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ANATOMICAL FEATURES IN HISTOLOGICAL SECTIONS OF CRASSOSTREA VIRGINICA (Gmelin, 1791) AS AN AID IN MEASUREMENTS OF GONAD AREA FOR REPRODUCTIVE ASSESSMENT

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ABSTRACT The relationship between gonad area in transverse histological sections of the American oyster Crassostrea virginica (Gmelin 1790) and body location from which the section was cut was studied in specimens collected from four stations in the James River, Virginia in 1984 and 1986. Gonad area, expressed as percentage of total body area, increases in an antero-posterior direction; this requires use of sections from the same body location in comparisons between oysters. Approximate body locations, identified according to the anatomy and arrangement of the internal organs in the sections, were grouped into five types with similar gonad area percentages. One of those types is uniquely suitable for identification of a specific body location because it includes an easily recognizable pair of H-shaped structures corresponding to the posterior appendix of the anterior stomach caecum; furthermore, the recommended section type can be readily found on the whole oyster because it is located close to the junction of the gills and the labial palps. Gamete volume fraction (GVF) was positively correlated with percent gonad area (PGA) in most of the section types at three of the stations, suggesting that either measurement may be used to estimate the relative gonadal development in oysters. Differences between collection dates at the fourth station indicated what external factors may disrupt the correlation. It is suggested that gonad area measurements from a series of selected histological sections could be combined with gamete density measurements to estimate total gamete production by an oyster.

KEY WORDS: Crassostrea virginica, histological sections, gonad area, anatomy

INTRODUCTION

Gametogenesis in oysters produces an increase in the transverse thickness of the gonad layer located between the mantle and the digestive diverticula (Coe 1932, Galtsoff 1938, Loosanoff 1942). The reverse process ensues as oysters spawn. Measurements of gonad thickness complement estimates of gamete maturation as indicators of the extent to which gametogenesis has progressed in an oyster. Several investigators measured changes in gonad thickness in whole unmounted transverse sections of Crassostrea virginica (Gmelin 1790) throughout the reproductive cycle in different years and locations (Loosanoff and Engel 1940, Loosanoff and Nomejko 1951, Hopkins et al. 1953, Loosanoff 1965). Kennedy and Battle (1964) modified those early attempts by measuring the width of the gonad in histological transverse sections of C. virginica and relating it to total body width in the section. A transverse section in C. virginica is defined here as the plane perpendicular to the antero-posterior axis of the body. The antero-posterior axis passes through the mouth and the adductor muscle or the anus (Jackson 1890, as cited by Yonge 1953).

More recently, other investigators have quantified gonad development in terms of the planar area occupied by gonad tissue in histological sections of oysters and other bivalve molluscs (Table 1). Use of gonad area measurements on histological preparations for comparative purposes requires specification of the location in the animal’s body from which the section was taken because gonad area changes with body location (Galtsoff 1964, Loosanoff 1965, Iwantsch 1970, Perdue 1983). Serious difficulties in interpretation of the data can arise if the sections from different animals come from widely separated parts of the body.

Examination of histological transverse sections of C. virginica oysters collected in 1984 from the James River, Virginia, indicated that variations in the area occupied by the gonad tissue were related to differences in the anatomical features of the visceral organs. The primary objective of this investigation was to identify the relationship between gonad area and the anatomy and arrangement of organs in transverse sections from different parts of the body. The relationship between gonad area and gamete volume fraction was also investigated.

MATERIALS AND METHODS

Oysters used in this study were collected on July 11 and 12 and August 21, 1984, and on August 12, 1986, from oyster beds in the James River, Virginia, the southernmost tributary of the Chesapeake Bay (Fig. 1). Fifty oysters having a shell height greater than 40 mm were selected at random in 1984 from each of four stations sampled (Nansemond Ridge, Naseway Shoal, Wreck Shoal and Horsehead Rock). Shell height is defined here as the distance between the hinge end of the shell and the opposite end. Transverse cuts were made with a scalpel through the mid-visceral region of each oyster (without prior determination of a precise location for the cuts) to obtain a segment approximately 5–8 mm thick; the segment was then placed in Davidson’s AFA fixative and embedded in paraffin after

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TABLE 1.
Literature references to use of gonad area, width or thickness in studies of the reproductive development of bivalve molluscs. Thickness refers to measurements made directly on unmounted transverse sections. Width refers to thickness measurements made on mounted transverse sections.

