An impression of the sampling station at E24 (Loch Etive)

SOME PHYSIOLOGICAL AND ECOLOGICAL ASPECTS OF THE PENETRATION INTO ~~BRACKISH~~ WATER OF REDUCED SALINITY BY CERTAIN MEMBERS OF THE OPHIUROIDEA.

By

Richard Michael Pagett

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Some physiological and ecological aspects of the penetration into brackish water by certain members of the Ophiuroidea. (Abstract)

by

Richard Michael Pagett

The problems of penetration into brackish water by echinoderms are reviewed. A comparison is made between Loch Etive and the Baltic Sea with respect to their individual hydrographies. Experiments indicate that ophiuroids behave as osmoconformers and that while *Ophiocomina nigra* is stenohaline, *Ophiura albida* is potentially euryhaline. It is argued that the perivisceral fluid of ophiuroids is similar, ionically and functionally, to the ambulacral fluids of asteroids and echinoids. Salinity tolerance experiments confirm that *O.albida* is potentially euryhaline. Differences in tolerance between *O.albida* and *Amphiura chiajei* are related to their modes of life. Evidence suggests that acclimatization by *O.albida* can extend the tolerance to diluted sea water. It is shown that *O.albida*, from Loch Etive, can be adapted to full strength sea water rapidly and without subsequent morphological deleterious effects. Possibilities resulting in the presence and maintenance of *O.albida* at the head of Loch Etive are discussed. It is considered that an increase in the percentage of skeleton in ophiuroids is important in the development of tolerance to reduced salinity. A proposal that *O.albida* from the Öresund may represent a more advanced stage than *O.albida* from Loch Etive in adaptation to brackish water is made.

Evidence suggests that smaller animals have higher Sr/Ca atom ratios than larger individuals of the same species. This may be manifested by a faster growth rate in young animals or by the physical constraints of the incorporation of the strontium ion into the calcite lattice. Data from *O.albida* (Loch Etive) demonstrates that there may be some concentrating mechanism in these ophiuroids. Discrimination ratios between the Sr/Ca atom ratio in ophiuroids and the ambient medium are calculated. In an elevated ambient strontium level ophiuroids may take up the 'extra' available strontium to different degrees which may partly reflect the original ambient strontium concentration to which the particular species are adapted.

To

My Parents

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PREFACE

> "... we creatures of the land are exiles, displaced organisms on the way from one element to another. We are still in the transit camp waiting for our visas to come through, and when they do and we go on out into outer space, where I suspect most of the human race will live for most of its history, we will regain most of the freedom we lost when we left the sea - a freedom which we try to recapture every time we sink beneath the waves ..."
>
> Arthur C.Clarke (1973)

It is fitting that the above words should precede this thesis since it was the yen for this concept of freedom which first brought me face to face with the small, red brittlestar known as Ophiura albida. This creature has been the subject of investigation for much of this thesis.

This having been said, it is also fitting to record my thanks, appreciation and debt to those who have assisted me in my research whether with advice and criticism, or during fieldwork or by their example.

The problems of echinoderms and the potential of some species for penetrating waters of reduced salinity were first brought to my attention by my supervisor, Dr.E.J.Binyon. During my research he advised me of, and guided me to, the substantial literature dealing with this subject. As the work progressed he suggested a number of approaches to the practical side of the research and later, as writing up began, discussed and criticised my draft. It is a very great pleasure to record my thanks to him.

Also at the Zoology Department of Royal Holloway College I should in particular like to acknowledge the practical assistance from Mr.Nigel Hurley, Mrs. Christine Waite and Mr Steve Horrell.

The Botany Department kindly allowed me extensive use of their atomic absorption spectrophotometer.

Part of the work for this thesis was carried out at the Dunstaffnage Laboratory of the Scottish Marine Biological Association. I am very grateful to Dr. J.D. Gage who courteously arranged bench space, boat - time and diving facilities for my visits. During my dives at station E24 in Loch Etive I was accompanied by the Chief Diving Officer of the Laboratory - Mr. Alan Gale - to him I extend my gratitude. It was also at this laboratory that I met Mr. Anton Edwards who gave generously of his time to explain the hydrography of Loch Etive, and to provide verbal and unpublished data of hydrographic interest. This data, together with rainfall information provided by the Meteorological Office, Bracknell, Berkshire, was used in conjunction with a mathematical model constructed by Mr. Anton Edwards and his colleague Mr. Duncan Edelsten to hindcast salinity at Bonawe during the last fifty years. The correlations between these various groups of data were established by the efforts of Miss Nicki Morgan. The results of her work have not been used directly in this thesis since they are not of my compilation. However, the information they have generated has influenced my thinking in regard to the presence of Ophiura albida in Loch Etive.

My visits to the Dunstaffnage Laboratory have been, without exception, happy and rewarding experiences which reflect the friendliness and helpfulness which I was accorded upon each occasion.

Since the ability to dive has largely determined the direction of this investigation it is a pleasure to acknowledge the diving prowess of Mr. Brian Cumming who, by his instruction, patience and most of all by his example has influenced my diving during the last five years.

A special note of gratitude is extended to Miss Helen Thomas. Helen was patient with my ramblings about brittlestars and provided encouragement and coffee when necessary - especially during the writing-up stage of the thesis. She was also responsible for the frontispiece - an impression of the sampling station E24 at the head of Loch Etive. Her mother, Mrs. Elizabeth Thomas

very competently typed out this thesis for which I am very grateful.

I must also thank Mr. Elvis Presley (deceased), Mr. Bob Marley, and the Hydrographer of the Navy. The first two produced the broad spectrum of vocal atmospheres necessary for my concentration, while the latter kindly gave permission allowing me to base my map of Lochs Creran and Etive upon the relevant Admiralty Charts.

Lastly, but by no means least, I must thank my parents. My debt to them cannot be met by words. As some small token I dedicate this thesis to them.

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(other abbreviations and symbols which are used are explained in the text).

$SrCl_2 \cdot 6H_2O$	- the hexahydrate of strontium chloride
Sr^{2+}	- strontium ion
Ca^{2+}	- calcium ion
µl	- microlitre
ml	- millilitre
mM	- millimole
mM/l	- millimoles per litre
mg	- milligram
mg/l	- milligrams per litre
mg/k	- milligrams per kilogram
g	- gram
mm	- millimetre
cm	- centmetre
m^2	- square metre
k^2	- square kilometre
%	- percentage or percent
r.p.m. (revs.per min.)	- revolutions per minute
°C	- degrees Centigrade
‰	- salinity, parts per thousand
ppm	- parts per million
$x10^3$	- "times one thousand"
$\bar{x}$	- mean
s	- standard deviation
R	- Regression coefficient
p	- probability
$\pm$	- "plus or minus"
$>$	- "greater than"
$\equiv$	- "equivalent to"
$<$	- "less than"

INTRODUCTION

Echinoderms, by convention, are regarded as typical stenohaline marine invertebrates. That is to say, they are intolerant of extensive fluctuations in the salinity of their habitat. Consequently they are not considered to be able to penetrate brackish water to any significant degree. During the past two or three decades there has been an increasing number of reports, occasionally with some supporting experimental observations, of echinoderms entering waters of reduced salinity. By the large numbers of such reports describing the occurrences of brittlestars in these environments, the Ophiuroidea are considered to be the most euryhaline of the Echinodermata.

Despite the abundance of such papers reporting the presence of ophiuroids in reduced salinities, such as are found in estuaries and brackish water lochs and seas, there is a dearth of physiological and ecological observations upon which to quantify the potential of ophiuroids to successfully tolerate hypo-osmotic environments. Often the size of some ophiuroid species and their renowned fragility has precluded the use of these echinoderms in, for instance, some of the physiological investigations which have been conducted upon some of the larger echinoderms.

There are two main approaches to be considered in the design of studies intended to explore the potential of brittlestars to tolerate lowered salinities. Firstly, natural distributions of the animals with associated limits can be assessed. Remane and Schlieper (1971) call this the 'ecological method' of study. Secondly, the tolerance of animals to reduced salinity can be experimentally determined. This has been termed the 'physiological method' (Remane and Schlieper, 1971).

The approach of the present study has been primarily concerned with the latter method, though not without due consideration to the benefits which the ecological method could bestow. The results gained by the physiological approach have been interpreted, closely, in terms of the ecology of the animals under investigation.

A fuller understanding of the ecological parameters which influence the distributions of marine communities and their continued existence has many consequences. A not unimportant possibility is that of predicting reactions, to some extent, in marine animal distributions and within the organisms per se due to the effects of processes, initiated by Man, which affect the chemistry and hydrography of the seas.

This concept provides the raison d'être for the strontium and calcium studies which comprise the latter part of the present work. Strontium 90 (half-life : 28 years), is a relatively long-lived fission product of uranium and so may remain in the environment for sufficient periods of time to be subject to the concentrating effects of food chains. Possible sources of oceanic contamination by strontium 90 are nuclear fallout and limited direct disposal of reactor wastes containing fission products (Mauchline and Templeton, 1964). Bowen (1956) estimated that, up to 1956, the total oceanic load of strontium 90 was approximately 0.8 megaCurie. Thus, the hazard from contamination of the ocean by radioactive strontium would seem to be fairly slight, assuming there to be a fair degree of dilution.

Since it has been demonstrated that several marine organisms have the ability to accumulate ions to much higher concentrations than are found in the surrounding sea water, it would be instructive to assess this ability in terms of the endemic total strontium. Subsequent studies could then relate the interaction between the total strontium and radioactive strontium to the possible effects upon marine organisms of an increase in the oceanic burden of radioactive strontium.

THE PROBLEMS OF PENETRATION INTO BRACKISH WATER.
(A review with special reference to echinoderms)

Various classifications of brackish water, based on physical considerations have been proposed, (these include Redeke, 1922, 1933; Välikangas, 1926; Remane, 1934, 1940; Ekman, 1953). Biological considerations have also been applied to the classification of brackish water, (Välikangas, 1933; Heiden, 1900). The latter graded regions of differing salinity on the basis of particular assemblages of diatoms. Recent reviews concerning the classification of brackish water have been published, (Remane and Schlieper, 1971; Symposium on the Classification of Brackish Waters, 1959).

Following Kinne (1964c), it suffices for the purposes of the present study to term any body of water whose salinity lies between 30°/oo and 0.5°/oo as brackish water. Most of the work described in this thesis considers waters whose salinities are no lower than 12°/oo. Where it is necessary, further quantitative qualification of the terms 'brackish water' and 'waters of reduced salinity' will be given.

The problems of penetration into brackish water by marine invertebrates have been the subject of much research in the past, (recent reviews include Remane and Schlieper, 1971; Prosser and Brown, 1961; Kinne, 1964b). However, most of this work has concentrated upon phyla other than the Echinodermata despite the number of species of this group which appear either to live wholly or partly in water with a depressed salinity. Where echinoderms have been the subject of physiological studies, (ASTEROIDEA - Loosanoff, 1942, 1945; Schlieper, 1957; Binyon, 1961, 1962, 1972, 1976, 1978; Pearse, 1967; Ellington and Lawrence, 1974; Stickle and Ahokas, 1974. ECHINOIDEA - Gezelius, 1963; Giese and Farmanfarmaian, 1963; Lange, 1964; Stickle and Ahokas, 1974; Lawrence, 1975. HOLOTHUROIDEA - Koizumi, 1932, 1935; Stickle and Ahokas 1974), the Ophiuroidea appear to have been studied very little.

It would clarify the contribution of the present work by summarising the physiological problems of survival encountered by marine, invertebrate species during euryhaline penetration and give some indication of previous work which has gone some way in describing the strategies employed by those echinoderms that do occur, to a lesser or greater degree, in waters of reduced salinity.

Probably the fundamental principle from which physiological problems can be considered is the effect which the lowered salt content of the environment has upon the animal. The integument of a typical soft-bodied, marine invertebrate is permeable to water (Bethe, 1930). The echinoderm integument is no exception. Early work by Frédéricq, 1901; Macallum, 1903; Botazzi, 1908 had shown that the surfaces of marine invertebrates act as semi-permeable membranes. However, Bethe, (1929) using the opisthobranch mollusc Aplysia, convincingly demonstrated that this was incorrect and that ions could pass through the integumental membranes.

Koizumi (1932, 1935) examined the permeability of the body surfaces of the holothurian, Caudina chilensis, and reported that univalent ions permeate faster than divalent ions. This selection of univalent ions is probably of general application (Remane, 1939). Remane and Schlieper (1971) further suggest that the permeability of the integument of species living in waters of reduced salinity should be lower for single ions only would be unlikely. They further propose that the rate of penetration of one ion should permit general conclusions to be made regarding ion-permeability and, perhaps, also for the passage of water. Krogh (1939) too, considers that the integumental permeability for ions and for water may be related. Employing sodium iodide, Ussing (1934) showed that stenohaline species are more permeable than euryhaline species. Euryhaline species and those that are largely independent of the external salinity are the least permeable.

There have been few permeability studies conducted on echinoderms. Subsequent to the studies of Koizumi (1935) the majority of these studies concern asteroids, (Kowalski, 1955; Schlieper, 1957; Binyon, 1961, 1962, 1972, 1976). The latter author investigated the permeability to water of Asterias rubens from the North Sea. It was demonstrated that this species is permeable to water and that this permeability remained unchanged during the year.

When a typically marine echinoderm, isosmotic with full strength seawater, is placed in diluted seawater, water passes through the integument into the animal by osmosis diluting the body fluids until a new, and lower, osmotic pressure equilibrium is achieved. It is likely that ions in the body fluid diffuse out of the animal down the concentration gradient between the body fluids and the medium and that this would also lower the osmotic pressure of the body fluid (Bethe, 1930).

So, the establishment of a new equilibrium is probably due to water movement into the animal and to salt movement in an opposite direction leading to a smaller water influx than would be predicted for a given dilution.

Kinne (1966) proposes that there are various compensatory mechanisms available to a marine invertebrate should the ambient salinity be depressed:-

(1) Escape
(2) Reduction of contact with the lowered salinity
(3) Regulation
(4) Adaptation

The ability to escape would require a reasonably efficient means of detecting lowered salinity. Pearse (1936) remarks that detection of slight changes in salinity by many aquatic invertebrates is known. Loosanoff (1945) suggests that asteroids, in general, and Asterias forbesi, in particular, are capable of such sensory ability.

Reduction of contact by echinoderms, when faced with a lowered ambient salinity, may be effected by, possibly at least three methods. Firstly, the production of mucus by Asterias rubens, found at the head of Loch Etive, may be a response to salinity (Binyon,1976). Secondly, Stancyk (1975) proposes that Ophiothrix angulata, from the Cedar Key region of Florida, has a shortened pelagic life which may enable it to avoid extremes of salinity. Finally, Turner (1974) proposes that selective post-metamorphic growth of the arms in the ophiuroid, Ophiophragmus filograneus may permit an early descent of the disc into the substratum, and thus remove it from contact with water of depressed salinity present in the surface layers. It has been recognised that the ability to burrow in marine invertebrates may enable them to avoid extremes of salinity (Topping and Fuller, 1942; Kinne, 1970). In echinoderms this method has been suggested for Ophiophragmus filograneus, Stancyk (personal communication).

As yet there does not appear to be any extensive ionic or osmo-regulatory system universal within the Echinodermata. Ellington and Lawrence (1974) report a degree of volume regulation in the asteroid, Luidia clathrata, which contrasts with the absence of any such regulation in Asterias rubens (Binyon, 1961). Earlier work on Asterias rubens (Bethe, 1934; Maloeuf, 1938) conflicts with these findings;

however it has been suggested (Binyon, 1961) that this is due to experimental design in the earlier work. Pearse (1967) reports some weight regulation (via inbibition into the supradorsal cavity) in the antarctic asteroid, Odontaster validus, while none was demonstrated by Giese and Farmanfarmaian (1963) in the echinoid, Strongylocentrotus purpuratus. It has been suggested that there may be an optimal coelomic volume to body volume ratio which would tend to be maintained (Freeman, 1966; Binyon, 1972a).

Some degree of ionic regulation has been described in Asterias rubens from the North Sea (Binyon 1962). There appears to be some accumulation and regulation of calcium ions in the perivisceral fluid, and of potassium ions in the ambulacral fluids of the water vascular system. Seck (1958) examined this species from the Western Baltic Sea and did not report any accumulation of calcium ions.

Isosmotic intracellular regulation is a phenomenon whereby cells do not take up as much water as is perhaps expected by the degree of dilution of the medium when a soft bodied marine animal is placed in diluted seawater.

It is thought that this is due to regulation at the cellular level rather than at the tissue level. This may be by a variation, perhaps, in the selective membrane permeability or shunting of various chemical components, e.g. amino acids, in order to maintain cellular osmotic pressure.

A response of this type is described in Asterias rubens (Jeuniaux et al, 1962; Binyon, 1972b) where there was found to be a smaller than expected tissue hydration during a lowering of salinity. This is thought to be indicative of the presence of an isosmotic intracellular regulation. Such regulation has also been reported for the echinoid, Strongylocentrotus droebachiensis (Lange, 1964)and in the asteroid, Luidia clathrata (Ellington and Lawrence, 1974).

In this type of regulation it is thought that amino acids are of paramount importance. Marine invertebrates which have a 'free amino acid pool' have been shown to regulate this pool in response to salinity change (Stephens and Schinske, 1961). Such responses have been investigated by several authors (Stevens and Virkar, 1966; Emerson 1969; Stickle and Ahokas, 1974; Stickle and Denoux, 1976). It has been demonstrated that the amino acid pool decreases as the ambient salinity decreases. Stevens and Virkar (1966) proposed that this is due to the incorporation of amino acids into polypeptides - a more economical process than excretion. Florkin (1962) has suggested that this represents an osmoregulatory response in the sense that a decrease in the size of the pool 'cushions' larger fluctuations in the other cellular constituents.

Stevens and Virkar (1966) demonstrated that the ophiuroid Ophiactis arenosa, was capable of removing amino acids from very dilute solutions in seawater, i.e . uptake may maintain the pool when the latter was being drained due to salinity depression. They suggest that the salinity decrease produces an increase in the incorporation of free amino acids into polypeptides and this causes a decline in the pool. Lange (1964) describes three stages in the isosmotic intracellular regulation of Strongylocentrotus droebachiensis - an isosmotic step, an intermediate step and, finally, a regulatory step where the amino acid pool is utilised.

Probably the main strategy employed by echinoderms, which penetrate waters of reduced salinity, is that of adaptation. This enables the organism to adjust to changes in the critical salinity. Such adaptation is of two main types: genetic and non-genetic (Kinne 1964c; Schlieper, 1967). The former governs increases in tolerance to fluctuating salinity where the main selection mechanism is selection acting upon the available genetic material. The latter, non-genetic adaptation, involves quantitative changes in the response mechanism of an individual which are not passed on, as such, to succeeding generations. These changes may lead to significant modifications with respect to lethal limits, activity, metabolism, reproduction and other functions.

Stancyk (1973) studied the ophiuroids and echinoids of the estuary at Cedar Key, Florida. He suggests that up to 75% of the inshore species display physiological, morphological and behavioural adaptations in response to the estuary conditions at Cedar Key. The ophiuroid, Ophiothrix angulata relies on high dispersal and the ability to colonise or recolonise disturbed habitats after local extinction due to rapid salinity depression. This is in contrast to two other ophiuroids, Ophiophragmus filograneus and Ophioderma brevispinum, which show adaptations which help the young stages avoid such stress conditions (Stancyk, 1973).

It has been proposed that egg size, in ophiuroids, correlates with development (Schoener, 1972) and that direct egg development would remove larvae from possible salinity depression in the surface waters (Pearse, 1969; Stancyk, 1973).

Schlieper (1957) compared various features - percentage water and ash content, and rates of metabolism in some of the soft tissues of Asterias rubens from the Baltic Sea and from the North Sea. He concludes that some of the differences may have arisen as responses to lowered salinity. Although Asterias rubens is able to reproduce in the depressed salinities of the Kieler Fjord, ($15^{o}/oo$), in the Western Baltic Sea he is not of the opinion that this is conclusive of a physiological race. He suggests that all the differences between Baltic and North Sea animals could be derived by a, more or less, lengthy process of adaptation. It should be mentioned that Asterias rubens at the extreme parts of its range in the Baltic Sea is unable to breed (Segerstråle, 1957, citing Brattström, 1941).

The ionic composition of seawater affects sperm motility and longevity. Timourian and Watchmaker (1970) used the echinoid, Lytechinus pictus, and demonstrated that the spermatozoa are capable of adapting to a reduction in the salt content of the ambient medium and therefore are able to fertilize eggs in lowered salinities.

The echinoid, Psammechinus miliaris, exists, off the Swedish coast, as two distinct forms - 'S' and 'Z' - which occur at different depths and thus live in different salinities. Gezelius (1963) attempted to characterise the optimum salinity regimes, of the two forms, by using the different cleavage rates for egg development. He demonstrated that adaptation to depressed salinity involved changes in tolerance range and cleavage rate. It was possible to cross-adapt these forms to the salinity range of the other form. The differences between these two forms should not be considered as permanent modifications or hereditary characters since they appear to be morphological forms which have developed in response to various regimes of salinity, light and temperature at which metamorphosis and early development occur (Gezelius, 1963).

A DESCRIPTION OF LOCH ETIVE

(With special reference to similarities with the Baltic Sea)

Loch Etive is a long, narrow sea loch situated on the fjordic coastline of Western Scotland a few miles north of the small town of Oban (National Grid Reference: NM 860 300). This loch runs inland in an easterly direction for some 13 kilometres and then bends round to the north east and continues for a further 16 kilometres (see map).

It is a triple basin system connected to the sea by a sill, 4 kilometres long, 300 metres wide and 10 metres deep (Edwards and Edelesten, 1977). The Falls of Lora mark the seaward end of this sill, while another sill is located at Bonawe some 11 kilometres further up the Loch. A third sill at Achnacloich divides the lower basin but this subdivision is considered to be of minor importance with respect to overall hydrography of the Loch (Edwards and Edelesten, 1977) so the loch will be treated as a functional twin basin system in the present work.

The elongate, upper basin has been subjected to glacial erosion (Charlesworth, 1954) and, as a consequence, has been over-deepened to the extent that the maximum depth attained is 153 metres. The lower basin has a maximum depth of 74 metres.

Gage (1972a, 1974) has described the features affecting the hydrography of the loch. The sills exert two effects upon the tidal regime operating within the loch. One effect is to reduce the tidal amplitude within the loch by as much as one third in comparison to the tides which impinge upon the open coast in the Firth of Lorne. The sills also cause a delay in tidal phase so that the times of high and low water may be delayed by as much as 1½ hours.

The surrounding topography of Loch Etive permits a rain-water catchment, (some 1400km^2), larger than any other Scottish fjord. The principal rivers draining into the loch are the rivers Awe, Etive and Kinglass. The River Etive enters at the head of the

Loch while the Kinglass enters on the east side between the Etive and the Awe (see map). The River Awe, which enters the Loch at Bonawe, flows through the Pass of Brander and drains Loch Awe.

The Awe flow is regulated by a hydroelectric scheme which has the potential to significantly modify the salinity of sill water at Bonawe and thus the renewal periodicity of the deep water in the upper basin of Loch Etive (Edwards, personal communication).

The salinity regime of the deep water mass of the upper basin has remained fairly steady with the salinity at about 27°/oo to 28°/oo (Edwards and Edelsten, unpublished data). The lower basin has a similar, or slightly higher, salinity value. Due to the large volumes of freshwater runoff, from the Loch Etive catchment, there is a pronounced freshening effect upon the upper layers of the water mass in the upper basin which manifests a relatively steep density gradient from the surface of the Loch down to about twenty metres below the surface. Gage (1972a) states that the salinity at the surface at the head of the Loch has varied from 1°/oo to 26°/oo and that the salinity tends to decrease from the seaward end of the Loch towards the head.

Lack of substantial tidal oscillation permits the establishment of a brackish layer of water, with a variable thickness, overlying the more saline, deeper water. The brackish layer, however, is not a packet of water where the salinity gradually increases from the surface to the halocline and then more rapidly. Rather, it is a layered structure comprising bands of water where salinity decrease is gradual, alternating with bands where the salinity changes sharply. The transition areas between these bands are sites of arrested sedimentation which appear as sheets of suspended particulate material which can be observed while diving through to the more saline, deeper water.

The bottom water of the upper basin stagnates for varying periods; from a few weeks to a year or more (Edwards and Edelsten, 1977). It is replaced by water of a greater density from the sill region, at Bonawe, which overflows and forms a turbulent plume and which then flows down the slope lifting the bottom water to sill level where it gradually loses its 'bottom water features'.

Such renewals of the deeper water mass are thought to be initiated by very low freshwater imput to the upper basin. The rate at which such flushing occurs is determined by the rate of supply of sill water by the flood tides. The minimum time to flush the basin below sill level is one week at spring tides (Edwards and Edelsten, 1977).

The high imput of freshwater to the Loch results in the establishment of a semi-permanent layer of brackish water which, although the salinity difference is of a stepped nature, is a distinct layer with a steep gradient separating it from the more saline water below. The depth, thickness and internal gradient of this layer is directly controlled by the recent history of rainfall on the Loch Etive catchment (Gage, 1972a).

There is a long term effect on the water below the halocline which has a consistently lower salinity than the open sea. This is thought to arise through a continued association between it and the brackish layer above. The characteristics of this more salty water lying below the halocline are probably more permanent (Gage, 1974).

The effects upon the animal communities within Loch Etive of the salinity conditions have been discussed by Gage (1974).

The hydrographic conditions found within Loch Etive are not as stable as those found in the brackish water Baltic Sea. They are, also, not rapidly fluctuating systems as observed in typical estuaries. The Late- and Post-Glacial history of the Baltic has included periods with fresh and marine waters (Segerstråle, 1957a). At the present time, the Baltic Sea is classified as 'brackish' (Segerstråle, 1957a).

Like Loch Etive, the Baltic Sea is diluted by a large freshwater influx from a widespread land area, via nearly 250 rivers (Segerstråle, 1957a), which is entrained due to the presence of narrow and shallow connections with the ocean. These areas are equivalent in function, with respect to effects upon the hydrography of the Baltic Sea, to the sills at Connel and Bonawe of Loch Etive. There are three outlets from the Baltic Sea; the Little and Great Belts and the Sound, (Öresund). Due to the width and depth of the Great Belt Sea there is a landward moving mass of salt water penetrating along the bed of the Great Belt into the Baltic. While on the surface, there is an outflowing mass from the brackish sea. Outflowing water also passes via the Little Belt and the Sound. Marine incursions occasionally penetrate through the latter outlets. The salinity of the landward flowing water does not exceed 15^o/oo - 20^o/oo, while that of the Little Belt and the Sound and the surface of the Great Belt is between 15^o/oo - 20^o/oo (Segerstråle, 1957a).

Occasionally the marine influx, probably under the influence of winds, builds up and enters the Baltic Sea beyond the thresholds and it is thought that such influxes are responsible for renewing the deep water east of the thresholds in the Baltic proper. These incursions have been described by Segestråle (1957a), as 'piston strokes' and are equivalent to the renewals of the upper basin in Loch Etive.

The echinoderms found in the Baltic Sea may occur as far to the east where the threshold salinities drop as low as 8^o/oo. However, though the conditions fluctuate in the Sound and the Belts as they do in Loch Etive, these fluctuations are by no means as frequent as those operating in Loch Etive. This allows echinoderms, principally, the asteroid, Asterias rubens, and the ophiuroid, Ophiura albida, to survive the reduced salinities, through the development of either physiological races or resistance to diluted seawater by the process of adaptation (Remane, 1959). It has been suggested by Gage (1974) that these species in Loch Etive are not able to tolerate such low salinities because of the wider salinity fluctuation over time scales which would not allow acclimatization.

DISTRIBUTION OF Ophiocomina nigra and Ophiura albida

(With special reference to their relative euryhalinities)

The genus Ophiocomina is known by only one species, Ophiocomina nigra (Abildgaard). Mortensen (1927) has described this species as occurring in British and European seas. In British waters O.nigra is common all round the south and west coasts and down to Durham on the east coast. Interestingly, it is not recorded from the open waters of the North Sea.

On the continent it is distributed from the Trondheim Fjord on the Norwegian coast down to the Azores and the Mediterranean. The bathymetrical distribution is 0 - 400 metres, though it is generally rare below 100 metres.

Nowhere in its range is Ophiocomina nigra reported to be found in waters of reduced salinity or waters which may periodically be diluted. During the following investigations described in the present study O.nigra is used as a representative of an oceanic, stenohaline brittlestar - one which does not tolerate any reduction in the salt content of its ambient medium.

The other species which is mainly involved in the present study is the ophiolepid, Ophiura albida (Forbes). Mortensen (1927) describes this species as occurring around all coasts and is distributed from East Finmark to the Mediterranean and the Azores. It is reported from the Western Baltic Sea, though not beyond Bornholm. In the North Sea it is 'very abundant' in the central part (Ursin, 1961). The distribution of this species has been exhaustively reviewed by Tyler (1976). It is a common echinoderm of the Öresund where its larvae are abundant in the autumn plankton. Larvae have also been recorded from the Wadden Sea (Smidt, 1951). Mistakidis (1951) cited by Ursin (1961) notes that O.albida occurs in the estuary of the River Roach in Southern England.

Records have described this species from either brackish water *per se* or from water which is subjected to some reduction in salinity on a more or less regular basis. Consequently, this species has been used as an example of a euryhaline brittlestar since it has a well documented ability to tolerate a reduction in the salt content of the ambient medium.

SOURCES OF ECHINODERM MATERIAL AND METHODS OF HUSBANDRY

Echinoderm material for experimental work, carried out at Royal Holloway College, was obtained from a variety of sources dependent upon the species required and/or the salinity regime under which the species lived.

(i) Ophiocomina nigra (from full salinity conditions:- $34^{o}/oo$) were sent by rail from the Marine Biological Association, Plymouth.

(ii) Ophiura albida (from full salinity conditions:- 33 - $34^{o}/oo$) were sent by rail from the University Marine Biological Station, Millport on the Isle of Cumbrae.

(iii) Ophiura albida, (Loch Etive), were trawled or hand collected (SCUBA diving), and transported by road.
See page 98 for the stations and their respective salinities sampled.

(iv) Ophiura albida from the Öresund (about $18^{o}/oo$) were sent by air preserved in 5% formalin.

(v) Ophiura albida from the Little Belts (about $18^{o}/oo$) were hand collected and subsequently transported by road and sea.

The procedure upon receiving live material was identical in all cases irrespective of mode of collection or transport. The glass or plastic containers in which the animals travelled were floated for 20 - 30 minutes in tanks containing seawater of the appropriate salinity. These tanks were maintained at a temperature of about 10 - 14°C. The bases of the stock tanks were lined with standard aquarium gravel in the case of Ophiocomina nigra; with sandy detritus - Ophiura albida from Millport; with mud - Ophiura albida from the Öresund and Loch Etive.

The water was circulated (30 gallons per hour) by either Nuova or Eheim power filters. The salinity was monitored every two days by silver nitrate titration (1975 - 1977) and by a Goldberg Temperature-Compensated Refractometer : Model No.10419 (1977 - 1978). The latter employs the principle of incremental changes in the refractive index of waters of differing salinity. The salinity was maintained ± 1.0°/oo. Adjustments to the salinity were made by evaporation (to increase salinity), or by the addition of glass distilled water (to lower salinity).

Although large aggregations of brittlestars have been reported in the literature (see review by Reese, 1966), there was some indication that some species of ophiuroid should be kept in low densities in vivo. This was particularly evident with Ophiura albida from Loch Etive. It was observed that after a week or so many of the brittlestars in the stock tank, containing individuals from station E24, of circa 150 - 200 per $0.5m^2$, had the last few distal segments, (5 - 6), of their arms missing. Observations by SCUBA diving did not indicate large numbers of 'cropped individuals' at this station. A crude, qualitative experiment was carried out to test the occurrence of cannibalism in Ophiura albida.

A few completely intact specimens of O. albida were kept in air for about five minutes in order to incur some degree of immobility in the animals from desiccation. These 'treated' individuals were then re-introduced to the original stock tank containing the rest of the ophiuroids from station E24. Within minutes, one or more of the 'normal' O.albida had moved onto the 'treated' animals. The method of attack was the same in all cases. The distal segments of the arm were held by the mouth podia of the 'normal' O.albida. When the 'normal' O.albida moved away from the 'treated' individuals, (after a variable time of 5 - 30 minutes), the most distal (5 - 6) segments of the 'treated' O.albida were clearly missing. It was observed upon two occasions that all five arms of a 'treated' individual had a 'normal' O.albida attached to them. Possible causes of this behaviour were not able to be tested. As a result of these observations all ophiuroids were kept in low densities - less than twenty per $0.5m^2$.

Every two weeks, thawed soft tissue of Mytilus edulis was broken up in the water whilst the circulation was switched off for one hour, (so as to allow for settling of the fragments). A week prior to use in experiments feeding was discontinued.

Following the above procedures ophiuroids could be maintained for up to periods in excess of a year.

From previous physiological studies it is possible to derive a scheme of investigation relating to the penetration of echinoderms into waters of reduced salinity, namely:-

1. Investigation of the ability of the species to regulate its weight in reduced salinity.

2. Investigation of the distribution of the major cations, sodium and potassium and of the chloride anion in the ambulacral and perivisceral body fluids and the subsequent adjustment, if any, to lowered salinities.

3. Investigation of the tolerance of the species under study to reduced salinity.

This scheme has largely been followed in order to investigate the potential which the ophiuroid, Ophiura albida, may have to penetrate waters of depressed salinity, with particular reference to the Scottish sea loch, Loch Etive.

CHAPTER ONE

INVESTIGATION OF THE ABILITY OF Ophiocomina nigra and Ophiura albida TO REGULATE THE BODY WEIGHT IN DILUTED SEAWATER.

1.1 Introduction:

Following the course of wet weight changes, if any, of a marine invertebrate when placed in diluted seawater permits observation of whether the invertebrate is osmo-conforming or osmoregulating when faced with dilution of its environmental medium.

In echinoderms, most work of this nature has been conducted upon asteroids (Bethe, 1934; Maloeuf, 1938; Binyon, 1961; Pearse, 1967; Ellington and Lawrence, 1974; Stickle and Ahokas, 1974).

Although the results of Bethe (1934) and Maloeuf (1938) both indicate evidence of weight regulation when Asterias rubens was placed in diluted seawater, it is the opinion of Binyon (1961) that this may have been due to experimental technique since these animals become very swollen in diluted seawater and the integument splits readily. Ellington and Lawrence (1974) considering this opinion ensure that this is not applicable to the results of their work upon the asteroid, Luidia clathrata which indicates a degree of volume regulation. Binyon (1961), using a cage method of weighing Asterias rubens, reports no weight regulation down to 50% sea water. Further dilution probably damaged the integument resulting in large salt losses producing a smaller than expected increase in weight as suggested by Binyon (1961). Pearse (1967) describes a regulation of coelomic water volume which maintains a seasonal fluctuation in the asteroid, Odontaster validus. This ability is thought to allow this shallow water asteroid to withstand hyposmotic conditions which occur when freshwater runoff enters the shallow waters of the habitat in summer. Stickle and Ahokas (1974) working upon the asteroid, Pisaster ochraceus, the holothurian, Cucumaria miniata and the echinoid, Strongylocentrotus droebachiensis concluded that there is little change in the percent body water during a twelve hour experimental tidal cycle, and suggest that changes in perivisceral fluid osmotic pressure were mostly due to ion movements.

It is of interest that no such work has been reported in the literature concerning weight change experiments using ophiuroids. It is one of the main objects of this work to determine whether any such weight regulation occurs when ophiuroids encounter water of reduced salinity.

Comparisons were made between the wet weights of two species, one considered to be stenohaline and one which is thought to be euryhaline due to its occurrence in the Baltic Sea. These were monitored during a seven hour period in varying dilutions of seawater.

Ophiocomina nigra was employed as an example of a solely oceanic ophiuroid, i.e. one which does not occur in reduced salinities. The other species was Ophiura albida which is to be found in full strength seawater at Millport where a salinity of 33°/oo to 34°/oo obtains. This species also occurs in reduced salinities in Loch Etive (16°/oo to 28°/oo).

1.2 Rationale of weighing method:

The common name for the Ophiuroidea - Brittlestars - reflects the inherent fragility of these echinoderms. It is this tendency to 'break up' which makes the repetitive weighing of these animals difficult. The tips of the arms are particularly liable to breakage and in this way leak body fluids when lifted out of the seawater which acts as a support. Consequently, it is desirable to be able to weigh these animals with the minimum of handling of the arms. It is when the arms of the brittlestar are out of the water that fracture and breakage is most likely to happen and so one of the main criteria to be considered in a weighing method is one which facilitates the continual support of the arms.

A cage method seemed appropriate. This technique had been used previously by Binyon (1961) who also recognised the need for continual physical support when weighing the asteroid, Asterias rubens. He used cages made of plastic which weighed about 90 grams and which, when using starfish of about 30 - 100 grams, gave results which were not unduly affected by the 'deadweight' of the cage. The brittlestars which were being used were, however, very much smaller - of the order of 0.1g - 2.8g - consequently such cage sizes would not give a sensitive enough indication of any weight change.

Various materials were tested:-

- with regard to proportion of cage weight to total weight of cage and animal
- for ease of drying to a constant weight
- for ease of handling
- for final reproducibility of results.

A cage made of muslin, (mesh size: 1mm in diameter) in which the animal was placed and the four corners of the muslin 'square' were brought together, secured by a paper clip attached to which was a length of cotton, was found to be suitable.

The procedure for weighing an animal was as follows:

The muslin cage containing the animal to be weighed was carefully lifted from the test solution and gently placed on a bed of tissue paper. The clip and cotton were removed and the muslin laid out flat on the tissue. Another tissue was used to gently pat the animal and the upper surface of the muslin. The muslin, still supporting the animal, was carefully gathered and placed in a weighing boat with another, inverted, boat acting as a lid. The total weight of the boat and lid was 4.0 - 5.0 grams. The weighings were found to vary considerably if a lid was not used, and this fact heavily outweighed any inaccuracy arising from the addition of more 'deadweight' to the method. The weighings were carried out on an Oertling balance (Model No. V 20), after which the muslin cage was taken out of the boat, remade with the clip and replaced in the test solution.

1.3 Test for reproducibility of weighing method.

(a) Method:

A series of weighings were made with both of the species under investigation in order to assess the reproducibility of the method. Great care was taken not to unduly expose the specimens to the atmosphere for any longer time than was absolutely necessary. Only animals with completely intact arms, or arms where regeneration had obviously resulted in a sealed apex were used. Breakage of arms could never be entirely eliminated and any results obtained before a fracture in an animal was identified were subsequently ignored. Animals were immersed in full strength seawater using the above method of caging, removed, dried, weighed, reimmersed and the operation repeated until one individual of each species had been weighed, consecutively, ten times.

(b) Results:

The results of this technique assessment are displayed in Table 1.1.

(c) Discussion:

There are two points of interest. Firstly, the method appears to be sufficiently reproducible despite an apparently large weight of cage. Secondly, Ophiura albida can be weighed with a consistently greater reproducibility than is possible with Ophiocomina nigra. It is thought that the more prominent spines of O.nigra would serve to entrap a more variable quantity of water than those of O.albida which are much reduced. Since a major potential source of error is the degree of patting which is applied, and which is difficult to regulate consistently, one would expect a longer spined animal to retain more water between the spines than a shorter spined animal after the same degree of patting had been applied to both species under similar conditions.

Before following the weights of animals in diluted seawater, it was necessary to first determine whether there was any natural oscillation in the wet weights of the two species, O.nigra and O.albida in full strength sea water.

Table 1•1 Reproducibility of weighing ophiuroids by a 'muslin - cage ' method•

SPECIES	NUMBER OF WEIGHINGS	PERCENT DIFFERENCE BETWEEN INITIAL AND SUBSEQUENT WEIGHTS AS A MEAN	STANDARD DEVIATION
Ophiura albida (Millport)	55	- 2•41	3•63
Ophiocomina nigra (Plymouth)	55	- 1•51	4•23

1.4 Test for natural oscillation of wet weight in full strength sea water.

(a) Method:

The wet weights of six individuals of both species were monitored during a seven hour period in salinities normal for the particular species. These salinities were as follows:

Ophiocomina nigra -	$34^{\circ}/oo$
Ophiura albida, (Millport) -	$33^{\circ}/oo$
Ophiura albida, (Loch Etive: E24)	$28^{\circ}/oo$

Six muslin cages were weighed without and then with specimens of the appropriate species. These cages were then lowered onto the gravel bottom of a 30 litre tank containing sea water of the appropriate salinity. The seawater was circulated through a Nuova power filter and was cooled by a Churchill cooling unit to 12°C. The salinity was checked by silver nitrate titration, using potassium chromate indicator, at the beginning and at the end of the experiment.

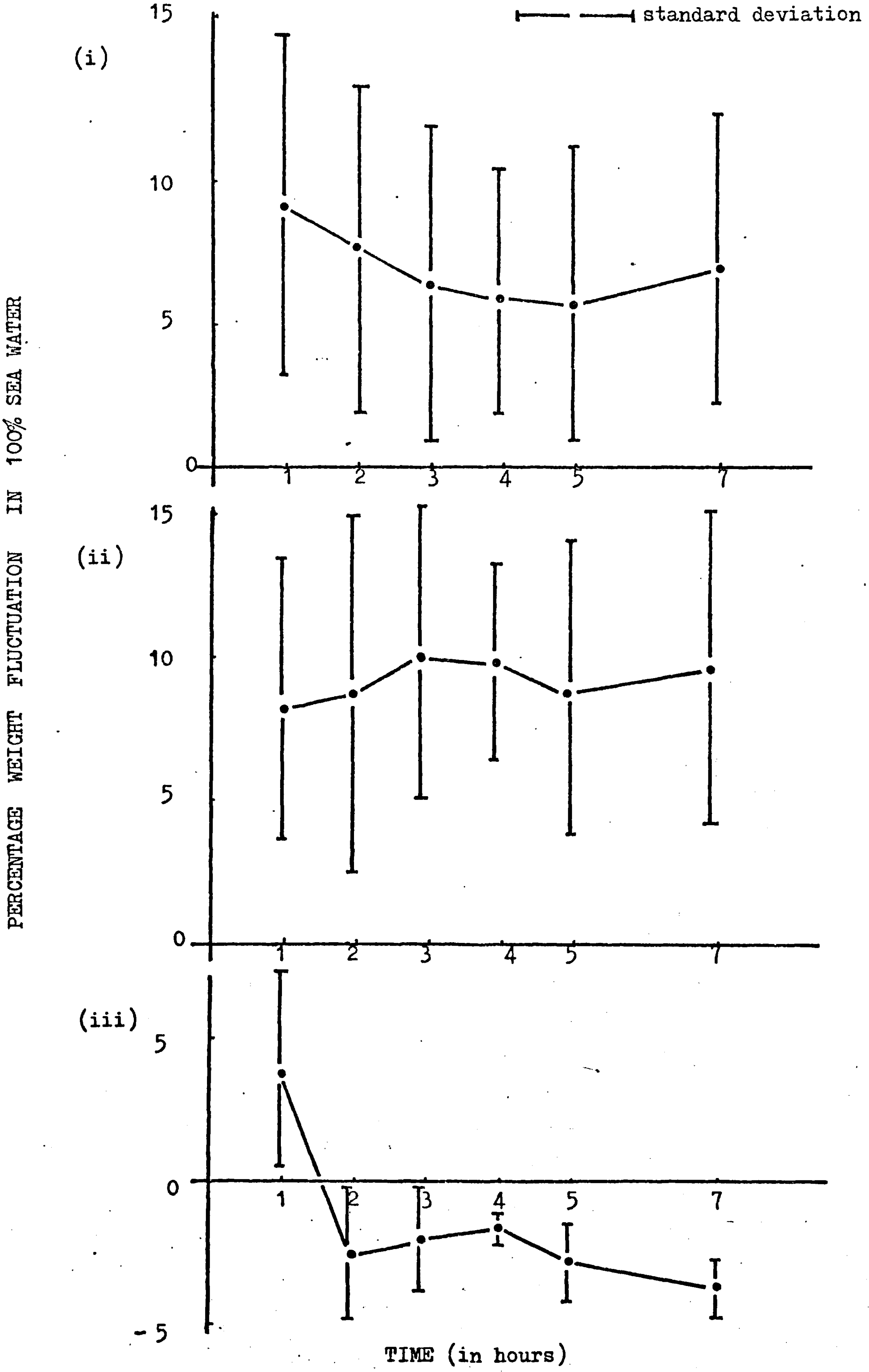
(A solution of silver nitrate containing 27.25 grams per litre when titrated against a 10 ml. sample of seawater gives a direct reading of salinity in parts per thousand, ($^{\circ}/oo$),).

