An Investigation Into Ageing Methods For Horse Clams (*Tresus nuttallii and T. capax*)

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Abstract

Campbell, B.N., Groot, J.B., and Mahannah, S.M. 2009. An investigation into ageing methods for horse clams (*Tresus nuttallii* and *T. capax*). Can. Tech. Rep. Fish. Aquat. Sci. 2765: iii + 25 p.

Age determination methods for the horse clams *Tresus nuttallii* and *T. capax* were first investigated by Zhang and Pegg upon these species being identified as candidates for Fisheries & Oceans Canada's new and developing fisheries program (Zhang and Pegg, unpublished). The present study reviewed this work and utilized their method of exposing the chondrophore cross-section to reveal the growth pattern for age determination. Horse clams (n=338) from Tofino, B.C. were aged with a high degree of precision between independent age readings and criteria for interpreting growth patterns was reviewed. Ages obtained ranged from 2–24 yrs. for *T. nuttallii*, and 4–29 yrs. for *T. capax*, which are respectively 2 and 11 years older than previously known maximum ages. Burning and staining techniques were investigated to increase contrast where annuli are closely spaced and difficult to differentiate.

Résumé

Campbell, B.N., Groot, J.B., and Mahannah, S.M. 2009. An investigation into ageing methods for horse clams (*Tresus nuttallii* and *T. capax*). Can. Tech. Rep. Fish. Aquat. Sci. 2765: iii + 25 p.

Zhang et Pegg (article non publié) sont les premiers à avoir étudié les méthodes de détermination de l'âge de deux espèces de fausse-mactre (*Tresus nuttalli* et *T. capax*) après que ces espèces ont été identifiées comme candidates pour le nouveau programme de pêche en développement du ministère des Pêches et des Océans. La présente étude a porté sur l'examen des travaux de Zhang et Pegg et sur l'utilisation de leur méthode d'exposition de la coupe transversale de chondrophores pour révéler le régime de croissance aux fins de détermination de l'âge. L'âge des fausses-mactres (n = 338) de Tofino (C.-B.) a été déterminé par différents chercheurs, indépendamment les uns des autres, et les résultats obtenus concordaient fortement. De plus, les critères d'interprétation des régimes de croissance ont été élargis. Les âges obtenus variaient entre 2 et 24 ans pour *T. nuttallii* et entre 4 et 29 ans pour *T. capax*, les âges maximums observés étant respectivement 2 et 11 ans supérieurs aux maximums observés par le passé. Les techniques de brûlage et de marquage ont été étudiées afin de déterminer leur efficacité à accroître le contraste quand les anneaux de croissance sont peu espacés et difficiles à distinguer.

Introduction

Since 1979, the horse clams *Tresus nuttallii* (Conrad 1837) and *T. capax* (Gould 1850) have been incidentally harvested alongside the geoduck fishery in British Columbia. Investigation into ageing methods for these species began in 2004/2005 as part of Fisheries & Oceans Canada framework for management of new and developing fisheries and the subsequent requirement for age data for management purposes. This research compared external and internal methods for ageing horse clam valves and developed criteria for interpreting growth patterns (Zhang and Pegg, unpublished). In 2005, the Sclerochronology Lab at the Pacific Biological Station received horse clam valves collected from Tofino, British Columbia with a request to determine if a method for producing age data from these species could be established. The investigators of the present study reviewed the work initiated and made available by Zhang and Pegg. Methods they had employed for processing horse clam chondrophores to reveal the internal growth pattern were examined and a preferred method was selected and applied to this study. Our study also reviewed Zhang and Pegg's criteria for interpreting annual growth patterns. Burning and staining techniques were briefly explored as a method of increasing contrast between growth zones where they appeared tightly spaced and difficult to differentiate.

Bi-valve Microstructure and Growth of Horse Clams

Growth increments in the microstructure of bivalve shells appear as alternating zones of opaque and translucent material. These zones are a manifestation of the relative proportions of conchiolin, a proteinaceous matrix and aragonite, one of three calcium carbonate polymorphs which comprise the shell (Rhoades and Lutz, 1980). Factors influencing growth (patterns) are water temperature, food supply, light, and other environmental variables. Relatively high temperatures and abundant food increase growth rates and create relatively wide incremental growth zones with proportionately greater amounts of aragonite. Winter temperatures and a decline in food result in a relatively narrow growth increment with proportionally less aragonite, reflecting a reduced growth rate. In this way, bivalve shell components are not unlike fish otoliths, however, growth zones in bivalves may be a result of a more complex process than material simply being deposited at seasonally variable rates (Rhoades and Lutz, 1980).

The chondrophore is an internal spoon-shaped shelf projecting from the hinge region which supports the ligament in some bivalves. Growth patterns viewed on the cross-section of the chondrophore from horse clams also appear as narrow zones of conchiolin within broader aragonite zones. When viewed under reflected light, the conchiolin zone appears translucent and the broad aragonite zone appears opaque. This study utilizes growth zones observed on the cross-sections of the chondrophore as a chronometer.

Literature indicates spawning occurs from April to August for *T. nuttallii* in British Columbia and mid-February to May for *T. capax* (Campbell, et. al., 1990). The larval veliger stage is estimated to be between 21 and 30 days, depending on water temperatures. Juvenile horse clams grow rapidly after settlement for the first three years. Thereafter, coinciding with the onset of maturity, growth slows and gradually decreases with age. The majority of annual growth occurs in late spring and summer, ceasing by October (Bourne and Smith, 1972). Growth rates for *Tresus spp*. are highly variable and dependant on water temperatures, food availability, and other environmental conditions. Growth may vary between subtidal and intertidal specimens within a bay and significant differences also occur between beds and between year classes in the same bed. Sexually mature *T. capax* from Seal Island, B.C. were found to be 70 mm. in length at 3 years of age, while 100 mm. clams were 5 years old (Bourne and Smith, 1972). Maximum ages of *T. nuttallii* and *T. capax* in British Columbia have been reported to be 22 years (shell length 220 mm.) and 18 years (shell length 180 mm.) respectively, interpreted by valve surface examination (Lauzier, et al., 1998).

Methods and Materials

Horse clams (315 *T. nuttallii* and 23 *T. capax*) collected from Tofino, British Columbia June 11, 2004 were submitted to the Sclerochronology Lab with a request to investigate ageing methods for these species. This study began by conducting a review of results reported and provided by Zhang and Pegg (unpublished). Structures from three processing methods of *Tresus* chondrophores processed by Zhang and Pegg were also made available to the investigators for review. The structures examined were: bisected chondrophores, thin sections of the chondrophore mounted on glass slides, and acetate "peels" of the growth pattern mounted on slides. Chondrophores bisected along the axis of greatest growth produced clearly visible growth patterns and required less time and materials than the other methods and therefore was adopted as the preferred method to examine growth patterns for the 338 horse clams aged in this study. The samples were divided among the authors for processing and age reading. Investigators initially collaborated on ageing the first 18 structures to ensure consistency in interpreting the growth patterns, thereafter ageing was done independently.

Valve Processing

Bisecting chondrophores

Preparing shells for age determination was a two-step process. The chondrophore is first excised from the valve to obtain a manageable structure and then bisected.

The umbo/chondrophore area from the left valve was excised using a hand-held Dremel® rotary tool (Dremel, Racine, WI, U.S.A.) under a fume hood to confine dust particles created during cutting (Fig. 1). Two blade types were used: #426 (reinforced cut off wheels) and #420 (heavy duty cut off wheels), either one effective.

Structures were then bisected through the centre of the umbo along the longest axis of the chondrophore (Fig. 2). This initially was done using a high-speed bone sectioning machine with a diamond tip blade, however further experimentation determined that using a low-speed Buehler® Isomet saw (Buehler Canada, Whitby, On., Canada) with a diamond tip blade allowed for more control while cutting (Fig. 3). A layer of plasticine on the chuck of the saw accommodated the various sizes and morphology of structures and also secured them for sectioning. Processing time and comments were recorded and a variety of shells were earmarked for photo-documentation of processing results. Sections were labelled and placed into coin envelopes for age determination.

