Age, Growth, Size at Sexual Maturity and Reproductive Biology of Channeled Whelk, *Busycotypus canaliculatus,* in the U.S. Mid-Atlantic

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VIRGINIA FISHERY RESOURCE GRANT PROGRAM

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Final Report for the Virginia Fishery Resource Grant Program Project 2009-12

Abstract

The channeled whelk, *Busycotypus canaliculatus*, was sampled from three in-shore commercially harvested resource areas in the US Mid-Atlantic: off Ocean City, Maryland (OC); Eastern Shore of Virginia (ES); and Virginia Beach, Virginia (VB). The largest whelk measured 230 mm shell length (SL) and was recorded from OC. Mean SL was largest in OC site (158.1 mm), followed by ES (137.6 mm), then VB (132.4 mm). Both VB and ES populations showed a unimodal length-frequency distribution with the single peak at shell length less than minimum landing size (MLS) for those regions, while OC population showed a bimodal (two peaks) distribution with the smaller peak at shell length less than the MLS for that region and larger peak at shell length greater than the MLS. Brody growth rate coefficient (k) was higher in males than females from all areas, and highest for both sexes in VB (Male 0.245, Female 0.155), followed by ES (Male 0.220, Female 0.151), then OC (Male 0.112, Female 0.100). The median size (SL) at 50% mature varied between resource area and sex. Males from ES and VB reached maturity at a smaller mean size (123 mm and 121 mm, respectively) than OC (134 mm). Females from VB reached maturity at a smaller size (148.9 mm) than ES (157.6 mm) and OC (158.6 mm). Recruitment to the fishery was estimated to occur at ~6 years for VB and ~ 7-8 years for ES and OC calculated from length at age estimates from the von Bertalanffy growth model. Under current MLS for each area, whelk harvested from VB recruited into the fishery at a much younger age to those from OC. The probability of females reaching MLS prior to sexual maturity is quite low given current MLS.

Introduction

Two species of large, predatory marine gastropods account for the large majority of whelk harvested in the US Mid-Atlantic, channeled whelk (*Busycotypus canaliculatus*), and the closely related knobbed whelk (*Busycon carica*), both members of the Melongenidae family found along the Atlantic coast of North America from Cape Cod, Massachusetts to Cape Canaveral, Florida (Abbott 1974, Edwards and Harasewych 1988). In Virginia, the knobbed whelk is largely fished within inshore estuarine habitats and channeled whelk within near-and off-shore marine habitats, though mixing is observed inshore along shallow waters of continental shelf. Channeled whelks are the focus of commercial fisheries throughout their range (Davis and Sisson 1988, DiCosimo 1988, Bruce 2006, Fisher and Fisher 2006, Eversole *et al.* 2008). In Virginia, the majority of channeled whelk is harvested by baited traps (pots) along the Virginia coast within near-shore state (within 3 miles) and off-shore (> 3 miles) federal waters. Whelk meats are removed from shells for distribution to various markets.

In the 1980s, the channeled whelk fishery in Virginia began as an unregulated fishery with fishermen working in state waters. Biological assessment information for the more accessible knobbed whelk was used for general whelk fishery guidelines. In the early 1990s experimental fishing permits were issued by Virginia's fisheries regulatory agency, Virginia Marine Resource Commission (VMRC) for pot fishing whelk (conch) with the objective to generate reproductive biology and fisheries stock information for the more marketable channeled whelk in state and federal waters. However, poor and inconsistent data was generated from these efforts and little information specific to *B. canaliculatus* was obtained. The channeled whelk fishery expanded within Virginia and throughout the Mid-Atlantic as fishermen diversified their efforts. Effort peaked in Virginia in the late 1990s, decreased in the early 2000s, and increased again in 2010 as shellstock (whole, live whelk) prices increased to levels 3 times higher than prices in the 1990s. In 2000, the Virginia conch pot fishery consisted of approximately 50 boats and 150 fishermen which supported processing, distribution and bait industries generating a total estimated economic value to Virginia in excess of $42 M (Manion, West, and Unsworth 2000). Each state with a developing whelk fishery enacted separate whelk management plans based on the data-poor Virginia whelk fishery, which used knobbed whelk biological assessment information. Further, states imposed different minimum landing size (MLS, shell length) requirements. Currently the MLS for Mid-Atlantic States are 5" (127 mm) in New Jersey, 6" (152.4 mm) in Maryland and Delaware, and 5.5" (139.7 mm) in Virginia. With declining size of individual landed whelk observed within various resource areas in the Mid-Atlantic, coupled with increased fishermen complaints

of undersize whelk being harvested and legally landed by fishermen from adjoining states, basic specie biological information governing the fishery was questioned by fishermen. Questions posed by industry relative to resource management centered on age/size at sexual maturity and relevancy of multiple MLS within the Mid-Atlantic whelk fishery. Upon review by lead author, it was found that little biological assessment information was available specific for *Busycotypus canaliculatus*.

With only modest amounts of published information available on *B. canaliculatus* biological assessment, research was initiated in 2009 within various resource areas in the Mid-Atlantic on channeled whelk aging, reproduction, growth through maturity, age at maturity, and population structure. Harding (2011) described larval to juvenile stage of growth of *B. canaliculatus*, but no information is available on age at sexual maturity, and growth rates through recruitment to the fishery for this species. Peemoeller and Stevens (2013) has since described age, size, and sexual maturity of *Busycotypus canaliculatus* in Buzzards Bay, Massachusetts, providing the first published biological assessment of this species.

The research findings presented here focuses on the *Busycotypus canaliculatus* fishery in the Mid-Atlantic with temporal and spatial variations in size and age structure of coastal populations as well as size at sexual maturity to assess the suitability of current minimum landing size regulations governing these populations. The information presented should provide building blocks for assessment within the currently data poor channeled whelk stock.

Aging Whelk

In recent years, aging gastropods has centered around interpreting marks deposited on animals non-shell hard structures, as with otoliths in fish, assuming discernible increments of growth associated with rapid and slow growing periods. The operculum, the corneous plate that closes the opening of the shell when animal is retracted, and the statolith, calcium carbonate structures (2 per animal) within gastropod sense organ for equilibrium (statocyst), are such structures. Numerous studies have validated the occurrence and use of annular stria (dark rings) formed on the operculum of gastropod species including *Buccinum undatum* (Santarelli and Gros, 1985; Kideys, 1996), *Buccinum isaotakii* (Ilano, *et al.*, 2004), *Babylonia japonica* (Kubo and Kondo, 1953), *Neptunea arthritica* (Miranda et el., 2008), *Coralliophila violacea* (Chen and Soong 2002), *Neptunea antiqua* (Richardson *et al.*, 2005b; Power and Keegan, 2001), and *Busycon carica* (Kraeuter *et al.*, 1989) with the assumption that stria are formed annually during periods of slow growth. However, no reported age estimate work has been done on *B. canaliculatus* prior to this study.

Statoliths have been used in estimating age through interpretation of growth increments (rings) radiating from a central nucleus (Barroso *et al.* 2005). Statoliths have been used in larval (Bell 1984, Grana-Raffucci and Appeldoorn 1997, Zacherl *et al.* 2003) and adult (Barroso *et al.* 2005b, Richardson *et al.* 2005a, 2005b) gastropods to better understand early life history and growth parameters. Annual ring periodicity was validated in the red whelk, *Neptunea antiqua* (Richardson *et al.* 2005b) and netted whelk, *Nassarius reticulatus* (Chatzinikolaou and Richardson, 2007) using laboratory-reared larvae and juveniles of known age, thus providing an accurate method of age estimation in gastropods.

This study explores aging techniques validated in other gastropod species to estimate age for *B. canaliculatus*.

Methods

Samples of *B. canaliculatus* were collected from 3 different near-shore resource areas in the Mid-Atlantic region (Figure 1); Maryland/Delaware boarder (OC), Eastern Shore of Virginia outside Hog Island Bay (ES), and Sand-
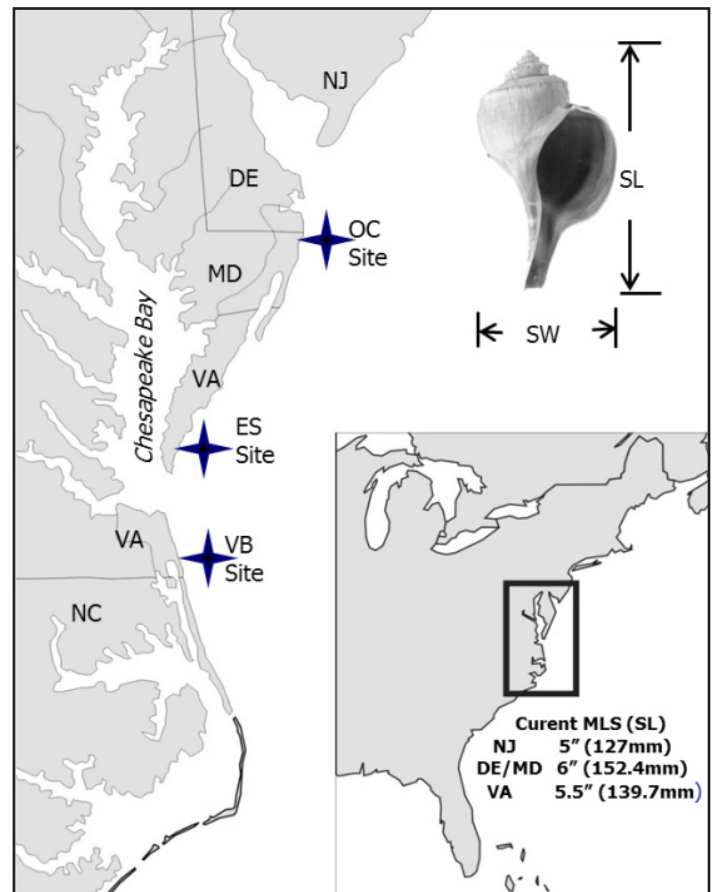

Figure 1. Whelk resource areas sampled; Maryland/Delaware boarder (OC), Eastern Shore of Virginia outside Hog Island Bay (ES), and off southern Virginia Beach, Virginia (VB). Whelk measurements; shell length (SL), shell width (SW). Channeled whelk current (2013) minimal landing size (MLS) for Mid-Atlantic States.

Figure 2. *Wood lathed traps (pots) used for commercial harvesting of whelk (conch) in the Mid-Atlantic.*

bridge, Virginia, south of Virginia Beach, Virginia (VB) during routine commercial fishing trips using traditional open-top, wood lathed whelk (conch) traps (58 x 58 x 28 cm) baited with horseshoe crab sections placed in bait bags (Figure 2). Sampling sites represented near-shore whelk resource areas [OC, 46-50 ft (14-16 m); ES, 28-35 ft (8.5-10.5 m); VB, 30-35 ft (9-10.5 m) depth range] targeted for commercial exploitation, but does not represent the full range of the fishery within Virginia. Commercial whelk trap design with lathes spaced 2.5 cm apart prevented retention of small whelk ~ <82 mm shell length (SL), thus juvenile whelk were not largely represented in sampling. Bottom temperature readings were made by inserting probe thermometer directly into foot of whelk immediately upon landing on–board. Initial collection from all 3 sites was conducted in November 2009 at bottom water temperatures of 12.5^0C (OC), 15^0C (ES), and 16.2^0C (VB). To better understand the population structure and reproductive potential of *B. canaliculatus* within these resource areas, additional limited seasonal sampling was performed from May 2010 through January 2011 within each resource area. Collected whelk was transported to VIMS for processing. A sub-sample of 20-57 fresh, live whelk from each sample collected were processed for reproductive histological analysis with the remaining whelk frozen whole at -2^0C for later analysis. All whole whelks were weighed for total weight (TW) and measured for shell length (SL; spire apex to the edge of siphonal canal) and shell width (SW; maximum straight-line distance measured across the shell perpendicular to shells length (coiling) axis) to nearest 1 mm (Figure 1) on a metric measuring board. Only collected whelk with in-tacked, unbroken siphonal canals was measured. In addition to commercially collected whelk samples, 2 live small (58.2 and 62.9 mm SL) *B. canaliculatus* whelk were collected from the beach in OC and used to aid in age estimation since early life history growth parameters for this species is lacking.

Fresh samples used for histology were processed by first removing bodies from shell: notching a hole in shell at the 2nd whorl up from aperture (ventral side) and in-line with coiling axis, then severing the columellar muscle from columella of shell, and gently pulling body from shell. Frozen samples were thawed at room temperature for 3-4 hrs and bodies removed from shell by first inserting finger up through aperture along columella and detaching columellar muscle from shell, then gently pulling body from shell with a twisting motion in the same direction of whorl.

Sex was determined by the presence or absence of a penis. Penis measurement (nearest 0.1 mm) was taken from the resting penis state (folded at right angle) from tip of penis to outside corner of fold (Figure 3). Penis index (PI = penis length/shell length) values were generated and used
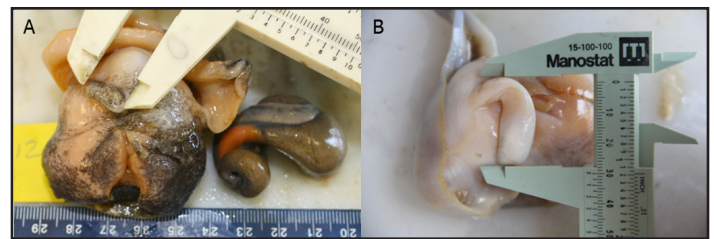

Figure 3. B. canaliculatus *penis (p) measurement; (A) fresh sample, (B) frozen-thawed sample.*

in evaluating male sexual maturity. Since size at maturity data is lacking for *B. canaliculatus*, and penis length was recorded from a relaxed penis state, which differs from studies in other whelk species, determining the relationship of penis size to SL at sexual maturity for *B. canaliculatus* was explored.

Total wet body weight to nearest 0.1g, and digestive gland and gonad weight to nearest 0.01g were recorded for gonadal somatic index (GSI) calculations.

GSI= gonad weight/somatic weight x 100

Where somatic weight was total wet body weight minus gonad weight.

Yield was calculated as body flesh weight minus gonad and digestive gland, but includes oviduct in females (though removed in commercial processing) and penis in males (which is part of commercially processed product). Whelk flesh yield was examined following research protocol determined for this study in which a sub-sample of whelk was necropsied live (fresh, non-frozen) for histological analysis while the larger portion of whelk were frozen for subsequent analyses. With concern over dehydration of frozen sample meat weights post freeze/thaw providing inaccurate yield information, yield was calculated from both frozen/thawed whelk and fresh, non-frozen whelk. Yield from fresh, non-frozen whelk may more accurately reflect

harvested and processed whelk product identity within the whelk fishery.

Macroscopic assessments were made on secondary sex organs to aid sexual maturity evaluations. Gonad size, color, and texture were noted in both sexes (Figure 4).
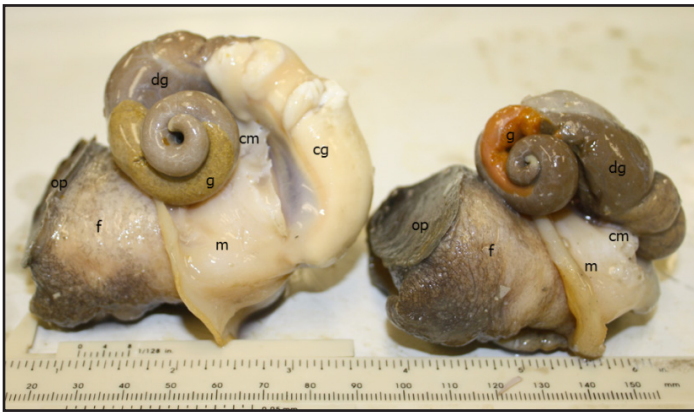

Figure 4. Female (left) and male (right) B. canaliculatus with shells removed. Cg=capsule gland, cm=columellar muscle, dg=digestive gland, f=foot, g=gonad, op=operculum, m=mantle.