Although salinity is a weight/weight relationship and not a volumetric concept, for which minor corrections can be made, (Harvey, 1955), it was not considered necessary to make such adjustments, since an accuracy of more than ± $1^{\circ}/oo$ for these experiments was not required. Also, over the duration of this experiment, the salinity was not found to vary significantly (i.e. by more than ± $1^{\circ}/oo$) from the initial value and so adjustment was not necessary.

The six animals were weighed at hourly intervals up to, and including, five hours and then finally at the end of the experiment (seven hours). This procedure was repeated with the other species.

Graph 1.1 Percentage weight fluctuation in 100% sea water in (i) Ophiocomina nigra, (ii) Ophiura albida (Millport) and (iii) Ophiura albida (Loch Etive, E24).

Each point represents the mean of six determinations ± 1 standard deviation



(b) Results:

From Graph 1.1 it is clear that individual fluctuation over the seven hour period for each species, after the initial increase, varies over the ranges:

Ophiocomina nigra:- 1.23% ±1.39 to 3.96% ±1.36
Ophiura albida (Millport):- 1.42% ±1.27 to 5.76% ±2.70
Ophiura albida (Loch Etive):- -4.70% ±1.28 to -1.01% ±2.03

The initial increase, which invariably happens, may vary from 3.4% to 19.5% in *O.nigra*; from 2.8% to 16.5% in *O.albida* (Millport); and from 0.2% to 13.2% in *O.albida* (Loch Etive). Once this initial, and often large, increase has occurred further fluctuation is small and relatively regular.

(c) In an attempt to explain this initial weight increase, the wet weights of five specimens of *Ophiocomina nigra* were recorded after one hour immersion following the method previously described, in water from their stock tank. The following day this procedure was repeated with the same animals under similar conditions.

(d) Results:

With reference to Table 1.2., the wet weight of an individual appears to change very little when weighed on consecutive days. The initial increase of any particular specimen after one hour is similar on successive days.

(e) Discussion:

The above results suggest that the increase is either indirectly of experimental origin or that it reflects some natural oscillation of wet weight inherent in the brittlestar. It is possible that this fluctuation in wet weight was prompted by the handling of the brittlestar which resulted in a sudden imbibition of water via some natural water uptake pattern. Water could be taken into the stomach or into the disc cavity. A regular 'rolling' of the aboral disc integument of *Ophiocomina nigra* was observed and

Table 1·2 The variation in wet weight of *Ophiocomina nigra* when weighed on successive days.

DAY 1			DAY 2		
WET WEIGHT (in grams)		%	WET WEIGHT (in grams)		%
INITIAL	AFTER ONE HOUR	DIFF.	INITIAL	AFTER ONE HOUR	DIFF.
1·7117	1·7738	3·63	1·8392	1·9000	3·31
2·1399	2·1829	2·01	2·2944	2·3460	2·29
3·0359	3·0932	1·89	2·9920	3·0518	2·00
2·4428	2·4999	2·34	2·5009	2·5641	2·53
1·5112	1·6747	10·82	1·2957	1·4025	8·25

% DIFF. = % DIFFERENCE

recorded on a physiograph (see Figure A). This regular movement was not apparently qualitatively related with any obvious environmental variable, such as light, salinity and temperature. There was some observation of similar disc integument movements in Ophiura albida.

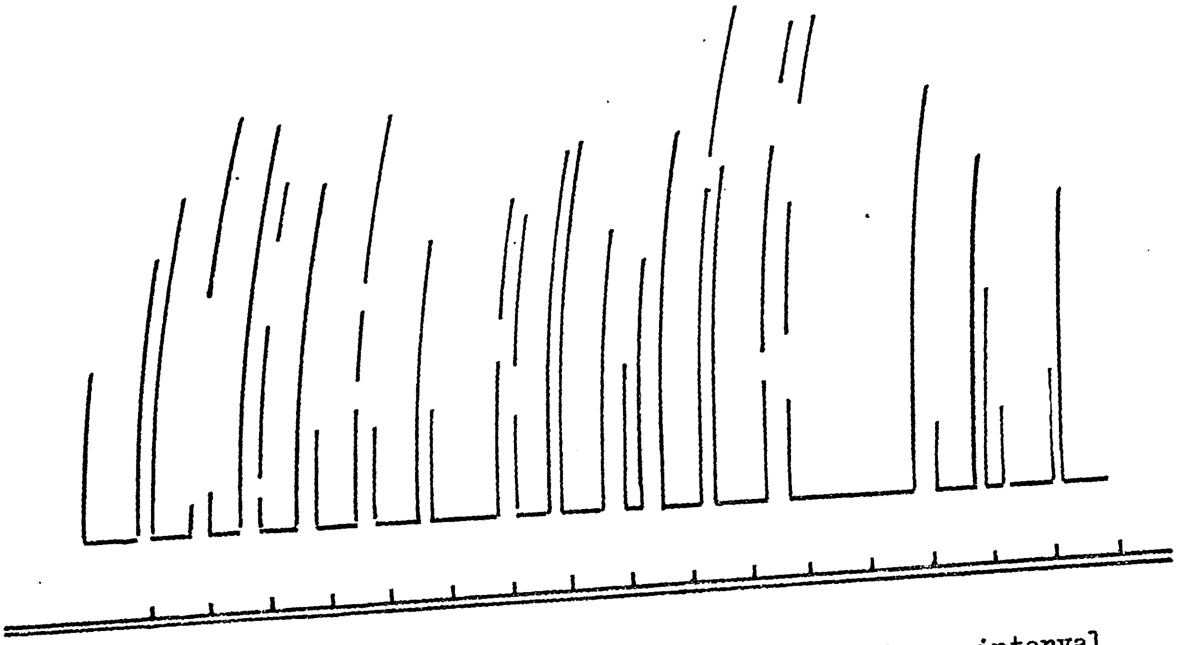
These movements were observed in animals that had been kept in aquaria for at least one week without food so there is the possibility that they are a response connected with feeding. Gemmill (1915) found a weight increase in the asteroid, Porania pulvillus when feeding. Pearse (1967) suggests a possible relationship between the regular weight fluctuation in the asteroid, Odontaster validus, and its flagellary-mucoid feeding activity. In the latter case the water is known to enter a supradorsal cavity; a structure which is not found in ophiuroids. It is also interesting to note (Graph 1.2) that there is some evidence that smaller animals have a greater fluctuation in wet weight than larger animals. Binyon (1972a) has suggested that asteroids have an optimum coelomic fluid volume which is maintained. Pearse (1965) reports Odontaster as holding water in the perivisceral coelom at a slightly positive pressure and suggests that this may be a form of coelomic fluid volume control. A regulation of the coelomic fluid volume has been reported in the holothurian, Opheodesoma spectabilis (Freeman, 1966).

From these preliminary studies it would seem that ophiuroids can be weighed regularly and with reasonable accuracy. However, since there is this, often large, initial rise in wet weight any conclusions concerning possible oscillations in wet weight of an ophiuroid, when in water of a salinity normal for the animal, should be by comparison of such values after the initial rise has occurred. That is to say, when the fluctuation pattern is consistent.

1.5 To test the effect of the diluting medium upon Ophiocomina nigra.

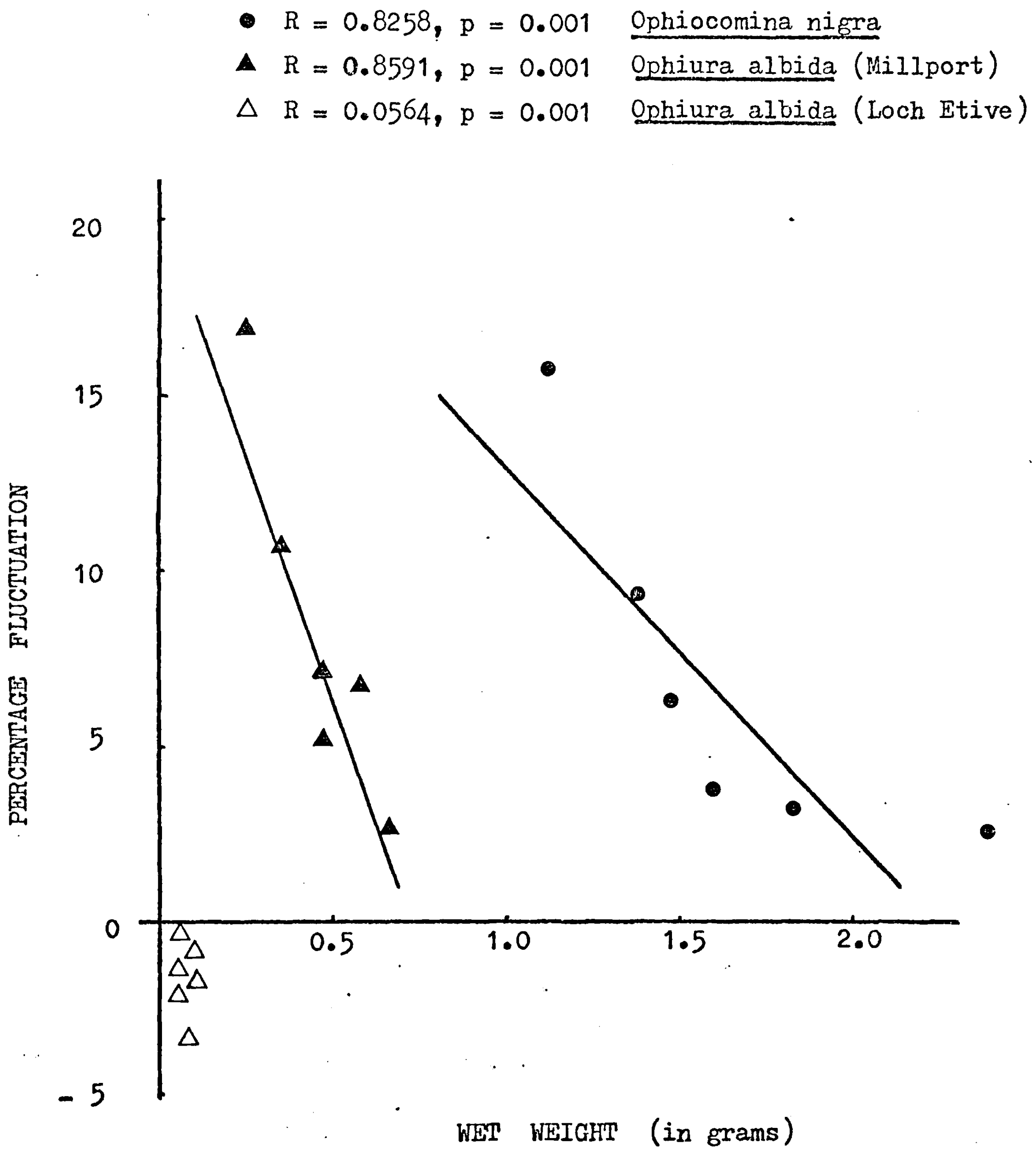
The test solutions were made up by diluting full strength sea water (34°/oo) with glass distilled to produce 90%, 70% and 55%

Figure A   A representation of some activity connected with the 'rolling' of the aboral disc integument of *Ophiocomina nigra* (redrawn from the original physiograph).



TIME (between each division is an interval of thirty seconds).

Graph 1.2 Comparison between *Ophiocomina nigra*, *Ophiura albida* (Millport) and *Ophiura albida* (Loch Etive) with respect to relative fluctuation of wet weight in 100% sea water.



Each point represents a value for one animal.

sea water with salinities of 30.6°/oo, 23.8°/oo and 18.7°/oo respectively. These solutions were placed in 1 litre beakers which were floated in a bath of circulating sea water maintained at 12°C. A set of solutions were also made up using M400 sodium hydrogen carbonate which does not change the pH of the sea waters, unlike glass distilled water, see Appendix 4 . There was no discernible behavioural difference between animals placed in solutions diluted by glass distilled water or by M400 sodium hydrogen carbonate. Also, there were no obvious differences in weight fluctuation between individuals placed in 70% sea water diluted by the two different methods (see Table 1.3). . Consequently, dilution by glass distilled was not considered to have any injurious effects when compared with a buffering dilutant and so this, more rapid, method of preparing dilutions was adopted throughout this study.

1.6 To test the effect of volume size of test solution upon Ophiocomina nigra.

To ensure that the 1 litre beakers were of sufficient capacity to preclude a large accumulation of metabolites and to allow an adequate volume of test solution such that further aeration was not necessary during the period of the experiment a range of animals of various sizes were kept, one per beaker, for 24 hours under similar conditions to those of the subsequent experiment. The condition and activity of these animals were qualitatively compared, by observation, with control animals of similar size in a stock tank. No obvious differences were detected between both batches of animals and so it was considered that 1 litre beakers were of suitable volume such that there would be no intrinsic injurious effects upon experimental animals arising from test volume size.

1.7 Effect of diluted sea water upon the wet weights of two species of ophiuroid.

Table 1·3 Comparison between diluting media with respect to weight changes of Ophiocomina nigra in 70% sea water·

(TIME in hours)	M400 Na_2HCO_3	GLASS DISTILLED WATER
1	6·63 ± 1·12	6·76 ± 1·19
2	7·10 ± 1·16	8·05 ± 0·73
3	10·97 ± 2·30	11·66 ± 1·46
4	16·46 ± 2·34	17·04 ± 2·39
5	17·35 ± 1·81	17·42 ± 1·51
7	17·91 ± 1·38	18·36 ± 1·69

(a) Method:

Six specimens of Ophiocomina nigra were placed, one per beaker, for each dilution: 90%, 70% and 55% sea water. Each animal was weighed at the following time-interval:

15, 30, 45 and 60 mins. during the first hour.
2, 3, 4, 5 and 7 hours during the subsequent six hours.

At the end of this period the animals were replaced in full strength sea water and any weight fluctuation monitored for a variable period.

The above experiment was repeated using Ophiura albida from Millport and Loch Etive (station E24). The latter group of animals tended not to be as active as those from Millport and so they were placed on small trays which were sunken into a series of bowls, each of which contained a particular dilution of sea water. The dilutions were made in relation to the ambient salinity from which they were taken. Consequently, the 70% sea water which was used for O.nigra was $23.8^{o}/oo$ which was similar to the ambient salinity at E24. The 70% sea water dilution for O.albida, from E24, was $16.8^{o}/oo$.

(b) Results:

When placed in 90% sea water, O.nigra increases in weight rapidly by 9%, which subsequently fluctuates slightly. This level is maintained until the animal is replaced in 100% sea water when the original weight is regained (see Graph 1.3). In 70% sea water O.nigra increases in weight gradually until an increase of about 17% has occurred. This level is maintained until the animal is replaced in full strength sea water when a return to the original weight is observed (see Graph 1.4).

This pattern of a more or less rapid increase in weight, followed by a regular fluctuation about the mean of the initial increase, and then a return to the original weight in full strength sea water holds for O.albida from Millport and from Loch Etive (see Graphs 1.5 and 1.6). The amount of initial increase varies according to dilution and species, being greater in O.nigra than

O.albida at comparable dilutions (see Graphs 1.3 to 1.6).

(c) Discussion:

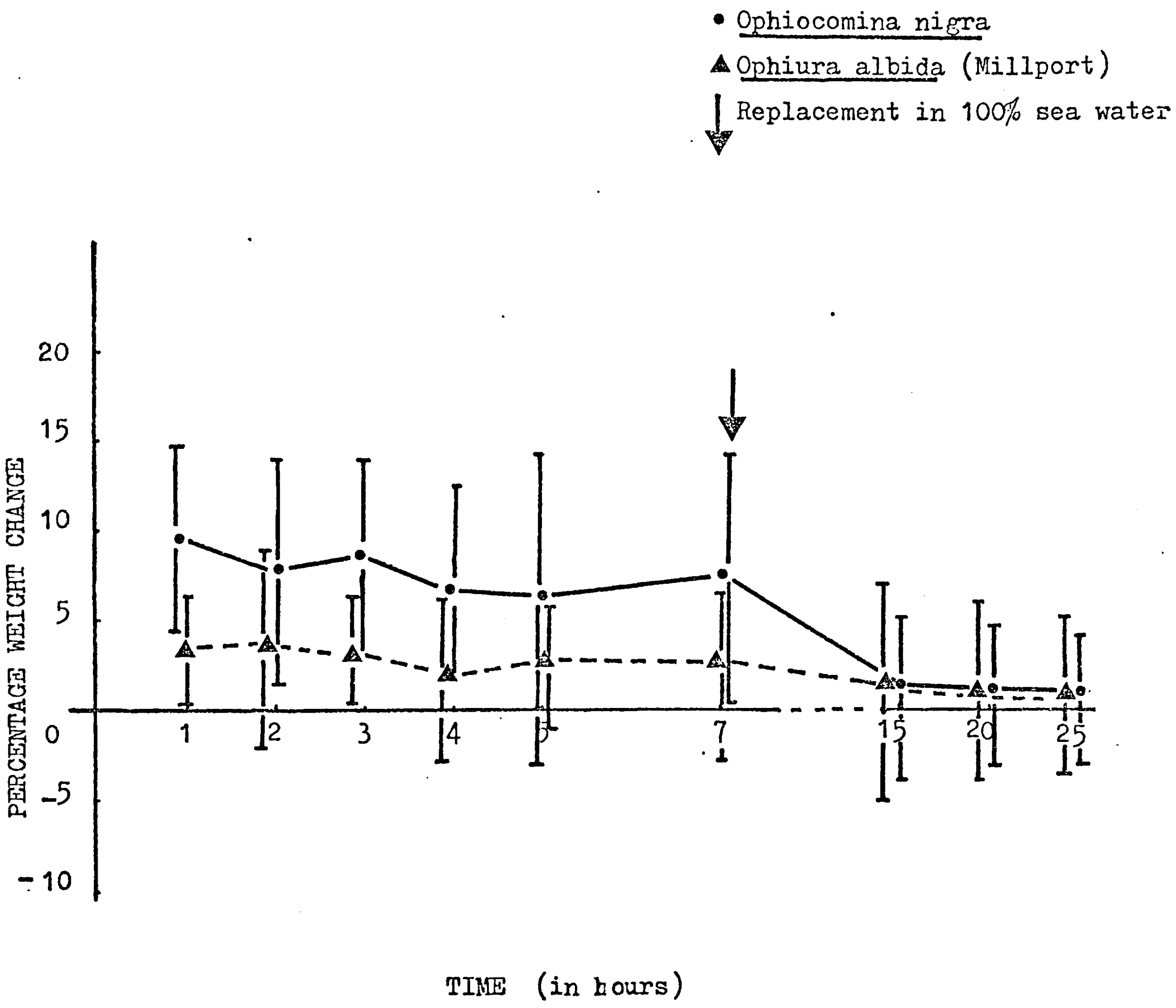
Ophiuroids appear to have a wet weight with a more or less regular fluctuation pattern. This periodicity is not correlated directly to any obvious environmental parameter, though it may be a feeding response or a method of coelomic fluid volume control.

Brittlestars appear to act as osmometers when placed in diluted seawater. They increase in weight in reduced salinity and this increase has not been found to be regulated. A return to the original weight only occurs when an animal is replaced in full strength sea water.

Ophiura albida does not increase in weight to the same degree as *Ophiocomina nigra* when in comparable dilutions of sea water. *O.albida* from the head of Loch Etive, E24, does not increase in weight as much as those from Millport, where normal salinities ($33/34^{o}/oo$) obtain. This may reflect the potential salinity regimes which these species may face. For instance, *O.nigra* is a typical stenohaline brittlestar and so would not normally face any reduction in the salt content of the ambient medium. Though *O.albida* from Millport is not considered to experience lowered salinities it may have the ability to tolerate some dilution of the surrounding sea water and must, therefore, be regarded as potentially euryhaline. This ability is manifested as smaller weight increases in comparison with *O.nigra* in comparable dilutions.

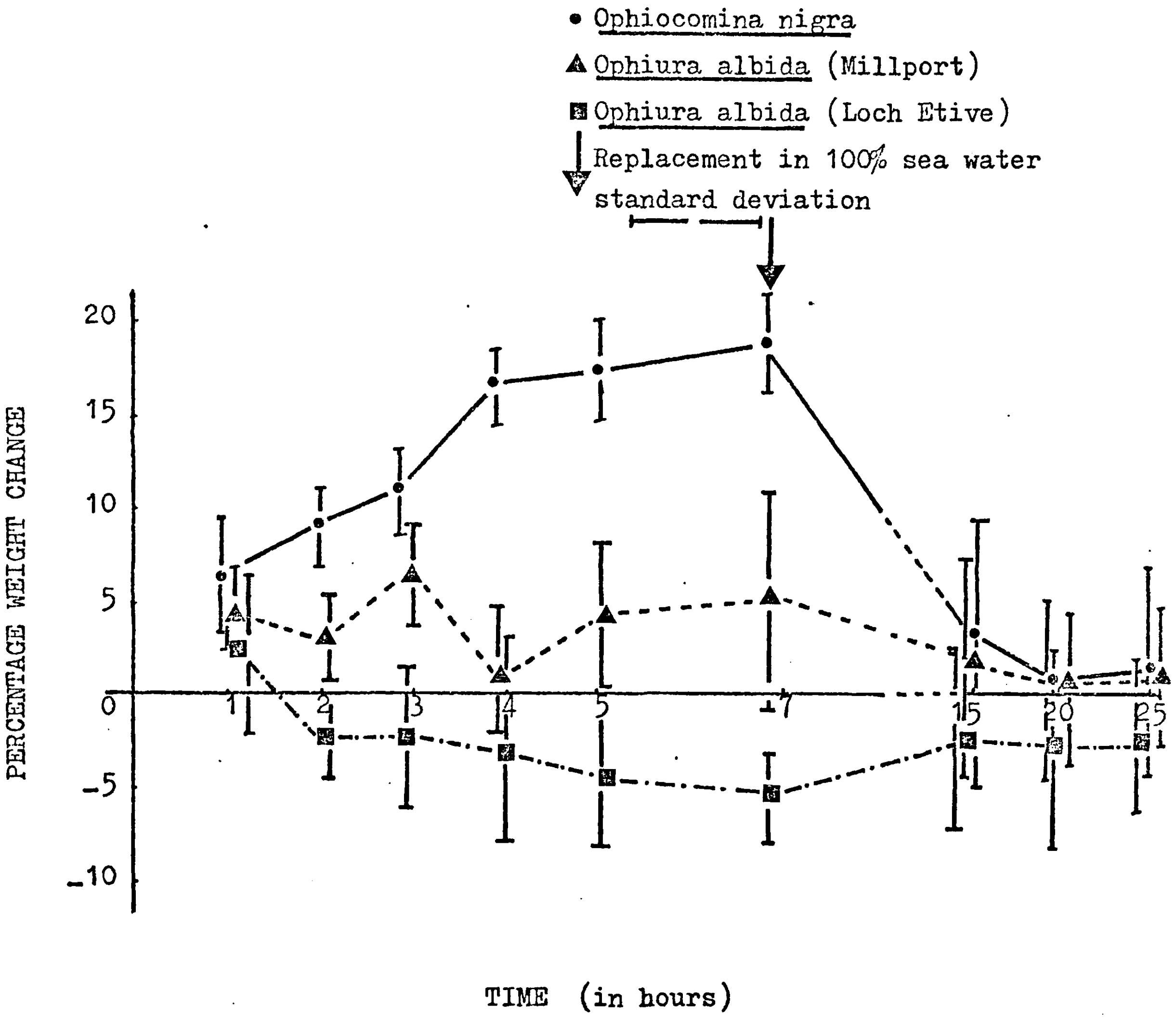
O.albida (Loch Etive) is from a brackish water environment and is thus continually faced with periodic dilution in its normal habitat. Consequently it is thought that these brittlestars from Loch Etive have developed the potential, which those from Millport appear to have, to tolerate extensive reduction in the salt content of their ambient medium. The ophiuroids from Loch Etive appear to be subject to less osmotic stress as evidenced by smaller weight increases than *O.albida* (Millport), and *O.nigra* in comparable dilution.

Graph 1.3 Percentage weight change in 90% sea water of Ophiocomina nigra and Ophiura albida (Millport) .



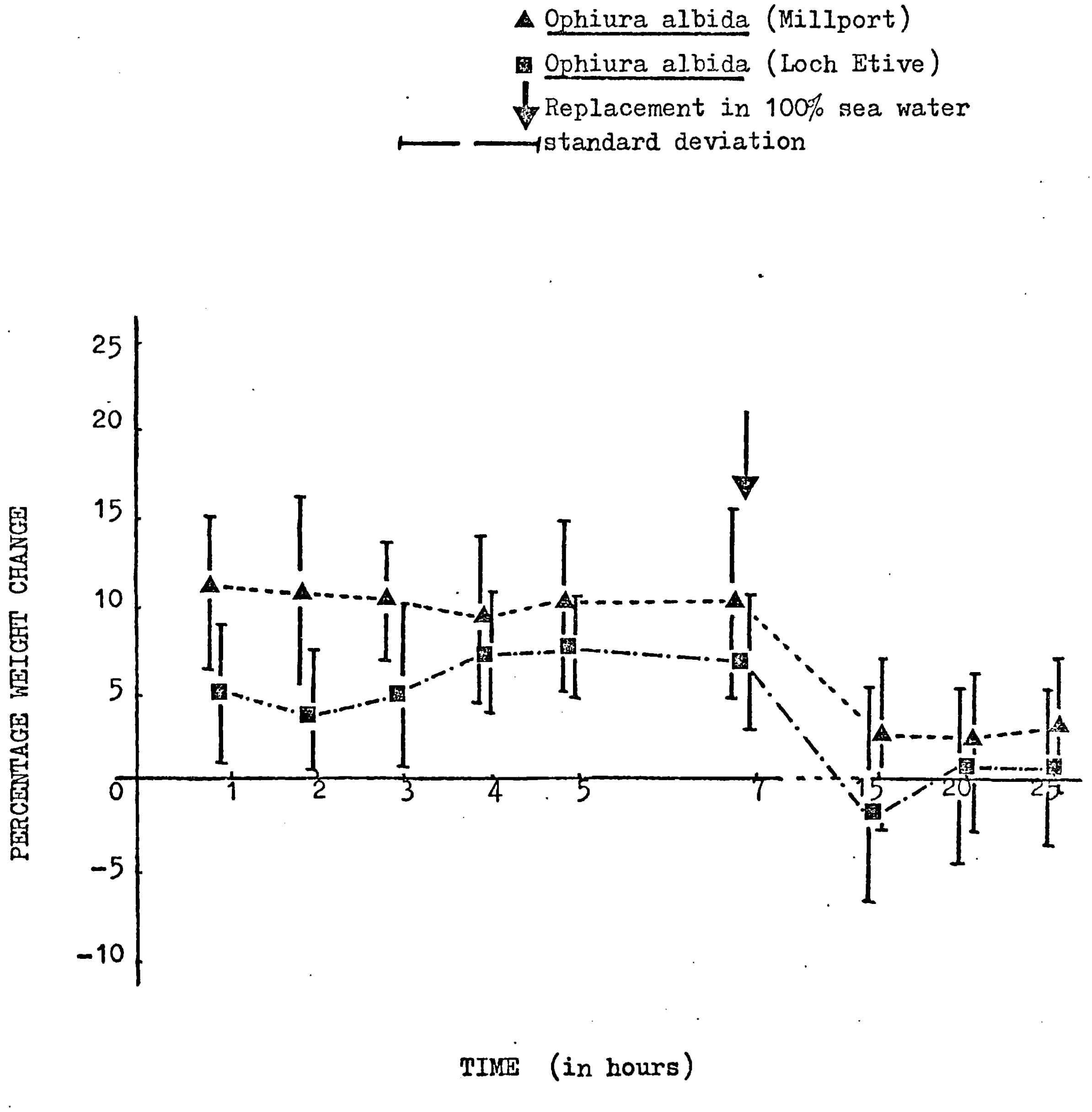
Each point represents the mean of six determinations ± 1 standard deviation

Graph 1.4 Percentage weight change in 70% sea water of <u>Ophiocomina nigra</u>, <u>Ophiura albida</u> (Millport) and <u>Ophiura albida</u> (Loch Etive) .



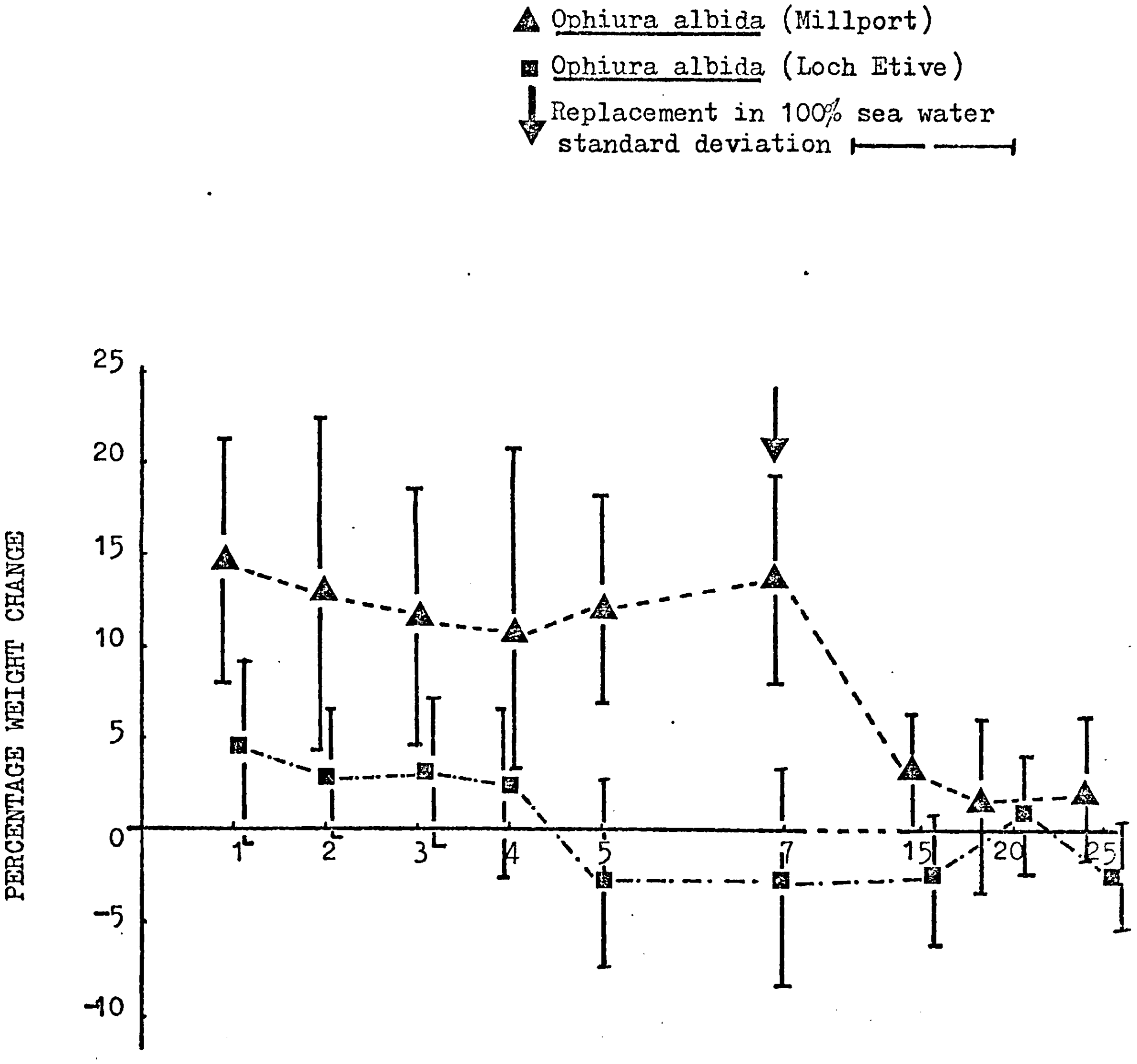
Each point represents the mean of six determinations ± 1 standard deviation

Graph 1.5 Percentage weight change in 60% sea water of _Ophiura albida_ (Millport) and _Ophiura albida_ (Loch Etive).



Each point represents the mean of six determinations ± 1 standard deviation

Graph 1.6 Percentage weight change in 50% sea water of Ophiura albida (Millport) and Ophiura albida (Loch Etive) .



Each point represents the mean of six determinations ± 1 standard deviation

CHAPTER TWO

INVESTIGATION OF THE SODIUM, POTASSIUM AND CHLORIDE CONCENTRATIONS IN THE BODY FLUIDS OF Ophiocomina nigra AND SUBSEQUENT ADJUSTMENT TO DILUTED SEA WATER.

2.1 Introduction:

Echinoderms are generally considered not to experience significant dilution of the surrounding medium in their usual marine habitats and so it is to be expected that, due to the absence of any obvious excretory organ, the ions in the body fluid would be similar in composition to those in the ambient sea water.

In general, this has been confirmed by previous workers for many species of echinoderms. Binyon (1966) lists the results of workers who have determined osmotic pressure and/or ionic concentrations in the perivisceral and/or ambulacral fluids of many echinoderms. He notes the following points of interest:-

Firstly, with reference to the perivisceral fluid:

1) In asteroids there is usually a small rise in the potassium concentration (9-16%) which is maintained in the more euryhaline species and in those acclimatised to reduced salinity. Calcium and magnesium concentrations may increase and decrease respectively.

2) In echinoids, calcium concentration increases significantly while there is little difference, (perhaps a small increase), in the potassium content.

3) In the holothurians there is little regulation of calcium or potassium ions.

Secondly, with reference to the ambulacral fluids:

The potassium concentration has been found to be 20% - 90% higher in the ambulacral fluids of three species of asteroid and an echinoid - Asterias rubens, Solaster papposus (Binyon, 1962, 1966), Marasterias glacialis (Robertson, 1949; Binyon, 1966), and Echinus esculentus (Robertson, 1949).

There have been fewer studies of changes in ionic composition in echinoderms in response to experimental dilution of the ambient medium (Robertson, 1939; Binyon, 1962; Stickle and Ahokas, 1974).

Binyon (1962) employing static dilution, demonstrated that in Asterias rubens the fluids of the perivisceral coelom and water vascular system are isionic with the medium down to at least 55% sea water. The regulation of potassium ions in the ambulacral fluids is maintained above the level of the experimental medium and falls in parallel with dilution (down to 55% sea water), temperature (0° - 20°C), sex of the animal or during the reproductive cycle. It was noted, however, that smaller animals have greater concentrations of potassium ions than larger animals.

Stickle and Ahokas (1974) using three species of echinoderm - Pisaster ochraceus, Cucumaria miniata and Strongylocentrotus droebachiensis - from estuarine conditions, subjected individuals to an experimental twelve hour tidal cycle. They demonstrated that the sodium and potassium concentrations in the perivisceral fluid of all the three species declined in conformation with the ambient salinity and subsequently returned to the original values in the asteroid and holothurian. It is suggested by Stickle and Ahokas (1974) that the shape of Strongylocentrotus droebachiensis does not facilitate a rapid ionic recovery because the perivisceral fluid compartment is located further away from the surrounding medium than in the other two species of echinoderm. Binyon (1961) has shown, in Asterias rubens, that water passes very quickly from the perivisceral coelom to the environment via the water vascular system.

One overiding feature arising from all previous work is the lack of any data concerning sodium, potassium and chloride concentrations in, possibly, the most euryhaline of the echinoderms - the Ophiuroidea.

2.2 To establish the reliability of the method for the determination of sodium and potassium ions.

The sea water samples and the samples of ophiuroid body fluids were analysed for sodium and potassium ions using an 'EEL' Flame photometer (see Appendix 1 for operational details) and a range of standard solutions containing known concentrations of sodium and potassium ions.

In order that the galvanometer deflection of the flame photometer would lie within the above ranges the sea water and body fluids were diluted 100 times for the potassium assay and a further ten times for the sodium determinations with glass distilled water. The use of such dilute solutions and extensive flushing of the atomiser of the photometer prevented the instrument becoming blocked with deposition of encrusting cations. The standard solutions were used to construct calibration curves the interpolation of which allows the determination of unknown concentrations. These curves were then checked periodically but insignificant drift was recorded.

(a) Method:

Ten, duplicated, separate determinations were carried out on samples taken from the same sea water stock using 50 μl pipettes.

Errors could be incurred in the diluting procedure at the following stages:

i) Removal of 50 μl sample with 50 μl pipette.
ii) Dilution of this sample with the use of small (1ml and 5ml) volumetric flasks.
iii) Subsequent subdiluting of the above for the determination of sodium ions.

(b) Results:

The results are presented in Table 2.1. The small sample sizes (50 μl) are responsible for most of the variation which is comparable with the standard deviations of 1 - 10% in Binyon (1961). The increased variation in the sodium assay probably reflects the additional dilution-step which is necessary to bring the sodium concentrations down over the range, 0ppm - 20ppm, where

Table 2·1 Reproducibility of technique using a 50 μl sample size.

CONCENTRATION (mM/l)	
SODIUM	POTASSIUM
611	16·8
622	14·3
676	14·8
712	14·0
611	15·6
709	15·3
742	16·1
676	14·3
655	15·0
698	14·3
$\bar{x}$ = 671	$\bar{x}$ = 15·0
± 45·7	± 0·90

the instrument is most sensitive.

2.3 Determination of sodium and potassium concentrations in the body fluids of Ophiocomina nigra in full strength sea water.

(a) Method:

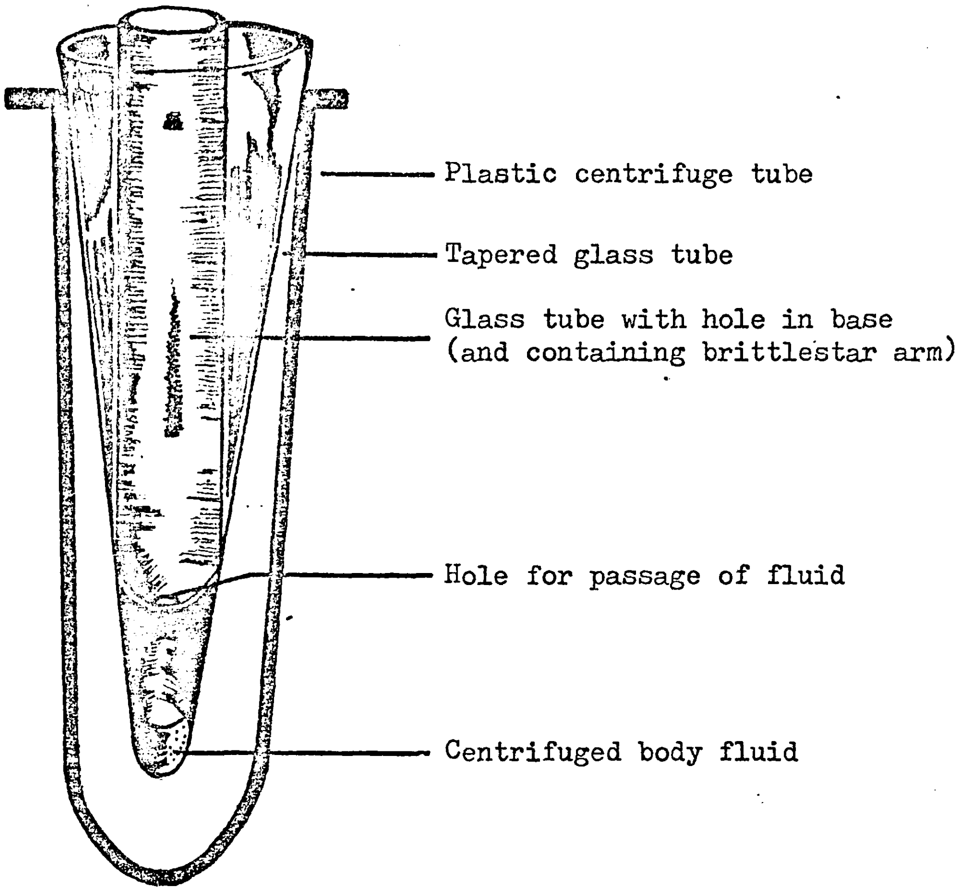
The body fluids were obtained by the following procedure. The brittlestar was superficially dried, as previously described (page 45), to remove, as far as possible, any sea water from the surface of the integument. This would minimise the possible contamination of the extracted fluids by the ions present in the sea water. The arm was then severed, with a sharp scalpel, from the disc. The first few distal segments were removed from the arm thus making the arm, in effect, an open-ended tube. The prepared arm was then inserted, proximal end first, into a glass tube which had a small hole (1mm in diameter) at the bottom. This tube was then placed into a slightly larger, tapering, tube sealed at the tapered end. This arrangement was then put into a plastic centrifuge cone and is illustrated in Figure B.

The first tube supported the arm during centrifuging so as to preclude extensive multiple fracturing of the sample. Also, since this tube rested on the taper of the ensheathing tube it prevented the extracted fluid, which passes through the small hole, from lying for any length of time in contact with any distrupted muscle fibres or cells. The plastic centrifuge tube ensured that the glass tube, containing the fluid, was protected during centrifuging.

High speed centrifuging using an electrically operated model had been found to be responsible for the disruption of the arm integrity (Binyon, personal communication). Such disruption would result in the release of muscle cells and fibres with their, presumed, high concentrations of potassium ions.

A hand propelled centrifuge (length of swing = 5.7 cm) was used in the present study. Various speeds were assessed in terms of the damage sustained by the arm musclature during centrifuging. Histological examination, using Mallory's Triple stain,

Figure B Diagram of brittlestar preparation prior to centrifuging.



of transverse and longitudinal sections of arms, before and after centrifuging, was carried out in order to assess any damage due to the action of centrifuging. It was found that by using 192 revs. per minute sufficient fluid could be extracted after two minutes. This speed, (192 revs per min.), was found not to disrupt the muscle blocks of the arm (see Photographs page 70). A photograph of a transverse section of an arm centrifuged at 480 r.p.m. is shown for comparison.

Another important consideration was that the glass components in use were not cracked or chipped. High, erroneous sodium ion concentrations were obtained occasionally and this was thought to be due to leakage of such ions from damaged glassware. The glassware in use was of the soda glass type and it was found that if a few millilitres of sea water were kept in a test tube containing a few fragments of soda glass the sodium ion concentration, analysed by the above method, of the sea water was greatly enhanced.

Approximately 60 - 70 μl. could generally be obtained from the arms of a medium-sized (3.5 - 4.0 grams) animal. If it was necessary, depending upon what was obtainable, to use smaller animals then two animals would be used as one sample.