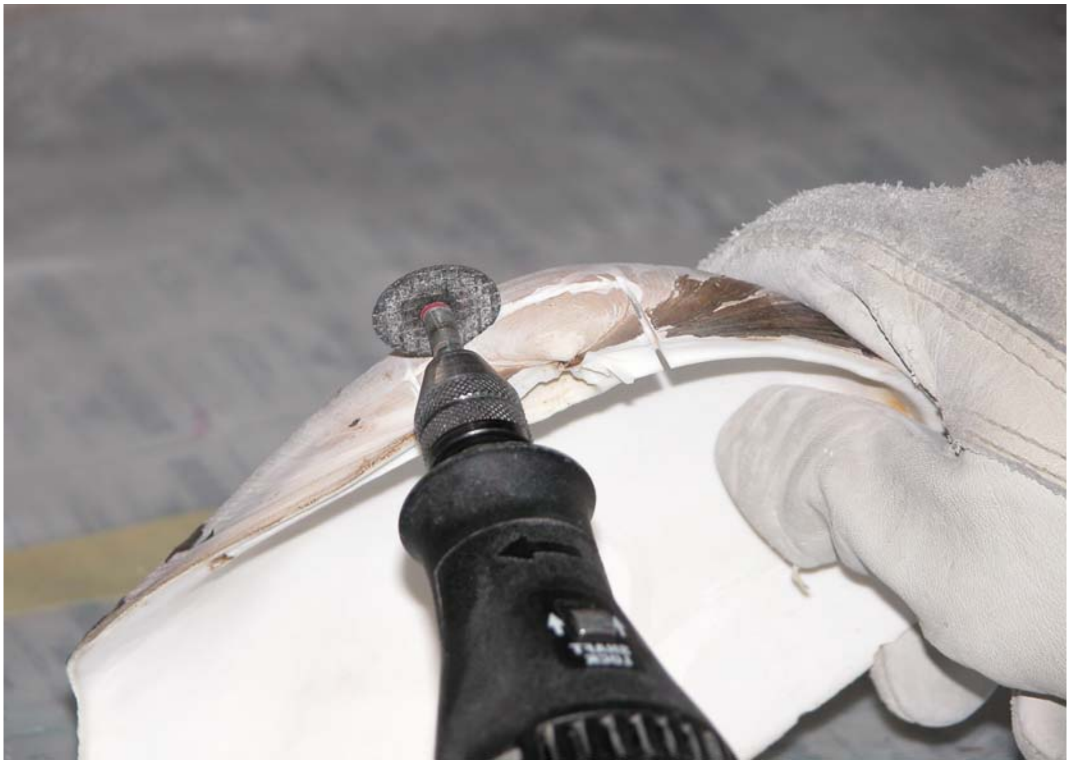

Fig. 1. Dremel® tool used to excise chondrophore from left valve.

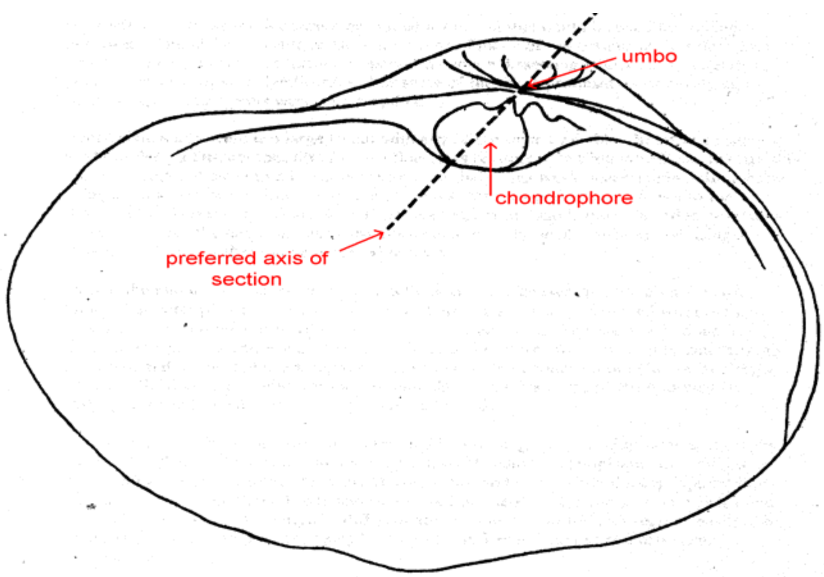

Fig. 2. Axis for bisecting chondrophore from Zhang and Pegg (unpublished).

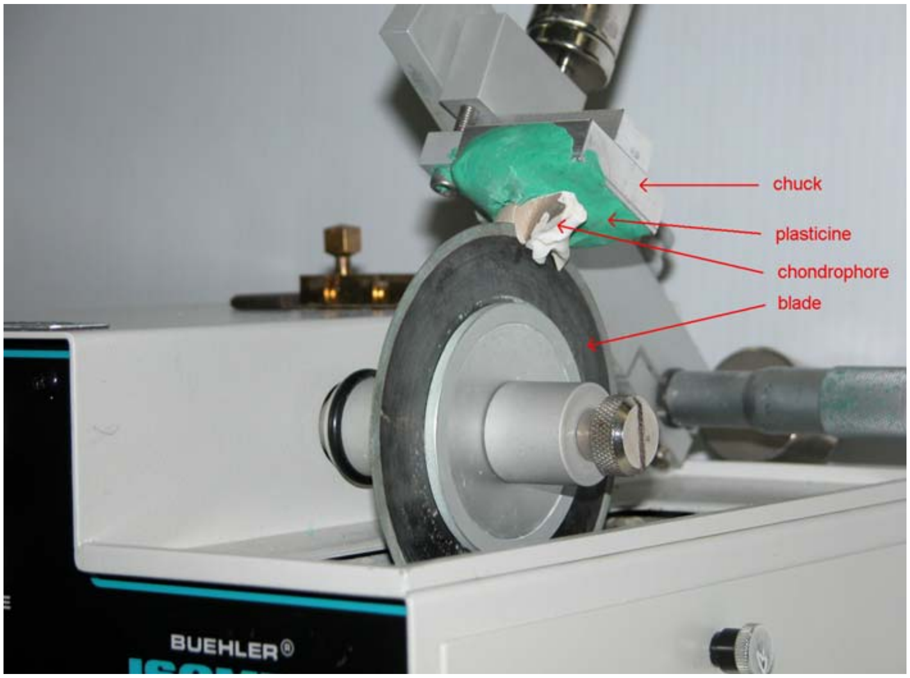

Fig. 3. Chondrophore being bisected with low-speed saw.

Pattern Enhancement

The translucent zones on older horse clam chondrophore cross-sections are often closely spaced and occasionally indistinct toward the growing edge of the structure. Our study experimented with four methods to increase contrast between the translucent and opaque zones where this occurred.

The first method utilized was to direct a flexible fibre-optic arm from the light source at roughly 90º to the structure to illuminate annuli from the side rather than from above.

Secondly we applied the “burnt otolith” technique used to emphasize annuli on many species of fish otoliths (MacLellan, 1997). This method involves waving the exposed cross-section over a 95% ethanol flame at several centimetres distance until an amber brown color is reached. The mineral differential of the shell microstructure results in the narrow translucent zones burning darker than the wider opaque zone, improving growth pattern contrast.

Following the same approach, other cross-sections were placed on a laboratory hot plate at a high setting with growth patterns face down for up to 30 minutes to determine if this would highlight annuli.

We also investigated various staining methods to increase contrast between growth zones (Albrechtsen, 1968; Bouain and Siau, 1988; Richter and McDermott, 1990).

The histology laboratory at Pacific Biological Station was consulted for recommendations and to determine what stains were available (Bennett, pers. comm.). Ten stains selected for their affinity to either calcium carbonate or proteins were obtained from the histology lab in powder or liquid form (Table 1). Stains obtained in powder form were mixed to a solution of 2% with distilled water in a fume hood. Stains in liquid form were used at the concentration already mixed. Ten structures were placed under a fume hood in a bed of plasticine to hold the structures upright. A small clean paint brush was used to coat each cross-section surface with a staining solution and the time of application was noted. Contact time ranged from one hour to over 72 hours. Structures were then rinsed with water and examined to assess absorption.

Table 1. Stains with concentrations utilized to highlight annuli.