In males, the visceral section of vas deferens (VD) was observed for coiling (convolution) and presence of sperm, indicating biological maturity. In large males, the anterior section of VD (prostate to penis) running across the dorsal body surface to the base of penis was routinely observed protruding from immediately below epithelial layer and noted as a possible characteristic of maturity. In females, capsule gland (CG) size, color, and condition (flaccid, plump) were recorded corresponding to maturity.

Aging

Age estimation in this study was obtained using both, the whelk operculum and paired statoliths. Two readers independently assessed age without knowledge of whelk shell length (SL). A McNemar test of symmetry about the main diagonal was used to test the null hypothesis that the readers were interchangeable against the alternative that there were systematic differences between the two readers (Evans and Hoenig 1998). Comparison between aging methods was evaluated.

Operculum aging

Opercula were detached from whelk foot (Figure 5), cleaned of attached tissue, placed flat under weighted board until dry, and retained for age estimations. The operculum of *B. canaliculatus* is of concentric type (Checa and Jimenez-Jimenez, 1998) with annular stria radiating outward from marginal (siphonal) nucleus (Figure 6). Only opercula with clearly defined stria were used for age estimation. Stria on the inside surface of operculum were more

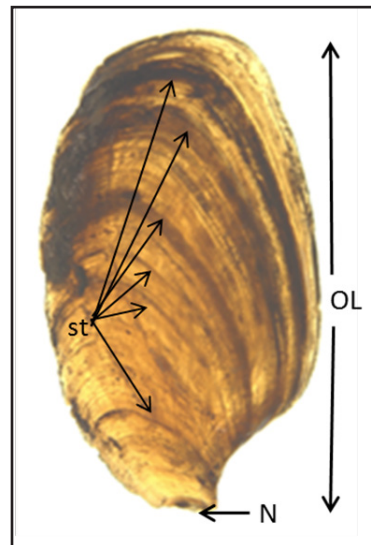*Figure 5. Operculum (op) attached to whelk foot (f).*

Figure 6. Operculum from B. canaliculatus with stria (st) and nucleus (N) as observed from inside (attached) surface. OL=operculum length.

visually defined than observed on outside surface, and therefore counted using back-lighting from stage light of dissecting micro-scope. Stria was further defined in this study as dark bands which extend completely from one lateral edge to the other. From close observation of opercula from 2 and 2+ year old B. canaliculatus, as aged from statoliths of juvenile whelk collected on the beach in Ocean City, Maryland, the first annular stria was observed to be 10-12 mm from nucleus but weakly represented. This first stria, as well as the second in lesser degree, is often inconspicuous in most *B. canaliculatus* operculum throughout subsequent age classes. Therefore, for *B. canaliculatus* opercula age estimations, readers were instructed to assign year one corresponding to an area 10-12 mm from nucleus and proceed counting stria outward from that point.

Statolith Recovery and Aging

Statolith removal from other reported gastropod species was either performed by tissue digestion using a 1M NaOh solution or by direct removal macroscopically. Neither of these methods proved appropriate or efficient for stato-lith removal of *B. canaliculatus*, therefore an exploratory method was developed.

To recover statoliths (2 per whelk), x-rays of differing sized *B. canaliculatus* were performed to initially identify biological positioning of statoliths within this species (Figure 7A). Statoliths are calcareous structures, therefore
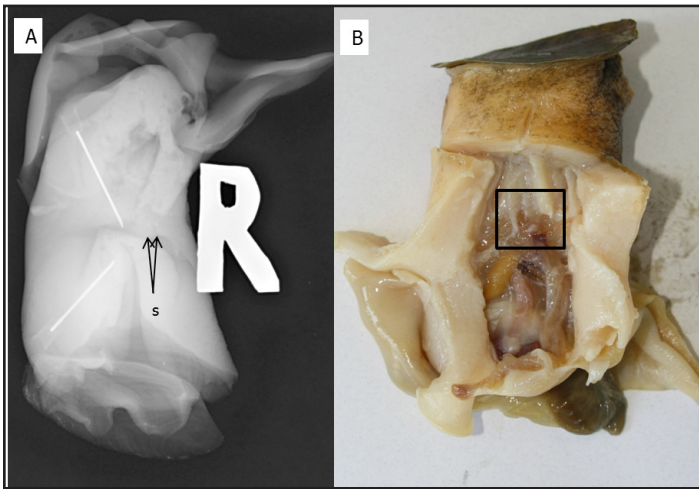

Figure 7. Location of statoliths in B. canaliculatus, (A) x-ray showing position of paired statoliths (s) in foot of whelk; (B) exposed cerebral-pedal ganglia complex (reddish-purple color within square) in which statoliths are associated with.

allowing x-ray imaging. Statoliths were confirmed occupying a posterior position to the pedal ganglia within the cephalopedal hemocoel of the whelk foot. Dissection for statolith recovery involved making 2 cuts into the whelk foot. With whelk foot (body) ventral side up, and starting from the operculum (anterior),a transverse cut is made across the ventral surface of foot at a point 1/3 the distance between operculum-foot and mantle skirt-foot attachment sites, and extending to a depth three-fourths through the diameter of foot. This cross-section incision reveals the centrally positioned extension of cephalopedal hemocoel which visually appears at this point as a small pore opening. The second cut is longitudinal extending entirely through (horizontally) the foot originating from, and at depth of the exposed hemocoel, and extending posteriorly to just beyond mantle-foot attachment site. Depth of longitudinal cut was aligned with ventral wall of hemocoel such as to expose cerebral-pedal ganglia complex (reddish-purple color) which encircles the esophagus mid-line to form the circumesophageal nerve ring (Figure 7B). The pedal ganglia are oriented anteriorly and slightly ventral of cerebral ganglia and are smaller in size. A single statolith is encased within a statocyst embedded within soft, spongie connective tissue surrounding each pedal ganglion (Figure 8). Macroscopic identification and recovery of statoliths was not possible. Statoliths were recovered by removing spongie connective tissue and related ganglia connectives posterior to each pedal ganglion (Figure 8B) to a petri dish and locating statocysts under dissecting microscope. Once
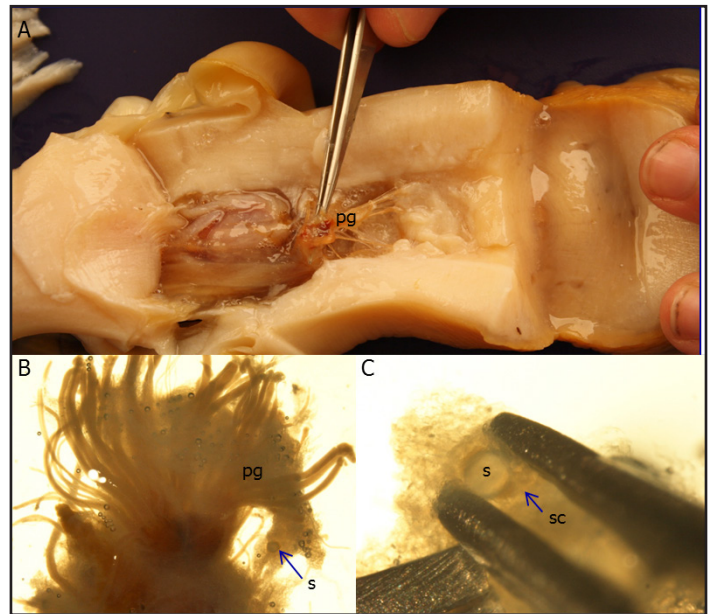

Figure 8. Statolith location in B. canaliculatus, (A) associated with pedal ganglion; (B) statolith within connective tissue adjacent to pedal ganglion; (C) statolith (320 μm diameter) within statocyst. s=statolith, sc=statocyst

located, statoliths were gently removed from statocysts with fine-point forceps (Figure 8C), rinsed with tap water to remove any tissue remnants, and then stored in 85% ETOH until mounted.

Each statolith was removed from alcohol storage, allowed to dry at room temperature (20^0C), embedded in a drop of heated CrystalBond™ 509 resins placed on glass microscope slide, then sectioned in half. Sectioning was performed by affixing a silicon suction cup to the back of slide with mounted statolith and gently passing the resin-bound statolith over progressively finer wet abrasive paper (600-1200 grit) under a dissecting microscope until the center of statolith (nucleus) was reached. The mid-point of statolith was verified under a compound scope viewing at 100x. After sectioning, statoliths were rinsed with distilled water to remove sanding debris. Two readers independently assessed age without knowledge of whelk shell length (SL) by counting concentric annular rings (100x magnification) observed on sectioned statoliths extending outward from an initial ring (settlement ring) which was validated to be laid down shortly after juvenile whelk emergence from egg casing (concurrent age methodology study). In this study, the term 'ring' references the dark concentric band observed between light concentric bands in statoliths which represents period of slow growth (Figure 5). This annual periodicity of growth ring formation in whelk statoliths was validated in *N. reticulatus* (Chatzinikolaou and Richardson, 2007).

5

Growth

An egg capsule string of *B. canaliculatus* containing live juveniles was collected from the beach at Buckroe, Virginia February 2010 during a high tide. The egg string consisted of capsules all but 3 without whelk. Within capsules containing whelk, one had the capsule pore still closed with 59 live juveniles inside. The other2 capsules had opened pores and contained 40 and 22 live juveniles each, suggesting that hatching was nearly complete. Twelve random juveniles from capsules with open pores were measured (average 4.01 mm +-0.1868) representing age 0. Statoliths from 8 of these juveniles were recovered to observe statolith diameter (average diameter 69.6 μm +/- 1.54 sd) and deposition of any markings (nucleus, settlement rings) to aid age estimations.

Growth parameters for each sex at the three sampling sites (OC, ES, and VB) were estimated from observed age at length data (statolith readings) using the three parameter von Bertalanffy growth equation:

$$L_t = L_\infty * (1 - e^{-k(t-t_0)})$$

Where L_t=whelk length in millimeters at age t, L_∞=asymptotic maximum length (mm), k=the growth rate parameter and t_0=the age of the animal at zero length. The three parameter function was fitted to the data using PROC NLMIXED in the SAS system v.9.2.

Histology

The gonads from male and female *B. canaliculatus* were processed for histological evaluation to determine female gonad synchrony, identify stages of gonadal development, and to assess maturity. All gonad samples were dissected from fresh live whelk (4-6 mm wide section), fixed in Davidson fixative, dehydrated in alcohol and embedded in paraffin using standard histological methods, sectioned at 6 μm thicknesses, and stained with Harris hematoxylin and eosin Y. Gonads from 3 female and 3 male channeled whelk >160 mm SH from all three collection sites were examined to determine if channeled whelk gonad development is synchronous (multiple developmental stages throughout the gonad), or asynchronous (only a single developmental stage throughout). Multiple visceral sections (gonad and digestive gland) were removed from the anterior, medial, and posterior areas of each gonad and evaluated. Synchrony within gonads was observed; therefore all histological sampling was performed using the medial section of gonads. Gonad developmental stages were evaluated across whelk size (SL) groups collected following Ilano *et al.* (2004), Cledon *et al.* (2005), Chung *et al.* (1993) and Chung and Kim (1997). Forty one whelks were found with large orange-red, granular appearing masses associated with the digestive gland-gonad visceral area and

histologically processed only for parasitology interest and were not included in reproductive analysis.

Sexual maturity was determined by histological and macroscopic evaluations of reproductive organs and gonadal and penis indices plotted against shell length. Maturity ogives were used to estimate the size at maturity (median SL at which 50% of the individuals are mature) and penis length at maturity. A logistic model using binomial maturity determinations (0=immature, 1=mature) as described above for both sexes was fitted to the data. Post hoc hypothesis tests were used to determine the significance of various levels of factors tested (ie. sex, area).

Results

In total, 8, 139 *B. canaliculatus* were collected for this study between November 2009 and January 2011. In November 2009 sampling, 1,920; 2,624; and 2, 757 live, undamaged whelk were collected from OC, ES, and VB resource areas, respectively. All of these whelks were used for length-frequency distribution analysis. From these whelks, random subsamples were taken for length-width relationship (1,490), histological reproductive biology evaluation (279), aging (214), and sexual maturity determinations (625). Not included in this study were 7.8% of OC, 9.0% of ES, and 6.3% of VB whelk landed on-board with broken siphonal canals, which were largely the result of culling practices on board commercial vessels (personal experience) and result in inaccurate length measurements. A one way anova was used to test for differences in the mean length of whelks from the three resource areas. Multiple comparisons indicate that the mean length significantly differed between all three areas, however the magnitude of the difference between OC (mean=158.1 mm, range 82-230 mm) and the other two areas (ES (mean=137.6, range 97-209 mm) VB (mean=132.2, range 90-190 mm)) was greater than the magnitude of the difference between ES and VB. By sex within each resource area, mean SL was: OC, female 152.6 mm (N=63), male 136.5 mm (N=117); ES, female 162.4 mm (N=63), male 137.5 mm (N=83); and VB, female 149.2 mm (N=134), male 125.6 mm (N=139). Percent sublegal (<MLS for give area) whelk caught per resource area was highest in VB (69%), followed by ES (60.1%), then OC (43%). The largest whelk collected measured 230 mm (3 females) and recorded from OC. The smallest whelk collected from commercial sampling was 82 mm SL from OC. The smallest whelk collected from the field for this study were 58.2 mm SL (female) and 62.9 mm SL (male), which were collected alive on beach in Ocean City, Maryland.

In 2010-2011 seasonal sampling, a total of 838 whelks were collected: May (n=107), June (n=127), and November (n=88) 2010 at OC site; May (n=168), and December

(n=98), 2010 at ES site; and June (n=116), and September (n=3), 2010 and January (n=131), 2011 at VB site. A sub-sample of 18-41 fresh, live whelks selectively representing available size classes from each sampling period (except September 2010 VB sample were only 3 whelks were collected) were processed for reproductive histological analysis.

From November 2009 sampling, length frequency distributions of whelk from VB and ES populations were very similar in structure, but quite different from the OC population (Figure 9). Whelk from VB and ES showed a

Area/sample period	Ave SL (mm) Male/Female	Sex ratio (M:F)	N
VB Fall 2009	136.5/152.6	1.03:1	273
VB Spring 2010	117.2/137.1	1.1:1	116
VB Fall 2010	124.3/129.8	1.1:1	132
ES Fall 2009	137.5/162.4	1.3:1	146
ES Spring 2010	125.3/140.6	1.5:1	162
ES Fall 2010	140.6/154.5	1.2:1	99
OC Fall 2009	136.5/152.6	1.3:1	206
OC Spring 2010	136.2/143.3	1.8:1	234
OC Fall 2010	131.5/145	2.0:1	88

Table 1. B. canaliculatus. Sex ratio and average size (SL) of whelks within each resource area over all sampling periods.

Figure 9. B. canaliculatus. Length-frequency distribution of whelk from 3 commercially targeted populations (OC, ES, VB) in the Mid-Atlantic, US. Vertical lines represent current minimal landing size (MLS) of 5.5" (139.7 mm) and 6" (152.4 mm).

unimodal (single peak) length-frequency distribution while the OC population showed a bimodal (two peaks) distribution. The single peaks in length-frequency distribution from ES (125-135 mm) and VB (120-135 mm) areas were at shell length less than minimal landing size (MLS) for that region (139.7 mm). In the OC bimodal distribution, the smaller peak (115-130 mm) was at a shell length less than the MLS (152.4 mm) for that region, while the larger peak (155-165 mm) was greater than the MLS.

The overall sex-ratio (M: F) of *B. canaliculatus* varied within each resource over all sampling periods (Figure 10). In November (fall) 2009, sampling favored males in all areas (Table 1), only slightly within VB (1.03:1), but more dominantly in both ES (1.32:1) and OC (1.31:1). Males dominated smaller whelk size in all areas, but more so in ES and VB. In spring and fall 2010 sampling, whelk distribution by sex remained similar to 2009 in VB and ES, but considerably more males were observed in OC (Figure 10). Though a large increase in males was observed in OC 2010 samples, average size of males remained similar (131.5-136.5 mm), at size of sexual maturity. Average whelk size

in both sexes from 2010 sampling was also observed to decrease from 2009 Fall sampling. Within all resource areas larger whelks were predominantly females.