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R = Text reference only; no measurements made.

dehydration and clearance through an alcohol-xylene series. Sections 6 μm-thick were cut, mounted and stained with Harris’ haematoxylin and eosin. No attempt was made to orient all segments in the same antero-posterior direction before embedding.

Serial sections were prepared from each of 10 oysters collected from the Wreck Shoal bed in August 1986. Five transverse segments were cut from each oyster. Four of the segments, located between the anterior end of the body (corresponding to the shell hinge location) and the pericardial cavity, were of approximately the same width in proportion to the size of the animal. The fifth segment, posterior to the pericardial cavity, was discarded. The cut between the first and second segments at the anterior end of the series was made at the junction of the labial palps and the gills. All segments were placed in the embedding containers with the posterior face up. Several 6-μm sections were cut from each of the segments, starting at the posterior end, usually at intervals of 0.3 or 0.6 mm. Additional sections were cut from some of the segments when needed for clarification of sequential changes in organ arrangement.

Sex of each oyster was recorded and reproductive condition of the gonad was assessed by estimation of the gamete volume fraction (GVF) using point-count volumetry (Chalkey 1943, Weibel et al. 1966, Bayne et al. 1978). Gonad and total body area of individual oysters were determined by projecting the section image (magnified 13 times) to a sheet of paper on a table and tracking separately the outlines of the body and the gonad with an electronic digitizing planimeter. The outline of the body was traced following the outer margin of the mantle and the inner margin of the epibranchial chambers at the posterior end. The outline of the gonad was traced following its outer and inner margins; interstitial spaces between follicles within the gonad were included only to the extent allowed by the precision of the planimeter’s tracking head. The proportion of the total body area occupied by the gonad (×100) was termed the percent gonad area (PGA).

The conventional characterization of the hinge area in oyster shells as dorsal (Galtssof 1964, Elston 1980) is disregarded here for specification of the directional relationships in the oyster body. Instead, we adopted the comparative anatomy approach advocated by Stasek (1963) and the attendant definition of the antero-posterior axis in C. virginica as the long axis passing through the mouth and the adductor muscle, or alternatively through the anus (Jackson 1890, cited by Yonge 1953; Fig. 2). The axis approximately perpendicular to the antero-posterior axis is then defined as the dorso-ventral axis. In accordance with Stasek’s proposal, the labial palps and most of the gills are part of the ventral half of the body and body areas in the opposite half of the body (including the rectum and the
promyal chamber) are part of the dorsal half. Based on these axis definitions, our sections were cut through the transverse plane.

Figures in Shaw and Battle (1957) and Galtsoff (1964) were used to identify visceral organs in the sections. Reference was made most frequently to the morphological descriptions of the stomach given by Shaw and Battle (1957) because they are more detailed than those of Galtsoff (1964). It was difficult to obtain a strict correspondence in details between the histological sections and Fig. 2; there-
fore, that figure was only used as a general guide to the gross anatomy of the viscera in *C. virginica*.

The serial sections from oysters collected in 1986 were arranged in an antero-posterior direction and grouped into 13 types. The sections of oysters from the July and August 1984 collections were compared with those 13 section types and assigned to the type to which they most closely corresponded. Only sections 2 through 10 were considered essential to this study and were the only ones subjected to analysis. The other sections were included in drawings and descriptions to serve as references. Among types 2 through 10 in the 1986 oysters, adjacent sections with little or no apparent difference in gonad area among them were grouped together as follows: 2–4, 5–6, 7–8, 9 and 10. Mean gonad areas of these groups were compared using one-way ANOVA and Scheffé’s multiple contrast test (Zar 1984). Sections from the 1984 collections were also combined into the same type groups for statistical analysis of gonad area and GVF measurements.

