A validation of growth structures and an annual growth profile in the Otoliths of Notothenia Coriiceps at signy island in the South Orkney Islands, Antarctica

Ashford, Julian Robin

A VALIDATION OF GROWTH STRUCTURES AND AN ANNUAL GROWTH PROFILE IN THE OTOLITHS OF Notothenia coriiceps AT SIGNY ISLAND IN THE SOUTH ORKNEY ISLANDS, ANTARCTICA. By JULIAN R. ASHFORD. M.Sc., 1992.

To investigate otolith growth over the year cycle in immature Notothenia coriiceps, a time-series of monthly samples was taken at Signy Island, Antarctica from June 1987 to June 1988. Attempts were also made to time-mark fish otoliths experimentally using tetracycline hydrochloride and acetazolamide. Light and scanning electron microscopy techniques were used to examine and compare the structure of sectioned otoliths. A method was developed for processing large numbers of otoliths for viewing using a scanning electron microscope (SEM); this permitted large sample sizes to be examined and, compared with similar light microscope techniques, gave enhanced resolution particularly for discerning edge structures. Using the SEM, six growth regions were identified in the otolith cross-sections. Evidence of micro-increments was found but these were not consistently clear across sections. A series of large micro-increments was also found around the central nucleus which may correspond to the growth phase of the pelagic fingerling stage in N. coriiceps. The timing and annual nature of growth of annuli visible using the SEM were validated using the time-series samples. Negative results were obtained from the marking experiments. The annuli revealed by SEM and light microscopy techniques were shown to correspond, supporting the hypothesis that annuli visible by using light microscopy represent one year in Antarctic fish. A profile of otolith growth was also constructed. Growth was found to fluctuate seasonally with a significant decrease between August and late November 1987. Fish continued feeding during winter. Conclusive evidence distinguishing between hypotheses for limitation of growth by seawater temperature or resource availability was not found.

A VALIDATION OF GROWTH STRUCTURES AND AN ANNUAL GROWTH PROFILE IN THE OTOLITHS OF *Notothenia coriiceps* AT SIGNY ISLAND IN THE SOUTH ORKNEY ISLANDS, ANTARCTICA.

JULIAN ROBIN ASHFORD

A thesis submitted to the University of Durham in candidature for the degree of Master of Science.

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DECLARATION

The work on which this thesis is based was performed while the candidate was employed by the British Antarctic Survey. None of the material offered has previously been submitted by the candidate for a degree from the University of Durham or any other university. All work was performed by the candidate except where otherwise acknowledged.

STATEMENT OF COPYRIGHT

1. INTRODUCTION

1.1 The Antarctic Marine Environment.

Antarctica consists of a polar continental land-mass surrounded by a large circum-polar ocean. Its northern boundary is delimited by the Polar Front, an area of oceanic upwelling marking the transition between cold southern surface water and the warmer sub-Antarctic waters. The circulation is predominantly in an eastward direction, the only major constriction being at the Drake Passage between South America and the Antarctic Peninsula (Fig. 1). An oceanic ridge forms the Scotia Arc running from the Antarctic Peninsula to the tip of Tierra del Fuego, of which the island of South Georgia and the archipelagos of the South Shetland, South Orkney, and South Sandwich Islands are part. A second current, flowing westward along the continental land-mass creates the large gyre in the Weddell Sea, circulating colder water along the southern edge of the Scotia Arc (Foster 1984).

The presence of a frozen continental land-mass allows temperatures within the Polar Front to drop far lower than in comparable northern latitudes. Water temperature remains low throughout the year; the amplitude of its range, as well as the annual average, decreasing with increasing latitude. Temperatures rarely rise above freezing point (-1.8^{o}C) in McMurdo Sound but, in the South Orkney Islands, range between -1.8^{o}C in winter and +1.5^{o}C in summer (Clarke 1988).

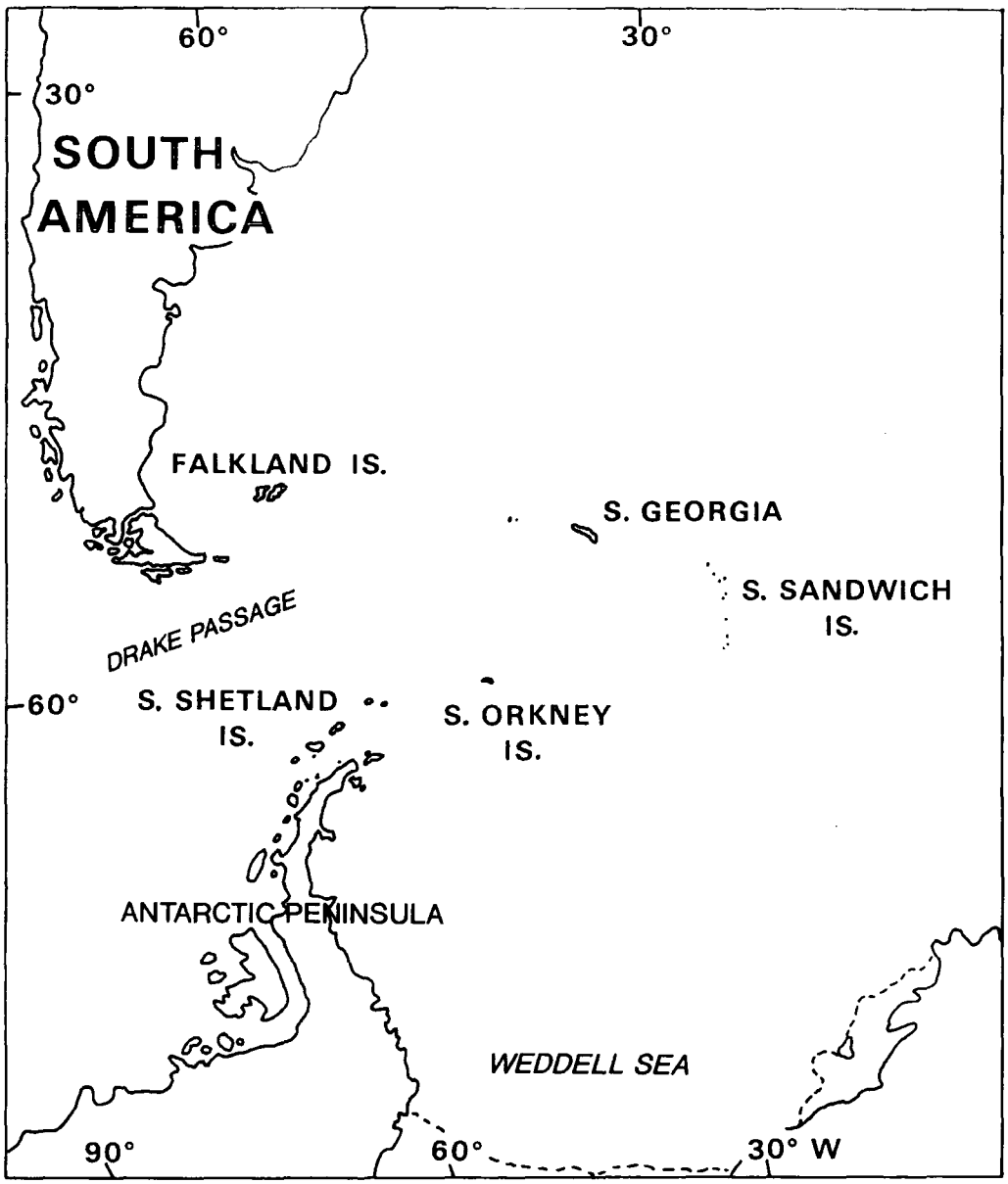

Figure 1. Location of South Orkney Islands.

The Antarctic marine environment is characterized by its seasonal nature. Given the high latitude, annual variations in photoperiod are large, while winter is marked by the formation of fast-ice inshore and the annual movement of pack-ice into more northerly latitudes. The sea-ice greatly affects the physical environment, creating a platform both above and below the surface for biota ranging from algae (Heywood and Whitaker 1984) to higher predators (Laws 1984); acting as a barrier to radiation and heat exchange; and altering parameters such as available light and turbidity (Foster 1984). A very dense bloom of diatoms usually occurs in the water column for 8-10 weeks during summer, typically reaching 8-10 mg/m^3 in open water (Sakshaug and Holm-Hansen 1984), and inshore occasionally reaching over 40 mg/m^3 at localities such as Signy Island in the South Orkney Islands (Clarke et al 1988). This declines abruptly to less than $1mg/m^3$ by April at Signy Island (Clarke et al 1988), with a low standing crop showing very little further productivity for the rest of the year.

1.2 The Antarctic Ichthyofauna

The Antarctic ichthyofauna is notable for a low number of species with a high degree of endemism: of the approximately 200 species, 88% are endemic. The dominant group of Antarctic fish are the Notothenoidei, comprising 53% of all species, of which the largest of its five families are the Nototheniidae (Andriashev 1987).

The majority of Antarctic fish do not have swim bladders and are demersal. A few are pelagic, the most notable being the Myctophidae, the notothenid Pleuragramma antarcticum, Dissostichus mawsoni, and the icefish Champsocephalus gunnari. Other fish may have a semi-pelagic life history as has been shown for the adults of Notothenia rossii (Burchett 1983a). The main prey consumed by non-demersal fish is krill (Euphausia superba). Demersal fish tend to have a diverse diet, feeding on macro-algae and an assortment of Amphipoda, Polychaeta, Isopoda and Mollusca. Many take fish, euphausiids and mysids on an opportunistic basis (Everson 1984). Growth rates tend to be low compared with temperate fish and individuals are often long-lived. Sexual maturity in most species is not attained until after five years old and gonad development and maturation in the adults of a population occurs synchronously resulting in a limited annual spawning period (Everson 1984).

The low range of temperature and its proximity to freezing have resulted in several adaptations. A series of macromolecules with anti-freeze properties have been identified that act in a non-colligative manner by lowering blood freezing-point without affecting the melting-point. Concentrations of haemoglobin and erythrocytes tend to be reduced in the blood of many species. The Channichthyidae are notable for their total lack of haemoglobin and any respiratory pigment; this may offset increased blood viscosity at low temperatures (Everson 1984). Stenothermy is marked among Antarctic fish with upper and lower lethal temperatures being little altered by periods of acclimation (DeVries 1978).

The complete life-history has only been ascertained in a few species. Burchett (1983a) has described that for N. rossii off South Georgia, showing that stages from larvae to young fingerlings are pelagic, the latter metamorphosing into blue-phase fingerlings which migrate to the near-shore environment, arriving between late January and February of each year. These then develop into brown phase fingerlings which in turn grow into demersal juveniles inhabiting the coastal beds of macroalgae in shallow water. At about five years of age, they migrate offshore to join the sexually mature adult fish population, remaining over the continental shelf areas of South Georgia where spawning takes place annually in April/May.

In an earlier project in the South Orkney Islands, Everson (1970) worked on the biology and population dynamics of Notothenia coriiceps (formerly described as Notothenia neglecta), using otoliths to age fish. He found growth to be slow, natural mortality to be high, and annual production low (0.34 g/g). Efficiency of transformation of energy to production was low (5%) which was concluded to be due to the effect of cold adaptation and because food may be energy limiting. Indications of seasonal movements were found although an offshore migration in adults, similar to that demonstrated for N. rossii, could not be proved.

Everson (1984) further demonstrated seasonal cycles in gonad size and maturation with spawning occurring in late May. Condition factor (where CF= 100 X weight/standard length^3) was found to be highest prior to spawning, then to decline to a post-spawning low; liver index (where LI= 100 X liver weight/total weight) was also found to correlate strongly with the gonad cycle. Twelves et al

(1975) also found thyroid activity to occur on an annual cycle. Everson (1984) related these to annual cycles of reproduction, feeding activity and predation; noting major differences between the sexes which he suggested to be associated with contrasting demands in reproduction.

1.3 Fisheries In The Scotia Sea.

After exploratory fishing during the 1960s, large-scale fishery activity in the South Atlantic sector of the Antarctic first started around South Georgia (United Nations Food And Agriculture Organisation Subarea 48.3) in 1969. This spread to the South Orkney Islands (FAO Subarea 48.2) in the summer of 1977/78 and to the South Shetland Islands and northern Antarctic Peninsula the following year (Kock et al 1984; Kock and Koster 1990). The nations involved commercially have been principally the former Soviet Union but also Poland, the former German Democratic Republic, Bulgaria and Chile.

The initial target species around South Georgia was N. rossii, yielding some 500 000 tonnes in two seasons between 1969 and 1971. With the collapse of this population, fishing was primarily directed at the channichthyid Champsocephalus. gunnari and to a lesser extent the nototheniid, Patagonotothen brevicauda. Catches of C. gunnari peaked at 240 000 tonnes in 1976-78, again in 1982-84 at 220 000 tonnes, and at 100 000 tonnes in 1986-88. By-catches have included Gobionotothen gibberifrons and Notothenia squamifrons. Presently the

myctophid Electrona carlsbergi is the most important catch, increasing from 23 623 tonnes in the year 1989/90 to 78 500 tonnes in 1990/91 .

In the South Orkney subarea (48.2), fishing effort has been directed at C. gunnari, peaking in the first year 1977-78 at 140 000 tonnes and falling dramatically thereafter. The principal by-catch has been G. gibberifrons but N. rossii has also been taken together with a large proportion of unknown species - the most extreme case being for 1982/83 with 12 500 tonnes unidentified fish of that year's catch of 18 500 tonnes.

Fisheries activity is currently regulated under the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) which came into force in 1982. Since 1984 annual assessments of exploited stocks are made by the CCAMLR Working Group on Fish Stock Assessment. The Working Group gives management advice to the Scientific Committee which in turn advises the Convention Commission. Conservation measures have been introduced increasingly in recent years against a background of resistance by fishing nations to what was seen as unwarranted interference in the industry.

However, in the South Georgia area, of a total allowable catch (TAC) of 26 000 tonnes for C. gunnari set by the Commission in 1990, only 93 tonnes were taken, over half in research vessel catches. Following this, a range of conservation measures were adopted in the 1991 CCAMLR meeting: direct fishing around South Georgia was limited to Dissostichus eleginoides, with a TAC of 3500 tonnes, and E.

carlsbergi, with a ceiling of 245 000 tonnes. New limits were placed on by-catches including for N. rossii (300 tonnes) and G. gibberifrons (500 tonnes); and the finfish fishery around the South Orkney Islands remained closed for 1991/92, following closure in 1990 due to insufficient information for proper management.

Research surveys indicate that some stocks in the South Orkney Islands sub-area are recovering from their severely depleted levels in the mid-1980s. Further conservation measures were instigated to promote adequate reporting of catch data for the area under CCAMLR auspices (Anon 1991).

1.4 Ageing Techniques.

Seasonal variations in growth, manifested in growth rings found in otoliths, scales, and skeletal structures, have been recognized and used in the ageing of fish for many years (eg. Tesch 1913; Thielemann 1916). Otoliths and scales have been the structures most widely utilized but Mugiya and Watabe (1977) have found that scales may be regenerated or resorbed. In contrast, neither process has been shown to occur in otoliths, which are therefore the more accurate structures for ageing (Six and Horton 1977; Campana and Neilsen 1985).

Seasonal growth rings in otoliths have, to date, been largely viewed using light microscopy and a great deal of confusion has been caused by inconsistent and conflicting terms describing the structures found. For this study, terminology therefore conforms to

that given by Everson (1981a) for Antarctic fish. The generalized internal structure of an otolith consists of a series of (usually concentric) bands, the annuli, proceeding away from a central nucleus. Although not defined as annual structures, annuli often correspond to one years' growth. Annuli are in turn divided into a hyaline zone of material which permits the passage of light and is usually formed in the winter or slow-growing period; and an opaque zone of material which obstructs light and is usually formed in the summer or fast-growing period. The first annulus is a special case as it consists of the nucleus plus the first true hyaline zone.

Otoliths have been extensively used for ageing in fisheries programmes. Williams and Bedford (1974) reported on an example, started in 1946, undertaken at the Fisheries Laboratory, Lowestoft: by 1973, this involved the annual collection and reading of 35 000 pairs of otoliths to monitor levels of forty separate stocks of North Sea fish.

Williams and Bedford (1974) further reviewed techniques available for viewing otoliths either whole or in section using light microscopy. For opaque otoliths, they found sectioning to be necessary, emphasizing that this must occur through the nucleus to allow an accurate count of annuli. The section can take the form of a thin slice of less than 0.5mm but this was found to be too costly in time for large-scale processing. Alternatively, for a quicker but cruder method, the otolith could be broken and illuminated from the side: the light reflected upwards through the otolith, lighting the cut surface from below. Reading could be facilitated by using a grinding machine to smooth the cut surface or by brushing the

surface with a suitable clearing agent such as xylene or cedar wood oil. Christensen (1964) found that lightly heating the cut surface of otoliths from sole (Solea solea) produced a clear pattern of alternating, narrow black and broad white zones, each combination corresponding to a year. Williams and Bedford (1974) found this particularly useful for species whose otoliths were too translucent to distinguish between opaque and hyaline zones.

More recently, a method for sectioning large samples of otoliths for use in light microscopy was developed by Bedford (1983) which reduced the time necessary to process each otolith. This allowed an accurate sectioning method to be used routinely in fisheries surveys.

Pannella (1971) used acetate peels to give replicas of otolith sections from three cold-temperate fish species etched with 1% HCl: micro-incremental structures were found within yearly annuli and were shown to correspond to a daily cycle. Micro-increments have been found to be a normal feature of the internal structure of fish otoliths and have been shown often to correspond to a daily cycle. They have therefore offered a method of assessing age and growth with greater accuracy and precision than was previously possible (Campana and Neilsen 1985). However, micro-increments are frequently too small to be resolved by light microscopy while scanning electron microscopy (SEM) techniques can overcome this problem but have the disadvantage of being time-consuming.

Validation of the timing of both micro-incremental and annular growth in the otoliths from a given species is a pre-requisite for

these techniques to be used with confidence for ageing (Williams and Bedford 1974; Beamish and McFarlane 1983; Campana and Neilsen 1985; Jones 1986). Structure has been found to vary between species and errors can be introduced by the failure to account for structures corresponding to sub-daily intervals or large scale checks induced by major events such as spawning.

Methods to validate annuli include obtaining a time-series of samples taken throughout the year and noting the type of growth present at the extreme outer edge. The timing of deposition of opaque and hyaline zones in temperate fish can then be ascertained (Christensen 1964; Williams and Bedford 1974).

Alternatively, chemical time-marks can be introduced by injecting fish with compounds such as tetracycline hydrochloride. This is incorporated into the otolith and leaves a mark corresponding to a known date that fluoresces under ultraviolet light, allowing subsequent growth over a known time period to be assessed (Williams and Bedford 1974; Campana and Neilsen 1985).

Micro-increments cannot be validated by the tetracycline method as the fluorescent mark does not show up using the SEM. Instead, fish can be injected or exposed to acetazolamide. This is a carbonic anhydrase inhibitor which temporarily interferes with the calcification process in the otolith resulting in a disruption mark corresponding to a known date. This can then serve as a reference location for study by SEM (Mugiya and Muramatsu 1982).

Management of Antarctic fish stocks is based upon age-dependent models that require good ageing techniques: small inaccuracies have been demonstrated to have large effects on parameters such as Yield/Recruit used in decision-making (Coggan et al 1990). Annular and micro-incremental growth structures have been found in the otoliths of Antarctic fish (Townsend 1980; Daniels 1983; Radtke et al 1989; Everson 1981a; North et al 1980) and have been used for ageing (eg. Burchett 1983b; Daniels 1983), although few studies validating these structures have been made (North 1988). The Biological Investigations of Marine Antarctic Systems and Stocks (BIOMASS) and CCAMLR have emphasized the importance of determining reliable ageing techniques and CCAMLR has organized a series of workshops held by the Working Group for Fish Stock Assessment (Anon 1989). However, Kock (1990) reported considerable variation in evaluations of samples from Antarctic fish and little evidence of greater agreement among experienced investigators than among experienced and less experienced ones: it was concluded that more validation studies were needed to clarify the nature of ageing structures. White (1991) concluded that the analysis of otoliths was the most accurate method for ageing of Antarctic fish, and reiterated the need for validation studies.

Radtke and Hourigan (1990) used tetracycline and acetazolamide marking techniques to validate otolith micro-increments as daily in Nototheniops nudifrons. Otoliths were etched with ethylenediaminetetraacetate acid (EDTA) before being viewed by SEM. Annuli were also found using the SEM but these were shown, using micro-increment counts, not to correspond to an annual period.