A strong linear length-width relationship for *B. canaliculatus* was demonstrated for all areas (Figure 11). Shell width in OC whelk increased slightly greater than ES and VB whelk (mm, inches) with increasing SL. There was no sexual dimorphism observed for whelk length-width relationship in OC and VB, the furthest geographically separated populations (Figure 12).

Yield

Yield was calculated from both fresh (n=143) and frozen/thawed (n=586) whelk. Flesh yield at shell length relationships for *B. canaliculatus* in this study was observed to be similar in frozen/thawed whelk between areas (Figure 13) and the sexes (Figure 14), and described by respective power functions. There was no sexual dimorphism in meat yield observed. Fresh whelk yield (Figure 15) was greater over shell lengths than frozen/thawed whelk, however, more variation between resource areas was observed with whelk from VB having the highest flesh yield over all sizes followed by ES then OC. This is supported by shell length-to-yield regression model results that indicated significant differences between each area. Multiple contrasts indicate marginal significance (p=0.0307) between VB and ES, while the differences between OC and the other two areas VB and ES, were highly significant at p<0.0001 and p=0.0034, respectively. Yield at governing MLS for VB and ES (5.5") was 72.8 and 68.6 g, respectively. Yield of OC whelk at MLS 6" was 85.9g. Relative to variable MLS, an increase in flesh yield of 41% was observed in VB whelk going from MLS of 5-5.5" (127-139.7 mm), and 37.2% from 5.5-6" (139.7-152.4 mm). Yield almost doubles (94% yield increase) in VB whelk going from MLS 5" (51.5 g) to 6" (99.5 g).
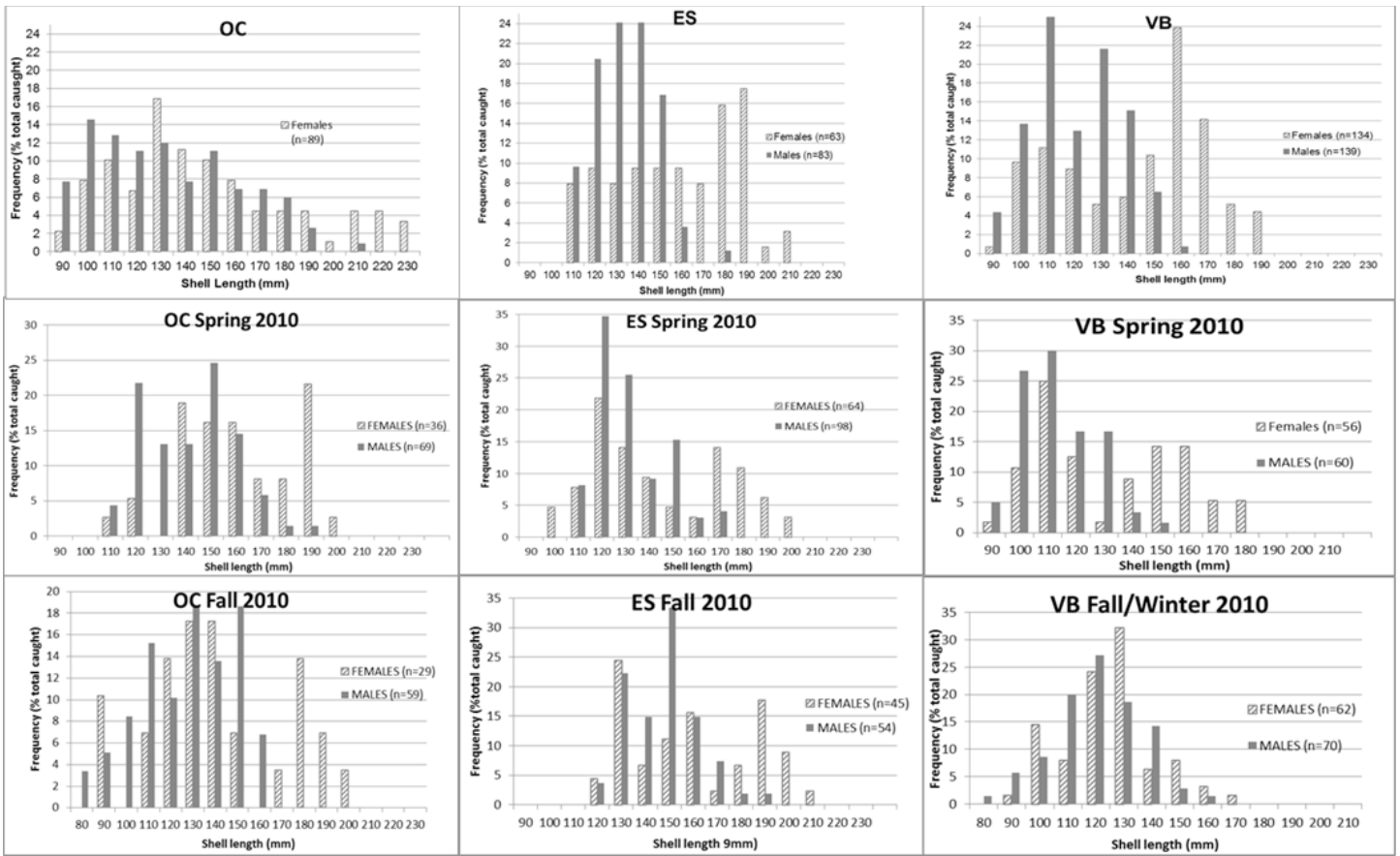
Figure 10. Sex ratio of B. canaliculatus within each resource area over all sampling periods (Fall 2009-winter 2010).
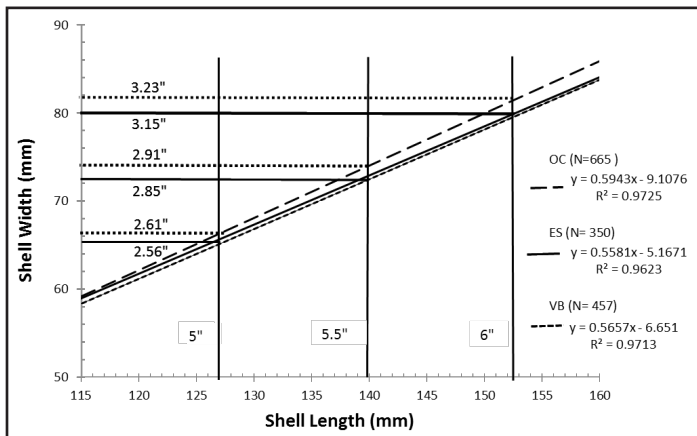

Figure 11. Relationship between shell length and shell width for B. canaliculatus from 3 resource areas with resulting shell width (inches, mm) given current minimal landing size (MLS; 5", 5.5", and 6") in shell length.
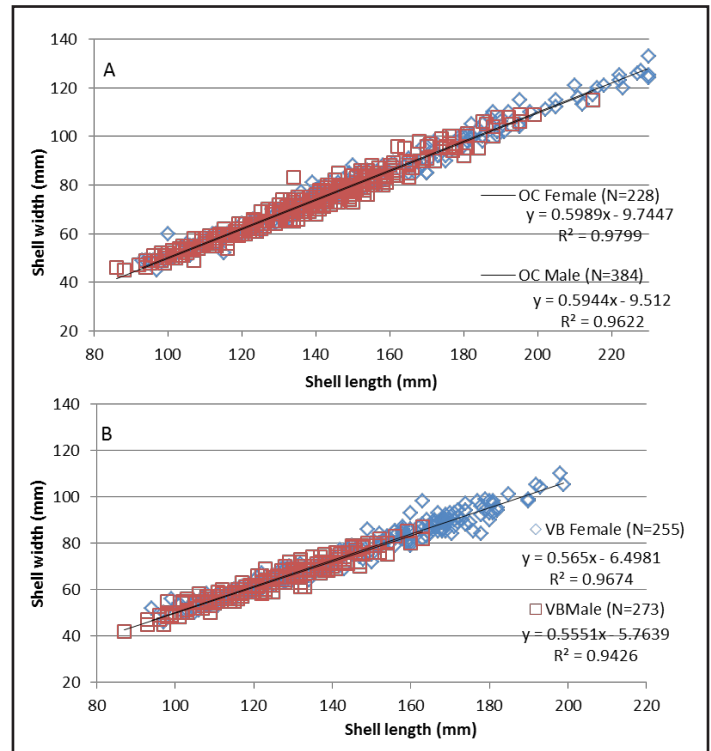

Figure 12. Shell length-shell width relationship by sex of whelk from OC (A) and VB (B) resource areas.
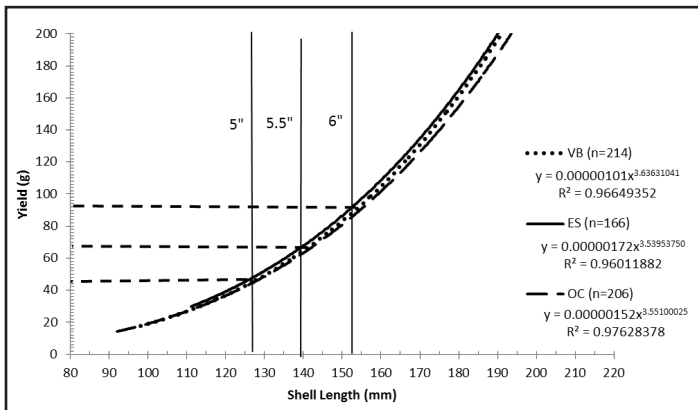
Figure 13. Flesh yield (g) of fresh <u>B. canaliculatus</u> from 3 resource areas. Vertical lines are current minimal landing sizes (MLS: 5", 5.5", 6") in shell length. Horizontal dashed line is yield for VB area.
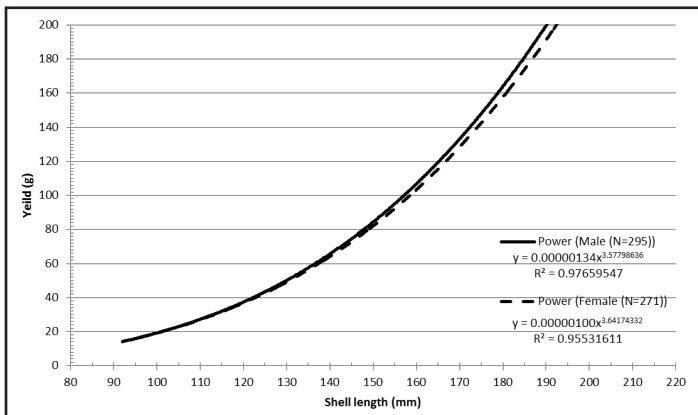

Figure 14. Flesh yield of frozen/thawed <u>B. canaliculatus</u> male and female whelk with power function from all areas combined.
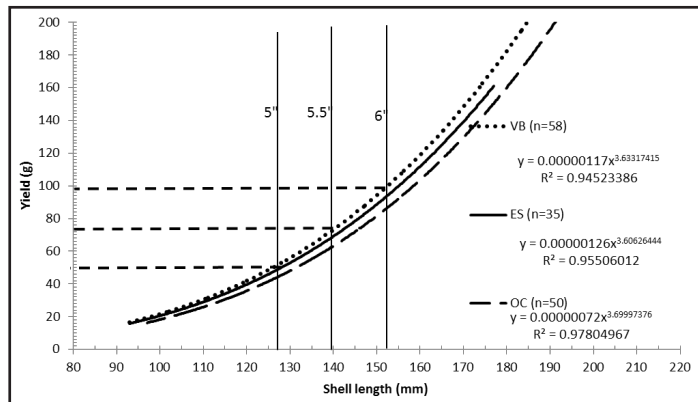

Figure 15. Flesh yield (g) of fresh, unfrozen <u>B. canaliculatus</u> from 3 resource areas. Vertical lines are current minimal landing sizes (MLS: 5", 5.5", 6") in shell length. Horizontal dashed line is yield for VB area.

Age Estimations

The majority of opercula and statoliths recovered from individual whelk demonstrated definitive markings (stria/rings) which corresponded well (Figure 16). Being internal structures, statoliths are not exposed to environmental erosion as operculum, and were observed to provide more consistent and discernible annular markings than operculum. Constructed of calcium carbonate within a spherical structure, with the transmission of light through statoliths discernible concentric rings are observed.
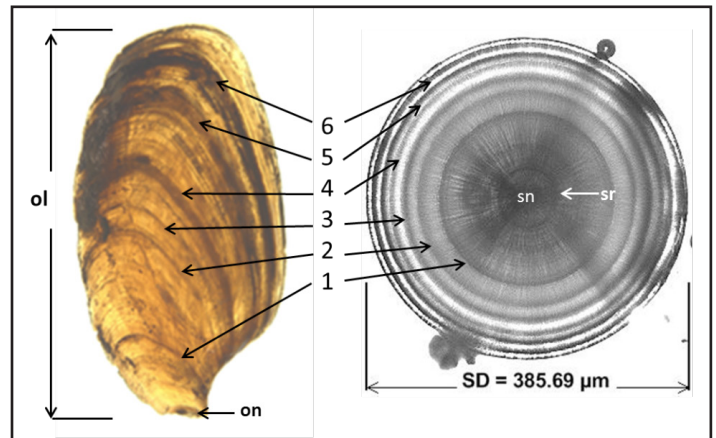

Figure 16. Opercula (left) and statolith (right) from the same whelk showing markings (numbered stria, rings, respectively) used to estimate age for <u>B. canaliculatus</u> in this study. ol=opercula length, on=opercula nucleus, SD=statolith diameter, sn=statolith nucleus, sr=settlement ring

Statolith: Whole vs. Sectioned

Whole and sectioned statoliths provided for gross enumeration of annuli, though differences in light refraction as the result of sectioning were apparent. Statolith nuclei where observed more visible in whole mounts than sectioned mounts, though dark annuli demarcation representing periods of slow growth were less defined (blurred) in whole mounted statolith (Figure 17). The light bands representing periods of rapid growth refract transmitted light, which results in more visually defined annular dark bands that
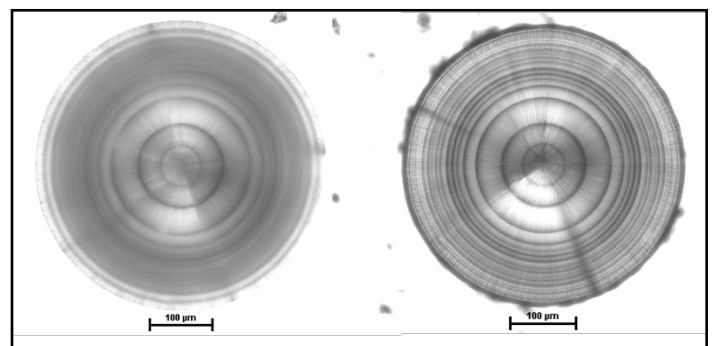

Figure 17. Pair of statoliths from a 210 mm SL female <u>B. canaliculatus</u>, viewed mounted whole (left) and sectioned (right) showing concentric rings counted for age estimation.

lay between them. Narrow light bands, as a result of slow growth due to advancing age and/or unfavorable environmental conditions, result in the stacking of annuli (dark bands) which minimizes light refraction between annuli and routinely blur stacked annuli and cause incorrect age estimations. With sectioning, more light refraction is attained, resulting in better resolution of closely spaced annuli. An observed difference in age estimate was detected by both readers between whole and sectioned statoliths as age increased. Whole statolith readings produced younger age estimates than sectioned statolith readings. Within both readers, no age estimates greater than age 11 were recorded using whole statoliths, but ages up to 16 were recorded using sectioned statoliths. It was observed that starting at ages >3, sectioned statoliths provided higher ages from both readers. Percent of estimates by the two readers in which whole readings were less than sectioned readings for the same whelk were 37.7% and 50.3%, while only 3.8% and 0.5%, respectively, of whole estimates were greater than sectioned estimates (Figure 18).

