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Growth of the European abalone (*Haliotis tuberculata* L.) *in situ*: seasonality and aging using stable oxygen isotopes

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Abstract

The ormer, *Haliotis tuberculata* is the only European abalone species commercially exploited. The determination of growth and age in the wild is an important tool for fisheries and aquaculture management. However, the ageing technique used in the past in the field is unreliable. The stable oxygen isotope composition ($^{18}O/^{16}O$) of the shell depends on temperature and oxygen isotope composition of the ambient sea water. The stable oxygen isotope technique, developed to study paleoclimatological changes in shellfish, was applied to three *H. tuberculata* specimens collected in North-West Brittany. For the specimens collected, oxygen isotope ratios of the shell reflected the seasonal cycle in temperature. From winter-to-winter cycles, estimates of the age and the annual growth increment, ranging from 13 to 55 mm per year were obtained. This study shows that stable oxygen isotopes can be a reliable tool for ageing and growth studies of this abalone species in the wild, and for validating other estimates.

Keywords

1. Introduction

Abalone biology and ecology have been widely studied worldwide mostly because of their high commercial value. These species generally live in shallow rocky subtidal habitats, although some can also be found in the lower intertidal either in temperate or tropical waters (Shepherd, 1973). Many abalone fisheries have collapsed due to overexploitation or infectious agents (Hobday et al., 2001; review by Leiva and Castilla, 2001; Richards and Davis, 1993; Shepherd and Rodda, 2001). In Europe, the only species found is *Haliotis tuberculata* L. reaching a maximum shell length of 14 cm in the best fishing areas of Brittany. Little has been done to conserve European abalone populations and more research is needed to understand stock dynamics (Huchette and Clavier, 2004). Determination of age at recruitment into the fishery stocks is a key tool for sustainable fisheries management (review by Day and Fleming, 1992; Heasman, 2006).

Abalone growth varies with seasons, food availability, population density, temperature, local hydrography of the reef and stress (review by Day and Fleming, 1992; Huchette, 2003). *H. tuberculata* growth is seasonal, growing faster in spring and summer (Clavier and Richard, 1986). As observed for many other gastropods and bivalves, growth of ormers is reduced at low temperature in winter. However, it seems not to stop completely (Clavier and Richard, 1986).

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Despite extensive research on the determination of abalone age and growth, ageing techniques are often not accurate enough, and until recently the most commonly used method was mark-recapture (review by Day and Fleming, 1992; Forster, 1967), but this technique presents several disadvantages: tagging may affect the growth of animals through handling stress and recapture rates are often low as long delays are required for accurate measurements before re-sampling (review by Day and Fleming, 1992). Other techniques such as measurement of growth check numbers - zones of shells deposited during slower growth periods - have been used to estimate the age of *H. tuberculata* shells (Forster, 1967). However, this method has been shown to be quite unreliable as these checks can be irregular or invisible due to shell erosion (Shepherd and Avalos-Borja, 1997; Shepherd and Turrubiates-Morales, 1997; Shepherd et al., 2000) or to variable individual rate of deposition (Forster, 1967). This technique requires a careful validation for each species to verify that deposition rates are regular and that deposition is occurring at the same time each year (review by Day and Fleming, 1992). The count of rings - prismatic layers of the shell deposited in the spire- of abalone shells under microscope or UV-light is another technique used to estimate the age of abalone (Forster, 1967; Proudfoot et al., 2008; Shepherd and Turrubiates-Morales, 1997) but has mostly the same disadvantages as the use of growth checks. The size frequency distributions have been also used in order to follow the growth of the annual cohorts and then to determine growth rate of juvenile abalones (Clavier and Richard, 1986). Fluorochromes markers is another technique used to 'mark' the shell. Growth rate is then calculated upon recapture (Day et al., 1995). Based on the same marking principle, artificial diets given to juveniles during few weeks in order to produce a contrasting shell band have been

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developed for assessing growth of the released individuals and assessing sea ranching experiments (Gallardo et al., 2003).

Recently, the use of stable oxygen isotopes has been developed, especially in order to study paleoclimatology changes. The stable oxygen isotope composition ($^{18}O/^{16}O$) of most shells is controlled by temperature and oxygen isotope composition of the ambient sea water (Grossman and Ku, 1986). One advantage of the shell isotope composition method is the preservation of seasonal variability of stable oxygen isotope ratio within accretionary structures (Dutton et al., 2002; Gurney et al., 2005; Jones et al., 2005; Thebault et al., 2007). Peak values of the stable oxygen isotope ratio correspond to parts of the shell laid down during the coldest time of the year (Gurney et al., 2005; Radermacher et al., 2009; Thebault et al., 2007). Although one of the first stable isotope oxygen experiments has been validated in *Haliotis* species (Urey, 1948), it has only recently been used to estimate growth in *Haliotis iris* in New-Zealand (Naylor et al., 2007) and Australia abalones (Gurney et al., 2005). Fitted growth models determine estimated parameters such as fecundity, mortality schedules and the age of recruitment to the fishery. The validation of accurate growth information is important because these parameters are key parameters in stock assessment models. Therefore, the use of stable oxygen isotope technique could be promising for abalone fisheries management (Gurney et al., 2005; Lee et al., 2002), even if it remains a costly analysis which severely restricts the numbers of shells that can be tested in studies.

