of these bathypelagic creatures.' Pelagic nemerteans off California are most abundant in moderate depths (625–1750 m) (Roe & Norenburg, 1998). Thus, on the premise that seasonal pulses of epipelagic plankton, light penetration, etc., should be even stronger at these depths than in abyssal depths, we examined our material for evidence of reproductive seasonality. This is the first study to address repro-

ductive biology of pelagic nemerteans with samples spanning most of the year.

Among pelagic species in which both sexes have been collected, females usually were more numerous than males, and females predominate among the remaining species known only from one sex (Coe, 1954). The gross morphology of ovaries and testes of shallow-water benthic nemerteans is basically similar for members of both recognized nemertean classes, the Anopla and Enopla. Ovaries and testes lie in extracellular matrix, arranged linearly along the sides of the gut or scattered and surrounding the gut, and usually alternate with gut diverticula when these are present. Ovaries first appear as aggregates of primordial cells. Each aggregate grows rapidly in size and number of cells, and a thin-walled sac, the definitive ovary, forms from some of these cells (Coe, 1905; Olivier, 1966). In anoplans, 4–10 oocytes mature per ovary, whereas in most enoplans usually only one relatively large oocyte matures per ovary (Bierne, 1983). Even in enoplans, however, total oocyte production can be quite large, since most animals have many ovaries (Bierne, 1983). Number of gonads, in both sexes, is greatly reduced in pelagic nemerteans, ranging from several to a few dozen as compared to up to hundreds in benthic forms of comparable volume. Among pelagic polystiliferans each ovary typically produces only a few mature oocytes (Coe, 1920); these oocytes are the largest known among nemerteans. For instance, Coe (1926) reported mature oocytes of a *Nectonemertes mirabilis* to be over 1 mm diameter and more than one-half the diameter of the worm, and Brinkmann (1917b) reported a *Dinonemertes investigatoris* (the largest recorded being 203 mm in length) that had about 50 pairs of ovaries, each with 6–8 huge oocytes up to 2.5 mm diameter. Many anoplans have a planktonic, feeding larva, the pilidium, that metamorphoses into adult form, whereas other anoplans and most enoplans have some form of direct development (Friedrich, 1979). The latter includes planktonic, typically non-feeding dispersal forms and 'crawl-away' forms – young worms that hatch from benthic egg masses or cocoons. There are no detailed observations on the development of pelagic nemerteans.

Nemertean oocytes typically contain yolk composed of protein, lipid and carbohydrate components (Bierne, 1983). In general, metazoans synthesize yolk in one of three ways: (1) autosynthesis within the oocyte, (2) heterosynthesis – synthesis of yolk precursors by other cells and subsequent transport to the oocyte, and (3) mixed synthesis, with both auto-

and heterosynthesis (Eckelbarger, 1994a). Pathways of yolk production in nemerteans have not been studied in much detail, but oocytes of some species can synthesize their own yolk (Bierne, 1983), whereas others may incorporate yolk produced elsewhere (Coe, 1905; Brinkmann, 1917b; Bierne, 1983; Stricker, 1986). According to Eckelbarger (1994a) four types of accessory cells are associated with invertebrate oocytes and apparently play trophic roles during vitellogenesis: (1) follicle cells, which are somatic in origin, completely encompass individual or groups of oocytes, and may have at least four distinct functions including synthesis of metabolites or yolk precursors; (2) nurse cells, which are abortive germ cells associated with oocytes via cytoplasmic bridges resulting from incomplete cytokineses – they generally replace or supplement synthetic activities of the oocyte; (3) nutritive eggs, which are germ cells that abort development and are phagocytized by definitive oocytes; and (4) miscellaneous accessory cells employed in different groups of invertebrates to obtain yolk. Stricker (1986) provided ultrastructural evidence suggesting a mixture of auto- and heterosynthesis in *Carcinonemertes epialti*, with adjacent somatic cells possibly helping to transport yolk derived from ingested crab eggs to the oocytes. In *Nemertopsis bivittata*, the morphology of somatic cells does not suggest transfer of yolk precursors (Turbeville, 1991). However, coated and smooth endocytotic pits and vesicles are common at the surface of vitellogenic oocytes, and an electron-dense substance that often is associated with coated pits also occurs between adjacent membranes of oocytes and somatic cells, suggesting the incorporation of yolk precursors into the oocyte. In some species, whole yolk cells, or aborted oocytes, apparently are absorbed into the cytoplasm of developing oocytes (Coe, 1905) in a process termed oocyte fusion by Bierne (1983). Oocyte fusion also was documented in the benthic hoplonemertean *Amphiporus lactifloreus* (Bierne & Rué, 1979).

In the first detailed description of oogenesis in pelagic nemerteans, Brinkmann (1917b) described follicle cells that surrounded and provided yolk and yolk precursors to the oocytes of *Nectonemertes mirabilis* via cytoplasmic bridges. He claimed that as an oocyte matures the volume of associated follicle cells diminished proportionately, and he concluded that when one oocyte approaches maturity, other oocytes are resorbed or expelled with the mature oocyte. In contrast, Coe (1926) believed that the follicle cells formed a syncytium and suggested that the most mature oocyte engulfed less mature oocytes. In this paper, we present

evidence that intercellular bridges extend between the oocytes and the accessory cells. Such an association is very unusual among metazoans and suggests to us that the follicle cells of pelagic polystiliferans most likely are nurse cells (Eckelbarger, 1994a).

Testes and ovaries in benthic nemerteans develop similarly. In some species they develop as a solid core of germ cells in the extracellular matrix with subsequent differentiation of the gonad wall, whereas in some other species the gonads first are recognized as a cavity, within which germinal cells subsequently multiply (Riser, 1974). Based on morphological description of testicular structure in *N. mirabilis* (Cravens & Heath, 1906; Brinkmann, 1917b; Coe & Ball, 1920) the testes of small males (with stubby tentacles) consist of thin-walled hollow sacs with spermatogonia imbedded in the testicular wall. As the testes enlarge they become heavily invested with circular muscle. The lumen of mature testes contains (a) immature sperm in spherical aggregates (cytophores), (b) mature sperm in bundles, and (c) cells in various stages of spermatogenesis toward the periphery. In addition to the conspicuously muscular wall of the testis in some species, the most striking feature is that in all known pelagic polystiliferans the testes are restricted to the anterior region, near the brain. Furthermore, the sperm ducts of *Phallonemertes cf. murrayi* elongate to form external 'penes' (Brinkmann, 1912), while males of *Nectonemertes* and *Balaenemertes* bear a pair of cephalic tentacles (cirri). These tentacles are a secondary sexual characteristic (Coe 1920; Coe & Ball, 1920). Brinkmann (1917b) found that, in a series of eight *N. mirabilis* from 21 to 40 mm long, tentacle length ranged from small protuberances to 10 mm and was 'perfectly correlated with the state of maturity of spermaries' (as translated by Coe, 1926) in four histologically examined specimens. Sperm with short and elongate heads are common (Franzén, 1983; Stricker & Folsom, 1998).

We report here on the depth distributions, sizes, reproductive seasonality, sex ratios and reproductive morphology at the level of light microscopy of pelagic nemerteans recently collected from California. The species we examined represent about half of the eleven recognized families of polystiliferous pelagic nemerteans and one specimen of a pelagic monostiliferan.

Methods and materials

Most collections were made during oceanographic cruises of the *R. V. Point Sur* and the *R. V. New Horizon* (directed by Dr James Childress, Univ. Calif. Santa Barbara). Collections were taken along a transect, 48–56 km long, north from Pt. Conception, paralleling the coastline 160 km off-shore, in Sept. 1992, Sept. 1993, Feb. 1994 and June 1994. Part of the June 1994 cruise was in Santa Cruz Basin, south of Santa Cruz Island. Some collections were made in and west of Monterey Bay, CA during research cruises by Monterey Bay Aquarium personnel (Sept. 1992, June 1993, Aug. 1993, Dec. 1993, Apr. 1994, Nov. 1994 and Dec. 1994). Miscellaneous specimens were also obtained from various studies done in and near Monterey Canyon by researchers from Moss Landing Marine Laboratories, Monterey Bay Aquarium Research Institute (MBARI) and Monterey Bay Aquarium (MBA) between 1989 and 1995. Collections were made with one of four types of mid-water trawls: (1) a modified Tucker Trawl with 10-m^2 opening, 6-mm net mesh, and a 30-l, thermally protected, cod end (Childress et al., 1978) – used on Childress and MBA cruises; (2) a 1-m^2 and (3) a 10-m^2 MOCNESS (Multiple Opening/Closing Net and Environmental Sensing System; see Wiebe et al., 1985) used only on Childress cruises; and (4) a standard Tucker Trawl with approximately 10-m^2 opening, used on MBA cruises and during miscellaneous studies. MBARI specimens were collected by the MBARI Remotely Operated Vehicle (ROV). Sampled depths ranged from 0 m (surface) to approximately 3800 m, and were determined from instrumentation connected with the MOCNESS, a time-depth recorder, or estimated from length of wire out.