In statoliths recovered from *B. canaliculatus* whelk at time of hatching, no settlement ring was observed (Figure 19A), indicating its formation occurs at some point after hatching and transformation to benthic life. Statoliths recovered from 2 wk old whelk (Figure 19B) revealed the formation of settlement ring (70.6 μm diameter) appearing at statolith circumference (71.9 μm diameter). By age 6 months, settlement ring (70.6 μm diameter) is clearly represented (Figure 19C) with a diameter consistent with statolith diameters of 2 wk old whelk. Mean statolith diameter at hatching (69.6 +-1.54 μm, n=8) was not significantly different from diameter of settlement ring (72.4 +-2.14 μm, n=87) in whelk over various size classes (Figure 20) from study areas combined. This relationship provided confirmation of SR demarcation in which to begin annuli counts for age estimation. The relationship between statolith diameter and shell length (3.7-201 mm SL) from areas combined is described by the power function Statolith diameter = 39.41600 x shell length$^{0.44049}$ (Figure 21).

In total, statoliths from 213 *B. canaliculatus* whelk were recovered for age estimation; 74 from OC, 50 from ES, and 89 from VB. Age estimates from sectioned statoliths ranged from 2-14 years. There was a significant difference between readers
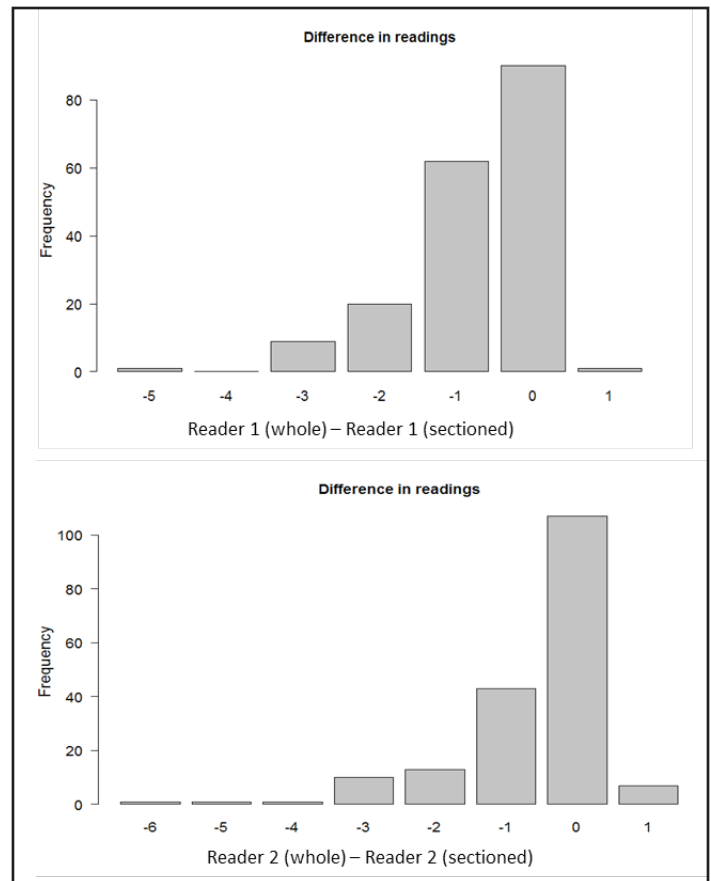

Figure 18. Difference in readings between readers of whelk statoliths, whole vs sectioned, for age estimations.

found ($\chi 2 = 6.744$, 1 df, p = 0.009) with one reading slightly higher than the other. Percent agreement between readers was 54.1% ±0 years, 35.0% ±0-1 years, 7.8% ±0-2, and 3.1% ≥3 years (Figure 22).
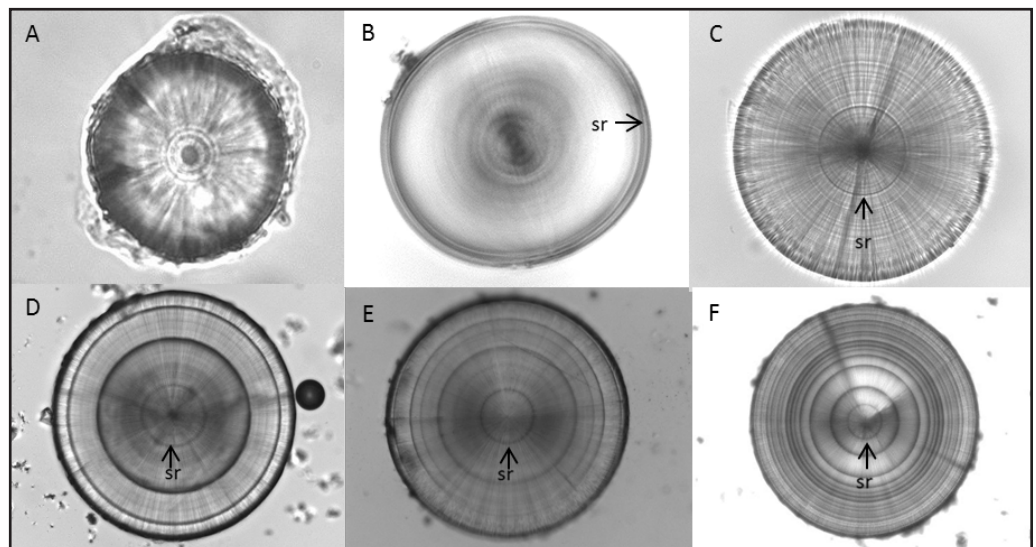

Figure 19. Statolithsfrom (A) at the time of hatching (67 μm diameter) with no settlement ring (sr) formed; (B) 2 week old (71.9 μm diameter), recently formed sr=70.6 μm; (C) 6 month old (206 μm diameter), sr=70.9 μm diameter; (D) 2+ year old (265 μm diamter), sr=70.3 μm diamter; (E) 4+ year old (304 μm diamter), sr=70.7 μm; and (F) ~12 year old (414 μm diamter), sr=69.6 μm diamter.
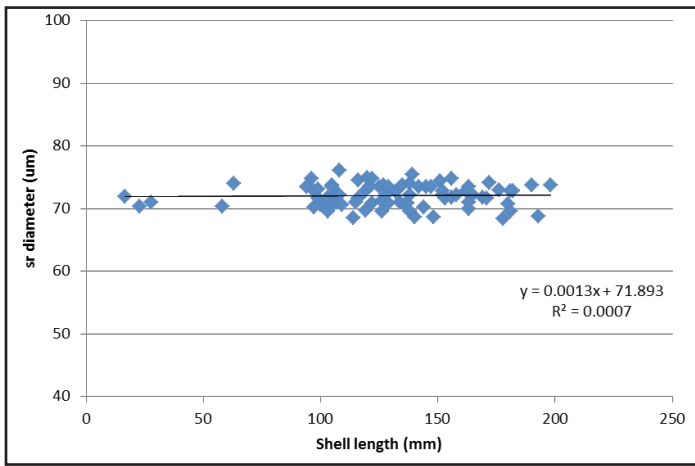
Figure 20. *Relationship between settlement ring (sr) diameter and shell length for* B. canaliculatus.
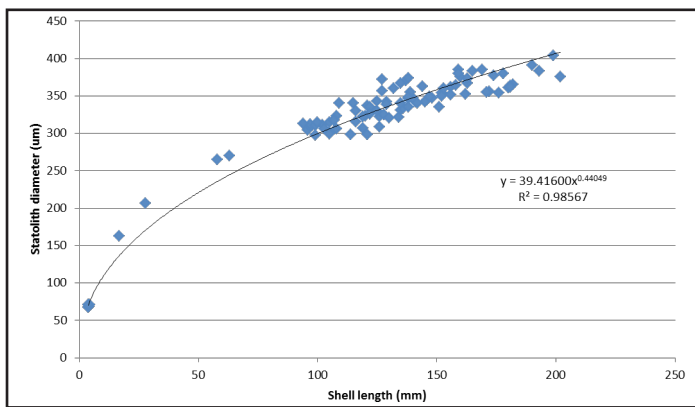

Figure 21. *Relationship between statolith diameter and shell length in* B. canaliculatus.
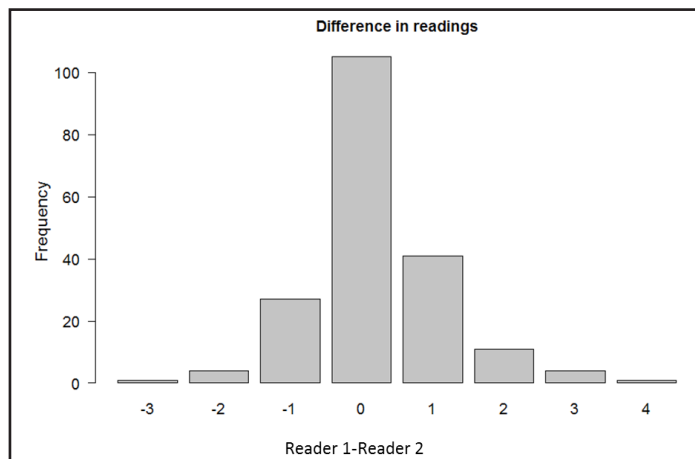

Figure 22. *Difference in readings between readers of sectioned statoliths for age estimations in* B. canaliculatus.

Figure 23. *Relationship between operculum length and shell length for* B. canaliculatus *(n=205).*
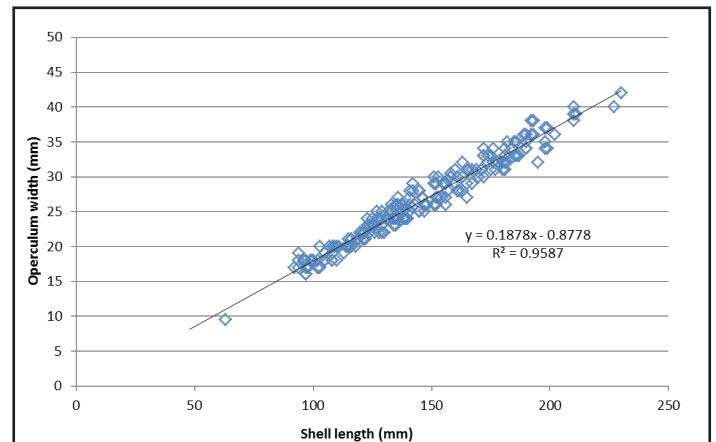
Figure 24. *Relationship between operculum width and shell length for* B. canaliculatus *(n=204).*
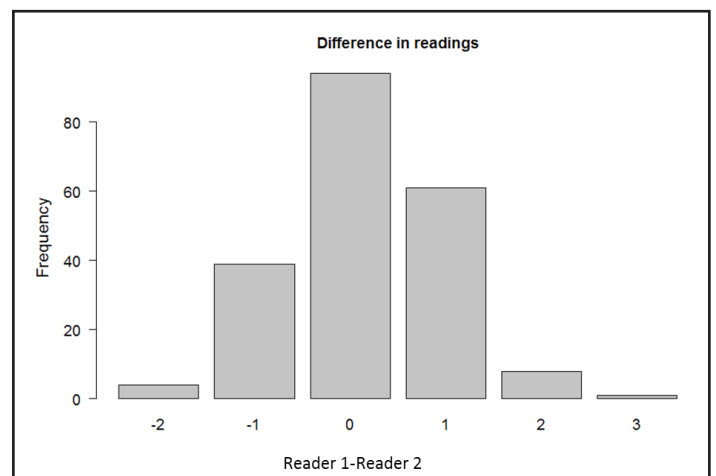
Figure 25. *Difference in readings between readers of opercula for age estimations in* B. canaliculatus.

In total, 214 opercula from *B. canaliculatus* were used for age estimation; 74 from OC, 51 from ES, 89 from VB. Linear relationships were observed between operculum length and shell length (Figure 23) and operculum width and shell length (Figure 24). Age estimates ranged from 3 to 14 years. There was 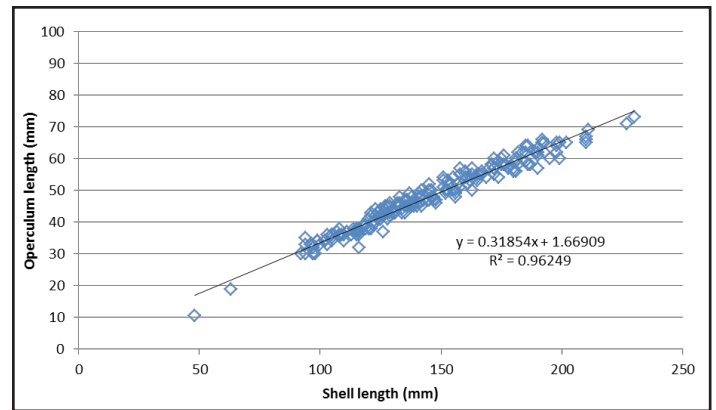a significant difference between readers found ($\chi2 = 5.929$, 1 df, p = 0.014) with reader difference of about 1 year observed. Percent agreement between readers was 45.3% ±0 years, 48.6% ±0-1 years, 5.7% ±0-2, and 0.5% ≥3 years (Figure 25).

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Age Tables

Age tables were created using both statolith (Table 2) and operculum (Table 3) age estimations for *B. canaliculatus* from all three resource areas. Statolith age estimations reported were for sectioned statoliths of male and female whelk. Sexes were combined for operculum age estimates. Operculum aging was observed to consistently underestimated age compared to statolith aging.

Growth

Length at age data fitted with von Bertalanffy growth curves are given for *B. canaliculatus* from each resource area (Figure 26, sexes combined) and by sex (Figure 27, areas combined). Growth rates of *B. canaliculatus* differed between study areas, with VB whelk observed to grow faster (k) than ES and OC, but reaching a smaller maximum length SL (L∞) than ES and OC (Table 3). Given maximum observed SL from each area (Table 2), the estimates for asymptotic maximum SL (L∞) were biologically reasonable for males and females from ES and VB, but overestimated for both sexes in OC. Overall, the size-at-age data indicate that male channeled whelk grow faster (k) and reach a smaller maximum size (L∞) than females. This sex growth dimorphism is observed within each resource area (Table 4) and within growth model esti-

Location	Age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
VB	Sexes Combined															
	Ave SL (mm)				103	117.3	135.6	146.8	168.5	183.2	195.5					
	SD				4.708149	8.253764	5.143398	10.2344	10.37075	4.32435	3.511885					
	n				13	26	11	15	15	5	4					
	Females															
	Ave SL (mm)				103.5	119.2	138	153	170.2	183.2	195.5					
	SD				5.75326	6.26564	5.09902	9.721111	10.08426	4.32435	3.511885					
	n				6	14	6	5	13	5	4					
	Males															
	Ave SL (mm)				102.5	115	132.8	143.7	161							
	SD				4.035556	9.899495	3.898718	9.42868	2.828427							
	n				7	12	5	10	2							
ES	Sexes Combined															
	Ave SL (mm)				115	127.57	132	141.28	161	177.33	175.5	189.75	202		198	210
	SD				2.54951	4.755949	5.656854	6.750661	9.268087	10.94836	14.84924	6.130525				
	n				5	7	2	7	13	6	2	4	1		1	1
	Females															
	Ave SL (mm)				114	129.5		142.2	164.77	181.2	186	192.3	202			210
	SD				2.645751	4.949747		4.949747	8.496731	6.140033		4.041452				
	n				3	2		2	9	5	1	3				1
	Males															
	Ave SL (mm)					116.5	126.8	132	140.8	153.5	158	165	182			
	SD					2.12132	5.01996	5.656854	7.823043	5.802298						
	n					2	5	2	5	4	1	1	1			
OC	Sexes Combined															
	Ave SL (mm)	48	60.5	98	102.3	122.5	136.5	142.8	163.1	186.2	193.3	198	210			230
	SD		3.535534		7.531983	6.587024	3.109126	5.303301	10.32523	7.596052	8.807464		17.52142			0
	n	1	2	1	13	10	4	8	17	5	7	1	3			2
	Females															
	Ave SL (mm)	48	58	98	105	126.25		144.5	168	190	198.33	198	219			230
	SD				9.165151	5.123475		3.785939	5.033223	12.72792	10.40833	198	11.31371			0
	n	1	1	1	3	4		4	4	2	3	1	2			2
	Males															
	Ave SL (mm)		63		101.5	120	136.5	141.25	161.61	183.66	192.33				192	
	SD				7.33712	6.60303	3.109126	6.652067	11.19924	3.21455	3.05505					
	n		1		10	6	4	4	13	3	3				1	