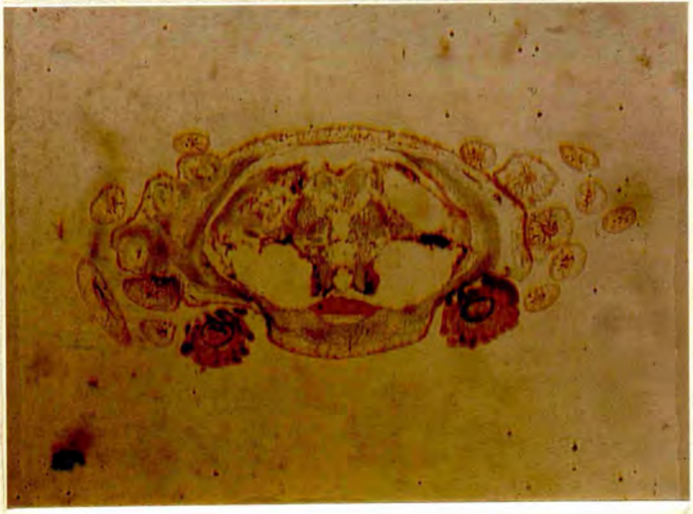
Examination of transverse sections of the arms of Ophiocomina nigra, stained stained with Mallory's Triple stain, showed that the diameter of the water vascular canal was considerably smaller than that of the perivisceral coelom (see Appendix 2). From this observation one would expect there to be a greater volume of perivisceral fluid than ambulacral fluid if the coeloms were of similar length. It seems likely that the 'body fluid' obtained by the method described above would mostly be of perivisceral coelom origin. There would, presumably, be some admixture of fluid derived from the water vascular system in the arm.

The concentration of sodium and potassium ions in the body fluids of Ophiocomina nigra, from full strength sea water, was determined by applying the above preparatory and diluting procedures. Six animals were used.

Photograph Transverse sections of the arm of Ophiocomina nigra.

Before centrifuging

After centrifuging at 192 r.p.m.



After centrifuging at 480 r.p.m.

Table 2·2 Concentration of sodium and potassium ions in sea water and the ophiuroid, *Ophiocomina nigra*.

CONCENTRATION (mM/l)			
SEA WATER		BODY FLUIDS OF *Ophiocomina nigra*	
SODIUM	POTASSIUM	SODIUM	POTASSIUM
464	10·6	435	14·4
467	10·3	402	14·6
445	10·7	391	14·6
459	10·5	391	15·1
447	10·5	391	13·0
460	10·5	413	13·8
$\bar{x}$ = 457	$\bar{x}$ = 10·5	$\bar{x}$ = 404	$\bar{x}$ = 14·2
± 9·01	± 0·13	± 17·6	± 0·74

(b) Results:

The results are given in Table 2.2. The sodium concentration of the body fluids is 404mM±17.6mM which is 11.5% lower than in the ambient sea water, (457mM±9.0mM). The concentration of potassium ions is 14.2mM±0.74 which is some 35% higher in the body fluids than in the surrounding sea water where it is at a concentration of 10.5mM±0.13.

(c) Discussion:

With the exception of the echinoid, Echinus esculentus (Bethe and Berger, 1931) and the holothurian, Cucumaria frondosa (Parker and Cole, 1940) the fluids of the water vascular system of echinoderms, analysed to date, have concentrations of sodium ions lower than in the ambient sea water. The perivisceral fluids of echinoderms are generally similar or slightly lower in sodium concentration than sea water; the two exceptions recorded so far are the asteroid, Solaster papposus (Binyon, 1966) and Echinus esculentus (Bethe and Berger, 1931).

In Ophiocomina nigra, the sodium concentration of the body fluid is lower than the ambient value and is thus considered to fall into the general level found in other echinoderms. However, for reasons discussed below, one cannot exactly equate, ionically, the result for the sodium concentration of the ophiuroid body fluids with data derived either from the water vascular system or the perivisceral coelom of other classes of echinoderm. Since it is generally true to say that both ambulacral and perivisceral fluids of echinoderms which have been analysed to date have sodium concentrations recorded in the literature which are lower or similar to the ambient medium then one can regard O.nigra as not being an exception to this trend.

The perivisceral fluids of echinoderms vary in the amount of potassium ions present; asteroids have 9% - 16% more than in the ambient sea water, while in echinoids and holothurians there is little increase. The potassium concentration in the water vascular fluids may vary between 20% - 90% higher than in the surrounding sea water (Robertson, 1949; Binyon, 1966). The potassium concentrations in the body fluids of Ophiocomina nigra were found to be 35% (14.2mM) higher than in the medium where it is 10.5mM. They thus resemble, ionically, the concentrations found in the ambulacral fluids of asteroids and echinoids rather than the perivisceral fluids of these echinoderms.

The high potassium concentration in the body fluids of the ophiuroid, Ophiocomina nigra, probably reflects the contribution of perivisceral fluid to these fluids. The volume of ambulacral fluids available for extraction is estimated at approximately 4.7μls (see Appendix 2). This figure assumes that the extraction method for this particular fluid is 100% efficient which is unlikely. The concentration of such fluids would have to be of the order of 66mM so as to produce a concentration of 14.5mM which has been shown to occur in the above determinations of body fluids. A concentration of 66mM for the potassium ion in the water vascular fluids seems unlikely since this represents a six fold increase over the value of potassium in the sea water.

The alternative possibility available, which is proposed by the present author, is that the high concentration of potassium is real and is due to the high concentration of this ion in the perivisceral fluids. This concentration would, presumably, be even higher if admixture with ambulacral fluids did not occur.

The enhanced potassium concentration of echinoid and asteroid ambulacral fluids have been explained in terms of the use of the water vascular system for locomotion via podial movements. In O.nigra the arms are the main locomotory organs and a high potassium concentration in the perivisceral fluids, which bathe the arm musculature, would, if the connection between locomotion and potassium concentration, as described for asteroids, is correct, be of similar importance in ophiuroids. In summary, the perivisceral coelomic fluid of O.nigra can be likened ionically and functionally, (in terms of its association with the locomotory muscles), to the ambulacral fluids of asteroids and echinoids rather than to the perivisceral fluid of these echinoderms.

2.4 The effect of diluted sea water, (90% and 70%), upon the sodium and potassium balance in the body fluids of Ophiocomina nigra.

(a) Method:

Twenty four specimens of the ophiuroid, Ophiocomina nigra were placed in 1 litre beakers, (two animals per beaker), containing 90% sea water. The temperature of the experiment was maintained at 12°C. Six animals were removed after:

3, 5, 7 and 10 hours

The concentrations of sodium and potassium in the body fluids were determined by the procedure described previously. This experiment was repeated with 70% sea water.

(b) Results:

The results of the sodium and potassium concentrations in the body fluids of O.nigra after immersion in these dilutions is given in Table 2.3.

When O.nigra is placed in 90% sea water, (Na^+:404mM), the sodium content in the body fluids drops by about 36% of its original value to 260mM ±67mM. The sodium concentration gradually increases during the next six hours reaching a level a little below the sea water value, (Graph 2.1). The potassium level in the perivisceral fluid in 90% sea water (K^+:9.2mM), falls, initially, by 32% in the first three hours to a value, (8.7±1.4mM), which is slightly below that of 90% sea water (9.2mM). The level of potassium then increases to a value, (14.0±0.9mM), which is similar to that when the animal was in 100% sea water, (see Graph 2.2).

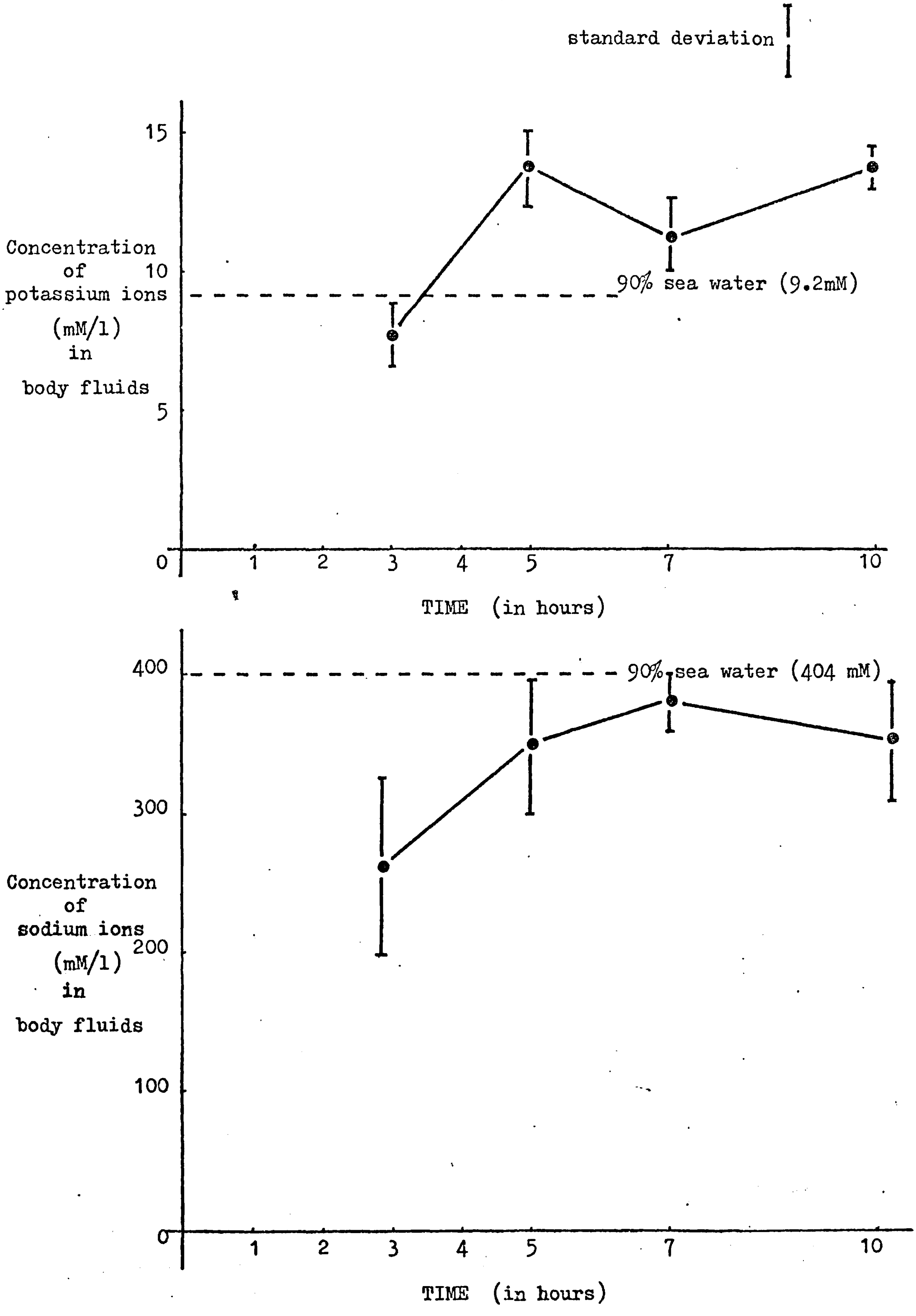
In 70% sea water, where the concentration of sodium ions is 315mM, the body fluids have a concentration of this ion which has fallen to 200mM±16mM; a fall of 50%. This is some 30% lower than in the ambient 70% sea water. During the following few hours the sodium concentration increases to a value only slightly below the 70% sea water value (see Graph 2.2).

The potassium concentration of the body fluids falls to 10.8±0.8mM which is nearly 50% higher than the potassium concentration in 70% sea water, (where it is 7.2mM). This level is maintained during the rest of the experimental period, (see Graph 2.2).

Table 2·3 The effect of diluted sea water upon the sodium and potassium concentrations in the body fluids of the ophiuroid, *Ophiocomina nigra* .

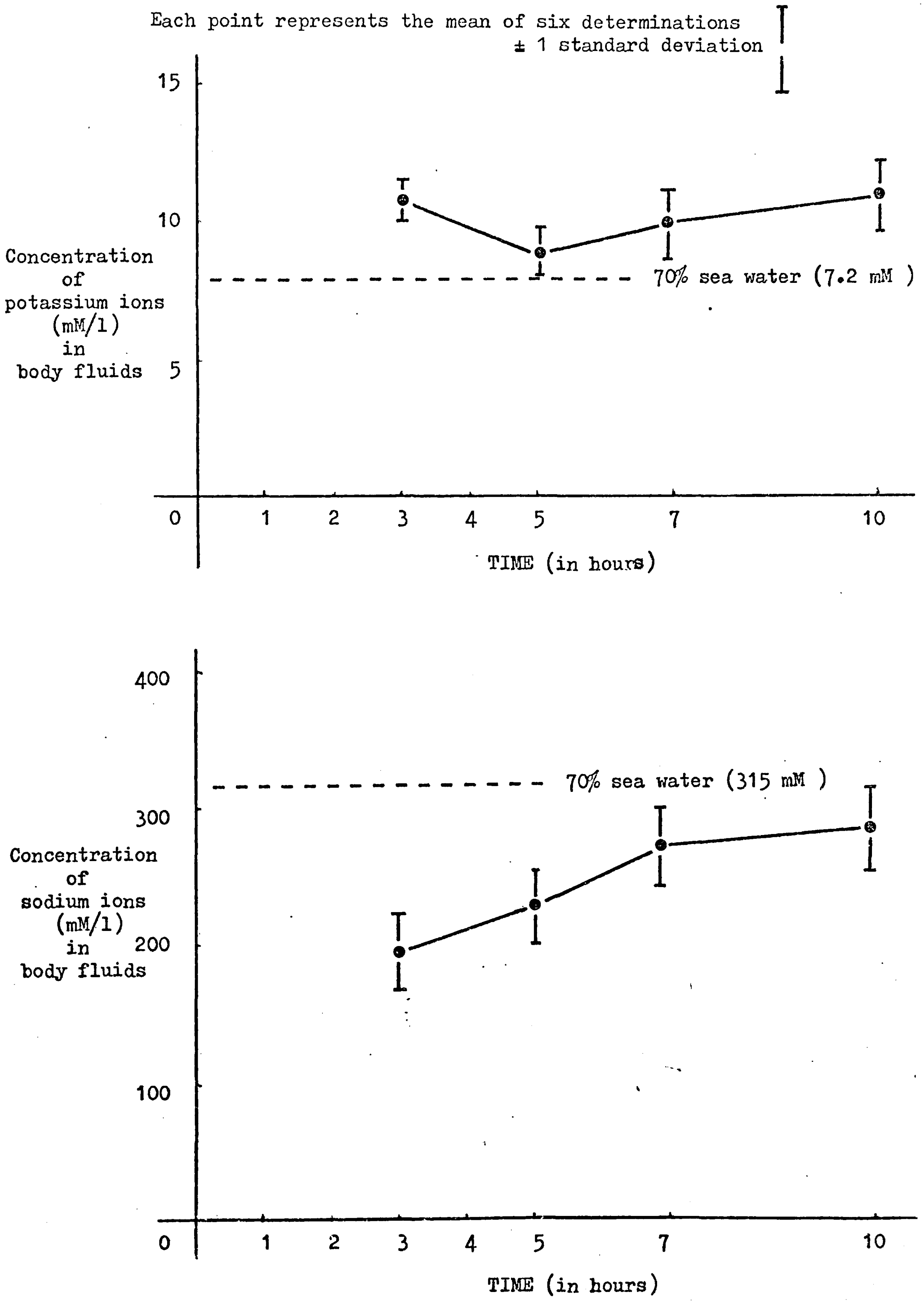
TIME in hours	CONCENTRATION IN BODY FLUIDS OF: *Ophiocomina nigra* (mM/l)			
	90% Sea water		70% Sea water	
	Sodium	Potassium	Sodium	Potassium
3	260 ± 67	8·7 ± 1·4	200 ± 26	10·8 ± 0·8
5	349 ± 48	14·3 ± 1·5	236 ± 25	8·6 ± 1·3
7	372 ± 29	11·2 ± 1·6	262 ± 32	9·4 ± 1·4
10	352 ± 40	14·0 ± 0·9	275 ± 39	10·3 ± 1·3
	CONCENTRATION IN AMBIENT MEDIUM (mM/l)			
	90% Sea water		70% Sea water	
	Sodium	Potassium	Sodium	Potassium
	404 ± 7·9	9·2 ± 0·3	315 ± 4·6	7·2 ± 0·3

Graph 2.1 Distribution of potassium and sodium ions in the body fluids of Ophiocomina nigra in 90% sea water.



Each point represents the mean of six determinations ± 1 standard deviation

Graph 2.2 Distribution of potassium and sodium ions in the body fluids of *Ophiocomina nigra* in 70% sea water.



Each point represents the mean of six determinations ± 1 standard deviation

(c) Discussion:

Binyon (1962) demonstrated that in diluted sea water (down to 55%) the concentration of potassium ions in the ambulacral fluids of Asterias rubens is maintained above the level of that in the experimental medium. This level falls in conformation with the dilution in the medium. He notes that the fluids of the perivisceral coelom are isionic with the medium down to at least 55% sea water.

Stickle and Ahokas (1974) showed, using the asteroid, Pisaster ochraceus, the holothurian, Cucumaria miniata and the echinoid, Strongylocentrotus droebachiensis, that the sodium and potassium ion concentrations in the perivisceral fluid fell in parallel with decreases of these ions in the ambient medium.

Results for Ophiocomina nigra are broadly similar to those for other echinoderms. The potassium concentration tends to be maintained in the body fluid despite dilution of the ambient medium down to at least 70% sea water. Sodium concentrations, however, are maintained at levels a little lower than the particular ambient level.

These results emphasise the suspected important physiological rôle played by the potassium ion in the body fluid.

2.5 Determination of the chloride concentration in sea water.

(a) Method:

Since only small quantities, (10µl-60 µl), of body fluids were available (obtained by methods described previously), a method based on Conway Microdiffusion technique (Conway, 1957) which employs the principle of isopieistic distillation was used. The chloride concentration of sea water, ($34^{o}/oo$), was first determined.

A standard microdiffusion unit (see Figure C) was used. Into the central chamber of the unit was run about 1ml. of a 20% potassium iodide solution. About 0.2 grams of pure potassium permanganate was placed in the outer chamber. Then, 1ml of sea water was run into the outer chamber. A square glass lid with a fixative (a mixture of paraffin wax and pure paraffin) smeared thinly on the contact surface was placed on the unit. 1ml of sulphuric acid solution, containing 24 - 30% by volume of pure sulphuric acid, (specific gravity: 1.84), was then introduced rapidly with a pipette. The lid was then returned to its completely sealed position. The contents were gently swirled to assist the mixing process and then left for one and a half hours at room temperature. Five similar units were also set up. As a check upon the free iodine content of the reagents used, a blank, omitting a sample of sea water, was run.

One and a half hours is sufficient for all the chloride in the sample to be oxidised to free chlorine. This liberated chlorine is subsequently absorbed by the potassium iodide. This absorption liberates an equivalent amount of iodine, see equations:

1) Sample (containing chloride) + H_2SO_4 + $KMnO_4$

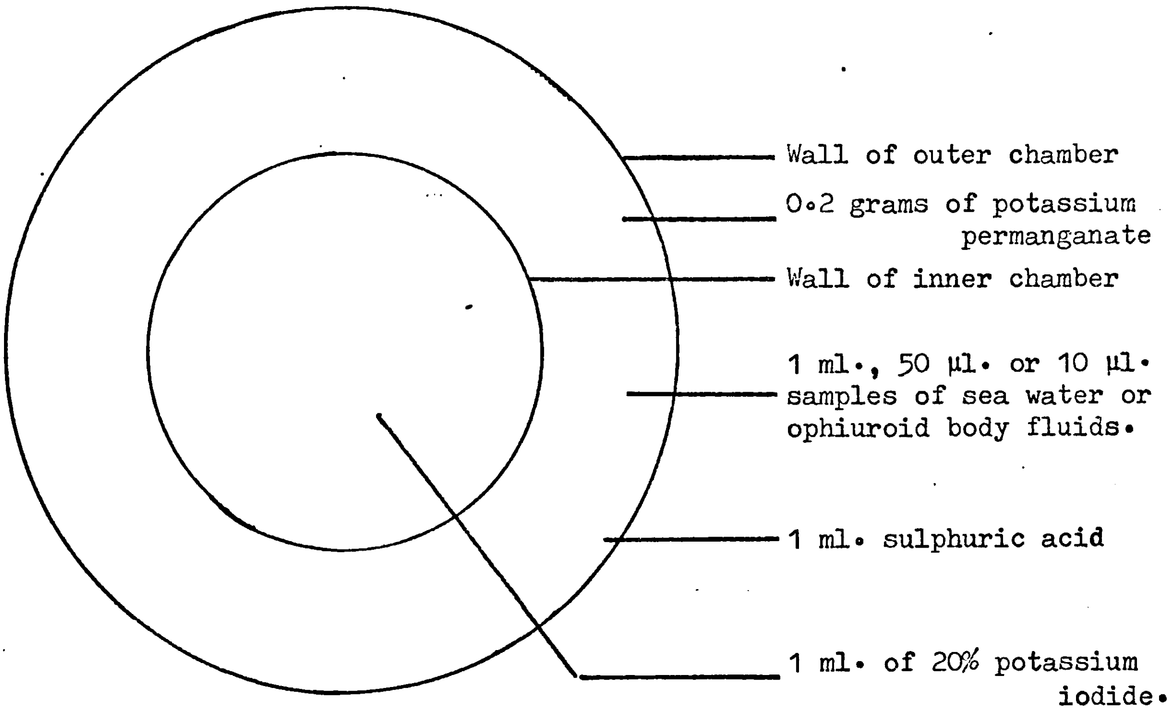
$\downarrow$

2) Sample + K_2SO_4 + $Cl_2\uparrow$ + $MnSO_4$ + H_2O

$$2KI + Cl_2 \longrightarrow 2KCl + I_2\uparrow$$

Titration of the liberated iodine with 0.05N sodium thiosulphate, using a Bang 2ml micropipette and using a starch indicator, allowed the quantitative determination of the chloride ion.

Figure C A microdiffusion unit for determining chloride ion concentrations.



PLAN VIEW

(b) Treatment of data:

i) The 'blank' reading was 0.09mls. This was the volume of sodium thiosulphate which was required to react with the free iodine present in the reagents using starch as an indicator. This value was subtracted from the volume of sodium thiosulphate which was required to react with the 'sea water units' thus giving a corrected volume. This corrected volume was then multiplied by the sodium thiosulphate equivalent for chloride.

ii) The chloride equivalent was determined by five separate titrations with 1ml aliquots of 0.0141N Hydrochloric acid which contains 0.5 mg of chloride. It was found that 0.57 mls of sodium thiosulphate was equivalent to 1 mg of chloride.

To ensure that the small quantities of the body fluids which were available were sufficient, chloride determinations based on 10 µl and 50 µl samples were carried out. The results for these small quantities were multiplied up to 1ml and compared with values obtained by using 1ml aliquots of sea water. No significant difference was found (see Table 2.4).

(c) Results:

The results for the determination of the chloride concentrations in 100% sea water are given in Table 2.5. Subsequent analyses of the chloride content of a range of diluted sea waters: 90% - 60% were also made. These results are also given in Table 2.6.

The value obtained, quoted in mM/litre, is a little lower than most published figures. Published figures for 100% sea water are, however, quite variable; from 492mM (Parker and Cole, 1940; and Cole, 1940) to 633mM (Robertson 1953). It is considered in view of the amount of variation in the literature that the current figures

Table 2.4 Comparison of concentrations of chloride ions in sea water as determined using 10 μl., 50 μl. and 1 ml. sample sizes.

10 μl.	50 μl.	1 ml.
449	451	448
453	448	447
447	448	449
454	445	451
445	448	448
444	451	446
$\bar{x}$ = 448 ± 4.1	$\bar{x}$ = 448 ± 2.2	$\bar{x}$ = 448 ± 1.7

Table 2•5 Concentration of chloride ions in varying dilutions of sea water ∘

% SEA WATER	CONCENTRATION OF CHLORIDE (mM/l)
100	448 ± 2•2
90	404 ± 5•9
80	384 ± 7•0
70	319 ± 8•0
60	267 ± 1•9

Each value represents the mean of six determinations ± 1 standard deviation are valid. Differences in chloride concentration in the literature may be due to variation in the salinity of the sea waters which have been analysed, or as a result of differences in technique accuracy.

2.6 Determination of the chloride content of the body fluids of Ophiocomina nigra from full strength and diluted sea water.

(a) Method:

1 litre beakers containing 100%, 90%, 80%, 70% and 60% sea water solutions were set up in a stock bath containing circulating sea water at 12°C. Six animals were placed in each dilution (two per beaker). After immersion in these solutions for ten hours the animals were removed and the body fluids extracted as described previously. Samples of the sea waters were also taken for analysis.

(b) Results:

The results of the analyses for the sea water chloride concentrations are given in Table 2.5, and for the body fluids in Table 2.6. Reference to Graph 2.3 indicates that the chloride concentration in the body fluids of Ophiocomina nigra is maintained at a value slightly below the value of the comparable sea water value.

(c) Discussion:

The concentration of the chloride ion in the body fluids of O.nigra is 434 ±18.4mM, which is some 3% lower than the corresponding value for 100% sea water (34.5°/oo) of 448mM. It has been stated previously (page 73) that the extracted body fluids of O.nigra are mostly from the perivisceral coelom but have, in terms of cation composition and probable function, greater analogy with the ambulacral fluids of echinoids and asteroids. This can now be further extended to consider a major anion of this fluid - the chloride ion.

The chloride concentrations of the perivisceral and ambulacral fluids have been determined in a range of asteroids, echinoids and holothurians by a number of workers (see review by

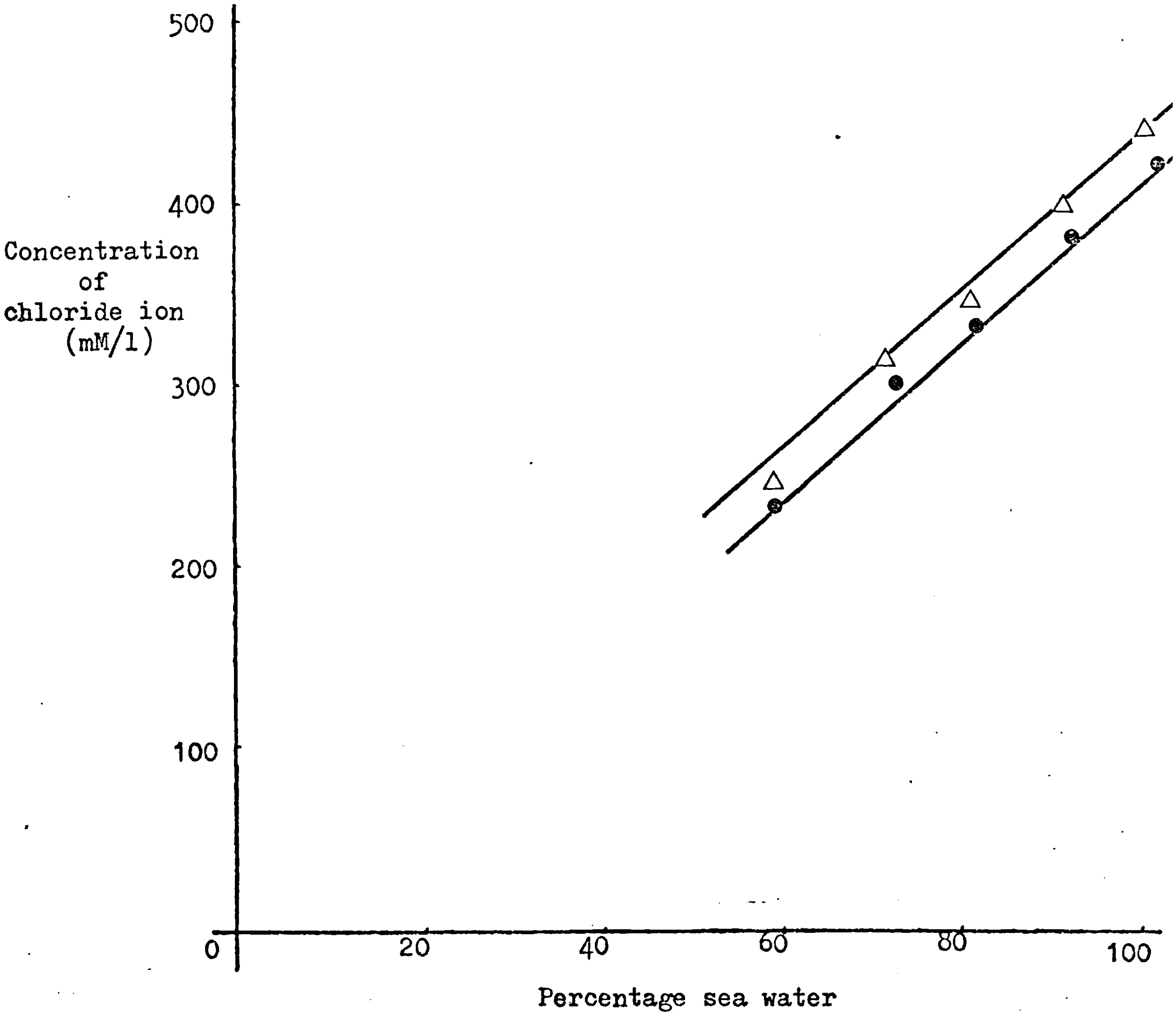
Table 2.6 Concentration of chloride ions in the body fluids of *Ophiocomina nigra* from various dilutions of sea water.

CONCENTRATION OF CHLORIDE (mM/l)	
SEA WATER	BODY FLUIDS
448	431 ± 18.4
403	394 ± 8.8
369	349 ± 6.3
318	311 ± 16.4
265	262 ± 2.2

(Each value represents the mean of six determinations ± 1 standard deviation)

Graph 2.3 Relationship between chloride concentration in the body fluids of <u>Ophiocomina nigra</u> and the ambient sea water .

△ R = 0.9970, p = 0.001 chloride concentration in sea water
● R = 0.9993, p = 0.001 chloride concentration in body fluids



Each point represents the mean of six determinations ± 1 standard deviation

Binyon, 1966).

The chloride content in the perivisceral fluids of asteroids may vary substantially. It has been recorded between 487mM (Asterias rubens) by Binyon (1962), and 659mM (Astropecten sp) by Bialaszewicz (1933). The chloride concentrations in the ambient sea waters were 494mM and 627mM respectively. Generally speaking, the chloride concentrations in the perivisceral fluids of asteroids are similar to or a little higher than the respective concentrations in the surrounding sea water (Myers, 1920; Bethe and Berger, 1931; Bialaszewicz, 1933; Parker and Cole, 1940; Cole, 1940; Binyon, 1962). In echinoids, the fluids generally appear to have chloride contents very similar to the ambient medium (Bialaszewicz, 1933; Robertson, 1949). An apparent exception to this is Echinus esculentus which has been reported by Bethe and Berger (1931) as having a concentration some 4% higher than the environmental concentration. Such concentrations in the few holothurians which have been sampled appear to have values a little higher than in the surrounding medium (Bethe and Berger, 1931; Bialaszewicz, 1933; Koizumi, 1935; Cole, 1940; Parker and Cole, 1940; Robertson, 1953).

There is less data for the ambulacral fluids. Binyon, (1962) reports a value (481mM) a little lower than the medium, (494mM) for the asteroid, Asterias rubens. Bethe and Berger (1931) give a figure (552mM for the chloride concentration in Echinus esculentus. The ambient sea water was 525mM. Parker and Cole (1940) and Cole (1940) report little difference between environmental and body fluid concentrations of chloride in the holothurian, Cucumaria frondosa.

It is clear that there is a large variation in the absolute values determined for various echinoderms not only between different workers but also between the same species, which probably reflects two considerations. Firstly, the different salinities of the various sea waters may have different chloride concentrations. Secondly, early workers probably used techniques for determining this ion that are less precise than those available to later workers.

Ophiocomina nigra, as the sole representative of the Ophiuroidea analysed to date in terms of its body fluid chloride concentration, appears to fall within the levels apparent in the other echinoderms so far investigated.

Generally speaking, with reference to the literature, the perivisceral fluids tend to have chloride concentrations similar to (echinoids and some asteroids) or a little higher than (holothurians and some asteroids) the concentrations in the surrounding medium.

Although there is less data for the ambulacral fluids, past work suggests that chloride concentrations are similar to or a little lower (asteroids and holothurians) or slightly higher (echinoids) than ambient concentrations.

The chloride concentrations which have been determined in Ophiocomina nigra and which are a little lower than those in the ambient medium have some similarity with the ambulacral fluids of asteroids and holothurians. It is considered that the above results fit into the concept that although these ophiuroid body fluids are almost certainly of perivisceral origin they have more analogy with the ambulacral fluids of other echinoderms with respect to ionic composition and at least some functions.

The chloride concentration in the body fluids of O.nigra remains slightly below the value of any given value of sea water down to at least 60% sea water. Asterias rubens, from the North Sea, when acclimated to Kiel salinities, has a chloride concentration in the perivisceral and ambulacral fluids which is a little lower (2.5%) than the concentration in the medium (Binyon, 1962). Within the range of sea water dilutions - 100% to 60% - O.nigra fluids contain chloride concentrations which vary no more than 5.4% from the corresponding sea water value (see graph 2.3).

CHAPTER THREE

INVESTIGATION INTO THE SALINITY TOLERANCE OF SPECIES OF OPHIUROID Ophiocomina nigra, Ophiura albida and Amphiura chiajei

3.1 Introduction:

Determination of tolerance to lowered salinity by echinoderms, especially of those penetrating water of reduced salinity, has been carried out on only a few occasions (Loosanoff, 1945; Binyon, 1961; Thomas, 1961; Stancyk and Shaffer, 1977;). Loosanoff (1945) using the asteroid, Asterias forbesi, determined the ability of this species to tolerate low salinities for short and long periods. However, he gave no indication in absolute terms of how the death-point was determined.

Binyon (1961) applied a 14 day LD50 to Asterias rubens and concluded that the lowest salinity which can be tolerated was approximately 22°/oo - 24°/oo. This value, as was also found with Asterias forbesi (Loosanoff, 1945), could not be extended by the more gradual lowering of salinity by 1°/oo per day.

Thomas (1961) reports the amphiurid brittlestar, Ophiophragmus filograneus, as living in 7.7°/oo in Whitewater Bay, Florida though he gives no additional experimental evidence as to absolute tolerance. More recently, Stancyk and Shaffer (1977) conducted salinity tolerance experiments upon Ophiothrix angulata from estuaries in Carolina and Florida. There is a greater salinity stress in the estuary in Florida and it was concluded that this is reflected in the greater tolerance to lowered salinity of Ophiothrix angulata from this estuary in comparison with those from the estuary in Carolina.

3.2 Sampling Methods:

(i) Ophiuroids

Two methods were used to obtain specimens for these experiments; trawling and SCUBA diving. Trawling has the advantage that large numbers can be obtained within a short time and it is a convenient way of sampling the deeper areas of the Loch. SCUBA diving permits in situ observations and sampling in shallow water (where a vessel may find it difficult or impossible to navigate). The R.V. Calanus of the Oban Laboratory of the Scottish Marine Biological Association, was used for trawling at the following stations within Lochs Etive and Creran (see Map page 91).

Loch Etive: E2, E4, E6, E11, E14, E24
Loch Creran: C12

Station E24 was further sampled by SCUBA diving.

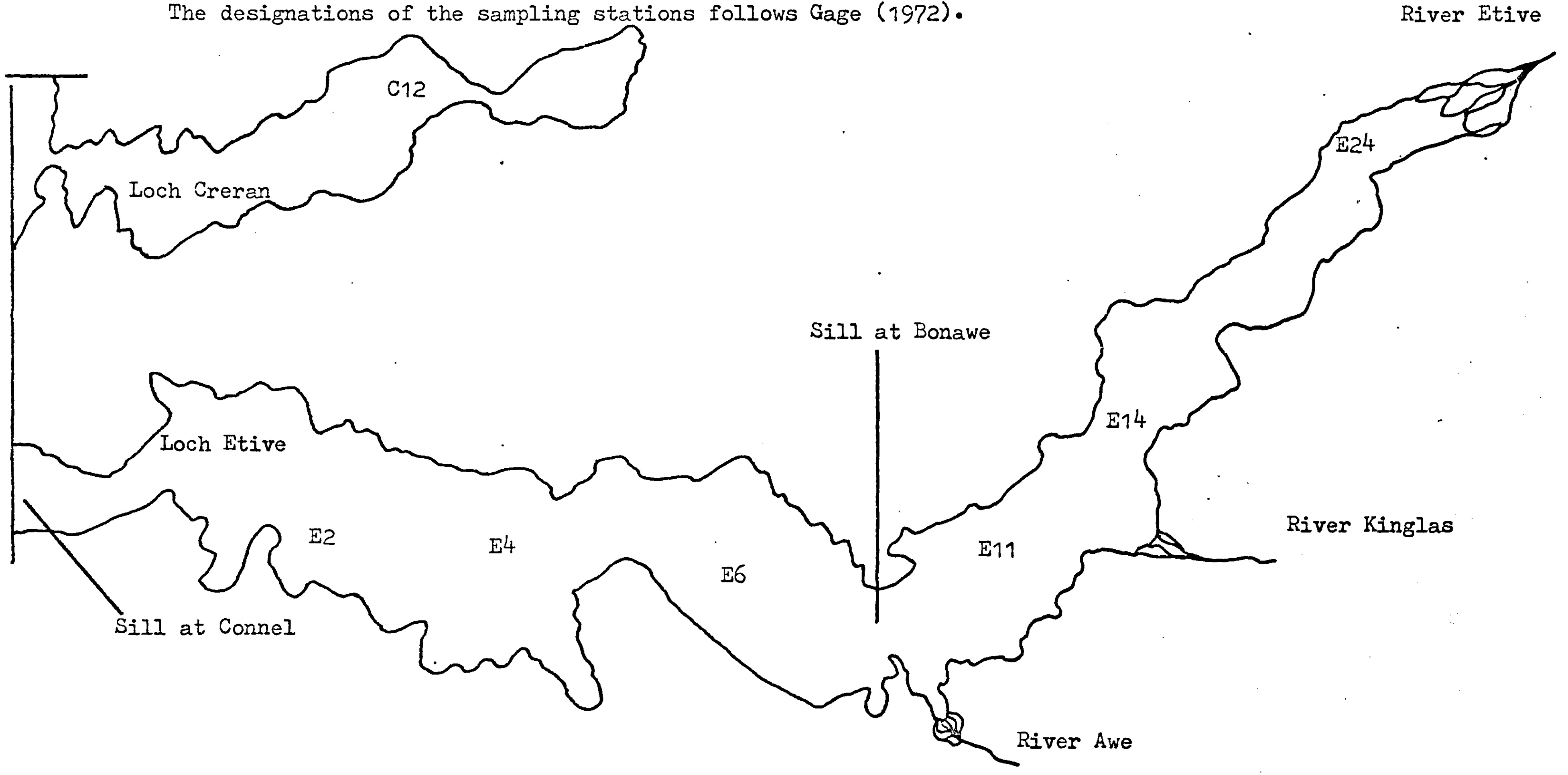
An Agassiz trawl was used in both Lochs. One of the problems associated with trawling for animals which are to be used in salinity tolerance experiments is that of the damage that can be sustained by the animals during capture. Diver observations have shown that as a trawl is towed it bounces along the sea bed ripping into the upper sections of the substratum and scraping up the surface animals. Ophiuroids, especially those with long, trailing arms such as Amphiura species, are particularly prone to damage in this way. Some amphiurids, for example Amphiura filiformis (O.F. Müller), have a tendency to cast off the aboral integument surface of the disc when trawled. It was for this reason that although A. chiajei (Forbes) and A.filiformis appear to occur synecologically only the former species was used since it tends not to lose its disc integument so readily.

It was found, during sorting, that those animals recovered from the innermost parts of the trawl were generally free from any extensive damage. This is possibly due to the support which the surrounding trawled material e.g. mud, detritus, and so on, would give to an animal. An animal which was more or less entirely contained within a clump of mud was noticeably less likely to be damaged than an animal clinging to the inside of the net meshes.

Another problem associated with trawling concerns the vertical, stratified salinity structure to be found in Loch Etive. Trawling was carried out on the bed of the Loch where, in this situation, the ophiuroids of any given section of the population are, and have been for some time, experiencing a specific salinity regime. This regime may vary according to the station which is

MAP OF LOCH CRERAN AND LOCH ETIVE (not to scale).

This map shows the approximate location of sills and sampling stations.
The designations of the sampling stations follows Gage (1972).



Produced from portions of British Admiralty Charts 2814^A and 2814^B with the permission of the Controller, H.M.Stationery Office and of the Hydrographer of the Navy.

being sampled. When the trawl is hauled to the surface the net is brought through the water column and the meshes of the net permit the continuous exchange of the water into and out of the trawl. This means that the brittlestars are subjected to rapid changes in the ambient salinity. These changes would be more or less severe depending upon the station which is being sampled. For instance, the difference in salinity between the bottom and the surface waters at station E2 would be less than at station E24. During sorting, only animals from the inner regions of the trawl were selected, not only because they were free from damage, but also because the surrounding detritus probably acts as a cushion against changes in the ambient salinity.

After sorting, on board ship, the animals were placed in large plastic bags containing water of the appropriate bottom salinity for the return journey to the Laboratory. The interval between capture in the Loch and the introduction to stock tank in the Laboratory was no longer than five hours. The stock tanks were of 30 litre capacity and contained sea water with the salinity adjusted according to the ambient salinity of the station from where a given batch of animals had been trawled. This water was circulated through Eheim power filters. Detritus collected from the trawl lined the base of the stock bath providing a food source and shelter.

Small, plastic buckets with snap-on lids were used when collecting animals by SCUBA diving. Animals were carefully picked off the mud surface and placed into the buckets. Due to the low temperature of the waters of the Loch only 15 - 20 minutes were spent under water. After the last animal had been placed into the bucket the lid was clipped on and this effectively prevented any water exchange occurring during the ascent. Since Amphiura chiajei lies just below the mud surface not only would it be difficult to locate their exact position (by the presence of arm holes on the surface of the mud) but also they would be difficult to collect due to the raising of mud clouds as the diver approaches the surface of the mud. Consequently, only O.albida which were on the surface of the mud were sampled by SCUBA diving.

(ii) Water samples.

At the stations where ophiuroids were sampled, water specimens were collected at the sampling depth, by 5 litre water bottle samplers on a static warp from the stern of the vessel. The salinity of these samples was determined by an inductively coupled salinometer.

It was necessary to assess the relative effects of trawling and SCUBA diving as methods of capture in terms of the damage and the deleterious salinity changes which could be sustained by the animals. Fifty animals caught by trawling and another batch caught by SCUBA diving, both from station E24, were kept in separate tanks. Nearly all the hand-collected specimens survived whilst about 10% of the trawled specimens died within a few hours after capture. After two days there were no apparent differences between the batches; righting reaction times were similar as was the general level of activity. On the basis of these observations it was considered that if animals from the inner part of the trawl were selected and if these were kept for at least two days before usage, then, if only fully intact animals were selected for experimentation, one could be reasonably sure that only the viable specimens were being used for the salinity tolerance experiments.

3.3 Determination of criteria for establishing the death-point in ophiuroids.

As with the other classes of echinoderms, it is difficult to determine at what point an ophiuroid is dead. Asteroids can cling tenaciously to the sides of an aquarium even when the disc is in a fairly advanced state of disintegration. Righting reaction which has been used successfully as a measure of activity in other echinoderms, (Percy, 1971; Ellington and Lawrence, 1974;), is not considered a sufficiently sensitive test with which to establish death in some ophiuroids. A small decrease in salinity, say $2^{o}/oo$ - $3^{o}/oo$, would almost completely prevent righting in some ophiuroids though it has been used for Ophiothrix angulata by Stancyk and Shaffer (1977). To establish a criterion or set of criteria fixing the death-point that would refect a true picture of the point of death, a preliminary salinity tolerance experiment was conducted using *Ophiocomina nigra* from Plymouth and *Ophiura albida* from Millport. The two species differ particularly with respect to the integrity of their integuments. One of the first signs of potential death is the occurrence of enlarging lesions of the integument.