Time-series techniques have not been widely used for Antarctic fish. Burchett (1983a) indicated the annual nature of annuli growth in N. rossii at South Georgia; while North (1988), looking at the timing of annuli formation in the otoliths and scales of an assemblage of fish species, found evidence to support the hypothesis that an annulus represented a year in the life history of Antarctic fish. Both of these studies used only light microscopy techniques and North (1988) noted the presence of a pseudo-hyaline edge; a zone of uncalcified material on the extreme edge of the otolith that appeared as hyaline even during the deposition of the opaque zone. This was calculated to introduce an error of about one month in reading the stage of growth of otoliths.

1.5 Environmental Influences On Otolith Growth.

Growth of N. coriiceps is a complex partitioning of available resources between organs, with indices for gonad, liver and condition factor varying over an annual cycle (Everson 1984). Otolith growth in turn is a function of this overall growth strategy of the fish, exhibiting its own annual cycle, but being cumulative in nature and leaving discernible structures that can be validated with respect to units of time. Otoliths therefore offer a potential record of growth and growth-related events during an individual fish's lifetime (Campana and Neilson 1985).

To interpret this record, it is important to understand the relationship of otolith growth, through the overall growth strategy of the fish, to the influence of outside variables. Studies in

species outside the Antarctic have shown otolith structure in certain species to be sensitive to changes in light, seawater temperature and food availability. For example, in the absence of a diel light-dark cycle, daily increments were not formed in the otoliths of young specimens of three species of Lepomis studied (Taubert and Coble 1977), or in Fundulus heteroclitus (Radtke and Dean 1982). Similarly, short-term fluctuations in seawater temperature could induce non-daily increment formation (Brothers 1981; Neilsen and Geen 1984) while young Chinook salmon (Oncorhynchus tshawytscha), fed more than once daily, produced significantly more increments than those fed only once daily (Neilsen and Geen 1982; 1984). The same may be true for Antarctic species: Targett et al (1987) showed that coupled photoperiod and sea temperature changes influenced feeding rates in Harpagifer antarcticus.

Clarke (1988) has observed that the Southern Ocean offers a highly seasonal environment where the annual variations in seawater temperature and primary production are uncoupled and it is possible to distinguish the effects of one from the other. At Signy Island, the locality for this study, nutrient levels have been shown largely to fluctuate around an annual cycle (Clarke et al 1988), while sea-ice plays a critical role with the annual northward extension of the pack-ice and temporary local freezing to form fast-ice. White (1977) has demonstrated the variability of ice cover between years and Clarke et al (1988) have shown similar considerable variation in the intensity and duration of the short algal bloom. Fish at Signy Island therefore are exposed to marked seasonal changes in environmental parameters with large variations between years and in

the sequence and timing between seasonal variables (Burren 1988; White and Burren 1992). Intervals between seasonal changes are stable and long-term.

Antarctic fish are characterized by slow growth rates (Everson 1984). This may be due to direct limitation by the low seawater temperatures found in the Antarctic; or to seasonal resource limitation with algal production concentrated into a short summer bloom. To choose between the competing hypotheses, Clarke and North (1991) showed that limitation of growth by temperature would be severely tested by the occurrence in Antarctic fish of a pronounced seasonality of growth in which maximum summer growth rates were similar to those of warmer water species.

The influence of temperature on growth may be both by its short-term fluctuations and by its overall baseline level. The first involves a temporary physiological adjustment to temperatures encountered within the annual range, the second may involve permanent physiological adjustment or evolutionary adaptation of species. Clarke and North (1991) tested the second of these temperature-related influences, pointing out that local seasonal variations may still have accelerating or decelerating effects on growth rate.

Data from fish larvae were used, where serial samples would show discernible change in length and not be influenced by reproduction on the growth rate. Comparisons were made between species from tropical, temperate and polar regions. These suggested a positive effect of temperature on growth rate in larval fish but

indicated that growth rates in the field may not represent actual capacity for rapid growth within an individual species.

Data from older life stages were not available to test the hypotheses. The test can, however, be applied to otolith growth studies in which the role of temperature and resource availability is of intrinsic interest. Furthermore, if otolith growth is directly indicative of somatic growth, hypotheses of growth limitation by temperature or resource availability can be tested by examining otolith growth rates during a year cycle.

1.6 The Study.

1.6.1 Signy Island

Sampling for the present study was undertaken between June 1987 and June 1988 from the British Antarctic Survey research station at Signy Island in the South Orkney Islands (60° 42'S, 45° 36'W) (Fig. 2).

The station is located on the eastern shore of Factory Cove, a small inlet that forms part of Borge Bay (fig 3). Mean depth of the bay is about 10m with a maximum of approximately 30m. The sea floor slopes gradually away into Orwell Bight with no submerged sill, allowing a free circulation of seawater (Clarke et al 1988). A number of rocky islets give some protection from pack-ice but rafts of smaller pieces frequently enter Factory Cove and shallow waters are subject to scouring by ice. The bottom varies from rock to fine

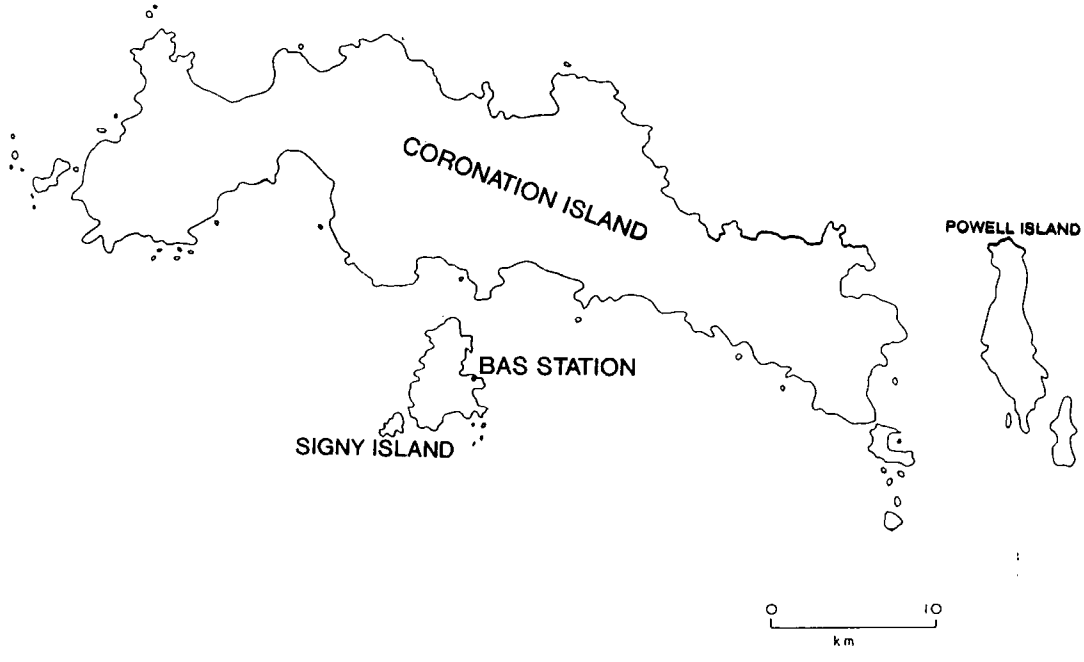

Figure 2. Western half of South Orkney Islands, showing location of Signy Island research station.

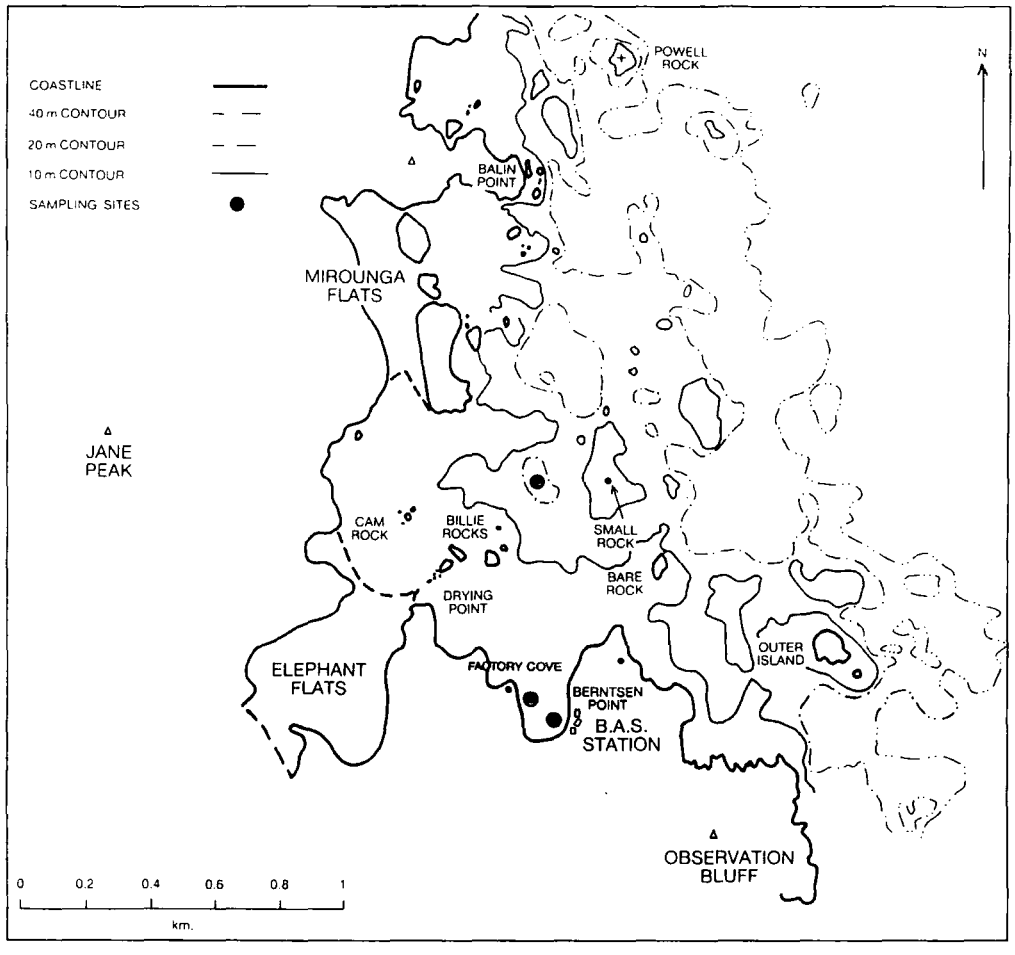

Figure 3. Detail of eastern side of Signy Island showing locality of research station, bathymetry, and major features in Borge Bay.

sediment and the nature of the substratum largely determines the distribution and abundance of benthic plants (Richardson 1979).

The research station has been in continuous use for over forty years. It is equipped with two Humber inflatables, a 7m launch and full facilities for diving in open water and under sea-ice. A small but adaptable wet laboratory with storage tanks and smaller aquaria is fed by open circulation of sea-water pumped from Factory Cove.

The latitudinal location of the site means seasonal changes in ambient light are pronounced without periods of 24-hour day or night, while sea temperatures regularly rise from -1.8^{o}C in winter to +1.5^{o}C in summer. A long-term environmental monitoring programme made it an appropriate site to study the influence of changing environmental variables on otolith growth.

1.6.2 Notothenia coriiceps

Nomenclature follows that of Gon and Heemstra (1990). N. coriiceps is also widely referred to as Notothenia neglecta.

N. coriiceps (Fig. 4) is a demersal species of nototheniid that is readily accessible during the whole year, including winter from under seasonal sea-ice, and which is dominant in terms of biomass in inshore habitats at Signy Island. Its biology has been well-documented by Everson (1970), who also conducted ageing studies at Signy Island by counting the annuli found in otolith cross-sections. The method, however, has not been validated for this

Figure 4. Immature Notothenia coriiceps.

species nor the nature of otolith growth, or the possible existence of micro-increments, elucidated.

Such a study has become increasingly important with the development of fisheries activity at the South Orkney Islands, especially given the history of over-exploitation and lack of information for management. Additionally, as stocks recover, political pressure for re-opening the fishery will increase in the near future. Several of the species that are subject to commercial-scale fishing, particularly N. rossii, are closely related to N. coriiceps; while Kock et al (1984) have found that the latter may represent an unknown proportion of catches from the Antarctic Peninsula, misidentified by fishermen due to its similar colouration and shape to N. rossii. These factors make N. coriiceps an appropriate species for this investigation.

1.6.3 Objectives And Approaches

1. **Validation of annular structures.** Two approaches were adopted: firstly, a time series of monthly samples was to be taken to allow comparisons to be made in the development of the outer annulus of otoliths over the annual cycle. Secondly, fish were to be injected with a solution of tetracycline hydrochloride to give a fluorescent mark of known date. Data could then be used to test North's (1988) hypothesis that an annulus represents a year in the life history of Antarctic fish.

2. **Validation of micro-incremental structures.** Fish were to be injected and exposed to acetazolamide, to give a disruption mark of

known date. Counts of micro-increments found could then be used to test the hypothesis that micro-increments correspond to a daily cycle in otolith growth.

3. **Description of annual growth of otolith.** Once annular and micro-incremental structures had been validated, an annual profile for otolith growth could be constructed using the time-series samples. This could then be used to apply Clarke and North's (1991) test; and to see if short-term fluctuations in seawater temperature, food availability and absolute light levels may be related to otolith growth.

2. METHODS

2.1 Capture Methods

Sampling was focused on immature individuals of N. coriiceps to avoid the influence of the reproductive cycle on otolith growth rate. Specimens were caught at two sites in Borge Bay (Fig. 3). The first was close inshore at 9m depth in Factory Cove and was selected for its accessibility, even during bad weather. It consisted of an interface between two habitats: a flat bed of silt and sand with clumps of free algae; and a sloping bank of boulders and smaller stones with clumps of fixed algae and crevices filled by sand and small pebbles. The second site was further out in Borge Bay at 18m depth, and was used as a result of falling capture rates at Factory Cove, to avoid possible over-fishing or learned avoidance by local fish. It consisted of a shallow bowl with a thick layer of algae covering a substrate of small cobbles lying over a sand/gravel mix. One sample for time-series analysis, taken on 25 June 1988, was collected by handline at 6m depth in Factory Cove, at a site directly in front of the research station jetty.

All specimens except those for the last monthly sample were taken with a 30m trammel net, of inner mesh size 9cm and outer mesh 17cm, set overnight and recovered early the next morning. This avoided both ensnaring any seabirds or seals and damage to fish by scavenging amphipods. In summer, an inflatable craft was used, one person deploying the net while the other directed the boat. In winter a line was swum between two holes in the fast-ice, the net

then suspended beneath the ice surface by two linesmen and dropped evenly to the sea floor. Checks by divers indicated this to be an efficient way of setting the net during the long periods of good sea-ice encountered during the austral winters of 1987 and 1988. Difficulties in reaching the sample sites were encountered during the formation and break-out of ice at the beginning and end of winter (April-June and November). Fishing with pack-ice in the vicinity was avoided as far as possible because of the risk of losing the equipment as a result of drifting ice snagging the net or surface marker.

Fish were extracted from the net by hand either on site or back at base depending on conditions, and placed in holding tanks as quickly as possible.

2.2 Time-Series Collections.

A monthly sample of ten fish was taken, and specimens sacrificed as soon as possible after collection. A full set of biometric data was taken including sex, total and standard length, total and eviscerated weight, gonad weight and an index for gonad stage. Otoliths were collected by section of the cranial area, exposing the brain and vestibular regions; sagittae were then extracted using forceps.

The otoliths were washed in a dilute solution of detergent, rinsed and left to dry (Fig. 5), before being stored in labelled vials. The BAS catalogue number and date were marked on each label.

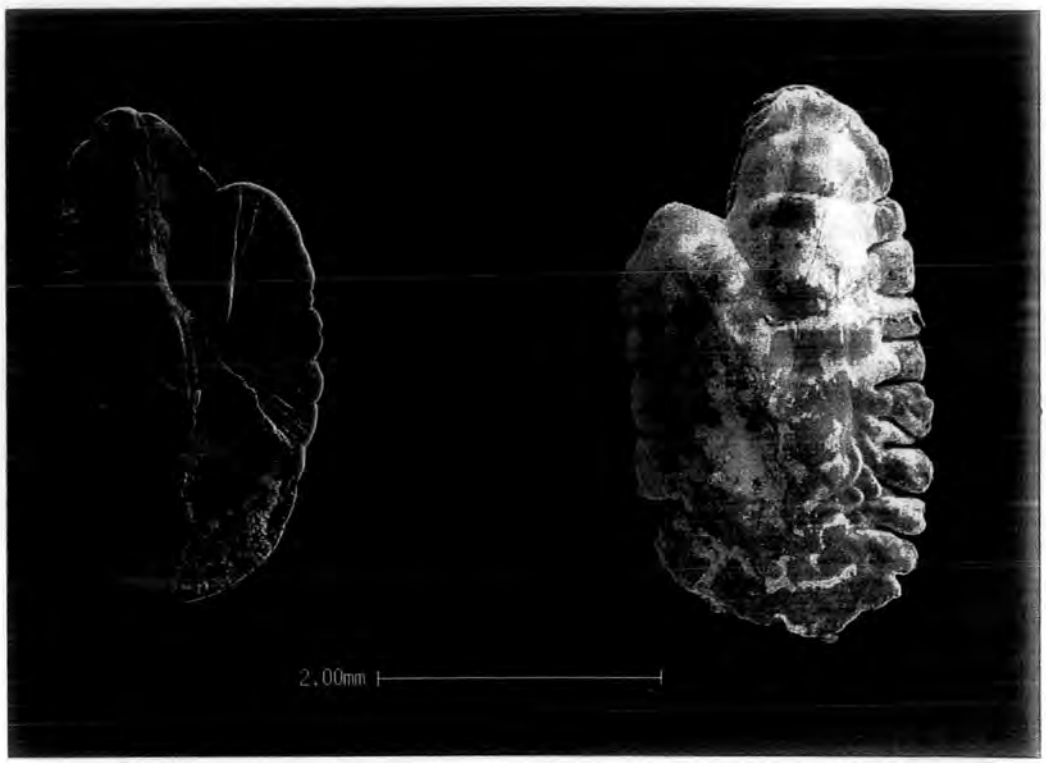

Figure 5. SEM photomicrograph of whole otoliths from two immature individuals of N. coriiceps after cleaning in dilute detergent. The interior (left) and exterior (right) faces are shown.

Samples were collected from early June 1987 to late June 1988 with a break between 4/2/88 and 3/5/88 when sampling was not possible due to other commitments. At temperatures of less than -20°C, all fish froze almost immediately on leaving the water.

Food availability was assessed using a Stomach Fullness Index (SFI), whereby the quantity of food in the stomach was estimated against its full capacity and classified on a scale of 0 (empty) to 4 (full). The advantage of this method over estimating prey levels directly lay in its speed and simplicity and that it assessed real food availability, taking into account variations in the behaviour of fish and prey that might act as a fluctuating barrier to a fish's foraging success.

Seawater temperature was determined twice a month at a single site in Borge Bay, from a moored boat during summer and through a hole in fast-ice during winter. Three Watanabe reversing thermometers mounted on a single NIO (National Institute of Oceanography) water bottle were used for each determination, with 15 minutes equilibration time *in situ* at 9m depth. Thermometers were allowed to equilibrate in the wet laboratory at the research station for at least one hour before being read and the mean value of the three readings was recorded.

Fast-ice break-out and formation in Borge Bay and Factory Cove, as well as major movements of pack-ice, was recorded by direct observation. Light data for April 1987 - April 1988 is taken from

Gilbert (1991) for light reaching the benthos at 8m depth in Factory Cove. Photoperiod was not recorded as otolith data could not be compared between years.

2.2.1 Viewing By Light Microscope

Otoliths were initially prepared for viewing under a light microscope using the technique developed by Bedford (1983) and used by Ministry of Agriculture, Fisheries and Food (MAFF) Laboratories, Lowestoft for fisheries surveys. Moulds were cleaned, greased with beeswax, the base coated with an initial layer of Strand Polyester Resin C mixed with 3% black dye, and left to set. The otoliths were then placed in rows, using nylon guidelines stretched across the mould above the resin, care being taken to avoid any positioning error due to parallax (Fig. 6). The right otolith was used, where possible, rostrum being positioned downwards and the sulcus side flat to the resin base. A string of dried spaghetti was placed to the left side of the rows of otoliths to act as a datum, and then a second layer of resin was poured to cover the otoliths until the nylon guidelines just caught a meniscus. The mould was then left overnight to set. A hardener was added to this second layer or the surface covered with a plastic sheet to allow it to harden fully.