Table 2. *Age estimates for* B. canaliculatus *from three resources areas by sex and combined. Sectioned statoliths used for aging.*

Location	Age (operculum_)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
VB	Ave SL (mm)				99.5	109.35	125.1	132.28	151.53	167.58	185	192				
	SD				4.41588	7.026211	10.06244	5.618846	13.29805	15.87868	16.44384					
	n				6	17	20	7	15	17	6	1				
ES	Ave SL (mm)						116.83	126.5	135.87	151.83	169.12	184	182	201.75	210	
	SD						5.036533		3	9.109453	9.907145	10.42576	6.377042	7.483315	5.909033	
	n						6	4	8	12	8	4	4	4	1	
OC	Ave SL (mm)					99.08	116.37	130.77	145.75	157.57	171.25	187	188.333	202.75	227	230
	SD					5.009083	6.02228	6.280481	6.757151	6.106203	6.453128	11.53256	5.454356	9.287088		0
	n					11	8	9	12	7	8	3	9	4	1	2

Table 3. *Age estimates for* B. canaliculatus *from three resources areas by sexes combined. Opercula used for aging.*
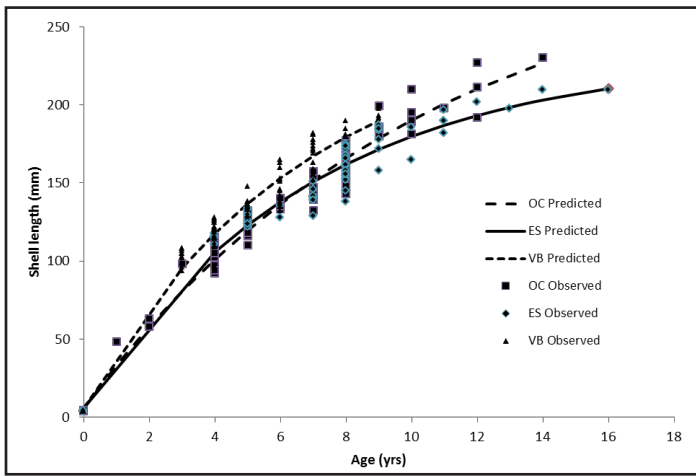
Figure 26. <u>B. canaliculatus</u>. Length at age data for three resource areas in the Mid-Atlantic with fitted von Bertalanffy growth curves. Age estimates from sectioned statoliths, sexes combined.
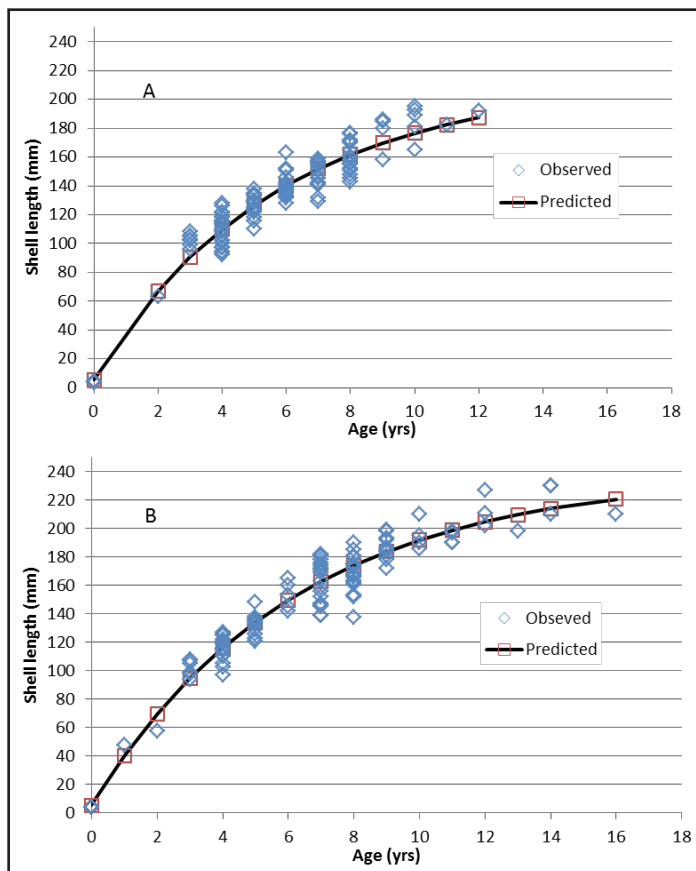
Location	Sex	L∞	k	t₀
Ocean City	Female	304.45	0.100	-0.178
Ocean City	Male	273.74	0.112	-0.140
Eastern Shore	Female	235.53	0.151	-0.122
Eastern Shore	Male	184.48	0.220	-0.105
Virginia Beach	Female	255.23	0.155	-0.116
Virginia Beach	Male	185.76	0.245	-0.093

Table 4. *Estimated von Bertalanffy growth parameters for male and female <u>B. canaliculatus</u> from three sampling regions are shown with values for estimated maximum length (L∞), growth rate coefficient (k), and theoretical time at length zero (to).*

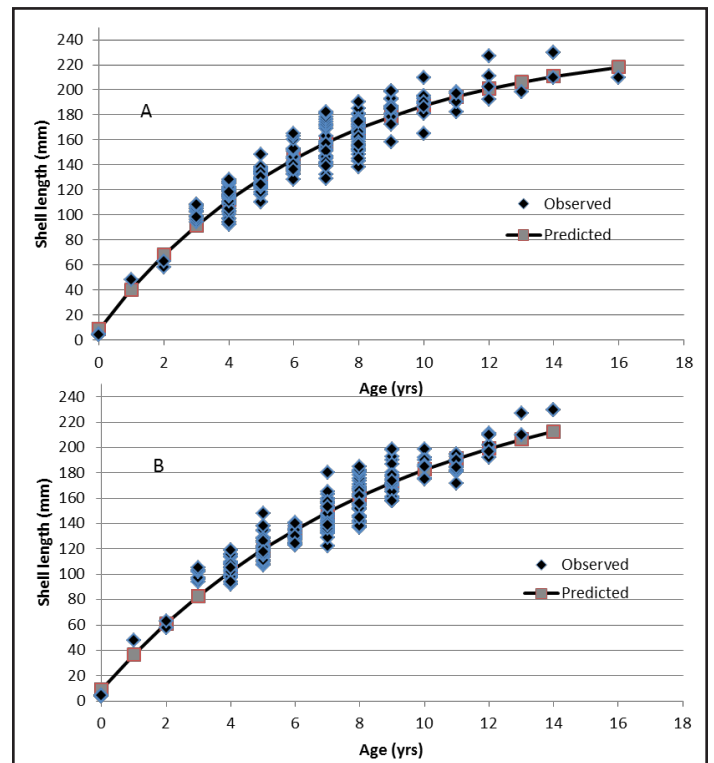**Figure 28.** *Growth curves for <u>B. canaliculatus</u> from all areas combined using statolith (A) and operculum (B) age estimations.*

Figure 27. *Growth curves from length at age date for male (A) and female (B) <u>B. canaliculatus</u>, resource areas combined.*

mates (Figure 27). Estimated growth using opercula for age estimates underestimated length at age compared to age estimations from sectioned statoliths (Figure 28). Further, in comparing average shell length at age from both aging methods (Tables 3 and 4), smaller average shell length over all ages is observed by opercula aging.

Sexual Maturity Stages

In fall 2009 sampling, 146 whelks were processed for histological evaluation of gonad developmental stages; 57 from VB, 35 from ES, and 54 from OC. An additional 133 whelk were sampled histologically from all three sites in spring (n=93) and fall (n-22) 2010, and January, 2011 (n=18). Sampling periods aided maturity assessment determination since whelk from all 3 sites (populations) were histologically determined to be at latter stages of gonadogenesis in sexually mature individuals; however, since some gonadic stages were not represented in these sampling periods (resting, and post-spawning), this report is limited to observations of gonad maturation and not

seasonal reproductive cycle. Extension of gonad, male and female, from dorsal side of digestive gland increased with whelk size and corresponded to stage of gonadal maturation. In females, early gonadogenesis was observed in whelk in which primary pre-vitellogenic oocytes (oogonia) appear in association with ovarian follicle germinal epithelium. Oocytes mature with accumulation of vitellogen. In males, the testis contains seminiferous tubules from which spermatogenesis takes place. This cell differentiation process starts with spermatogonia (germ cell) in the germinal epithelium of tubules undergoing maturational changes into mature spermatozoa (sperm). Histological stages of maturity are presented in Figure 29.

Immature

The female gonad first appears as an off-white and translucent band of tissue, then begins to extend slightly outward from digestive gland, accumulating mass as follicles thicken and oogonia develop into primary pre-vitellogenic oocytes ($<10\,\mu m$) along the germinal epithelium (Figure 29A). Pre-vitellogenic oocytes have a large nucleus/cytoplasm ratio and basophilic cytoplasm and granular nuclei. A significant amount of connective tissue is present separating ovarian follicles. The capsule gland (Nidamental gland) is thin, light beige and non-conspicuous. The male gonad first appears a thin, light band of tissue with few seminiferous tubules. Gonad starts developing a light to pale orange color as spermatogenesis begins with tubule wall thickening. Only spermatogonium (7-$9\,\mu m$) is present at this stage (Figure 29E). The vas deferens is inconspicuous throughout its length.

Maturing (Early to Late)

This stage consists of a wide developmental period centered on progression and extent of oocyte vitellogenesis in females and spermatogenesis in males. In females, gonad enlarges with increasing number of elongated ovarian follicles with increasing number of vitellogenic oocytes which are characterized by cytoplasm accumulating yolk granules and prominent, round basophil nucleus (Figures 29B and 29C). Yolk granules stain bright red with eosin and become oval in shape within mature ova. In early developing stage, follicles contain oocytes (50-$150\,\mu m$) with small, granulated appearing yolk granules. With maturity, oocytes enlarge (300-$500\,\mu m$ diameter) with accumulation of large (20-$30\,\mu m$) yolk granules (globules), however not at the same rate, with various stages of oocyte vitellogenisis observed within follicles. Connective tissue remains significant and distributed between follicles, but connective tissue-follicle ratio decreases with maturity. Female gonad is a light, pale orange in color which changes to a varying shade of yellow-brown as maturity progresses. Enlarging follicles begin to give gonad a coarser texture macroscopi-
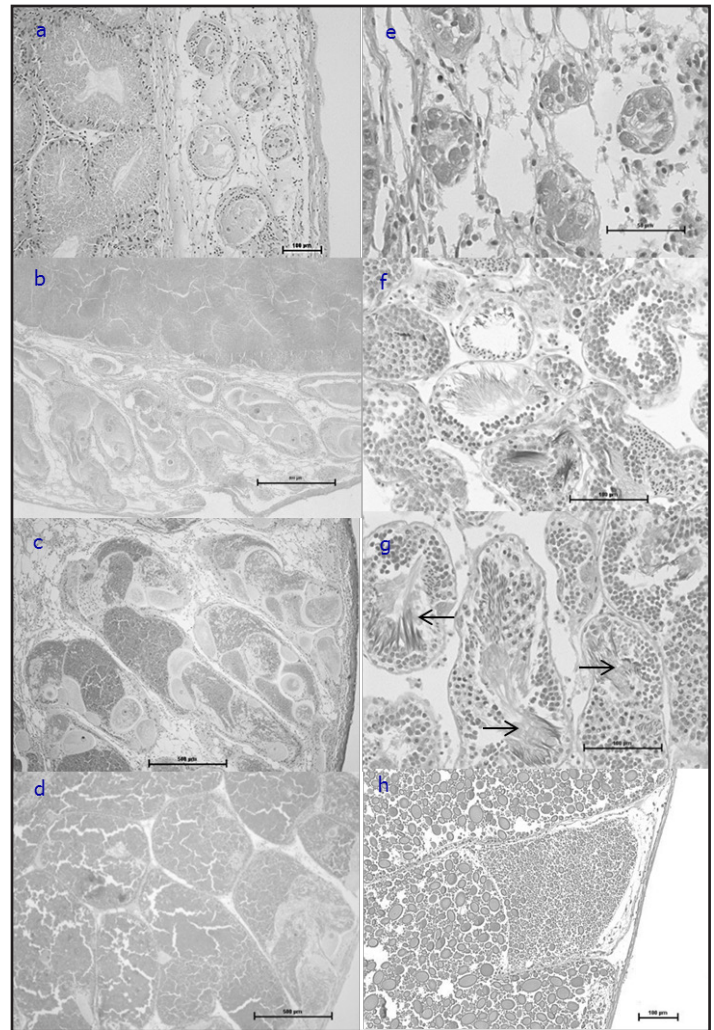

Figure 29. *Photomicrographs of* <u>B. canaliculatus</u> *showing histological stages of gonad development. Immature stage: female (a) showing only pre-vitellogenic oocytes (oogonia) within follicles, and male (e) showing only spermatagonia in seminiferous tubules. Early maturing stage: female (b) showing pre-vitellogenic and vitellogenic oocytes with prominent nuclei and small yolk granules. Late maturing stage: female (c) showing varying size vitellogenic oocytes with increasing number of mature ova with large yolk granules, and male (f) showing late maturing stage with all reproductive cells present in tubules but only few with mature spermatozoa (arrows). Mature stage: female (d, h) showing large, cuboidal shaped follicles with ova (300-$500\,\mu m$) filled with large, oval yolk granules, males (g) showing most tubules with mature spermatogonia densely accumulating in tubules lumen (arrows). Scale bars; a, f, g, h, $100\,\mu m$; b, c, d, $500\,\mu m$; e, $50\,\mu m$.*

cally, becoming slight granular nearing maturity. Capsule gland is beige in color and flaccid in structure, but firms as it enlarges with maturity with glandular tissue inside becoming snowy-white (Figure 30).

In the early stage of male gonad maturation, only sperm precursors (spermatogonia, spermatocytes, spermatids) are
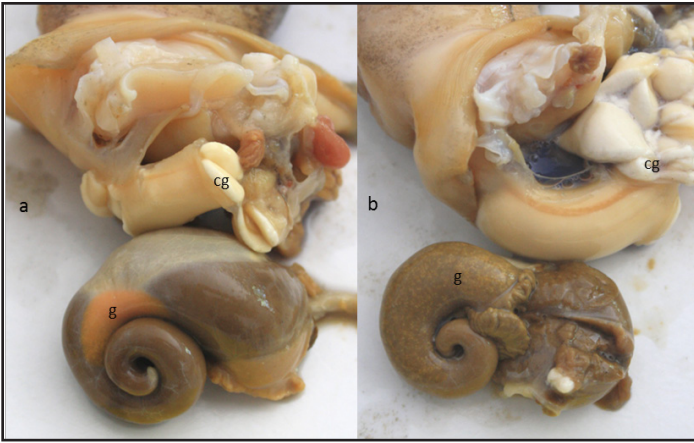
Figure 30. Maturing (a) and mature (b) female B. canaliculatus from VB area both measured at 144 mm SL but at different stages of maturity. Mature female with larger, ripe gonad (g), and larger capsule gland (cg) viewed ruptured containing bright white tissue.

Figure 31. Gonad of a mature female B. canaliculatus (SL=174 mm) showing granular exterior appearance (large arrows) and columnar arrangement of follicles (small arrows).

observed. As maturity progresses, all development stages of spermatogenesis are observed with zones of sparmatogonia, spermatocytes (4-6 μm), spermatids (elongated cells ~2x8 μm), and some spermatozoa (sperm), when nearing maturity, extending from the tubule wall to the lumen (Figure 29F). With maturity, a larger proportion of tubule's are observed containing mature spermatozoa. The visceral section of vas deferens is visible on the ventral side of digestive gland. It appears as a very narrow, straight tube in immature whelk, then thickens and becomes convoluted (coiled) as maturity is reached. Maturing male gonad color ranges from pale orange to orange.