Nine 10 litre bowls were filled as follows:

Ophiocomina nigra	*Ophiura albida*
1. 100% sea water (34.5^{o}/oo)	5. 100% sea water (33.5 ^{o}/oo)
2. 90% sea water	6. 90% sea water
3. 80% sea water	7. 80% sea water
4. 70% sea water	8. 70% sea water
	9. 60% sea water

The 100% sea water bowls acted as controls. The water was aerated continuously and partly changed every two days to prevent the accumulation of metabolites. Five specimens of *Ophiocomina nigra* were placed in each of the bowls 1-4, and five specimens of *Ophiura albida* were placed in each of the bowls 5-9. The salinity was checked and adjusted, if necessary, every two days. The following were observed and assessed every day:-

1. The general level of activity
2. The colour of the integument
3. Arm breakage
4. Tube foot movements

Animals were removed when dead to prevent pollution of the water. The sequence of events is shown in Figure D . This sequence of events is irrespective of the species or individual under test though the time to undergo the sequence varies presumably due to individual variability in resistance to the effects of lowered salinity, and the degree of dilution of the medium.

The podia guarding the mouth on the oral surface of the brittlestar were found to show the last sign of a response to

Figure D Sequence of events when *Ophiocomina nigra* and *Ophiura albida* are exposed to reduced salinity.

I. Immobility and horizontal curling of the arm tips.

II. Spine loss particularly at the base of the arms.

III. Disc lesions which enlarge rapidly.

IV. Colour loss which begins at the base of arms and spreads towards the tips.

V. Breakage of the arms.

VI. Decrease in podial response.

VII. Mouth podia fail to respond to stimuli.

mechanical stimulation (by a seeker), and when they failed to respond this was taken as the point of death. As a precaution, the animals which had reached this stage were left for a further week in order to ensure that there was no recovery. Such long recovery periods are known for Ophiothrix angulata (Stancyk, 1975).

3.4 Experimental method:

Salinity tolerance experiments have been carried out on the asteroid, Asterias rubens,(Binyon, 1961; Erikson et al, 1975) and on the ophiuroid, Ophiothrix angulata, (Stancyk and Shaffer, 1977). Binyon and Eriksson applied the LD50 criterion of tolerance but there are few details available concerning the criteria used for determining the death-point of the experimental animals or the subsequent methods of analysis.

Sprague (1969) reviewed the current techniques for measuring the lethal level of test solutions for aquatic organisms. He recommends the incipient LC50 (lethal concentration for 50% of the individuals on long exposure to die) as the most useful single criterion. He suggests a standardisation of methodology and notes that tolerance experiments commonly incorporate the following features:

(a) A series of test containers with a different but constant concentration of solution.
(b) At least ten specimens should be used since individuals may vary in susceptibility. It is better to measure an average response rather than a minimum or a maximum.
(c) Observations on mortality during exposure.
(d) Results should be expressed as concentrations tolerated by median individuals.

There are currently two main methods of determining the reaction of the median individual. In the first method the mortality times are recorded for each individual. The time to obtain 50% mortality is estimated for each test dilution. The series of median lethal times is generally used to estimate an appropriate threshold concentration for a lethal effect. In the second method the mortality is recorded at only the following time-intervals: one, two and four days. The concentration lethal to half the individuals in each of these time-intervals is estimated. The former has the advantage of complete observations and has been adopted in the following work.

The methodology of such experiments has been discussed by Doudoroff (1950) and APHA et al (1965). There are three main points to consider:

(1) There should be sufficient volume of test solution so that there is no fouling or oxygen depletion.
(2) Careful and accurate diluting.
(3) A period prior to experimentation for acclimatizing so as to reduce the influence of stress on the animals under test.

In the following set of experiments ten litres of test solution were used. Previous experiments have shown that such volumes would be sufficient to eliminate the accumulation of toxic excretory products and prevent the oxygen concentration from falling below the level necessary for the brittlestars as long as the water was partially changed every two days. The diluting medium was glass distilled water and the salinity was monitored with an inductively coupled salinometer. The ratio obtained from the salinometer was converted to a salinity value in parts per thousand by the use of International Oceanographical Tables. The salinity of each test solution was maintained within $\pm 0.5^{\circ}/oo$. All specimens to be tested were kept for two days prior to use. This was considered to be a reasonable time to allow for adjustment to stock tank conditions. As far as possible there was no selection of particular individuals other than fully intact specimens.

The species used in these experiments are listed below with the station of capture and the particular ambient salinity:

Loch Creran - (C12); 32°/oo *Amphiura chiajei*
Ophiura albida

Loch Etive - *Amphiura chiajei*: E6, E11, E24
Ophiura albida: E2, E6, E11, E14, E24

E2	24.1°/oo
E4	26.3°/oo
E6	25.9°/oo
E11	29.3°/oo
E14	28.5°/oo
E24	24.1°/oo

The following LD50 experiments were set up:

SPECIES	STATION OF CAPTURE	DILUTIONS TESTED (% sea water)
A.chiajei	C12	80, 70, 60, 50
O.albida	C12	80, 70, 60, 50
A.chiajei	E24	60, 50, 40
	E11	70, 60, 50, 40
	E6	70, 60, 50, 40
O.albida	E24	70, 60, 50, 40
	E14	70, 60, 50, 40
	E11	70, 60, 50, 40
	E6	70, 60, 50, 40
	E2	70, 60, 50, 40

The conditions of these experiments were similar to those of the preliminary experiments except that ten specimens were used for each dilution tested.

3.5 Treatment of data:

If the times when individuals in given test solutions die are recorded, data is obtained which can be expressed by a curve relating cumulative per cent effect to time. Such curves may or may not be truncated depending upon whether or not all the individuals in a given dilution react. Litchfield and Wilcoxon (1949) and Litchfield (1949) have developed a method for the graphic solution of such curves:-

a) Each observation time is plotted against the cumulative per cent effect on logarithmic-probability paper

b) A straight line is fitted to these plotted points, particularly those between 10% and 90%. The 'goodness of fit' can be tested by the $(\mathrm{Chi})^2$ test.

c) The times which intercept 16%, 50% and 84% are noted. The Median Effective Time is ET_{50}. The times at 16% and 84% are used since, mathematically, they can be related to a standard deviation of one. From these times the slope function, S, can be calculated:-

$$S = \frac{ET_{84}/ET_{50} \quad - \quad ET_{50}/ET_{16}}{2}$$

d) Using 'S' and the total number tested (as a percentage) i.e. 100% unless not all the individuals reacted, it is possible to derive a value, 'f', by using a series of nomographs (given in Litchfield, 1949). This value 'f' can be used to determine the 95% confidence limits of the parameter, ET_{50}

so that, parameter x 'f' = upper limit
and parameter ÷ 'f' = lower limit

e) Using 'ET_{50}', '$f_{ET_{50}}$', 'S' and 'fs' for each line the time-percent effect curves can be compared.

f) The Reaction Ratios, 'RR', can be determined thus,

$RR = ET_{50_i} / ET_{50_{ii}}$ (where ET_{50_i} is the larger value)

From the nomograph and using f_{ET50_i} and $f_{ET50_{ii}}$

f_{RR} can be read off. The value of 'RR' will exceed the value of f_{RR} if the two median reaction times being compared are significantly different. The 95% confidence limits are given by:-

$$RR \times f_{RR} = \text{upper limit}$$

$$RR \div f_{RR} = \text{lower limit}$$

3.6 Results:

Two species of ophiuroid were tested from station C12 in Loch Creran where full salinity conditions occur. Ophiura albida appears to have a greater tolerance to diluted sea water than Amphiura chiajei. (Graph 3.1)

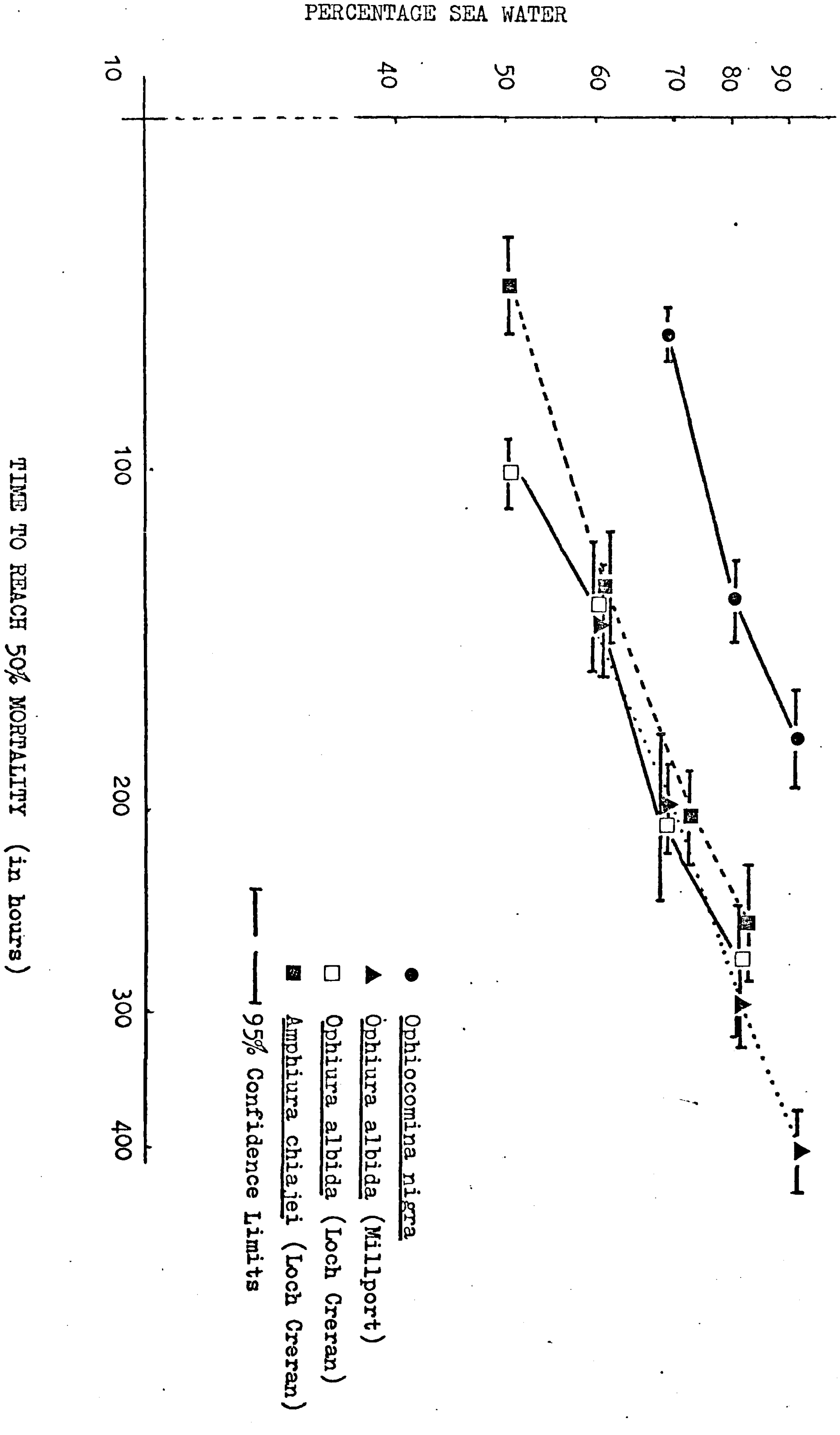
O.albida, C12, and O.albida from Millport, both from environments with full strength sea water have similar tolerances to lowered salinity. (Graph 3.1)

Amphiura chiajei from E24, E11 and E6 in Loch Etive were tested. Those animals from E24 show a greater tolerance than those from E11 and E6. Individuals from E24 have a substantially greater tolerance than those from E6 with respect to 70% sea water. (Graph 3.2)

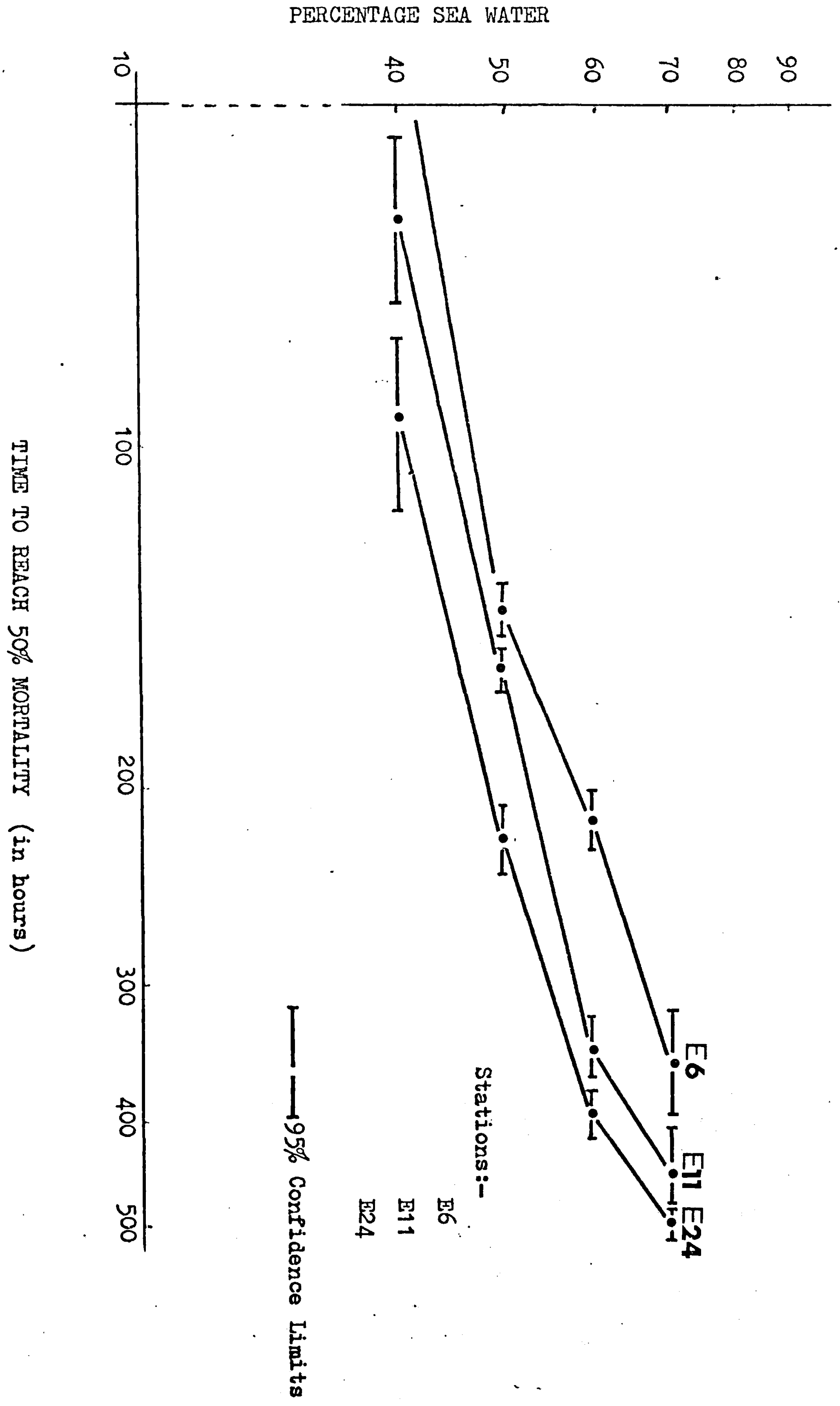
Ophiura albida from station E24 are already living at 70% sea water and only one animal died at 60% during the experimental period. At all other dilutions there is a decrease in tolerance to a particular dilution over the range of animals from the stations within Loch Etive, to the extent that animals from E24 have greater tolerance than those from E14, E14 $\equiv$ E11 and E11 $>$ E6. (Graph 3.3) Animals from E6 and E2 are approximately similar in their tolerances to diluted sea water.

O.albida has a greater tolerance at any given dilution than A.chiajei from the same sampling station.
Data for Effective Mortality times, 'S', '$f_{ET_{50}}$', 95% Confidence Limits, RR and f_{RR} are given in Data Tables on pages 248 - 250.

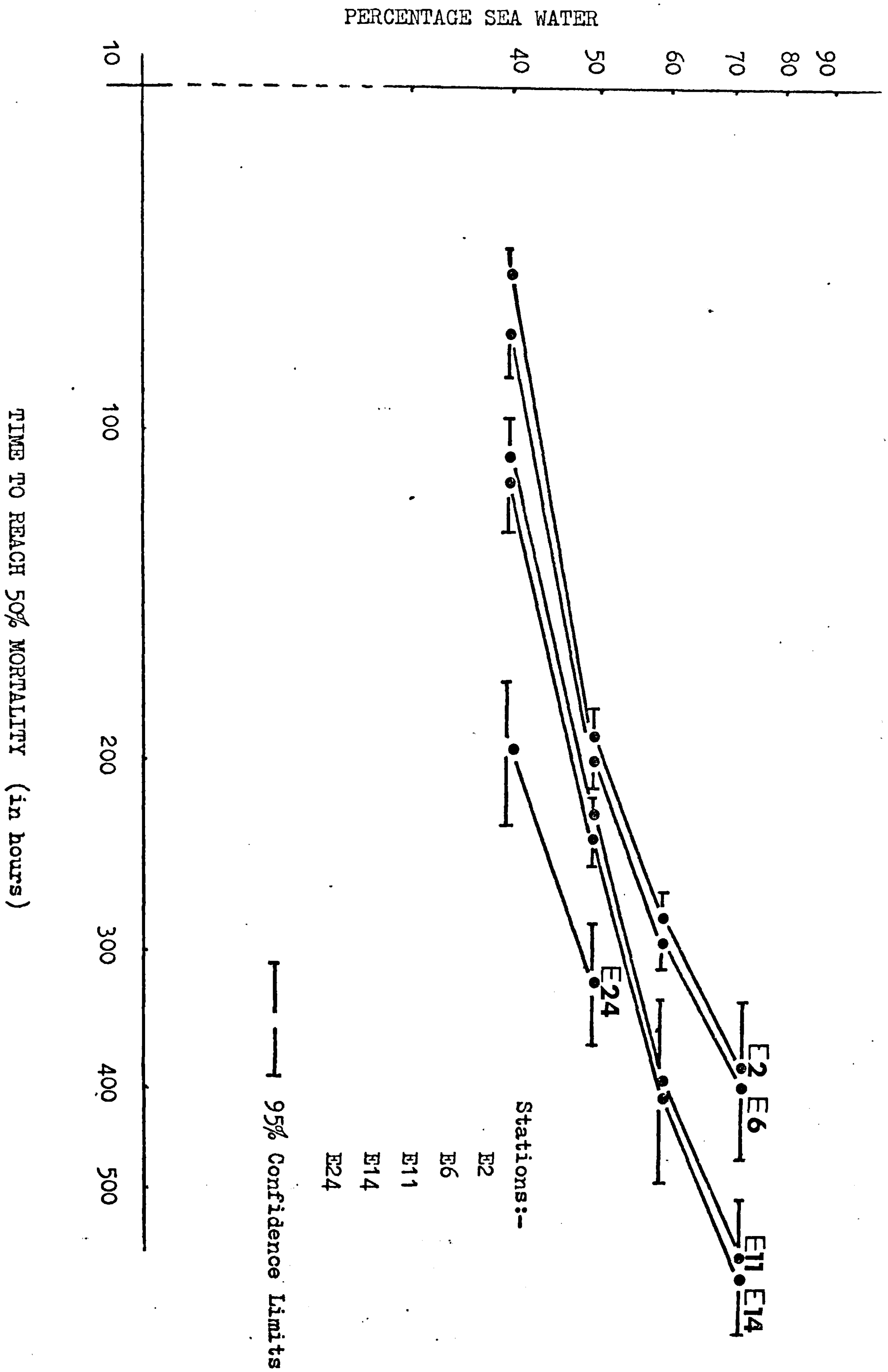
Graph 3.1 Comparison of salinity tolerances between Ophiocomina nigra, Amphiura chiajei, Ophiura albida (Millport) and Ophiura albida (Loch Creran).



Graph 3.2 Comparison of salinity tolerances between Amphiura chiajei from different stations within Loch Etive.



Graph 3.3 Comparison of salinity tolerances between Ophiura albida from different stations within Loch Etive.



With the exception of the data for E24, only 95% Confidence Limits for each set of stations have been inscribed (for the sake of clarity).

3.7 Discussion:

Individuals of Ophiura albida from Millport and Loch Creran, where full salinity conditions obtain, show a greater tolerance to reduced salinity than others such as Ophiocomina nigra also from full strength sea water conditions. Only the upper layers of Loch Creran could be subject to inundation to the extent that the surface salinity would be substantially depressed. Because of the tidal nature of this Loch, Gage (1972a), there is little likelihood that such depression would actually occur. In the event of lowered salinity, O.albida would probably not be affected since this species lives on the bottom of the Loch. Despite this unlikely possibility of a lowering of the salt content of the Loch waters O.albida has the ability to tolerate some dilution of the ambient medium. Though O.albida from Millport would most likely never experience diluted sea water, these animals still appear to have a similar potential to the members from Loch Creran where, though highly unlikely, salinity may very occasionally, be depressed. Ophiocomina nigra from Plymouth, where full salinity also obtains, is intolerant of any extensive dilution of the medium and is considered to have no ability to penetrate waters of reduced salinity.

In general, the salinity of the bottom water of Loch Etive decreases with increasing distance from the seaward entrance, Gage (1972a). However, it must be borne in mind from what depth the animals under test were taken. For instance, station E6 has a salinity of 25°/oo while E11 has a salinity of 29.3°/oo. The anomaly, since one would expect (see page 34) E11 to be less saline than E6, is due to the depth at which trawling took place. E6 animals were trawled at 35 metres, while those at E11 were taken from a depth of 125 metres.

Amphiura chiajei from stations E24, E11 and E6 have substantially different tolerances to diluted sea water. Individuals of this species from E24 (salinity of 24.4°/oo) are living in, more or less, 70% sea water, and when placed in an experimental dilution of this strength survived for the period of the experiment - four weeks. A.chiajei from E11 and E6 survived 18 days and 15 days respectively though one would expect them not to experience such dilution, especially those from E6. Animals from E11 and E6 have the potential to tolerate dilution whereas those from Loch Creran do not have this ability. In the other dilutions, 60%, 50% and 40% sea water, the animals from E24 again show a greater tolerance than animals from other stations. In the most extreme dilution, 40% sea water, the animals from E11 and E6 have a similar tolerance while those from E24 have a higher tolerance. The animals from E24 may occasionally experience such depressed salinity while those from E11 and E6 would never, and this is presumably reflected in the different tolerances to this dilution by the animals from stations E24, E11 and E6.

Inspecting the results for Ophiura albida it is clear that, like A.chiajei, animals from nearer the head of the Loch have a greater tolerance to reduced salinity than those nearer the sea. Animals from stations E11 and E14 have similar tolerances while those from E24 have a greater tolerance. Station E24 is a much shallower part of the Loch than E11 and E14 and experiences greater salinity depression. Animals from E6 and E2 have similar tolerances yet are less tolerant than those from E14 and E11. Lying between stations E11 and E6 is one of the sills (Bonawe) and the different tolerances of animals from E11 and E6 probably reflect the different salinity regimes prevalent at these stations. Stations E2 and E6 are situated in the lower basin, while E11 and E14 are located in the upper basins. Stations E2 and E6, on the whole, would be more saline than stations E11 and E14.

If the results for O.albida are compared with those for A.chiajei it is clear that individuals of both species, from the same station, have different tolerances; in each case O.albida has the greater tolerance to reduced salinity than A.chiajei. This is emphasised by animals from E24; Ophiura albida tolerates 60% sea water ($20.7^{o}/oo$) very well - only one animal died in this dilution. In the same dilution A.chiajei has a much lower tolerance. At station E24 there is similar salinity regime acting upon both species on a broad scale.

However, the two different modes of life of these species may result in their being subjected to different salinity regimes. *Ophiura albida* is a fairly mobile, predatory species capable of moving over the surface of the substratum (mud at station E24) and is rarely completely buried, though the lower part of the disc may sometimes sink into the substratum. *Amphiura chiajei* generally lies below the mud surface with only its arm tips protruding. Looking at their relative positions at station E24 in Loch Etive by SCUBA diving it is possible to suggest how the different tolerances to diluted sea water and the two modes of life of the two species are related. *O.albida* tends to range up the sides of the Loch, presumably in search of food, and thus may penetrate water of considerable dilution (due to the stratified nature of the water in Loch Etive) for relatively short periods. *A.chiajei* is more or less restricted in its depth distribution and would not normally experience such rapid changes in salinity as *O.albida* as a result of the relative depths at which the two species occur. Also, being buried may help *A.chiajei* to withstand depressed salinity by the cushioning effect of the substratum. Smith (1955) has proposed that this cushioning effect by the substratum is an important factor in the distribution of the errant polychaete, *Nereis diversicolor*, in the intertidal muds of Kames Bay, Millport. Also, Stancyk (personal communication) has suggested that a cushioning mechanism is responsible for the presence of the ophiuroid, *Ophiophragmus filograneous* in Whitewater Bay, Florida where very reduced salinities obtain.

So, in effect, there is probably a greater salinity stress acting upon *O.albida* than upon *A.chiajei* which may be reflected in *O.albida* by its greater tolerance of diluted sea water.

There is evidence to suggest that acclimatization to a lower salinity will extend the tolerance to experimentally diluted sea water. *Ophiura albida* taken from station E24 at a depth of about 10 metres, where the salinity was 19.3^{o}/oo, showed a much greater tolerance to diluted sea water than *O.albida*, also from E24, but taken at a depth where the salinity was 24.4^{o}/oo. This acclimatization must be a fairly gradual process. At the end of

October, 1977, diver observations at station E24 from 0 metres to 21 metres revealed very few living O.albida. There was an extensive mortality, probably due to the recent fresh water inundation of the Loch. It had been raining almost continuously for two days prior to the dive. The River Etive, the major fresh water input to the head of the Loch, was in full spate. Most living O.albida were found below 20 metres. At about 12 metres there were more dead O.albida than at 16 metres. It is possible that at 12 metres the salinity was lowered so quickly that it prevented any escape response by the O.albida at that depth. At 16 metres the salinity would not be depressed so rapidly due to the cushioning effect of the mass of water lying above, and thus the animals may have been able to escape to the more favourable salinity conditions below. This would imply some sort of sensory perception of lowered salinity was necessary. It has been suggested that this is possible in the asteroid, Asterias rubens (Loosanoff, 1945; Castilla and Crisp, 1973).

The brittlestars which were found living at the 16 metre mark had, presumably, acclimatized or, at least, had the ability to tolerate the depressed salinity until acclimatization was completed. This was considered to be correct because some of these animals remained in experimental tanks at the 16 metre salinity of 18.3°/oo for the subsequent four months. The observations were discontinued after that period since it was thought that acclimatization was complete. Evidence for this opinion arises from the regeneration of broken arms which suggests that there had been no long term deleterious effect on the brittlestars due to the extensive freshening of the waters at the head of Loch Etive. Indeed, it is perhaps indicative of the physiological flexibility which Ophiura albida has and which can be employed when faced with sudden and often extensive salinity depression.

CHAPTER FOUR

CROSS ADAPTATION EXPERIMENTS USING Ophiura albida FROM NORMAL AND DILUTED SEA WATER ENVIRONMENTS

4.1 Introduction:

The differences in tolerance to hyposmotic salinity stress in two populations of the same species from differing salinity regimes have been considered to be either due to a more or less lengthy process of adaptation or due to the selection of favourable mechanisms leading to the production of physiological races.

Physiological races are known for many species. These include:-

Gasterosteus aculeatus (Heuts, 1947)
Gammarus duebeni (Beadle and Craig, 1940a)
Tisbe furcata (Battaglia, 1967)
Palaemon squilla (Pora, 1946)
Dikerogammarus haeobaphes (Beliaev and Birstein, 1944)

The occurrence of physiological races has not yet been convincingly demonstrated in those echinoderms which have penetrated brackish water.

Gezelius (1962) quoting the work of Horstadius (1925) on the echinoid, Paracentrotus lividus, notes that adaptation to different salinity regimes has much the same characteristics as the adaptation to new temperature ranges involving changes in tolerance and cleavage rate of fertilised eggs. He suggests that the two processes facilitating such adaptations are not too distantly related physiologically. Beadle (1943) postulates that the extension of the tolerance range is due to the development of a new mechanism not originally functional.

That a relatively long and gradual period of acclimatization is required to adapt a species from one salinity to another, lower, salinity value is indicated by previous work (Loosanoff, 1945; Schlieper, 1957; Gezelius, 1962).

Loosanoff (1945) carried out experiments on Asterias forbesi in an attempt to acclimatize this asteroid from its usual salinity of 27°/oo to a much lower one. The tolerance, for 100% survival, could not be extended beyond 18°/oo despite lowering the salinity by a gradual 1.5 - 2.0°/oo per day.

Smith (1940) using the asteroid, Asterias vulgaris, established a lower limit of 14°/oo within a 6 - 10 day period for adaptation. However, Loosanoff (1945) points out that such periods are too short. He cites his own work as evidence in which he maintained Asterias forbesi at 16°/oo for a similar period of adaptation 10 days, as Smith (1940). It was noted that deleterious effects of this lower salinity only became evident after two months at this new salinity.

Gezelius (1962) states that about 50 days are required to achieve a complete adaptation between the 'Z'(shallow) and 'S' (deep) forms of the echinoid, Psammechinus miliaris. Schlieper (1957) found that Asterias rubens can be transferred between the Baltic and North Sea salinities by a progressive adaptation over several weeks.

It has been recognised that adaptation to a new salinity must be apparent in all stages of the life cycle, not merely in the adult form. Schlieper (1957) found that Asterias rubens could not reproduce at the extreme parts of its range within the Baltic Sea. The population of this species depends upon larval recruitment from the Kattegat populations for maintaining the adult presence at these extreme salinity limits (8°/oo).

Timourian and Watchmaker (1970) using the spermatozoa from the echinoid, Lytechinus pictus, demonstrated that the mobility of the spermatozoa depended upon the salinity in which the male echinoid had been kept. The eggs of this species could be fertilised at all dilutions in which the sea urchin could survive. However, it was the mobility of the spermatozoa that was important in determining the success of fertilisation. Gezelius (1962) using the 'Z' and 'S' forms of Psammechinus miliaris studied the salinity optimum and range of stages other than the adult. He found that the cleavage rate of the egg was dependent upon the salinity at which the parent was adapted. He also noted that the temperature, and not the salinity, was the major criterion determining the beginning of the reproductive period. Davis (1958) states that the salinity range of oyster larvae depended upon the salinity in which the maturation of the gonads had occurred. Gezelius (1962) points out that adult Psammechinus miliaris can only adapt within certain limits. These limits are those to which the sex cells can adapt, though the adults of P.miliaris may be able to exist in areas, e.g. the littoral zone, where they are sometimes exposed to salinities outside the tolerance limits of the sex cells.

Much work, mostly on the Asteroidea and the Echinoidea, has been concerned with tolerance to differing salinities in the assessment of whether two populations of a species are separate physiological entities.

Schlieper (1957) states that there is no indication that the two populations of Asterias rubens, namely from the Baltic and North Seas, are genetically different races. He proposes that the differences which do occur, (including: water content, body size, ash weight, tissue metabolism, activity and gonad development time), are a result of a lengthy process of adaptation.

Gezelius (1962) kept 'S' and 'Z' forms of P.miliaris in salinities of the other form for more than a year and noted that there were no major morphological changes in habitus. He comments that the occurrence of morphological transformation between the two forms "must be rare".

Borei and Wernstedt (1935) state that it is unlikely that the habitat of the 'S' form is populated by individuals of the 'Z' form. Gezelius (1962) concludes that the two morphs, 'S' and 'Z', are developed each into its particular habitus due to the effects of salinity, temperature and light intensity operating at the time of metamorphosis and the subsequent early development of the sea urchin.

The ultimate test of a physiological race is the production of viable forms from the cross fertilisation of individuals of both 'entities' since an inherent feature of speciation is that of reproductive isolation (Lack, 1947).

There are differences in salinity tolerance and weight fluctuation in reduced salinities (see Chapter 1) between Ophiura albida from Millport and station E24 in Loch Etive. To investigate further the nature of the population of Ophiura albida in Loch Etive, in terms of whether these animals are tending towards a physiological isolation distinct from the O.albida living off the west coast of Scotland, a cross adaptation experiment was carried out.

(a) Method:

Twenty animals from Loch Creran, where full salinity conditions obtain (33°/oo), were used in an attempt to assess their ability to tolerate and survive an extensive reduction in the salt content of the ambient medium. The animals were obtained by trawling as described previously (page 89). Since the loch is fully tidal (Gage, 1972a) the salinity is considered to be equivalent to that off the southern tip of Lismore (Long. $50^{\circ}36'0''$W, Lat. $56^{\circ}27'30''$N) where the nearest population of O.albida from fully marine conditions occurs (Pearson, personal communication). Prior to use the animals were kept in stock baths for a few days (see page 93). To effect the adaptation, the animals were placed in a large stock tank filled with sea water of the appropriate initial salinity, 33°/oo, and aerated by two airstones. Lining the base of the tank was various detrital material obtained from Loch Creran by trawling.

Animals for adaptation to full salinity conditions were obtained from E24 by hand collection using SCUBA diving. The relative viabilities of animals caught by diving and by trawling have been discussed (see page 93). Twenty animals from this station, with a salinity of 24.4°/oo, were set up in similar conditions as the above. Two 20 litre bowls containing Loch Creran animals and E24 animals were set up and acted as controls, there being no salinity change in these bowls.

The reduction in salinity was effected by the addition of glass distilled water and produced a decrease in salinity of 1.4°/oo per day in the experiment with the Loch Creran animals.

In the experiment with the E24 animals the salinity was raised from 24.4°/oo to 33°/oo by the process of evaporation at a rate of 1.0°/oo per day.

(b) Results:

In falling salinities, the animals from Loch Creran soon began to show signs of stress such as curling of arms, loss of colour and lower level of activity. They began to die at 26°/oo (the eleventh day of the experiment) and were all dead after a further eight days at 24.9°/oo.

The O.albida from station E24 at the head of the Loch withstood the experimental increase in salinity and were maintained at the final slainity of 33°/oo, which was reached in nine days, for a further five months to ensure that there were no long term morphological deleterious effects (see Loosanoff, 1945). After five months, extensive regeneration of the distal segments of the arms had occurred and it was considered that the animals had adapted to the new salinity.

There were no obvious morphological differences between these animals and those which normally live in salinities of 34°/oo, for example, those from Millport. Furthermore, differences in colour and level of activity were not apparent between these newly adapted animals and those from Millport, strengthening the evidence leading to the conclusion that these animals had not suffered any apparently deleterious effects due to the increased salinity. It was not, however, possible to test whether these animals could be reproductive in these new salinities.

(c) Discussion:

From the above experiments it seems that the population of O.albida from E24 are able to readjust quite quickly to fully marine conditions. The Loch Creran animals apparently either, find it is impossible to survive the reduction in salinity or, they require a substantial time and gradual salinity decline in order to allow the adaptation to a reduced salinity. That O.albida from E24 are able to adapt quite quickly may, perhaps, reflect the degree of euryhalinity that is possible in Ophiura albida when its salinity tolerance, as instanced by its occurrence in brackish water has, at least, been modified and extended.

4.2 A discussion concerning the possibilities resulting in the presence of Ophiura albida at the head of Loch Etive.

It is instructive, at this point, to consider how Ophiura albida may have become established in the Loch, particularly at the head where very reduced salinities obtain. From the above experiment there is an indication that relatively long periods of time are required in order to allow the adaptation to reduced salinities which occur within Loch Etive. Such periods may have occurred during the colonisation of the Loch. The possible routes of establishment of O.albida within the Loch all have their attendant problems, which will now be discussed.

Thorson (1957) has discussed the importance of the larval stage for dispersal of marine invertebrates. Larval dispersal is a possible method to be considered. A pelagic larva, Ophiopluteus paradoxus, has been described by Mortensen (1927) and is thought to be the larva of Ophiura albida. The presence of a brackish water layer on the surface waters of Loch Etive would seem to be an osmotic and ionic barrier to larvae. To avoid such conditions it is possible that the larvae could travel in subsurface currents where salinity is presumably more favourable. Gage (1972a) has shown that the speed of the bottom currents decreases with depth. Although Fell (1948) reports that Ophiura albida has an eight armed larva (prior to metamorphosis) which would indicate that it has a fairly long larva stage, and Thorson (1957) has reported that some members of the genus Ophiura can defer metamorphosis by as much as three days, it is not considered that this would be sufficient to allow significant penetration of the Loch. Also, the sills would tend to reflect water of subsurface currents. It seems likely that only surface waters, and some subsurface waters during 'renewals' would flow over the sills up towards the inner reaches of the Loch. The reasons governing the presumed absence of larvae from the surface waters would, it is assumed, also apply to potential egg transport within the surface waters. If colonisation via larvae and/or eggs is impractical, then this would imply that adult penetration would be necessary.

There are two possible routes of colonisation of Loch Etive by adult O.albida. Firstly, animals from the Firth of Lorne could establish populations of Ophiura albida progressively further up the lower basin of the Loch. From these stations, colonisation could extend up towards the head of the Loch commensurate with increases in tolerances to lowered salinities and rates of movement. Though ophiuroids, particularly those of the genus Ophiura, are thought to be the most agile of the echinoderms, little quantitative work has been done on rates of movement. Most work has been confined to the asteroids (Romanes and Ewart, 1881; Galtsoff and Loosanoff, 1939; Smith, 1940; Reese, 1966). The latter author summarises the current state of knowledge, particularly with reference to the motivation of certain directional movements in echinoderms. In the asteroids, the rates of movement are very variable, and Binyon (1976) thinks that they are not sufficient for Asterias rubens, which also occurs at station E24, to establish a population in Loch Etive. It seems unlikely that, relatively, a more rapid process of colonisation could occur by O.albida. It must be remembered, however, that a rapid colonisation is not necessary. Indeed, a slow process of establishment would enable a lengthy process of adaptation, which appears to be a prerequisite for survival in the upper reaches of the Loch, to occur. One problem with this scheme, not yet discussed, is that of the geological barriers which are present in the Loch.

These barriers, which could hinder progression into and up the Loch, are the sills; one at Connel and the other at Bonawe. These sills rise to within seven and ten metres, respectively, of the surface; a distance from the bed of the Loch to the tops of the ridges of thirty metres at Connel and seventy-two metres at Bonawe. Such obstacles would have to be surmounted in order to establish populations of O.albida in the lower and upper basins. Though the sill at Connel cannot be readily associated with any particular geotectonic incident, it is clear from the survey work of Anderson (1937) that, at Bonawe, the sill lies along the fault line running through the Pass of Brander.

Such obstacles are not, initially, critical problems in the process of colonisation of the Loch by O.albida in the second route. The second route requires that Loch Etive was, formerly, fully marine. As such, O.albida could be present throughout the Loch by larval dispersal, since there would be no brackish water layer problem. Then, geomorphic movements could upthrust the sills thus confining the animals. Presumably it would take some considerable time before the upper basin waters were diluted to their present condition since there would be renewals of marine waters from the lower basin where, it is supposed, dilution would take even longer. Such long periods for dilution to have effect would allow the marine O.albida to withstand the lowered salinities. If glacial over-deepening of the upper basin had subsequently occurred, wiping out any resident populations, re-population could take place, after the recession of the glacier. This is speculation however. Although it is known that glaciation of Etive occurred during the last Ice Age (Charlesworth, 1954) it is by no means clear whether this was pre- or post-sill appearance. One might suppose that since it was possible to adapt the O.albida from station E24 quite rapidly to fully marine conditions, animals at the head of the Loch, and thus, it is likely, from within the whole Loch, have been derived fairly recently from marine stock. From this it could be thought that the O.albida in Loch Etive are of post-sill appearance. This still leaves, however, the unsolved problem of the mode of their original penetration.

4.3 A discussion concerning the possibilities resulting in the maintenance of the presence of Ophiura albida at the head of Loch Etive.

Maintenance of the Loch Etive Ophiura albida poses similar problems to those of original colonisation. Such maintenance could be via larval and/or adult recruitment from nearby marine waters or by the resident population undergoing a complete reproductive cycle in the reduced salinity on the Loch floor.

Larval recruitment, which would rely upon the larvae tolerating the lower salinity of the brackish layer of the surface waters of Loch Etive, and upon their transfer, eastwards, up the Loch by the currents would mean that larvae would need to traverse the sills. The problems inherent in this have been discussed. Similarly, larvae spawned by the resident population would need to tolerate the salinities of the brackish water layer, or stay in the more saline water below. Wood _et al_ (1973) have demonstrated that there are blooms of diatoms, dinoflagellates and μ-flagellates occurring within the euphotic zone - the upper ten metres. So, presumably the larvae would have to rise in order to feed upon these phytoplankters since it may be that such micro-organisms would be unlikely, due to the obvious buoyancy problems, to penetrate and descend below the halocline which separates the brackish layer and the deeper, more saline water. Also, since these plants are photosynthetic, there would seem little reason to descend out of the euphotic zone.

Another method of recruitment to the population is that arising from adult movements. This would need a large contingent of adults moving into the lower basin, over the sill at Connel, and thence into the upper basin, over the sill at Bonawe. In both instances it would require large numbers of animals moving into, initially, unfavourable conditions. Not only that, the sills would have to be traversed with an unlikely regularity.

If the lower basin animals do not reproduce then adults would have to be recruited from the nearest _O.albida_ population. The nearest population is about 12 kilometres from the entrance narrows of Loch Etive. This distance would seem to preclude adult recruitment. It would also seem unlikely that larvae would have a delayed metamorphosis great enough to permit recruitment from this distance. It has been estimated that a packet of water from this area would take two or three months to reach the Bonawe narrows (Edwards, personal communication).

To assess the occurrence of reproduction of _Ophiura albida_ within the confines of the Loch, plankton hauls were taken at the end of July, 1977 when it was considered, with reference to known breeding populations around Brtain of this species, that ophioplutei would be present in the plankton (Tyler, personal communication). It was not possible to follow the reproductive cycle histologically. Binyon (personal communication) examined the gonads of Asterias rubens, from station E24, and found them ripe and ready to discharge gametes into the water. He also observed that the eggs showed germinal vesicle breakdown.

Plankton hauls were taken at the following stations: E2, E11, E24. At these stations oblique tows which sampled the whole water column were taken. In case ophioplutei were at the level just below the halocline a horizontal tow was taken at station E24, at six metres depth.

No echinoderm larvae were found. This is inconclusive, however, of evidence of a non-breeding population of Ophiura albida because it is possible that trawling was carried out at the wrong time. It has been demonstrated in some echinoderms, for example, Ophiocomina nigra, that spawning is confined to the production of discrete packets of gametes over a very short period of time (Gorzula, 1976).

The presence of small specimens (1.0mm to 1.5mm in diameter across the disc) of Ophiura albida in the trawl net at station E24 during the Easter of 1977 suggests either that the breeding season was extremely early that year or that the animals were derived from the spat of the previous year. It is known that in some echinoderms, e.g. Asterias rubens, the occurrence in brackish water slows down the maturation of the gonads (Schlieper, 1957). From the newly metamorphosed size and with reference to reproductive studies of O.albida off the Gower Peninsula, South Wales (Tyler, 1977) it seems likely that these small brittlestars were spawned in the summer of 1976 and subsequently overwintered. It seems unlikely that these small brittlestars, if spawned in the lower basin, could move from this basin into the upper basin and thence to station E24 at the head of the Loch. Assuming that as larvae they could not tolerate the salinities of the brackish water layer, it seems plausible that the larvae are derived from animals within the upper basin.