Stain	**% conc.**	**Comments**	**Shell #**
Alcian Blue	1		T789
Alizarin Red S	2	with acetic acid (required to dissolve powder)	T588
Carbol Fuchsin	.005	5% phenol/10% alcohol	T793
Eosin	.5		T717
Fast Green	1		T574
Gentian Violet	2	with acetic acid (required to dissolve powder)	T619
Giemsa	1		T676
Jenners	.4		T723
Methylene Blue	2		T666
Toluidine Blue	2		T598

Age Determination

A Leica MZ7.5 dissecting microscope with 10X eyepieces and 3-arm fibre-optic lighting (Fig. 4) was used to examine the chondrophore cross-section for annuli. An annulus is defined as a growth zone which forms once a year (Chilton and Beamish, 1982). The narrow slow-growing translucent zone formed in winter is typically counted in assessing age. This zone and the preceding wider opaque zone together comprise the annual zone (Fig. 5).

Fig. 4. Leica dissecting microscope with Leica light source.

Digital callipers were used to measure the distance from the origin of the umbo to the end of the first and second annulus where the zones converge onto the concave surface for a minimum of 81 *T. nuttallii* and 23 *T. capax* (Fig. 6). These two annuli were established by identifying prominent translucent zones appearing closest to the measurements obtained by Zhang and Pegg for the first and second year (3.5 mm. and 6.2 mm. respectively). Measurements obtained in the present study were then used to calculate the average distance for the first and second year for each species and compared with measurements from Zhang and Pegg's findings. Where annuli were difficult to distinguish, fibre-optic light was directed to highlight the growth pattern. Occasionally specimens were burned over an alcohol flame to further enhance contrast near the margin tip.

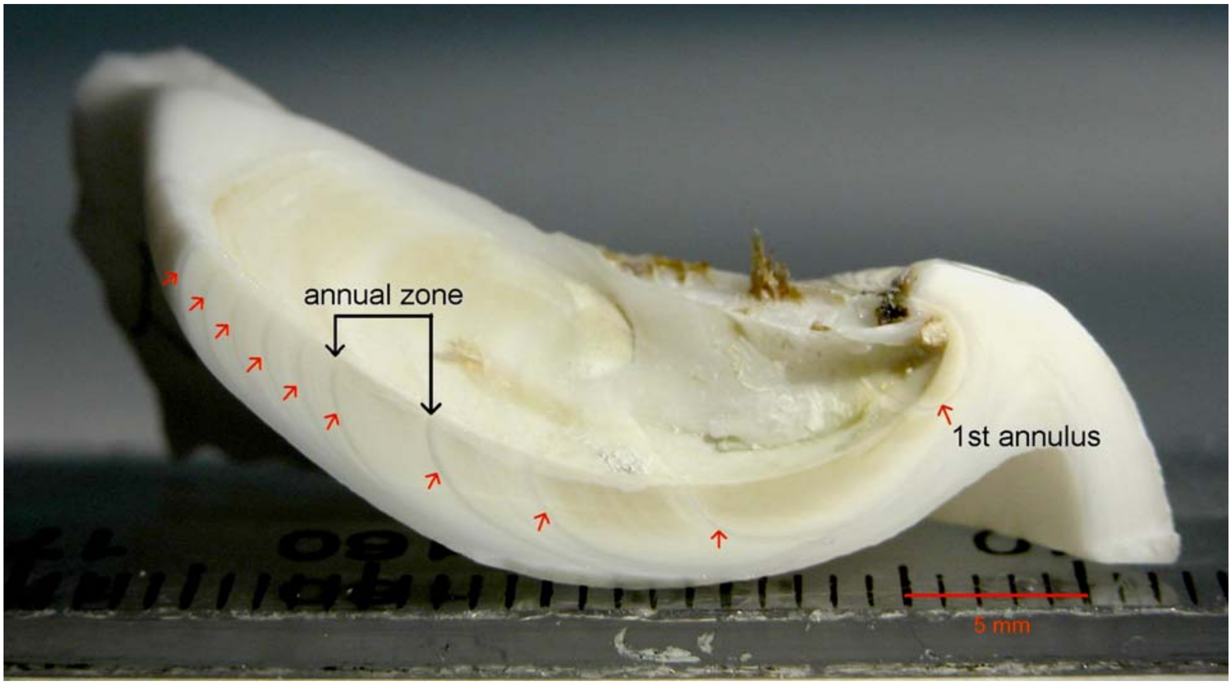

Fig. 5. Growth pattern on *T. nuttallii* chondrophore cross-section with 10 annuli (translucent zones) indicated by red arrows.

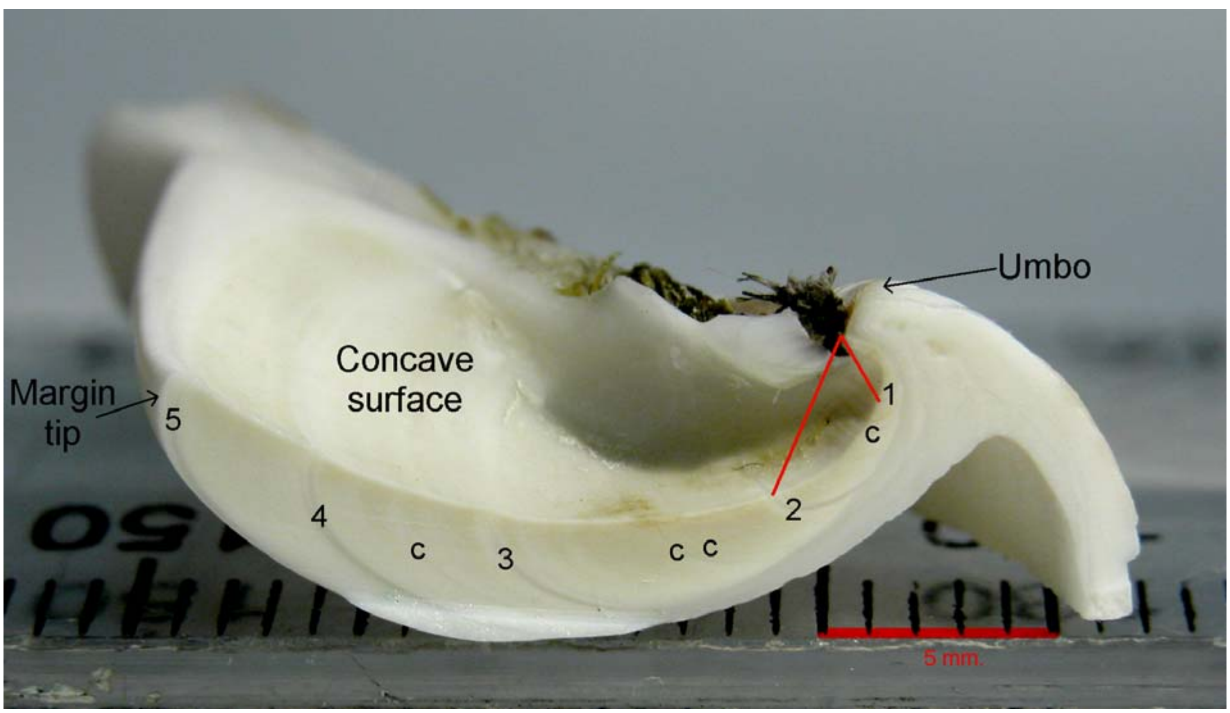

Fig. 6. Chondrophore cross-section showing measurement axes (red lines), margin tip, umbo, faint checks (c) and 5 annuli.

Growth patterns were analysed and the following information recorded for each specimen: number of annuli, a qualitative assessment of the growth pattern regularly applied to groundfish species aged in the Sclerochronology Lab (good, fairly good, fair, or fairly poor), the presence of edge growth (growth beyond last annulus), significant transitions in growth rate, presence of checks, uneven and even growth patterns, faint annuli, absence of first annulus, and any other notable characteristics of the growth pattern. Where one or more annuli were questionable, ages were annotated with a “plus or minus X number of years”, indicating an age range for the specimen, a standard method for capturing uncertainty in the Sclerochronology Lab.

After the first age reading by an investigator a minimum of 20% of the specimens were randomly selected for independent ageing by a second reader to obtain precision results, a method of quality control routinely used in collecting age data (Morison, et.al. 2005). Because of the small sample size, all remaining *T. capax* were independently aged by a second reader after the precision test was completed. Average percent error (APE; Beamish and Fournier, 1981) was calculated for *T. nuttallii* where there were 2 independent ages (first reading and precision test ages). This was done for two groups of cohorts, 0-5 years and over 5 years, as well as for all cohorts collectively. A single APE was calculated for all *T. capax*. Any differences in age designation were resolved by the readers re-examining the structure and reaching a consensus on interpreting the growth pattern. Age and length frequency distribution were graphed as well as age versus shell length, for *T. nuttallii*.