Identifications are best approximations based on examination of all relevant literature and comparison with museum specimens. However, we consider available museum material to be of inadequate quality for making definitive identifications. Except for some miscellaneous specimens from the Monterey Bay area and all specimens for March 1993, the sex, length, width, and occasionally wet weight of specimens were determined on shipboard while animals were alive. Live specimens were prepared for light or electron microscopy as described by Norenburg & Roe (1998). Specimens from the Point Conception transect in March 1993 were collected for us by Karen Light. We identified, sexed and measured these specimens several weeks post-fixation. Likewise, miscellaneous
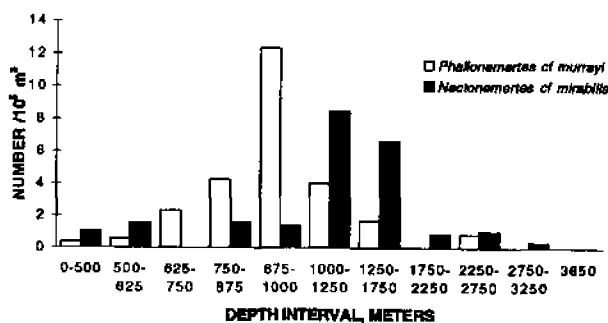
Figure 1. Abundance of *Phallonemertes cf. murrayi* and *Nectonemertes cf. mirabilis* by depth intervals along a transect 160 km west of California called the Point Conception transect. Abundance is in number of individuals per 10^5 m³ water filtered through trawl net.

specimens from Monterey were analyzed post-fixation. Paraffin-embedded, serially sectioned voucher specimens are deposited in the National Museum of Natural History (USNM), Washington, DC, USA.

Species and number of specimens sectioned for gonad analysis:

Family Nectonemertidae – *Nectonemertes cf. mirabilis*, 6 males, 10 females (USNM #174017, 174018, 174025–174038). We accept provisionally Coe's (1954) synonymies and use *N. cf. mirabilis* to refer to the most common *Nectonemertes* off California; our observations (unpublished) to date suggest that further study may resurrect the name *N. pelagica* for the California specimens in our study.

Family Phallonemertidae – *Phallonemertes cf. murrayi*, 1 male, 5 females (USNM #174019–174024). This species previously was known only from the Atlantic Basin; a definitive identification awaits availability of, and comparison with, suitable Atlantic specimens.

Family Armaueriidae – 13 specimens (USNM #174039–174051); probably all *Proarmaueria pellucida*, but possibly including other closely related species.

Family Pelagonemertidae – *Cuneonemertes elongata*, 2 females (USNM #174052, 174053).

Family Planktonemertidae – two specimens (USNM #174058–174059), possibly both *Crassonemertes robusta*.

Suborder Monostilifera – one unidentified specimen (USNM #174056); clearly pelagic, and presumed to be undescribed.

Results

Depth ranges

Phallonemertes cf. murrayi and *Nectonemertes cf. mirabilis*, together comprised 59% of all nemerteans collected along the Point Conception transect, and 70% and 73% of nemerteans collected from the Monterey Bay area and Santa Cruz Basin, respectively (Roe & Norenburg, 1998). The depth ranges of these two species overlapped extensively. However, *P. cf. murrayi* was most abundant at about 875–1000 m, whereas *N. cf. mirabilis* was most abundant at about 1000–1750 m (Figure 1). Depth for 30 intact armaueriids, collected from the Point Conception transect, Monterey Bay areas and Santa Cruz Basin, ranged from 2200 m to about 700 m, with 23 from 700–1050 m, five from 1150 m and one each from 1650 and 2200-1750 m. Twenty-six specimens of *Cuneonemertes elongata* were collected along the Point Conception transect and Santa Cruz Basin. The depth range was approximately 800–1250 m for 14 specimens for which data were available, whereas the monostiliferan was from a depth interval of 1750–1250 m. Depth information for the two planktonemertids (*Crassonemertes robusta*?) was lacking.

Sizes and weights

Mature males of *Phallonemertes cf. murrayi* were wider and longer than mature females (Table 1). Individuals lacking distinct gonads were smaller than were mature females and males, especially in width and weight. For *P. cf. murrayi* the correlation of length to weight was 0.863, length to area was 0.891, and area to weight was 0.921. In *N. cf. mirabilis*, mature males (judged by relative length of tentacles) averaged approximately the same length as did mature females (judged by ovary size and color). However, these males were broader and weighed more than did mature females (Table 2). Long-tentacled males were larger than those with short or stubby tentacles; mature females were larger than those with small ovaries; and individuals of both sexes were larger in all measurements than animals without evident gonads (Table 2). Weight at onset of maturity was similar for both sexes. For *N. cf. mirabilis*, the correlation of length to area was 0.764, length to weight was 0.832, and area to wet weight was 0.930. Sizes of other species in this study are shown in Table 3. The monostiliferan, at

Table 1. Mean sizes (± SD) of *Phallonemertes cf. murrayi* specimens from a transect 160 km west of Pt. Conception, CA, Sept. 1992 – June 1994

Gender[a]	Length (mm) $\chi \pm SD$	Width (mm) $\chi \pm SD$	Area (mm^2) $\chi \pm SD$	n	Weight (g) $\chi \pm SD$	n
Mature Males	61 ± 9.4	7.7 ± 1.2	476.2 ± 139.7	6	–	0
Immature Males	38.3 ± 9.0	6.3 ± 0.58	244.3 ± 68.6	3	0.52	1
All Males	53.4 ± 14.3	7.2 ± 1.2	398.9 ± 164	9	–	0
Mature Females	46.3 ± 11.5	6.6 ± 1.5	310.9 ± 134	6	0.45 ± 0.07	3
Immature Females	38.6 ± 6.9	5.1 ± 1.0	199.1 ± 65.7	16	0.43 ± 0.16	10
All Females	39.5 ± 8.0	5.9 ± 1.3	237.0 ± 88.5	43	0.43 ± 0.14	13
Sex Not Apparent	33.1 ± 7.8	4.1 ± 1.4	141.4 ± 72.1	76	0.20 ± 0.12	13

[a] Most females were recorded only as females, not as mature or immature.

Table 2. Mean sizes (± SD) of *Nectonemertes cf. mirabilis* specimens from a transect 160 km west of Pt. Conception, CA Sept. 1992 – June 1994

Gender	Length (mm) $\chi \pm SD$	Width (mm) $\chi \pm SD$	Area (mm^2) $\chi \pm SD$	n	Weight (g) $\chi \pm SD$	n
Mature Males	47.7 ± 8.2	7.2 ± 1.3	342.8 ± 85.3	13	0.57 ± 0.08	4
Immature Males	39.2 ± 7.3	4.7 ± 0	185.2 ± 44.7	18	0.41 ± 0.06	4
All Males	42.7 ± 8.7	5.8 ± 1.6	251.3 ± 102	31	0.49 ± 0.11	8
Mature Females	47.0 ± 4.2	5.4 ± 1.1	257.3 ± 63.0	18	0.41 ± 0.06	4
Immature Females	44.4 ± 6.7	5.0 ± 1.4	226.1 ± 75.4	16	0.42 ± 0.15	6
All Females	44.9 ± 7.2	5.3 ± 1.2	242.6 ± 69.8	34	0.41 ± 0.11	10
Sex Not Apparent	33.9 ± 9.0	3.1 ± 1.1	110.7 ± 60.8	38	0.15 ± 0.07	17

about 2 mm long, was among the smallest of all known nemerteans.

Seasonality

Phallonemertes cf. murrayi and *Nectonemertes cf. mirabilis* were abundant enough off California to permit the study of reproductive seasonality. In addition, males of *N. cf. mirabilis* in various stages of maturity, and mature males of *P. cf. murrayi* were recognized easily. Similarly, mature ovaries of both species could be seen through the body wall without the aid of a dissecting microscope.

At Point Conception *P. cf. murrayi* had a reproductive peak in spring–early summer (Figure 2), with the collection rate of reproductively mature individuals rising from 42% in February to 80% in June. However, at least a few mature *P. cf. murrayi* were collected at almost all times of the year when data were included from the more coastal areas of Monterey Bay and Santa Cruz Basin (Figure 3). Fall (September with only 14.3%) and winter months had the lowest proportion of the population in a reproductive state (Figures 2, 3).
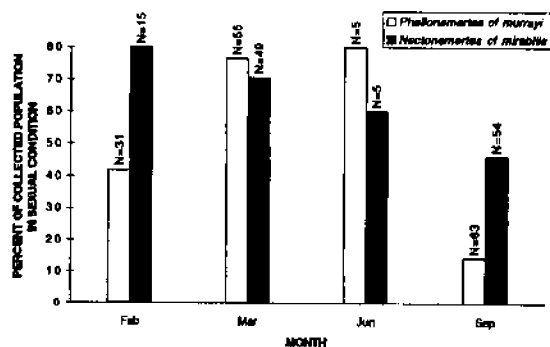

Figure 2. Percent of *Phallonemertes cf. murrayi* and *Nectonemertes cf. mirabilis* along Point Conception transect with visible gonads. N = total number specimens collected.