Furthermore, it is considered that, on the basis of likely rates of movement and improbabilities of larval transport in the surface layers, as discussed previously, Ophiura albida is capable of reproducing at the head of Loch Etive. It is, perhaps, unlikely that these small animals merely travelled up the Loch from, say, stations E11 or E14 since, if this were so, then these young animals with a possibly lower tolerance to reduced salinities than the adult stages, would be moving into less favourable conditions which seems implausible for such young, and presumably more physiologically sensitive, animals.

CHAPTER FIVE

INVESTIGATION INTO THE ROLE OF MUCUS IN THE PENETRATION OF LOCH ETIVE BY Ophiura albida

5.1 Introduction:

The secretion of mucus is a well documented phenomenon found in all classes of echinoderms. These secretions may emanate locally, or from over the whole body surface. Hyman (1955) points out that the Ophiuroidea, in which an epidermis is generally lacking, are unusual in that, with few exceptions, the exudation of mucus is confined to the tube foot region. It is, perhaps noteworthy to add that members of the Holothuroidea do not appear to liberate mucus from the tube foot epidermis.

There is a vast literature concerning the work describing the types of cells and glands which are known to secrete mucus. Such descriptions have been summarised by Boolootian (1966).

Many functions have been ascribed to the production of mucus:-

1. It acts as attraction or adhesive agent during the locomotion of some asteroids (Paine, 1926).

2. It serves to trap food particles in some of the Crinoidea and the Ophiuroidea (Nichol, 1966).

3. It acts as a lubricant for the teeth of the Aristotle's Lantern in the echinoid, <u>Echinus esculentus</u> (Reese, 1966).

4. Asteroids, echinoids and ophiuroids use it to line their burrows (Boolootian, 1966).

5. The attachment of eggs to the adult integument

is known in Cucumaria curata (Smith, 1962).

6. It may confer some protection from predators, e.g. in some crinoids (Fell, 1966). It has been suggested (Binyon, 1976) that there may be some cause and effect relationship between mucus in echinoderms and the apparent, virtual absence of ectoparasites.

7. Light production, as phosphorescence, may be related to mucus production (Gotto, 1963).

The most recent, extensive work concerning mucus and its occurrence in ophiuroids is that of Fontaine (1964) in which Ophiocomina nigra was investigated. It was shown that:-

a) An aid to locomotion was the primary role of mucus from the tube feet.

b) Mucus was used in the form of a mucus trap slung between two arms and across a current (Fontaine, 1965).

c) As has been suggested for some of the Crinoidea it is thought that mucus may act in a defensive role against predation. O.nigra is notably absent from the list of Northeastern Atlantic species that are preyed upon by such fish as cod and haddock.

Binyon (1976) has described the occurrence of mucous glands in the integument of Asterias rubens, from the head of Loch Etive, in Scotland. Experiments demonstrated that this secretion did not form a permeability barrier to the lowered salinity prevalent at the head of the Loch. Since the Baltic Sea population of Asterias rubens, from a similar salinity to those from station E24 at the head of the Loch, did not show any abundance of mucous glands it was considered that these glands may be a response to a less stable lowered salinity. Binyon (1976) suggests that because the Baltic Sea population may have had a greater period of time in which to adjust to the reduced salinity and also because there was less seasonal fluctuation than in Loch Etive at the head, it was possible that the number of mucous glands had declined in the Baltic Sea animals. Schlieper (1957) has described these latter asteroids as '..less well sclerotised...' ; this may mean that they have a similar appearance to those from E24 in Loch Etive (Binyon, 1976).

To assess further whether mucous glands and reduced salinity were connected the following material was examined histologically using Alcian blue staining techniques.

5.2 Materials:

Ophiura albida	- Millport,
	- Loch Etive - E4, E6, E24
	- Loch Creran - C12
Amphiura chiajei	- Loch Etive - E4, E6
	- Loch Creran - C12

Ophiura albida, from station E24, was subjected for twelve days to a dilution of 40% sea water ($13.6^{o}/oo$). Animals were removed at 24 hour intervals fixed and subsequently examined for the presence of mucus.

5.3 Histological Procedure:

The material for sectioning was placed in Bouin's sea water fixative for two weeks and then transferred to 70% alcohol for storage. Since ophiuroids are highly calcareous (up to 50%) and standard sectioning methods require the complete absence of all skeletal material it was necessary to decalcify all material by immersing the ophiuroid samples in several changes of EDTA (Ethylene diamine tetra acetic acid) over a period of six weeks.

The material was rendered suitable for embedding in paraffin wax by dehydrating the samples with an ascending series of alcohols (30% to absolute). The tissues were subsequently cleared using several changes of toluene.

Block making and trimming were carried out using the methods described in Mahoney (1966). A rotary microtome set at 10µ was used for the section cutting. Only transverse sections were made. Attachment of sections to slides and the subsequent treatment for storage in the unstained state followed Mahoney (1966).

The Alcian blue method of staining for acid mucopolysaccharides as described in Gurr (1963), was used.

5.4 Results:

No mucous cells or glands were found in Ophiura albida from Millport or from those occurring within Loch Etive. This absence was also noted for Amphiura chiajei from Loch Creran and Loch Etive. Ophiura albida from the Öresund did not display any mucous cells or glands.

There was no evidence to suggest that any development of mucous glands or cells occurred when individuals of Ophiura albida (E24) were kept in 40% sea water. Similar changes in the morphology of the integument such as the appearance of lesions, loss of colour and general necrosis as have been described for Asterias rubens, when placed under similar conditions (Binyon, 1976) were observed in Ophiura albida.

5.5 Discussion:

One might have expected a similar presence of mucous glands in the integument of Ophiura albida, as has been described for Asterias rubens from Loch Etive by Binyon (1976), if a reduction in salinity was of absolute importance in influencing the development of such glands in this asteroid.

It must be remembered, however, that Asterias rubens from the head of the Loch has a distinctly different morphological appearance in comparison with the same species from normal salinities ($34^{\circ}/oo$). These individuals are very soft and drip with large amounts of mucus when removed from water. The appearance of these starfish would seem to be analogous to that of Asterias rubens from the Western Baltic Sea which have been described by Schlieper (1957) as '.... less well sclerotised ....' but which have relatively few mucous glands (Binyon, 1976).

There are no such differences in morphological appearance between Ophiura albida from Millport ($34^{\circ}/oo$) and those which occur in the brackish water of Loch Etive.

It must be remembered, of course, that Asterias rubens is an asteroid and an analogy between this species and the two ophiuroids, Ophiura albida and Amphiura chiajei, in terms of penetration into waters of reduced salinity, should, if at all, be drawn with care. However, it would appear that neither O.albida nor A.chiajei rely upon mucus production for penetration into waters of reduced salinity.

CHAPTER SIX

COMPARISON OF WATER AND ASH BETWEEN OPHIUROIDS FROM DIFFERING SALINITY REGIMES

6.1 Introduction:

In 1953, Vinogradov exhaustively summarised the current state of knowledge concerning the elementary chemical composition of the phylum - Echinodermata. In this review, he quotes Petersen who claimed that the water content of an echinoderm varied from 56.6% to 75%. He gave figures for the water content of two ophiuroids, Ophiopholis aculeata and Ophiothrix fragilis as 56.6% and 68.8%, respectively, (Meyer, 1914; Weigelt, 1891).

Giese (1966) notes that the asteroid, Pisaster ochraceus is 71.4% water while the Antarctic asteroid, Odontaster validus, has a water content of 75% to 80% (Pearse, 1964).

Riley and Segar (1970) give percentage water contents in a range of echinoderms. A cursory examination of these data shows that there is a wide variation between classes and between species. They gave a value for an unidentified ophiuroid of 46%.

Schlieper (1957) compared the water contents of Asterias rubens from the North Sea and the Baltic Sea. He quotes a figure of 83.1% and of 76.8% for animals from the Baltic and North Seas respectively.

Thompson and Chow (1955) analysed a wide range of echinoderms and gave values for the percentage of organic matter in whole animals. There is a variable percentage of organic material present in an echinoderm, primarily depending upon the extent of the calcareous skeleton. The amount of organic matter ranges from 1.2% to 84% (Thompson and Chow, 1955). The Echinoidea appear to have the least, presumably due to the presence of the heavy test. Thompson and Chow (1955) gave figures for the Echinoidea - 1.2% to 9.3% (in Heterocentrotus trigonarius and Strongylocentrotus fragilis respectively); for the Holothuroidea - 12.2%, (Psolous chitinoides), to 84%, (Cucumaria curata); for the Asteroidea - 19%, (Mediaster aequalis), to 39.4%, (Asterias forbesi); for the Ophiuroidea - 11%, (Ophiura sarsii), to 33.4%, (Ophiothrix spiculata). The single crinoid analysed, Antedon sp., had an organic content of 13.3%.

Vinogradov (1953) states that nearly 50% of the dry weight of an echinoderm is ash, except in the case of the Holothuroidea where, due to the rather insubstantial spicule skeleton, it is somewhat less. Mauchline and Templeton (1966) gave the ash weights as a percentage of the dry weight and their results indicate that it is variable with the echinoids having the greatest percentage, presumably due to the presence of the extensive test.

Giese (1966) notes the presence of a certain variability in the percent water content between species. However, it appears that the percent water content of analogous bodily components in different species are similar. He gave the value for the arms of Ophioderma panamensis of 44.5% and that the arms constitute 78% of the whole animal.

Often the above results have been obtained as an indirect consequence of extensive biochemical investigations. Since these studies would require large animals in order to obtain sufficient experimental material there has been a bias of results towards the larger species and the larger individuals of these species. Even so, it is evident that 'a representative echinoderm', in terms of percent water, organic matter and ash, has very little meaning.

6.2 Materials and Methods:

The percentages of water, organic matter, skeleton and ash were investigated in the following species:

Ophiocomina nigra - Plymouth
Ophiura albida - Millport, Loch Etive, Öresund
Amphiura chiajei - Loch Etive

The percentages of water and skeleton were determined by the drying and potassium hydroxide procedures, respectively. These procedures have been described elsewhere (see Page 150). The organic matter comprising salts, muscles, reproductive and visceral material was estimated by subtraction.

For some of the determinations the animals were partitioned into disc, arms and regenerating parts of arms. This latter division, namely the subdivision of the arm into the older portion and the regenerating section, was carried out for *Ophiocomina nigra* only. *Ophiura albida* from Millport and the Öresund were partitioned only into discs and arms. The percentages of skeleton was determined only in *Ophiura albida* from Millport; and *Ophiocomina nigra* from Plymouth.

Since some material was obtained in a preserved state (*Ophiura albida* from the Öresund) it was necessary to assess the effects of the preservative, (formalin), upon the water and ash contents before using data from such sources. This is particularly necessary in view of the possibility that formalin may, over time, dissolve calcium carbonate skeletons.

For this test, twelve specimens of *Ophiocomina nigra*, ranging in wet weight from 0.98 grams to 3.67 grams were kept in 5% formalin for two months. These were then treated as for non-preserved animals, and the percentages of water and ash determined.

These results were compared with those obtained for specimens of *O.nigra* which had not been subject to preservation (see Table 6.1). The percent water content in the preserved individuals appears to be a little higher, while the amount of ash is about the same as in the fresh material. It is considered that percentage ash data from animals preserved in formalin may be used in comparisons. The value of results for water content must be assessed in the light of the differences as shown in Table 6.1.

The results for all the analyses are presented in tabular form and diagrammatically for ease of general comparison,(see Table 6.2 and Figure E).

6.3 Results:

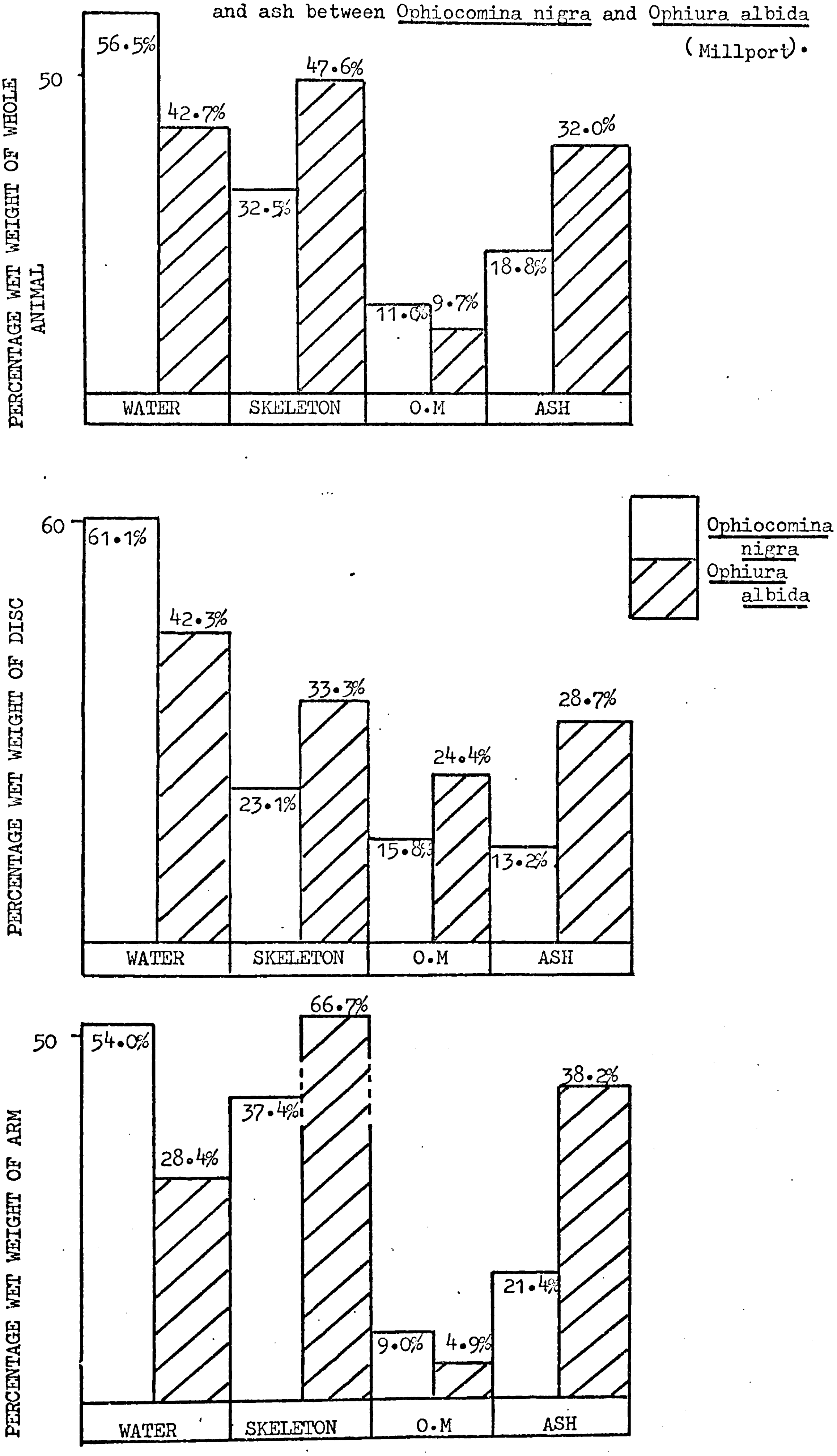
Whole specimen analysis

Whole specimens of *Ophiocomina nigra* have a greater percentage water content than whole specimens of *Ophiura albida*

Table 6·1 Comparison of percentage ash and percentage water contents in preserved and non-preserved (fresh) specimens of Ophiocomina nigra ·

CONDITION	% ASH	% WATER
Fresh	18·8 ± 1·3	56·5 ± 2·4
Preserved (in 5% formalin)	17·1 ± 5·9	60·6 ± 2·5

Figure E Comparison of percentage water, skeleton, organic matter(O.M) and ash between <u>Ophiocomina nigra</u> and <u>Ophiura albida</u> (Millport).



from Millport, (see Table 6.2).

From this Table it appears that *O.nigra* has a smaller percentage of its wet weight as skeleton than is recorded for *O.albida*. The percentage ash content, too, in the latter is greater than was found in *O.nigra.*

Disc analysis

The discs of *O.nigra* contain nearly 20% more water and about 10% less organic matter than those of *O.albida*. It was found that *O.albida* had slightly more ash as *O.nigra*.

Arm analysis

With reference to Table 6.2, the arms of *O.nigra* contain 54% water while those of *O.albida* contain about half this amount - 28%. Also, *O.albida* has about half the organic matter when compared with *O.nigra*. The larger ash content of *O.albida* in contrast to *O.nigra* probably reflects the larger percentage of skeleton recorded in *O.albida*.

If the disc and arms data are examined in terms of their contributions to the total percentages of ash, water and skeleton of the whole animal it is clear from Table 6.3 that in *Ophiocomina nigra* the disc contributes 30% water, 15% skeleton and 19% ash, while the arms contribute 70% water, 85% skeleton and 81% ash. *Ophiura albida* is broadly similar. The disc supplies 40% water, 18% skeleton and 24% ash, while the arms contribute 59% water, 82% skeleton and 76% ash (see Table 6.3).

Graph 6.1 shows water contents of *O.albida* sampled from different stations in Loch Etive, thus representing different salinity regimes. There seems to be little evidence of any water changes due to the influence of different salinities. This also applies to percentage ash as shown in Graph 6.2. Graph 6.3 displays the water contents for *Amphiura chiajei* sampled from stations E4, E6, E11 and E14 in Loch Etive, and indicates a higher percentage of water than *O.albida*. The ash content as shown in Graph 6.4 is of a similar level to that of *O.albida* - 24.3% and 27.9% for *A.chiajei* and *O.albida* respectively.

The *O.albida* from the Öresund have 35.8% water. This is lower than in those from Loch Etive, despite possible preservative

Table 6•2 Comparison of percentage water, skeleton, organic matter and ash in Ophiocomina nigra and Ophiura albida (Millport).

COMPONENT	Ophiocomina nigra				Ophiura albida			
	% water	% skeleton	% O•M	% ash	water	% skel -eton	% O•M	% ash
Whole animal	56•5 ± 2•4	32•5 ± 4•6	11•0	18•8 ± 1•4	42•7 ± 5•6	47•6 ± 8•2	9•7	22•0 ± 4•4
Disc	61•1 ± 3•3	23•1 ± 3•7	15•8	13•2 ± 1•6	42•3 ± 5•0	33•3 ± 2•3	24•4	18•7 ± 5•5
Arm	53•6 ± 3•2	37•4 ± 2•3	9•0	21•4 ± 1•5	28•4 ± 6•5	66•7 ± 2•3	4•9	28•2 ± 5•2
Regenerating part of arm	51•7 ± 4•3	36•0 ±3•9	12•3	ND	ND	ND	ND	ND

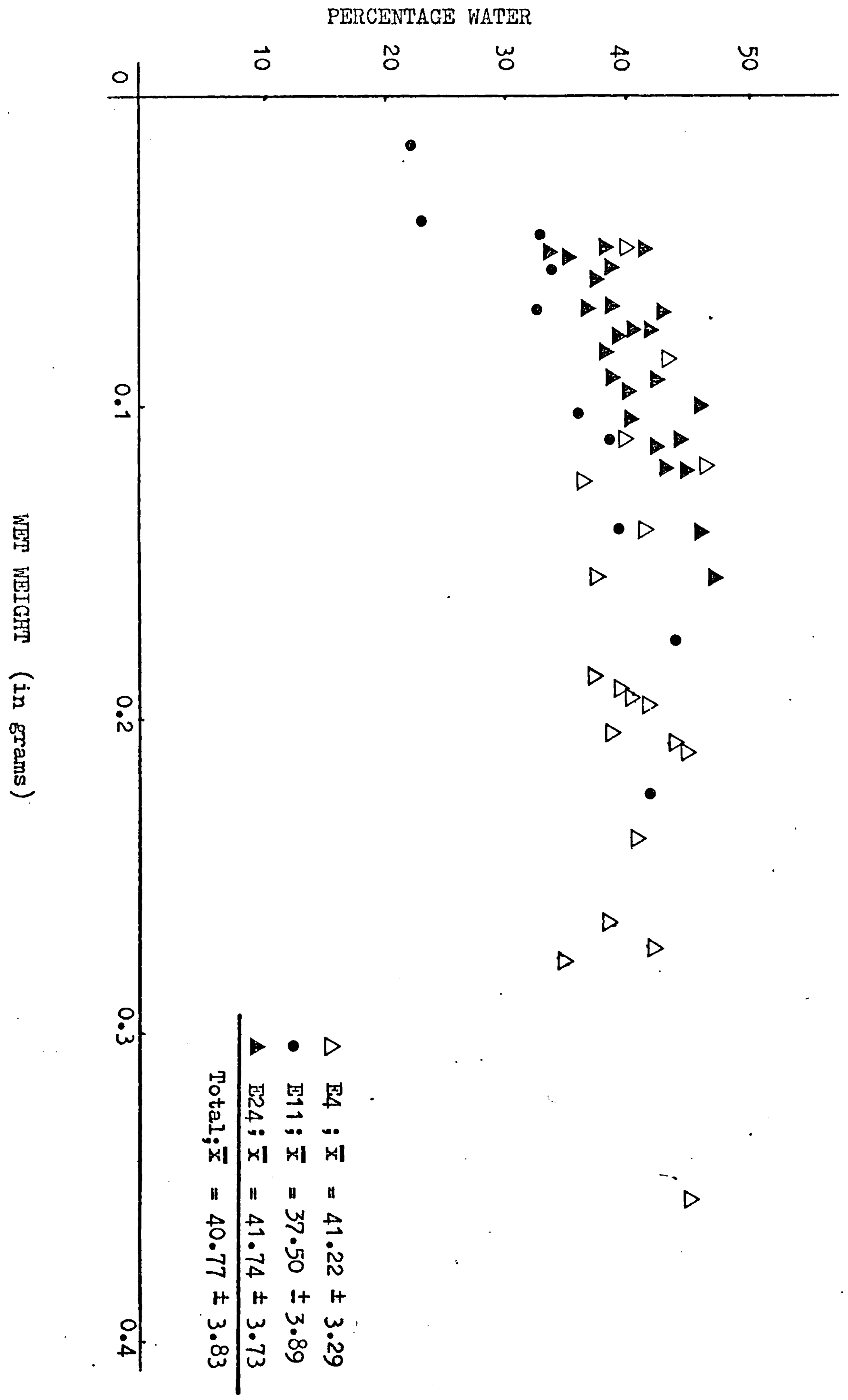
ND - Not determined

O•M - Organic matter

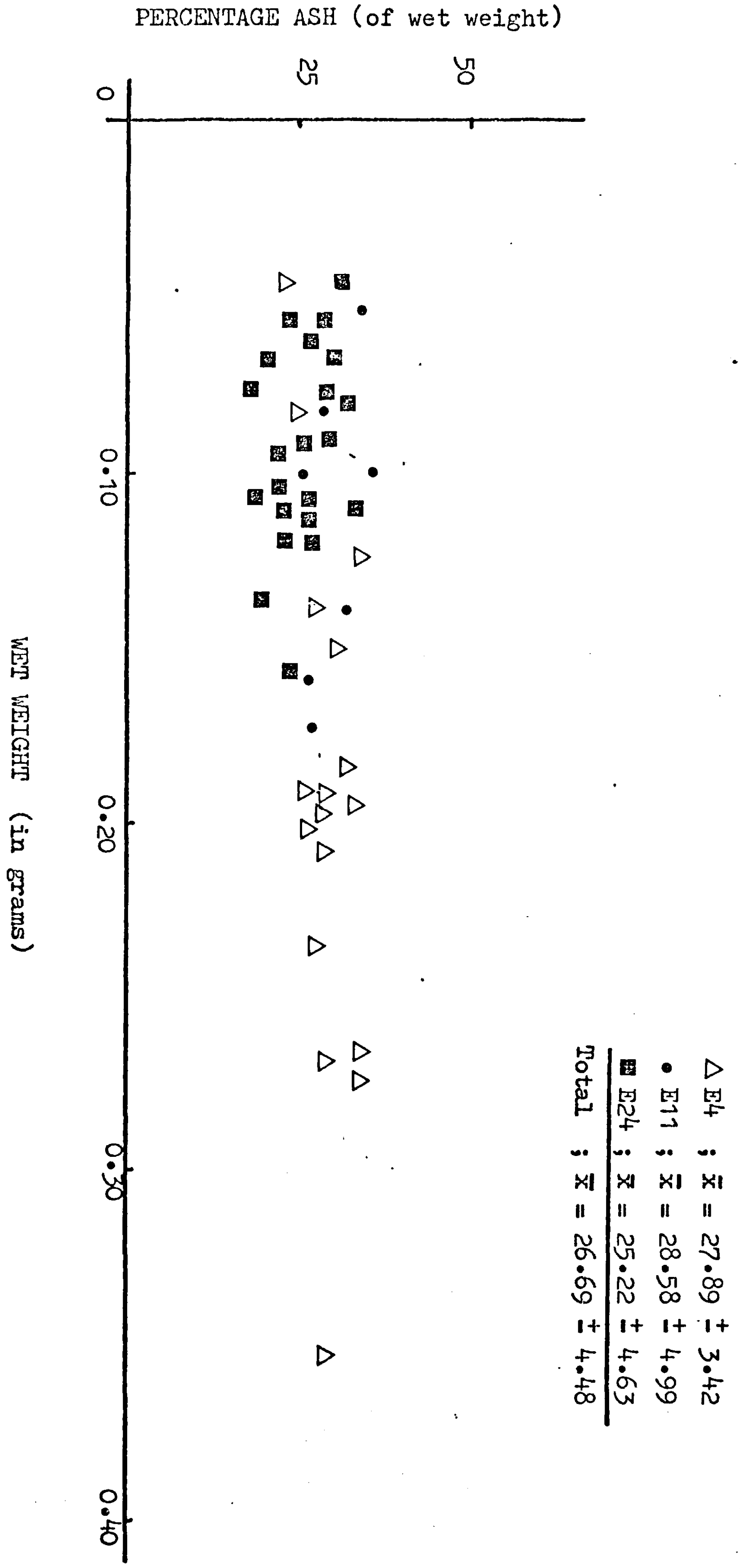
Table 6.3 Comparison between *Ophiocomina nigra* and *Ophiura albida* with respect to percentage water, skeleton and ash in the disc and in the arm .

COMPONENT	*Ophiocomina nigra*			*Ophiura albida*		
	% water	% skeleton	% ash	% water	% skeleton	% ash
DISC	29.56 ± 4.16	14.70 ± 3.93	19.17 ± 2.33	40.45 ± 5.79	17.80 ± 2.06	24.00 ± 1.64
ARM	70.40 ± 4.16	85.27 ± 3.93	80.80 ± 2.33	59.44 ± 5.56	82.19 ± 2.06	75.99 ± 1.64

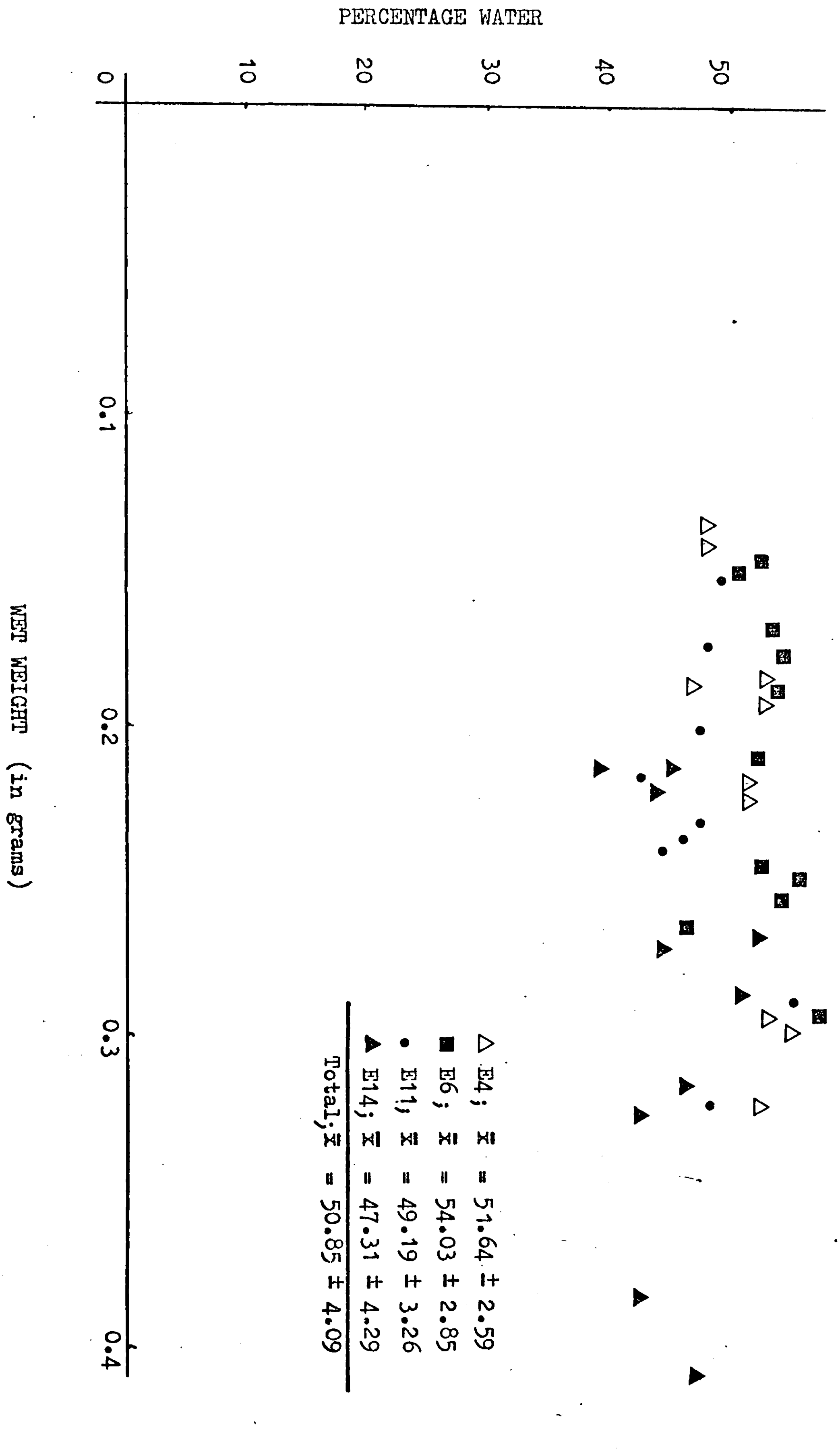
Graph 6.1 Percentage water content in <u>Ophiura albida</u> from different stations within Loch Etive.



Graph 6.2 Percentage ash content in _Ophiura albida_ from different stations within Loch Etive.



Graph 6.3 Percentage water content in Amphiura chiajei from different stations within Loch Etive.



Graph 6.4 Percentage ash content in Amphiura chiajei from different stations within Loch Etive.

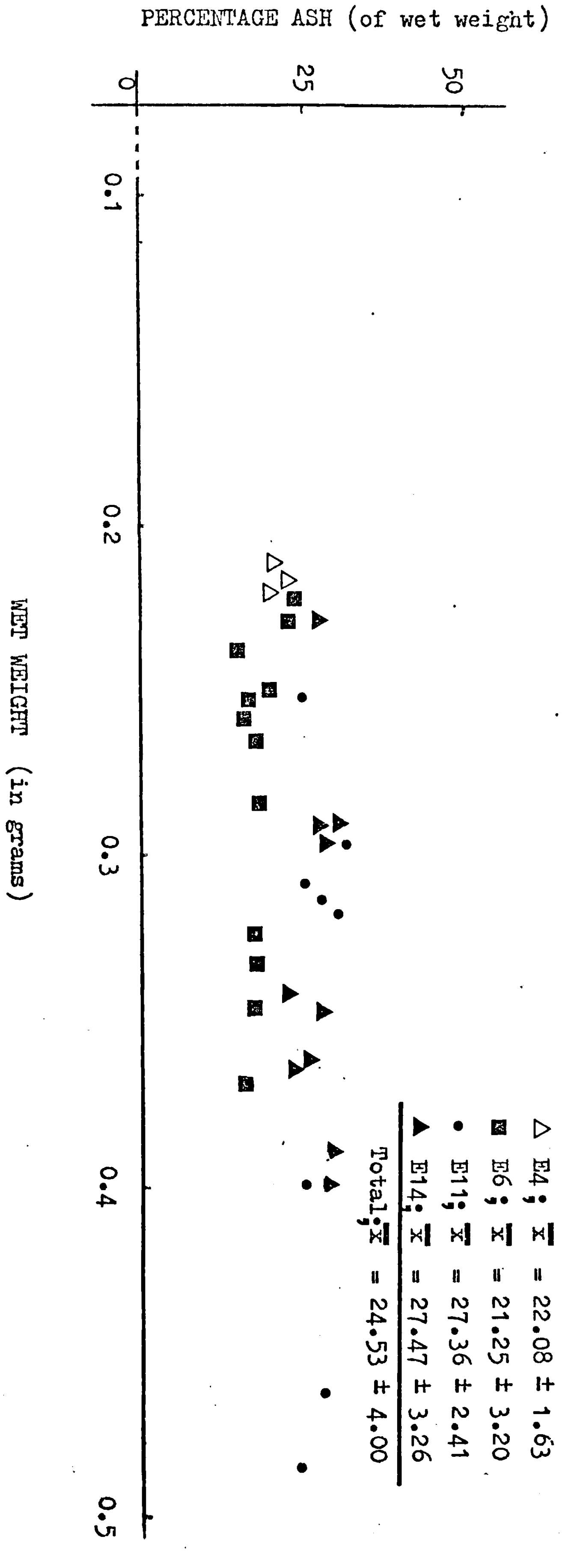


Table 6.4 Comparison of percentage water and ash between _Ophiura albida_ populations from Loch Etive and the Öresund.

LOCATION	% WATER	% ASH
Loch Etive	40.77 ± 3.38	26.69 ± 4.48
Öresund	35.27 ± 4.59	33.34 ± 3.48

effects. Individuals from the Öresund have an ash content which is greater than in the Loch Etive specimens - 32.2% and 27.9% respectively (see Table 6.4).

6.4 Discussion:

Schlieper (1957) noted differences in percentage water and ash contents between the Baltic and North Sea individuals of Asterias rubens. It would be pertinent to examine the results in the light of the salinity regimes experienced by the various species.

Firstly, it is necessary to compare an oceanic brittle-star, represented by Ophiocomina nigra from Plymouth, with one which is considered to be euryhaline - Ophiura albida from Millport. It is immediately clear that O.nigra has a greater water content in the whole animal than does O.albida , whilst it has less skeleton and ash. From this, one might conclude that in order to tolerate reduced salinities it would be advantageous to increase the less osmotically active component in order to reduce the degree of hydration which might result from lowered salinity.

Ophiura albida, from Millport, has a slightly higher amount of organic matter and 13% more skeletal material. This additional skeletal component is presumably osmotically and ionically inactive while the higher organic matter probably represents the additional musculature necessary to articulate the extra skeleton.

If this proposed correlation between salinity and the percentage of osmotically inactive component is correct, then one would expect to find evidence of a similar trend, at least, in O.albida from Loch Etive and the Öresund. Amphiura chiajei, too, should show similar characteristics.

In O.albida, from Loch Etive, it is found that there is an overall water content which is similar or slightly lower than in O.albida from Millport. The latter population has similar or slightly lesser amounts of ash than those from Loch Etive. Amphiura chiajei, which is not considered to experience quite the same degree of hyposmotic salinity stress as O.albida (Loch Etive) has more water than this species but less than O.nigra. This would be consistent with the view that the Loch animals are recently derived from a fully marine population and with the concept that A.chiajei is less likely, by virtue of its burrowing habit, to be subjected to the lowered salinities which may be experienced by the more mobile O.albida.

Ophiura albida, from the Öresund, comes from conditions where, it is thought, the salinity reduction and fluctuation have been over a longer time scale resulting in a greater stability in salinity than is found in Loch Etive. These animals have less water and more ash than the Loch Etive O.albida.

One might suppose from this that the Öresund animals may represent a more advanced stage than the Etive animals in adaptation to reduced salinity.

GENERAL DISCUSSION :

The Echinodermata are, perhaps, not the rigidly stenohaline group of marine invertebrates which has been the view concluded by the classical textbooks. There are many examples of echinoderms which are known to penetrate or tolerate water of reduced salinity. Most work has been confined to considerations of the Asteroidea and the Echinoidea with some concerning the Holothuroidea. However, despite the abundance of reports regarding such penetration by ophiuroids, little work has been carried out upon these particular echinoderms. The present study considers the ophiuroids with respect to penetration into waters of lowered salinity and provides a platform for future work.

Like some asteroids, for example, Asterias rubens, the two ophiuroid species, Ophiocomina nigra and Ophiura albida, behave as osmometers when placed in diluted sea water. However, when in comparable dilutions of sea water the wet weight of Ophiura albida from Millport does not increase to the same extent as that in Ophiocomina nigra. This is possibly a reflection of the potential of O.albida to tolerate some decrease in salinity.

If the population of O.albida from Loch Etive is considered in these terms it is clear that these ophiuroids from brackish water have developed more fully the potential which appears to be present in O.albida from Millport, namely the ability to tolerate lowered salinity. Presumably, continuous exposure to depressed salinities could induce O.albida from Loch Etive to further develop the potential displayed by individuals of the same species from full strength sea water.

It is proposed that in terms of euryhalinity Ophiocomina nigra is less euryhaline than Ophiura albida from Millport which is less than Ophiura albida from Loch Etive.

Further evidence for this view is obtained if the percentages of water and ash of these ophiuroids are considered in the light of the salinity regimes experienced by these various species. The O.albida from Millport contain less water and a greater percentage of skeleton than O.nigra. This is in accord with the present scheme that O.albida is more euryhaline than O.nigra. The skeleton is presumably less osmotically active and so, if an ophiuroid has a greater percentage of skeleton, which would reduce the degree of hydration of the ophiuroid, then there is less material in the body to be affected by changes in the external osmotic pressure.

That O.albida from Loch Etive would have more skeleton than the same species from Millport may pre-suppose a better extraction efficiency for calcium ions. Certainly, as the ambient salinity decreases so does the available calcium (see Table 7.1). It will be noted from Graphs 7.2 to 7.36 that similar calcium concentrations were found in all the ophiuroids assayed irrespective of external concentration, species or bodily component analysed.

This concept of increase in percent skeleton as the salinity falls is further supported by the data for the group of O.albida from Loch Etive which have less water and more ash than the O.albida from Millport. So again evidence is provided for an order of euryhalinity between these three groups of ophiuroids, namely:

O.nigra < O.albida (Millport) < O.albida (Loch Etive)

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O.nigra < O.albida (Millport) < O.albida (Loch Etive)

In the Western Baltic Sea, Ophiura albida are found in salinities which are generally lower than those experienced by the same species in Loch Etive. It is of interest that the individuals from the Baltic Sea have less water and more ash than those from Loch Etive. This is also consistent with the view that an increase in the less osmotically and less ionically active component of the body material and a decrease in the water content is advantageous when penetrating brackish water. One might further extend the scheme of relative euryhalinity thus:

O.nigra < O.albida(Millport) < O.albida(Etive) < O.albida(Baltic)

Thus, O.albida from the Baltic Sea may represent a more advanced stage than those from Loch Etive in adaptation to a hyposmotic environment.

Salinity tolerance experiments confirm the above scheme in terms of the proposed relative euryhalinities. It has been experimentally established that in order of tolerance to lowered salinity the first three stages are correct.

It would appear that O.albida from Loch Etive, by virtue of their greater tolerance, have exploited the potential indicated by the species from Millport in order to withstand the salinities prevalent in Loch Etive. It is of interest that the lower and upper basins of the loch have slightly different salinity regimes and this is reflected by variation in the tolerances shown by individuals from the lower basin (E2 and E6) and from the upper basin (E11 and E14). These differences, however, are not reflected by changes in percentage water and ash between the various stations in Loch Etive. There would seem to be at least two possibilities related to this point. Either the differences in salinity between stations are not sufficiently defined to be associated with changes in water and ash or, there has been an insufficient period of sojourn under the present Loch conditions in which water and ash changes could be developed.

Amphiura chiajei, which would appear to occur under similar salinity conditions as Ophiura albida in Loch Etive, has a different salinity tolerance when compared with the latter which probably reflects the differences in position, within the Loch, of these two species. Whereas O.albida lives on the surface of the mud, A.chiajei lies buried, often several centimetres, within it and is thus considered to be removed from the salinity stresses encountered by O.albida. Further evidence for this view is suggested by the relative percentages of water and ash of these two species. A.chiajei has more water and less ash than O.albida from Loch Etive. There could be as much as 5 - $6^{\circ}/oo$ difference between the salinity of the water interstitially and overlying the mud - such differences are thought to occur in Whitewater Bay, Florida (Stancyk, personal communication); A 5 - $6^{\circ}/oo$ difference could be sufficient to induce the water and ash differences between A.chiajei and O.albida.

The differences in percent water and ash, weight fluctuation and salinity tolerance between O.albida from normal salinities and from Loch Etive pose the question of the existence of a physiological race. Adaptation experiments in which O.albida from Loch Creran (with a salinity regime equivalent to that of Millport) were not able to be acclimatized to a salinity equivalent to that of station E24 in Loch Etive. O.albida from E24 could be adapted, quite rapidly, to full salinity conditions which suggests that the Loch Etive O.albida could be derived from populations experiencing normal salinities. Furthermore, they are surviving the salinities of Loch Etive by adaptations which extend those capacities present in O.albida from normal salinities. It is not considered that these adaptations have led to the production of a physiological race. No cross breeding experiments between O.albida from reduced salinities (Loch Etive) and normal salinities (Loch Creran) were able to be carried out; such experiments would throw more light upon whether or not the O.albida in Loch Etive are becoming physiologically isolated from the fully marine O.albida, such as from Loch Creran or Millport.

In conclusion, penetration into the brackish waters of Loch Etive by Ophiura albida appears to be associated with changes in water uptake, percentages of body water and ash, and increases in tolerance to reduced salinities.

CHAPTER SEVEN

INVESTIGATION INTO THE CONCENTRATIONS OF STRONTIUM AND CALCIUM IN Ophiocomina nigra, Ophiura albida AND Amphiura chiajei.

7.1 Introduction:

Strontium, which was named after the village of Strontian in Argyllshire, Scotland, where it may have been first discovered by Kirwann and Klaproth in 1793 (Partington 1946), was later isolated by electrolysis by Davey in 1808 (Weast, 1964). It was shown to occur in marine organisms in the last century (Moretti, 1813; Vogel, 1814; Forchammer, 1852). Accurate quantitative determination of small amounts of strontium has, however, presented many difficulties due to the similarities between this element and calcium which make chemical separation difficult. Vinogradov (1953) has collected and reviewed analyses of marine animals and plants. These data are often of limited value with respect to strontium concentration due to the insensitive, analytical procedures which were then available. The early spectographic methods and the nitrate method (Miyake, 1939b) gave values for strontium concentration in sea water which are now considered to be too high, probably due to the interference effects of the ions calcium and barium. More recent methods include:

1. Radiochemistry and flame photometry (Smales, 1951)
2. Radioactivation and ion exchange separation (Böwen,1956)
3. Isotope dilution and radioactivation techniques (Hummel and Smales, 1956)
4. Flame photometry and oxalate precipitation (Odum, 1957; Sugawara and Kawasaki, 1958)
5. Flame photometry using an 'internal standards' technique (Thompson and Chow, 1955)
6. Emission spectroscopy and absorption spectroscopy (Culkin and Cox, 1966; Riley and Segar, 1970; Gibbs and Bryan, 1972).

In this study, atomic absorption spectroscopy was employed using lanthanum chloride to suppress the effects of interference due to the presence of anions such as phosphates.