Abalone shells are made of calcium carbonate ($CaCO_3$) in 2 main crystal forms: calcite and aragonite, embedded in protein often called conchiolin. Just under the periostracum is

a prismatic layer ranging from ca. 0.2-1 mm in thickness depending on the part of the shell. The prismatic layers of *H. tuberculata* consist mostly of calcite and aragonite (Mutvei, 1985). The nacre layer of the ormer is mostly composed of tiles of aragonite that are interleaved with an organic matrix (Lin et al., 2008). The fact the inner aragonite layers are deposited at a different time from prismatic layers and the isotope ratios differ between aragonite and calcite make the nacre layer unreliable for the determination of temperature determination. Therefore it is important to collect only the outer prismatic layer of the shell for the stable oxygen isotope technique.

The present study aimed at demonstrating that the stable oxygen isotope technique can be used for *H. tuberculata* determination of age. This is important for European abalone fisheries management as it will help setting minimum harvest sizes for sustainable fishing, to ensure that most females are allowed to breed adequately before becoming available for the fishery. This experiment also aims at giving valuable information to compare abalone growth measured in the wild to growth in aquaculture in order to improve growth conditions in aquaculture husbandry (Huchette and Clavier, 2004). Finally, it compares the results of stable oxygen isotope method with the growth check measurements, a rapid and non-expensive technique on the field.

2. Materials and methods

2.1. Field collection

Three adult abalones (S, M, L) were collected by SCUBA divers on the 01/05/2002 directly outside of the small port of Primel in North-West Brittany, France (Fig. 1). Relatively clean shells with few parasites such as barnacles or boring polychaetes were chosen to reduce the risk of contamination of calcium carbonate samples by skeletal elements of other organisms. Weekly Sea Surface Temperatures (SST) were obtained from SOMLIT of the Roscoff marine experimental station (Fig. 2).

2.2. Isotope sample collection

Each shell was carefully cleaned and external parasites were removed using successive acetic acid bathing and gently scraping of the shell surface with a scalpel blade under a binocular magnifier. In addition, only non-damaged shells were studied as thin aragonite layers can be deposit to repair damage to the calcite growing margin (Hawkes et al., 1996). The laboratory bench sampling area was maintained clean with pressurized air to blow dust away and acetic acid sprayed over the bench and wiped dry with clean paper towels. The superficial calcite layer of the shell was ground off the shell surface using a DREMEL drill with 0.5 mm grinding tip. Rotating speed was adjusted according to the hardness of the shell. Samples were taken following the fine growth lines formed as the abalone grow (Cochard, 1980) to ensure that the entire sample had been deposited within a short period of time (Fig. 3). Growth lines were followed until a sufficient amount of material for the analysis was obtained (0.5-1 mg). This technique allowed large samples to be collected without drilling too deep and contaminating the sample with aragonite

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from the nacre. The calcite was collected on non-static weighing paper and transferred into a 3 ml Eppendorf vial. Sampling towards the shell spire tip – i.e. the older part of the shell – did not always produce enough material because the surface area available was too small and the calcite layer too thin. In addition, for the larger shell, some samples were missing due to the difficulties in sampling near the edge.

2.3. Isotope sample analysis

Samples were analysed for stable oxygen isotope ratio using an automated Finnigan MAT Kiel III carbonate device coupled to a Finnigan MAT 252 isotope ratio mass spectrometer at Stanford University. The results were reported in per mil (‰) deviations relative to V-SMOW (Vienna Standard Mean Ocean Water) and V-PDB (Vienna Pee Dee Belemnite) carbonate standard. When enough powder was available, it was divided into two or three sub-samples and the analysis was run several times to check for accuracy. The overall analytical precision (1 S.D.) for $\delta^{18}O$ and $\delta^{13}C$ of calcite samples were ≤0.12‰ and ≤0.20‰ respectively, based on analyses of 2 sub samples of the same sample and ≤0.033 ‰ and ≤0.076‰ based on analyses of internal laboratory standards run concurrently with all the samples.