Results

Valve Processing

Bisecting chondrophores

Approximately 25 – 30 chondrophores per hour could be excised from valves using the Dremel® tool. Approximately 20 – 25 chondrophores per hour could be bisected to reveal the internal growth pattern using the Isomet low-speed Isomet saw.

Pattern Enhancement

Transmitting light through the structure from the side proved the most practical method to differentiate annuli near the margin.

Burning structures over an alcohol flame produced inconsistent results. Annuli were emphasized to various degrees on some structures but not all.

The hot plate method did not highlight annuli. After more than a half hour, very little to no increase in contrast was observed.

Staining of *T. nuttallii* shells to differentiate annuli on older specimens also produced generally unsatisfactory results in improving contrast between the seasonal growth zones on the cross-sections. Some areas appeared heavily pigmented with stain while other areas of the same structure appeared pallid, resulting in little zone distinction (Fig. 7). Eosin did display some affinity for conchiolin and was the most uniformly absorbed of the stains, however, it did not result in substantially improving annuli clarity (Fig. 8). Results were inferior to what was obtained by either fibre-optic light manipulation or by slight burning of the structure over an alcohol flame.

Age Determination

Our study found annual growth patterns were typically quite clear particularly on specimens under 10 years and ages were generally assigned with little difficulty. The first annulus usually appeared quite small followed by 3 or 4 relatively large years. Growth then slowed either considerably or more gradually after 4 or 5 years and annuli frequently became closely spaced by 12 or 13 years. Annuli could be quite crowded on some specimens by the late teen years. A minimal amount of edge growth was observed on the large majority of specimens. Several displayed a moderate amount of edge growth and some showed none. We also noted the same challenges reported by Zhang and Pegg associated with locating the first annulus, differentiating between checks and annuli, and distinguishing annuli near the margin on older specimens where they became crowded.

The first annulus in this study measured on average 2.2 mm. ± 0.6 (n=81) for *T. nuttallii* (Table 2), and 2.4 mm. ± 0.6 (n=23) for *T. capax* (Table 3). Occasionally the first annulus was absent. Our measurement to the second annulus averaged 6.2 mm. ± 1.4 for *T. nuttallii* (n=91) and 6.2 mm. ± 1.1 for *T. capax* (n=23).

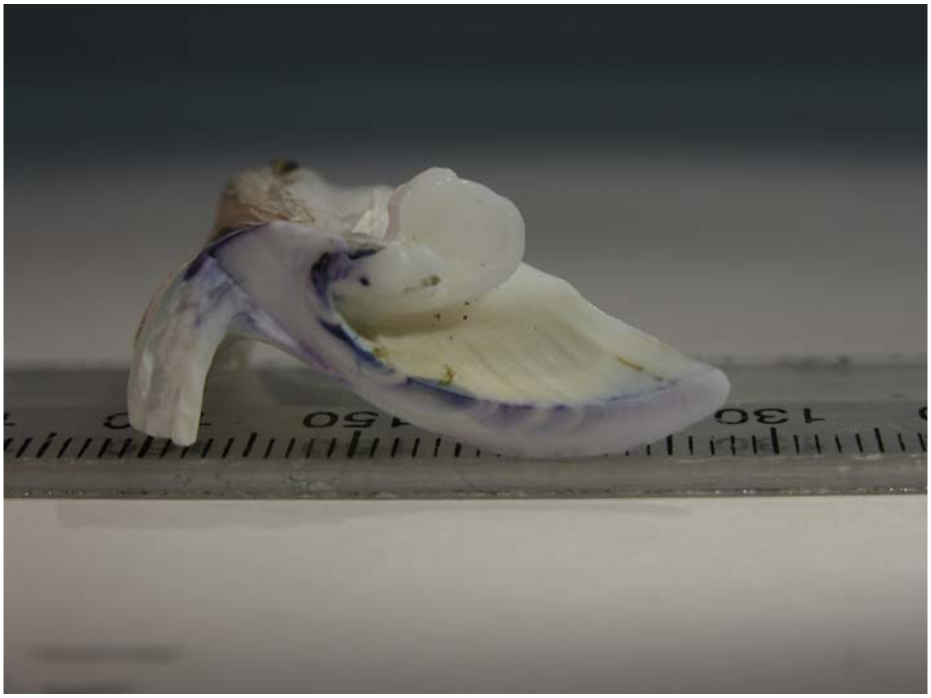

Fig. 7. Chondrophore T598 stained with 2% Toluidine Blue solution.

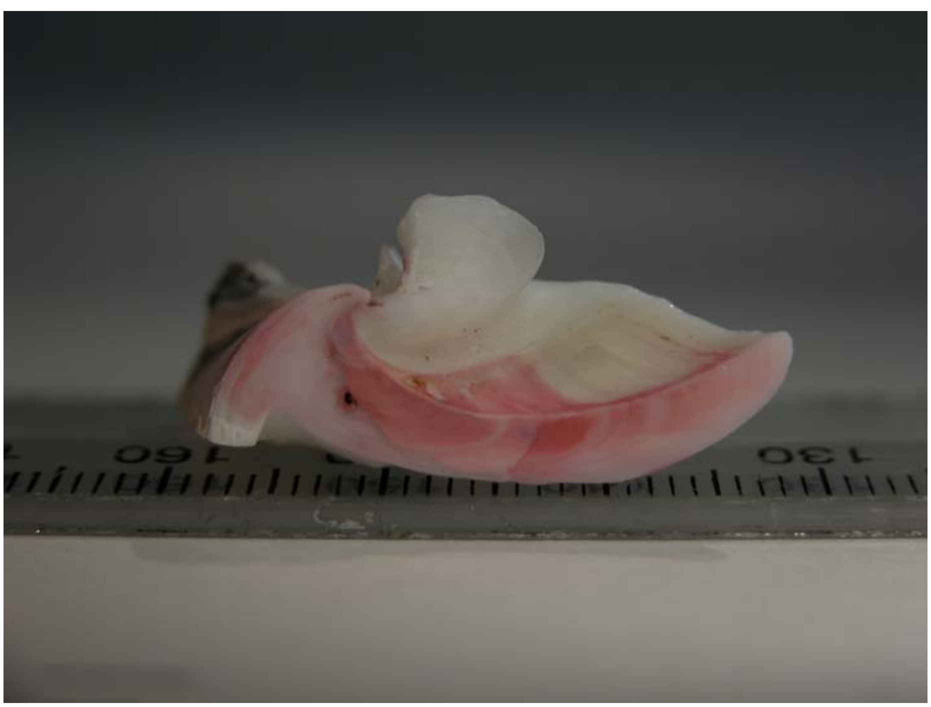

Fig. 8. Chondrophore T717 stained with .5% Eosin solution.

Table 2. Distance (mm.) from umbo to 1st and 2nd annulus for *T. nuttallii*.

Clam number	Age (yrs.)	1st year	2nd year	Clam number	Age (yrs.)	1st year	2nd year
T501	6	3.49	6.41	T552	10	2.36	8.02
T502	9	2.58	7.34	T554	9	2.61	6.43
T503	5		6.41	T555	13	2.89	7.64
T504	10	3.53	9.11	T556	16	1.47	5.41
T505	8	3.02	6.88	T563	6	2.49	7.10
T506	7	2.83	6.80	T564	6	2.53	7.27
T507	12	2.83	7.52	T565	6	2.90	6.92
T508	6	1.31	5.81	T566	4	2.18	7.28
T509	7	2.35	7.59	T567	5	1.23	3.81
T510	3	2.94	7.88	T569	4	2.19	7.33
T511	7	1.89	6.28	T570	4	2.86	8.06
T512	5	1.09	6.12	T572	4	2.38	7.92
T513	13	1.90		T575	10		7.14
T514	13	3.20	6.50	T576	10	1.86	5.53
T515	14	2.89	6.99	T579	6		5.89
T516	9	3.07	6.90	T581	5	2.42	5.83
T517	6	2.17	7.33	T585	10		7.33
T518	14	2.09	6.08	T592	5	0.90	5.05
T520	10	3.37	8.45	T594	4		7.07
T521	5	1.76	7.11	T595	9	3.82	8.43
T522	5	1.20	5.04	T596	6	1.26	4.77
T523	3	2.58	7.54	T597	14	1.38	4.27
T524	4	2.50	7.40	T601	18		4.07
T525	3	2.39	7.67	T603	5	1.07	5.60
T526	9	2.35	5.06	T604	5	1.98	6.97
T527	5	2.83	7.56	T612	8	1.78	5.55
T528	14	1.16	4.30	T615	6	2.97	7.20
T529	16	2.40	6.15	T616	5	1.90	6.00
T530	14	2.08	6.10	T617	5	1.70	6.17
T531	6	2.66	5.97	T620	5	1.36	6.00
T532	6	2.81	7.24	T627	4	2.64	8.18
T534	11	1.99	5.97	T628	6	2.50	6.27
T535	13		5.98	T629	5	0.94	4.47
T536	5	1.81	5.88	T630	4	1.72	5.58
T537	6	3.46	7.34	T632	3	2.97	7.64
T539	9	3.12	7.49	T636	14	1.55	5.00
T540	16	1.97	5.35	T638	13	1.40	4.58
T542	5		4.96	T639	23	1.51	4.25
T543	5	2.04	6.47	T640	3	2.54	5.81
T544	7	1.88	7.11	T642	4	1.36	6.55
T545	4	0.75	6.91	T690	5		6.38
T546	4	1.88	7.11	T703	5		5.68
T547	3	1.98	6.81	T737	7	3.96	8.90
T548	3	2.40	6.39	T744	9	2.91	8.59
T550	4	3.43	9.04	T810	4	2.52	7.10
T551	10	2.83	6.97	T816	7		7.49