Reproductive individuals of *Nectonemertes cf. mirabilis* from the Point Conception transect appeared to have a winter–early spring peak, with 80% of the population showing some development of gonads in February, 71% in March, 60% in June and 46% by September (Figure 2). In the Monterey Bay area a high percentage of reproductive animals was found in December (85.7%), and some mature or maturing

Table 3. Mean sizes ($\pm$ SD) of species of pelagic nemerteans considered in this study, excluding *Nectonemertes cf. mirabilis* and *Phallonemertes cf. murrayi*

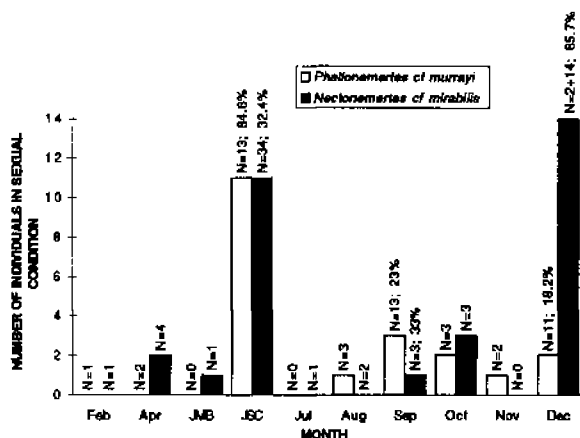| Species	Length (mm) $\chi \pm$ SD	Width (mm) $\chi \pm$ SD	Area (mm^2) $\chi \pm$ SD	n	Weight (g) $\chi \pm SD$	n
Armaueriidae	13.8 $\pm$ 3.4	4.3 $\pm$ 1.6	62.7 $\pm$ 35.3	20	0.10 $\pm$ 0.06	4
Cuneonemertes elongata	15.9 $\pm$ 3.9	2.9 $\pm$ 0.9	48.2 $\pm$ 24.0	20	0.04 $\pm$ 0.01	5
Specimen 266	15	3	45	1	0.05	1
Specimen 180	28.3 $\pm$ 3.5	8.7 $\pm$ 0.6	245.7 $\pm$ 36.7	3	–	0
Monostiliferan	2	0.5	1.0	1	–	0

Figure 3. Number of individuals with visible gonads collected from miscellaneous and quantitative samples along the California Coastline, excluding Point Conception transect. Bars = number of individuals designated sexually mature; N = total number specimens available in miscellaneous collections, for which the actual total collected is unknown (N for September, December and JSC samples is total number individuals collected); % is the proportion of reproductively mature individuals. JMB = specimens collected near Monterey Bay in June; JSC = specimens collected in Santa Cruz Basin in June, 1995. No specimens were available from Monterey Bay areas for January, March or May. The 14 specimens of *Nectonemertes cf. mirabilis* in reproductive condition in December include 2 of a total of 2 from miscellaneous collections and 12, or 85.7%, from a quantitative sample of 14.

individuals were found throughout most of the year (Figure 3).

Since the testes of the armaueriids were not visible without histological sectioning, such specimens were initially recorded as 'female' or 'no sex.' Female armaueriids were observed in all collections from: (1) the Point Conception transect during February, June and September; (2) Santa Cruz Basin during June, and (3) Monterey Bay during August. However, of the three specimens collected from Monterey Bay during December, none was a female.

Gonads were not readily apparent in living specimens of *Cuneonemertes elongata* except in June, when three of four specimens collected were clearly mature females. Seven of 11 preserved specimens from March 1993 were also identified as females. In addition, two sectioned specimens from September 1992, whose sex was not apparent in the living condition, were female. The planktonemertids (USNM #174054 and 174055) and the single monostiliferan specimen were opaque when alive, but sections revealed all of these to be female.

Sex ratios

Males of *Nectonemertes cf. mirabilis* were readily recognizable, with short tentacles identifying even immature males. We identified 32 males and 42 females from the Point Conception collection of 74 *N. cf. mirabilis* specimens, and there were 13 males and 11 females from the Santa Cruz Basin & Monterey Bay samplings of 24 sexually mature individuals. The ratios of males to females for these were 0.76 and 1.18 respectively.

Mature males of *Phallonemertes cf. murrayi* also were readily identified when they bore external phalli. However, these were lacking in immature males and may have been missing in recently spawned males. We identified 48 females and only 20 males from the 68 living specimens collected along the Point Conception transect. In the March 1993 collection, which was analyzed post-fixation, a total of 17 males and 25 female were identified, along with several immature males.

Of 30 armaueriids collected, 19 initially were identified as female and 11 as 'no sex' based on external examination. Five of seven putative females were confirmed as females following sectioning, whereas two appeared to be hermaphroditic. One of these two (USNM #174043) had moderately mature ovaries posteriorly and a possible mixed gonad anteriorly. The

other (USNM #174040) had moderately mature ovaries posteriorly and eight small anterior gonads that appear to be lined by spermatogonia. At least one of these anterior gonads opened laterally, as did the definitive testes in this species, in contrast to the ventral pore of ovaries. Among the six 'no sex' specimens that were sectioned, three were unambiguous males, two were young females, and one (USNM #174041) was hermaphroditic, with small ovaries posteriorly and testes anteriorly. However, two of these 'testes' also contained immature oocytes. Among 26 specimens of *Cuneonemertes elongata* examined, including two that were sectioned, twelve females and no males were identified.

Ovarian morphology

In each polystiliferous species studied, ovaries were arranged linearly and alternated more or less regularly with intestinal diverticula, from the foregut region to near the posterior end of the body. Ovaries in all of the species opened more or less ventrolaterally, peripheral to the lateral nerve cords. The ovaries extended dorsally over and close to the lateral nerve cords. The ovarian musculature lay primarily along the long axis of the ovary or formed a meshwork. In all species studied, the ovarian musculature was thin, with relatively widely spaced fibers. In most cases, there was a relatively well-developed dorsoventral muscle in close proximity to the ovaries.

Oogenesis appeared to be synchronous within an individual, with all of the ovaries of a given individual possessing a similar range of oocyte maturation. Oocytes in all species studied attained large sizes, ranging from 0.5 to 1 mm diameter. Each ovary usually had only one, or rarely a few, oocyte(s) of the largest size class. Oocytes seemed to develop more or less sequentially from the ovarian wall near the gonopore; i.e., smallest/youngest oocytes were usually near the gonopore and mature oocytes were near the intestine (e.g., Figures 4 and 8). We could not discern a distinct epithelial lining in the ovary.

Small cells lining the ovarian wall appeared to provide yolk or yolk precursors to maturing oocytes via cytoplasmic bridges (e.g., Figures 11 and 13). These cells had small nuclei, and their cytoplasm paralleled changes in the cytoplasm of the adjacent maturing oocyte. Thus, we take these to be nurse cells. They appear in association with oocytes after the oocyte nucleus had enlarged; this was especially evident in *Cuneonemertes elongata*. There was no evi-

dence that young, previtellogenic oocytes were diverted to become follicle or nurse cells. Based on estimated counts of nuclei, the number of these nurse cells associated with an oocyte appeared to reach an equilibrium early during oogenesis. (Although we refer occasionally to apparent vitellogenic products as 'yolk,' we have no chemical characterization of these products.)