To determine the maximal shell length corresponding to each drilled sample, the longest dimension of the shell was calculated for each given sample. Using an image analyzer program Visolog 5.1.1 (Noesis, Paris, France), X,Y values (i.e. abscissa and ordinate) of each drilled sample - position taken at the outside edge of a respiratory pore point were

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measured (Fig. 4). For example, X_1, X_2 and Y_1, Y_2 values were obtained for the drilled sample 1 and 2. These (X,Y) values allowed us to calculate the shell distance (S) between 2 points using the Pythagoras hypotenuse equation (for example $S_{1,2.} = \text{root}((X_1 - X_2)^2 + (Y_1 - Y_2)^2)$. Calculation was performed between all the points and the maximal shell distance was kept.

Growth checks were evaluated by 3 independent experimenters in order to compare these measurements with age obtained by isotope technique.

3. Results

Sampling details and shell length at sampling for the 3 specimens are provided in Table 1. The stable oxygen isotope values showed a clear pattern in function of the shell length (Fig. 5), reflecting the seasonal cycle in temperatures (Fig. 2). Three, three and four and a half full oscillations for the winter-to winter cycles were observed for the shells S, M and L respectively. The first coldest time of the year - peak of stable oxygen isotope value, corresponding to the coldest time of the year - for the shells S and L took place at a shell length estimation of 7 and 9 mm (Table 2). For shell M, it took place at 14 mm. Annual growth increment before the first winter oscillation cannot be calculated due to the uncertainty of the time of spawning. In addition, the stable oxygen isotope values from samples before this first winter oscillation were very variable suggesting that these samples were unreliable.

Starting from the first winter oscillation, annual growth increments were calculated using the winter-to-winter oscillations - the higher stable oxygen isotope value for each seasonal cycle (Table 2). The first full annual growth increment was the largest with 44 mm in average. However, the range was quite large, from 35 mm for shell S to 55 mm for shell M. Moreover, when approaching a size of 90 mm, annual growth increment of shells M and L decreased sharply (Table 2).

Most of the growth checks described by the 3 experimenters were at the same position as the peak of stable oxygen isotope values (in 57 % of the case). For the 3 specimens no growth checks were observed at the first highest peak of stable oxygen isotope. However, 1 or 2 supplementary growth checks were observed per abalone (Fig. 5), and some discrepancies were found between the experimenters. The supplementary growth check corresponded either to growth accident (front part of the shell broken) or probably to reproductive events in summer at a period where stable oxygen isotope ratios were lower.

4. Discussion

In these three H. *tuberculata* specimens, marked oscillations in the stable oxygen isotope ratio were observed. Indeed, based on the inverse relationship between the stable oxygen isotope ratio and temperature, the age and growth of the specimens can be estimated.

The stable oxygen isotope technique has several advantages compared to the techniques usually used in the field. Firstly, this technique is less susceptible to the bias due to the lack of deposition of growth checks or rings if drill samplings are finely spaced. Indeed, undetectable or missing growth checks are common, especially in old animals (Day and

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Fleming, 1992; McShane and Smith, 1992). In *H. tuberculata* in Guernsey, Forster (1967) observed that a single growth check was present per year in 90% of the shells in small abalones. In larger individuals, 78% of the shells had a single growth check, the remaining 22 % having either two growth checks or none per year. The absence of clear correspondence between the peak stable oxygen isotope values and the observed growth checks confirms the difficulties in correctly evaluating the presence or absence of growth checks due to discrepancies between experimenters. Second, this method does not require the use of tagging, capture and recapture of abalones. Tagging as well as natural disturbances such as El Niño events were found to cause extra growth checks (Forster, 1967) or abnormally prominent ones (Shepherd and Turrubiates-Morales, 1997).

However, this technique has some limitations. Stable oxygen isotope ratios were highly variable under 10 mm in size probably due to erosion and difficulties in obtaining enough sample material for accurate analysis in the shell spire tip. A contamination of the sample with the inner nacreous layer was also highly probable due to the thinness of this shell region. The size of 10 mm probably corresponds to the size reached before the first winter as it matches the size of *H. tuberculata* individuals raised in nurseries in winter after a spawning in June-July the previous year, although smaller sizes are observed in nurseries in winter from later summer spawnings (Huchette, unpublished). As only 3 animals were collected in Primel, the results of this study cannot be extrapolated to all *H. tuberculata* populations. Shell length increments obtained in this study appeared extremely high in comparison with growth measured on the same species with mark-recapture experiments in Guernsey where a uniform growth rate of 15-16 mm per year

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was observed with a progressive decline after 55 mm (Forster, 1967). In Saint-Malo, a growth rate of less than 15 mm was observed the first two years, reaching 20 mm in average the 3rd and 4th year of age, and thereafter decreasing to 10 mm the 5th year to reach a size of 65 mm (Clavier and Richard, 1986). In offshore cages rearing abalone, with algae fed *ad libitum* at high densities, abalone have an average growth of about 20 mm per year, with some individuals reaching 30 mm per year (Huchette, unpublished). This differential shell growth could result from several parameters including parasitism, such as a boring sponge which damages the shell. At least 70% of the shells were infected in Guernsey (Forster, 1967), whereas a very low incidence of shell infection was observed in Primel. Density can also affect growth significantly (Huchette, 2003). In addition, the availability of food is a key factor affecting growth rate (review by Day and Fleming, 1992).