Mean = 2.2 mm. ± 0.6 (n=81) for 1st year and 6.2 mm. ± 1.4 (n=91) for 2nd year.

Table 3. Distance (mm.) from umbo to 1st and 2nd annulus for *T. capax*.

Clam number	Age (yrs.)	1st year	2nd year	Clam number	Age (yrs.)	1st year	2nd year
T500	13	1.04	4.33	T792	9	3.4	5.31
T574	29	1.62	3.96	T793	21	1.38	5.16
T634	4	1.64	6.63	T794	9	2.52	6.4
T721	5	1.93	6.38	T795	9	2.22	6.17
T723	15	3.34	7.87	T796	9	3.08	6.56
T724	9	1.78	5.02	T797	16	2.06	6.92
T725	5	1.91	6.87	T798	9	3.18	6.83
T726	6	2.45	4.99	T831	6	3.49	7.33
T788	6	3.16	6.31	T832	10	3.79	7.28
T789	15	2.7	7.64	T833	7	1.36	6.71
T790	6	2.26	5.06	T844	9	2.44	5.96
T791	18	2.43	6.41				

Mean = 2.4 mm. ± 0.6 (n=23) for 1st year and 6.2 mm. ± 1.1 (n=23) for 2nd year.

Discontinuities occurring within the growth pattern were determined to be "checks" if they did not appear to form annually (Fig. 6). Checks by definition do not form seasonally but indicate physiological or environmental changes which are reflected in the growth pattern (Chilton and Beamish, 1982). They were not uncommon in horse clam growth patterns particularly in the juvenile years. These interruptions usually appeared irregularly spaced and indistinct but were occasionally more prominent. Some specimens displayed "double annuli", that is two prominent and closely-spaced narrow translucent zones representing one year (Fig. 9). Typically several or more double annuli appeared in succession to create a distinctive repeating pattern of two close translucent zones and a large opaque zone.

It was observed that the growth patterns from the small sample of *T. capax* were overall less clear and displayed more checks than those of *T. nuttallii*.

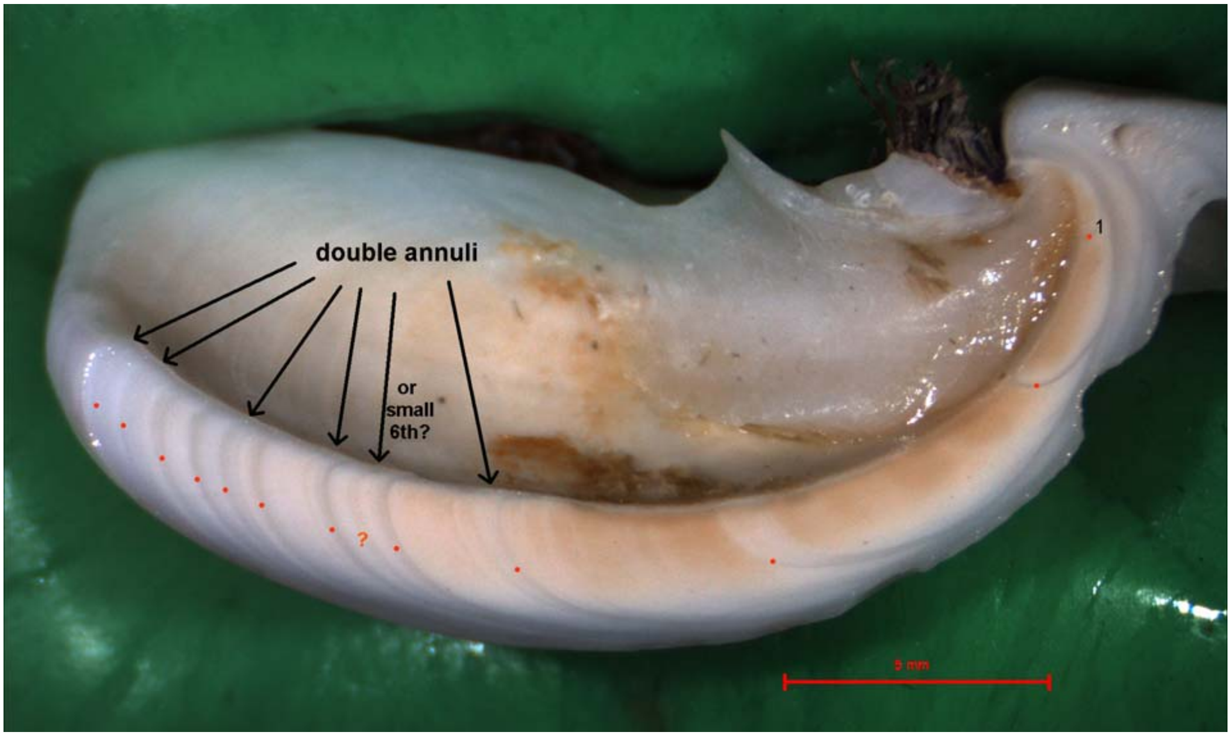

Fig. 9. *T. nuttallii* chondrophore cross-section displaying “double annuli”, age 12 yrs.

Our study recorded ages ranging from 2 – 24 years for *T. nuttallii* (n=315), and 4 – 29 years for *T. capax* (n=23). A relatively strong year class of 5 year old *T. nuttallii* appeared from the 1999 spawn comprising 19.4 % of the sample (Fig. 10). Shell lengths from 170-185 mm. comprised 35.0% of the total sample (Fig. 11) and represented an age range from 5 years to over 20 years old (Fig. 12). Length data was not available for 4 of the 315 *T. nuttallii* shells aged.