Data on the strontium levels in the calcareous and soft tissues of various members of the Echinodermata have been reported, (Banbury *et al*, 1950; Thompson and Chow, 1955; Mauchline and Templeton, 1966; Riley and Segar, 1970).

Results of strontium determinations have been presented in two main ways - as a concentration of the element, for example ppm/dry weight *per se*, or, in the form of a ratio of the number of atoms of strontium to those of calcium. In the latter presentation, the atomic weights of each element must be considered. Presentation as a ratio demonstrates more clearly the relationship of strontium to calcium. Since strontium and calcium have similar but quantitatively different solubility products and ionization constants, their respective behaviours vary in many phases of their cycles. One of the elements may act as a control upon the other, for the ratio changes only when a process acts upon the elements specifically and differentially, for example during the incorporation of the ions within, say, echinoderm ossicle. Subsequent presentation of the Sr/Ca atom ratio in the form of an atomic accumulation or discrimination ratios immediately allows observation as to whether a given element is being concentrated or rejected with respect to the same concentration of the element in sea water.

Black and Mitchell (1952) define an accumulation factor (A) as

$$\frac{\text{ppm element in the dry organism}}{\text{ppm element in the ambient sea water}}$$

while Spooner (1949) uses a more complex formulation based upon the effective dilution of sea water within the element per gram of the organism.

Bowen (1956) defines a discrimination factor 'D' for the uptake of a pair of related elements 'X' and 'Y' as

$$\frac{\text{X/Y in the dry organism}}{\text{X/Y in the ambient sea water}}$$

so that

$$D\ (X,\ Y) = A(X)/A(Y)$$

Odum (1957) calculated a distribution factor but does not say what units were used to calculate this factor.

Mauchline and Templeton (1966) use a concentration factor derived from the expression

$$\frac{\text{mg of the element/gram wet weight}}{\text{mg of the element/ml of sea water}}$$

Direct comparison of earlier work is possible to a certain extent though authors have presented data using different units. A few authors have given accumulation factors for the organisms they have analysed (Bowen, 1956; Odum, 1957; Mauchline and Templeton, 1966). The latter two have reported factors for some members of the Echinodermata. Calculations of such factors from the data supplied by other workers is generally a little imprecise since one has to make an assumption as to the concentration of strontium in the water from which the organisms were sampled. This has been done for the work of Riley and Segar (1970) and Banbury _et al_ (1950).

A discrimination ratio has been employed in the present study and is given by:

$$\frac{\text{Atom ratio of Sr/Ca of the sample}}{\text{Atom ratio of Sr/Ca of the sea water}}$$

so that, clearly, if the value obtained is less than one then the tissue is rejecting strontium, and if the value is greater than one then the tissue is selecting for the strontium ions.

Levels of strontium in echinoderms, which have been reported in the literature, appear to be fairly uniform. Banbury et al (1950) provide one of the first reports of a Sr/Ca atom ratio to be determined in echinoderms. Thompson and Chow (1955) carried out an extensive analysis of echinoderms and report an Sr/Ca atom ratio for the calcareous components of between 2.56 and 2.74 for the Ophiuroidea. Such figures are to be taken as meaning that there are between 2.56 and 2.74 atoms of strontium for every 1000 atoms of calcium.

Discrimination ratios for the hard tissues of the ophiuroids are given as 0.30 by Thompson and Chow (1955). Odum (1957) does not find such a uniform series of ratios for the Echinodermata. He reports a Sr/Ca atom ratio of 3.47 and gives a 'distribution factor' of -2.6 which gives an equivalent discrimination ratio, using the Sr/Ca atom ratio for sea water quoted in Odum (1957a) of 0.35.

Mauchline and Templeton (1966) present similar findings and give a Sr/Ca atom ratio for whole echinoderms within the range 2.3 to 2.8. Concentration factors are given for strontium and calcium which, when reinterpreted, give a ratio of 2.8 for ophiuroids. The mean value of Sr/Ca atom ratios for sea water cited in their work has been used to calculate a discrimination ratio for the ophiuroids of 0.32.

Riley and Segar (1970) carried out multi-element analyses of some echinoderms and noted that the strontium contents of the two halves of the asteroid Solaster papposus were significantly different, though the calcium concentrations were of the same order. The Sr/Ca atom ratios of 0.54 to 1.80 are much lower than those presented in earlier work. One unidentified ophiuroid was assayed and had a Sr/Ca atom ratio of 1.07 giving a discrimination ratio, calculated from data provided in the paper as 0.12, which is much lower than the discrimination ratio that has been reported previously.

Factors which may affect the Sr/Ca atom ratio and have been investigated by many workers include:-

1. Phylogeny - Thompson and Chow (1955); Turekian and Armstrong (1960).

2. Skeletal mineralogy - Clark and Wheeler (1922); Hariss (1965); Lowenstam (1954); Thompson and Chow (1955); Odum (1957b).

3. Temperature - Pilkey and Hower (1960); Dodd (1965); Lerman (1965); Hallam and Price (1968).

4. Salinity - Rucker and Valentine (1961); Pilkey and Goodell (1963).

5. Intertidal position - Pilkey and Hariss (1966).

6. Growth rate - Swan (1956).

Despite the many factors which may influence the Sr/Ca atom ratio of marine organisms there seems to be some correlation between the Sr/Ca atom ratio of the calcareous skeleton and that of the environment, (Kulp et al 1952; Dodd, 1967; Odum, 1951a, 1951b, 1957b).

Kulp et al (1952) state that the main factor determining the Sr/Ca atom ratio is the Sr/Ca atom ratio of the water from which the calcium carbonate is laid down. The ratio is ultimately related to the source of the mineral and the salinity of the water. However, Thompson and Chow (1955) found that the marine organisms living in the same ecological niche accumulate calcium and strontium in their calcareous skeletons in very different proportions. They suggest that a very definite relationship exists between the atom ratio and the phylogenetic classification of the organism. They analysed a series of echinoderms ranging from intertidal to deep water habitats and found a variation in the Sr/Ca atom ratio from 2.63 to 2.89. This is a remarkably uniform value despite the variation in habitats and they suggested that certain marine organisms will have a constant Sr/Ca atom ratio in their calcareous skeletons regardless of habitat. They do not, however, provide data concerning the ambient Sr/Ca atom ratio of the various habitats.

Odum (1957a) showed that the calcarous shell of Physa, a pulmonate gastropod, is directly proportional to a range of artificial sea waters with a varying concentration of strontium. Samples of waters from the various oceans have a constant ratio between strontium and chlorinity and between calcium and chlorinity. Thompson and Chow (1955) state that the Sr/Ca atom ratio would be constant despite the ambient salinity and suggest that a lowering or raising of the salinity (within an acceptable range to the organism) would not be of major importance in determining the ratio of calcarious skeletons. Gibbs and Bryan (1972), sampling in the coastal lagoons of Barbuda showed that the ratios vary with chlorinity according to the relative effects of fresh water run-off and evaporation in the various localities sampled.

Riley and Segar (1970) provide the only data of strontium concentrations which occur in the soft tissues of some echinoderms. With the exceptions of gonad and pyloric caecae the Sr/Ca atom ratio is fairly similar to that which is found in the skeleton.

Besides the chemical composition of the environment, it is considered that the mineralogical character of the species is important to the Sr/Ca atom ratio which is developed by the animal (Thompson and Chow, 1955). These authors state that there is always more strontium present in a calcarous skeleton when the calcium carbonate exists as aragonite rather than as calcite. Kulp et al (1952) states that this is because the crystal lattice of calcite is "less amenable" to strontium than the lattice of aragonite crystals.

For instance, a pelecypod mollusc which lays down calcite has a Sr/Ca atom ratio of 1.22, while a gastropod mollusc which lays down aragonite has a Sr/Ca atom ratio reported as 10.0 (Thompson and Chow, 1955).

All echinoderms which have been mineralogically analysed to date lay down calcium carbonate in the form of calcite (Thompson and Chow, 1955). Since only echinoderms have been considered in the present study little attention has been paid to mineralogy when comparing different ophiuroid species with respect to Sr/Ca atom ratios.

7.2 Materials:

In this study of strontium and calcium distribution in ophiuroids the following species were used:

Ophiocomina nigra (Plymouth)

Ophiura albida (Millport, E24, E11, E4)

Amphiura chiajei (C12, E14, E11, E6, E4)

Stations E- refer to the sampling stations in Loch Etive and Station C12 refers to the sampling site in Loch Creran (see Map, page 91). Water samples were obtained at these stations at the sampling depth by the use of a five litre water bottle.

7.3 Treatment of samples prior to analysis:

Unless stated otherwise, only intact animals, i.e. those with a full complement of entire arms, were used for these analyses. These animals were kept for at least two days to allow a period of adjustment to the new conditions. This period of adjustment facilitates the voiding of any waste matter and digestion of food particles present in the gut which may otherwise unduly affect or mask the actual Sr/Ca atom ratio. Failure to do this may account for anomalous results in previous work.

After two days, the animals for analysis were placed upon a bed of tissue paper and lightly dabbed with another tissue to remove excess moisture. The effectiveness of this method of removing excess water has been assessed previously (Page 45). The animals were then weighed on an Oertling balance (Model No.V20) to give a wet weight for the ophiuroid.

There are three main methods for obtaining a dry weight (Holmes and McIntyre, 1971):-

1. Heating to 100°C at atmospheric pressure

2. Drying in vacuo at 60°C

3. Freeze drying

A compromise between drying at too low a temperature, which may permit some tissues to slowly autolyse, and too high a temperature, which may facilitate a loss of the more volatile components of the soft, organic tissues was necessary (Paine, 1964). Heating to 85°C at atmospheric pressure was thought to be suitable. Drying to constant weight was complete between ten and fifty hours depending upon the size of the sample under test.

The dried sample was then either sealed in plastic bags and stored in a deep freeze at -20°C until required, or was immediately assayed. Most of the Scottish sea loch and all the Baltic Sea material was stored initially.

The next stage in the procedure, prior to assaying, was ashing. The samples were ashed in silica crucibles by burning off the organic matter in a muffle furnace. Before use, the crucibles were scraped, washed in distilled water and heated to constant weight for six hours. Heating for six to twelve hours at 600°C was sufficient to render the sample in its ash form. The actual time depended upon the initial sample size.

With the possible exception of the echinoids, the skeleton cannot be mechanically separated from the organic fraction so chemical separation is necessary which unfortunately destroys the soft tissues. To obtain skeletons free of organic matter the sample, after drying and weighing, was gently heated in a solution of 10% by weight of potassium hydroxide at 90°C for thirty to forty hours. The resulting liquor was poured off and the skeleton, which had been reduced to individual ossicles, was washed in distilled water and left to dry on filter paper. Any residual organic matter, after such treatment, would be insignificant in its effect upon the final result.

After muffling, the samples were left to cool and then dissolved by adding hydrochloric acid (specific gravity 1.18) drop-wise until dissolution was completed. The resulting solution was rinsed, with distilled water, into a volumetric flask. The capacity of the flask was determined by the final concentration which was required such that when the volume was made up to the mark with distilled water the concentration of strontium in the unknown lay within the range 0ppm - 30ppm, where the calibration curve was linear. Subsequent dilution brought the concentration of the unknown within the range 0ppm - 50ppm with respect to calcium. Prior to making the volume up to the mark, a certain volume of lanthanum chloride was added so as to produce a final concentration of 6000 ppm of lanthanum chloride. A concentration of 6000 ppm Lanthanum chloride has been found to be sufficient to reduce interference to negligible proportions (Unicam Instruments Manual, 1966).

7.4 Interference in atomic absorption spectroscopy

In this study, strontium and calcium ion concentrations were determined by atomic absorption spectroscopy using an SP90 Unicam atomic absorption spectrophotometer (see Appendix 3 for specification and operational conditions).

Because no two elements have resonance lines of the same wavelength, atomic absorption spectroscopy is one of the most specific analytical methods. Although each element is different, the response of one element may well be quantitatively affected by the presence of others, particularly anions. The effect of such interference is to decrease or increase the number of available free atoms undergoing energy transformations (forming temperature stable compounds) in the flame.

There are four main methods of reducing and eliminating such interference (Unicam Instruments Manual, 1966).

1. By the addition of an element which forms a stable compound with the interfering anion

2. By the use of complex agents, such as EDTA, which inhibit the formation in solution of compounds stable at high temperatures

3. By adding an excess of the interfering element to the samples and the standard solutions and thus cancelling the effect of interference

4. By removing the interfering constituents, for example by an ion exchange column

Anions, such as phoshate, sulphate, silicate and aluminate all depress the absorption and emission of the alkaline earth metals because of the stability of their compounds. The depressive effect is less with acetylene (used in the present work) than with, say, propane since the degree of dissociation of these compounds is greater at higher flame temperatures. However, if another element, which also forms a stable compound with the interfering anion is added to the solution in excess, the required element is then released as atoms which are able to absorb their resonance energy. For example, if the concentration of phosphate is high enough to depress the absorption of calcium ions a large excess of lanthanum is added. Lanthanum phoshate is more stable than calcium phosphate and is formed preferentially preventing the depressive effect due to the phosphate anion (Unicam Instruments Manual, 1966).

In the present study method 1 has been adopted. However, since a high lanthanum concentration may have a slight suppressive effect upon the absorption of calcium, lanthanum chloride was added to both standard solutions as well as the unknown solutions.

7.5 Preparation of standard solutions:

To prepare a solution containing 10ppm of strontium, 1ml from a stock solution containing 1000 ppm was pipetted into a 100ml volumetric flask. To this flask was added x mls of lanthanum chloride to produce a concentration of 6000 ppm. The volume was made up to the mark with glass distilled water. The following strontium solutions were made up:-

2, 4, 6, 8, 10, 20 and 30 ppm in 6000 ppm lanthanum chloride.

A similar procedure was employed for the preparation of the calcium standard solutions which were:

10, 20, 30, 40 and 50 ppm in 6000 ppm lanthanum chloride.

These standard solutions were used to construct calibration curves. See Graph 7.1 for the amount of shift of such curves. A new curve was drawn for every twelve samples assayed. The linear parts of the curve were: for strontium 0 to 30 ppm, and for calcium 0 to 50 ppm. The unknown samples were diluted so that the spectrophotometer readings lay within the linear parts of both the strontium and calcium curves.

7.6 Treatment of Data:

(a) To determine concentration of strontium in an unknown sample.

Dry weight of sample = 1.7464 grams
Reading from calibration curve = 8 ppm $\equiv$ 8 mg
Solution of ash contains = 8mg/l
Volume of volumetric flask = 25 mls
Actual weight of strontium in ash

$$\frac{25}{1000} \times 8 = 0.2 \text{ mg } Sr^{2+}$$

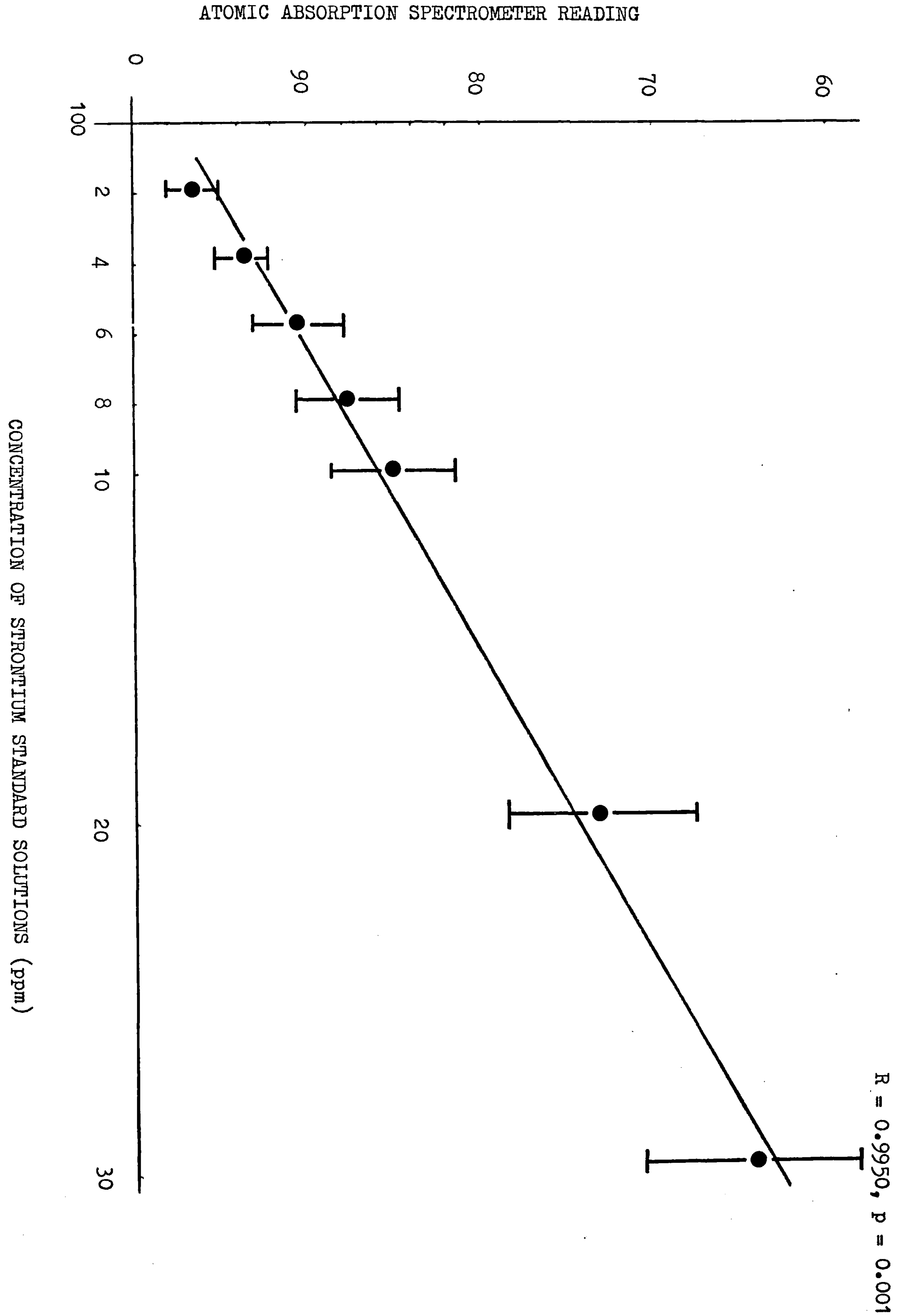
1.7464 gram animal contains

$$\frac{0.2}{1.7464} \times 1000 = 114 \text{ mg/k}$$

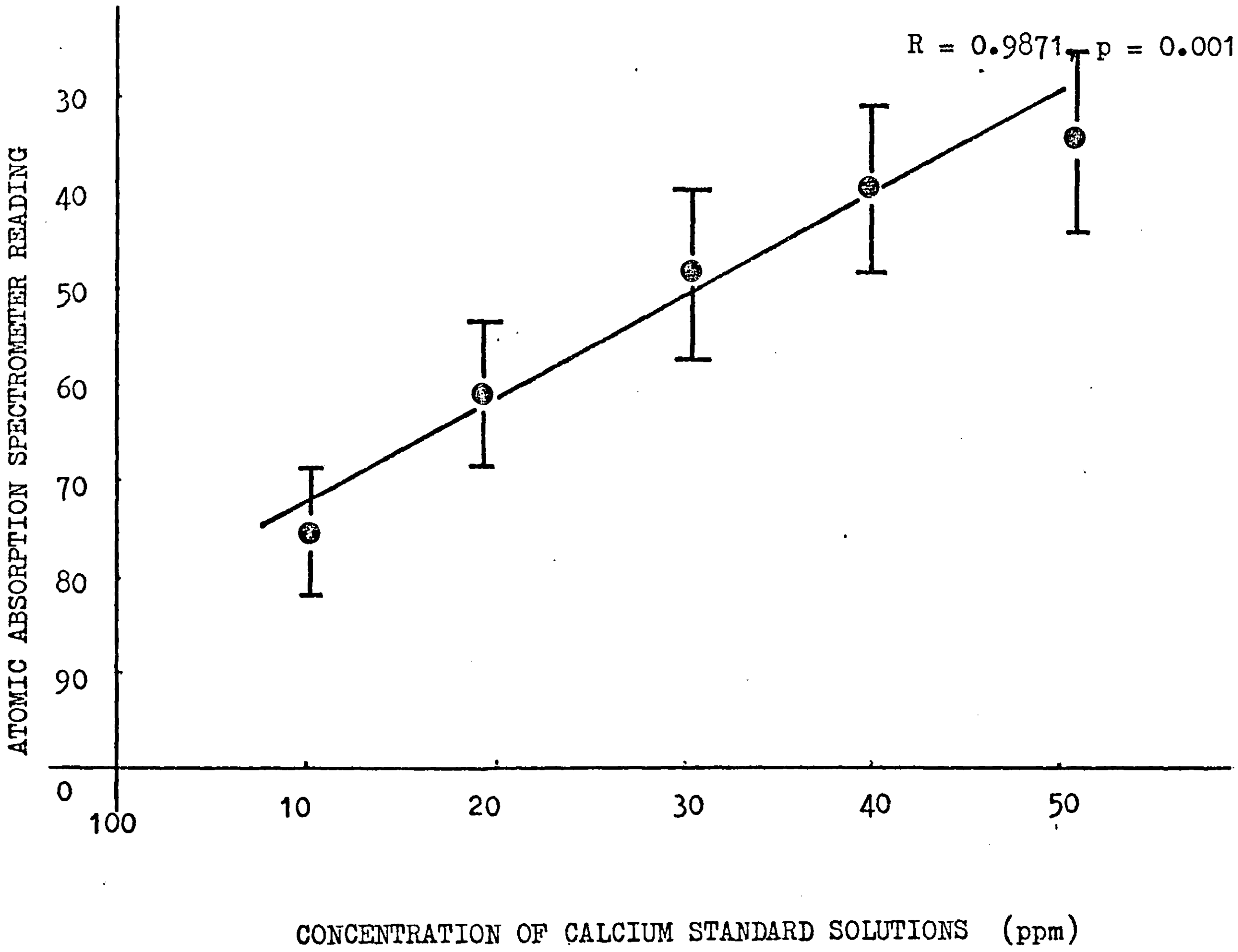
(b) To determine the concentration of calcium in an unknown sample.

Reading from calibration curve = 32 mg/l.

Graph 7.1(a) Calibration curve for strontium.



Graph 7.1(b) Calibration curve for calcium.



Volume of volumetric flask = 50mls, containing 1 ml of the solution, above.

Actual weight of calcium in ash =

$$\frac{50}{1000} \times 32 \times 25 = 40\text{mgCa}^{2+}$$

1.7464 gram animal contains

$$\frac{40}{1.7464} \times 1000 = 22{,}904 \text{ mg/k}$$

(c) To determine the strontium/calcium atom ratio per 1000 atoms of calcium.

Atomic weight of Strontium = 87.63

Atomic weight of Calcium = 40.08

$$\frac{114.52 / 87.63}{22{,}904 / 40.08} \times 1000 = 2.28$$

(d) To determine the Accumulation or Discrimination ratio:

$$\frac{\text{Sr/Ca atom ratio of sample}}{\text{Sr/Ca atom ratio of sea water}} = x$$

If 'x' is greater than one then the tissues are selecting for strontium and the converse if 'x' is less than one.

7.7 Results:

(a) <u>Ophiocomina nigra</u> - analysis of the calcareous parts showed that the Sr/Ca atom ratio is variable and is inversely proportional to the weight of the calcareous ossicles (Graph 7.2) which suggests that as the ossicles increase in size they incorporate less strontium. Comparing the contributions of the strontium and calcium concentrations it can be see (Graph 7.3) that, while the strontium level varies inversely with the dry weight, the calcium concentration remains fairly constant.

The arm ossicles have a Sr/Ca atom ratio which, too, is inversely related to the weight of the ossicles (Graph 7.4). This reflects the concentration of strontium which shows an inverse relationship to ossicle weight while the calcium content remains steady (Graphs 7.5).

The ossicles of the disc do not show a Sr/Ca ratio variation in response to an increase in dry weight (Graph 7.6). The levels of strontium and calcium are fairly uniform over the range of dry weight sampled (Graphs 7.7).

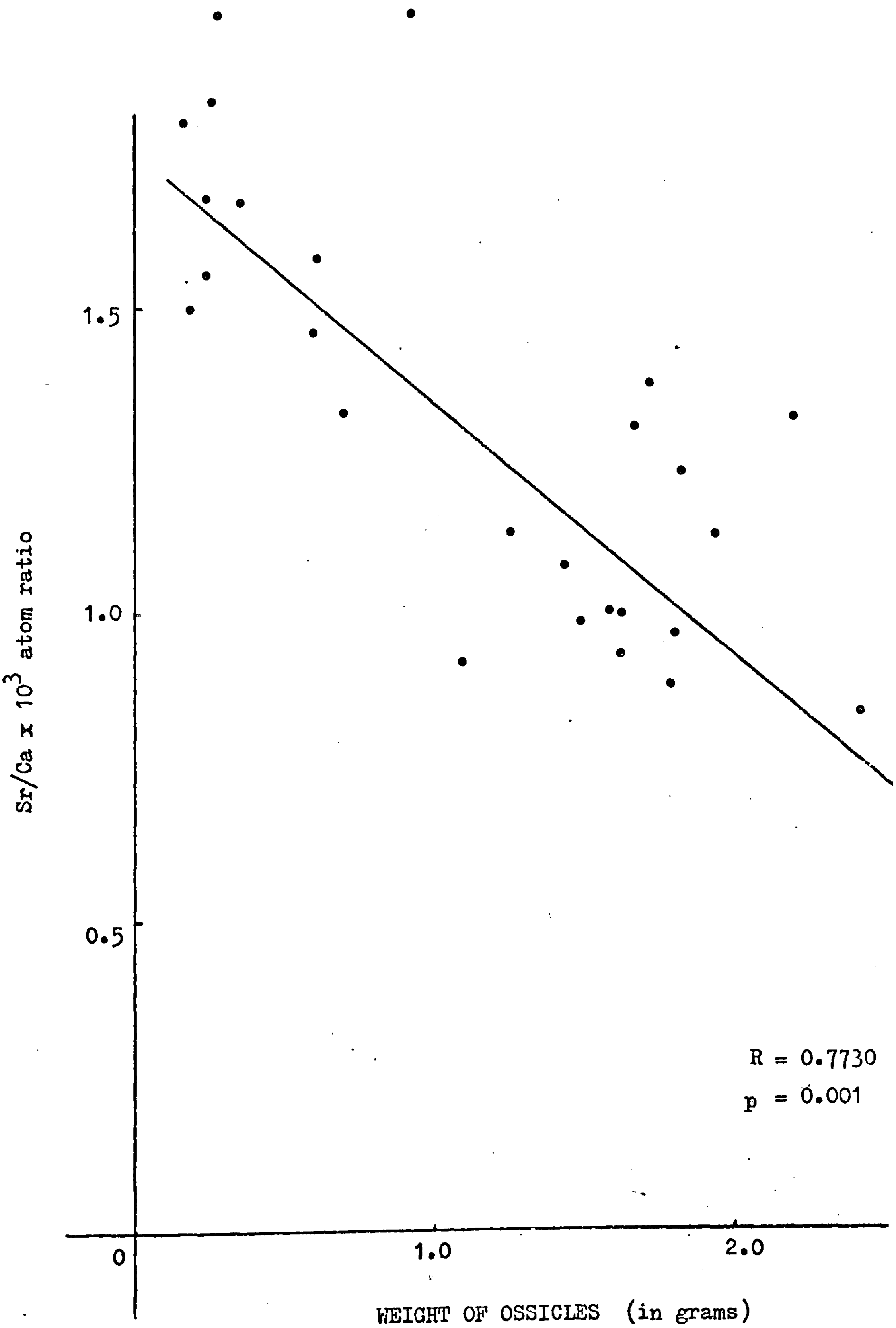
Illustrating the above results in graphical form it is clear that the Sr/Ca atom ratio of the arm ossicles and the ossicles as a whole are similar if equivalently sized animals are considered. The Sr/Ca atom ratio of the disc is a little higher. The strontium content of similar sized animals are equivalent in terms of whole and arm analyses. O.nigra generally has a similar concentration of strontium in the disc ossicles and in arm ossicles. The calcium contents of the arms and of the disc are of similar values.

Analysis of whole specimens of O.nigra from which the organic matter had not been chemically removed showed (Graph 7.8) that the Sr/Ca atom ratio varied inversely with the dry weight of the specimen. Strontium levels of whole O.nigra (Graph 7.9) are also inversely related to increasing dry weight while the calcium content is fairly uniform.

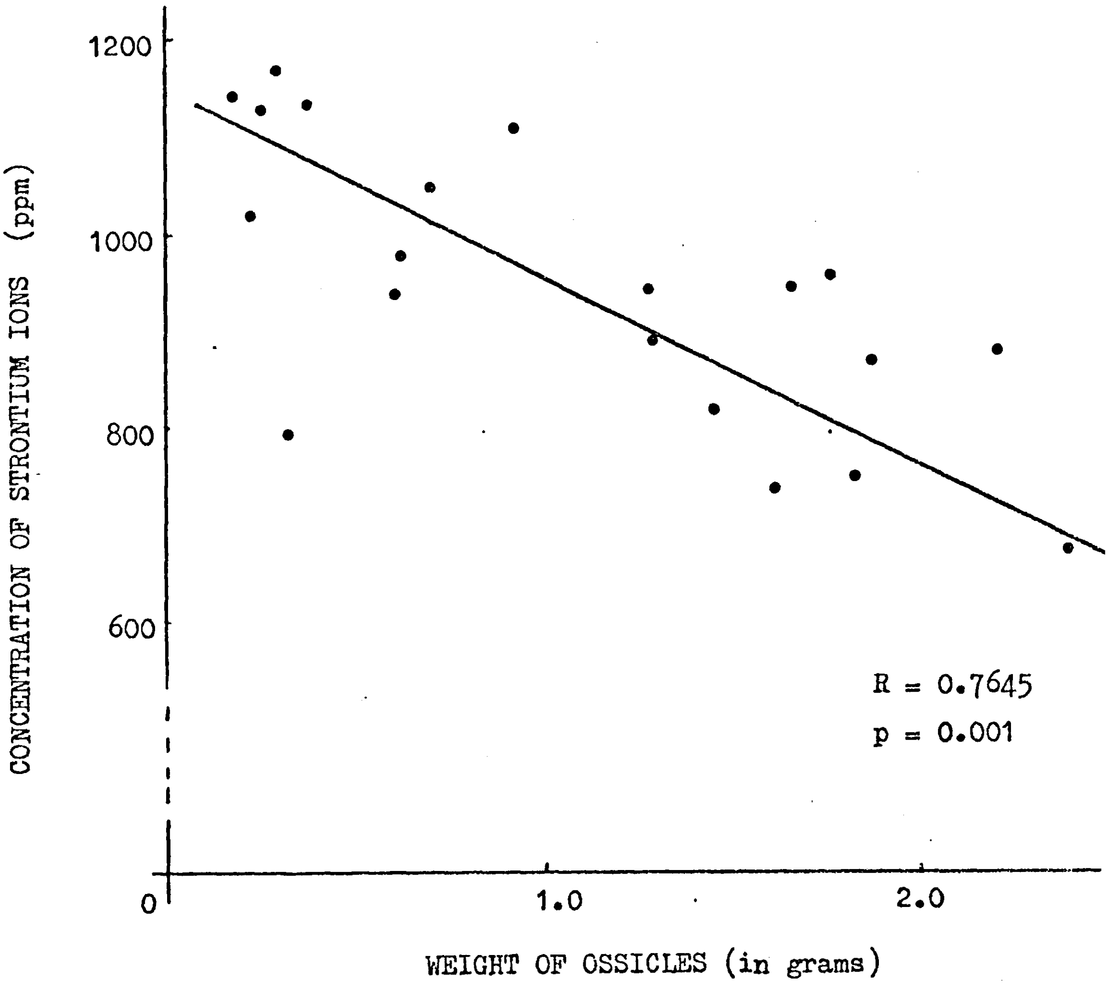
Comparison between the contribution of the disc and the whole animal in terms of the Sr/Ca ratio shows (Graph 7.10) that there is no inverse relationship with dry weight. Strontium and calcium concentrations of the disc do not appear to be related to dry weight (Graphs 7.11).

In contrast to the Sr/Ca atom ratio of the disc, the arms (Graph 7.12) show a relationship with dry weight; this reflects the inverse relationship of the strontium concentration and the uniform content of the calcium ions within the arm (Graphs 7.13).

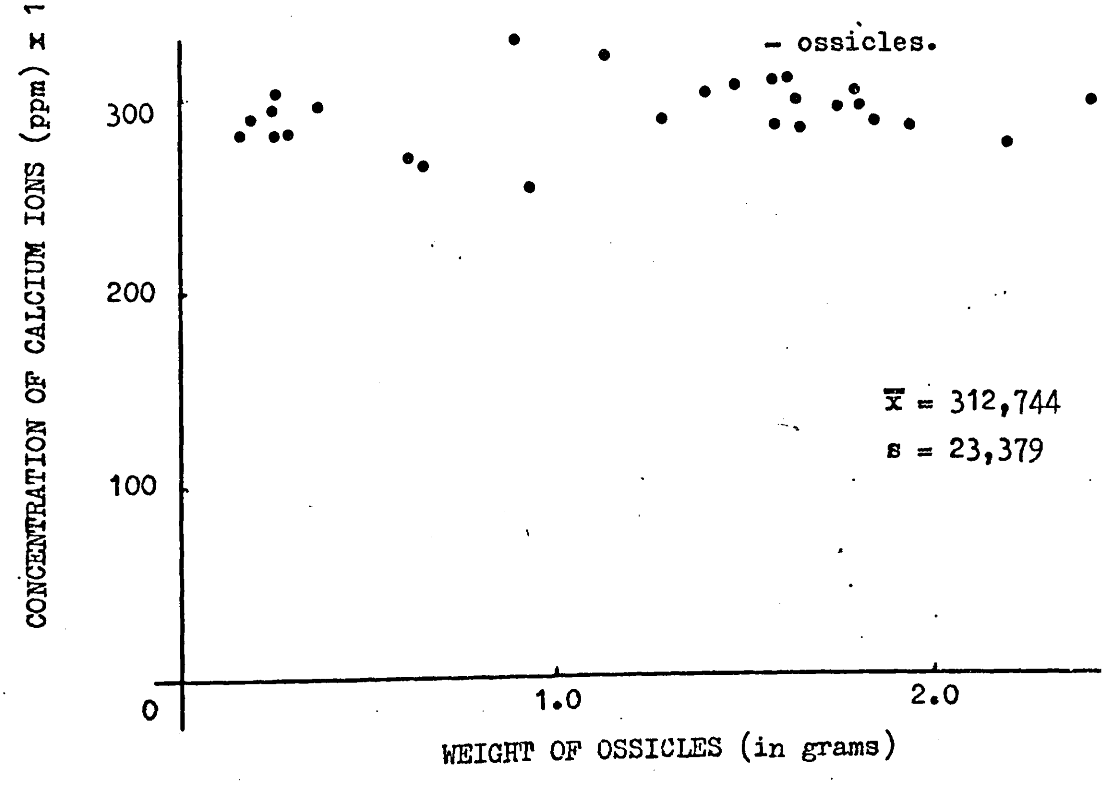
Graph 7.2 Sr/Ca x 10^3 atom ratio in <u>Ophiocomina nigra</u> - ossicles.



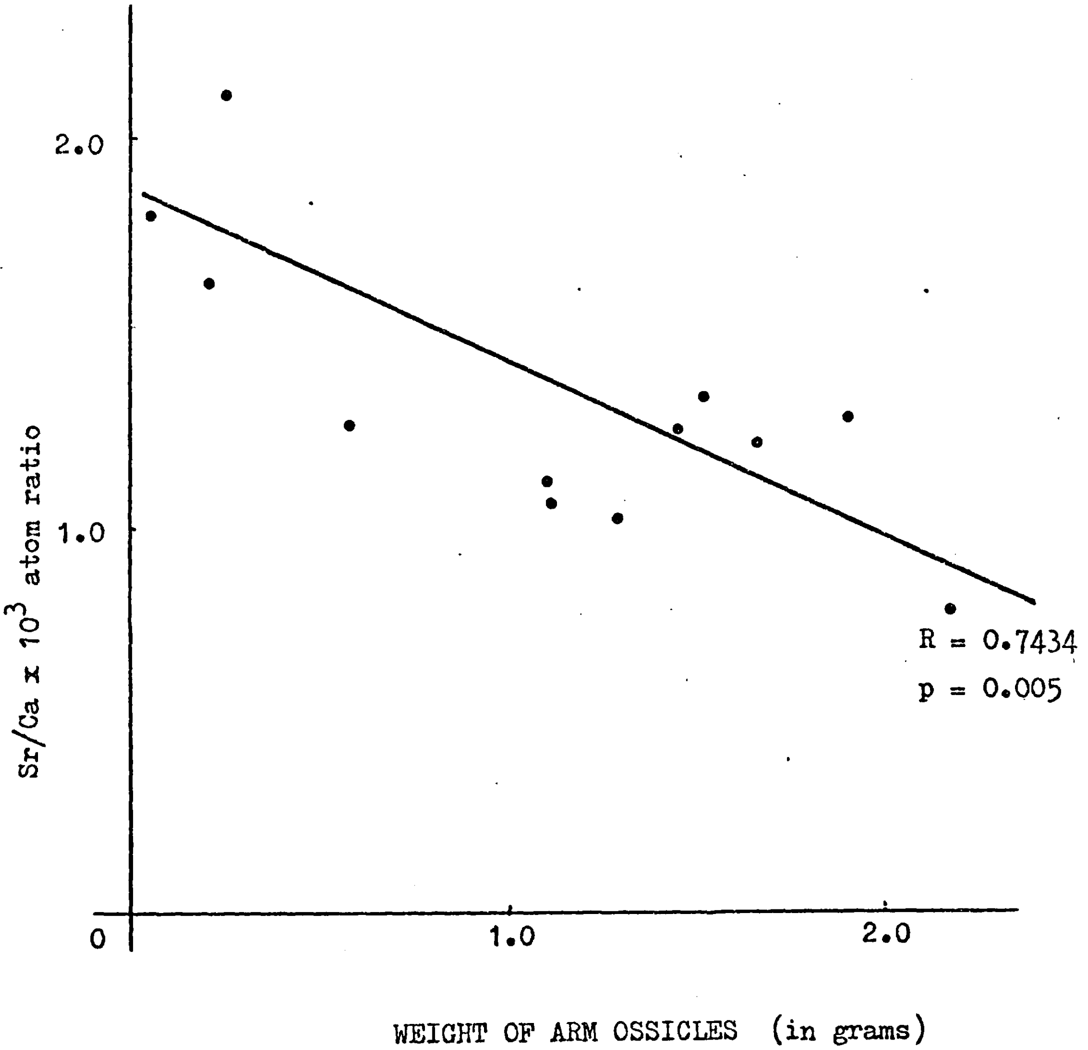
Graph 7.3 (a) Concentration of strontium in <u>Ophiocomina nigra</u> - ossicles.



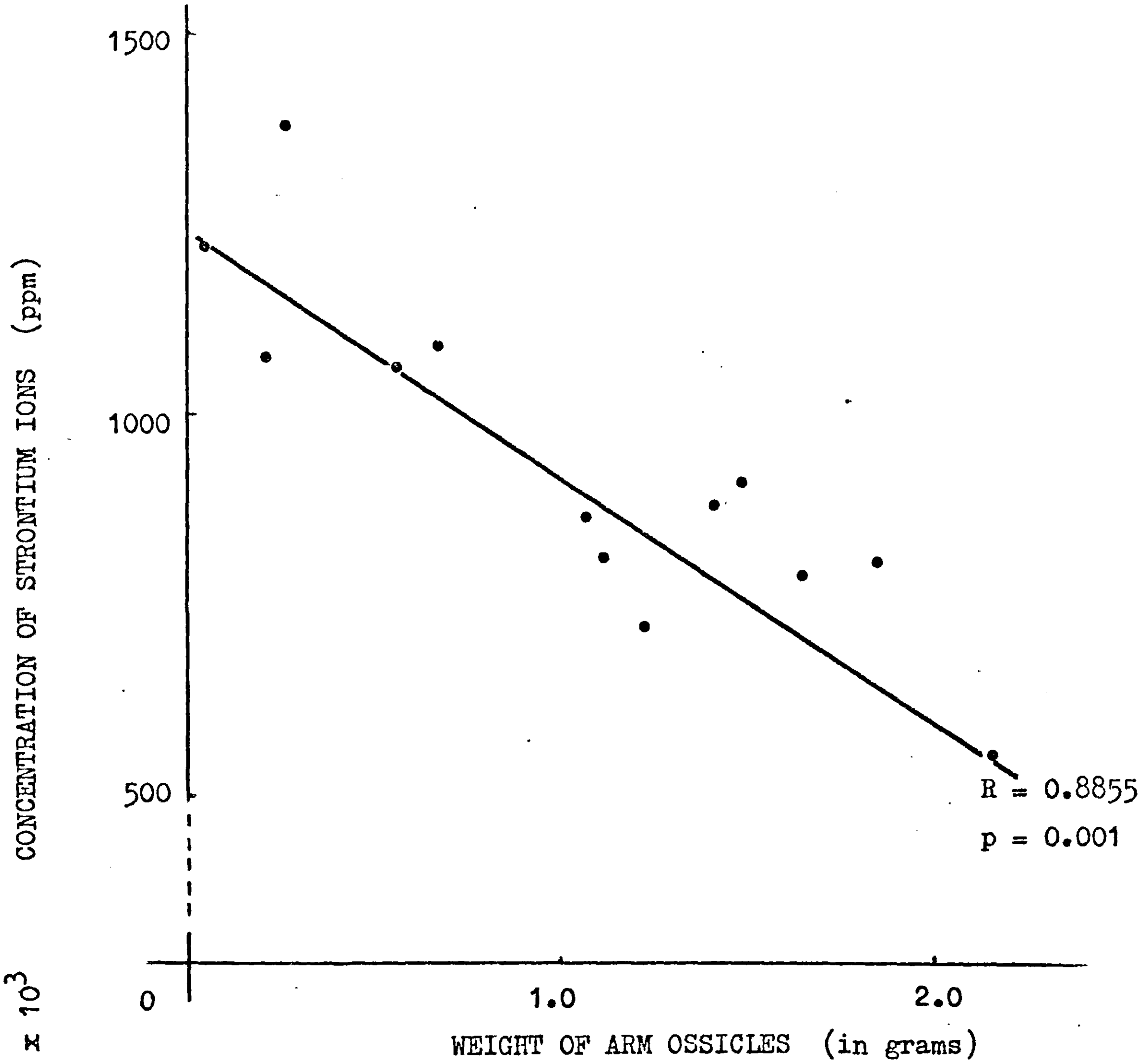
(b) Concentration of calcium in <u>Ophiocomina nigra</u> - ossicles.