The resin casts thus formed were released from the mould and sectioned at the MAFF Labs at Lowestoft using a high speed diamond-disc saw. A cut was made through the nuclei of each row of otoliths; a second cut behind then gave a strip of transverse sections 0.5mm

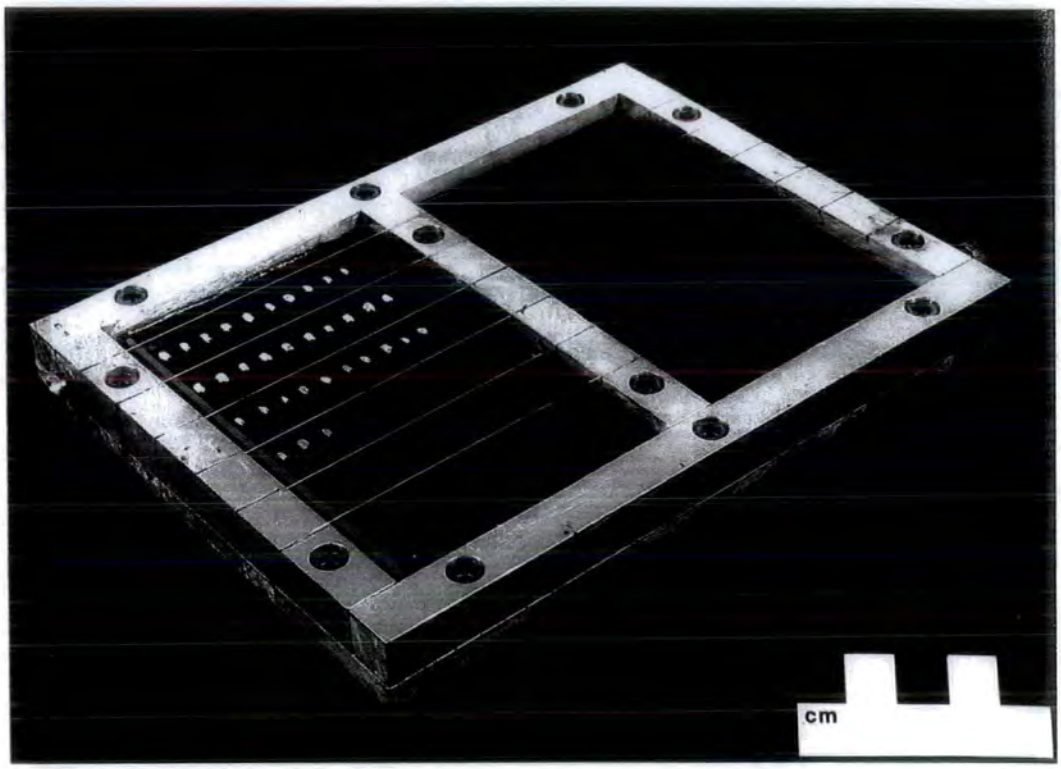

Figure 6. Mould constructed after Bedford (1983) showing otoliths aligned on black-pigmented polyester resin.

thick. Up to three strips were then mounted in clear casting resin under a coverslip on a glass slide measuring 7.5cm by 5cm, and left to set.

The slides were viewed under a binocular microscope at magnifications up to 50 times, and development of the outer annulus assessed. North (1988) used five categories for the stage of growth reached by the outer annulus: for this study, his system was adapted to four categories. These are given in Table I. Repeat readings were made blind and where notable discrepancies occurred in samples, a further observation was made.

Table I. Categories used to assess stage of growth reached by the outer annulus using light microscopy.

Stage	Zone	Zone Width
1	Hyaline	Narrow
2	Hyaline	Well Developed
3	Opaque	Narrow
4	Opaque	Well Developed

2.2.2 Adaptation Of Bedford's (1983) Technique For Viewing By SEM

Results from the light microscopy method were considered to be equivocal so transverse cross-sections of all otoliths from monthly

samples were viewed using an SEM. To achieve this in the time available, a method for processing otoliths in large numbers had to be developed.

In applying Bedford's (1983) technique described above, one face of the cut through the nuclei of each row of otoliths was left unused, forming residual blocks. These were then divided into two and the surface containing the sectioned otoliths lapped and polished. A number of protocols were tried, initially encountering the problem of contamination by small chips of otolith leading to gouging on the sample surface. Eventually, a consistent method was arrived at whereby lapping was performed by hand on a glass surface using aluminium oxide paste of grit sizes 600, 800 and 1000. They were then polished on a Kent 3 Lapping And Polishing Machine, held by hand and using 3um and 1um grit paste with Engis Hyprocel PSU polishing cloths, and finally completing the process using 0.3um grit alumina paste with Engis Lamplan Type 410 polishing cloths. Both cloths and blocks were regularly rinsed with runnning tap water to remove any fragments breaking off the otoliths during polishing. An alternative method was attempted whereby otoliths were mounted individually in epoxy blocks and ground down to the nucleus, but this was found to be a time-consuming process. The large discrepancy between hard matrix and softer material, together with a more vigorous polishing action, also frequently resulted in the samples being damaged during polishing.

Otoliths were then etched using 4% EDTA for five minutes followed by rinsing in distilled water and a further immersion of at least five minutes. This was found to be the most suitable protocol

after a series of tests using EDTA and HCl at different concentrations and for different durations. Variability between otoliths was found to be the greatest factor in producing satisfactory etching. However, EDTA was found to give a better relief across the whole otolith section while the concentration was an optimum between that needed to show micro-increments, and over-etching of larger scale structure.

Blocks were dried after etching, mounted on SEM stubs with araldite or double-sided adhesive tape, coated with gold using a BIORAD SC502 sputter-coating unit, and earthed to the stub using colloidal silver (Fig. 7). They were then viewed with a Leica Cambridge 360 SEM (Fig. 8). For each otolith, the SEM image processing facility was employed to produce stored images using the line integrate function. Working distance was set at 7-10mm. Each line was scanned 32 times at a scan rate of TV/32. Videoprints were taken, using a Mitsubishi P68B videoprinter, of the whole otolith at times 75 magnification and another at times 200 of a previously selected edge region. In this way 60 otoliths were processed on the SEM in a single day.

Finally, the videoprints for each sample were examined for the stage of growth reached by the outer annulus. Four categories were used, based on the two types of etching observed within each band and then sub-divided into 'narrow' and 'well-developed'. Videoprints and evaluation were made 'blind', without reference to the date of sacrifice. Data were then pooled into sets of three months for analysis of distribution by chi-square test.

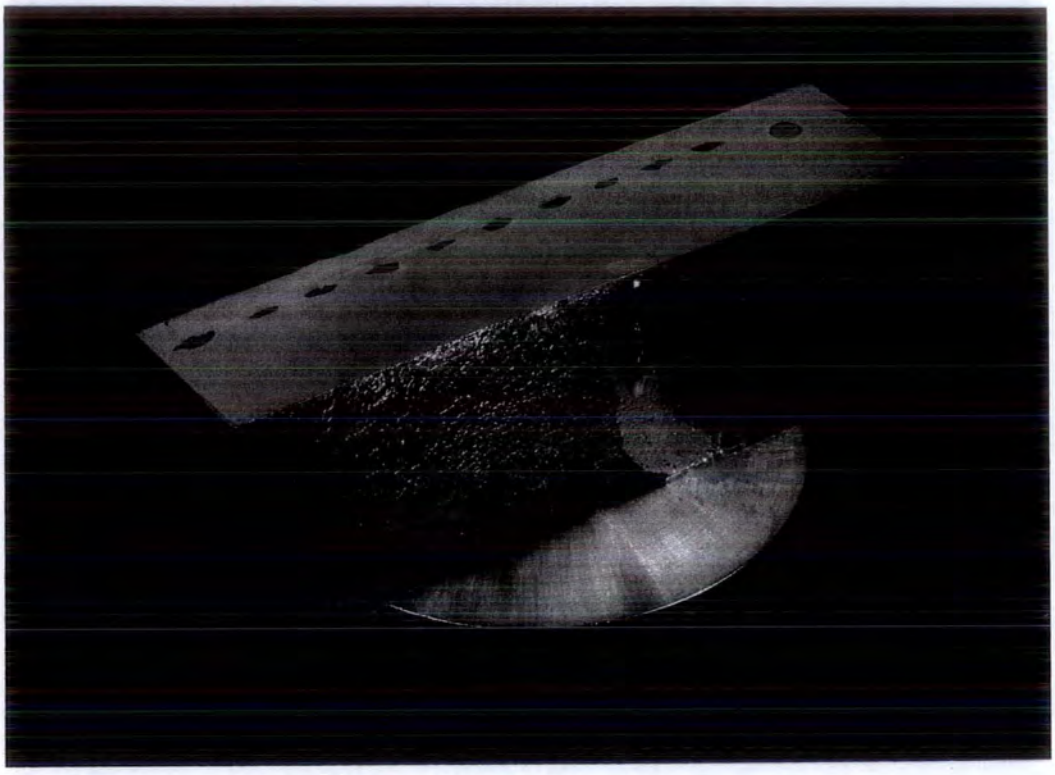

Figure 7. Cut block showing sectioned otoliths ready for examination by scanning electron microscope.

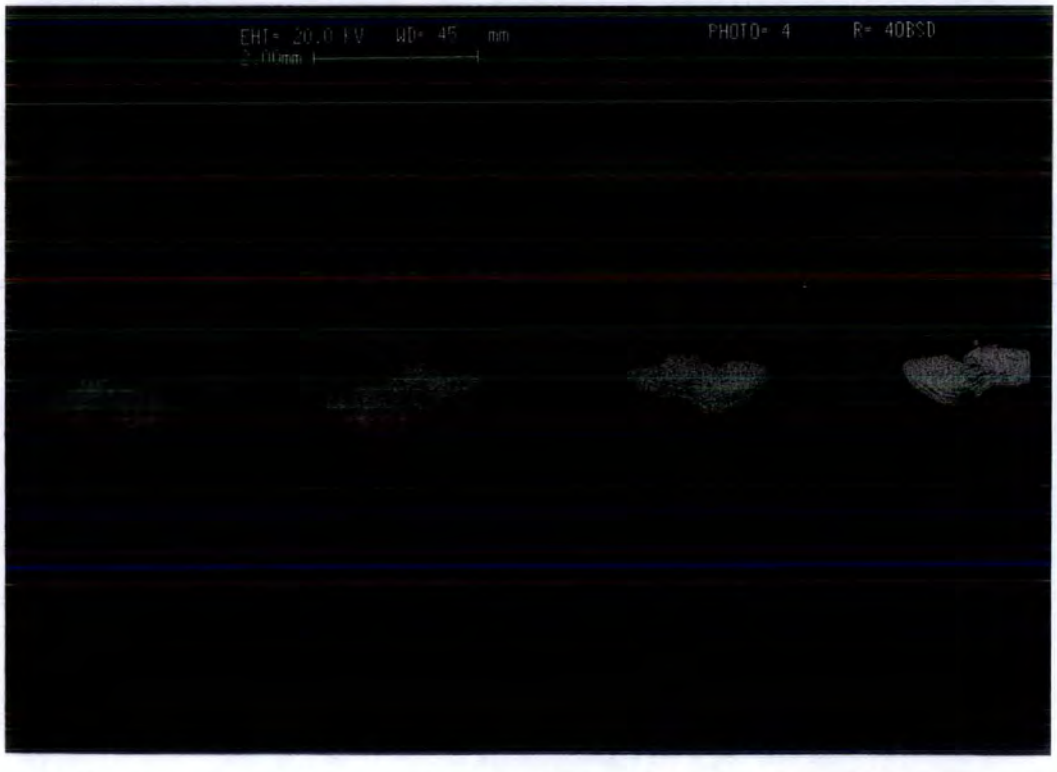

Figure 8. Low magnification SEM photomicrograph of otolith sections embedded in polyester block.

Two sub-samples of ten and five otoliths were viewed using the SEM. In the first, a series of widely-spaced micro-increments present in the first annulus was measured and the mean size of micro-increments taken. The inner margin of the series was found to be difficult to define for comparison between otoliths, so the distance measured was that between the centre of the nucleus and the outer edge of the series. The equivalent number of micro-increments present was then calculated.

For the second sub-sample, the number of micro-increments occurring in each annulus was calculated. The width of outer annuli and the mean size of micro-increments within each was measured. Micro-increments were found to be rarely well-defined through an entire annulus, so the mean size was based on those visible.

2.3 Marking Experiment Using Tetracycline.

Specimens were caught between 3 and 16 June 1987 under newly-formed ice. They were then left in holding tanks in the wet-lab for a minimum of two weeks to acclimatize to laboratory conditions. The fish were fed on amphipods entering with the water supply, supplemented with diced canned pork.

On 28th June, an injection trial using Young's saline solution (NaCl 13.5g/l; KCl 0.60g/l; CaCl2 0.25g/l; MgCl2 0.35g/l) was performed on three fish. On 1 July, once no ill effects had been observed, the first injections of tetracycline were given to the remaining fish.

Eight groups of three N. coriiceps each were established in four holding tanks, each divided in two by moveable partitions. The inflow and outflow of a tank were on opposite sides of the partition, ensuring a good circulation of water to both groups of fish.

Dosage rates from 30mg/kg to 100mg/kg body weight of fish have been used successfully to give a fluorescent mark in otoliths (Mugiya 1977; Campana and Neilsen 1985; Ralston and Miyamoto 1983). Doses of 25mg Tetracycline/kg body weight of fish were therefore administered to the two groups in one tank and doses of 50mg/kg and 100mg/kg to individuals in another two tanks. The fourth tank contained the laboratory controls consisting of uninjected fish and those injected with Young's solution on 28 June. A wild control was also used, consisting of that month's time-series sample. Solutions of 5mg Tetracycline/cm^3 Young's solution were used for the former, 10mg/cm^3 and 20mg/cm^3 for the latter ensuring a constant ratio of injection volume to fish weight for all injections given. Specimens were taken from the tanks individually and weighed using a small net hanging from a spring balance.

The required volume of the appropriate solution was then calculated and injected into the perivisceral cavity. The method used was to insert the syringe needle into the ventral body wall, raising the muscle layer to avoid injecting the intestinal tract, before sliding the point into the abdominal cavity. One group from each experimental tank was given a second series of injections nine days later on 10 July.

The fish were maintained until 22 and 23 September before being sacrificed, their otoliths and a scale sample collected and a full set of biometric data taken. Eleven fish died during this period, including all the injected (but not the uninjected) controls, indicating the possibly adverse effect of the injection protocol. Otoliths and biometric data were collected from fish that died during the experiment as well.

Otoliths were examined back in Cambridge in October 1991 under ultraviolet light using a Nikon Diaphot binocular light microscope with ultraviolet filter. Otoliths were previously sectioned by Bedford's (1983) technique but using a single cut, leaving two halves instead of two cuts giving a thin section: this allowed direct exposure of the otolith surface to ultraviolet light. One of the halves was then washed, polished and examined for evidence of fluorescent marks, using two viewing protocols: dry and under a drop of water.

2.4 Marking Experiments Using Acetazolamide.

Two separate experiments were performed during different periods of the year and using different methods.

2.4.1 Experiment 1

Specimens were caught between 16 and 21 October 1987 and left in holding tanks for approximately three weeks to acclimatize to

laboratory conditions. They were fed either amphipods, diced Laternula sp. or diced pork but it was increasingly obvious that while some fish adapted quickly to the new feeding regime, others ate practically nothing during much of the experiment.

Ten individuals were exposed in a single tank to a solution of 125ppm acetazolamide (Ralston and Miyamoto 1983) on 9 November: in a volume of 175 litres of water, 21.88g was dissolved, the inflow suspended and aeration increased to maintain dissolved oxygen levels. Fish were then transferred from their holding tanks at 17.00 hours and left in the solution until 12.30 hours the next day before being transferred back to natural seawater. A control group of seven individuals in another tank was similarly treated, but exposed to seawater only. All fish were then left until being sacrificed on 17 February: biometric data was recorded, and otoliths taken. Seven fish died prematurely, two before the beginning of February. All but one of those dying prematurely had been exposed to acetazolamide, indicating the possibility of some adverse effects of the treatment. Full biometric data and otoliths were also taken from these fish.

2.4.2 Experiment 2

Specimens were caught between 16 and 25 June 1988 and left in holding tanks to acclimatize to laboratory conditions, with a similar feeding regime to the previous experiment. Again, there were notable differences in feeding behaviour between fish, some feeding very little.

Ten fish were injected on 20 July with a solution of 10g acetazolamide dissolved in a litre of Young's saline solution. Dosage rate was at 50mg/kg body weight of fish (Mugiya 1977), and a further five fish were injected with Young's saline solution as a laboratory control. Additionally, seven fish were injected with acetazolamide and placed in an enclosure on the seabed at 6m in Factory Cove to act as a semi-wild control. The latter was unsuccessful, the fish dying within two weeks for unidentified reasons and their corpses immediately scavenged by amphipods so that all otoliths were unrecoverable. Laboratory fish were sacrificed on 1 September, seven fish dying prematurely; again, all but one had been injected with acetazolamide, possibly indicating some adverse effect of the treatment. Biometric data and otoliths were collected for all fish.

Otoliths from both experiments were washed, dried, stored in labelled vials and sent to Cambridge. They were examined using the method of Bedford (1983) adapted to SEM use as outlined above. A single cut was made through the nuclei of each row of otoliths and the resulting blocks prepared for viewing by SEM. Videoprints were taken of whole otoliths at magnifications of times 60 and times 200 for up to four selected regions within each otolith. The videoprints were then analysed for any disruption marks caused by the acetazolamide.

2.5 Growth Profile.

The set of videoprints of otolith cross-sections taken at a magnification of 200 times for the time-series validation were viewed using a Panasonic TV Camera and a Seescan Solitaire Plus image analysis system. Measurements were taken of the width of the annulus corresponding to growth during 1985/86 (a) and the width of all succeeding growth (b). The ratio b/a was then calculated as the growth index (GI) for each otolith for the period June 1987 - June 1988. Mean of GI for each sample was then plotted against time and regression equations fitted to the data produced. An analysis of covariance was then performed to test for differences between the regression equations.

3. RESULTS

3.1 Biometric Data

Data for monthly means of standard length, total weight, and eviscerated weight are presented in Fig. 9, showing standard deviations. All but three specimens were immature fish with mean gonad weight of 1.60g; the three mature fish, all females, had gonad weights of 25.2g, 96.0g and 110.0g.

The data for standard length, total weight and eviscerated weight showed some evidence of increasing size of fish caught up to 1 February 1988, with a decline in all parameters on 3 May and 2 June, before a sharp increase for the final sample on 25 June. However, interpretation is unreliable due to the small size of each sample and variability of data.