Nectonemertes cf. mirabilis had about 30–60 ovaries, depending on size of the animal. Ovaries usually were lacking in the last 6–10 posterior-most interdiverticular spaces. As oocytes enlarged they became completely surrounded by nurse cells. The youngest oocytes were near the gonopore and the most mature oocyte was always distal to it (Figure 4). Three oocytes that were tracked through serial sections of one specimen were each associated with 120–150 cytoplasmic bridges. Each cytoplasmic bridge was associated with about ten nuclei in a reticulated cluster of nurse cell cytoplasm, but it was not possible to determine if these represented syncytia or clusters of individual nurse cells. A conspicuous, more or less aligned fibrous component, possibly microfilaments or actin bundles, extended through the cytoplasmic bridges (Figure 5). The cytoplasm of the oocyte and nurse cells at early stages of oogenesis was finely granular and relatively homogenous, but a fibrous or lamellar organization (presumably, rough endoplasmic reticulum) was the first evidence of cellular differentiation. This was followed by the more obvious formation of relatively large non-staining vesicles. These appeared to be translocated from nurse cells to oocyte, as they were found in cytoplasmic bridges and occasionally appeared to be aggregated in the oocyte near bridge sites. Next, small homogenous globules began to appear, and there was a progressive increase in the number of larger globules as the oocyte enlarged. In the largest oocyte and its nurse cells there was a heterogeneous population of globule sizes. Staining affinity of globules suggests a significant proteinaceous component, consistent with the inference that these contained yolk. At or near oocyte maturity there was a relatively larger proportion of non-staining vesicles in the nurse cells than in the oocyte, and the proportion of nurse cell to oocyte volume decreased. The maturing ooplasm of *N. cf. mirabilis*, unlike that of other species examined, appeared to have a polyhedral substructure owing to ordered association of the non-stained and stained vesicles. Vitellogenic vesicles appeared to coalesce as they matured, eventually forming a large pool of presumed yolk in the oocyte. As this pool enlarged, the oocyte nucleus moved to a peripheral position, near the
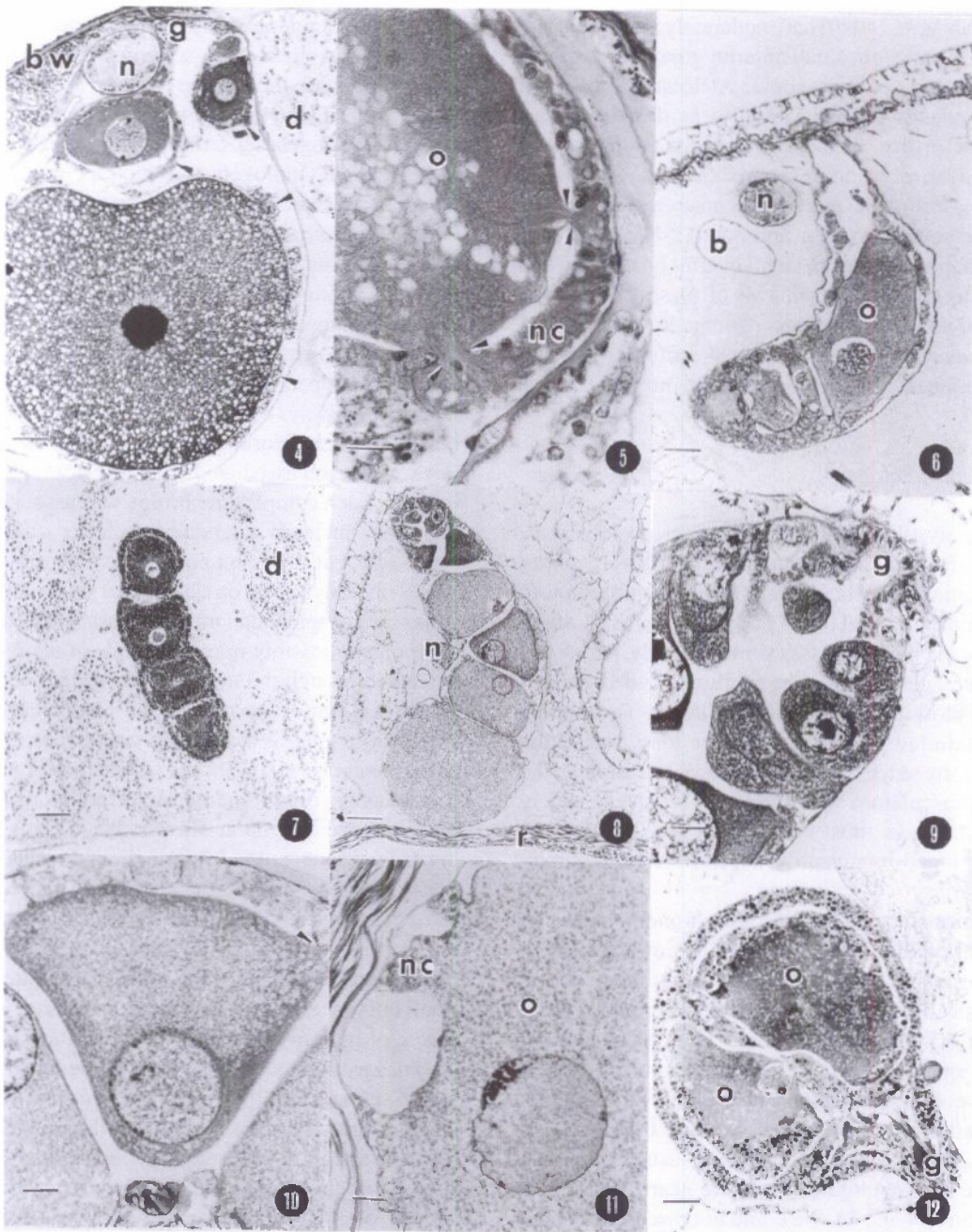
Figures 4–12. Photomicrographs of 8-μm histological sections, stained with modified Mallory procedure. *4, 5. Nectonemertes cf. mirabilis* (USNM #174031). *4.* Transverse section of body, showing ovary with full range of developing oocytes, each surrounded by nurse cells (arrowheads); scale = 100 μm. *5.* Oocyte in early vitellogenesis, showing nuclei in nurse cells and lamellar arrays extending through cytoplasmic bridges (arrowheads) between nurse cells and oocyte; scale = 20 μm. *6, 7. Phallonemertes cf. murrayi* (USNM #174019, #174020). *6.* Transverse section of body, showing linearly arrayed oocytes, in early vitellogenesis, with nurse cells located only along ovarian wall; scale = 100 μm. *7.* Ovary with four oocytes in equally advanced vitellogenic condition; scale = 250 μm. *8–11. Cuneonemertes cf. elongata* (USNM #174052). *8.* Transverse section of body, showing ovary with full range of developing oocytes, each accompanied by nurse cells along ovarian wall, except for oocytes near future gonopore; scale = 100 μm. *9.* Previtellogenic oocytes near future gonopore, scale = 25 μm. *10.* Early vitellogenic oocyte with adjacent nurse cells, and cytoplasmic bridge (arrowhead); scale = 25 μm. *11.* Most advanced vitellogenic oocyte from Figure 8, showing cytoplasmic continuity between nurse cell and oocyte; scale = 25 μm. *12. Proarmaueria cf. pellucida* (USNM #174039); transverse section of body, showing two oocytes arranged side by side in advanced vitellogenic condition, with prominent zone of nurse cells lining ovarian wall and peripheral cortex of oocytes containing similar multi-colored vesicles; bulk of oocytes filled with amalgam of secretory product; scale = 100 μm. Abbreviations: b, lateral blood vessel; bw, body wall; d, digestive tract; g, gonoduct; n, lateral nerve cord; nc, nurse cell; o, oocyte; r, rhynchocoel wall.
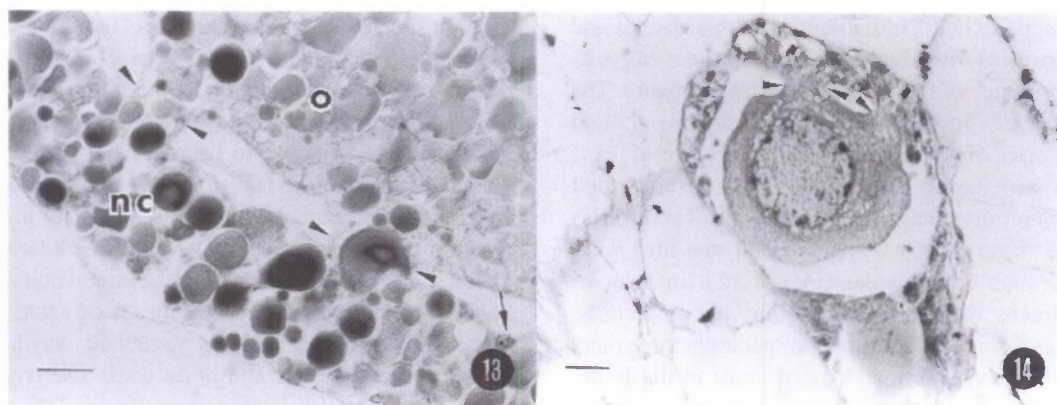
Figures 13–14. Photomicrographs of 8-μm histological sections, stained with modified Mallory procedure. *13. Proarmaueria cf. pellucida* (USNM #174039); detailed view of ovary in Figure 12, showing nurse cells and cortex of oocyte linked by cytoplasmic bridges (arrow heads); note solitary nucleus (arrow) among nurse cell secretory vesicles; nc, nurse cell; o, oocyte; scale = 20 μm. *14. Crassonemertes cf. robusta* (USNM #174055); transverse section of body showing tubular ovary in transverse section; early vitellogenic oocyte with cytoplasmic bridges (arrow heads) to nurse cells lining ovarian wall; scale = 25 μm.

cell membrane, that was farthest from the ovarian wall. Late in the maturation process a mucoid (weakly staining acid-mucopolysaccharide) coat was occasionally seen around the oocyte. After attaining a diameter of about 500 μm, the cytoplasmic appearance of oocytes remains relatively constant. There rarely was more than one oocyte per ovary larger than 500 μm, and the largest oocytes observed were about 1 mm in diameter. Females with very mature ovaries appeared to have the beginning of a new series of ovaries developing in interdiverticular spaces between mature ovaries. Development in these ovaries was not synchronous; whereas some contained modestly developed oocytes, others contained small cells barely recognizable as oocytes.

P. cf. murrayi also had about 30–60 ovaries. One specimen had an average of five oocytes per ovary, each oocyte measuring 300–400 μm in diameter. These oocytes were linearly arrayed and resulted in the ovaries appearing tubular (Figures 6 and 7). Another specimen had 3–5 oocytes per ovary, with each oocyte about 200–250 μm in diameter. Most of the ovaries in a third specimen had two oocytes that were about 1-mm in diameter. This caused the ovary to have a dumbbell shape that often was evident in living and in cleared, preserved specimens. Nurse cells incompletely surrounded most of the maturing oocytes, but were lacking between adjoining oocytes. The majority of stained vesicles in nurse cells and in the periphery of the oocyte were stained red with the modified Gomori stain. Some vesicles had a blue core and seemed to be transitional to the larger, predominantly blue vesicles. These vesicles became increasingly prominent deeper

in the cortex of the oocyte and appeared eventually to coalesce, forming a pool of blue-stained material. In many cases, oocytes with a large pool of blue material appeared to be ruptured and partially surrounded by the same material. It was unclear if this was due to specimen handling or was related to a resorptive or autolytic process.

Cuneonemertes elongata had about 26–30 ovaries. Each demonstrated a full range of development among oocytes (Figures 8 and 9), with ovaries containing up to 22 oocytes, including up to 3–7 relatively mature oocytes. Oocytes were arranged linearly in more or less tubular ovaries. Nurse cells in *C. elongata* completely lined the ovarian wall and appeared to be nonsyncytial. Such cells were lacking between adjoining oocytes (Figure 10). In three medium sized oocytes that were tracked through serial sections, there were 15–25 bridges per oocyte and generally there seemed to be only one nucleus associated with each cytoplasmic bridge. The most mature oocytes and mid-size oocytes in the same specimen had similar numbers of associated nurse cell nuclei, indicating that there was no additional proliferation of nurse cells during the later phase of vitellogenesis. Sequential development of oocytes was especially evident in this species, with the most immature oocytes near the gonopore and the most mature oocytes situated farthest from the gonopore (Figure 9). Neither of the two sectioned female specimens of *C. cf. elongata* appeared fully mature. The largest oocytes were about 385 μm in diameter and had nuclear diameters of about 85 μm. We know from observation of living specimens that

oocytes approached 1 mm diameter in this species and that the ovaries often had a dumbbell shape, suggesting the presence of two mature oocytes per ovary. The largest oocytes in both sectioned specimens contain small vesicles of presumed yolk/lipid (Figure 11).