The high cost of this technique is another limitation which reduces the numbers of abalone studied. Some techniques with more limited costs could be an interesting alternative such as the one developed by Shepherd and Avalos-Borja in 1997. Using scanning electron microscopy, this experiment showed that the thickness of aragonite crystals in the *Haliotis corrugata* shell followed the seasonal temperature change pattern.

Abalone, like many other shellfish species have a huge plasticity in growth and maximum shell length at maturity can vary from one environment to another (Huchette, 2003; Saunders et al., 2008). Although the European abalone gene pool variability is low between geographic locations (Van Wormhoudt et al., 2009), the asymptotic length (L_∞)

can be variable from one site to another, e.g. 108 mm in a sublittoral site, and up to 119 mm for a shore population in Guernsey (Forster, 1967).

Moreover, by reducing significantly the density of the stock, fishing will affect the growth of unfished animals (Dixon and Day, 2004). Studies by Forster (1962; 1967), and Clavier and Richard (1986) were conducted before the massive reduction in abalone wild population stocks that occurred in the late 1990s due to fishing (poaching, recreational and professional fishing) and the outbreak of *Vibrio harveyi* epizooty (Nicolas et al., 2002). At the time much higher densities were found in the wild.

In this context, stable oxygen isotope technique will be valuable to obtain accurate and precise data between sites, to evaluate changes over time and to allow a more sustainable management of the fisheries.

5. Conclusions

Stable oxygen isotope techniques have been used with molluscs as a tool to validate age, growth measurements and verify if the growth bands or muscle scar rings correspond to age or growth season (Gurney et al., 2005; Richardson et al., 2004; Surge et al., 2001). Even if this technique remains quite expensive and cannot be applied to a large number of animals, it provides very accurate measurements compared to others techniques, and could be used to provide precise measurements of age and growth of abalone.

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Fig. 1. Sampling site of *H. tuberculata* in Primel in the North-West part of Brittany (France)

Fig. 2. Seawater temperatures between January 1997 and December 2002 recorded by SOMLIT of the Roscoff marine experimental station

Fig. 3. Shell from abalone S showing sampling grooves from milling procedure to collect shell powder for stable oxygen isotopic analyses.

Fig. 4. Using an image analyzer program Visolog 5.1.1 (Noesis, Paris, France), X,Y values (i.e. abscissa and ordinate) of each drilled sample were measured. Grey dots represent the position taken at the respiratory pore in continuation of the sampling point. The black dots represent the outside edge of the respiratory pore in continuation of the sampling point. The black dots were used for the calculation of the hypotenuse.

Fig. 5. Stable oxygen isotope ratio (°/°°) in function of the maximal shell length (mm) of 3 H. *tuberculata* shells (A) shell S, (B) shell M, (C) shell L. White arrows indicate where the samples with the highest peaks (winter samples) were taken on each shell, and $\triangle$ $\bigcirc$ + symbols indicate growth checks described by 3 experimenters. The Y-axis was reversed in order to match the temperature oscillations.

Table 1: Shell size and sampling details of shells S, M and L of *H. Tuberculata.*

	Shell S	Shell M	Shell L
Maximum shell length (mm)	92	112	123
Number of serial samples	96	139	130
Average sample interval along the spiral length[+] (mm)*	1.5 ± 0.05	1.3 ± 0.04	1.6 ± 0.05
Range of sample interval along the spiral length[+] (mm)	0.3 – 2.8	1.0 – 2.8	0.4 – 3.3

* Mean ± s.e. are presented - [+] spiral length = distance measured from the apex, along the respiratory holes up the anterior margin of the shell

Table 2: Maximum shell length estimate (mm), annual growth increment (mm) for each winter-to-winter oscillation and stable oxygen isotope values *(‰)* at the lowest winter-to-winter temperature oscillation for shell S, M and L of *H. tuberculata* collected in the North-West part of Brittany (France).