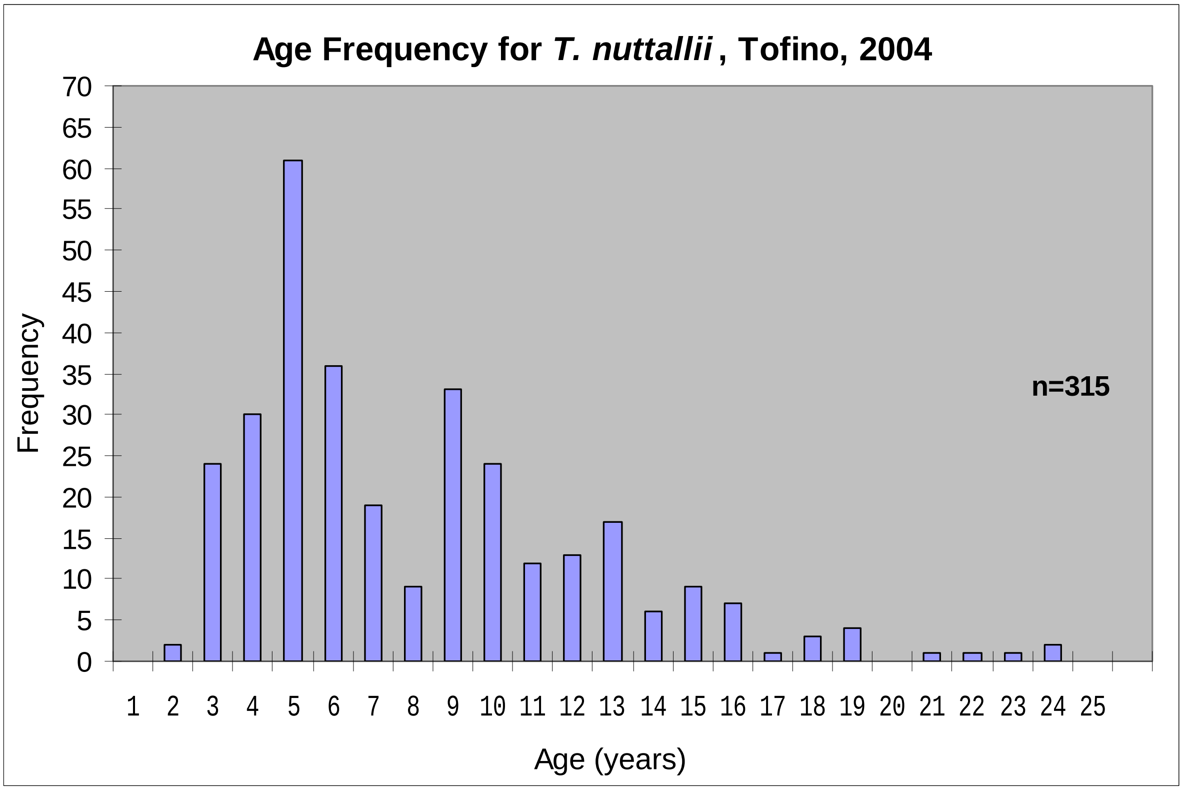

Fig. 10. Age frequency distribution of *T. nuttallii*.

Precision test results (n=61) between the three age readers showed an average agreement for *T. nuttallii* from the combined sample of 315 specimens of 82% ± 0, and 98% ±1 year, indicating independent reader agreement for 82% of *T. nuttallii* specimens and within 1 year for 98% of the sample. *T. capax* precision results (n= 10) were 57% ± 0, 82% ± 1 year, and 85 % ± 2 years for the sample of 23 animals. Average percent error for *T. nuttallii* collectively was 0.8% (n=61), 0.0% for specimens 0 to 5 years old (n=25), and 1.4% for *T. nuttallii* over 5 years old (n=36). *T. capax* had an overall APE of 1.1% (n=23). The greatest single difference in age designation between any two readings was 8 years for a *T. capax* specimen which was resolved at 29 years old.

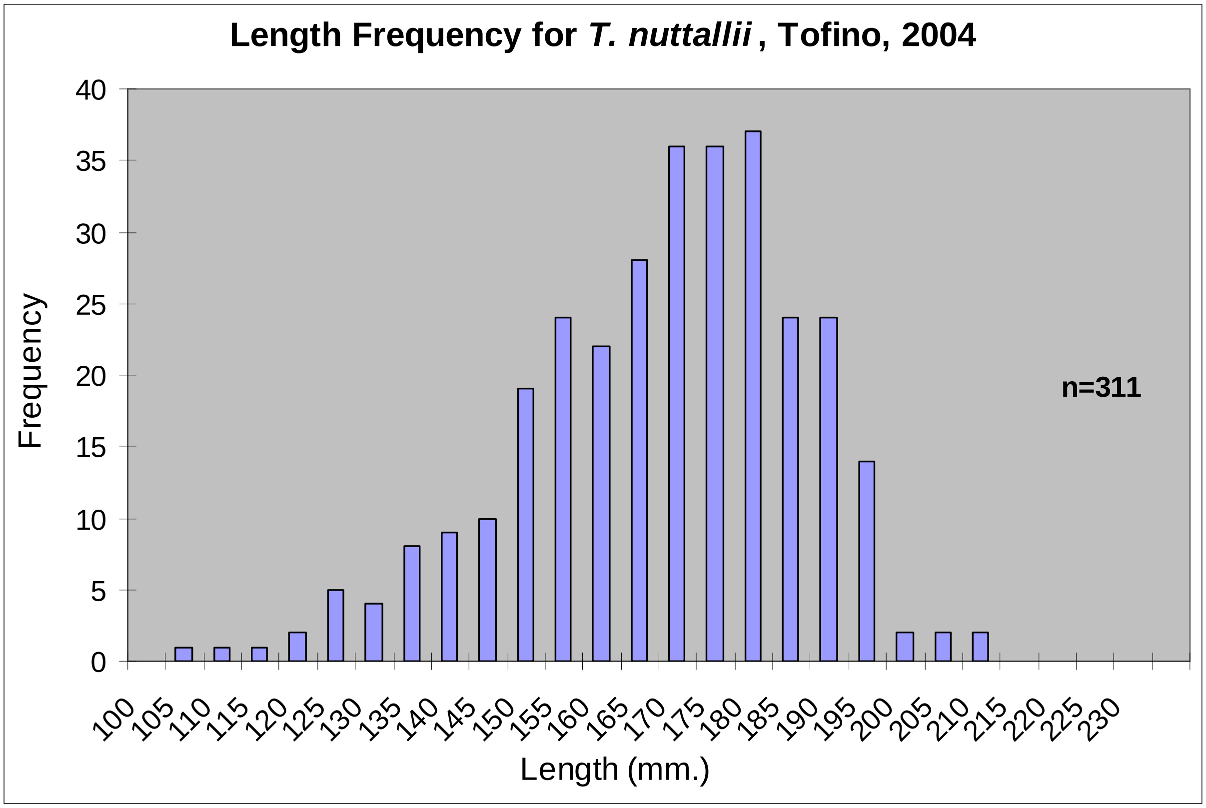

Fig. 11. Length frequency distribution of *T. nuttallii*.

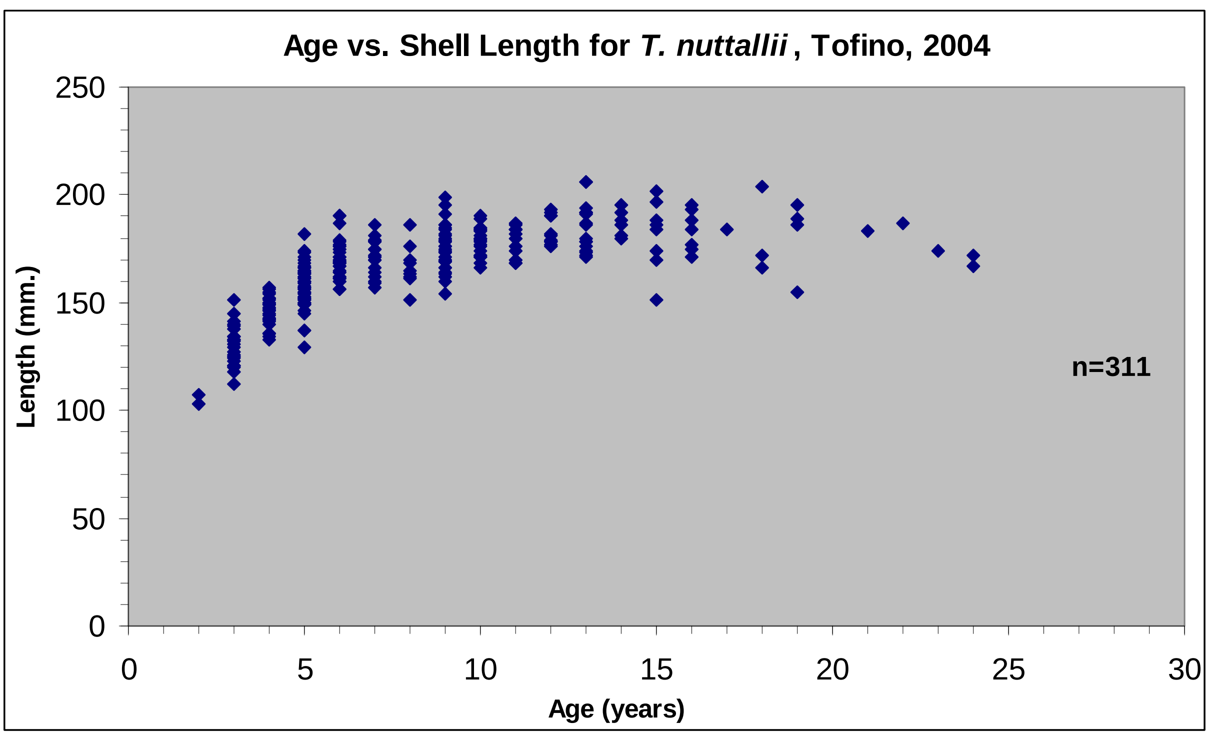

Fig. 12. Age vs. shell length for *T. nuttallii*.