Mature

Female and male gonads become plump, fully extended laterally and longitudinally from digestive gland. Ovarian follicles in mature female gonads are compacted together each cuboidal in shape (in transverse section) with mature oocytes (ova) filled with large (300-500 μm, oval-shaped yolk (vitellus) globules (Figures 29D and 29H). A few smaller oocytes in earlier stages of vitellogenesis may be present, but were observed to largely occupy the dorsal periphery of gonad. At late maturation, ova nuclei become inconspicuous as yolk globules dominate ova cytoplasm and individual ova become difficult to distinguish. Minimal connective tissue is present in inter-follicle space. Ripe female gonads macroscopically range from pale orange to yellowish-brown in color and appear granular as viewed through an increasingly thinning germinal epithelium (Figure 30). When ruptured, elongated follicles are observed arranged in columns, extending outward from digestive gland (Figure 31). Capsule gland large, plump lightening in color by underlining creamy white glandular tissue observed through thinning gland epithelial Figure 30B).
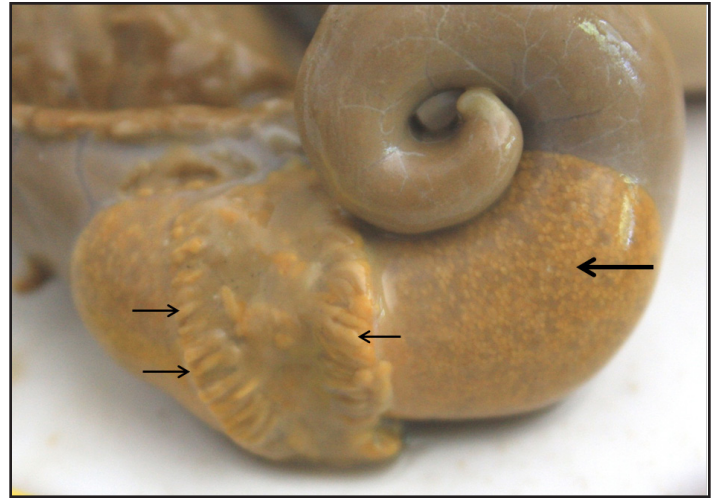
In the mature male gonad, seminiferous tubules still contain all developing stages of reproductive cells, but the majority of tubules become full of mature spermatozoa accumulating in dense masses in lumen. With all stages of spermatogenesis represented, continuous and/or protracted production of mature sperm is suggested. The visceral section of vas deferens becomes highly convoluted (coiled) along digestive gland (Figure 32), with convolution frequently observed to progress from gonad anteriorly towards
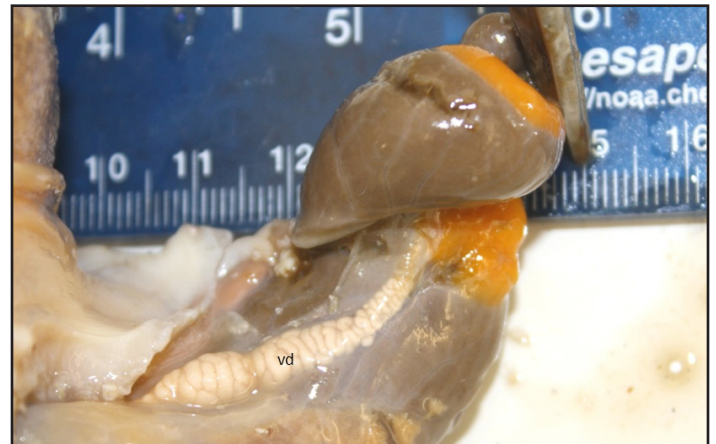

Figure 32. Convoluted (coiled) visceral section of vas deferens (vd) full of mature spermatozoa.

penis with maturity. If ruptured, mature sperm (spermatozoa) freely flows from vd. The most anterior section of vas deferens is often visible on large mature whelk running along dorsal surface (immediately below epithelial layer) of body to the base of penis (Figure 33). Gonad color darkens to an orange-red (Figure 34). Penis size increases through maturation, with a rapid increase in size observed as maturity is attained (Figure 35).
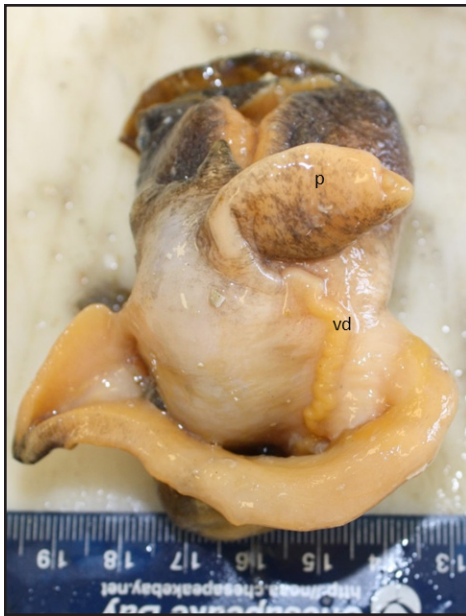
Figure 33. *Anterior section of vas deferens (vd) visible along the dorsal surface of mature male B. canaliculatus providing mature sperm to penis (p).*

Figure 34. *Male B. canaliculatus gonad (g) color: a) older mature male (149 mm SL) darker reddish color; b) younger mature male (125 mm SL) lighter, orange color. Whelk from VB area.*

Figure 35. *Increase in penis size and darkening of gonad color as maturity is reached in male B. canaliculatus; a) immature, b) early mature, and c) mature. Whelk from OC area. g=gonad, p=penis.*

Gonadal Somatic Index (GSI) Results

An increase in gonad weight was observed with increasing shell length in both sexes and correlated with advancing gametogenesis. This relationship was observed plotting the gonadal somatic index (GSI) over increasing shell lengths. Since sampling occurred only during two seasonal periods (fall 2009, spring 2010, fall 2010), only snapshots of gonad condition at these time periods were obtained, thereby not allowing comparison of gonad developmental stages between seasons within sexually mature whelk. However, late stage of gametogenesis was represented in sexually mature whelk from all sampling periods, thus allowing for correlation between whelk sizes (SL), GSI, and biological sexual maturity. Overall GSI values by sex were similar between resource areas sampled, likely due to the similar water temperatures in respective environments at time of sampling. In fall 2009 sampling male GSI values were observed to increase starting at SL between 115-120 mm for VB and ES males, but not until ~130 mm SL for OC males (Figure 36). In females, GSI values for VB females were also observed to increase at smaller sizes (140-150 mm SL), however, GSI for ES females were observed to closely coincide with values for OC females, both showing increases starting between 155-160 mm SL (Figure 37). Mature sex products in whelk
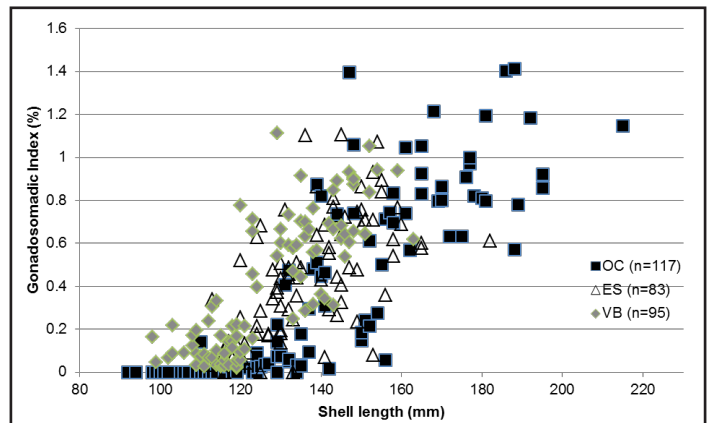

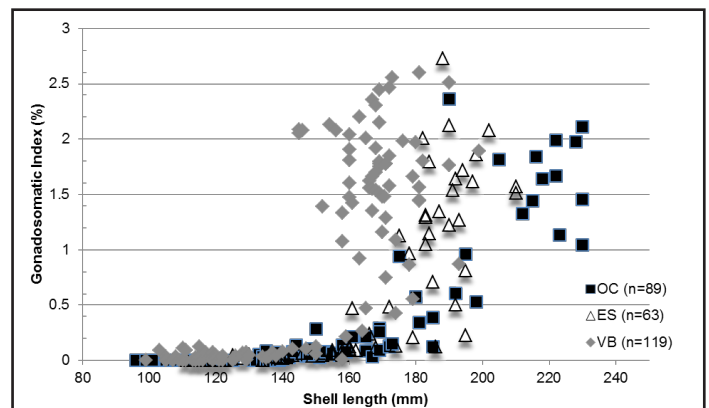*Figure 36.* *Male B. canaliculatus gonadal somatic index (GSI) by resource area late fall 2009.*

Figure 37. *Female B. canaliculatus gonadal somatic index (GSI) by resource area late fall 2009.*

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gonads were observed at GSI values >0.1 in females, and >0.2 in males. The GSI-shell length relationships for both sexes from all sampling areas in spring 2010 and fall 2010 sampling (Figures 38, 39 and 40) mirrored fall 2009 GSI-shell length relationships, indicating spawning occurring in both fall and spring for *B. canaliculatus*.

Penis Length

Penis length progressively increased with increasing shell length with a more rapid growth observed beginning between 110 mm and 115 mm SL in VB and ES males and between 120 mm-125 mm SL in OC males (Figure 41). This rapid increase in penis length correlated to shell lengths (PI range 0.10-0.15. Figure 42) in which rapid increases in GSI were observed and likely signify the onset of male sexual maturity for respective areas. Penis index (PI) values were generated (Figure 42) and used in evaluating male sexual maturity.

Maturity stages for *B. canaliculatus* were defined by gonad histology, evaluations of secondary sex indices, and gross anatomy and are summarized in Table 5. Female sexual maturity was determined using the following criteria: GSI > 0.2; ova 300-500 μm; CG medium to large, plump; and gonads granular in appearance. Male sexual maturity was determined using the following criteria: GSI >0.2; VD convoluted with sperm present; and PI=>0.15. Smallest mature and largest immature whelk found in this study is reported in Table 6.

Figure 39. B. canaliculatus. *Seasonal gonadal somatic index (GSI) from ES resource area, a) male, b)female. (ES=fall 2009, ESS=spring 2010, ESF=fall 2010)*
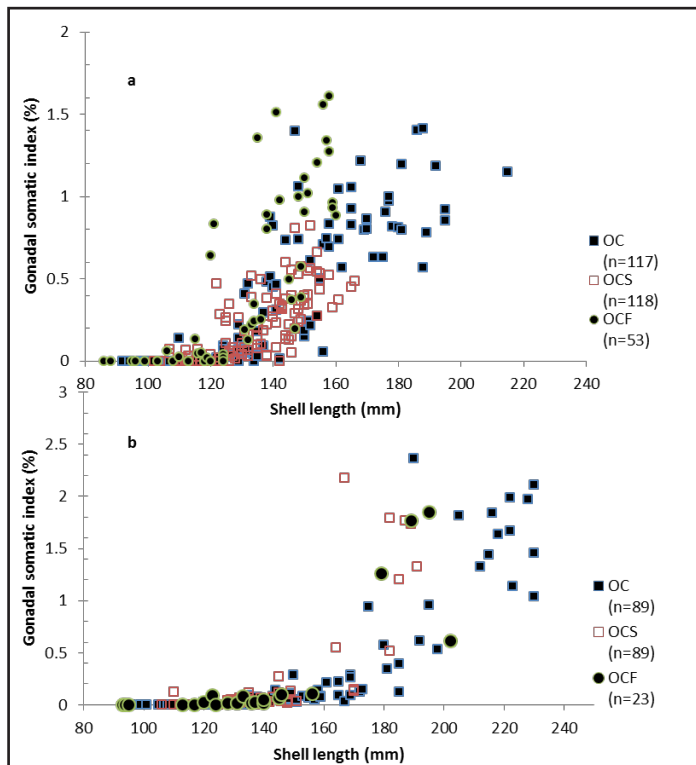

Figure 38. B. canaliculatus. *Seasonal gonadal somatic index (GSI) from OC resource area, a) male, b)female. (OC=fall 2009, OCS=spring 2010, OCF=fall 2010)*
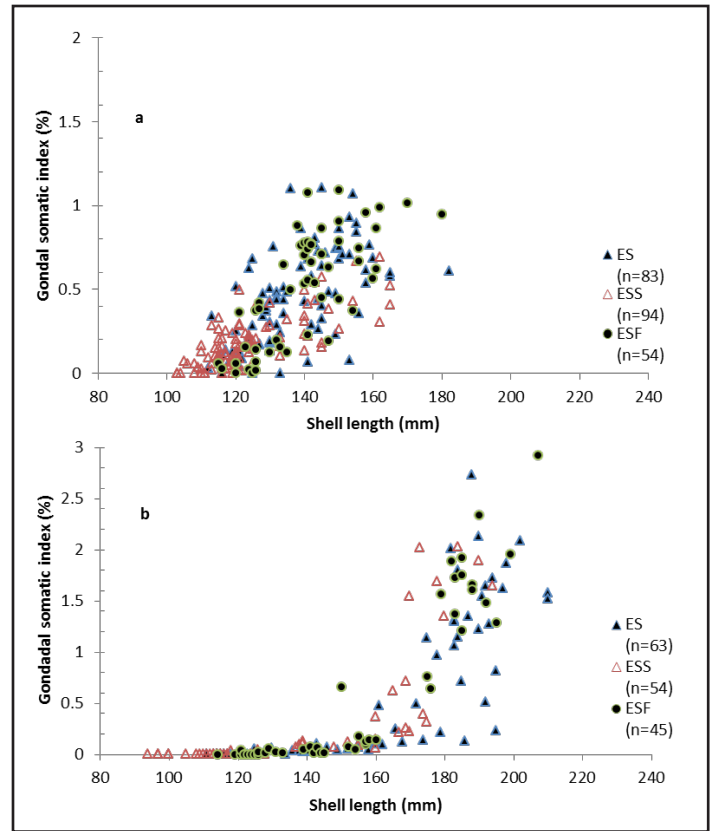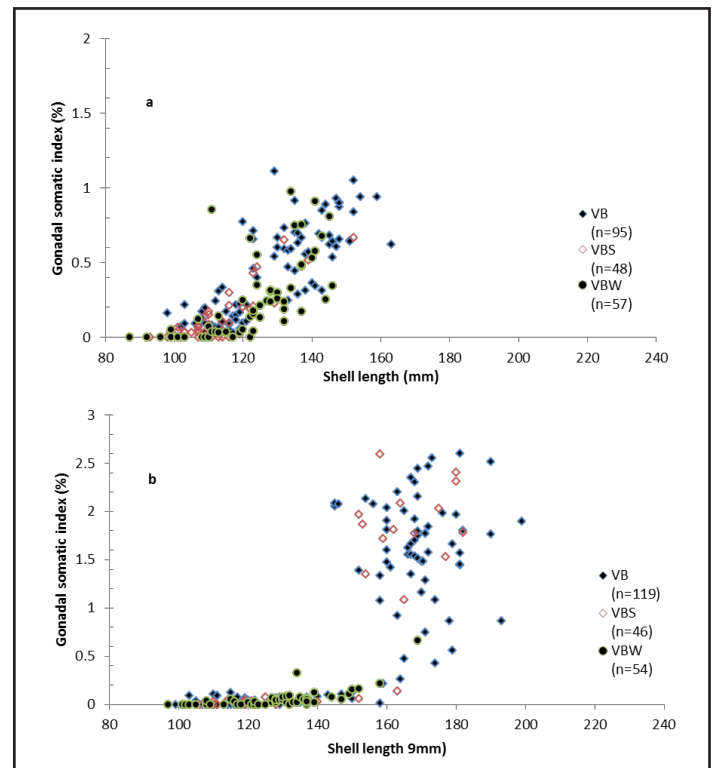