Individual plots of GVF against PGA in male and female oysters collected in 1984, separated by date of collection and histological section type, were drawn on the same figure for each station (Figs. 6 and 7). Those figures should not be viewed as a unit but as a composite of up to ten separate sets of data (five section-type groups and two sampling dates). The strength of the relationship between GVF and PGA in these data sets was analyzed using Pearson product-moment correlation analysis (Zar 1984). July and August data were combined for three of the stations; however, the data for the two months were analyzed separately for Horsehead Rock because the plots for female oysters in July were drastically different from those in August.

**RESULTS**

The anatomic elements used to identify the relative location of a section in the antero-posterior axis of the oyster were the stomach, diverticula, intestinal branches and posterior appendix of the anterior caecum of the stomach (Figs. 2 and 3). The caecum appendix is of major importance in this study. Descriptions of the appendix presented here are based on the work of Shaw and Battle (1957). The appendix appears as a pair of H-shaped structures on the left ventral quadrant in transverse sections of oysters taken near the junction of the gills and the labial palps when the section is viewed in an antero-posterior direction (Fig. 3). The appendix is a compressed band-like structure that coils upon itself one-and-one-quarter turns. The paired H-shaped structures represent the juxtaposed sides of the sectioned coil and their arms are formed by invagination into typhlosoles of the appendix walls. Occasionally, three of the H-
The diverticula surround the stomach except at the dorsal end of the section; the area covered by the diverticula on the right side is over twice as great as that on the left side. The ascending branch of the intestine is displaced toward the right dorsally, immediately inward of the gonad, and is separated from the stomach by connective tissue. The descending branch of the intestine is located on the left side of the body at the ventral end, between the diverticula and the gonad.

**Section type 2:** Located just posterior to, or at, the gills-palps junction. The ventral end is occupied by the gills, although parts of the palps may also be present. The gonad, if present, may extend around the whole body but may also be absent ventrally. The stomach is elongated and the two H-shaped structures are present in a partially distorted form. The diverticula extend around the ventral end but the area occupied on the left side is narrower than in section type 1. Diverticula are absent between the H-shaped structures and between the stomach and its appendix on the left side.

**Section type 3:** Located posterior to but still close to the palps-gills junction. The ventral part of the section may be occupied exclusively by the gills or may also include parts of the palps. The gonad may or may not extend completely around the ventral end of the section. The stomach is elongated with one major and several smaller ducts leading into the right mass of the diverticula. The two H-shaped structures are separate but may be distorted. The diverticula are almost completely absent from the left side but project slightly leftward between the stomach and the H-shaped structures. There is little change in arrangement of intestine sections.

**Section type 4:** Located posterior to the palps-gills junction. Very similar to Section 3 except that the gonad extends fully around the ventral end of the section and the ventral end of the body is occupied exclusively by the gills. H-shaped structures are clearly formed. Connection between the stomach and ducts to right side diverticula is absent. Diverticula extend into the left side between the stomach and the H-shaped structures. There is little change in location of the intestine sections.

**Section type 5:** Stomach appears relatively wider than in previous sections. There is a major duct from stomach to diverticula ventrally and other smaller ducts scattered throughout, particularly on the right side. The H-shaped structures appear fused together, marking the posterior end of the caecum appendix. There is little change in location of the intestine sections. The distance between section types 1 and 5 was approximately 1.2 mm in the paraffin-embedded segment.

**Section type 6:** Stomach still relatively wide with a major duct to the diverticula extending rightward and ventrally; a partly-connected large duct is directed leftward and anteriad. A few other ducts are visible. The H-shaped structures are no longer present. Diverticula occupy whole
right side but are absent from most of the left dorsal quadrant. There is little change in location of the intestine branches.