3.2 Otolith Morphology

When viewed using light microscopy, transverse sections of otoliths of N. coriiceps showed a series of concentric annuli around a central nucleus (Fig. 10). These were almost entirely opaque on the exterior, dorsal and ventral sides, characterized by a sudden break between the outer edge of the opaque zone and the succeeding narrow hyaline. The latter then graded into the next opaque. On the interior side of the otolith, the annuli were comparatively

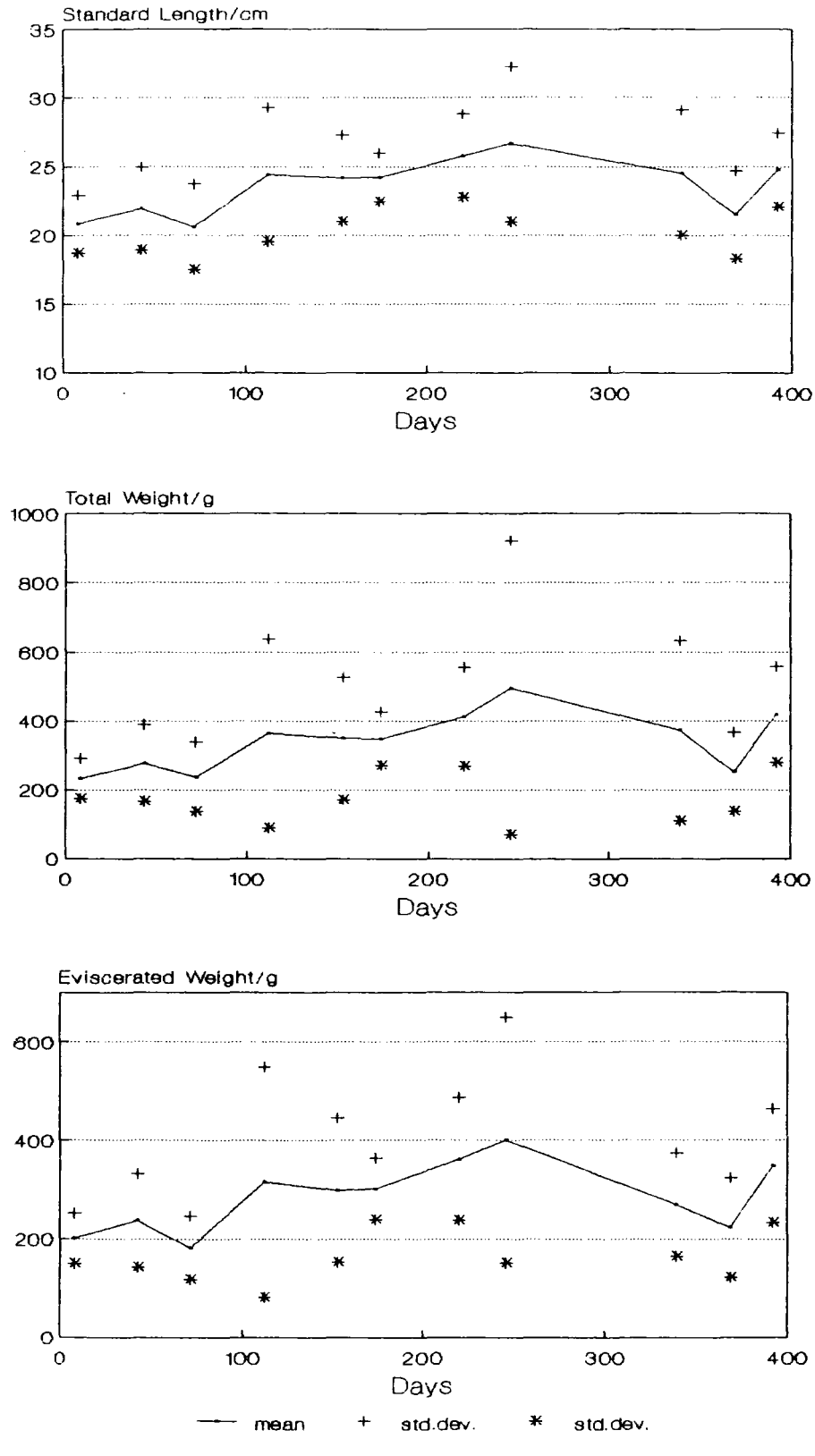

Figure 9. Annual variation in standard length, total weight, and eviscerated weight, from samples collected for the time series study.

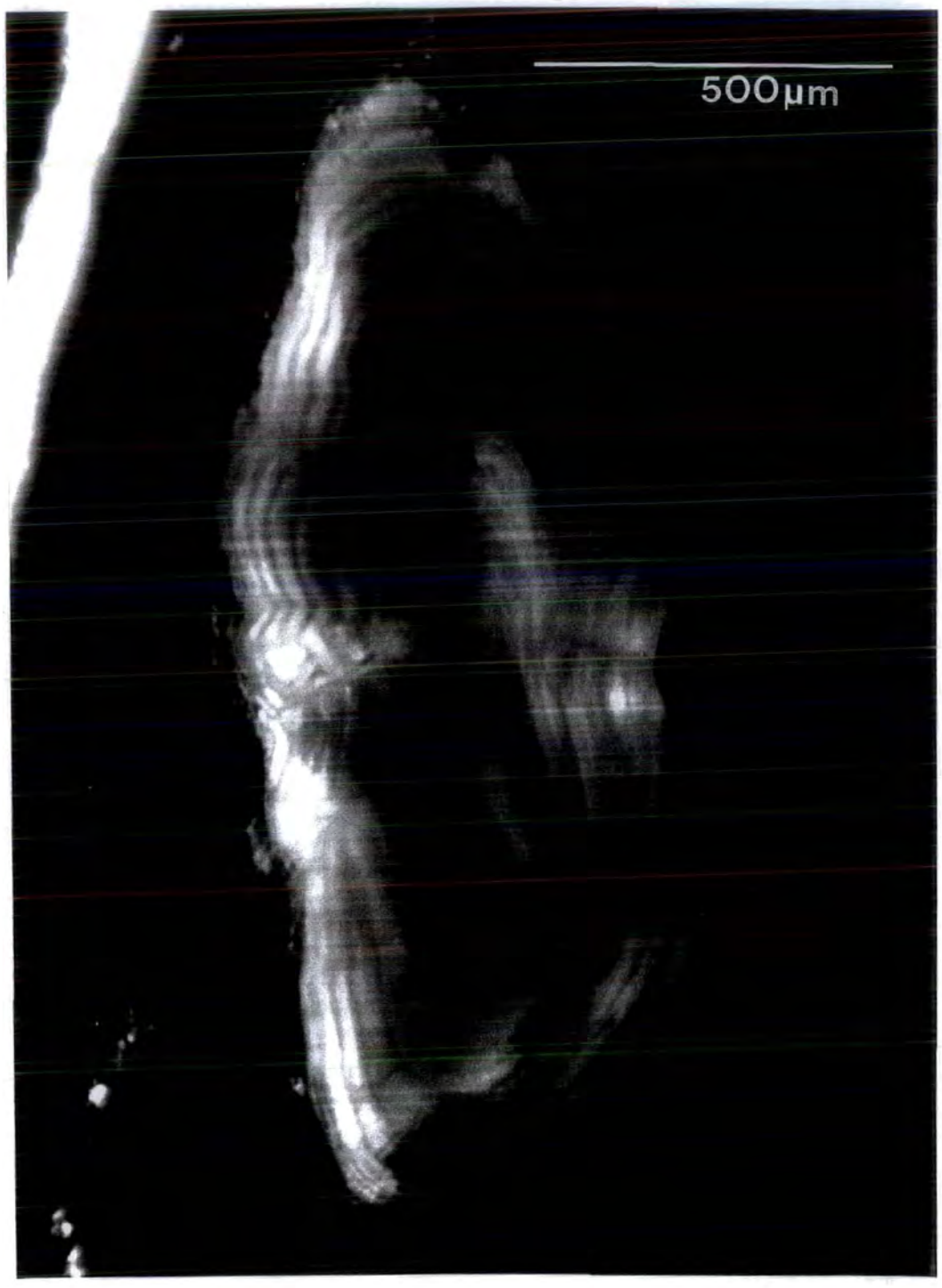

Figure 10. Otolith cross-section, using transmitted light and showing well-defined annuli.

translucent, each becoming more opaque toward its outer edge, with a sudden break between it and the succeeding hyaline.

When viewed using an SEM, transverse cross-sections of otoliths were found to have a highly differentiated structure (Fig. 11). Typically, six regions could be distinguished, radiating out from the centre. Concentric annuli were also found and were characteristically modified within each region. Boundaries between regions were marked either clearly by a fusion line or by a gradual transformation of growth patterns (Fig. 12). Within the first annulus was a discrete nuclear core with no sign of regional boundaries, separated from the rest of the otolith by a distinct nuclear check (Fig. 13a).

Two regions (Fig. 11, regions 1 and 2) radiated from the centre of the otolith dorsally and ventrally with wide annuli divided into large lightly-etched and smaller heavily-etched bands. Another two (Fig. 11, regions 3 and 4) radiated interiorly from the nuclear core on either side of a fifth (Fig. 11, region 5) formed by the bridge of the sulcus, and were characterized by narrow annuli in which the lightly-etched bands were frequently of a similar size to the heavily-etched ones. The sixth zone was on the exterior side with narrow annuli divided into wide lightly-etched and narrow heavily-etched bands. The width of annuli decreased from the centre to the outer edge of the otolith.

Using the SEM, micro-incremental structure within the annuli was evident but rarely well-defined over a large area. The width of micro-increments was found to vary from 0.4um to 1.9um. Immediately

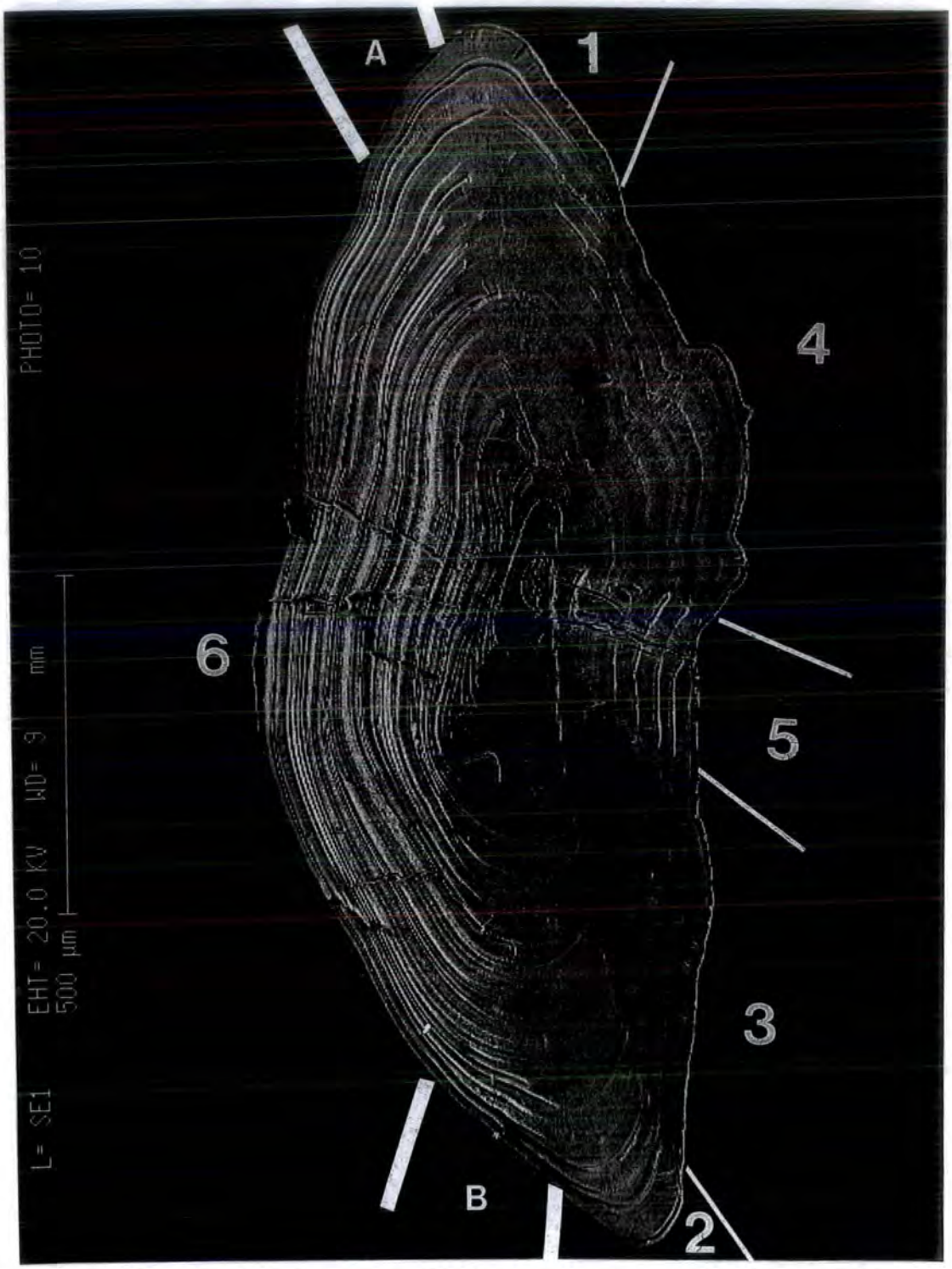

Figure 11. SEM photomicrograph of transverse section of otolith from Notothenia coriiceps. Thin lines denote fusion lines between regions of growth on inner side of otolith. A and B mark boundaries between regions demarcated by a gradual transformation of growth patterns. Thick lines denote limits of these changes in pattern. For regions 1-6, see text.

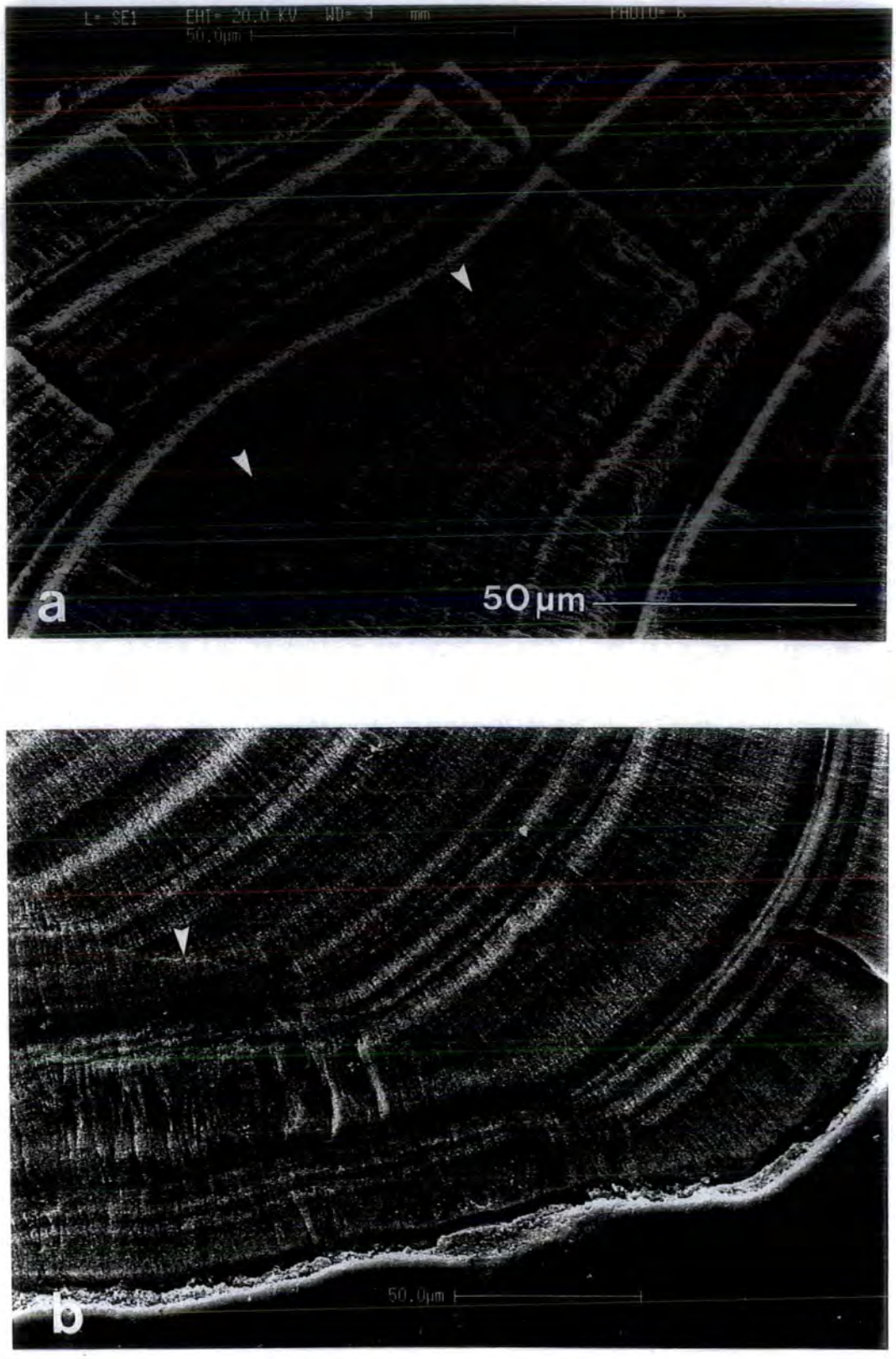

Figure 12. SEM photomicrographs showing boundaries between regions: a) gradual transformation of growth patterns; b) fusion line.

around the nuclear core there was, however, a medially compressed area with consistently well-defined micro-increments (Fig. 13). This was composed of a series (A) of wide increments close to the nuclear check, followed by a second series (B) of narrow increments becoming gradually more obscure, with the transition occurring rapidly between the two series (Fig. 14).

Table II. Sub-sample I: no. of equivalent micro-increments between centre of the nucleus core and outer edge of series A.

Specimen	Centre nucleus - outer edge series A	Mean width of micro-increments	Equivalent no. days
1	165	1.59	104
2	204	1.60	127.5
3	248	1.49	166
4	178	1.87	95
5	177	1.82	97
6	209	1.42	147
7	215	1.46	147
8	154	1.42	108
9	-	1.34	-
10	174	1.09	160

In a sub-sample of ten otoliths, the distance from the centre of the nuclear core to the outer edge of series A was found to be between 154um and 248um with a mean of 191.5um (Table II). The inner

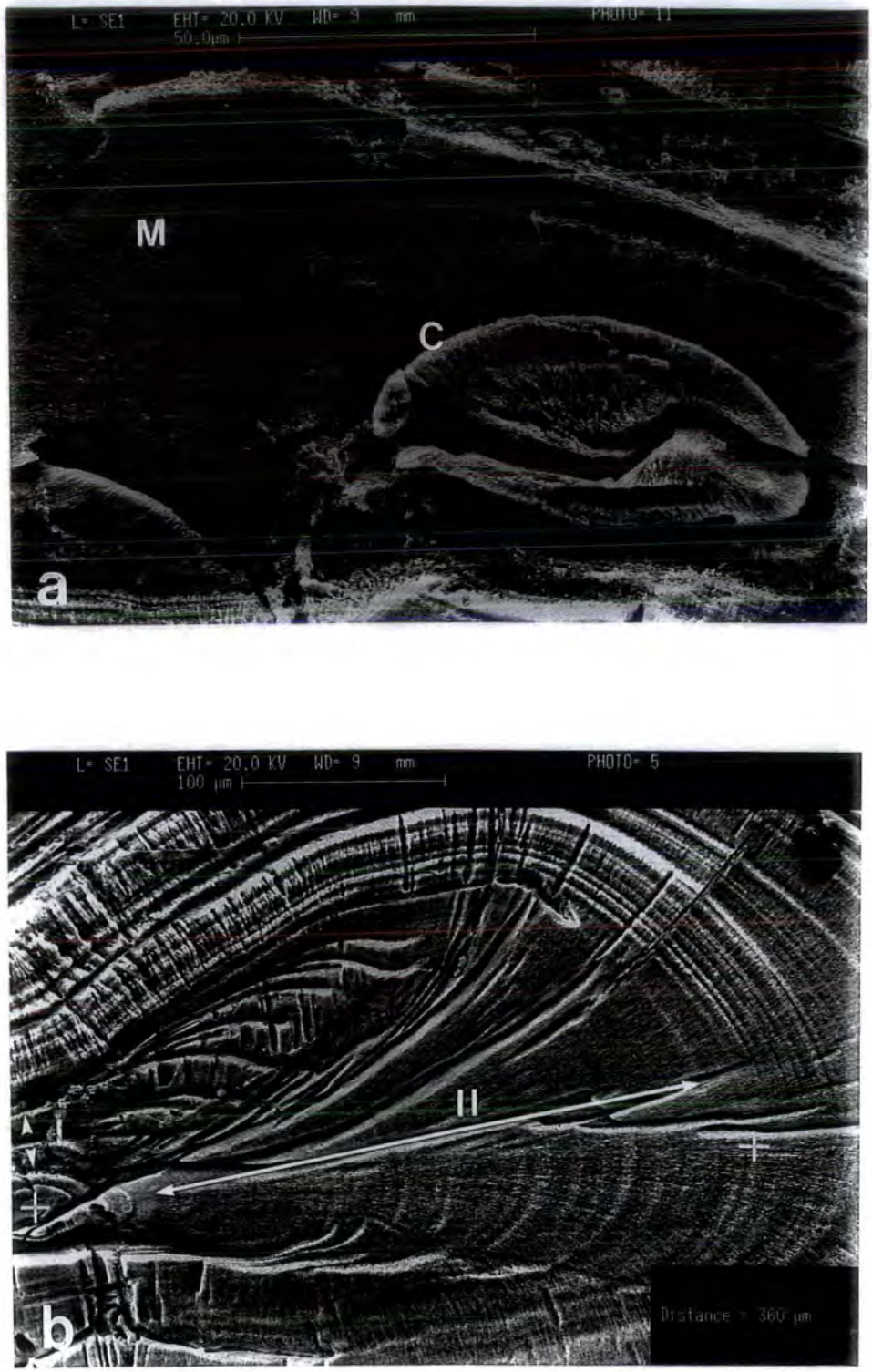

Figure 13. SEM photomicrographs of a) area surrounding nuclear core, showing micro-increments (M) and check (C) separating nuclear core from rest of otolith; and b) area of clear micro-increments, showing compressed pattern (I) and full pattern (II).