Figure 40. B. canaliculatus. *Seasonal gonadal somatic index (GSI) from VB resource area, a) male, b)female. (VB=fall 2009, VBS=spring 2010, VBF=fall 2010)*
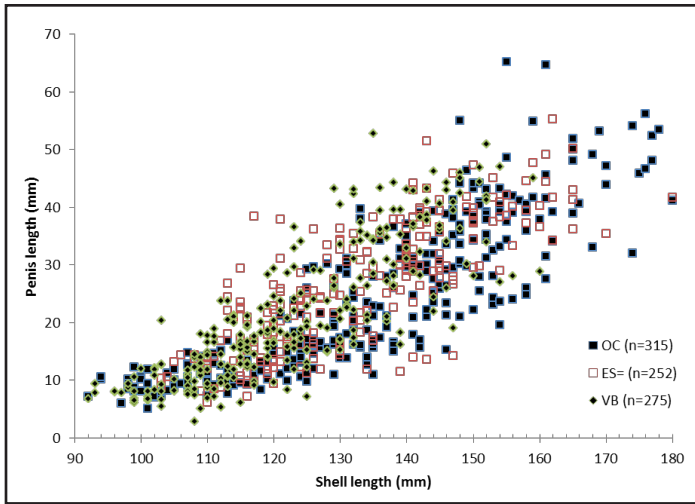
Figure 41. *B. canaliculatus.* *Male penis length-shell length relationship by area.*
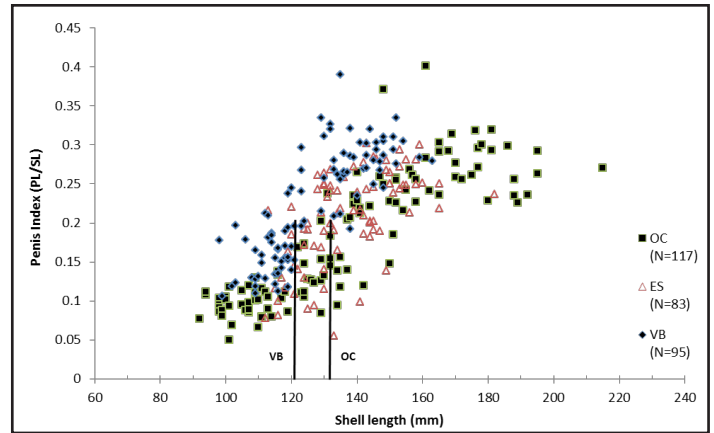

Figure 42. *B. canaliculatus.* *Relationship between shell length and penis index for male whelks from three resource areas (fall 2009). Vertical lines estimated 50% mature for VB and OC.*

	Immature	Maturing	Mature
Male	GSI <0.1	GSI 0.1-0.2	GSI >0.2
	PI < 0.1	PI 0.1-0.15	PI >0.15
	Vd; visceral section thin, straight, anterior section not visible	Vd; visceral section thickening, slight coiling during late stage, anterior section becoming slightly visible	Vd; visceral section highly coiled, full of sperm, anterior section prominently visible on body surface
	Gonad color light/pale orange	Gonad color pale orange	Gonad color dark orange/red
Female	GSI <0.01	GSI 0.01-0.1	GSI >0.1
	Gonad mostly CT appearing as a light band of tissue; Follicles small with pre-vitellogenic oocytes (oogonia) <10 μm	Early: follicles rounded with significant CT between, gonads lacking of texture, vitellogenic oocytes 50-150 μm with prominent nuclei and small yolk granules. Late: follicles expanding with increasing number of large ova (300-500 μm diameter) with large oval yolk granules (20-30 μm), texture firming, becoming granulated in appearance	Follicles columnar, densely packed with ova (300-500 μm) full of large, oval yolk granules and inconspicuous nuclei; gonads highly granular in appearance
	CG thin, beige, non-conspicuous	CG: early; small, beige, flaccid late; medium, beige, becoming firm	CG large, plump, light beige
	Gonad color off-white, translucent	Gonad color light, pale orange progressing to yellow/brown	Gonad color ranging from orange-yellow to yellow-brown

Table 5. *Definition of maturity stages of* B. canaliculatus. *(CG=capsule gland; CT=connective tissue; GSI=gonadal somatic index; PI=penis index; Vd=vas deferens)*

Area	Sex	Smallest Mature	Largest Immature
OC	Male	SL=122mm, GSI=.221, PI=.15, PL=19.8	SL=142mm, GSI=.015, PI=.12, PL=19.8
	Female	SL=144mm, GSI=.137, CG=medium/plump, firm	SL=159mm, GSI=.076, CG=medium/semi-flaccid
ES	Male	SL=113mm, GSI=.339, PI=.22, PL=24.4	SL=122mm, GSI=.089, PI=.14, PL=17.1
	Female	SL=143mm, GSI=.101, CG=small/plump, firm	SL=162mm, GSI=.089 CG=medium/flaccid
VB	Male	SL=103mm, GSI=.216, PI=.19, PL=20.3	SL=120mm, GSI=.094, PI=.17, PL=20.4
	Female	SL=143mm, GSI=.102, CG=medium/semi-firm	SL=158mm, GSI=.008, SG=medium/flaccid

Table 6. B. canaliculatus. *Smallest mature and largest immature whelks recorded with maturity measures. SL=shell length, GSI=gonadal somatic index, CG=capsule gland (relative size/condition), PI=penis index, PL-penis length.*

Maturity Ogives

The relationship between size and sexual maturity is indicated by maturity ogives for male and female *B. canaliculatus* from all three resource areas (Figure 43). The predicted
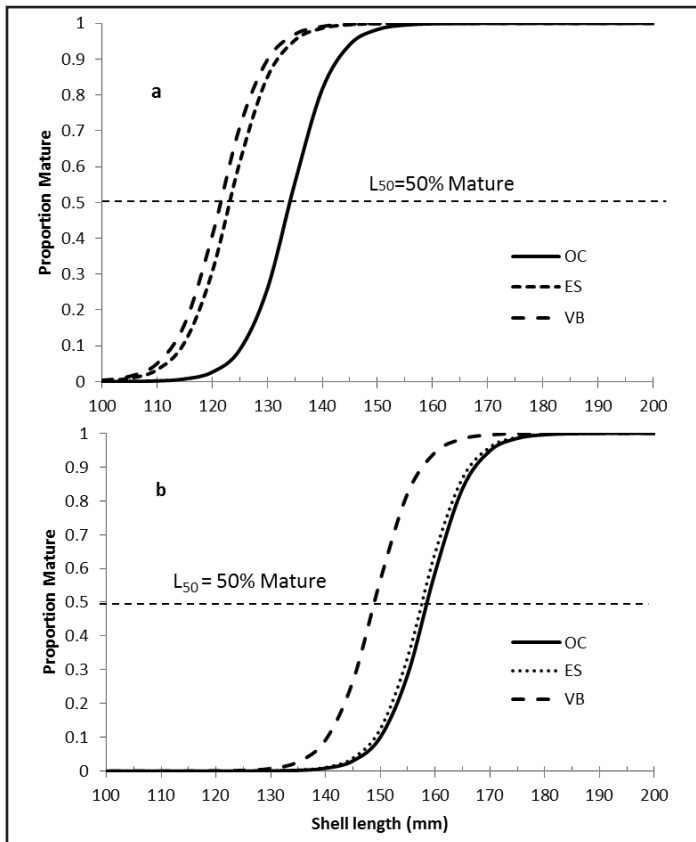

Figure 43. B. canaliculatus. *Maturity ogives for (a) male and (b) female by resource area.*

median shell length at 50% maturity for male and female with bootstrap 95% confidence intervals (Efron and Tibshirani, 1993) is represented in Table 7 for all areas. Males reach sexual maturity at smaller shell lengths than females within each area. Based on non-overlapping confidence intervals of the Bootstrap L50, as well as multiple contrast generated from logistic regression models, significant differences in size at maturity within areas are observed. Males from OC matured at a significantly greater shell length (134.05 mm) than both ES (122.51 mm) and VB (121.03 mm) males. Females from VB (149.21 mm) matured at a

significantly smaller shell length than ES (157.65 mm) and OC (159.28 mm) females. Penis length at maturity was predicted by a maturity ogive for males from each resource area (Figure 44). Predicted penis length at 50% maturity was 18.8 mm for ES males, 21.2 mm for OC males, 22.1 mm for VB males, and 21.1 for males from all areas combined.
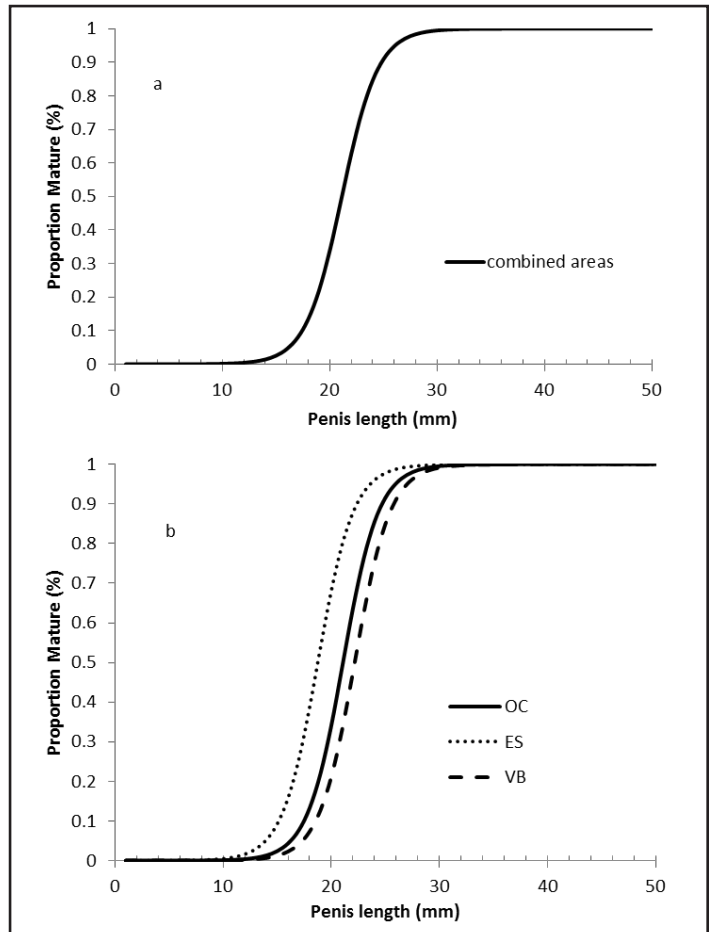

Figure 44. B. canaliculatus. *Maturity ogive of male whelks penis length for a) areas combined, and b) by area.*

Discussion

Both unimodal and bimodal distributions within whelk populations from different geographic locations have been reported (Shelmerdine, *et al.* 2006, Fahy *et al.*, 2000). In the bimodal distribution observed in OC of this study, the first peak (~120 mm well below MLS) suggests large number of recruits followed by high mortality, with the second peak (~160 mm above MLS) suggesting decrease in mortality with increase size. The first peak shows potential for recruitment to the fishery, though at size/age under that of estimated sexual maturity. Unimodal distributions were observed in ES and VB with peaks (~120-135 mm) less than MLS of 139.7 mm (5.5") for that resource area. A unimodal distribution reflects high recruitment followed by high mortality, as from predation or fishing pressure. If from

Resource area	Sex	Size at maturity (mm)	Lower Limit (95%)	Upper Limit (95%)
OC	F	159.28	153.09	165.25
	M	134.05	130.57	137.26
ES	F	157.65	154.28	161.80
	M	122.51	117.59	126.10
VB	F	149.21	145.17	153.03
	M	121.03	118.91	123.02

Table 7. *Predicted size at maturity for* B. canaliculatus*; area, sex, size at maturity, lower and upper confidence limits*

higher predation, morphological differences, as shell thickness (Thomas, M. L. H., *et al.* 1988) would be expected in *B. canaliculatus* whelk from these regions, but no such differences were observed. Greater fishing pressure, relative to smaller MLS in VB and ES may also contribute to this mortality. The strong similarity in whelk size distribution observed in VB and ES populations may suggest more synchronized spawning and recruitment than observed in the OC population, possibly related to more similar environmental conditions shared by ES and VB. The similarity observed in gametogenesis occurring in both sexes (macroscopically and histologically) in all three resource areas in fall and spring periods (2009-2011), coupled with GSIs indicative of maturing and mature whelk, also suggests synchrony in gonadal development in both sexes of *B. canaliculatus* over extended periods. With mature, ripe whelks present in both fall and spring seasons, multiple annual cohorts seem plausible.

When length frequency distributions are viewed in 1 mm bin size (Figure 45), the multiple peaks observed over SL are very distinct (high amplitude peaks with low amplitude between), initially giving appearance of strong annual cohort recognition. Many of these peaks are also observed with a secondary peak with less amplitude. However, the strength of, and distance (length) between the peaks, would suggest much slower growth than that determined in this study if representing annual cohorts. Multiple peaks over relatively short lengths suggest multiple annual cohorts. By binning size groups to reduce frequency "noise" over shell length, major peaks were identified which matched up with size ranges corresponding with whelk age estimates from this study (Figure 46).

In *B. canaliculatus* with SL > MLS of 5.5" (139.7 mm), 50.5%, 55.3%, and 76% were females in OC, ES, and VB areas respectively (fall 2009). In whelk with SL > MLS of 6" (152 mm), 52.1%, 74.1%, and 95% were females in OC, ES, and VB areas respectively. Though the sex ratio (M:F) was more similar within VB (1.03:1) than ES (1.3:1) and OC (1.3:1) areas, significantly more females comprised older, larger whelk
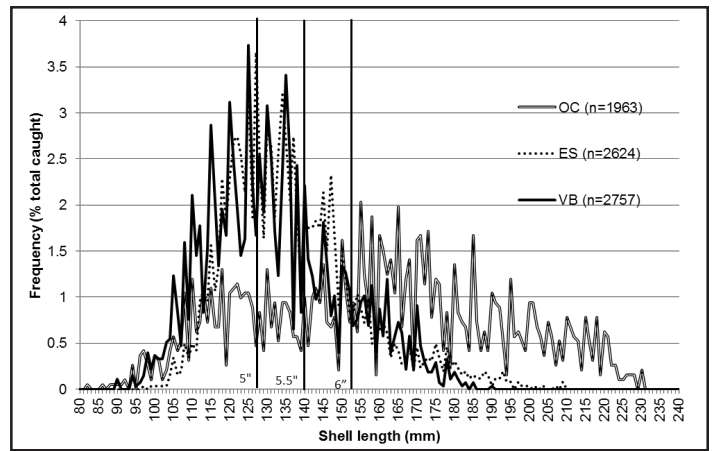