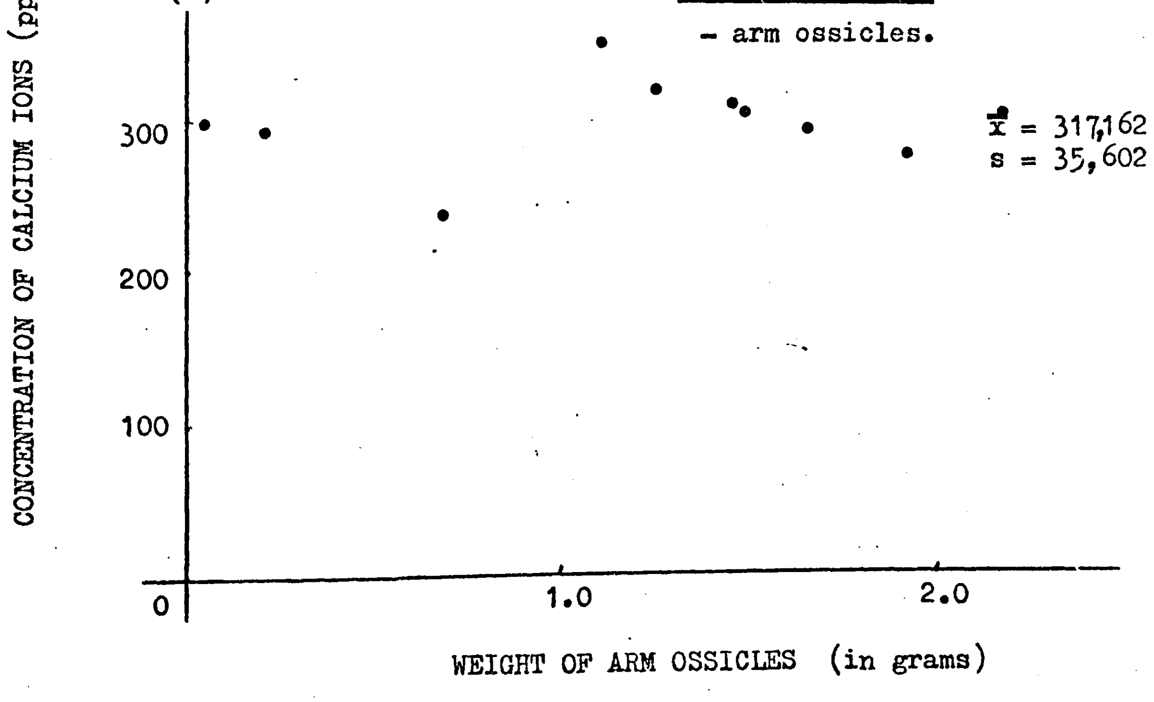
Graph 7.4 Sr/Ca x10^3 atom ratio in Ophiocomina nigra - arm ossicles.



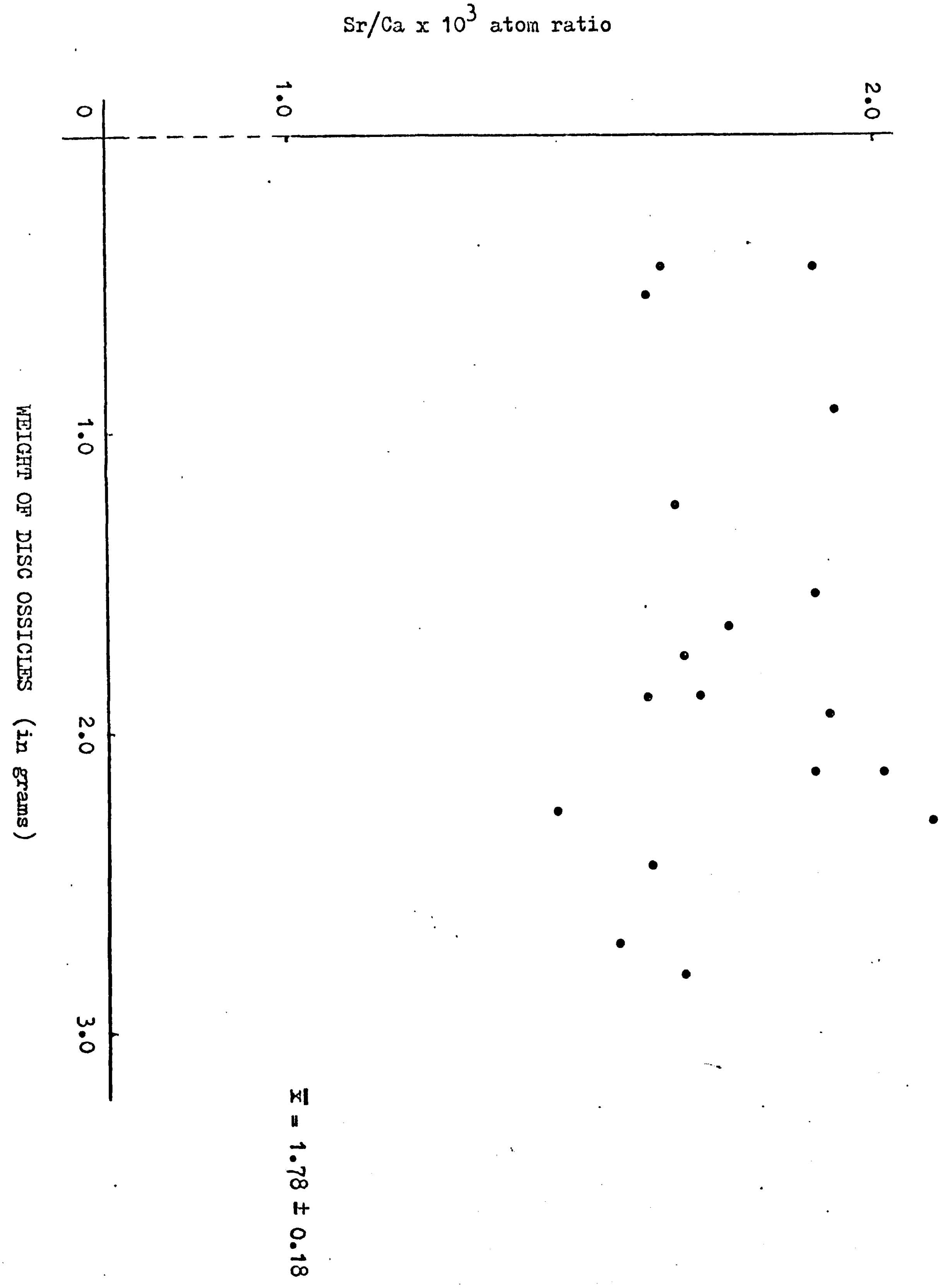
Graph 7.5 (a) Concentration of strontium in Ophiocomina nigra - arm ossicles.



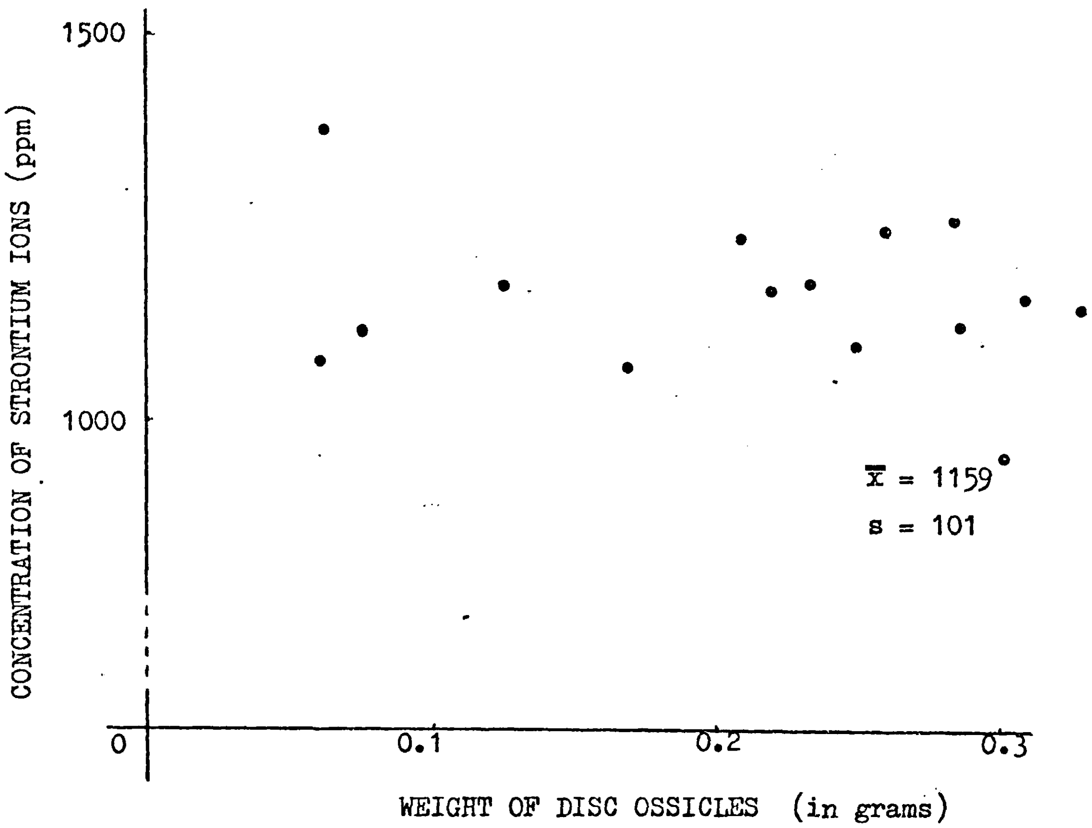
(b) Concentration of calcium in Ophiocomina nigra - arm ossicles.



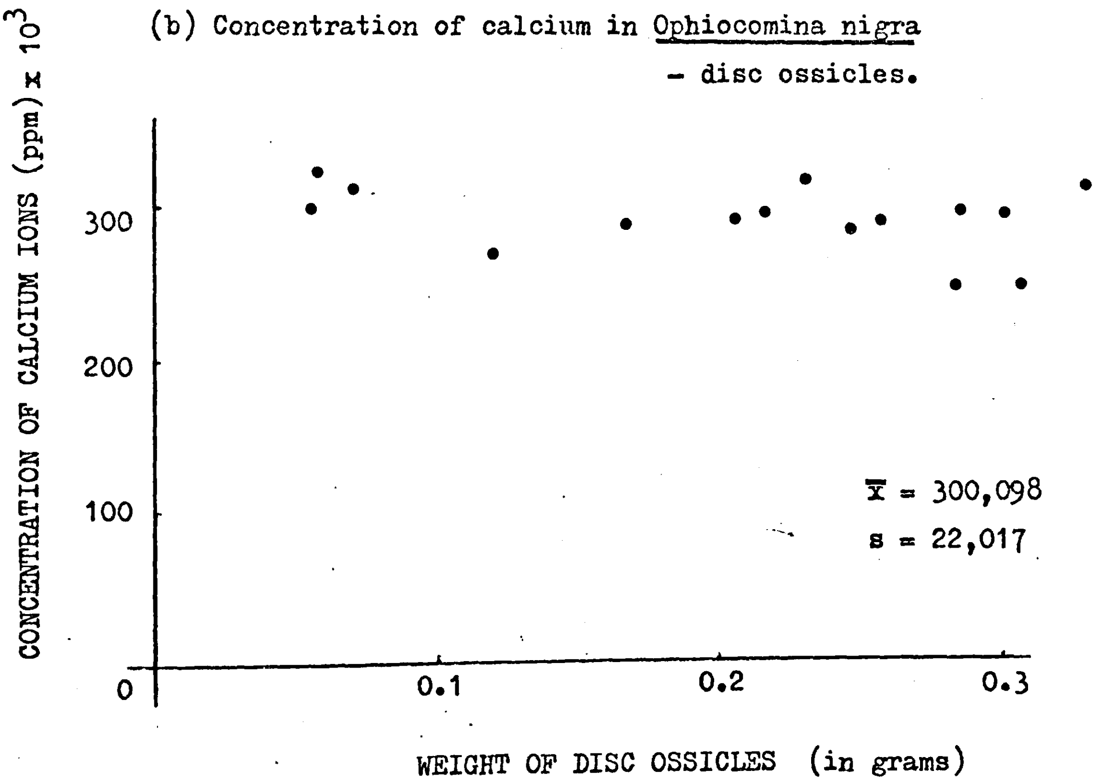
Graph 7.6 Sr/Ca x 10^3 atom ratio of Ophiocomina nigra - disc ossicles.



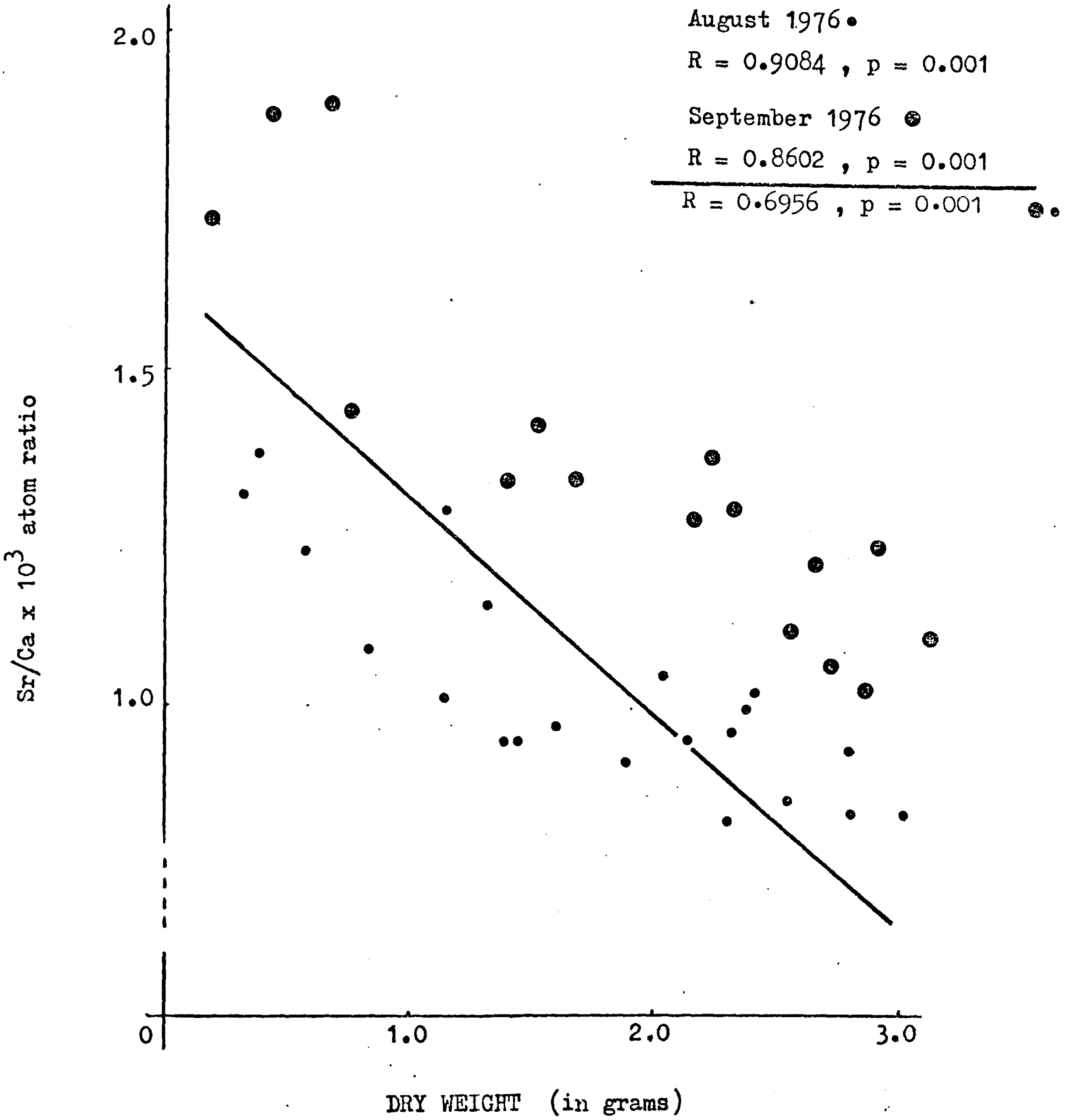
Graph 7.7 (a) Concentration of strontium in *Ophiocomina nigra* - disc ossicles.



(b) Concentration of calcium in *Ophiocomina nigra* - disc ossicles.

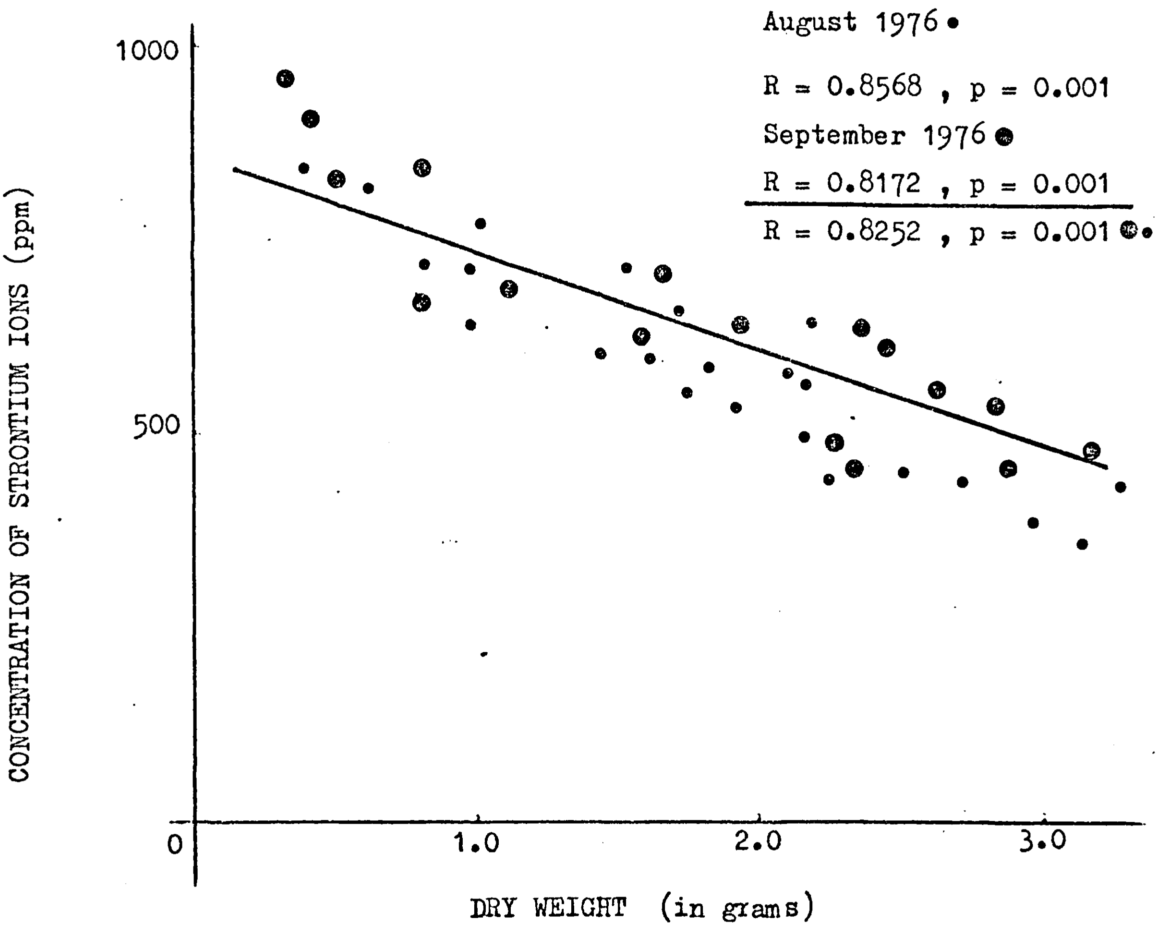


Graph 7.8 Sr/Ca x 10^3 atom ratio in *Ophiocomina nigra* - without removal of organic matter.

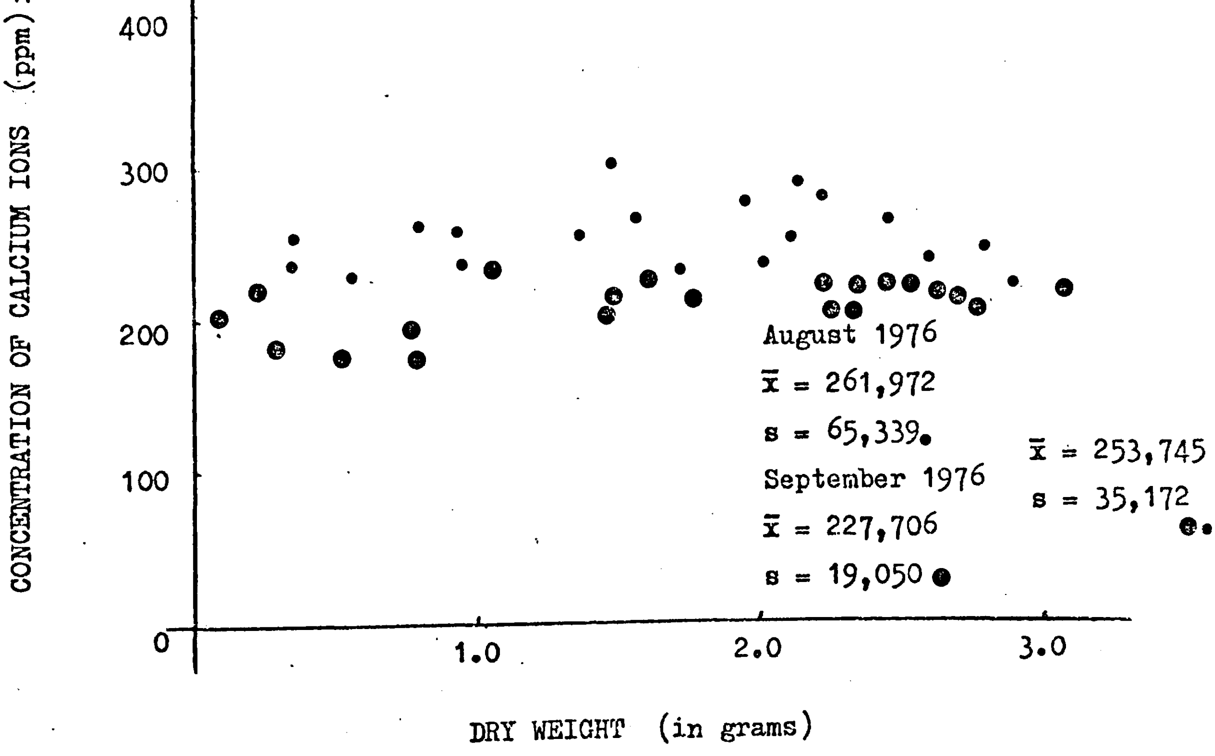


Please note - Although on this graph (and some following ones) data is presented with reference to two months, all results are treated by assessing the samples as a whole. This is done since monthly sampling was not possible. Most data points were obtained from September 1976 unless otherwise stated.

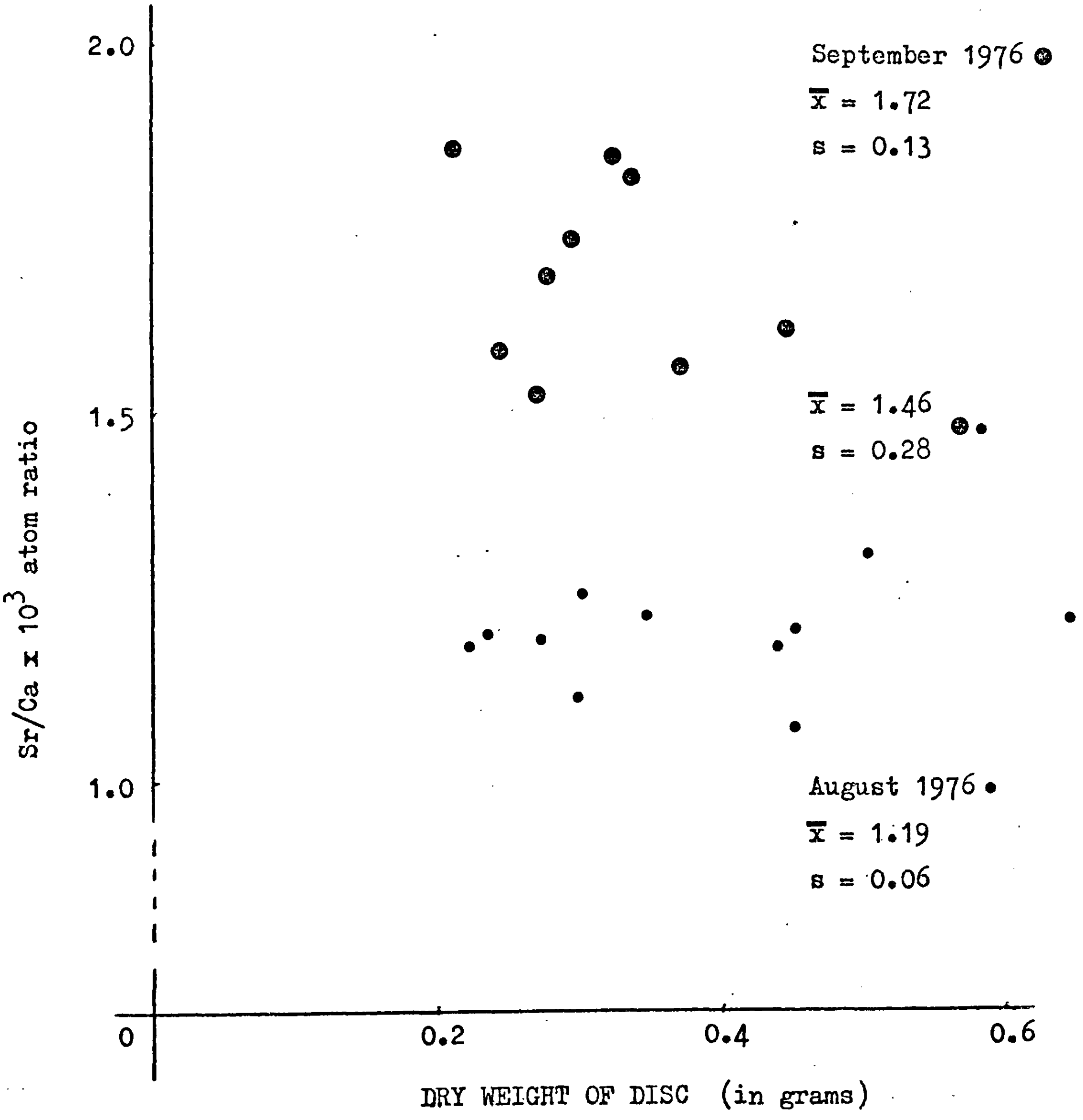
Graph 7.9 (a) Concentration of strontium in Ophiocomina nigra - without removal of organic matter.



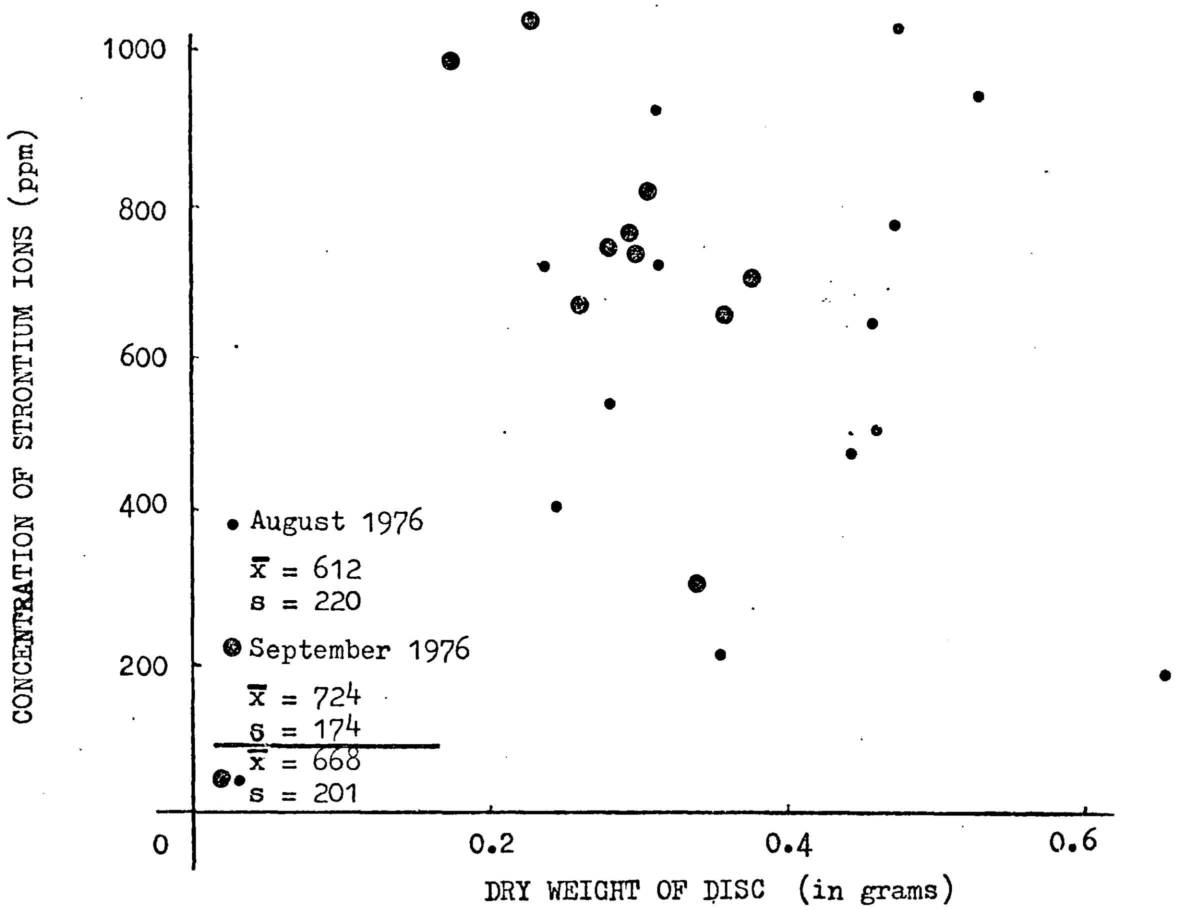
(b) Concentration of calcium in Ophiocomina nigra - without removal of organic matter.



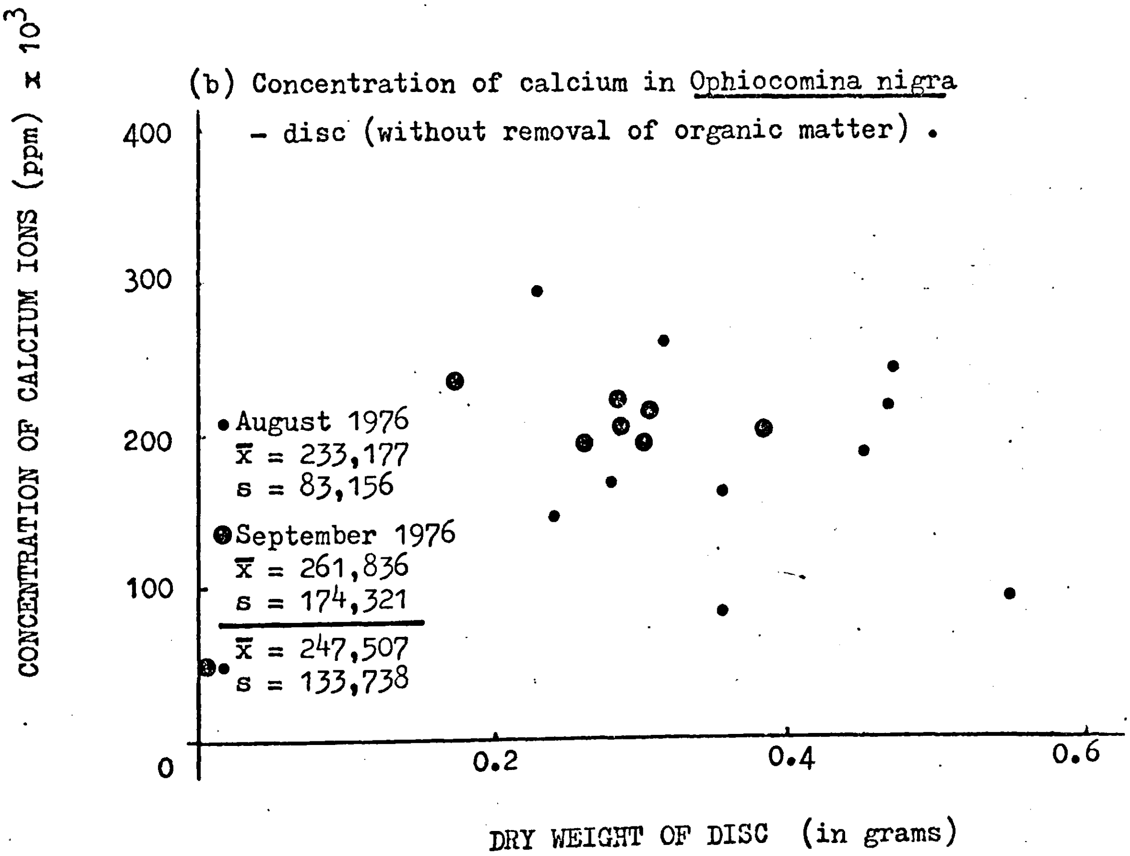
Graph 7.10 Sr/Ca x 10^3 atom ratio in Ophiocomina nigra - disc (without removal of organic matter) .



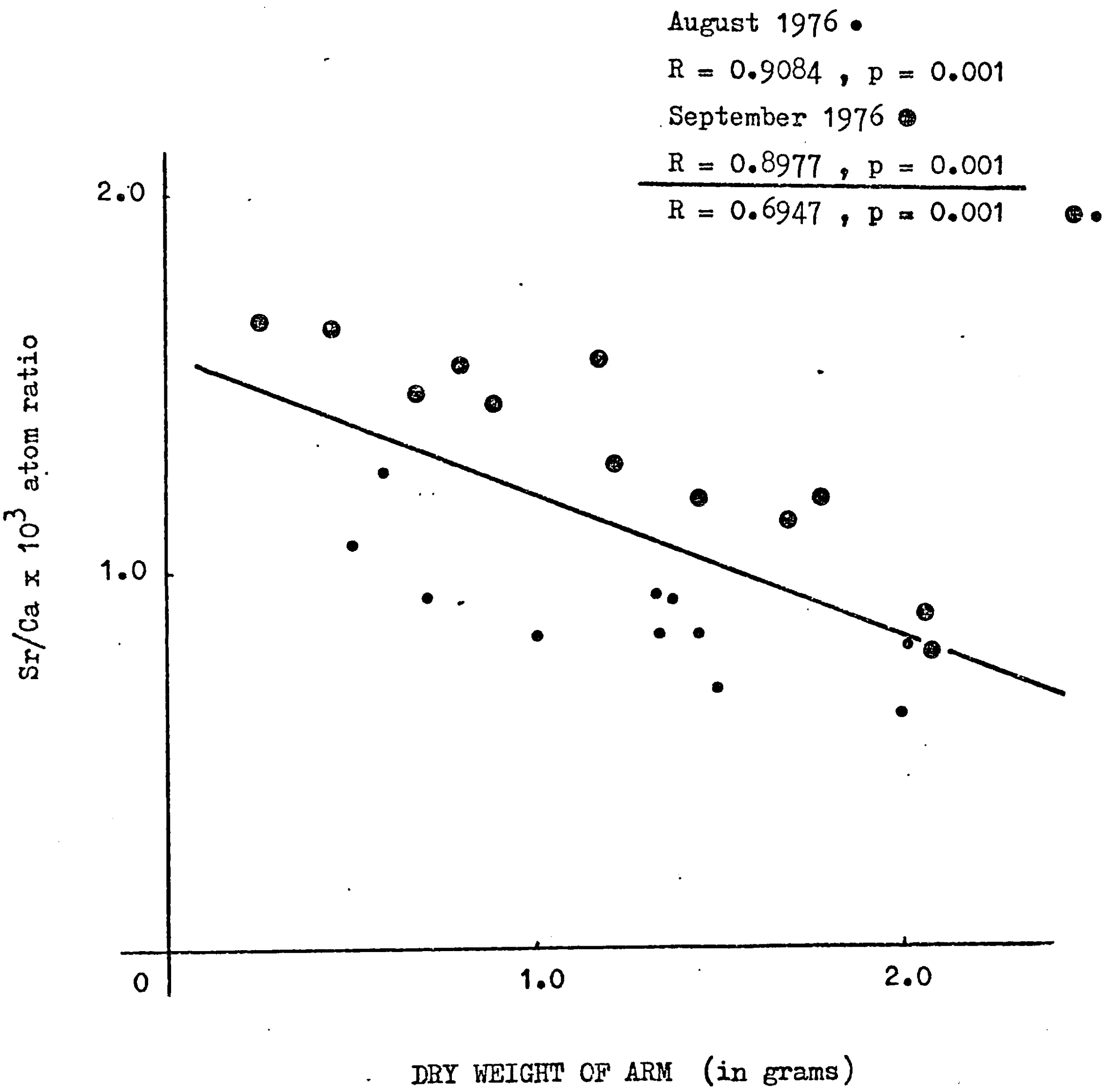
Graph 7.11 (a) Concentration of strontium in *Ophiocomina nigra* - disc (without removal of organic matter).



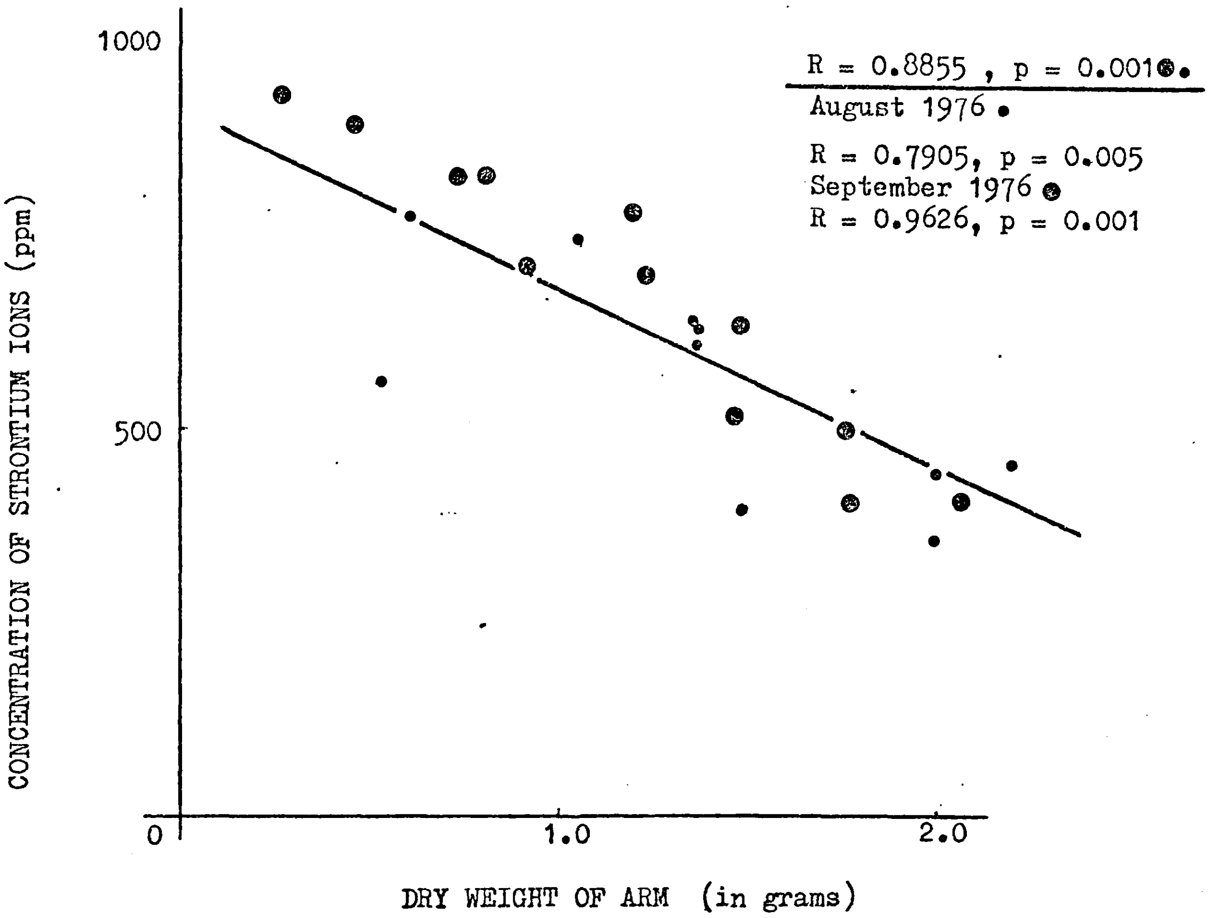
(b) Concentration of calcium in *Ophiocomina nigra* - disc (without removal of organic matter).



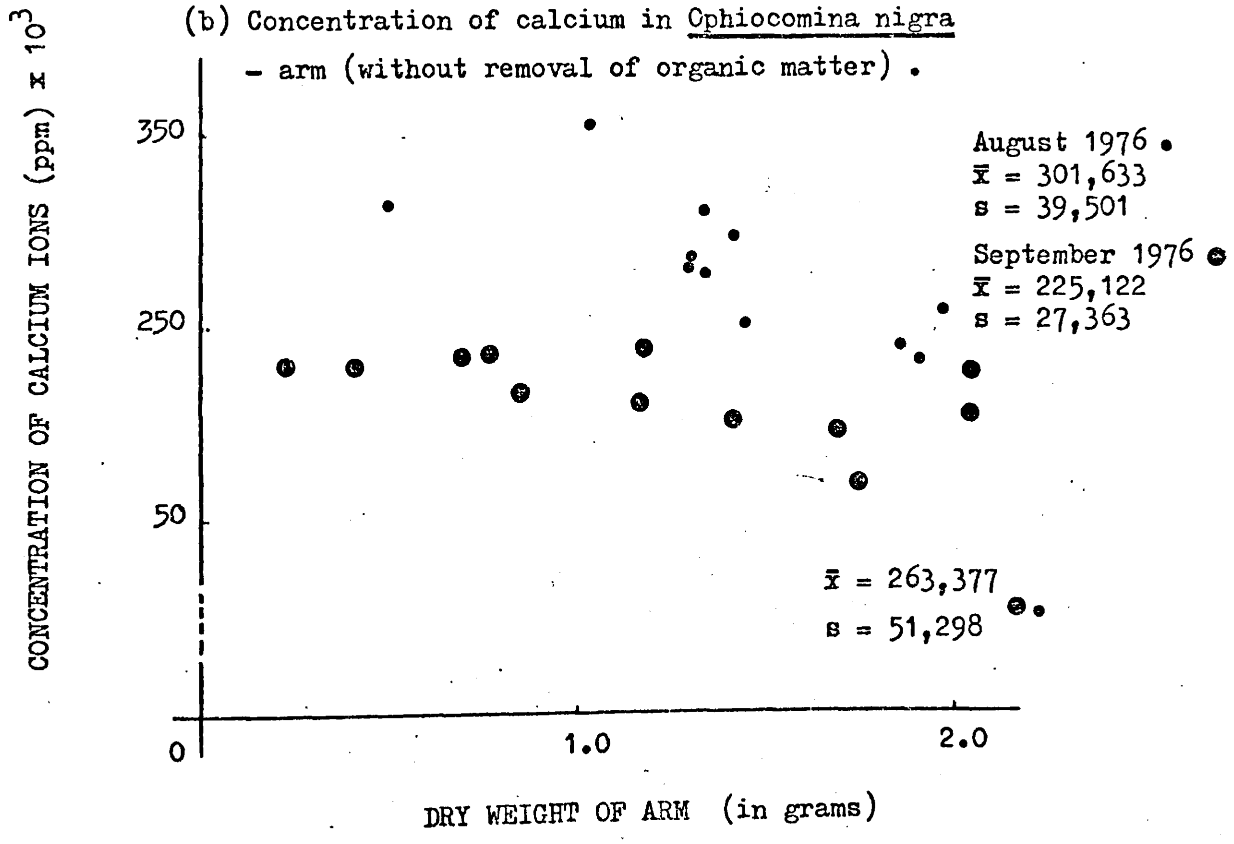
Graph 7.12 Sr/Ca x 10^3 atom ratio in Ophiocomina nigra - arm (without removal of organic matter).



Graph 7.13 (a) Concentration of strontium in *Ophiocomina nigra* - arm (without removal of organic matter) .



(b) Concentration of calcium in *Ophiocomina nigra* - arm (without removal of organic matter) .