Section type 7: Stomach is rounded. There is a large detached stomach duct or appendix toward the left side and ventrad; several smaller ducts radiate in the same direction. At this point the central cavity, which corresponded to the stomach in the preceding sections, actually includes part of the mid-gut toward the right side and dorsal end of the body and part of the crystalline style sac toward the left side and dorsal end. These organs can be separated by the morphology of their epithelial lining (Shaw and Battle 1957). From here on we will refer to this cavity as the central cavity. The diverticula are evenly distributed from right to left with the widest part on the ventral end. There is little change in location of the intestine branches.

Section type 8: Similar to section type 7 but central cavity is slightly elongated diagonally. Remnants of major stomach duct or appendix from section type 7 are present. The central cavity includes the style sac at the left and dorsal end and the mid-gut at the right and ventral end. The area occupied by the diverticula on the right side is significantly smaller than in type 7.

Section type 9: Central cavity elongated and narrower than in previous sections and no longer includes the stomach. The dorsal one-third is part of the style sac and the small crescent-shaped projection at the ventral end is part of the mid-gut. The section between the style sac and the mid-gut is bounded by two intestinal typhlosoles (Shaw and Battle 1957). A major disconnected stomach duct to the diverticula appears on the right side and several smaller ducts are scattered throughout the diverticula. The diverticula on the right and left sides are separated from each other; the area occupied on the left side is several times greater than that on the right side.

Section type 10: Most of the central cavity consists of the style sac; the narrow projection leftward is the only part occupied by the mid-gut. The diverticula occupy only the
left side of the body. The descending branch of the intestine is located almost half-way along the left side and the ascending branch appears distorted and is located on the right side.

Section type 11: There is little change in position or morphology of the central cavity. The area occupied by the diverticula on the left side is substantially smaller than in section type 10. The descending branch of the intestine is located half-way along the right side and opposite the ascending branch. This displacement of the intestinal branches in section types 10 and 11 is the result of their being closer to each other in the vicinity of their cross-over point at the posterior end of the diverticula (see Fig. 2).

Section type 12: Central cavity resembles a mushroom on its side. Only the tip of the narrow projection to the left of the section is part of the mid-gut; the sides of the projection are made up of the two intestinal typhlosoles mentioned under section type 9. The wider part of the cavity is the style sac. The descending branch of the intestine is near the dorsal end on the left side. Gonad, when present, separates almost completely the descending branch of the intestine from the pericardial cavity. Ascending branch of intestine still located half-way along the right side. No diverticula present.

Section type 13: Identical to section type 12 except that the descending branch of the intestine is completely surrounded by the gonad (when present) near the dorsal end. The gonads are distinguishable as a small left gonad posteriorly dorsally and a much larger right gonad ventrally, connected by a short neck. The right gonad surrounds the central cavity and the ascending branch of the intestine.

There is a progressive increase in gonad width (and hence in area) in the serial sections in an antero-posterior direction (Fig. 4). PGA means for grouped adjacent section types in three oysters collected in 1986 (Fig. 5) were compared using one-way ANOVA and Scheffe's multiple contrast test after arcsine transformation (Zar 1984). There was no evidence of a difference between section types 2–4 and 5–6 nor between section types 5–6 and 7–8 (P > 0.05); however, mean PGA for section types 2–4 was significantly smaller (P ≤ 0.05) than for section types 7–8 and 10. The mean for section types 7–8 was significantly smaller than the mean for type 10 (P ≤ 0.05, Fig. 5). Lack of sufficient data for section type 9 prevented adequate comparisons between that type and most others; it was designated a separate type because gonad area appeared intermediate between types 7–8 and 10.

The highest PGA values among the oysters collected in 1984 were recorded in the sections that corresponded to types 9 and 10 regardless of sex, station or collection date (Figs. 6 and 7). Although those two section types were not always associated with the highest PGA values, they accounted for most of the PGA values greater than 30 percent. Less than 25% of the sections in types 7–8 showed PGA values higher than 30. Almost every oyster in types 2–4 and 5–6 had PGA values under 30.