Each armaueriid had about 8–14 ovaries that opened ventrally via a short gonoduct. The ovaries were like those of *N. cf. mirabilis*, in that they were bulbous rather than tubular (Figure 12). In most of the specimens, the ovaries started fairly far posteriorly, near the end of the foregut. In one specimen three pairs of immature ovaries were located close to the brain, and more posterior mature ovaries were separated from these by a conspicuous gap. Most small oocytes were near the oviduct, but they occurred elsewhere as well, usually along the ovarian wall or occasionally a nestled between large oocytes. The ovaries of our most mature specimen contained two to four large oocytes (500–550 μm in diameter) that were equally mature. However, these were situated side by side, in a clovelike arrangement, relative to the gonopore. Three of these oocytes that were tracked through serial sections bore about 100–125 cytoplasmic bridges to the nurse cells. Nurse cells encapsulated most of the oocyte, but a non-yolky, membranous extension of the nurse cells appeared to separate large oocytes. Thus, the vitellogenic portion of nurse cells was only between ovary wall and oocyte. Nurse cells formed a more or less continuous lining within which multiple nuclei were evident but cell boundaries were not. Presumed yolk vesicles in these sectioned armaueriid specimens were (1) richly multicolored, (2) generally larger, and (3) appeared much more heterogeneous than those of the other species examined (Figure 13). This difference in staining affinity appeared to hold for all fixation procedures. There were relatively few of the non-staining vesicles that were present in maturing oocytes of the other species. Equivalent ranges of vitellogenic vesicles were seen in both nurse cells and large oocytes, and vesicles at all stages of differentiation appeared to be translocated through the cytoplasmic bridges. As vesicles migrated toward the interior of the oocyte they appeared to coalesce, resulting in a single large, monocolored pool. The largest oocytes contained a large pool of yolk and a cortex of multicolored yolk vesicles occupying about half of the oocyte nearest the ovary wall. The germinal vesicle in these large oocytes was always situated near the cell membrane closest to other oocytes and was surrounded by the pool of yolk.

The two putative planktonemertids (*Crassonemertes robusta*?) had about 40 and 20 ovaries. Ovaries of both specimens were more or less tubular and about 100 μm in diameter. The ovaries of one specimen were relatively short, each containing 4–6 oocytes (55–70 μm in diameter), whereas the ovaries of the other specimen were up to 1.3 mm long, and each contained 15–17 oocytes (60 to 90 μm in diameter). The ovaries of the latter specimen lay primarily in a horizontal orientation immediately above the lateral nerve cords, which were 1.5 mm from the lateral body wall in this 7-mm wide specimen. A ventrally directed gonoduct extended from each ovary about half-way between the lateral nerve cord and body wall. The ovaries in both specimens clearly were immature and the oocytes occupied about two-thirds or less of the luminal volume. Developing oocytes appeared to be randomly distributed along the ovarian wall and were attached via small plaques of putative nurse cells (Figure 14). Some young oocytes occurred near the oviduct, but a distinct germinal zone was not evident. Each oocyte had about 4–10 cytoplasmic bridges, but these were closely spaced and difficult to distinguish. The larger oocytes were full of large yolk vesicles.

USNM #174056 was a minuscule, unidentifiable pelagic monostiliferan that was not fixed well. Remarkably, it bore four ovaries, each with several immature oocytes. The ovarian wall was indistinct, but there was no evidence of nurse cells.

In living armaueriids, both the well developed ovaries and the oviducts were dark brown. The ovaries or oocytes of what we considered very mature females of *P. cf. murrayi* and *N. cf. mirabilis* frequently were dark brown, but not in some individuals, although these had comparably sized oocytes. In those females of *P. cf. murrayi* and *N. cf. mirabilis* in which only a few scattered large oocytes were present, i.e. those we considered to be post-reproductive or nearly spawned out, the remaining ovaries/oocytes were nearly always dark brown. We were unable to correlate the brown color in living specimens with anything in sections; however, the dark color most likely reflected the mature phase of vitellogenic material.

Testicular morphology

The testes of all males studied were distributed in rows or clusters at the anterior end of the animal in close proximity to the lateral nerve cords and near the cerebral ganglia. Sperm development in any given animal was approximately synchronous for all testes, with only one or two stages of spermatogenesis prominent at a time. In our material mature sperm were present only

in *Nectonemertes cf. mirabilis* and *Phallonemertes cf. murrayi*; sperm in both have an elongate head of the so-called modified type (see Stricker & Folsom, 1998).

In specimens of *N. cf. mirabilis*, the gonopore of mature testes was at the tip of a small papilla formed by a thickened cushion of specialized, glandular epidermis. In contrast, in *P. cf. murrayi*, the gonopore of each mature testis was at the tip of an external phallus – a tubular, external extension of the vas deferens. The phallus consisted primarily of a thick dermal connective tissue, which was the principal structural element attaching the phallus to the body wall. In addition, the phallus contained a few, primarily circular, muscle fibers. The internal epithelium of the phallus was squamous and continuous with that of the testis. An outer epidermis was missing on the phalli of our sectioned specimens.

Testes of mature *N. cf. mirabilis*, with long tentacles, and *P. cf. murrayi*, with external phalli, were both heavily muscularized with a sheath of fibers that ran primarily parallel around the long axis of the testis (Figure 15). A web-like matrix, with small round cells at the nodes, traversed mature testes and compartmentalized maturing sperm aggregates. Spermatocytes appeared to be distributed throughout the testis. Sperm in *P. cf. murrayi* were aggregated into spherical rosettes containing from a few dozen to hundreds of sperm (Figure 16). Sperm in *N. cf. mirabilis* were arrayed parallel in bundles containing a few to many dozens of sperm (Figure 17). The lengths of the heads were about 16 μm and 25–30 μm for *P. cf. murrayi* and *N. cf. mirabilis* respectively (see also Stricker & Folsom, 1998).

Nectonemertes cf. mirabilis specimens with short, stubby tentacles appeared to be less mature, and were smaller (Table 1), than those with elongate (up to 2 cm in living specimens) tentacles. Four specimens, designated A-D (USNM #174025–174028 respectively), were selected as representing a sequence of maturity based on tentacle length, as proposed by Brinkmann (1917b). Specimen A had short, stubby tentacles; specimen B had tentacles about 1 mm in length; specimen C had long tentacles but not many testes were visible; and specimen D had long tentacles and many testes were visible. Histological observations of these specimens were at variance with our expectations. Specimen A had about 20 testes per side, arrayed in approximately three rows lying almost against the body wall (Figure 18). The testes were small empty chambers, approximately 110–150 μm in diameter, with dispersed spermatocyte-like cells lining the walls and a few scattered in the lumen. There were only a few iso-

lated muscle fibers and the gonoducts appeared to end blindly in the dermis. Epidermal papillae were lacking. However, three testes on one side of the body each contained a few, but distinct clusters of mature sperm. Specimen B had about 15 testes per side in a very irregular row, not against the body wall (Figure 19). The circular musculature of these testes was distinct, but was thin and dispersed. The gametes mostly were at the spermatid stage, and were suspended among filaments of the web-like matrix. Gonoducts ended blindly in dermis and their inflated appearance suggested that the specimen was exposed to high internal pressure. The epidermis was missing, so state of papillae was unknown. Testes of specimens C (Figure 20) and D (Figure 21) contained spermatids around the periphery, interconnected by threads of matrix, and mature sperm in the lumen. Specimen D appeared to be partially spawned. The testes of both specimens had a well-developed sheath of circular musculature about 3 μm thick. Specimen C had about 10–12 testes per side, each about 350 μm in diameter, whereas specimen D had about 15 testes per side, each 230–340 μm in diameter. In both specimens, the testes were not against the body wall and both had epidermal, glandular cushions associated with the gonopores (Figures 22 and 23). The epidermis of these cushions was up to 95 μm tall, as compared to 32 μm for nearby, well-preserved epidermis of the body-wall. The glandular cells constituted most of the volume of the cushion, with each cell containing a large, elongate 'goblet' consisting of very regular, small (< 0.5 μm) spherules packed tightly together. Thus, these cells were of the bacillary cell-type described by Norenburg (1985), which he noted often occurs in association with special functions.