Number of oscillations	0	1	2	3	4	5
Maximum shell length estimate (mm)						
Shell S	6.9	42.5	68.7	91.3		
Shell M	14.2	69.6	98.5	111.7		
Shell L	9.1	51.2	86.0	108.1	115.6	123
Mean ± s.d.	*10.7 ± 3.74*	*54.2 ± 13.84*	*84.4 ± 14.96*	*103.7 ± 10.89*	-	-
Annual growth Increment (mm)						
Shell S	-	35.6	26.2	22.6		
Shell M	-	55.4	28.9	13.1		
Shell L	-	42.1	34.8	22.1	7.5	7.4
Mean ± s.d.		*44.4 ± 10.10*	*30 ± 4.40*	*19.3 ±5.35*		
Highest stable oxygen isotope values (‰)						
Shell S	33.33	33.35	32.91	33.14		
Shell M	32.79	33.15	32.97	33.43		
Shell L	33.44	33.43	33.44	33.33	33.03	33.12

Fig. 1

Fig. 2

Fig. 3

sampling
grooves

1 cm

Fig. 4.

The figure shows a scatter plot with X-values on the horizontal axis (ranging from 0 to 1800) and Y-values on the vertical axis (ranging from 0 to 1000). Two points are labeled "point 1" (near X_1, Y_1) and "point 2" (near X_2, Y_2). A line connecting the two points is labeled with the equation:

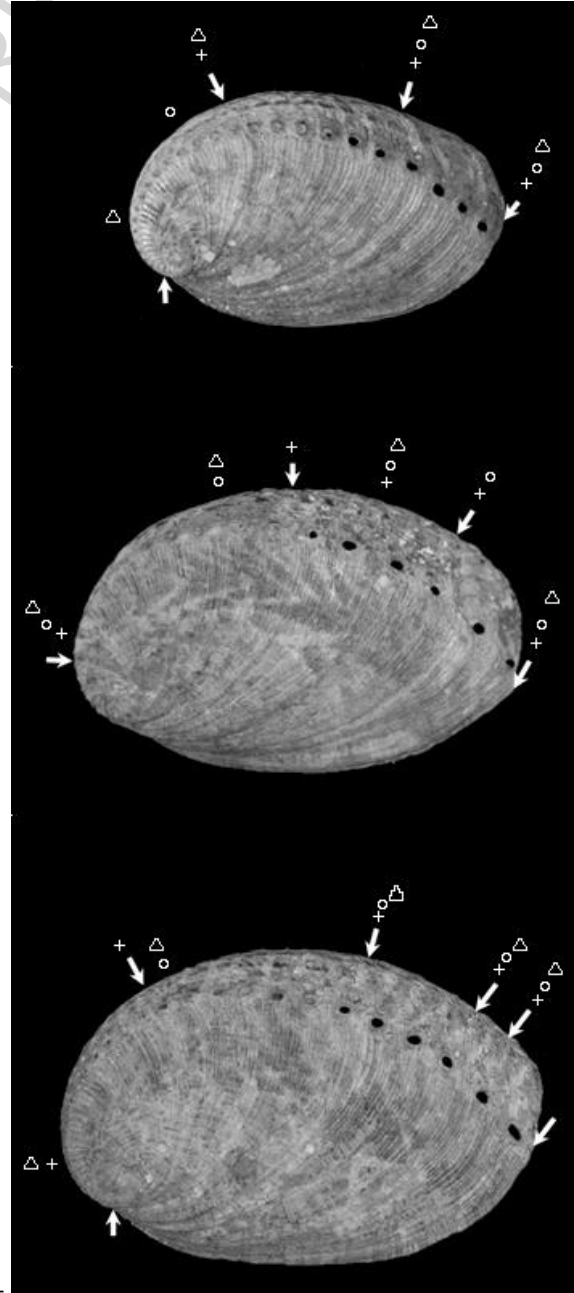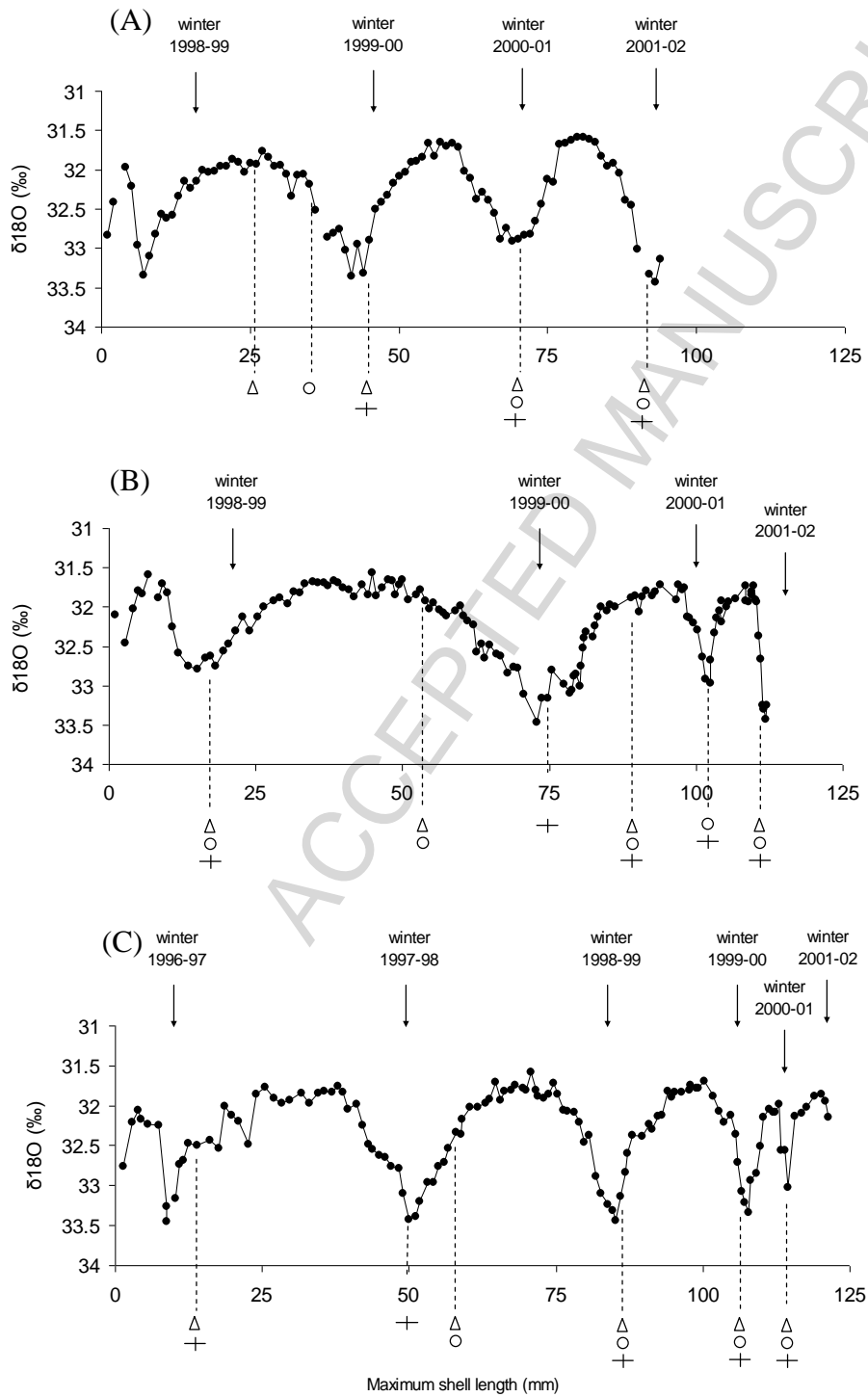$$(S_{1,2})^2 = (X_1 - X_2)^2 + (Y_1 - Y_2)^2$$
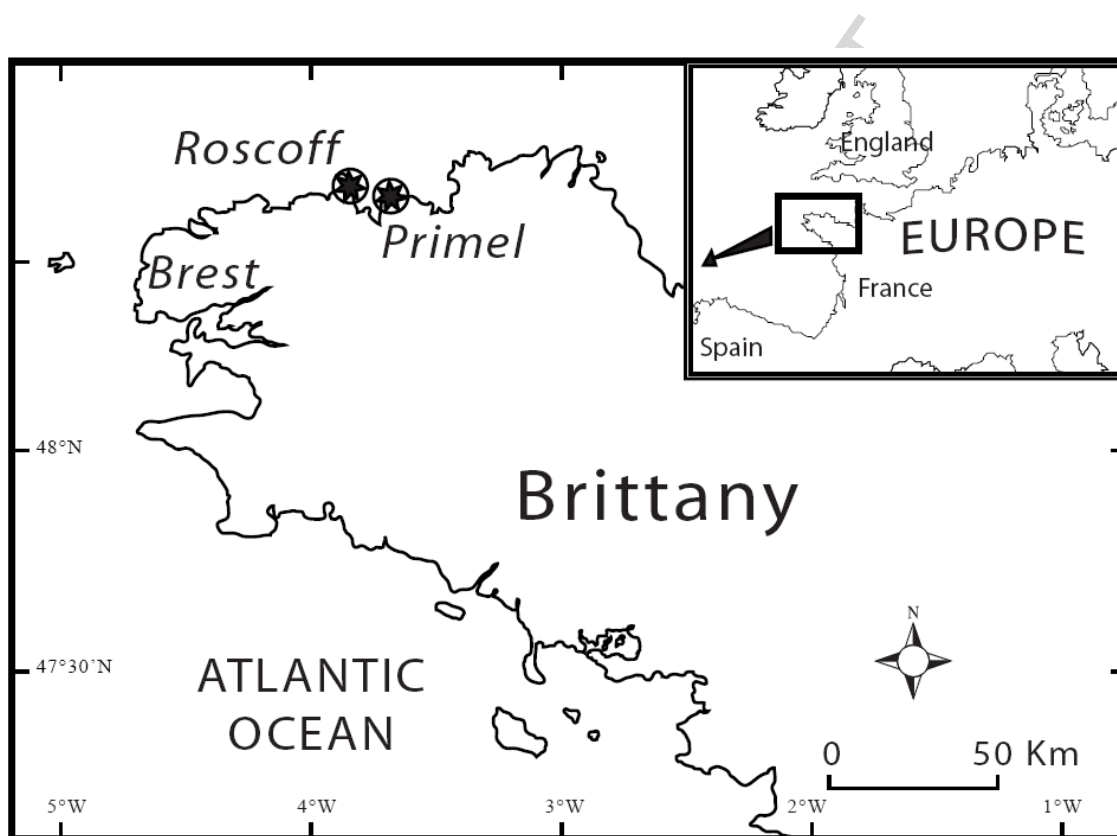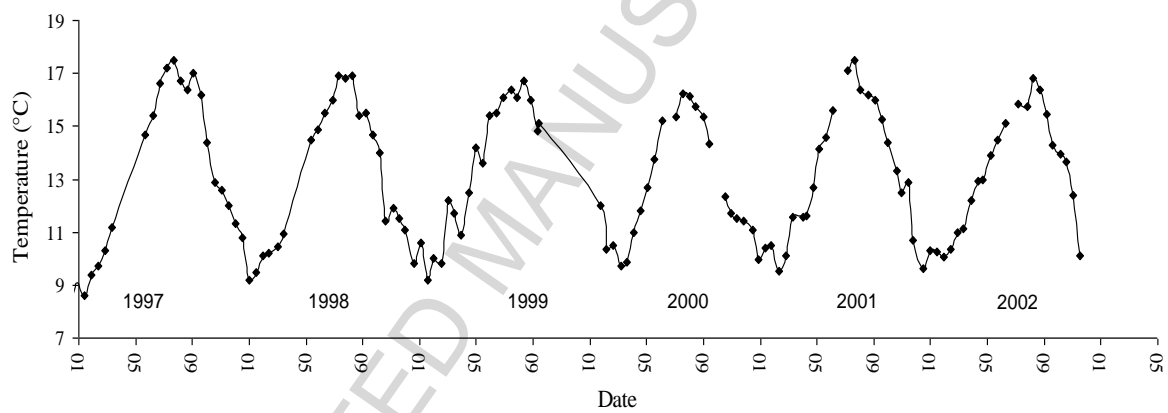