Discussion

Valve Processing

Bisecting chondrophores

After examining structures previously processed by Zhang and Pegg, our study established bisected chondrophores were preferable to view the growth pattern for age determination. This method of processing requires fewer materials and less time than either the thin section or ‘acetate peel’ methods and in most cases produces equal or better results. Removing a thin section from chondrophores for mounting on glass slides involves the most cutting and introduces handling concerns as thin sections are quite delicate. The “peel” method utilizes 5% hydrochloric acid, acetone and entails prolonged work under a fume hood but may be worthwhile for a subset of samples. We recommend using the bisected chondrophore for ageing these species on a production basis.

Shell morphology and differences in individual dimensions made securing the shell in a rigid vice to excise the chondrophore awkward. The rotary tool was reasonably efficient in excising the chondrophore and holding the shell by hand offered more manoeuvrability and control. Wearing a heavy leather glove offered protection and the process was regarded as safe.

The high speed bone cutting saw initially used for bisecting the chondrophore was discontinued due to the difficulty of aligning shells in an inflexible chuck in order to obtain a cut along a predetermined axis. We found the low-speed saw was more efficient and although slightly slower at cutting, it ultimately saved time as less manipulation was required to position the structure for sectioning. The malleable plasticine chuck accommodated individual differences in the structure’s size and its placement provided the operator with a superior view of the structure. The blade could be aligned directly against the umbo resulting in a more precise cut along the preferred axis.

Pattern Enhancement

We recommend directing the flexible fibre-optic gooseneck of the light source to illuminate the structure from the side as a first effort in highlighting the growth pattern. In many cases this was effective in providing the necessary contrast to distinguish annuli near the margin.

Burning the structure over an alcohol flame was an efficient technique and increased contrast quite well for some specimens. However, results were inconsistent, possibly due to the difficulty in precisely controlling the burning process or slight individual differences in elemental ratios. This method could be used when manipulating fibre-optic light does not adequately differentiate crowded annuli.

Our experiments with stains were largely unsuccessful. The duration of contact between stain and cross-section surface was initially one hour. After rinsing and examination revealed no trace of absorption, stains were re-applied and contact time was gradually extended several times, eventually lasting over 72 hours. Examination of structures after this period also showed unsatisfactory results for all stains. Absorption varied across the surface of the cross-sections and none fixed consistently to either the conchiolin or aragonite zone. The technique was often least effective near the margin where contrast between zones was most desired. This study tested stains readily available from labs at the Pacific Biological Station and should be considered a cursory experiment. Further investigation could perhaps reveal a more appropriate stain and refine the technique.

Zhang and Pegg etched cross-sections with hydrochloric acid and created acetate peel replicates of growth patterns to view under a projecting microscope. They found this method (Ropes, 1984), also used to prepare geoduck shells for ageing, increased detail and the large image facilitated in distinguishing crowded annuli. Our review of structures prepared by Zhang and Pegg agreed that this technique is superior for elucidating crowded annuli at the margin. It is recommended when other methods are inadequate but would be unnecessary for all structures on a production ageing basis.

Age Determination

The annual growth patterns of *T. nuttallii* and to a slightly lesser extent, *T. capax*, were typically clear and supported early life history findings from past growth studies. Where occasional complications in interpreting growth patterns emerged, uncertainty usually occurred with establishing the first two annuli and/or identifying checks.

Identifying 1st and 2nd annuli

Our investigation employed criteria developed by Zhang and Pegg to position the first two annuli on the growth pattern. They had determined a combined average distance of 3.5 mm. for the first year for *T. nuttallii* and *T. capax* from the Comox area of the Strait of Georgia, B. C., and 6.2 mm. for the second year. These measurements were utilized as ‘range finders’, thereby assisting in the precise location of these years for our sample. Our measurements to the first and second annuli then helped to corroborate Zhang and Pegg’s results in return.

The size of the first annulus in this study varied, perhaps a function of protracted spawning dates, variable growth rates, and possible deviation from the origin of the umbo during the sectioning process which could potentially exclude the first year entirely. These factors as well as two diverse sample sites likely contributed to mean differences for the size of the first year between this study and Zhang and Pegg’s. Occasionally we noted the first annulus was missing as the first prominent growth zone occurred where the second annulus was consistently located according to measurements. In this case, the first year was assumed and counted. Our result of 6.2 mm. to the second annulus for both species and also between our samples and

Zhang and Pegg's samples proved a reliable reference point and led to considerable confidence in identifying the second year and counting a first annulus when it wasn't prominent or was absent.

Identifying checks

Checks are characteristically weak in appearance relative to the more prominent annuli, often appear in close proximity to annuli, and typically do not occur with the regularity of annuli on the growth pattern. Distinguishing between checks and annuli is based on relative prominence of the zone in question, the spacing of prospective checks in relation to the annual pattern, and the experience of the age reader. These factors were involved to varying degrees in identifying checks in the fast growing juvenile years, the transition area where growth is slowing, and the slow growing adult years where annuli are relatively closely spaced (Fig. 13).

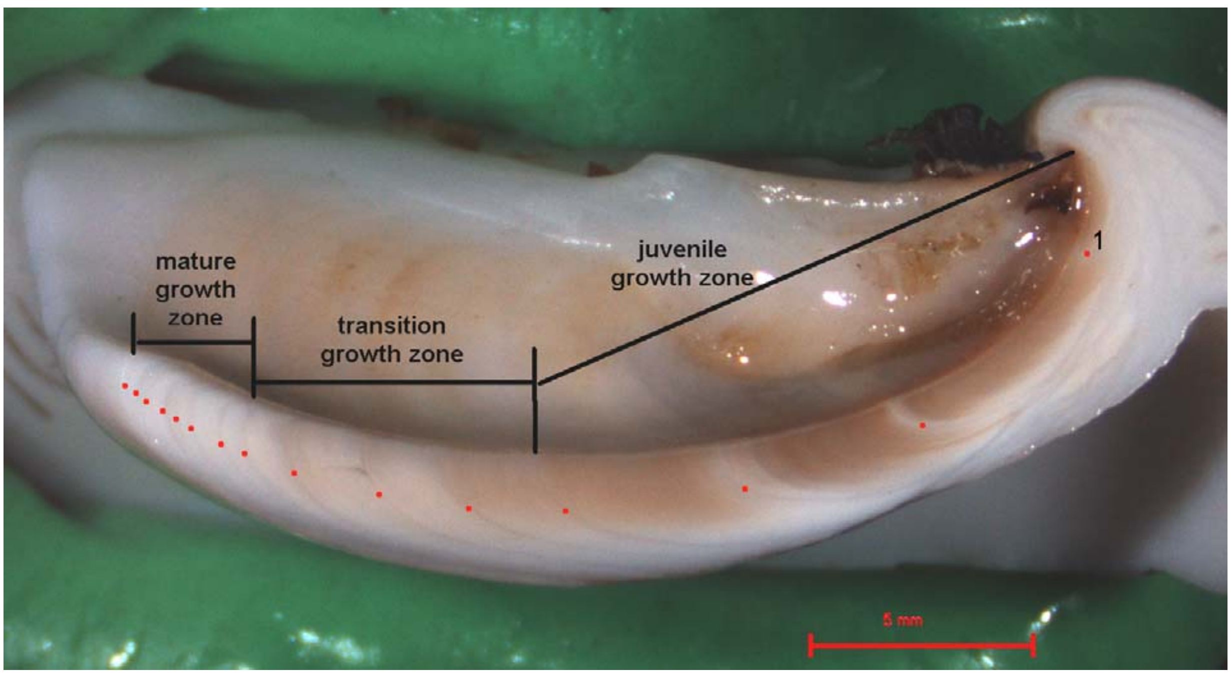

Fig. 13. *T. nuttallii* chondrophore cross-section showing the three regions of the growth pattern, age 15 yrs.

In the Tofino samples, checks occurred throughout the growth pattern regions but were most prevalent in the juvenile years. Weak checks can usually be identified and discounted because they are faint. However, we also noted prominent checks and these were most common in the first 3 years. Often they could be discounted by measuring from the umbo; just as measurements assisted in identifying annuli in the first two years they were also useful in discounting checks. Checks also tended to be more conspicuous in the fast growing juvenile years because they are a narrow interruption in a relatively wide summer growth zone, therefore proportion is inconsistent within the overall pattern. Once the first two years are established and checks within those zones are identified, the annual growth pattern rhythm begins to emerge. We generally had less difficulty identifying checks in the juvenile years than in the transition region of the growth pattern.