 Gonad Volume Fraction and PGA were positively correlated with r values ≥ 0.6 and P ≤ 0.05 in 20 of 28 tests on the combined data for July and August at three of the four stations (Nansemond Ridge, Naseway Shoal and Wreck Shoal; Table 2). The relationship between GVF and PGA appeared different at Horsehead Rock. Plots of the relationship for female oysters at Horsehead Rock in July were similar to those at the other three stations and high positive correlations were found for three of the five section types (Fig. 6, Table 2). In August, however, the data were radically different; almost all GVF values were higher than 0.8 regardless of the PGA value and only one of four comparisons showed a correlation coefficient higher than 0.6. Most of the male oysters also showed GVF values higher than 0.8 regardless of PGA value at Horsehead Rock in July and August and only two of seven computed r values were higher than 0.6 at P ≤ 0.05 (Fig. 7, Table 2). Most of the individual component plots in Figs. 6 and 7 appeared to be linear on visual examination while a few appeared to be curvilinear. The data, however, were insufficient for an accurate determination of the nature of the relationship.

Figure 5. Individual measurements, mean and 95% confidence interval of percent gonad area in serial sections from each of three Wreck Shoal oysters (RM 3–5) collected in August 1986; sections combined into five groups of adjacent types as identified in Figure 5.
DISCUSSION

Seasonal reproductive development in bivalve molluscs has been studied primarily in terms of descriptive characterization of gametogenic stages (for example, Kennedy and Battle 1964, Brousseau 1978, 1984, Kennedy and Krantz 1982, Mann 1982, Manzi et al. 1985, Dudgeon and Morton 1983). Quantitative measurements, however, have been used frequently. Such measurements include determination of gamete volume fraction (as in Bayne et al. 1978, Newell et al. 1982, Sundet and Lee 1984, Pipe 1985, Kennedy 1986), gonad area (Table 1), and gamete number and size (as in Keck et al. 1975, Brousseau 1978, Lannan 1980, Barber and Blake 1983, Wilson and Simons 1985, Gustafson et al. 1987). Gravimetric gonad measurements, which follow the increase in weight as the gonad matures and the decrease that occurs on spawning, have also been used but almost exclusively with pectinid species, whose gonad can be separated from the rest of the body (as in Sastry 1966, Ansell 1974, Shalee 1981, MacDonald and Bourne 1987); however, they have also been used a few times for other species (Fox and Coe 1943, Griffiths 1977, Thompson 1979, Bayne and Worrall 1980, Peterson and Fegley 1986).

Quantitative estimates are preferable to descriptive stage characterization because they eliminate the subjectivity and semantic problems associated with the descriptions (Brousseau 1978) and tend to provide ecologically meaningful information. Nevertheless, several investigators have used stage characterizations as supplementary information to quantitative measurements (Keck et al. 1975, Tinsman et al. 1976, Brousseau 1983, Dinamani 1987). Histological examinations that include quantitative measurements of gonadal material should be a part of any study on reproduction of bivalve molluscs because they provide detailed information not available otherwise, as was suggested by Benninger (1987) and MacDonald and Bourne (1987).

The positive correlation between gamete volume fraction and percent gonad area found at three of the four sta-
tions on the two collection dates indicates that they are comparable estimates of the same parameter. Apparently, either may be used to monitor progression of reproductive development in bivalves; however, the discrepancies in correlation of GVF and PGA found at Horsehead Rock between females collected in July and August suggest that the relationship between the two may change under some circumstances. These discrepancies may indicate that none of the oysters at Horsehead Rock had spawned at the time of collection in August, possibly due to extremely low water salinities recorded during that time period, but those facts could not be verified. Nevertheless, plots of GVF against PGA may call attention to factors affecting the reproductive biology of oysters and other bivalves.

Gonad volume fraction is a precise indicator of seasonal changes in gametogenic development and incidence of spawning in bivalve populations because it accounts for the continuity in stages of development (Newell et al. 1982). It does not, however, provide a gamete count (Chalkey 1943), nor does it give an estimate of the quantity of gonadal material produced (Hilbish and Zimmerman 1988). Bayne et al. (1982) and Lowe and Pipe (1987), however, combined the volume of the mantle in Mytilus edulis with GVF measurements to obtain estimates of total volume of gametes and Hilbish and Zimmerman (1988) estimated the proportional gamete weight of an individual M. edulis using body and mantle weights and GVF measurements. Yankson (1986) also used the change in GVF due to spawning as an estimate of fecundity and spawning efficiency in two species of Cerastoderma.