Among other males of *N. cf. mirabilis* in our study, USNM #174034 had very short tentacles that were not evident in sections, but it had well-formed, empty testes (most about 170 μm in diameter) that lay along the ventral body wall muscle. These testes were lined by undifferentiated cells, small spermatogonia, and very few muscle fibers. All had gonoducts ending blindly in the dermis and there were no epidermal papillae. At a few testes, the epidermis above a gonoduct appeared to be undergoing reorganization. USNM #174033, with very well-developed tentacles, had large testes (up to 350 μm in major dimension) pressed against the body wall. These testes were sheathed in thick musculature and they were more or less packed with evenly distributed spermatocytes. A few scattered bundles of elongate spermatids also were present. Another specimen considered to be immature
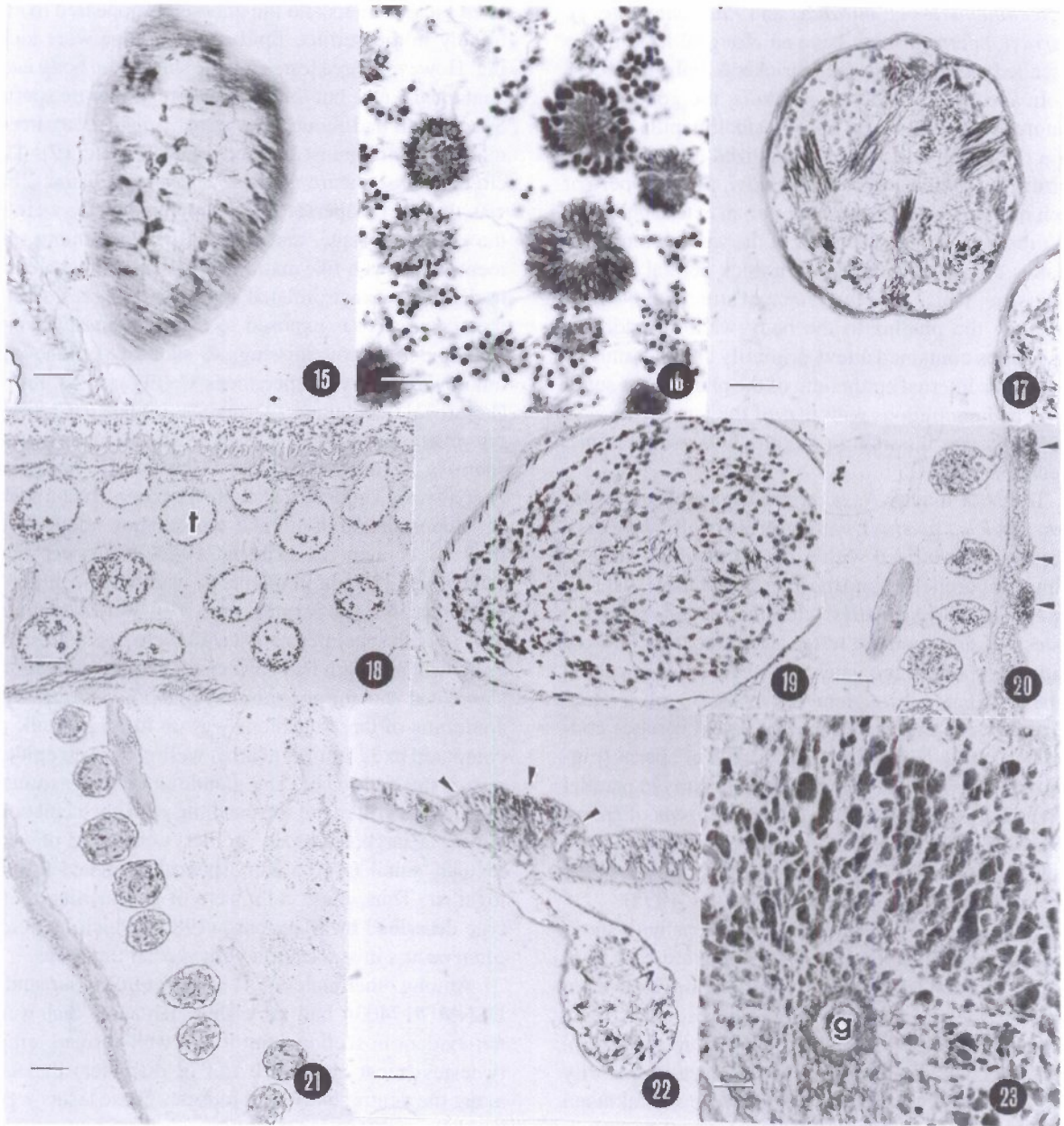
Figures 15–23. Photomicrographs of 8-μm histological sections, stained with modified Mallory procedure. *15. Nectonemertes cf. mirabilis* (USNM #174027); grazing section of testis along long axis of testis, showing muscular wall; scale = 25 μm. *16. Phallonemertes cf. murrayi* (USNM #174021); cytophores of early to advanced spermatid stages; scale = 20 μm. *17–23. Nectonemertes cf. mirabilis. 17.* (USNM #174028) mature testis with muscular wall, arrays of parallel mature sperm, and filamentous meshwork traversing lumen, and peripheral cells (early spermatids?); scale = 50 μm. *18.* (USNM #174025) longitudinal section of male predicted to be immature based on tentacle length; showing numerous, scattered, testes that were mostly empty and lined by non-muscular walls; scale = 100 μm. *19.* (USNM #174026) male predicted to be moderately mature; section through testis, showing sparsely developed muscle of wall and lumen filled with presumed spermatocytes or early spermatids that appear loosely connected to each other; scale = 25 μm. *20.* (USNM #174027) male predicted to be almost mature; linear array of testes with moderately muscular testes, sperm development primarily in the range of spermatids to mature sperm; epidermal papillae (arrow heads) surrounding gonopores; scale = 25 μm. *21.* (USNM #174028) male predicted to be most mature; linear array of testes with overall development of sperm more advanced than that in Figure 20; walls of testes with strong musculature; scale = 250 μm. *22.* (USNM #174027) testis connecting to epidermal, glandular epidermal papilla (arrowheads); scale = 100 μm. *23.* (USNM #174028) section parallel to body surface cutting transversely through glandular papilla, with testicular gonoduct at center; scale = 25 μm. Abbreviations: g, gonoduct; t, testis.

had mature sperm, with elongate heads and flagella, in the testes. Katherine Pearson (Evergreen State College, pers. comm.) reported a very long (8 cm) male with long tentacles but with empty testes.

The testes in the armaueriids lay ventromedial to the lateral nerve cords and opened laterally or sublaterally via a relatively long gonoduct that passed underneath the lateral nerve cord and upward along the body wall. Testicular musculature consisted of a weak, dispersed meshwork. Developing sperm occurred separately or in rosettes and appeared to be held in the lumen of the testis by a fibrous matrix. The sperm in all specimens examined were immature. In two specimens (USNM #174041 and #174049), in which most testes were packed with rosettes of presumed primary spermatocytes, a few testes had a single cluster of advanced spermatids with elongated nuclei (Figure 24). In addition, as noted earlier, three putative ovaries, each with small oocytes, were present much farther posteriorly in USNM #174041 (Figure 25), while two of the putative testes in this specimen contained anomalous oocytes (Figure 26). As mentioned earlier, two other specimens (USNM #174040 and #174043) also showed signs of hermaphroditism. One of the presumed testes in USNM #174043 included an early oocyte among what appeared to be an accumulation of primary spermatocytes (Figure 27).

Discussion

Seasonality and spawning

Among the species in this study, both *Phallonemertes cf. murrayi* and *Nectonemertes cf. mirabilis* appeared to show evidence of seasonal reproductive activity. The percentage of *P. cf. murrayi* specimens with visible gonads rose from 42% in February to 80% in June, before decreasing to 14.3% in September (Figure 2). At least some reproductive individuals were found during most months of the year (Figure 3), but in months when sampling was relatively thorough, the percentage of reproductive specimens was low except in early spring-early summer. *Nectonemertes cf. mirabilis* showed a maximum of reproductive activity in winter-early spring, but also maintained relatively high reproductive activity during the remainder of the year (Figures 2, 3). Clearly, more data are needed for poorly sampled segments of the year. It is not unusual for benthic nemerteans that have a clear seasonal spawning peak to have some reproductive individuals throughout

the year. For example, reproductive individuals of the benthic intertidal hoplonemerteans *Paranemertes peregrina* and *Pantinonemertes californiensis* were found in low numbers during much of the year (Roe, 1976, 1993).

Seasonality may manifest itself in life histories as the result of environmental pressures such as seasonal excess in energy input, e.g., spring plankton blooms, or because of adaptive advantages conferring increased reproductive success (Olive, 1992). Hypotheses of adaptive significance to seasonal spawning include 1) selective advantage for larvae, e.g., by insuring maximum food supply for larvae or increasing survivorship to predation by simultaneous production of large numbers of larvae; and 2) synchronized spawning maximizes fertilization rate and increases outbreeding or mixing of gametes (Olive, 1992). The California coast does have upwelling episodes and plankton blooms, and at least the number of pelagic nemertean specimens is much larger than, for example, off Hawaii (Roe & Norenburg, 1998). We expect that seasonality in the productivity of upper waters would be more noticeable at moderate deep-sea depths, where *P. cf. murrayi* and *N. cf. mirabilis* were found, than in deeper waters, although we have no evidence that either species responds to seasonally abundant foods. In fact, we do not know the diets of either species (see Feller et al., 1998). Seasonal spawning by the benthic nemertean *Paranemertes peregrina* ensures that settling larvae encounter the maximum availability of juveniles of the preferred prey of the adult nemerteans (Roe, 1976).

Based on current phylogenetic estimates (Sundberg, 1990; Norenburg, unpub. obs.) it seems reasonable to infer that pelagic nemerteans have 'direct' development, which is the plesiomorphic state for the rest of the known enoplans. However, the oocytes of pelagic polystiliferans are the largest known among nemerteans and much more yolky than those of benthic hoplonemerteans, such as *P. peregrina*. Yolk content of the oocytes should permit young of pelagic polystiliferans to grow substantially before needing food. We know nothing about predation on larvae or adults of pelagic nemerteans, but we do know that predation on adult benthic nemerteans is exceedingly rare (pers. obs.), apparently because of noxious compounds in the epidermal mucus (Kem, 1985). Overall, it seems unlikely that either synchronous or seasonal spawning would significantly reduce predation on larvae of *P. cf. murrayi* or *N. cf. mirabilis*. In contrast, the prolonged seasonal reproductive peak seen for these two species
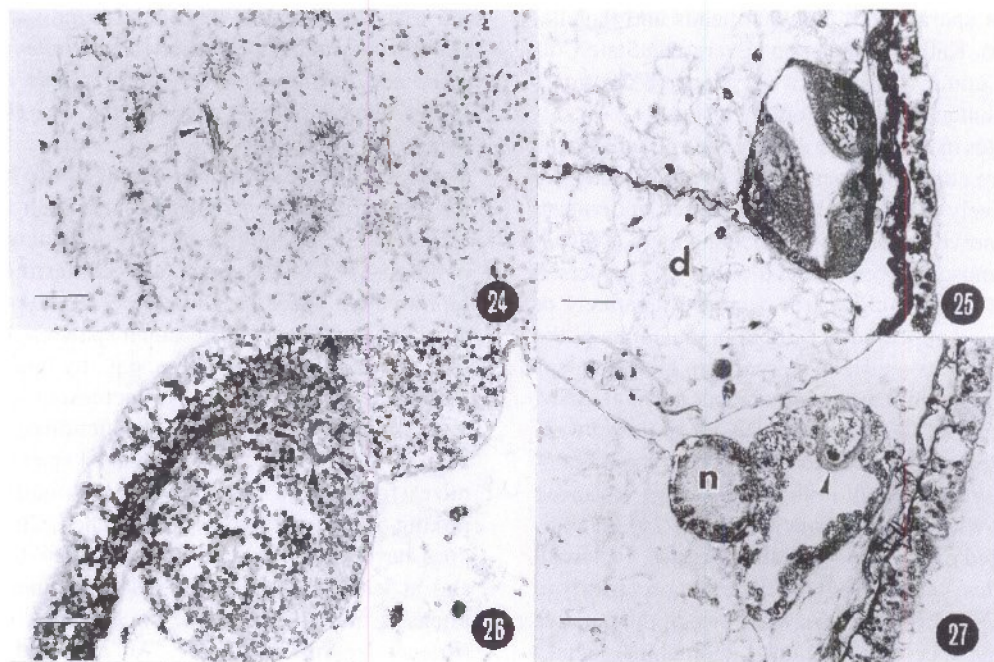
Figures 24–27. *Proarmaueria cf. pellucida*; photomicrographs of 8-μm histological sections, stained with modified Mallory procedure. *24*. (USNM #174049) testis with numerous individual spermatocytes or early spermatids, a few poorly organized cytophores with early spermatids, and very few clusters of mature sperm (arrowhead); scale = 50 μm. *25*. (USNM #174041) transverse section in posterior half of body, showing ovary with young oocytes and no nurse cells; scale = 50 μm. *26*. (USNM #174041) transverse section of body near posterior of cerebral ganglia, showing presumed testes filled with spermatocytes, and showing one anomalous oocyte (arrowheads); scale = 50 μm. *27*. (USNM #174043) transverse section of body near posterior of brain showing presumed immature testis with spermatocytes and two oocytes (one marked with arrow head) developing along wall; scale = 100 μm. Abbreviations: d, digestive tract; n, lateral nerve cord.