Figure 45. <u>B. canaliculatus</u>. *Length frequency distributions by area. Vertical lines represent current minimal landing sizes (MLS) for the region.*

in VB than ES or OC. Size dimorphism between sexes in whelk has been widely reported (Magalhaes, 1948; Weinheimer, 1982; Anderson *et al.*, 1985; Sisson and Wood, 1988; DiCosimo, 1988, Shalack 2007; Walker *et al.*, 2008). With the largest whelk within a population dominated by females, the question of sequential protandrous hermaphroditism within this species arises. However, no evidence
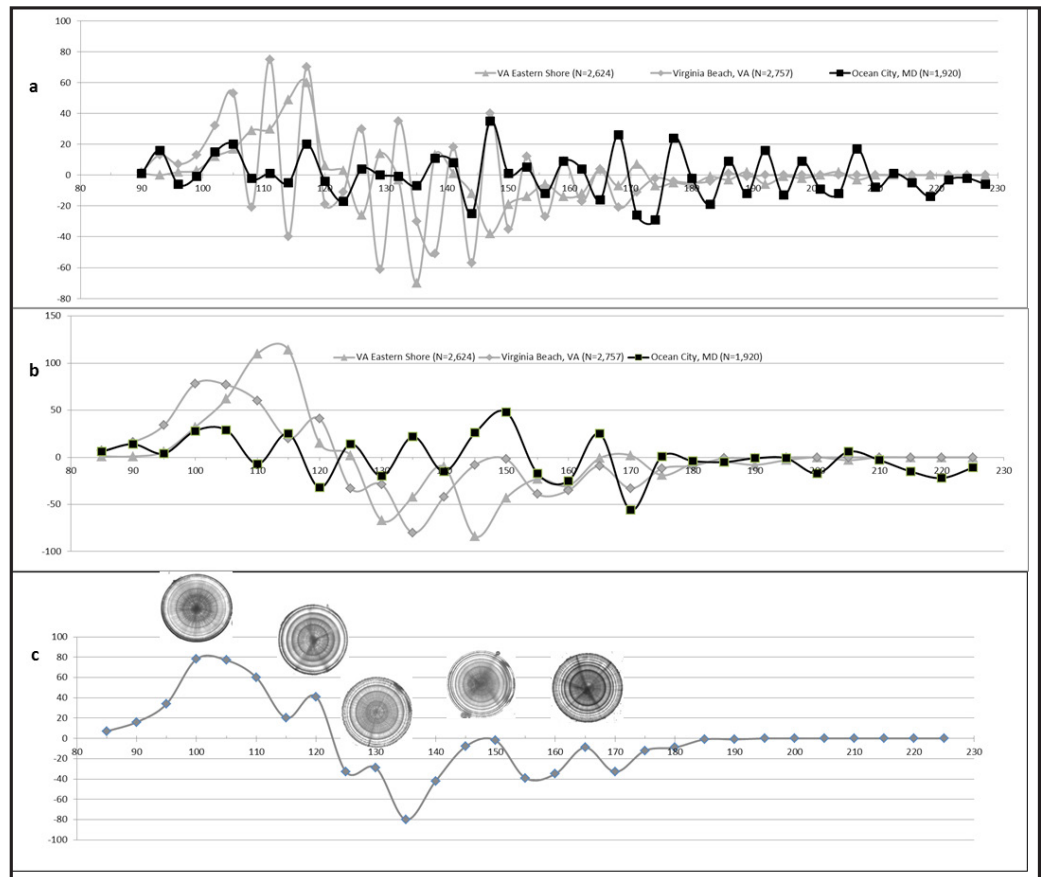

Figure 46. <u>B. canaliculatus</u>. *Length frequency distributions by area with shell lengths binned by a) 3 mm, and b) 5 mm, with c) showing 5 mm binned length frequency distribution of whelk from VB resource area and statoliths with estimated ages (4-8 yrs, left to right) corresponding with shell length ranges spanning distribution peaks*

of this reproductive variation was observed within this study, with all male and female *B. canaliculatus* possessing respective sex organs and secondary sex characteristics throughout life stages of growth and development (i.e., gonochoristic). Predominance of smaller whelk may be due to differences in growth rates and management MLS which favors the larger whelk. Observed size dimorphism within these populations may be a result of spatial and/or temporal variations at time of sampling. Habitat use within *B. canaliculatus* for any given life history parameter is poorly understood.

As in other carnivorous whelk, the possibility of sampling bias from selectivity of baited traps during periods of breeding and varying feeding rates between the sexes may exist (McQuinn *et al.* 1988, Power and Keegan 2001). From spring and fall 2010 sampling, *B. canaliculatus* distribution by sex of harvested whelk from baited traps remained similar to fall 2009 sampling in VB and ES, but significantly more males were observed in OC (Table 1). Though a significant increase in males was observed in OC 2010 samples, average size of males remained similar at size of sexual maturity. Average whelk size in both sexes from the 2010 sampling was also observed to decrease from the 2009 fall sampling. With similar states of reproduction (GSI and histological evaluation) observed in both sexes from all areas and sampling periods in this study, attraction to baited traps, and therefore sampling bias attributed to altered feeding rate associated with state of reproduction, was not discernible. Monthly sampling using non-selective sampling methods would likely provide a more accurate indication of *B. canaliculatus* sex ratio within each resource area. Catchability (q) is not evaluated as part of this study.

Age and Growth

Size at hatching (age 0) of *B. canaliculatus* in this study (4.01 mm+-0.18 mm SL) agreed with size at hatching (3.84 mm +- 0.13 mm SL) reported by Harding (2011), providing reasonable confidence for including time 0 for growth model estimation. The term "settlement ring" references marking on statoliths formed when larvae, from whelk species which larvae are released from capsules into the water as planktonic veliger larvae to complete development outside the egg capsule, reach a certain density in which they settle out, or metamorphose, from a pelagic life to a benthic life as juveniles. In *B. canaliculatus*, larval development into free crawling juveniles is completed within the egg capsule, hatching juveniles directly to benthic existence. In statoliths recovered from *B. canaliculatus* at time of hatching, no settlement, or hatching ring was observed, indicating its formation occurs at some point after hatching and during transformation to benthic life. Observing average statolith diameter of juveniles upon hatching (69.6 μm, n=8) and average settlement ring

diameter of adult *B. canaliculatus* over various size classes (72.4 μm, n=87) from all 3 study areas, the "settlement ring", or "hatching ring" can be considered to reference the period of time in which *B. canaliculatus* juveniles acclimate to benthic life shortly after hatching (observed between day 7 and 14 in this study), and at which demarcation enumeration of subsequent rings for aging commences. Similar environmental conditions between studied resource areas at time of hatching is suggested since no geographical variation in settlement ring size at formation was observed.

Obtaining age estimate for *B. canaliculatus* was most easily performed counting opercula stria, however, more precise aging was observed through annuli counts from sectioned statoliths. Operculum aging in this study underestimated age in comparison to aging results from sectioned statolith, even though readers were directed to account for year 1 stria which is less discernible in adult whelk and routinely not observed by readers during age estimation.

The lack of small, juvenile whelk collected in this study was largely the result of sampling gear selectivity where commercial trap design allows for escape of small whelks; however, since the incidental capture of small whelk <90 mm is rare in the fishery (only 4 whelk <90 mm out of 8,137 caught in traps during this study through various seasons, 82-89 mm), the possibility exists that juvenile *B. canaliculatus* do not share the same habit as adults. Further, these juveniles may also inhabit areas different from that of hatching since they have been rarely observed in the same habitats in which their egg strings have been found (Kraeuter *et al.*, 1989, Castagna and Kraeuter 1994, Power *et al.*, 2002).

In this study, *B. canaliculatus* are observed to mature at different sizes under different management MLS. Male *B. canaliculatus* growth rate was greater than females in all three resource areas, with males reaching sexual maturity at a smaller size and younger age. Males maturing before females is consistent with most dioecious prosobranchs (Runham, 1993), where females are reported to invest more energy in reproduction which delays maturation (Kideys *et al.*, 1993, Brokordt *et al.*, 2003). Spatial variation in size reached at sexual maturity has been reported for *Buccinum undatum* (Martel *et al.*, (1986, Kideys *et al.*, 1993; Kenchington and Glass, 1998), *Strombus gigas* (Avila-Poveda and Baqueiro-Cárdenas, 2006), and *B. caricas* (Power *et al.* 2009). In this study, size at sexual maturity between areas was observed to differ, but not consistently between the sexes. Males from VB and ES reached sexual maturity at smaller sizes than males from OC. This relationship may be expected since males from ES and VB grow considerably faster (higher k values) than OC males. However, in observing female growth rates (k) and size at maturity, VB females reached sexual maturity earlier than OC and ES, even though growth rates for VB

and ES females are similar. Rate of maturation may further be associated to the discarding of sub-legal whelk during commercial fishing. In this study percent sublegal whelks retained in traps, and therefore percent discard whelks, was much higher in VB (69%) than OC (43%) even though VB is regulated under the a smaller MLS. This situation is consistent with reports from Duncan *et al.* (1989) and Valentinsson *et al.* (1999) that sexual maturation is reached at smaller size in *Buccinum undatum* populations in which large whelk are heavily exploited. Investigation into alternative trap designs which allow for greater escapement of sublegal whelk may be warranted. Within the Virginia whelk fishery, the size composition of landings has decreased since the fishery's development, resulting in fewer large whelk meats (size grades) in the market. Smaller size composition reduces the unit value of the output from the fishery. Although shellstock prices are currently at record levels, they are a function of size composition and would be higher with a larger size composition. From yield observations in this report, increasing the MLS from 5.5" to 6" (139.7-152.4 mm SL) could result in a 42% increase in meat yield; re-establish market diversity and increasing whelk value. This study did not evaluate the size composition of the commercial catch to determine the corresponding loss of landings associated with a change in MLS.

Penis length 50% of SL is used for sexual maturity determination in the commercially exploited specie *Buccinum undatum* (Ilano *et al.*, Santarelli-Chaurand, 1985, Martel *et al.* 1986a, Lanteigne and Davidson 1992). Penis length measurements in the literature are not collected by a consistent method. For example, reports do not state if the penis is at rest or somewhere in between rest and fully extended when measured. In one example, the penis was first removed from body then measured (Power *et al.*, 2009). Throughout sampling of male *B. canaliculatus* in the current study the attached penis was consistently observed only at rest, folded once along its length with fold occurring near the base of penis. This resulted in the majority of penis length easily accessible for measuring, thus providing a consistent, more accurate and easily performed method of sampling. Estimate of 50% maturity using this method of penis measurement for *B. canaliculatus* ranged from 18-22% between areas sampled.

In this study, 28 males were observed with proportionally small penises. Though penis atrophy in gastropods has been associated to senility (Gendron 1992), penis atrophy in this study was observed in both mature and immature male whelk (101-176 mm SL, ave 133.6 mm) infected with digenetic trematodes, macroscopically observed as orange colored masses of cells (Figure 47) on the ventral side of digestive cecum (opposite and more anterior of gonad). Observed artifacts of infection by this parasite in male

Figure 47. *Male B. canaliculatus (141 mm SL) with large, orange mass of parasitic cells (m) on digestive gland. Note small size of penis (p).*
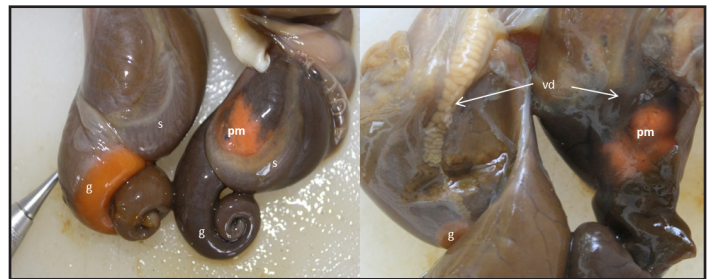

Figure 48. *Dorsal (a) and ventral (b) views of visceral sections of two mature male B. canaliculatus. Whelk on right in each view (145 mm SL) contains a parasitic mass (pm) resulting in vestigial vas deferens (vd) and gonad (not visible), and atrophied penis (not shown). Whelk on left (164 mm SL), not infected with parasite, with functional vd and gonad. g=gonad, s=stomach*

whelk were penis atrophy and non-functional (vestigial) gonads and vas deferens (Figure 48). At 50% mature, male penis length ranges from 118-122 mm in normal, non-infected whelk. Average penis length of parasitized males at SL>50% mature (n=20) was 8.0 mm. Infection by this parasite was also found in immature and mature females (113-185 mm SL, ave 145.6 mm), which resulted in less developed capsule glands in infected whelk. Histologically, the masses contain sporocysts with developing cercaria (Figure 49), suggesting that *B. canaliculatus* serves as an intermediate host for this parasite. In total, 41 of 1604 (2.5%) *B. canaliculatus* observed in this study were infected with this parasite (13 female, 28 male). Infected whelks were found in all 3 areas during each sampling period (fall, spring) and were not included in age, growth, or reproductive maturity evaluation in this study.

Though breakage of the siphonal canal may result due to feeding strategies of whelk (Malgalhaes 1948; Kraeuter *et al.*, 1989), it routinely results from on-board handling/
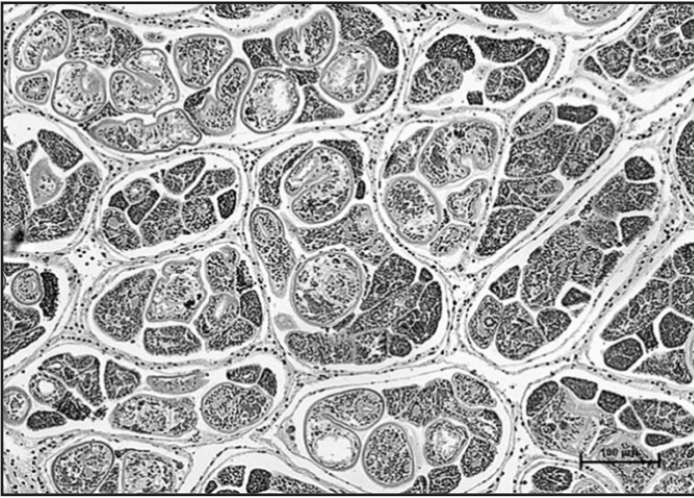
Figure 49. *Histological section of parasitic mass showing sporocysts with developing cercaria of digenetic tremotods.*

culling practices (personal experience) in the *B. canaliculatus* fishery, thereby preventing accurate SL measurement and giving rise to management interest in SW measurement for regulatory MLS. An accurate and efficient means to measure channeled whelk SW would need to be clearly defined since maximum nominal linear shell diameter cannot accurately be measured by traditional low-profile cull rings due to the conical-irregular shape of *B. canaliculatus*. Whelk of a given SW diameter can easily be passed through a cull ring of same diameter by changing the angle of whelk orientation to the ring as whelk is passed through the ring. A 3" (76.2 mm inside measurement) cull ring will readily allow up to a 90 mm (linearly measured) channeled whelk to pass through. As a possible alternative, a tubular cull devise with length equal to or greater than the whelk SL would be need to maintain whelks perpendicular orientation to tubes opening (plane of linear measurement), thereby prevent manipulation of whelk linear maximum width.

Under current MLS for each area, whelk harvested from VB recruited into the fishery at a much younger age to those from OC (Table 7). The probability of females reaching MLS prior to sexual maturity is quite low given current MLS (Table 8).

Location	Sex	MLS (mm)	Calculated Age @MLS	Probability of Maturity @MLS
Ocean City	Female	152	6.74	0.157
Ocean City	Male	152	7.12	0.990
Eastern Shore	Female	139.7	5.85	0.010
Eastern Shore	Male	139.7	6.32	0.985
Virginia Beach	Female	139.7	5.00	0.086
Virginia Beach	Male	139.7	5.60	0.990

Table 8. <u>B. canaliculatus</u>. *Estimated age and probability of maturity at which whelks reach minimum landing size (MLS) as calculated from von Bertalanffy growth parameters by sex and area.*

This work has shown that regional variations of growth and maturity exist in *B. canaliculatus* within the Mid-Atlantic region of the US. Other aspects of *B. canaliculatus* life history characteristics require consideration for conservation management, mainly their sedentary existence, long life span, and significant sexual dimorphism in age at maturity. Being sedentary benthic animals in which free crawling juveniles emerge from egg capsule directly to benthic existence (lack of planktonic larval stage), limited dispersal occurs, creating localized stocks which could be easily overexploited. Males maturing at sizes below MLS are allowed to reproduce prior to legal harvest; however, successful recruitment to a fishery is dependent on female reproduction capacity, and females have been shown in this study to reach maturity at size greater than current MLS.

In fisheries management, the intent of most MLS is to identify a harvestable size which allows for juveniles to survive and spawn at least once. The information provided in this report for channeled whelk resource areas in the Mid-Atlantic region, in particular size at sexual maturity, sex composition, and vulnerability to overfishing, provide for harvesting conservation considerations for management within the *B. canaliculatus* fishery. Results indicate that current MLS's are inappropriate for sustainable management of the *B. canaliculatus* resource. Size at first maturity as calculated in this study indicate that immature whelks are removed from the fishery under current MLS, impacting recruitment success of the populations and increasing risk of overexploitation. This report strongly suggests that MLS regulations and fishery management plans for individual resource stocks of *B. canaliculatus* should be reevaluated relative to their reproductive biology and population dynamics in order to ensure the sustainability and productivity of the fishery and the resource.

Acknowledgements

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