It was more complicated to identify checks in the transition area because decreasing growth rates result in more closely spaced annuli. With less summer growth separating annuli, checks may appear less disproportionate and therefore less obvious than in the juvenile region. Examining the spacing of adjacent annuli will frequently indicate whether to count the zone in question since growth in the transition area often declines fairly steadily, resulting in a somewhat uniform pattern. If the zone in question is inconsistent within the overall growth pattern, it may be discounted. However, it must also be kept in mind that growth rates of horse clams may be quite variable; uniform shell growth can not always be assumed therefore some variation in proportion is to be expected. Our study criteria relied on lack of prominence to a large degree, with spacing as an influential secondary consideration to identify checks, particularly in the first 5 years. Therefore, a prominent zone could be counted when it shared characteristics of other annuli, even if the pattern reflected some uneven rates of growth, particularly if the disproportion was not very great. Assessing growth patterns for age determination has subjective elements. Experience in pattern recognition was valuable when balancing the prominence and spacing of zones appearing on a growth pattern.

Double annuli occasionally appeared and were also more challenging to interpret as growth declined during the transition between the juvenile and adult growth phases. In faster growing patterns, double annuli were fairly easy to recognize as two closely spaced translucent zones between relatively large opaque zones. In these cases, spacing prevailed as the over-riding criteria. Where growth was transitioning from rapid early growth to slow adult growth, the double annuli became closer, and the disproportion became less obvious. Summer growth zones separating double annuli decreased in incremental width, resulting in successive double annuli appearing closer together as the pattern progressed, until eventually all prominent zones appeared equally spaced, whereupon our study counted each prominent zone as an annulus. Deciding where to begin counting all prominent zones where a double annuli pattern appears could be difficult and these specimens had a higher degree of uncertainty recorded with the age designation.

In identifying checks, the investigators also found it useful to follow the zone in question onto the concave surface of the chondrophore where annuli typically appeared more prominent than checks. Checks were often discontinuous or merged with an annulus on this surface.

In the slow growing adult years, checks became infrequent and did not generally hinder age assessment. Where zones became very tightly spaced at the margin, few checks were identified. Zones appeared consistent in prominence and spacing and generally all were counted, the challenge in this area being to distinguish between them.

The development of edge growth was generally consistent with expectations for samples collected early to mid-June, and also unsurprising are a few specimens with more and less edge growth.

This study was successful in ageing horse clams from Tofino, British Columbia with a high degree of precision between experienced age readers, indicating that high quality age data can be obtained by examining the growth pattern of the chondrophore. Average percent error, which may be even more meaningful in evaluating repetitive age estimates as it relates any difference in age assessment to the year class of the specimen, was very low and absent in the case of young *T. nuttallii*. We found *T. capax* patterns generally displayed more checks but were otherwise similar to *T. nuttallii*, although a larger sample size would be required to detail comparisons between the species growth patterns (Fig. 14). All *T. capax* were given a fair to fairly poor assessment of the growth pattern, while there were many *T. nuttallii* assessed as having fairly good growth patterns for age determination and few which were fairly poor. The lower precision results and higher overall APE for *T. capax* were attributed to more ambiguous growth patterns and the small sample size.

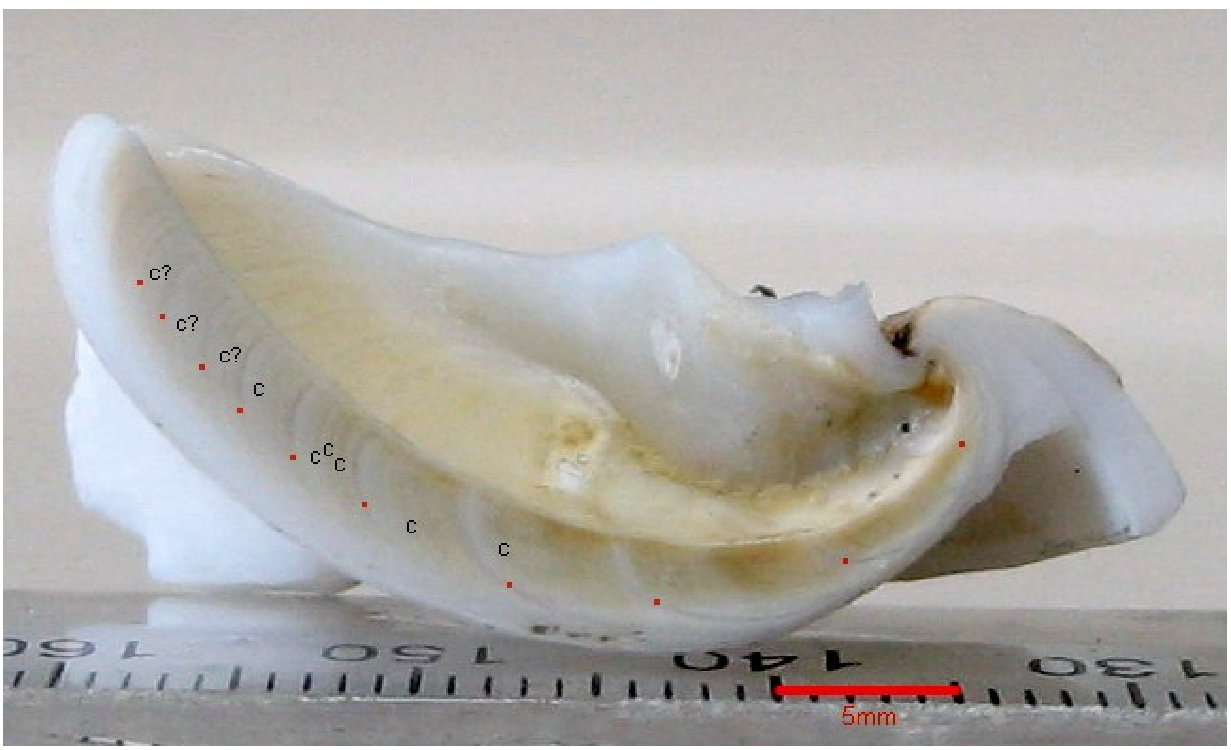

Fig.14. Cross-section of *T. capax* chondrophore showing multiple checks (c), age 10 yrs.

This study noted a strong *T. nuttallii* year class in 1999 and thereafter a sharp decline for the following 3 years but did not speculate on contributing factors. Plotting age versus shell length illustrated wide variation in length at age, demonstrating size alone to be a poor indicator of age even at 3 years of age.

Zhang and Pegg determined a maximum age of 20 years in a combined sample of both species by examining the chondrophore growth pattern. This study determined ages which are respectively 2 and 11 years older than previously estimated maximum ages recorded for *T. nuttallii* and *T. capax* (Lauzier, et al. 1998) derived from valve surface examination. As the chondrophore is not in direct contact with the external environment, it may be less perturbed by environmental noise and more accurately reflect the growth response to major environmental changes than the external shell surface. It would be useful to examine the external growth pattern on the samples used for the present study to compare with the chondrophore cross-section ages. The unprocessed valves from our study animals were kept with this purpose in mind. The necessity for validating criteria is acknowledged.

Criteria Summary

Criteria development for ageing horse clams is a work in progress. This study agreed with general criteria reported by Zhang and Pegg to age horse clam chondrophores, is consistent with results from growth studies, and expands upon the bivalve ageing literature. Our criteria may be summarized as follows:

- Location of 1st annulus is determined using measurements; it varies in size or may be absent.
- Location of 2nd annulus is determined using measurements and appears consistently located.
- Checks within the juvenile years may be discounted using the same measurements and an annual rhythm begins to emerge.
- Prominence and proportion are considered in identifying checks, particularly in the transition region of the growth pattern. Examine the spacing of adjacent annuli when deciding between an annulus and check but recognize variable growth rates mean patterns do not always display uniform growth.
- Following the zone in question onto the concave surface of the chondrophore may be useful to distinguish checks from annuli.
- All prominent, tightly spaced zones near the margin are interpreted as annuli but may be difficult to distinguish. Techniques to enhance the growth pattern in this area may be employed to highlight annuli.

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