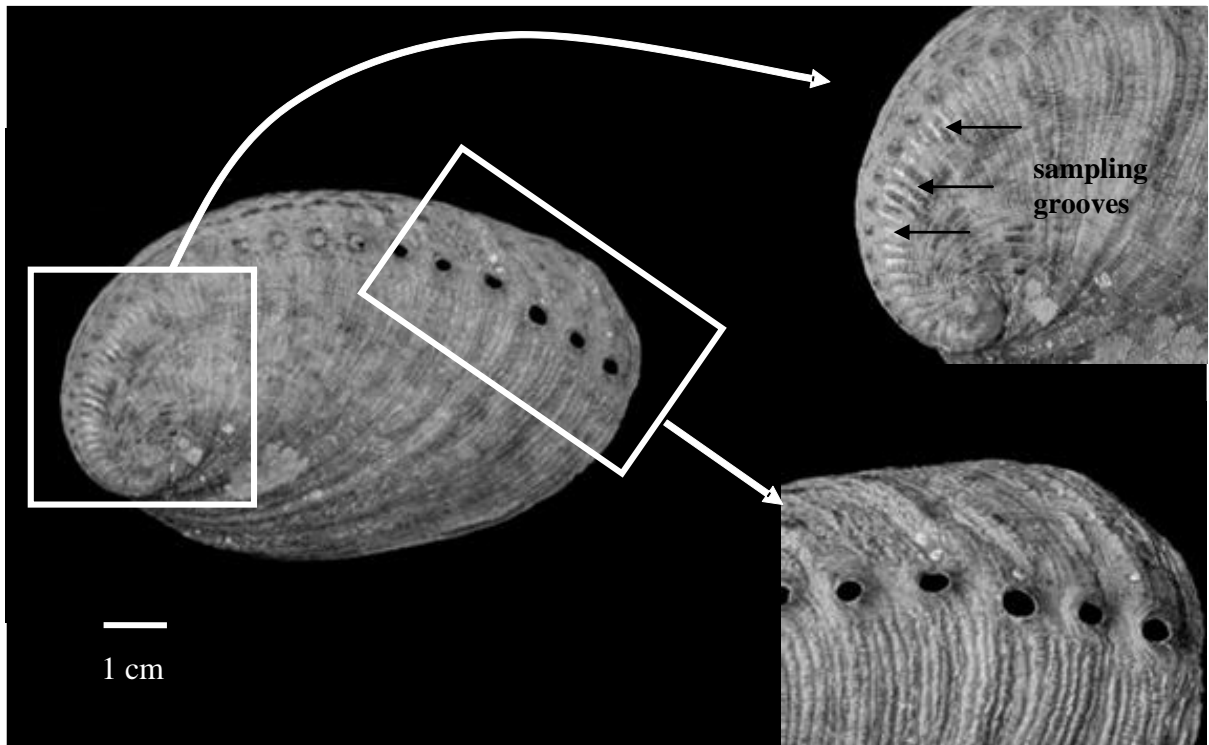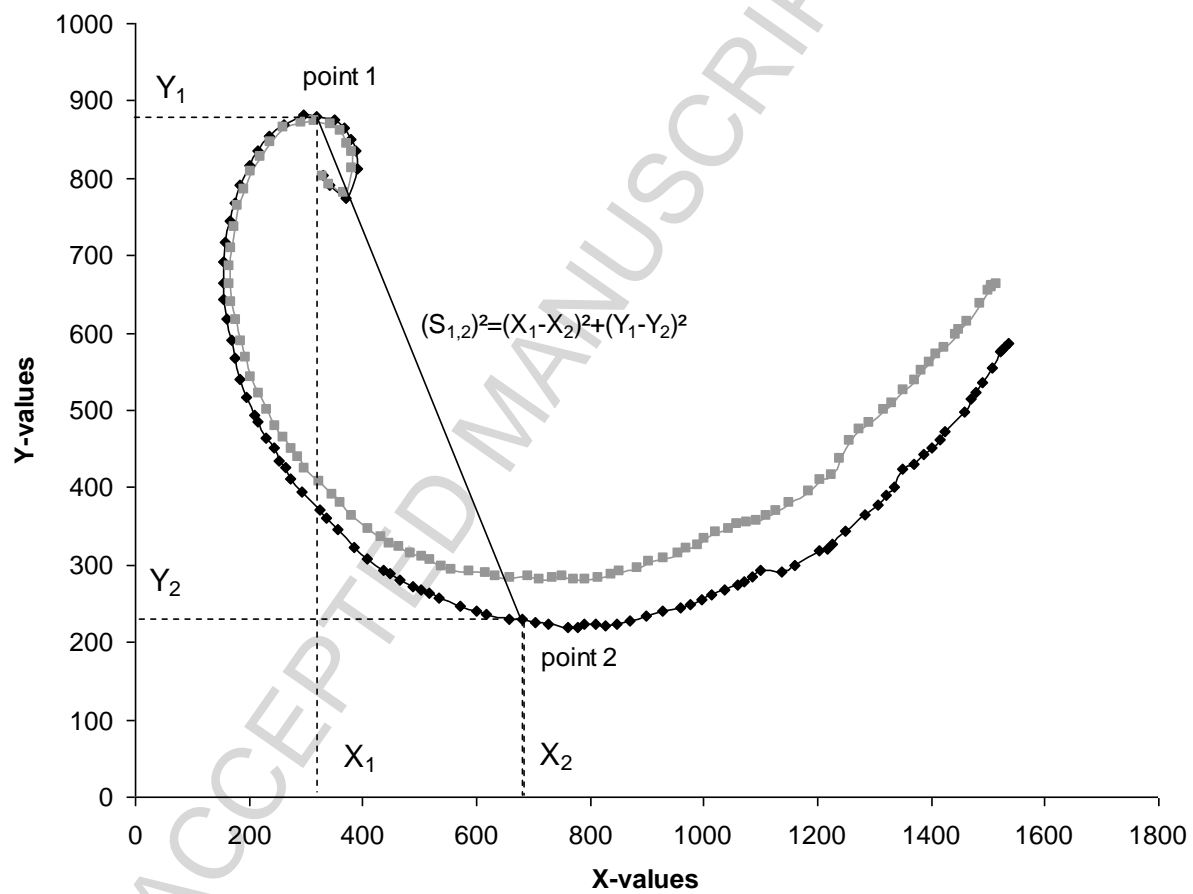
Fig. 5

Research Highlights

Oxygen isotope ratios of the *H. tuberculata* shell reflect the seasonal cycle in temperature

It can be used with *H. tuberculata* to validate other growth measurement techniques

Stable oxygen isotope technique is less susceptible to bias than growth check measurements

High costs of the stable oxygen isotope analysis reduce the numbers of abalone studied