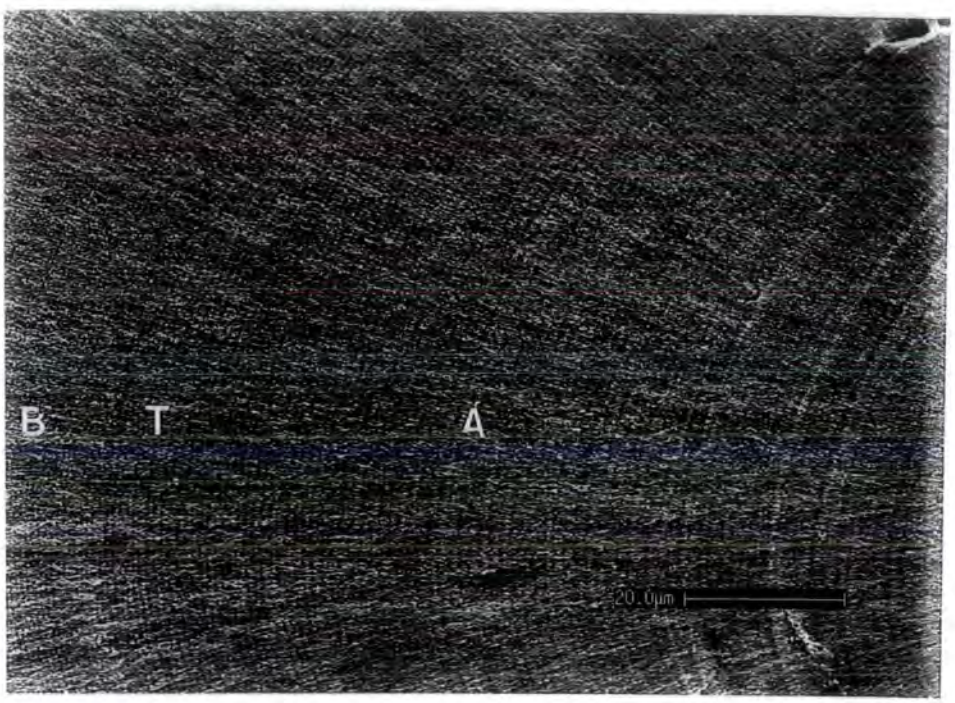

Figure 14. SEM photomicrograph showing area of clear micro-increments: an inner series of large micro-increments (A) and transition (T) to beginning of outer series of smaller micro-increments (B).

edge of the series was found to be difficult to define, the centre of the nuclear core giving a more consistent point for comparison of measurements between otoliths. Mean width of series A micro-increments within each otolith was found to vary between 1.09um and 1.87um with an overall mean of 1.51um. The equivalent number of micro-increments between the centre of the nuclear core and the edge of the series was then calculated, varying between 104 and 166 micro-increments with a mean of 128.

3.3 Validation Of Annular Structures - Time Series.

Observation of the edge of otolith sections viewed using light microscopy was prone to confusion: the small size of the otoliths, the narrowness of the annuli, light aberrations at the otolith edge and the frequent presence of a pseudo-hyaline edge made possible several different interpretations.

Eight monthly samples were examined using light microscopy: a number of otoliths (11 out of 82, or 13%) were unreadable; and of those read, 36 (51%) were considered doubtful in at least one repeat reading. Variations between readings were high (29 out of 71, or 39% varying by one category or more) with dramatic variations between monthly samples: all repeat readings for otoliths taken on 13 July agreed, whereas for those taken on 6 January, no repeats agreed where the otolith was readable. Data is given in the appendices.

Images taken using the SEM were more readily interpreted. In addition, comparative SEM images indicated recurring infusion of the

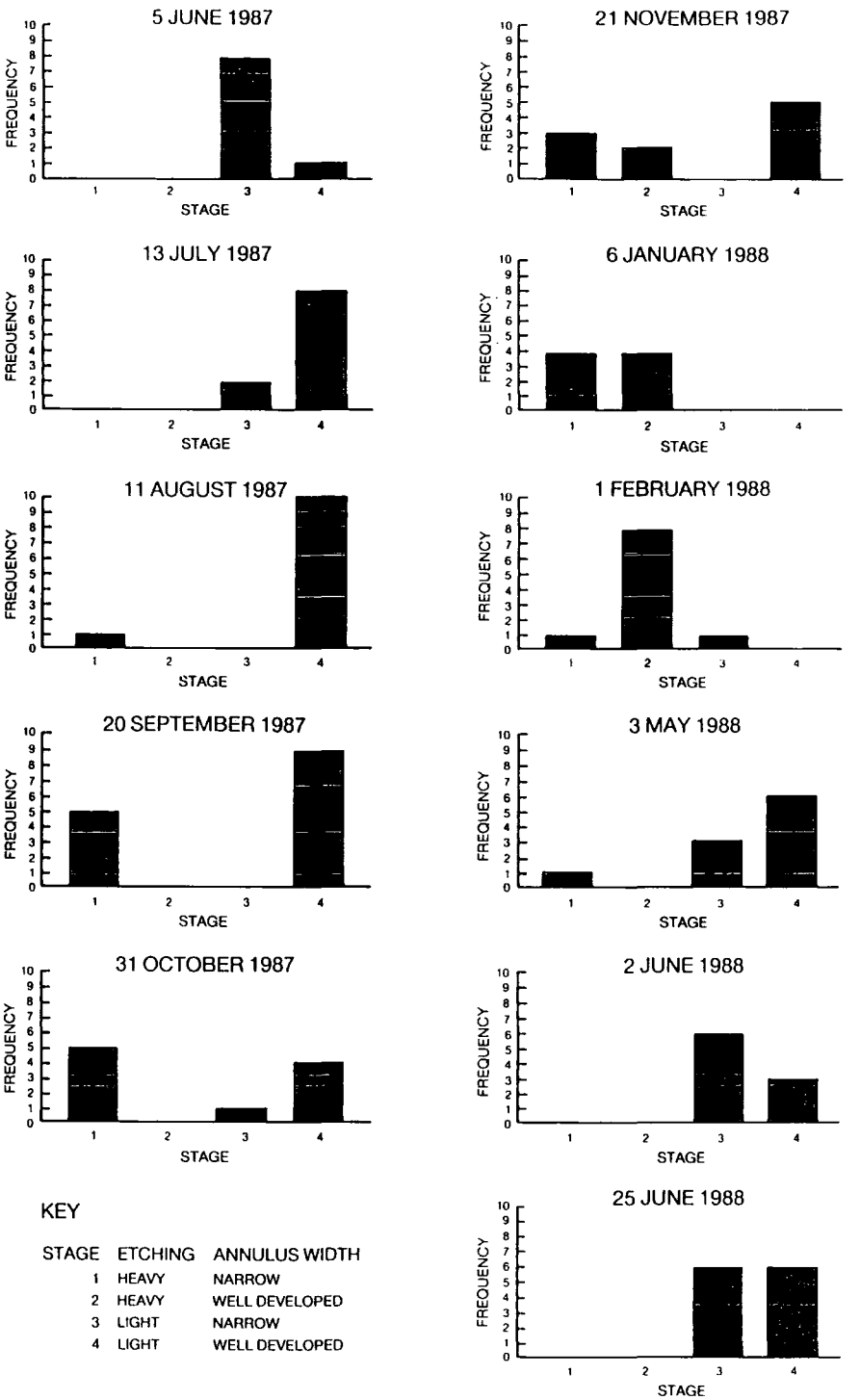

Figure 15. Histograms for growth stage of outer annulus for monthly time series of samples of otoliths from Notothenia coriiceps, June 1987 to June 1988.

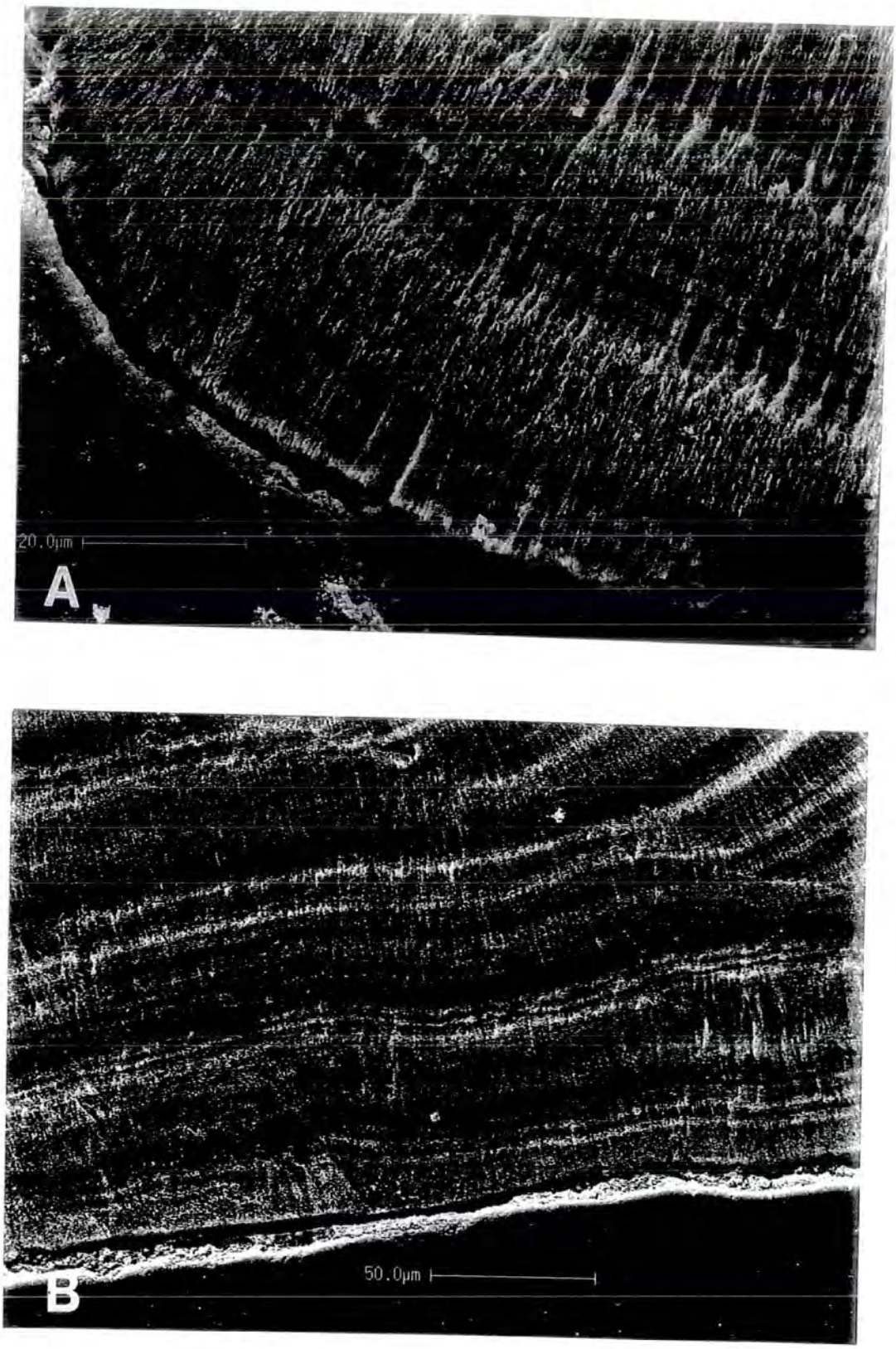

Figure 16. SEM photomicrograph comparing growth stage of the outer annulus from Notothenia coriiceps. A shows otolith at end of stage 2; B shows otolith at stage 4.

outer otolith edge with stained polyester matrix, creating an artificial otolith edge at a varying distance inside the true boundary. Confirmation of the true edge was achieved by examining calcium levels using the energy-dispersive X-ray analysis function of the SEM.

SEM analysis of the outermost annulus in region 3 among the time-series samples gave clear results. The progression of stages through the year is shown in Fig. 15, and examples of stage 2 and 4 in Fig. 16. The distinctive heavily etched bands were deposited at the edge of the otoliths from 20 September to 1 February. The lightly etched band started to be deposited at the periphery from between 1 February and 3 May in 1988, and was almost exclusively present from 5 June 1987 when sampling began until 11 August 1987, and from 3 May 1988 until sampling ended on 25 June 1988.

Table III. Frequency of SEM growth stage in outer annulus of otoliths from time-series samples, using pooled data.

	Stage			
Period	1	2	3	4
June - August 1987	1	0	10	19
September - November 1987	13	2	1	18
January - February 1988	5	12	1	0
May - June 1988	1	0	15	15

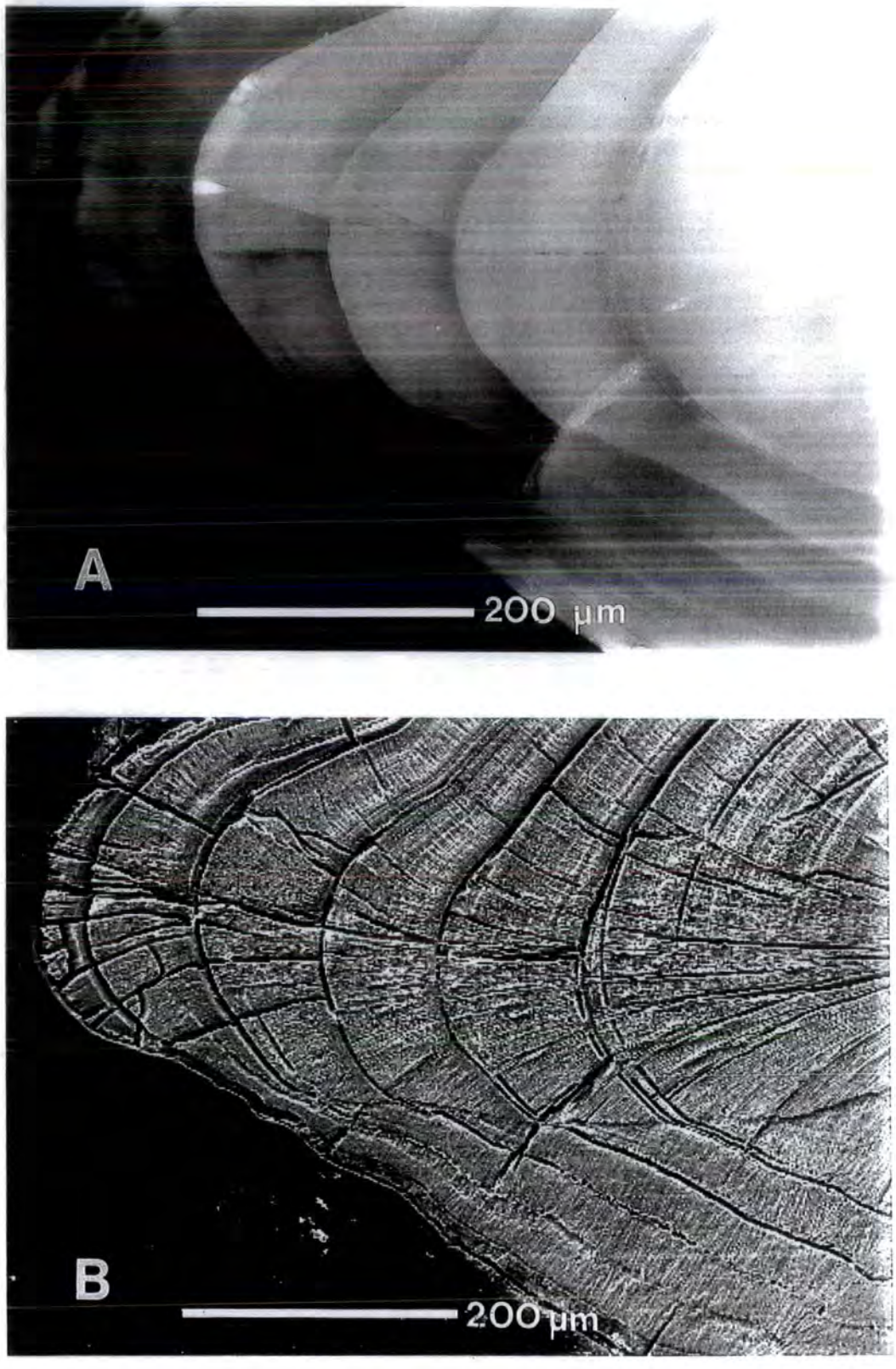

Figure 17. Structures observed for same otolith from Notothenia coriiceps using light microscopy (A) and scanning electron microscopy (B).

The distribution of data for stages was pooled for analysis (Table III) and was shown to be significantly different from a uniform distribution according to a chi-square test (9 degrees of freedom, $p<0.001$). This consistent seasonal development demonstrated that the annuli visible using the SEM each represented a year's growth.

In turn, there was an obvious general coincidence between annuli observed using light microscopy and those observed using the SEM (Fig.17), demonstrating that the former were also deposited annually. An attempt was made to establish exact correspondance by super-imposition of light and SEM images, and by measurements taken from defined marks visible in both images. However, the narrowness of the hyaline zone in the light images meant that sufficiently accurate super-imposition was not possible as a result of slight variations between light and SEM images; due either to the process of etching or the differential refractive properties of light and electrons in the microscopes used. Similarly, measurements from a common mark were not sufficiently accurate. Based on features common to both images (eg. edge and cracks in otolith) and the approximate distances between, interpretation of the structures revealed using light microscopy was that the narrow hyaline zone corresponded to the margin between SEM stages 4 and 1; and the wide opaque zone corresponded to the remaining growth of stages 4 and 1, and all growth of stages 2 and 3.

In the second sub-sample, the number of micro-increments occurring within an annulus was calculated for seventeen annuli (Table IV). The number of micro-increments found per annulus varied between 72 and 293 with a mean of 143.3. Some indication was also

found for decreasing mean width of micro-increments and number of micro-increments/annuli with increasing distance from the nucleus. Some bias towards larger micro-increments was likely when selecting for measurement, and the sample is too small to be reliable. The data, however, illustrates that the number of micro-increments per annulus may be significantly less than the 365 expected if annuli and micro-increments correspond to intervals of one year and one day respectively.

Table IV. Sub-sample 2: no. of micro-increments/annulus against growth year.

		Micro-increments/annulus			
Specimen	Year	1983/84	1984/85	1985/86	1986/87
1		125	98	88	92
2			208	161	164
3		171	72	107	100
4		293	159	218	172
5				134	75

3.4 Validation Of Annular Structures - Marking Experiment.

No consistent fluorescent marks were observed in the otoliths of fish injected with tetracycline, compared with control fish. A slight general increase in fluorescence levels was observed in the

former and a broad band of fluorescence frequently occured on the edges of both. The inner areas of the otoliths showed low levels of fluorescence with higher levels around cracks or fault-lines in the surface of the cross-section. In two experimental otoliths only, there was possible evidence of fluorescent marks close to the otolith edge. However, the fluorescence was not well-defined, and the marks were inconsistent around the otolith margin.

3.5 Validation Of Micro-Incremental Structures.

No consistent disruption marks were observed by SEM in the otoliths of fish exposed to acetazolamide in either of the two experiments, compared with those of the control fish. Possible evidence of disruption marks occurred in several otoliths but frequent growth marks occurred in those of both experimental and control fish, such that identifying those structures due to acetazolamide would have been difficult. There was no evidence of a distinct signature pattern that could have been attributed to the effect of acetazolamide.

3.6 Growth Profile

Fig. 18 shows mean Growth Index (GI) plotted against time, giving a growth profile. As data in the form of ratios may not be normally distributed (Sokal and Rohlf 1981), and the full size range may be skewed by a few extreme results, spread of data is indicated by the inter-quartile range. The increased spread in later samples

may reflect the cumulative effects of changes in growth rate between years in individual fish. The regression equation for all mean data points is

$$Y = 0.715 + 0.00281X \quad (1)$$

where Y = Growth Index and X = Time (R^2=95.4%).

The plot is better fitted by two separate regression lines with equations

$$Y = 0.728 + 0.00344X \quad (2) \qquad (R^2=99.2\%)$$

$$Y = 0.492 + 0.00353X \quad (3) \qquad (R^2=97.3\%)$$

Given that distribution of data may not be normally distributed, an analysis of covariance was performed to test for differences between (2) and (3). The slopes were not found to be significantly different (F-ratio=0.01; DF 1,7; P-value=0.912). The intercepts were significantly different (F-ratio=18.93; DF 1,8; P-value=0.002). The slope for regression equation (1) is less than for (2) and (3) but a statistical comparison would be meaningless given that the data are not independent from those for (2) and (3). The equivalent difference in time between the latter equations is 72 days, achieved by extrapolation back to Y=0.