Gonad area measurements, on the other hand, may be used to estimate the quantity of gonadal material produced by bivalve molluscs, such as C. virginica, whose gonad weight or volume cannot be measured directly. They could be combined with measurements of areal density of gametes on a set of serial sections and integrated into an acceptable estimate of total gamete production. Brousseau (1978) combined visceral displacement volume, oocyte numbers per unit volume, and relative gonad size in serial sections to estimate fecundity in Mya arenaria. Tinsman et
TABLE 2.
Correlation coefficient (r) and probability values (P) for the relationship between Gamete Volume Fraction and Percent Gonad Area in histological sections from different parts of the body (as indicated by section types) of oysters collected in July and August, 1984, at four stations in the James River, Virginia; July and August data combined for three of the stations. Section types explained in text.

\[ n = \text{number of sections.} \]

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<thead>
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al. (1976) and Dinamani (1987) computed indices based on gonad area and follicle coverage per unit area for C. virginica and Crassostrea gigas, respectively. It may also be possible to relate such estimates to whole animal weight to arrive at gravimetric estimates of gonadal production.

Gonad area measurements involving bivalve molluscs may require careful attention to the body location from which histological sections are prepared, depending on the objectives of the study. Body locations have been identified in previous publications involving a variety of bivalve species only in general terms (usually 'the mid-ventral region') and without any explanation of the degree of correspondence between individual transverse sections from different individuals. This presents no serious problem where gametogenesis is uniform throughout the gonad and where the only interest is establishment of the seasonal cycle or spawning incidence; it can, however, lead to serious difficulties in investigations dealing with gonad area comparisons. Perdue (1983) studied the relationship between gonad area and body location in C. gigas and concluded that a section from within 8-10 mm of the base of the labial palps would result in similar gonad area measurements. Our observations, however, allow a more precise specification of the section to be used for gonad area measurements in terms of the anatomy and arrangement of the internal organs included in the section.

The description of changes in morphology and arrangement of the internal organs in a series of transverse sections of C. virginica presented here permits standardization of the location from which sections are taken in a particular study or in a series of studies with that species. Similar descriptions for other species would be useful in the same manner. Usefulness of the sections and descriptions does not lie in precise identification of body locations but in allowing recognition of the features of specific sections so that only similar sections are used in comparative studies of gonad area. The exact arrangement of organs in the sections illustrated here may not be identical to that in sections prepared by others; variations will occur at body locations intermediate to those shown here and distortions due to shrinkage during fixation are possible. Similarities between other sections and our illustrations, however, will be found readily.

Generation of oyster sections with similar features can
be simplified considerably if efforts are directed toward obtaining sections showing the organ configurations illustrated by section types 2–4. These sections are characterized by the easily recognizable H-shaped structures corresponding to the posterior appendix of the stomach caecum. The possibility exists, however, that some of those sections (especially those very close to the gills-palps junction) may not show a gonad that completely surrounds the visceral mass because part of the ventral side is occupied by the labial palps and they appear to preclude the presence of the gonad at those sites. The presence of a fully circumferential gonad is required for gonad area measurements to represent the maximum obtainable for that section of the body. Therefore, a section similar to type 4, in which the ventral side is occupied exclusively by the gills should be sought. Section type 4 can be located easily because it is found close to the gills-palps junction, a distinct gross feature of the oyster anatomy. A section similar to type 4 is obtainable by scanning the sequence of sections generated with the microtome, it should be found within 1–2 mm posterior to the gills-palps junction in the paraffin-embedded oyster segment. The ease with which such a readily identifiable section can be found in Crassostrea virginica should encourage its use as a standard for studies of reproductive development involving gonad area comparisons in that species.

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