may result in increased outbreeding. Females probably spawn relatively few oocytes at a time, and each clutch probably is fertilized by a single male, unless multiple males swarmed around spawning females. Females may also be capable of spawning more than once in a season, but there is no direct evidence that they do. If they do in fact retain immature oocytes between spawning events, they could increase the number of males with which they mate.

Coe (1920) postulated that internal fertilization occurred in *P. cf. murrayi* and *N. cf. mirabilis*. More recently, sperm with elongated heads have been considered to be indicative of internal fertilization (Franzén, 1956, 1983), but there also may be a correlation between elongated sperm heads and large, yolky oocytes in some groups (Eckelbarger, 1994b), although this has not been found to be the case among nemerteans (Stricker & Folsom, 1998). We have seen no evidence of internal fertilization. The relatively few, but synchronous, mature oocytes produced by an individual does suggest that these are spawned simultaneously in response to some exogenous cue, per-

haps requiring close proximity of a sexually competent male. Pelagic nemerteans are common in some samples, but it is evident that even at their greatest abundance they may be very sparsely distributed. In such populations, reproductive seasonality should confer a selective advantage to species that increase the likelihood of encounters between reproductively competent individuals.

Sex ratios

There was a preponderance of females in the samples examined in this study, as well as in the remainder of our collections, which includes many species not addressed in this study (unpub. obs.). Coe (1954) reported a similar dominance of females. Perhaps the very large oocytes produced by pelagic polystiliferans take so much longer to produce than do mature sperm that at any one collecting time there are more obvious females than males, or there actually may be a selective skew toward females. Clearly, there was also a bias in our ability to recognize females. In contrast to mature

ovaries, which were fairly opaque and often orange or brown, mature testes typically were inconspicuous in preserved or living specimens. *Nectonemertes cf. mirabilis* was the only species in this study that approaches a 1:1 sex ratio. Males of *N. cf. mirabilis*, with their tentacles, are more easily recognized than are the males of other species, especially in the immature state. Males of *Phallonemertes cf. murrayi* are not evident until they are mature, which is when external phalli become apparent. Among our 13 sectioned armaueriids, three males and one hermaphroditic functional male were identified from the six initial 'no sex' specimens. Furthermore, two of the seven specimens initially identified as female were hermaphroditic. Among the remaining unsectioned armaueriids, five are 'no sex' and 12 are putative 'females.' We have identified 12 females and no males among 26 specimens of *Cuneonemertes elongata*. Perhaps the species is dimorphic and we have not yet recognized males. However, among a relatively large pool of putative species that are as of yet unidentified, we have not recognized a unique morphotype consisting only of males. Despite this, we believe that there is insufficient good data to either reject or affirm the hypothesis that sex ratios of most pelagic nemertean species approach 1:1.

Oocytes and ovarian morphology

Ovaries of the pelagic nemerteans studied here differ from those of benthic enoplans in (1) the presence of nurse cells, (2) a reduction in number of ovaries and (3) the sequential development of oocytes. In all species we studied here, except the pelagic monostiliferan, there is evidence, obtained with light microscopy, that putative nurse cells manufacture and pass vitellogenic products to developing oocytes via cytoplasmic bridges. This phenomenon had been documented for several species by Bürger (1909), Brinkmann (1917a) and Coe (1926), but interpreted in a variety of ways. We have found no evidence for the assertion of Brinkmann (1917a) that the nurse cells are abortive oocytes being phagocytized by the dominant oocyte, nor for Coe's (1926) conclusion that they are follicle cells being absorbed by the oocyte. Very small, young oocytes are easily distinguished by their large nuclei from the nurse cells, or so-called follicle cells. We also see no evidence for Coe's (1926) claim that less mature oocytes are aborted and absorbed by the definitive oocyte. The strongest indication that developing oocytes do not become nurse cells is the sequential production of oocytes from a proliferative zone near

the gonoduct in *Cuneonemertes elongata*. This is in sharp contrast to the situation in benthic monostiliferan hoplonemerteans, such as *Amphiporus lactifloreus*, for which oocyte fusion has been postulated based on the observation that each gonad produces a single, synchronous clutch of oocytes, but eventually yields only a single oocyte (e.g., Bierne, 1983).

By currently accepted definitions, the presence of intercellular cytoplasmic bridges suggests that the cells so associated with oocytes are nurse cells, and therefore should be derived from the germ line (Eckelbarger 1994a), but we have no data for the origin of these cells. In all of the Pelagica studied, vitellogenesis appears to have a significant heterosynthetic component, with vitellogenic products being transferred to oocytes via cytoplasmic bridges. It appears that at least some of the vitellogenic products continue to mature in the oocyte, but our material cannot demonstrate actual autosynthesis of yolk in the oocyte. However, in the early stages of vitellogenesis, even with light microscopy, the cytoplasm of oocytes and nurse cells had a lamellate sub-structure characteristic of rough endoplasmic reticulum – thus, we expect that there is autosynthesis and that yolk production in the worms examined here is of the 'mixed' type as defined by Schechtman (1955). The oocyte nucleus migrated to a cortical position farthest from the ovarian wall, and the synthesis of yolk, with its change in size and texture, as well as the migration and fusion of yolk vesicles occurred to some extent in the more mature oocytes of all species we studied except *Phallonemertes cf. murrayi*, where yolk remained as vesicles even in mature oocytes.

Heterosynthesis (especially via nurse cells) is often associated with rapid oocyte development and short intervals between reproductive episodes, whereas autosynthesis and mixed synthesis usually characterize slow oocyte growth and relatively long periods between reproductive episodes (Eckelbarger, 1994a). The pelagic nemerteans in this study have much larger oocytes than do their benthic relatives and live in such cold temperatures that vitellogenesis may be slow even with extensive heterosynthesis. Long-lived, shallow-water invertebrates usually have mechanisms for slow oocyte production consistent with predictable (e.g., seasonal) or continuous (even if low) food supplies and relatively stable environments (Eckelbarger, 1994a). These animals typically are long-lived iteroparous species adapted to stable environments and often are also characterized as being relatively 'K-selected,' and by relatively large body size, high food reserve storage, late sexual maturity, low brood fre-

quency and high fecundity (Eckelbarger, 1994a). The parameter 'size' could be based on volume, weight, or a combination of them. Although we provide both kinds of size data here (Tables 1 and 2), such data are not available for other nemerteans, thereby precluding strict comparisons. It is evident that the much more muscular benthic nemerteans have greater mass than the relatively gelatinous pelagic nemerteans of equivalent volume. We suspect that compared to benthic species the pelagic nemerteans in this study have a higher ratio of intestinal storage (= food reserve) of volume to number of sperm or oocytes, but not necessarily to oocyte volume. The larger specimens in this study, especially *P. cf. murrayi* and *N. cf. mirabilis*, appeared to have relatively large body size for nemerteans, most of it filled with digestive tract that nearly always was full of lipid food reserves. These nemerteans also appear to be well-adapted to their food supply. For these nemerteans a mixed yolk synthesis and extensive food reserves are in keeping with our expectation that these worms are relatively long-lived and iteroparous.

What happens to immature oocytes as some oocytes mature or are spawned remains unanswered but critical to understanding the life history of pelagic nemerteans. In this study, we saw no evidence of absorption of immature oocytes by other oocytes, nor did we observe evidence that younger oocytes were resorbed, but we cannot rule out either process. Apparent lysis of oocytes was evident in only one specimen of *P. cf. murrayi* (USNM #174020), which was scored as 'post-reproductive' because only a few large ovaries or mature oocytes were observed in the living animal. Lysing of at least one oocyte was evident in four of ten ovaries examined histologically. However, each ovary had 3–6 oocytes, each from 300 to 450 μm, which was about half the expected maximum diameter. Lysis in our specimens and in those of previous accounts could be as well a consequence of the collecting process as of reabsorbtion. In histological samples in the present study almost all ovaries with mature oocytes have one or more oocytes well on the way to maturation. The immature oocytes usually were between the gonopore and mature oocytes. Immature oocytes may be expelled at spawning, resorbed or phagocytized prior to spawning, or they may be retained and continue to mature after spawning of mature oocytes. Coe (1926) also noted that small 'aborted' ova may remain after the definitive ovum was spawned and wondered if these might form new ovaries. The expulsion of nearly mature oocytes along with mature oocytes could constitute a considerable waste of energy and would

increase the time required between spawnings. However, in the *P. cf. murrayi* with 1-mm oocytes there were indeed only a few very small oocytes in addition to the two or three large oocytes in each ovary. Thus, only about half as many oocytes remain as were present in ovaries with oocytes at 250–450 μm. In one of our mature *N. cf. mirabilis*, small, apparently new, ovaries appear to have been developing in the interdiverticular areas between successive mature ovaries. Thus, we still have only indirect evidence that number of oocytes per ovary may be reduced as some oocytes achieve maturity. However, it seems evident that multiple generations of oocytes are produced and that these worms are iteroparous.