The analysis suffers from a number of problems: the data are variable and a long way from the origin while ranges do not overlap. Additionally differences between the two regressions (2) and (3) could be explained by movements in the fish population. The latter,

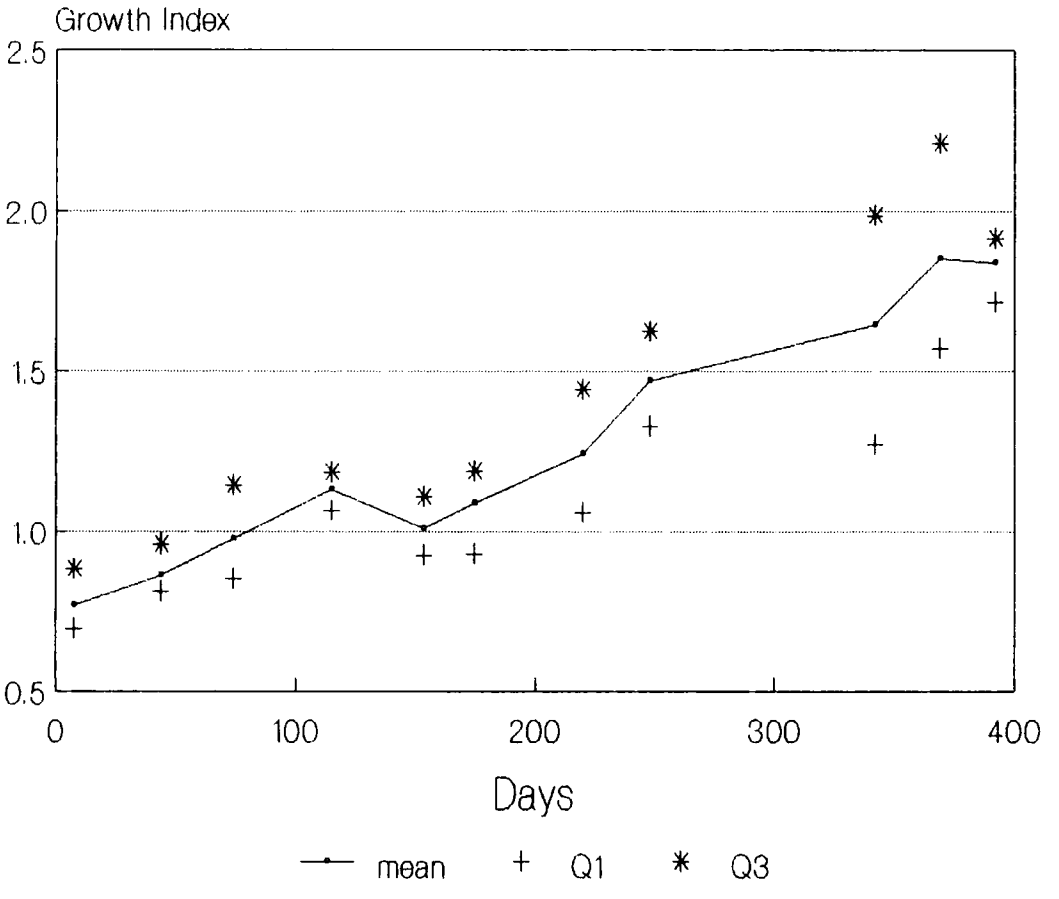

Figure 18. Seasonal variation in otolith growth, using mean Growth Index (GI), and showing inter-quartile range. Day 1 corresponds to 1 June 1987.

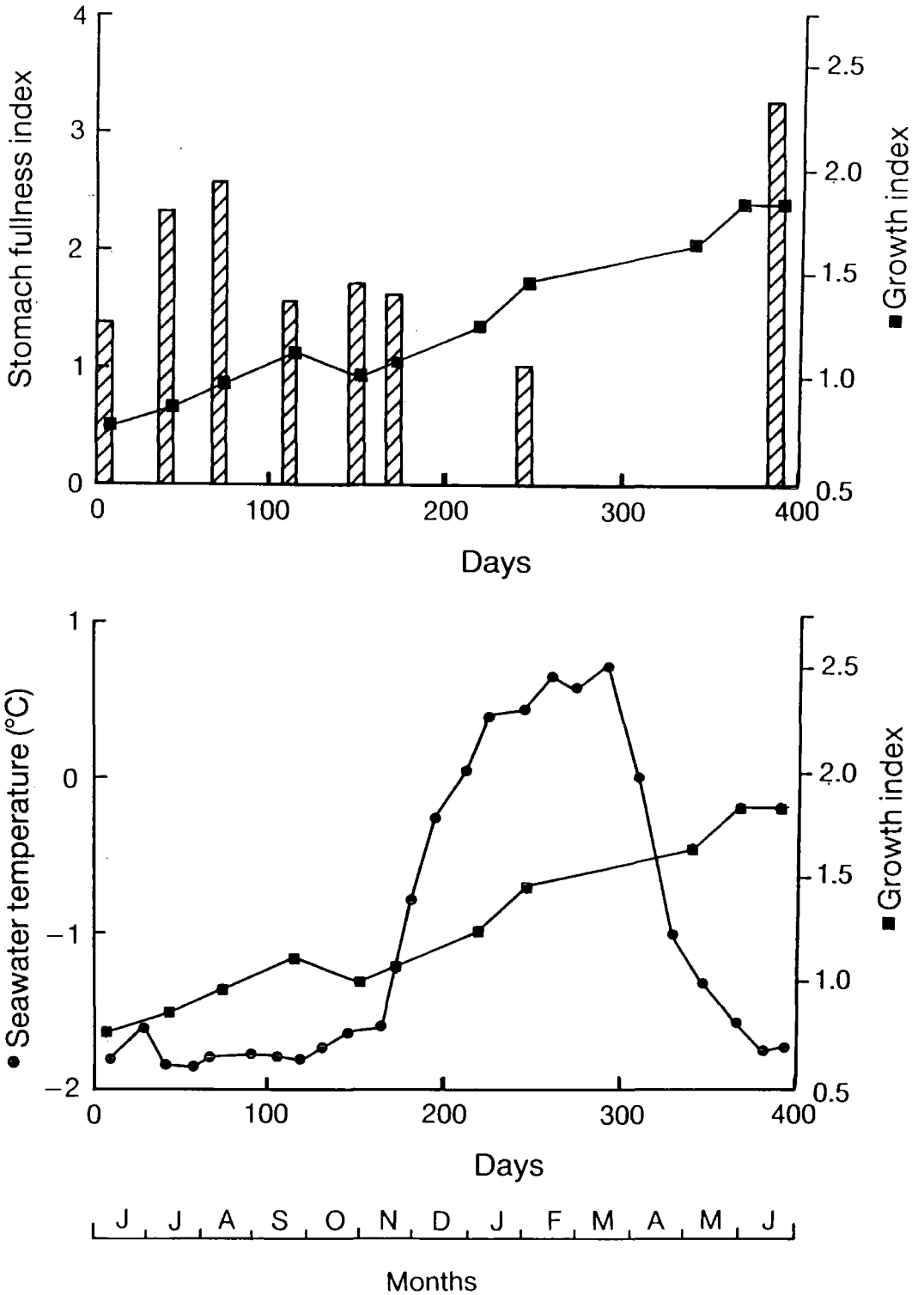

Figure 19. Seasonal Variation in a) stomach fullness and b) seawater temperature in Borge Bay.

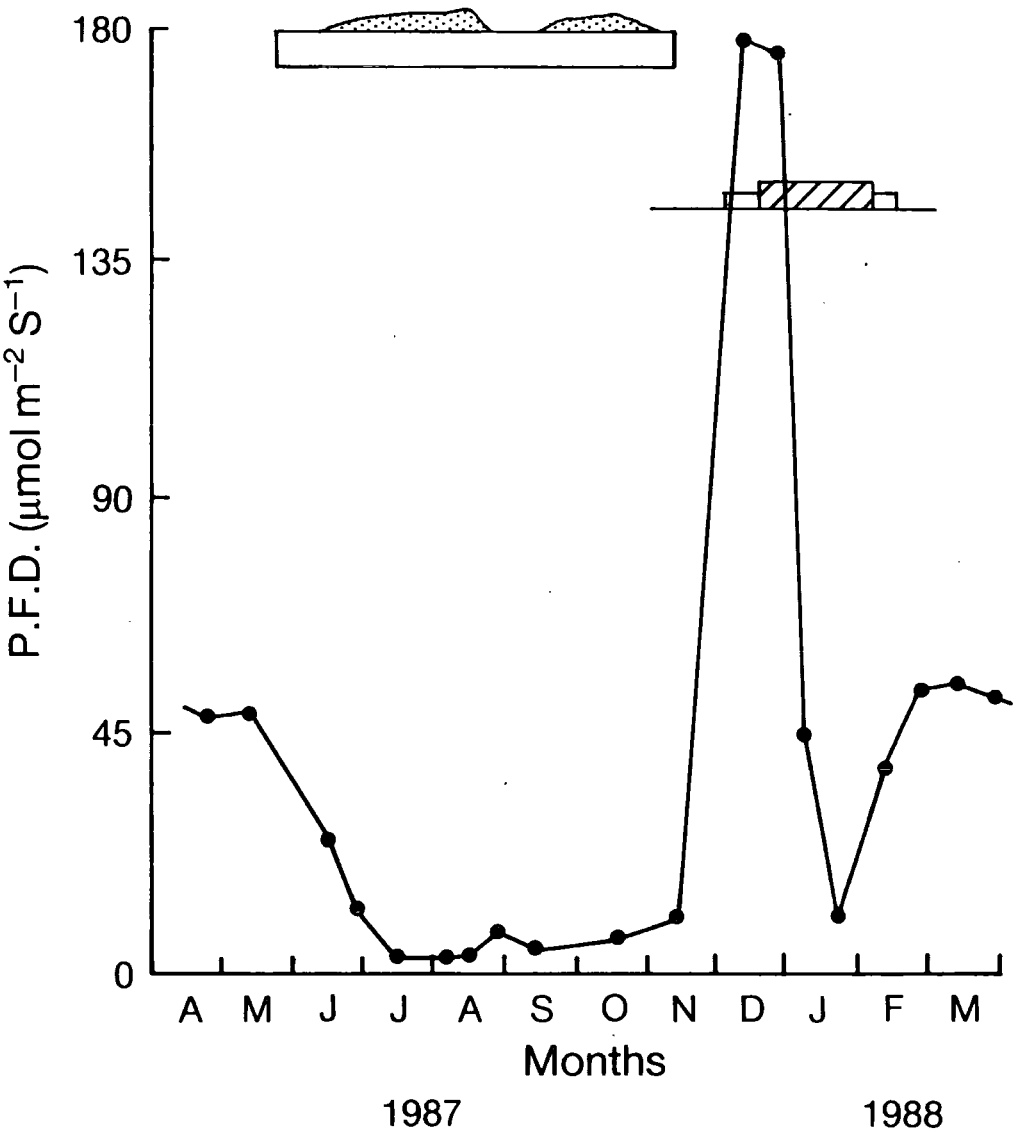

Figure 20. Seasonal variation in photon flux density reaching the benthos at 8m in Borge Bay (adapted from Gilbert (1991a)). Bars at the top of the graph indicate sea-ice and snow cover (stippled). Bars on the graph represent the duration and intensity of a phytoplankton bloom for an average year (from Clarke et al, 1988). Single line= >1mg chlorophyll m^{-3}; open box= >5mg m^{-3} and hatched box= >10mg m^{-3}.

however, is unlikely given the recapture rate of marked animals achieved by Everson (1970) and R. Coggan (pers. comm.), and the analysis serves to illustrate that growth changed significantly at some time between 11 August and 6 January, a period of 148 days. The value of 72 days serves only as a very rough estimate of this change, suggesting that overall growth rate during this period may have declined by 50%.

Fig. 19 shows environmental data during the period of sampling. Data for stomach fullness was used only when the fish was sacrificed within 36 hours of capture. Although gut evacuation rates are slow (R. Coggan, pers. comm.), some stomach emptying would have occurred during this period so the results are likely to be an under-estimate. However, the results illustrate that between 5 June 1987 and 1 February 1988, the immature fish normally had food in their stomachs, a very low proportion of fish sampled having empty guts (5 out of 72, or 7%). Stomach fullness was highest initially on 11 August with evidence of falling levels between 20 September and 21 November, continuing to 1 February. High levels were again found on 26 June 1988 when fish died immediately on capture due to exposure to low ambient air temperatures.

Sea temperature stabilized at about -1.8^{o}C by 11 July 1987, having fallen to this level by 9 June. It had begun to rise again by 24th October and was rising rapidly on 29th November, peaking on 27th March at 0.8^{o}C and falling to winter levels again by 15 June.

Absolute light levels at 8m depth in Borge Bay (Fig. 20) show that a strong pulse of light, peaking at just under 180 mol m^{-2} s^{-1},

occurred when the ice broke out between 19 November and 21 November. Light levels fell rapidly again due to the algal bloom, before partially recovering in late January. Fast-ice had reformed by 14 June, light levels the previous year falling from about 45 to close to 0 mol m^{-2} s^{-1} within two months of the advent of fast-ice.

4. DISCUSSION

4.1 Adaptation Of Bedford's (1983) Technique For Use With Scanning Electron Microscopy.

The SEM technique used in this study overcame difficulties inherent in examining otolith edges using light microscopy. This is partly due to higher resolution and the avoidance of light artefacts but also due to the effect of etching at the otolith periphery. A clear pattern to the edge of the outer annulus was thus visible in specimens viewed using the SEM, whereas this was often not present when viewed using the light microscope due to the pseudo-hyaline edge.

Compared with Bedford's original technique, preparation time was the same to cut the resin-embedded otoliths but an additional day was required for polishing and etching each cast. The rate of preparing and examining video-prints was, however, comparable with rates for direct examination using a light microscope: the high resolution of SEM methods resulted in less interpretation being needed, reducing time taken and increasing precision of each reading.

Light microscope techniques are generally employed by fisheries researchers because conventional SEM techniques are comparatively slow for processing the large numbers of otoliths taken in fishery studies and may be more costly. Using the adapted technique, however, 60 otoliths were examined with the SEM during a

single day. This rate was achieved with little previous experience and without development of a regular routine: thus once performed routinely with experienced personnel, the rate of evaluation could be increased considerably.

Rates for analysing the age of otoliths using light microscopy at MAFF Laboratories, Lowestoft can be as high as 500 d^{-1} for an experienced operator examining samples which are easily read from species such as Gadus morhua (North Sea cod); or as low as 150 d^{-1} for a difficult to read species like Merlangius merlangus (whiting) (G. Howlett and M. Eazy, pers. comm.). However, due to eye fatigue when using a light microscope, operators are rarely able to read otoliths for more than half of any working day. The SEM technique **should** compare favourably therefore, particularly when used for more difficult species and for younger life stages, with advantages of greater accuracy for evaluating edge structure. This may be especially true for species with small otoliths, such as N. coriiceps.

Another advantage of the technique compared to previous preparation methods for SEM is in increased precision in orientation: otoliths are laid at the exact angle desired and the cut made at the same angle through all otoliths. Variation in the angle achieved by hand-grinding leads to inaccuracies in comparative measurements between otoliths (Williams and Bedford 1974). Using the adaptation of Bedford's (1983) method reduces the problem to operator consistency when laying otoliths in the moulds.

The technique may thus allow the SEM to be utilised for the large-scale processing of otoliths performed in fisheries studies. In experimental SEM investigations, it also makes possible the use of larger sample sizes for less effort with consequent implications for experimental design.

4.2 Otolith Morphology

The differentiation of growth structures into regions in otolith cross-sections has not been investigated in Antarctic fish.

The structure and width of annuli were different in each region of the transverse otolith sections observed, all showing evidence of micro-increments. The differences imply that growth must vary between regions: daily growth may be less in regions where annuli are narrow or the period of faster growth may be initiated later or terminated earlier, or be more irregular in these regions. Age determinations using the outer annulus may therefore be influenced by the region selected while the micro-incremental structure of each region needs to be validated before it can be used reliably in ageing. Another implication is that comparisons between otoliths in N. coriiceps need to be based on corresponding regions.

Similar questions about the nature of otolith growth arise over the progressive reduction in width of the annuli further from the nucleus: do the annuli grow more slowly, more irregularly, or for a shorter period of fast growth? If the latter, the age of older fish will be under-estimated by one year relative to younger fish

during the interval between onset of fast growth in each, unless the pattern of growth with age is known.

The regions observed in N. coriiceps otolith cross-sections may be derived from different primordia in the nuclear core. There was, however, little direct evidence for this although the area of clear micro-increments immediately outside the nuclear check showed differential growth assuming a characteristic compressed pattern (Fig. 13b).

Radtke and Kellermann (1991) noted that the nuclear check is assumed to mark the time of hatching in Antarctic fish; hence the concentric micro-increments radiating outward from the nuclear core are laid down by free-swimming larvae. Kellermann (1991) found eggs of N. coriiceps to hatch in late November/early December near the Antarctic Peninsula. White et al (1982) recorded pelagic blue-phase fingerlings of N. coriiceps inshore at South Georgia from late December while they are found in greatest numbers in late February off Signy Island (M. G. White, pers. comm.).

Otolith microstructure around the nuclear core may therefore reflect this timing. A pelagic phase of approximately 90 days for fingerlings arriving inshore at Signy Island conforms well with the figure of 128 for mean equivalent number of micro-increments found between the centre of the nuclear core and the outer edge of the series A of wide increments (Fig. 14), once the radius of the nuclear core is accounted for. If series A can be demonstrated to correspond to the pelagic phase in N. coriiceps fingerlings, this would support the hypothesis that micro-increments are daily.

Furthermore, it follows that the series B of narrow increments would indicate growth of the demersal stage; and the transition between the two series, the period of transformation between pelagic and demersal stages. The regular pattern of the earlier micro-increments may in that case indicate the homogeneous environment encountered during the pelagic life when compared with that once a demersal lifestyle is adopted.

Kellermann (1991) hypothesized that the year class of pelagic fingerlings may divide; most changing to an inshore demersal habit during the first summer while some remain in pelagic mode overwinter to join the demersal population the following summer. This can be tested using otolith microstructure if series A can be demonstrated to correspond to the pelagic fingerling stage. If juveniles stayed offshore into a second summer, a much greater number of micro-increments deposited would be predicted. No such pattern was in fact observed in the otoliths examined, indicating that if series A does correspond to the pelagic stage, either the second year pelagic group does not exist; or it must be in very small numbers; or important locally in areas other than that sampled in this study.

4.3 Validation Of Annular Structures - Time Series

In an earlier study of N. coriiceps at Signy Island using the 'crack and char' technique of Christensen (1964), Everson (1970) found opaque zones were laid down in spring and early summer while hyaline zones were formed in early winter. Daniels (1983) found that an opaque border appeared in otoliths of Harpagifer antarcticus

between July and September off the Antarctic Peninsula. Burchett (1983a) indicated that a true hyaline edge was present between May and November in the otoliths of N. rossii at South Georgia. North (1988) used light microscopy techniques to examine the edges of otoliths from an assemblage of fish species mostly from South Georgia, and found opaque zones present from January to June, while hyaline ones were found during July to January.

In this study, SEM observations showed that the structure equivalent to the hyaline zone started to form one or two months later than that described by North (1988); the equivalent structure to the opaque zone had started to be deposited by early January, at the same time as shown by North. This may be the result of differences between the study sites although a shorter growing season has been shown generally to be associated with higher latitudes (eg. Williams and Bedford 1974). Alternatively, it may be due to improved resolution and removal of light artefacts by using the SEM technique. The differences are in any case close to North's calculated error for the pseudo-hyaline edge. For a more reliable conclusion, direct SEM comparisons between similar assemblages from South Georgia and the South Orkney Islands should be undertaken.

Furthermore, both this study and that by North (1988) investigated fish from several year classes. In two year old cod from the North Sea, Williams and Bedford (1974) found that opaque zones may start to be laid down in February while the otoliths of older, mature fish may show no sign of it until June. A seasonal pattern of zone development that may be clear in a sample of cod of the same age could therefore be obscured in a sample containing

several age-groups. Given the potential for earlier growth of the opaque zone in young N. coriiceps outlined in Section 4.2, the variation found in both this study and that of North (1988) may therefore be significantly reduced by examining animals from a single year class; furthermore, comparison between the two studies would need samples to be of fish of similar age.

Radtke and Hourigan (1990) found annuli that were visible using the SEM not to correspond to yearly intervals in N. nudifrons. They suggested this may be because Antarctic marine habitats undergo smaller fluctuations in temperature which may result in a lack of distinct annual growth increments. However, structures were not shown to correspond between light and SEM images, so the annuli identified by Radtke and Hourigan (1990) using the SEM may not be comparable to those observed by other investigators using light microscopy. If the structures are homologous, the presence of yearly annuli in other species exposed to a limited range of temperatures suggests large variations between species, and indicates that otolith growth in Antarctic fish may be considerably more complicated than either North's (1988) or Radtke and Hourigan's (1990) hypotheses suggest. There is therefore a need for a hypothesis of annuli formation in Antarctic fish which can accomodate these finer distinctions.

Radtke and Hourigan's (1990) observations, however, were achieved by counts of micro-increments and may be the result of other factors. In the second sub-sample used in this study, numbers of micro-increments/annulus found were similarly less than 365, despite the validation of annuli as annual in nature. This may

indicate non-daily micro-incremental growth, but alternatively may be due to suspension of growth, either seasonally or on an irregular basis during the year, so that, although each micro-increment may correspond to a single day and may be useful for ageing over a short period when growth is regular, the technique may be open to error over longer periods. Additionally, some increments may be too small to be revealed properly by etching, introducing further potential for error. The usefulness of micro-increments in the validation of annuli, in certain Antarctic fish at least, may therefore be limited.

4.4 Validations Of Annular And Micro-Incremental Structures - Marking Experiments

The negative results from the marking experiment with tetracycline can be explained in several ways.

Firstly, the fluorescent marks may have faded between the time of the experiment and viewing of the otolith sections, a period of four years. This is unlikely as fluorescence has been found to last up to at least four years (Campana and Neilson 1985). Additionally, higher levels of fluorescence were observable on the experimental otoliths than on the control, indicating that the overall effect of the tetracycline had persisted.

A second possibility is that the experimental treatment interrupted otolith growth so that the tetracycline could not be deposited while still in sufficient concentration to leave a clear

mark. The high number of deaths during the experiment, including of the injected controls, also indicated the stressful nature of the injection protocol. There was, however, no evidence of a disruption mark in experimental or control animals so this explanation is also unlikely.

Other forms of stress could also have played a part: aquarium temperatures were higher and fluctuated more than ambient sea temperature. Occasional icing up of the aquaria may have reduced oxygen levels at times. Crowding may have had an effect, several animals showing signs of bite injuries. Finally, the diet schedule was atypical and may have led to heightened levels of stress or feeding discrepancies between animals. However, all uninjected controls survived and N. coriiceps has been found to be a very robust species that survives well in aquarium conditions over large periods of time (M. G. White, pers.comm.), so this is again an unlikely but possible explanation.

Finally, insufficient growth may have occurred between the dates of marking and sacrifice for the mark to be distinguishable from the fluorescence found around the edge of otoliths. This is considered to be the most likely explanation given the amount of growth that occurred between early June and September, shown in the growth profile. However, the experimental protocol was established before the potential seasonality of otolith growth in N. coriiceps was elucidated, and had further to take into account constraints on available laboratory space during the busy summer season.

To avoid these pitfalls, any future marking study using tetracycline with N. coriiceps should be undertaken for longer during the period when growth is fastest to ensure sufficient growth to distinguish any mark from fluorescence occurring at the edge of the otoliths. A treatment either by branchial uptake through exposure to a solution of tetracycline (Neilson and Geen 1985) or intake through the diet (Weber and Ridgway 1967) could be included to circumvent the problem of stress during treatment. A frequent feeding schedule of natural prey should be incorporated into the protocol, and animals may be kept in individual, temperature-controlled tanks.

For the micro-increment validation experiments using acetazolamide, the negative results may also have been due to several reasons. Firstly, the acetazolamide may disrupt otolith growth but this would have been during the period of minimal or suspended growth in the first experiment. Any disruption mark may thus have been masked.

Secondly, discrepancies in feeding between fish may have had adverse effects on otolith growth, so fish that were not feeding would have produced narrower or fewer increments. This may also have had a masking effect on deposition of any disruption mark. It would also have led to inconsistencies between fish in the location of disruption marks observed and therefore compounded problems in their identification.

The main problem, however, for a future study lies in identifying the disruption mark in a background of very similar

marks. The problems outlined above can readibly be overcome by undertaking experiments during the fast growth period and holding fish separately with individual food supplies. Identifying a disruption mark is more complex and may involve sequential marking to create a signature pattern, or cross-marking with another technique.

In addition, it should be noted that data taken in the acetazolamide experiments would have been flawed if growth were suspended naturally at any stage. The correlation between number of micro-increments and days of the experiment would in that event have been disrupted. The technique may therefore have limited use for Antarctic fish, if pronounced seasonality with suspension of growth during winter is shown to be a widespread phenomenon.

4.5 Growth Profile

4.5.1. Limitation Of Growth By Sea Temperature Or Seasonal Resource Availability?

Williams and Bedford (1974) have drawn attention to inaccuracies of measurement of otolith sections. As well as increased precision allowed by the adaptation for the SEM of Bedford's (1983) method (see Section 4.1), the problem was overcome by calculation of growth as an index ratio. This compensated for variations between sections due to angle and site of cut.

Furthermore, by using only the outer three annuli, inaccuracies due to discontinuities in growth across a section were kept to a minimum.

Clarke and North (1991) showed that the hypothesis of growth limitation by sea temperature in Antarctic fish would be severely tested by the occurrence of pronounced seasonality of growth in which summer rates were similar to those of warmer water species. With regard to the first aspect of their test, the change in rate of Growth Index (GI) between August and late November found in this study could be explained by two hypotheses: firstly that growth was suspended for the estimated period; and secondly that growth slowed for a longer period. In either case, significant seasonal variation in growth was evident, but distinguishing between them is not possible from this data, and the full degree of seasonality cannot be realistically quantified.

With reference to the second aspect of Clarke and North's (1991) test, there is evidence of comparatively high summer growth rates in the difference between the slopes of regression equations 2, 3 and 1. Additionally, there may be a short period of rapid growth during the summer that is not adequately described by sampling at monthly intervals - the sharp increases between samples taken on 6 January and 1 February, and between 3 May and 2 June may be evidence of brief periods of rapid growth.

The range of sea temperature between winter and summer was very low (2.6^{o}C), making changing Q_{10} unlikely as a simple explanation for the seasonality and summer growth rates observed.

However, if N. coriiceps has undergone evolutionary adaptation to low ambient temperatures, response to small temperature changes may be highly sensitive and more complex than a direct linear Q_{10} relation.

Thus, although there is some evidence for pronounced seasonality and high summer growth rates, the hypothesis of otolith growth limitation by low sea temperatures in N. coriiceps was not disproved by this data.

Another possible explanation for the difference between equations (2) and (3) is a change in sampling site. However, the two sites were separated by less than 800m with no geographical barriers between; the difference between the sites reflects only a small variation in the depth and habitat encountered by the species; while data for standard length pooled from each site for the samples between 20 September and 6 January showed no significant difference between the sample sites (two sample t-test: n=20, 24; P=0.27). Additionally, rhythmic fast and slow growing zones have been found in the otoliths and scales of a number of other Antarctic fish species (eg. Olsen 1954, 1955; Wohlschlag 1961; Hureau 1970; Daniels 1983), and Everson (1970, 1984) found growth parameters to vary seasonally in N. coriiceps. The shift in intercept between regression equations (2) and (3) is therefore unlikely to be due to a change in sampling site.

The degree of seasonal variation in growth found may have been obscured by the number of year classes in the sample. Rates of the growth shown in Fig. 18 will be a composite of differences among

year classes, blurring the edges between the periods of minimum and maximum growth, as well as peak rates of growth during summer. Additionally, Clarke and North (1991) found that field data did not necessarily show maximum growth rates achievable under optimum conditions. Laboratory experiments using excess food are therefore needed to establish the maximum growth rate of which immature individuals of N. coriiceps are capable.

To overcome the problems outlined, future sampling needs to be at more frequent intervals and the analysis based on individual year classes. Additionally, sampling needs to begin as early in the season of fast growth as possible to minimize the distance of data from the origin (Fig. 16).

4.5.2. Seasonal Environmental Influences: Stomach Fullness Index, Seawater Temperature, Absolute Light.

The data for Stomach Fullness Index (SFI) demonstrated that N. coriiceps juveniles were feeding during the winter months. For the first six samples the Growth Index (GI) profile appeared to be parallel to that for SFI with a lag of one month. However, the rate of gut turnover was not reflected in these data and the SFI may be highly dependent on the time lapse between capture and sacrifice. This was emphasized by the last sample when sacrifice was immediate and the highest SFI value was obtained.

The rise in seawater temperature in November approximately coincided with the resurgence of otolith growth. However, GI increased from June 1987 before levelling out between August and

November with temperature remaining constant throughout. Growth again continued with little modification after water temperatures fell in April 1988. Otolith growth in N. coriiceps therefore appears not to be directly dependent on annual fluctuations in sea temperature but the latter may be involved in the onset of faster summer growth.

Similarly, absolute light levels may be involved, the pulse resulting from break-out of sea-ice coinciding with an acceleration in GI. Decreasing light levels due to sea-ice formation in June 1987, however, were not associated with a fall in GI. Therefore, the factors responsible for initiating growth may be independent of those causing its reduction or cessation.

4.5.3. Growth In Relation To The Environment.

Everson (1984) found high SFI values in N. coriiceps during summer which, combined with the results for SFI in winter found in this study (Fig. 19), indicate that fish sampled were feeding throughout the year. Richardson (1975) showed that amphipoda comprise the largest proportion of the diet of N. coriiceps. Amphipod biomass in the sediment of Borge Bay can be up to 111.3 g/m^2 (Hardy 1972), which remains as a permanent supply for year-round feeding. Any evidence for limitation of otolith growth by resource availability therefore must be explained by other means than the simple availability of suitable prey. Changes in food quality may occur as the summer primary production is partitioned through the food chain or the fish may be inhibited from feeding freely by other factors. Everson (1970) has suggested that changes

in feeding activity in spring in N. coriiceps may be associated with the return of Weddell seals to inshore waters. Water clarity under winter sea-ice may also increase the risk of predation, forcing fish to concentrate feeding on a small area near cover and perhaps thereby reducing diet quantity and quality.

It should be noted however that the presence of an unlimited supply of prey for N. coriiceps in Borge Bay may not be a constant phenomenon between habitats. Hardy (1972) found a patchy distribution of amphipods within Borge Bay, the highest levels occurring in substrates consisting of sand and silt mixed with larger particles; other substrates, notably soft flocculent ooze or silt, supported lower amphipod biomasses of 12.0 g/m^2 and 38.1 g/m^2 respectively. Amphipod populations may therefore be lower in areas with a predominance of low quality substrates. High quality substrates predominated at the sample sites in Borge Bay, and the large number of amphipods available may have reduced the extremes of seasonality in N. coriiceps that would be present in populations living in lower quality habitats. On the other hand, the amplitude of the seasonal pulse of primary production may be reduced when transferred through the food chain. Given the different habitats occupied by N. coriiceps during its life history, the pattern of resource requirements and availability is likely to change considerably through an individual fish's lifetime. Younger pelagic stages, for instance, feeding on prey at lower levels in the food chain, may show higher summer rates and greater seasonality of otolith growth.

Otolith growth may be an interaction of environmental factors. Campana and Neilsen (1985) have indicated the importance of environmental variables in masking a hypothetical endogenous rhythm in daily incremental growth. In immature N. coriiceps, otolith growth at the end of summer may slow due to changes in diet, indicated by apparent reductions in SFI. However, a similar timing of changes in seasonal environmental variables and otolith growth rates does not demonstrate a direct influence of one on the other; this may be mediated by other factors or be simply a coincidence. Thus, at the end of winter, rising sea temperature and the pulse of light associated with ice break-out may be linked to increases in otolith growth either by an increase in metabolic activity in the case of temperature or by acting as cues to influence an endogenous growth rhythm. Alternatively, the behaviour of N. coriiceps or its prey may be influenced by seasonal changes. For example, perception of prey and prey behaviour may alter as a result of more light and higher temperatures, allowing fish greater selection of prey with a consequent improvement in diet quality. This would coincide with a change of predation on N. coriiceps by Weddell seals, dispersing after breeding, to blue-eyed shags which take smaller fish of size range 30-500g (Everson 1970) in open water. The manner in which these interacting factors may play a part has yet to be fully investigated.

Otolith growth may not be directly coupled with somatic growth. Several studies have reported a strong correlation between the two (Campana and Neilsen 1985), but Wright et al (1990) have reported uncoupled growth rates in first year Atlantic salmon parr Salmo salar that would subsequently smolt in their third year. Secor

and Dean (1989) showed that slower growing groups of larval and juvenile striped bass Morone saxatilis had larger and heavier otoliths relative to fish length than groups which grew more quickly. Campana (1984) reported uncharacteristic otolith growth relative to fish growth as a result of abnormal photoperiod while otolith growth has been found to continue after cessation of linear fish growth during periods of starvation (Brothers 1981; Campana 1983). To extrapolate from otolith growth to overall somatic growth, the relationship between the two in N. coriiceps needs to be validated to check that otolith growth is representative of somatic growth and exhibits a similar response to environmental factors.

Cross-sections of N. coriiceps otoliths were shown to exhibit distinctly differentiated regions and these introduce a complication in interpreting growth patterns. In this study, data was taken using the region (3) with the most clearly defined break between consecutive years growth. If growth varies between regions, not all regions can correspond to somatic growth. The relationship of each region to overall somatic growth thus needs further clarification.

This study demonstrates that SEM techniques can greatly improve the understanding and accuracy in interpretation of seasonal increments as well as microstructure in otoliths of Antarctic fish. High resolution SEM methods can facilitate fisheries work by adding to knowledge obtained using more usual light techniques - providing the correspondence of structures between light and SEM images is properly established. Using the technique outlined in this study, SEM equipment can also be of use in fisheries surveys for the large-scale processing of otoliths.

4.5.4. The Antarctic Laboratory.

In the year of this study, increasing sea temperatures and the pulse of absolute light coincided. The following year, ice break-out occurred on 20 December instead of 21 November at a time when the intensity of the phytoplankton bloom would be more than 10mg m^{-3} in an average year (Fig. 20). The pulse of light would therefore have been much reduced or cancelled out. During winter 1989, fast ice occurred from 9-30 June only, with pack ice prevalent for the rest of the season: these conditions allowed light to penetrate to the seafloor of Factory Cove. This variation between years would allow the effects of the light pulse to be uncoupled from those of sea temperature. Similarly, the size and duration of the phytoplankton bloom varies considerably between years, independently of sea temperature, allowing for an <u>in situ</u> comparison between years in its effects on growth (White and Burren 1992).

Sea temperature varies at Signy Island from approximately -1.8°C to 1.5°C. This range is low in comparison with temperate waters and the variation should therefore have minimal effect on fish growth. However, it reflects the extremes of a fish's exposure; temperature related events in fish found in lower latitudes may be mediated by a narrow band of temperature in the Antarctic. The variation may be particularly important given that Antarctic fish have a marked stenothermy (DeVries 1978). Additionally, the lower limit of the temperature range is that at which seawater freezes, which presents a range of physiological risks during winter that would not be normally encountered during summer. Sea temperature varies even less further south, for example rarely rising above -

1.8°C in McMurdo Sound and varying by about 0.25°C. Growth profiles for N. coriiceps at a site further south may thus allow the effects of short-term fluctuations in seawater temperatures to be separated from resource availability. Comparisons with profiles from Signy Island may give an insight into effects of such temperature fluctuations on otolith growth.

The essentially stable nature of the marine environment combined with its marked, well-defined shifts in equilibrium for seasonal environmental variables means the Antarctic offers considerable scope as an in situ laboratory.

5. CONCLUSIONS

1. Annuli in Notothenia coriiceps were annual.

2. Opaque zone was deposited from January to July.

3. Scanning electron microscopy was very much better than light microscopy for viewing structures in otolith sections.

4. Otolith cross-sections of Notothenia coriiceps when viewed using SEM had a differential structure with varying growth patterns between regions. Age determinations may be influenced by the region selected.

5. Annuli became narrower with increasing distance from the centre of the otolith. Age determinations may be influenced by variations in timing of growth with age.

6. Micro-increments occurred in annuli laid down by immature Notothenia coriiceps.

7. A series of large well-defined micro-increments occurred near the nucleus.

8. Otolith growth rate was seasonal in immature Notothenia coriiceps.

9. Immature Notothenia coriiceps continued to feed underneath winter fast-ice.

10. Increasing light levels and sea temperature coincided with the onset of fast otolith growth in Spring.

11. Otolith marking experiments need to be carefully timed to coincide with otolith growth phases and to avoid interference by edge structures.

6. QUESTIONS FOR FURTHER INVESTIGATION (Fig. 21)

1. In regions where annuli are narrow, do annuli grow: proportionately less per day; more irregularly during the year; or for a longer season of slow or suspended growth?

2. Do annuli grow more slowly; more irregularly; or for a shorter period of fast growth, with increasing distance from the nucleus?

3. Is the change in growth rate between August and late November in N. coriiceps due to slowing or suspension of growth?

4. Is the series of large, well-defined micro-increments near the nucleus in otoliths of N. coriiceps laid down during the pelagic life-stage of fingerlings?

5. Can micro-increments be validated as daily in N. coriiceps; for all otolith regions; and throughout the year?

6. Is otolith growth influenced by fluctuations in seawater temperature; absolute light; photoperiod; or food availability?

7. Is otolith growth limited by seawater temperature; seasonal resource availability; endogenous growth factors; or a combination of these?

8. Is the timing of seasonal variation in otolith growth similar between South Georgia and the South Orkney Islands, or does it vary with latitude in Antarctic fish?

9. Is annuli formation similar in the otoliths of other Antarctic fish species?

10. Is otolith growth coupled to somatic growth, and how does this relate to growth of otolith regions?

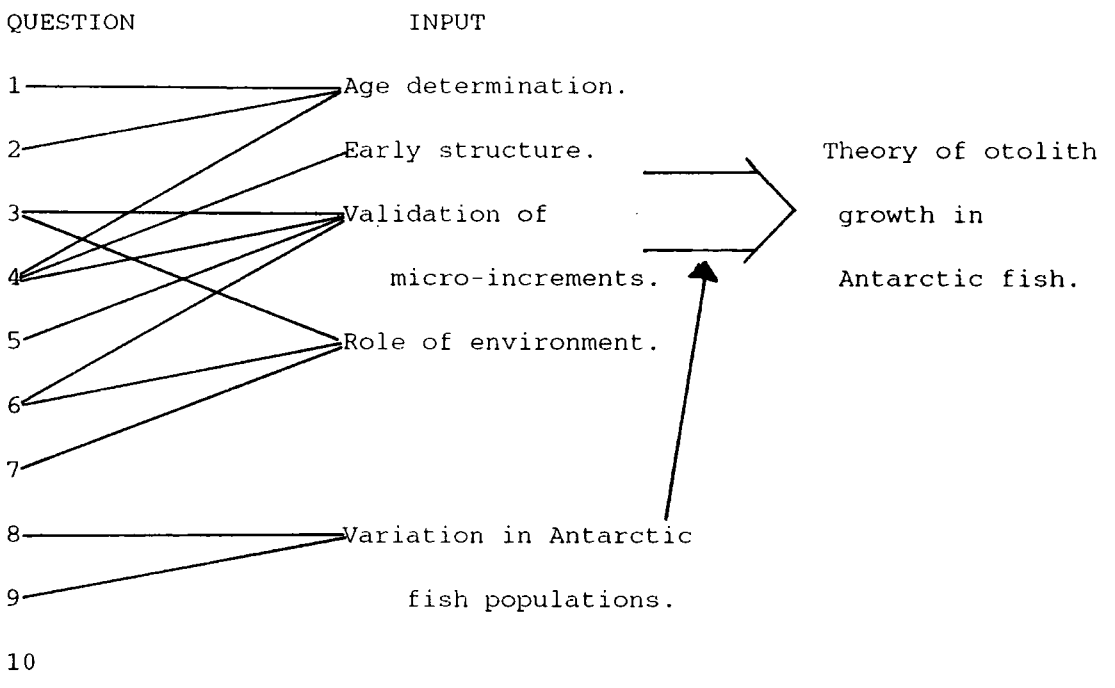

Figure 21. Questions for future research on ageing and growth in the otoliths of Antarctic fish.