We were unable to recognize a discrete epithelial lining to the ovary wall, as described for the monostiliferan *Amphiporus lactifloreus* by Rué (1973, in Bierne, 1983). Nor can we speculate on whether there is a relation between that and the complete or partial lining of 'nurse' cells seen in ovaries of our pelagic polystiliferans. There were clear species differences in the number and distribution of nurse cells. In the absence of a phylogeny for pelagic nemerteans it is not possible to determine to what extent these differences are determined by functional requirements of the individual species or by ancestry. However, an unidentified benthic, cratenemertid monostiliferan (belonging to the *bimaculatus* group) also appears to have a plaque of nurse cells attaching each oocyte to the ovarian wall (pers. obs.). In contrast, the pelagic monostiliferan examined in this study lacked nurse cells and is thus in accord with what appears to be the condition for most monostiliferans. The oogenesis described and figured by Coe (1939) for the terrestrial monostiliferans *Geonemertes pelaensis* Semper, 1863 and *Pantinonemertes* (formerly *Geonemertes*) *agricola* (Willemoes-Suhm, 1874) appears to be very similar to those of the pelagic nemerteans. We have been able to confirm the similarity directly in Coe's *P. agricola* material (USNM 51985, slide G2D). Coe (1939) considered the cytoplasmic bridges in the latter two species to be pseudopodia of the oocyte that extend to follicle cells lining the ovary. Surprisingly, he does not reference his previous, virtually identical descriptions for pelagic nemerteans. Riepen (1933) described a stalk-like connection between oocyte and ovarian wall in *Malacobdella grossa*, as did Gibson (1990) for *Pheroneonemertes dianae* Gibson, 1990, considering this stalk to be 'unique' among nemerteans. Both of these observations are consistent with Bierne & Rué's (1979, Figure 5) description of a mature oocyte of

A. lactifloreus apparently absorbing ('capture by suction') other oocytes. Thus, this stalk may be much more widespread among monostiliferans than previously recognized. Though simpler, the stalk may be a phylogenetic antecedent or derivative of the plaques of nurse cells observed in pelagic polystiliferans in this study. Because the presence of nurse cells, or at least heterosynthetic vitellogenesis, does not appear to be an autapomorphy of the Pelagica, the elaboration of nurse cells may be an adaptation to a condition that characterizes but is not necessarily an intrinsic property of the pelagic realm, for example, an unpredictable or scarce food supply. Whether or not the expression of this character within the Pelagica is apomorphic, plesiomorphic or homoplasious must await much more extensive phylogenetic analyses than currently available.

Testicular morphology

Compared to benthic species, males of pelagic nemerteans differ dramatically in their anterior placement and the smaller number of testes. Coe (1920) thought that the reduction in number of testes and sperm (as well as the reduction in the number of ovaries) should lead to 'conservation of gametes by adaptation for securing maximum fertilization.' One such adaptation could be internal fertilization, which Coe (1920) and Brinkmann (1912) suggested as possibilities for *N. cf. mirabilis* and *P. cf. murrayi*. Brinkmann (1917a) had perhaps the most interesting hypothesis for spawning by *Nectonemertes*. He suggested that males, which had been reported with tentacles wrapped around deep-sea fishing lines, wrap the long tentacles around a female and in this way strip her oocytes while discharging sperm.

We found sperm of both *P. cf. murrayi* and *N. cf. mirabilis* to be of the so-called modified type (Franzén, 1956), with an elongated head. Although this type of sperm often has been inferred to be associated with internal fertilization, other factors are now recognized to be important as well, e.g., (1) the release of oocytes and sperm in close proximity to each other, (2) an unusually yolky content of the oocyte (Franzén, 1983; Eckelbarger, 1994b) and/or (3) the presence of extracellular coats around the spawned oocytes (Stricker & Folsom, 1998). We found no evidence of internal fertilization in any of our female specimens. However, pelagic nemerteans do have large oocytes, e.g., 1 mm diameter in *P. cf. murrayi* (this study) and *N. cf. mirabilis* (Coe, 1926) and 2.5 mm in *Dinonemertes*

investigatoris (Brinkmann, 1917a). We agree with Coe (1926) that the small number of oocytes and sperm available should put a high premium on proximity between males and females during spawning. Multiple spawning periods, or iteroparity, also would be adaptive in these circumstances.

Coe (1926) described testes and ovaries as developing with the cavity forming first, which is very different from benthic nemerteans, where the gonadal cavity, if it forms at all, develops after clusters of germ cells start dividing (Coe, 1905; Riser, 1974). Our observations on the development of the tentacles and testes in *N. cf. mirabilis* coincide with those of Brinkmann (1917a, 1917b) in that individuals with short, stubby tentacles generally have small testes with an empty lumen and very little muscle surrounding the testis. As tentacles increase in length, testes enlarge and become invested with substantial muscle, and later stages of sperm development fill the testicular lumen. However, in our sequence of four specimens, we did not find the number of testes to increase with length of tentacles, as reported by Brinkmann (1917a). Also, we found one or two stages of spermatogenesis to predominate, rather than several as reported by Coe (1926). In specimen A, which was one of the two immature specimens with very short tentacles, three testes contained only a few, fully mature sperm, suggesting that this animal had already spawned. This specimen also had more testes and these were in much closer proximity to the body wall than in the three larger specimens of the sequence. However, the gonoducts ended blindly in the dermis. The simplest explanation might be that there had been a precocious development of some sperm. Alternatively, young specimens, with short tentacles, may be capable of precocious spawning, thereby uncoupling testis from tentacle development. Of course, the possibility of a cryptic species of *Nectonemertes* cannot be precluded. The presence of long tentacles on mature males does provide an opportunity to determine if males are iteroparous. If they are, one might expect to find a proportion of males with long tentacles to have testes with mostly early stages of spermatogenesis. Alternatively, they could have testes that are mostly filled with mature sperm but are still producing a continual supply of spermatocytes peripherally. This could be one implication of Coe's (1926) observation of a full range of spermatogenesis in at least one large male. Accordingly, testes in young males appeared to be highly synchronous, whereas they may become progressively less so in older males. At least one 8-cm long male had empty testes, but seemed otherwise healthy (K. Pear-

son, pers. comm.). Too few males of this species have been sectioned to understand fully spermatogenesis or to assess the likelihood of iteroparity. However, the size distribution seems to suggest that individuals of at least this species live more than a year.

Several authors have noted that the male gonopores of *Nectonemertes* often are associated with a papilla (Cravens & Heath, 1906; Brinkmann, 1917a; Coe & Ball, 1920), but none had observed the glandular epidermis of that papilla. Coe & Ball (1920) found enlarged gonoducts in association with the papillae in *Nectonemertes*, which they considered to be so unique that they referred to them as seminal vesicles. However, it was evident in two of our large specimens of *Nectonemertes* with mature testes that the gonoduct normally is not inflated and the dermis protrudes little. The dermis protruded as a papilla in only one of our specimens, which also was the one in the worst condition, and it seems evident that inflation of the gonoduct and formation of the papilla was an artifact (a herniation) resulting from pressure applied to the testes. The raised nature of the papillae in well-fixed specimens is due to the thickened glandular epidermis. The presence of bacillary glandular cells comprising these epidermal papillae provides support for the hypothesis that the males spawn in close proximity to females. The papillae could be used either to adhere to a female or to provide a viscous medium that inhibits rapid dilution and dispersal of sperm. Interestingly, Bürger (1909: 211, Figure VII: 3) described papillae consisting of a thickened cushion of epidermal glandular cells around the gonopores of male *Balaenemertes chuni* Bürger, 1909. This description is indistinguishable from our observations of the papilla in *Nectonemertes*. However, Bürger's (1909) observation was not mentioned in the subsequent papers by Brinkmann (1917a) or Coe (1926) in discussions of *Nectonemertes* papillae. The similarity of the papillae and the fact that males of both species bear similar cephalic tentacles suggests that the relationship of these two taxa, and the characters that 'diagnose' their respective families, should be carefully reconsidered.

Two armaueriid specimens, and perhaps a third, that we had independently concluded were highly likely to be *Proarmaueria pellucida*, show evidence of hermaphroditism. Coe (1926) describes hermaphroditic gonads for *P. pellucida*. However, our observations on additional specimens indicate that gonadal activity in this species (or group of species) is complex and cannot be interpreted simply. The data does support that *P.*

pellucida is to some extent a sequential hermaphrodite and, therefore, is likely to spawn more than once.

Conclusion

The polystiliferous hoplonemerteans examined in this study represented a wide variety of the principal pelagic nemertean taxa. The representatives appear to be similar to each other and different from most benthic hoplonemerteans in key parameters of their reproductive biology, especially vitellogenesis and the number of gametes produced. We infer that these pelagic nemerteans may be relatively long-lived and iteroparous. Spawning in these pelagic forms probably occurs with male and female in close proximity and results in relatively few, very large oocytes being shed at a time. *Nectonemertes* and *Phallonemertes* may have seasonal reproductive peaks, thereby increasing the chance of reproductively competent individuals meeting. *Proarmaueria pellucida* appears to increase its reproductive chances by adopting hermaphroditism. However, to obtain a better understanding of the life-history dynamics of even the most abundant and identifiable of these pelagic species still requires additional anatomical studies and sampling.

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