APPENDIX 1: Biometric Data For Time-Series Samples.

Section 1

A	B	C	D	E	F	G	H	I	J
841731	50687	30687	7	9	*	7	*	22.1	18.9
841732	80687	80687	7	9	1	7	5	22.7	19.8
841733	80687	80687	7	9	1	7	5	22.1	19.2
841734	80687	80687	7	9	2	7	5	23.5	20.3
841735	80687	80687	7	9	1	7	5	28.5	25.4
841736	80687	80687	7	9	1	7	5	24.0	20.9
841737	80687	80687	7	9	2	7	5	26.7	23.1
841738	80687	80687	7	9	1	7	5	23.1	20.0
841739	80687	80687	7	9	1	7	1	21.8	19.6
841749	130787	130787	7	9	2	7	3	30.2	26.7
841750	130787	130787	7	9	1	7	5	28.7	25.0
841751	130787	130787	7	9	1	7	2	21.6	18.6
841752	130787	130787	7	9	1	7	2	28.8	24.8
841753	130787	130787	7	9	1	7	1	21.9	18.7
841754	130787	130787	7	9	2	7	1	23.8	20.7
841755	150787	130787	7	9	2	7	1	22.6	19.8
841756	150787	130787	7	9	2	7	1	23.5	20.3
841757	150787	130787	7	9	1	7	1	22.9	19.8
841758	150787	130787	7	9	2	7	2	28.5	24.6
841779	110887	110887	7	9	2	7	1	25.1	21.5
841780	110887	110887	7	9	1	7	1	20.2	17.4
841781	110887	110887	7	9	2	7	2	27.6	24.1
841782	110887	110887	7	9	1	7	1	20.7	24.3

841783	110887	110887	7	9	1	7	1	20.3	17.6
841784	110887	110887	7	9	2	7	1	24.5	21.5
841785	110887	110887	7	9	2	7	1	21.1	17.8
841786	160887	160887	7	9	2	7	2	30.3	26.2
841787	160887	160887	7	9	2	7	1	21.9	19.0
841788	160887	160887	7	9	2	7	1	21.8	18.5
841789	160887	160887	7	9	1	7	2	21.8	18.7
841820	200987	190987	7	9	1	7	1	30.0	25.9
841821	200987	190987	7	9	1	7	1	26.8	23.1
841822	200987	190987	7	9	1	7	1	29.0	25.3
841823	200987	190987	7	9	1	7	1	32.2	27.8
841824	200987	190987	7	9	1	7	2	35.1	30.8
841825	210987	190987	7	9	2	7	2	42.1	37.3
841826	210987	190987	7	9	1	7	1	25.8	22.7
841827	210987	190987	7	9	2	7	1	25.5	21.9
841828	210987	190987	7	9	2	7	1	23.2	19.8
841829	210987	190987	7	9	2	7	1	25.1	22.1
841830	280987	280987	7	9	1	7	1	20.2	23.1
841831	280987	280987	7	9	2	7	1	19.9	22.8
841832	280987	280987	7	9	1	7	1	22.3	19.6
841833	280987	280987	7	9	2	7	1	22.6	19.6
841872	311087	301087	6	18	1	7	1	30.9	26.8
841873	311087	301087	6	18	2	7	1	28.9	25.5
841874	311087	301087	6	18	2	7	1	28.9	25.1
841875	311087	301087	6	18	1	7	1	27.6	24.1
841876	311087	301087	6	18	1	7	1	24.9	21.5
841877	311087	301087	6	18	1	7	2	35.1	30.7
841878	311087	301087	6	18	2	7	1	27.6	24.0
841879	311087	301087	6	18	1	7	1	24.2	20.7

841880	311087	301087	6	18	2	7	1	23.4	20.1
841881	311087	301087	6	18	1	7	1	26.5	22.8
841918	211187	201187	7	9	2	7	1	24.3	20.7
841919	211187	201187	7	9	1	7	1	27.6	23.6
841920	211187	201187	7	9	2	7	2	30.6	26.5
841921	211187	201187	7	9	2	7	1	26.8	22.9
841922	211187	201187	7	9	1	7	2	27.8	23.9
841923	211187	201187	7	9	2	7	1	30.4	26.1
841924	211187	201187	7	9	1	7	2	28.5	24.5
841925	211187	201187	7	9	1	7	2	26.4	22.8
841926	211187	201187	7	9	2	7	2	29.2	25.4
841927	211187	201187	7	9	1	7	2	29.8	25.4
841939	60188	20188	6	18	1	7	1	26.1	22.8
841940	60188	20188	6	18	1	7	2	30.4	25.9
841941	60188	20188	6	18	2	7	1	34.2	29.7
841942	60188	20188	6	18	1	7	1	28.4	24.6
841943	60188	30188	6	18	1	7	2	33.0	28.6
841944	60188	30188	6	18	1	7	1	28.5	25.0
841945	60188	30188	6	18	2	7	1	32.2	28.4
841946	60188	30188	6	18	1	7	1	25.9	22.3
841947	60188	40188	6	18	2	7	1	24.7	21.4
841948	60188	40188	6	18	2	7	2	33.1	29.1
841968	10288	210188	6	18	2	7	1	30.5	26.5
841969	10288	210188	6	18	1	7	2	31.8	27.9
841970	10288	210188	6	18	1	7	1	25.2	21.5
841971	10288	210188	6	18	2	7	1	30.7	26.4
841972	40288	40288	6	18	1	7	2	32.4	28.2
841973	40288	40288	6	18	1	7	1	26.6	22.9
841974	40288	40288	6	18	1	7	2	33.9	29.8

841975	40288	40288	6	18	2	7	1	25.8	22.2
841976	40288	40288	6	18	1	7	1	24.2	21.1
841977	40288	40288	6	18	2	7	4	45.5	40.0
842018	60588	10588	6	18	2	7	1	29.4	25.6
842019	60588	10588	6	18	1	7	1	23.0	20.3
842020	60588	10588	6	18	2	7	1	24.3	21.4
842021	60588	10588	6	18	1	7	1	28.7	24.8
842022	30588	10588	6	18	1	7	1	24.1	20.5
842023	30588	10588	6	18	1	7	1	29.9	26.1
842024	70588	10588	6	18	1	7	1	22.2	19.3
842025	70588	10588	6	18	2	7	1	29.4	25.5
842026	70588	10588	6	18	1	7	1	31.0	26.9
842027	70588	10588	6	18	2	7	3	40.1	34.8
842038	20688	240588	7	9	1	7	2	30.7	27.0
842039	20688	290588	6	18	2	7	2	27.7	24.0
842040	20688	290588	6	18	1	7	1	23.4	20.1
842041	20688	290588	6	18	1	7	1	22.4	19.1
842042	20688	290588	6	18	1	7	1	24.3	21.1
842043	20688	290588	6	18	2	7	2	28.8	25.0
842044	20688	290588	6	18	1	7	2	24.0	20.7
842045	20688	290588	6	18	2	7	1	22.7	19.3
842046	20688	290588	6	18	2	7	1	19.1	17.0
842047	250688	250688	15	6	1	7	2	31.4	27.6
842048	250688	250688	15	6	2	7	1	28.5	24.9
842049	250688	250688	15	6	1	7	1	30.8	26.7
842050	250688	250688	15	6	2	7	1	24.7	21.2
842051	250688	250688	15	6	2	7	1	28.7	24.7
842052	250688	250688	15	6	1	7	1	25.3	22.0
842053	250688	250688	15	6	1	7	2	33.0	29.3

842054	250688	250688	15	6	1	7	2	31.0	27.0
842055	250688	250688	15	6	2	7	1	28.9	25.1
842056	250688	250688	15	6	2	7	1	23.7	20.4
842057	250688	250688	15	6	2	7	1	27.1	23.5
842058	250688	250688	15	6	2	7	1	28.6	24.7

Section 2

A	K	L	M	N	O	P	Q	R	S
841731	*	*	*	*	350510	3	0	3	0.516
841732	200	171	0.2	1	350511	3	*	3	0.690
841733	191	165	0.5	1	350512	4	*	3	0.867
841734	220	186	1.4	1	350513	*	3	3	0.797
841735	320	284	0.3	1	350514	3	3	3	0.745
841736	210	184	0.1	1	350515	*	3	3	0.703
841737	330	280	3.1	2	350516	3	3	3	0.896
841738	207	181	0.4	2	350517	4	3	3	0.739
841739	181	158	0.1	2	350518	4	3	4	0.964
841749	460	390	8.8	3	350601	3	3	4	0.968
841750	400	350	0.2	3	350602	4	3	4	0.828
841751	184	158	0.1	1	350603	3	*	4	0.817
841752	380	320	0.2	2	350604	3	*	3	0.621
841753	178	156	0.1	3	350605	3	3	4	0.794
841754	208	177	1.0	2	350606	3	3	4	0.975
841755	184	155	1.3	1	350607	3	3	4	0.909
841756	200	170	1.6	2	350608	*	3	4	0.845
841757	204	176	0.2	1	350609	3	3	3	0.920
841758	370	320	3.4	2	350610	3	*	4	0.956

841779	267	227	2.0	1	360101	*	*	4	1.216
841780	153	126	0.2	3	350619	3	*	4	0.796
841781	380	325	6.5	1	350618	3	*	4	0.855
841782	269	225	0.3	3	350617	3	3	4	0.905
841783	149	127	0.2	3	350616	3	4	1	1.186
841784	250	210	1.6	1	350615	3	3	4	0.943
841785	164	135	1.4	2	350614	3	3	4	1.131
841786	455	*	4.1	4	*	*	*	4	*
841787	191	150	1.4	3	350613	*	3	4	0.851
841788	166	141	0.7	4	350612	3	4	4	0.923
841789	173	151	0.3	3	350611	3	3	4	0.941
841820	400	360	0.3	1	360102	*	3	4	1.074
841821	283	249	0.2	2	360103	*	*	4	1.180
841822	350	300	0.2	1	360104	4	*	4	1.070
841823	550	490	0.7	3	360105	4	3	4	1.056
841824	680	600	1.3	0	360106	4	3	1	1.093
841825	1160	980	25.2	0	360107	3	*	4	1.250
841826	241	215	0.3	0	360108	4	*	4	0.955
841827	231	203	1.2	1	360109	*	*	4	1.187
841828	211	179	1.2	2	360110	*	*	1	1.136
841829	241	205	1.6	3	360111	3	3	1	1.157
841830	190	163	0.7	2	360112	*	3	4	1.105
841831	199	168	1.3	1	360113	*	*	1	1.375
841832	168	147	0.4	3	360114	*	4	1	1.042
841833	180	158	1.1	1	360115	4	3	4	1.187
841872	420	360	0.9	2	360116	*	3	4	0.861
841873	350	310	2.8	1	360117	4	*	1	1.169
841874	460	390	2.7	0	360201	*	4	3	0.728
841875	340	280	0.3	3	360202	*	*	4	1.087

841876	214	192	0.7	1	360203	*	*	1	1.172
841877	780	650	2.4	3	360204	3	3	1	0.949
841878	300	260	2.8	2	360205	*	4	1	1.047
841879	186	168	0.3	0	360206	*	*	1	1.028
841880	187	154	0.9	4	360207	*	*	4	1.021
841881	249	222	0.5	1	360208	*	*	4	1.032
841918	214	190	1.6	1	360209	*	*	4	1.059
841919	252	228	0.5	1	360210	*	*	4	1.192
841920	480	410	9.4	2	360211	*	4	4	1.186
841921	310	280	1.4	1	360212	3	4	2	1.151
841922	360	320	1.6	2	360213	2	*	4	0.903
841923	400	350	1.1	0	360214	*	*	4	0.883
841924	380	300	0.5	2	360215	0	4	1	0.938
841925	320	280	0.5	4	360216	*	3	1	1.303
841926	410	350	4.6	1	360217	*	4	1	1.118
841927	350	310	0.9	2	360218	*	4	2	1.154
841939	247	223	0.4	3	350501	2	3	2	1.111
841940	420	380	1.5	1	350502	4	3	2	1.216
841941	610	550	6.9	2	350503	0	3	4	1.503
841942	330	290	0.4	2	*	*	*	*	*
841943	530	460	5.2	1	350504	1	*	1	0.778
841944	420	360	0.7	3	350505	3	*	1	1.535
841945	500	430	4.0	4	350506	2	2	2	1.378
841946	260	233	0.8	1	350507	3	2	1	1.010
841947	215	190	1.7	1	350508	*	*	*	1.309
841948	580	500	7.0	2	350509	4	3	1	1.352
841968	380	340	3.0	0	360301	3	3	1	1.308
841969	440	400	0.8	0	360302	*	*	2	1.183
841970	238	214	0.3	0	360303	*	*	2	1.349

841971	450	400	3.6	0	360304	2	1	2	1.641
841972	520	470	2.4	1	360305	1	1	2	1.493
841973	285	256	0.5	2	360306	3	3	2	1.432
841974	570	510	1.9	1	360307	2	3	2	1.797
841975	231	207	1.9	1	360308	*	1	2	1.601
841976	198	181	0.1	0	360309	*	*	2	1.410
841977	1650	1030	96.0	1	360310	1	1	3	*
842018	340	300	3.5	0	600208	*	*	4	1.926
842019	157	144	0.1	0	600209	*	*	3	1.493
842020	200	181	1.3	0	600210	*	*	4	1.955
842021	360	330	0.3	0	600301	*	*	3	1.292
842022	213	194	0.6	0	600302	*	*	4	1.209
842023	450	400	0.3	0	600303	*	*	1	2.075
842024	167	143	0.1	0	600304	*	*	4	1.578
842025	340	330	2.6	0	600305	*	*	4	1.435
842026	440	400	0.6	0	600306	*	*	3	2.287
842027	1050	*	111.0	0	600307	*	*	4	1.173
842038	440	390	0.3	0	600308	*	*	3	1.728
842039	410	360	4.1	0	600309	*	*	3	2.302
842040	184	166	0.1	0	600310	*	*	3	1.595
842041	168	152	0.1	0	600401	*	*	4	1.842
842042	206	186	0.1	0	600402	*	*	3	1.786
842043	340	300	2.1	0	600403	*	*	4	2.661
842044	196	176	0.2	0	600404	*	*	4	2.123
842045	186	166	1.3	0	600405	*	*	3	1.542
842046	131	111	0.6	0	600406	*	*	3	1.057
842047	530	450	0.6	3	600407	*	*	3	1.753
842048	420	350	4.2	3	600408	*	*	3	1.710
842049	540	450	0.2	3	600409	*	*	3	1.721

842050	244	203	1.7	4	600410	*	*	4	2.476
842051	420	350	4.6	3	600501	*	*	3	1.526
842052	272	232	0.2	3	600502	*	*	4	1.745
842053	710	580	1.3	3	600503	*	*	4	1.854
842054	480	400	0.6	4	600504	*	*	3	1.667
842055	450	380	4.8	4	600505	*	*	4	1.929
842056	220	182	1.2	3	600506	*	*	4	2.068
842057	350	280	4.6	3	600507	*	*	3	1.761
842058	390	320	4.9	3	600508	*	*	4	1.824

KEY

A BAS specimen number

B Sacrifice date

C Capture date

D BAS site code

E Depth (m)

F Sex (1=male, 2=female)

G BAS life stage code

H BAS gonad stage code

I Total length (cm)

J Standard length (cm)

K Total weight (g)

L Eviscerated weight (g)

M Gonad weight (g)

N Stomach fullness index

O SEM block code

P Outer annulus growth stage - first reading using light microscopy

Q Outer annulus growth stage - second reading using light microscopy

R Outer annulus growth stage - reading using SEM.

S Growth Index

APPENDIX 2: Biometric Data For Tetracycline Experiment

A	B	C	D	E	F	G	H	I	J	K	L
1	120787	2	7	2	23.4	20.1	229	192	1.6	100	2
2	120787	2	7	2	22.8	19.9	170	152	0.6	50	1
3	120787	1	7	2	21.3	18.1	160	142	0.3	100	1
4	120787	2	7	1	23.2	19.9	178	160	0.3	100	1
5	120787	1	7	2	22.9	19.7	174	154	0.2	0	1
6	260787	1	7	2	22.1	19.1	129	111	0.2	100	2
8	280887	2	7	2	22.4	19.2	173	152	1.4	0	1
9	280887	1	7	1	27.2	23.7	263	239	0.3	0	1
10	280887	2	7	2	30.1	26.5	370	340	2.8	100	2
11	310887	2	7	1	21.5	19.0	139	123	0.7	50	2
12	220987	2	7	1	22.9	20.0	171	151	1.0	*	0
13	220987	2	7	1	22.5	19.0	155	142	0.9	*	0
14	220987	1	7	1	22.5	19.1	159	144	0.2	*	0
15	220987	1	7	1	28.7	24.8	286	253	0.5	50	2
16	220987	1	7	1	22.4	18.7	148	129	0.1	50	2
17	220987	2	7	1	30.4	26.2	400	360	1.9	25	2
18	220987	2	7	1	25.0	21.7	218	197	1.3	25	2
19	220987	2	7	1	22.4	19.2	151	136	1.3	25	2
20	220987	1	7	1	20.8	17.7	115	102	0.2	25	2
21	220987	1	7	1	23.8	20.2	176	157	0.2	25	1
22	220987	1	7	1	23.6	20.2	180	162	0.3	25	1
23	220987	1	7	1	29.8	26.2	350	310	0.5	50	1
24	220987	2	7	1	23.8	20.2	171	155	1.0	50	1
25	220987	2	7	1	29.0	25.1	330	292	5.2	100	1

APPENDIX 3: Biometric Data For First Acetazolamide Experiment

A	B	C	D	E	F	G	H	I	J	M
1	271287	2	7	1	24.4	21.0	184	163	1.2	1
2	80188	2	7	1	26.4	23.0	250	213	1.9	2
3	20288	*	7	1	*	*	*	*	*	*
4	110288	2	7	1	29.0	25.8	450	390	6.0	1
5	110288	1	7	1	28.4	25.1	360	330	0.6	1
6	110288	2	7	1	25.5	22.8	272	246	2.7	1
7	110288	2	7	1	21.7	19.0	157	142	1.0	1
8	170288	2	7	1	26.1	22.3	268	252	1.9	1
9	170288	1	7	1	24.5	21.3	198	180	0.2	1
10	170288	2	7	1	23.7	20.6	163	146	1.8	1
11	170288	2	7	1	23.1	19.6	169	151	1.6	1
12	170288	2	7	1	28.5	24.9	310	280	4.3	2
13	170288	1	7	1	25.6	22.2	236	217	0.3	2
14	170288	2	7	1	23.5	20.1	214	193	2.2	2
15	170288	2	7	1	29.4	25.7	350	320	3.8	2
16	170288	2	7	1	31.3	26.9	410	360	6.0	2
17	170288	2	7	1	24.3	20.8	216	194	1.9	2

APPENDIX 4: Biometric Data For Second Acetazolamide Experiment

A	B	C	D	E	F	G	H	I	J	K
1	40888	1	7	2	27.5	23.3	287	254	0.30	50
2	40888	2	7	1	25.5	21.6	236	213	1.65	50
3	80888	2	7	1	22.4	19.4	179	157	0.90	50
4	80888	2	7	1	22.0	19.8	181	160	1.30	50
5	110888	1	7	2	33.2	29.3	510	450	1.00	50
6	110888	2	7	1	24.6	21.1	217	194	1.70	50
7	160888	1	7	2	33.4	29.3	600	510	1.00	0
8	10988	2	7	2	32.3	28.1	550	490	7.00	0
9	10988	2	7	2	33.2	29.2	550	500	9.60	0
10	10988	2	7	2	35.2	30.8	600	540	7.00	0
11	10988	2	7	2	32.1	28.1	500	450	10.20	0
12	10988	1	7	1	28.1	24.2	320	280	0.40	50
13	10988	2	7	1	22.6	19.3	159	144	1.40	50
14	10988	2	7	1	26.4	22.8	216	191	1.60	50
15	10988	2	7	1	16.8	14.5	65	57	0.50	50

KEY TO DATA FOR MARKING EXPERIMENTS USING TETRACYCLINE AND ACETAZOLAMIDE

A Specimen no.

B Sacrifice date

C Sex (1=male, 2=female)

D BAS life stage code

E BAS gonad stage code

F Total length (cm)

G Standard length (cm)

H Total weight (g)

I Eviscerated weight (g)

J Gonad weight (g)

K Injection dosage (mg/g)

L Number of injections

M Exposure to acetazolamide (1=exposed, 2=control)

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