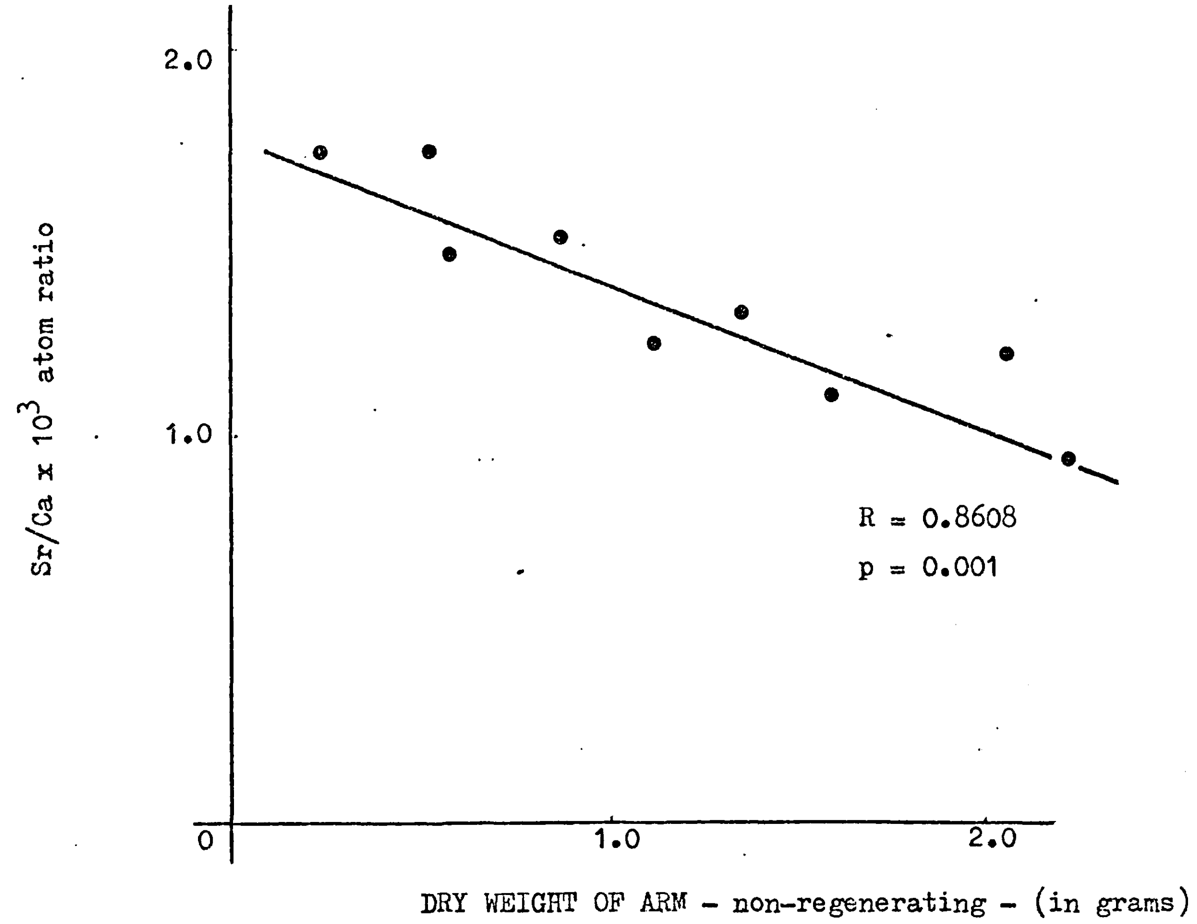
Where an arm has been damaged and is undergoing regeneration it is possible to analyse the arm as a two-compartment structure; the older portion (the basal region) and the regenerating part. It can be seen (Graph 7.14) that the older section of the arm shows the familiar inverse relationship between Sr/Ca atom ratio and dry weight. The regenerating portion, however, has a fairly constant value over the range of dry weights sampled. Looking at these differences in terms of strontium and calcium it is clear that the regenerating part of the arm and the older portion (Graphs 7.15) have similar values for calcium while the strontium is of a uniform concentration in the regenerating part and inversely related to dry weight in the older section of the arm,(Graph 7.16). In the latter, it has a slightly lower content than in the other younger part. It will be noticed that the regenerating parts of the arm which were analysed were assayed whole. That is to say, without the removal of the organic matter. The relative amount of soft tissue to ossicle was very small and was not considered to affect the Sr/Ca atom ratio result if the ratio was used to describe the situation in the calcareous parts of the regenerating part of the arm.

(b) Ophiura albida - analysis of the ossicles of the whole animal (Graph 7.17) in contrast to those of O.nigra, does not show any relationship between Sr/Ca atom ratio and ossicle weight. However, if equivalently sized animals of the two species are compared then similar Sr/Ca atom ratios are obtained. The strontium and calcium contents (Graphs 7.18) are fairly uniform over the range of weights assayed in O.albida, and are broadly equivalent to those found in the ossicles of O.nigra (Graph 7.3).

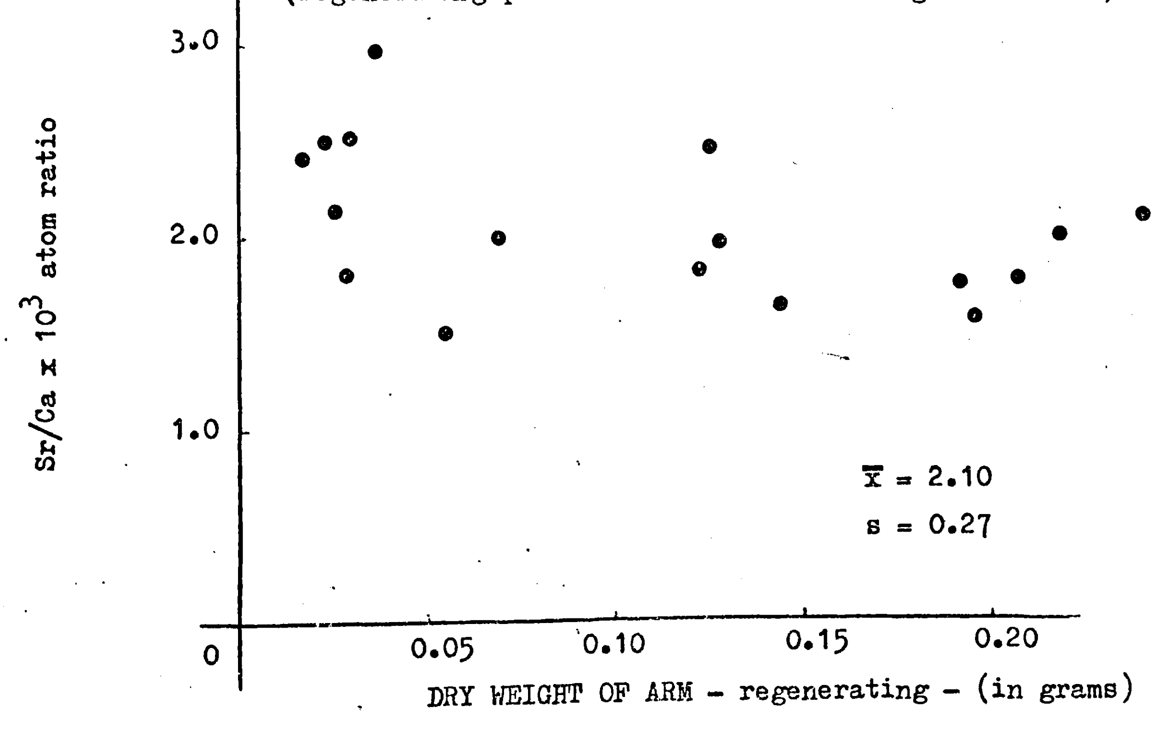
The ossicles of the disc have fairly uniform Sr/Ca atom ratios (Graph 7.19) which reflect the uniform concentrations of strontium and calcium. These ratios are similar to those of O.nigra. The strontium and calcium concentrations of O.albida are uniform (Graphs 7.20) and, over the appropriate size scale, are similar to those of O.nigra.

The Sr/Ca atom ratio of the arm ossicles (Graphs 7.21) is

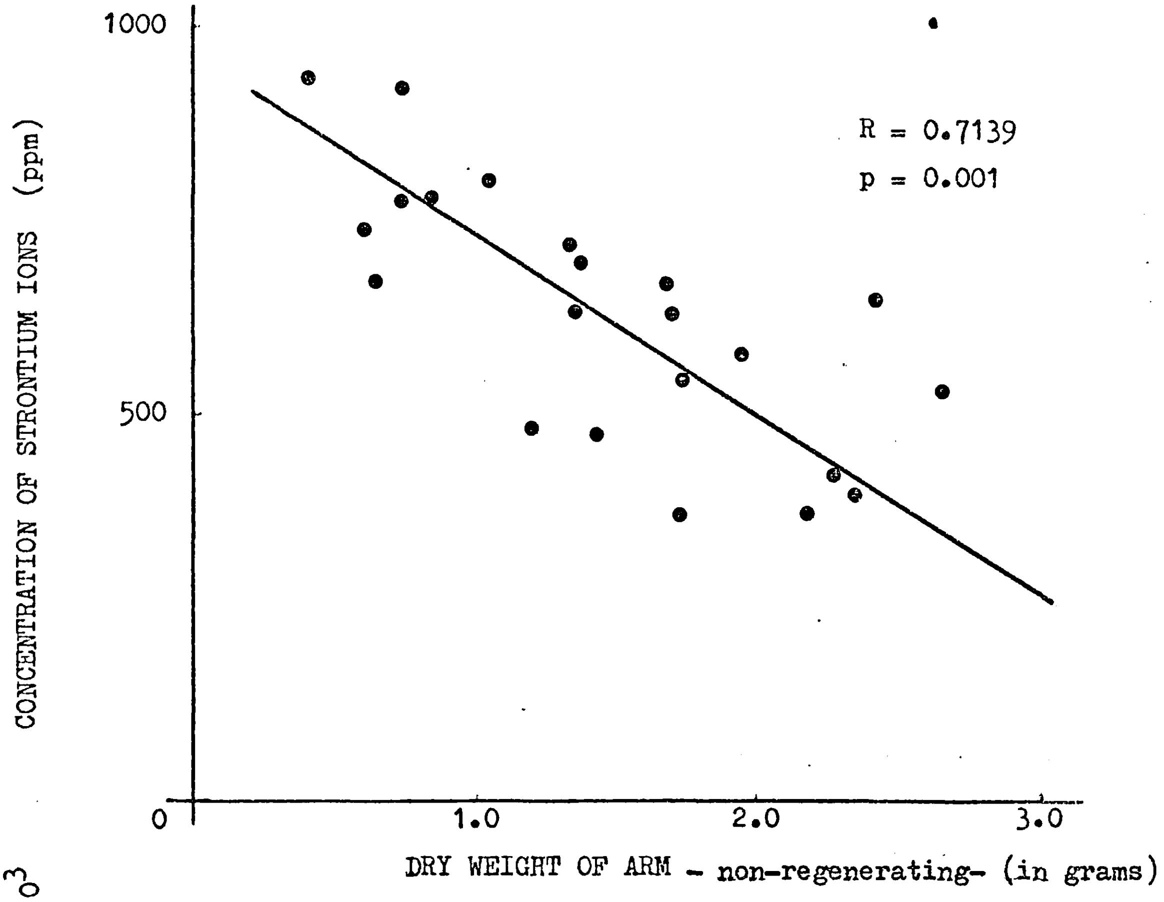
Graph 7.14 (a) Sr/Ca x 10^3 atom ratio in *Ophiocomina nigra* - arm (non-regenerating part: without removal of organic matter).



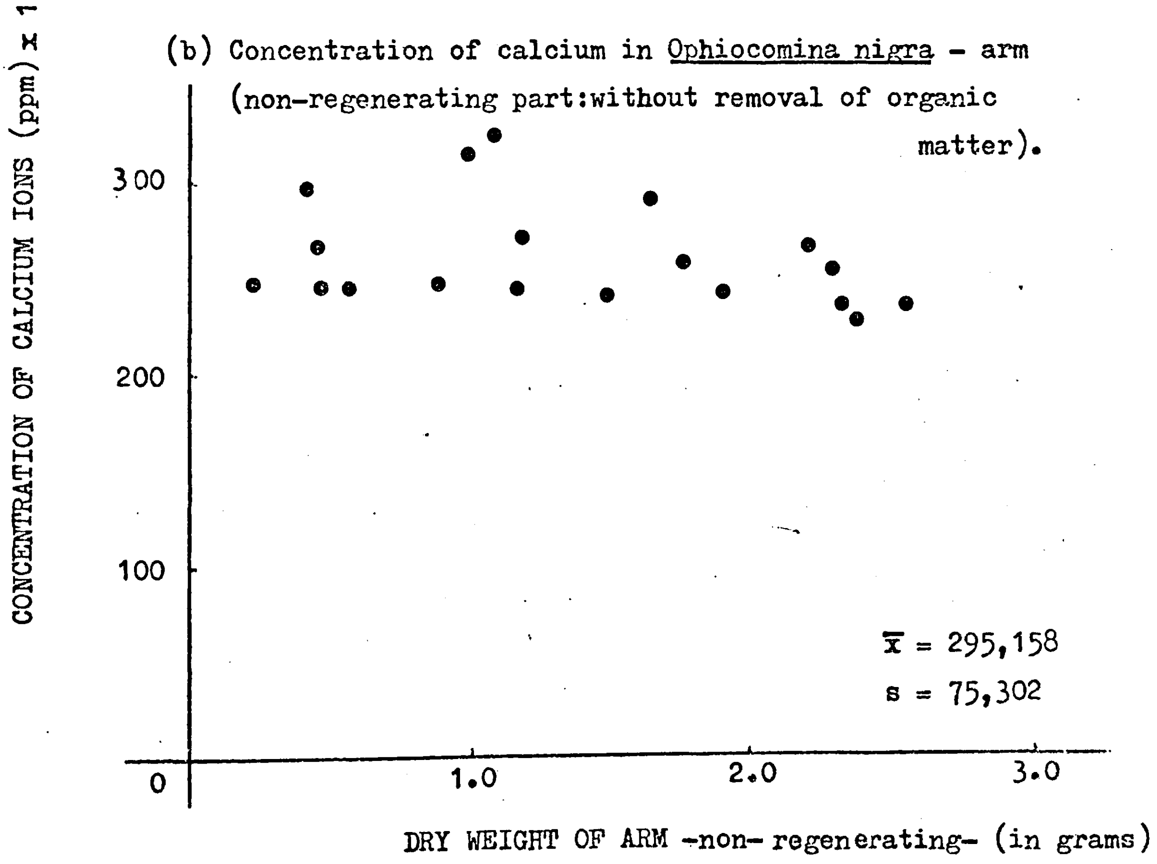
(b) Sr/Ca x 10^3 atom ratio in *Ophiocomina nigra* - arm (regenerating part: without removal of organic matter).



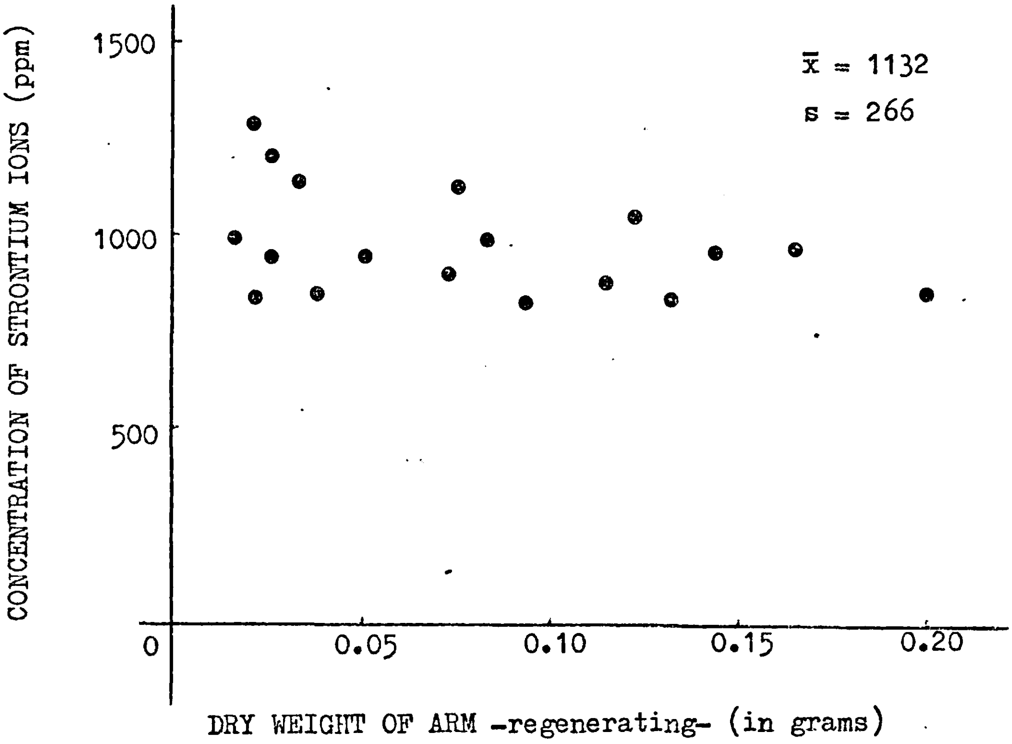
Graph 7.15 (a) Concentration of strontium in Ophiocomina nigra - arm (non-regenerating part:without removal of organic matter) .



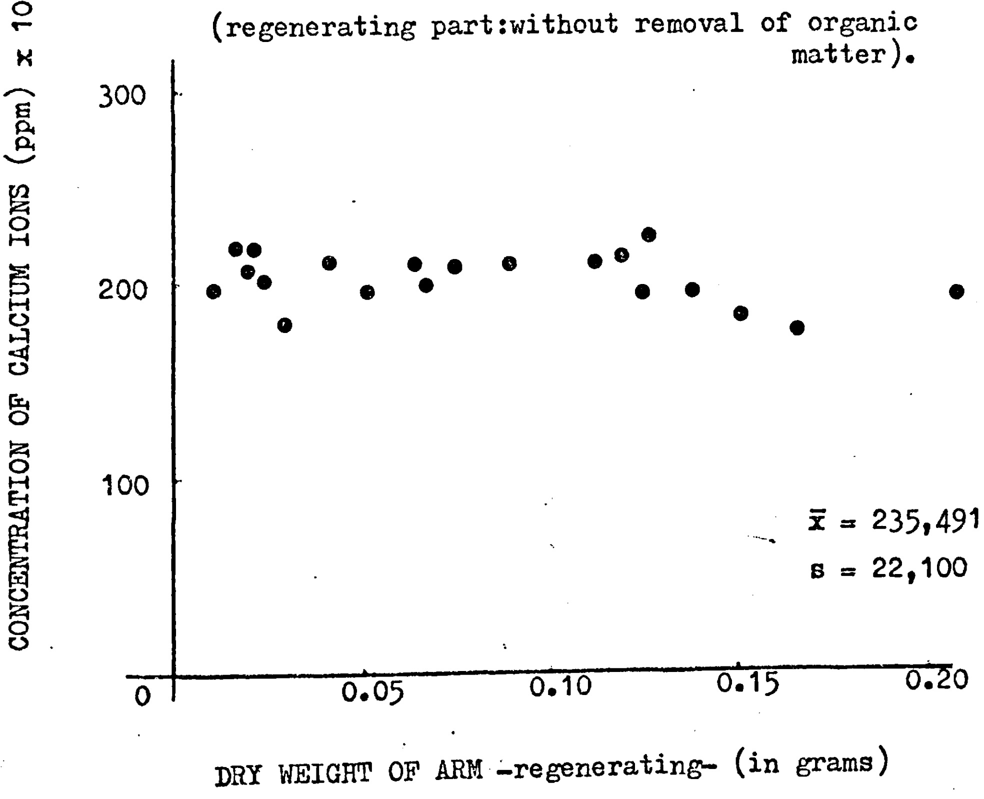
(b) Concentration of calcium in Ophiocomina nigra - arm (non-regenerating part:without removal of organic matter).



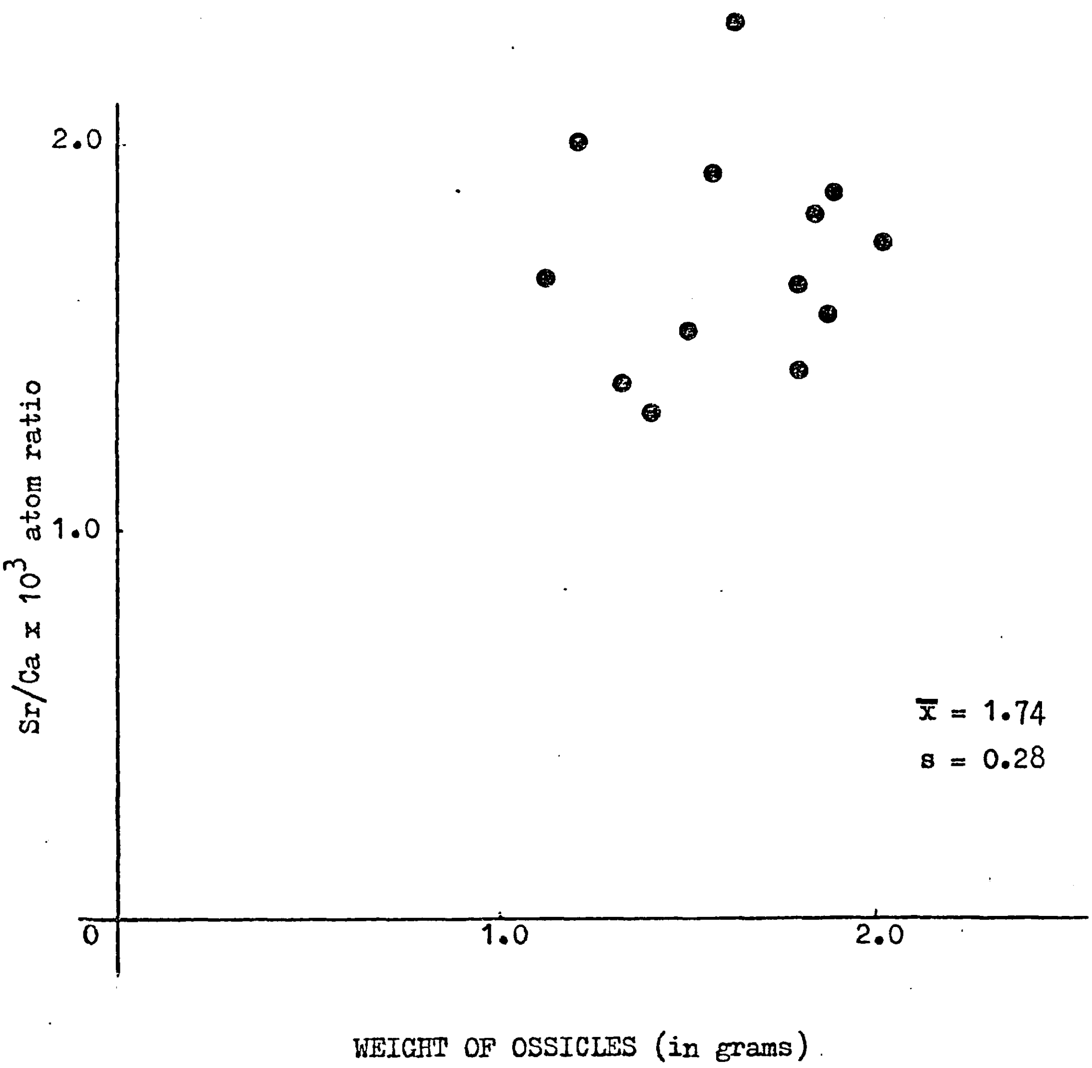
Graph 7.16 (a) Concentration of strontium in Ophiocomina nigra - arm (regenerating part:without removal of organic matter) .



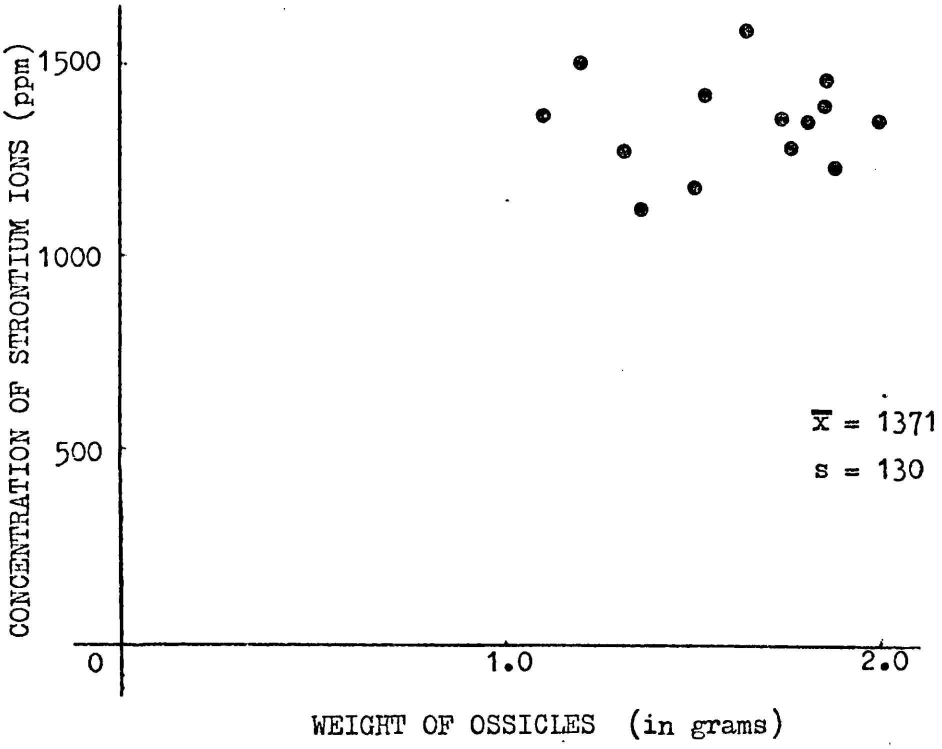
(b) Concentration of calcium in Ophiocomina nigra -arm (regenerating part:without removal of organic matter).



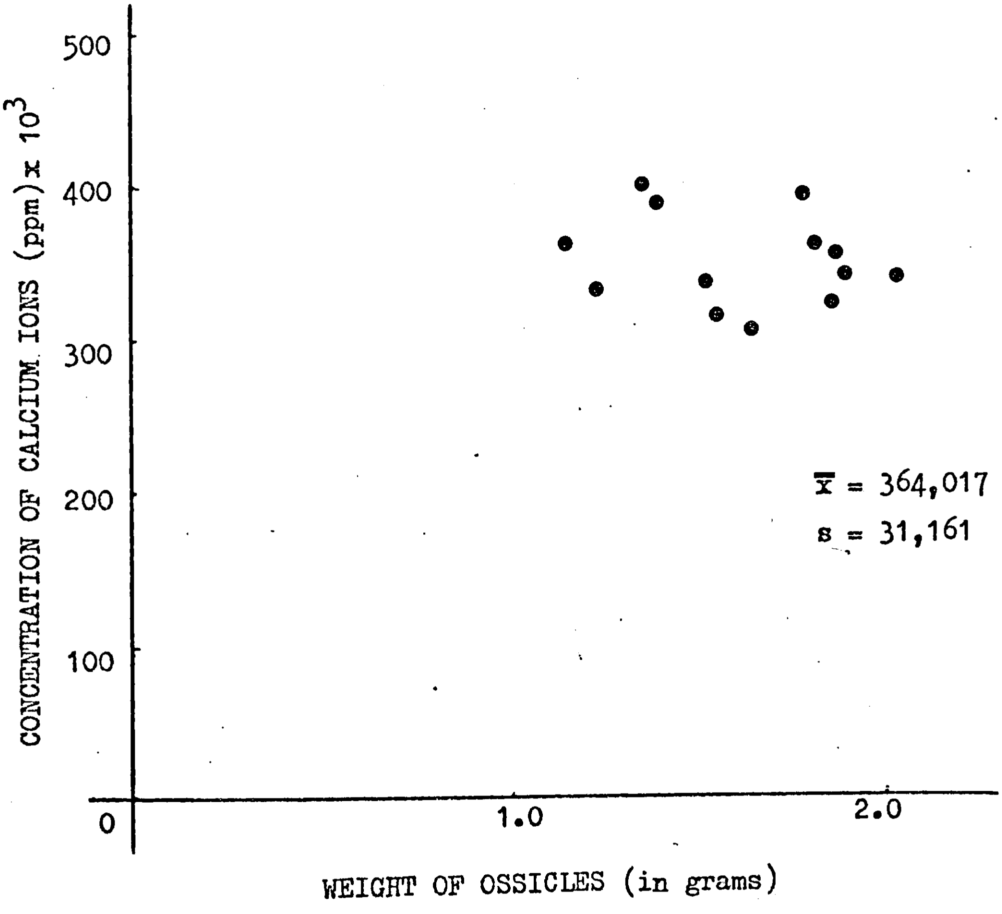
Graph 7.17 Sr/Ca x 10^3 atom ratio in <u>Ophiura albida</u> (Millport) - ossicles.



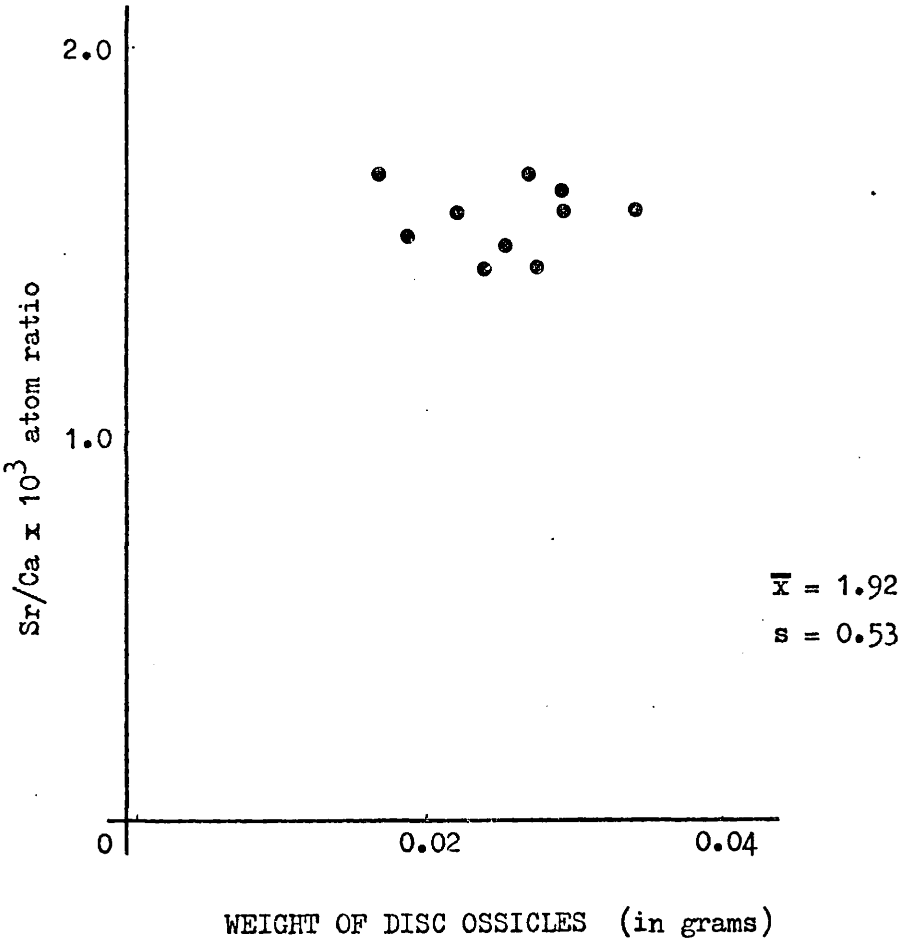
Graph 7.18 (a) Concentration of strontium in <u>Ophiura albida</u> (Millport) - ossicles .



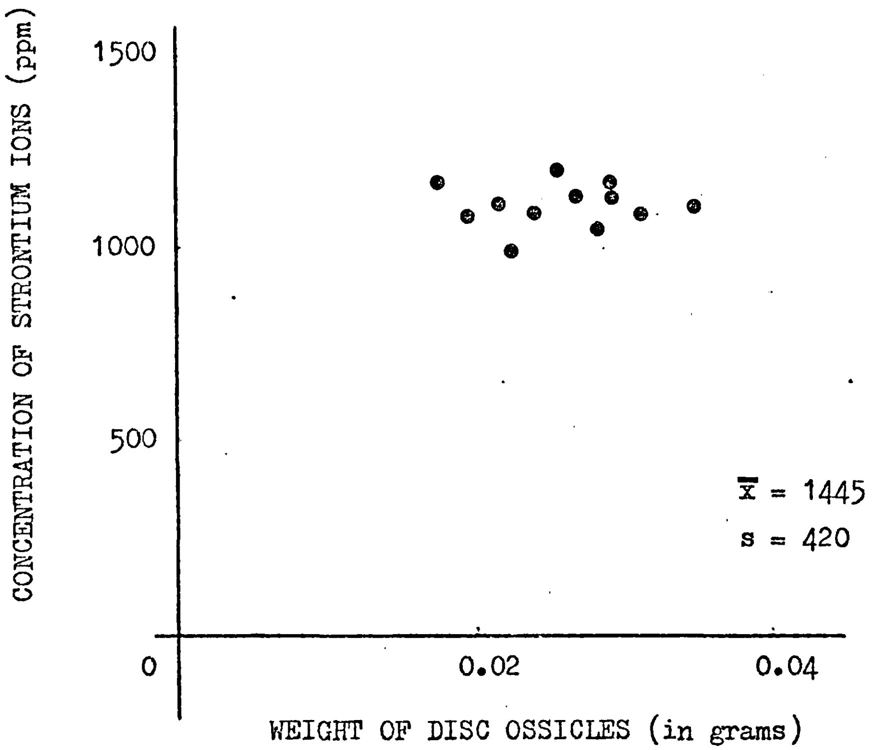
(b) Concentration of calcium in <u>Ophiura albida</u> (Millport) - ossicles.



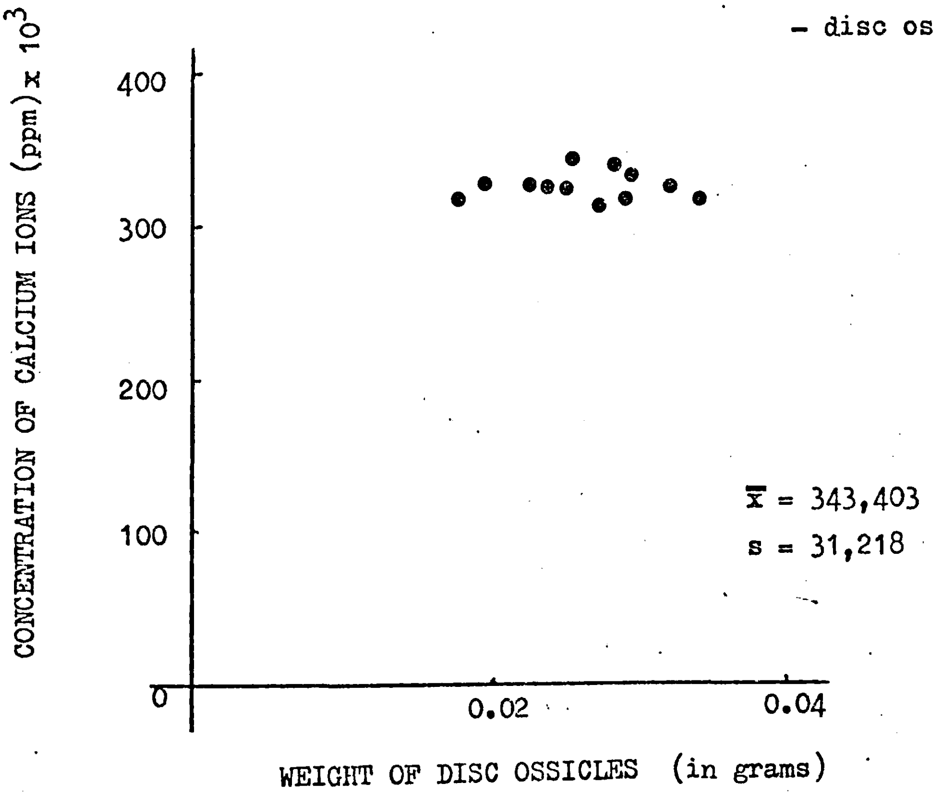
Graph 7.19 Sr/Ca x 10^3 atom ratio in <u>Ophiura albida</u> (Millport) - disc ossicles .



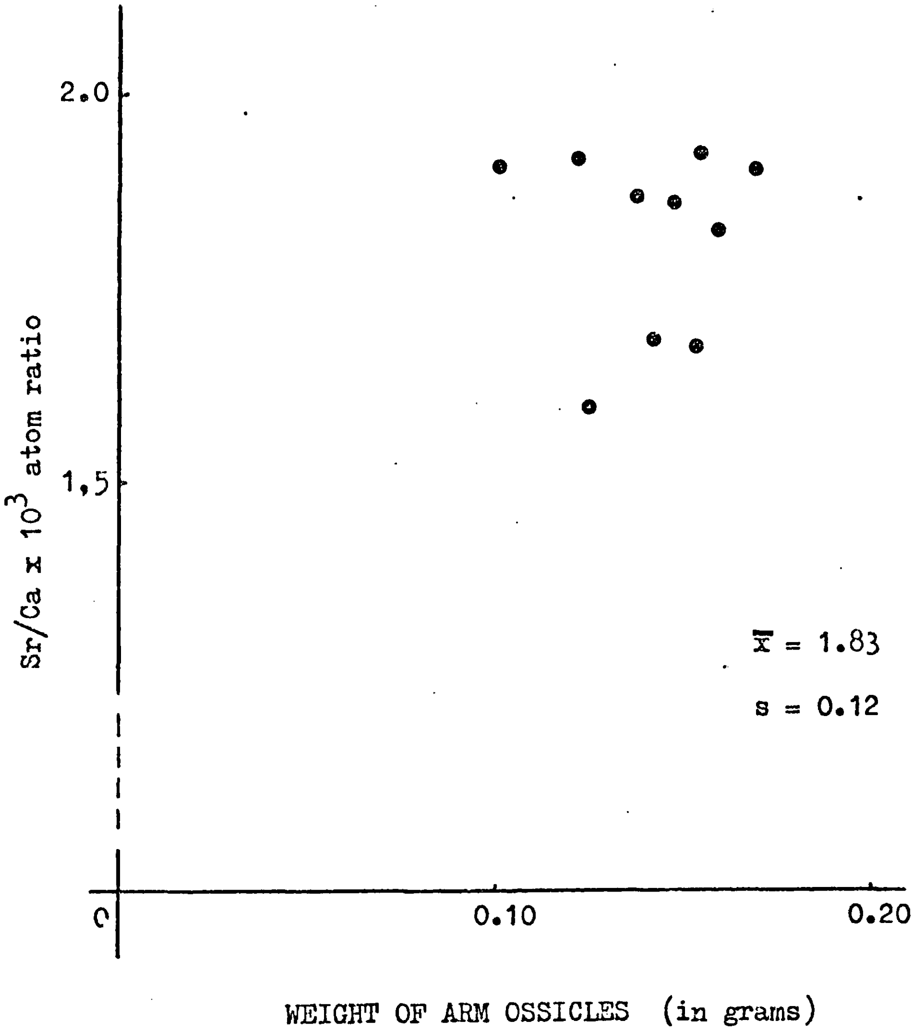
Graph 7.20 (a) Concentration of strontium in Ophiura albida (Millport) - disc ossicles .



(b) Concentration of calcium in Ophiura albida (Millport) - disc ossicles.



Graph 7.21 Sr/Ca x 10^3 atom ratio in Ophiura albida (Millport) - arm ossicles.



uniform and at about the same level as the inversely dry weight-related Sr/Ca ratio range of O.nigra within an equivalent dry weight scale. The strontium content (Graphs 7.22) is also uniform though it is much lower than in comparably sized samples of O.nigra. The concentration of calcium in the arm ossicles of O.albida is a little higher than in O.nigra arm ossicles.

The Sr/Ca ratios and the individual strontium and calcium concentrations are similar over comparable size ranges in O.albida.

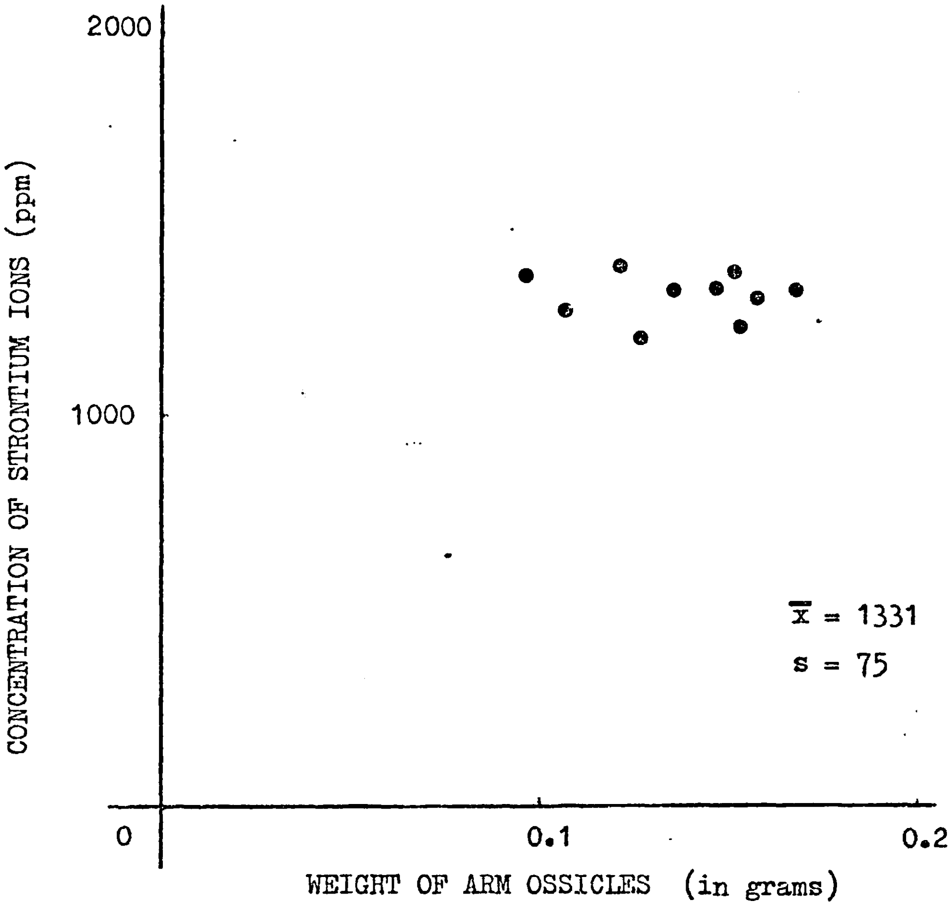
The analyses of O.albida specimens without the removal of the organic matter demonstrated a tendency for the Sr/Ca atom ratio to be directly proportional to the dry weight (Graph 7.23) which is the converse of the situation in O.nigra (Graph 7.8). There is an overall trend of a uniform strontium value in O.albida (Graph 7.24), in contrast to the inversely weight-related values in O.nigra. Comparing similar sized specimens of these two species there is evidence to suggest that O.albida has a slightly higher strontium concentration. Broadly speaking, the calcium content of O.albida is a little higher than O.nigra.

The discs of O.albida appear to have similar Sr/Ca atom ratios though not as high as in O.nigra (Graph 7.25). The strontium and calcium concentrations of both species are uniform and fairly similar (Graphs 7.26).

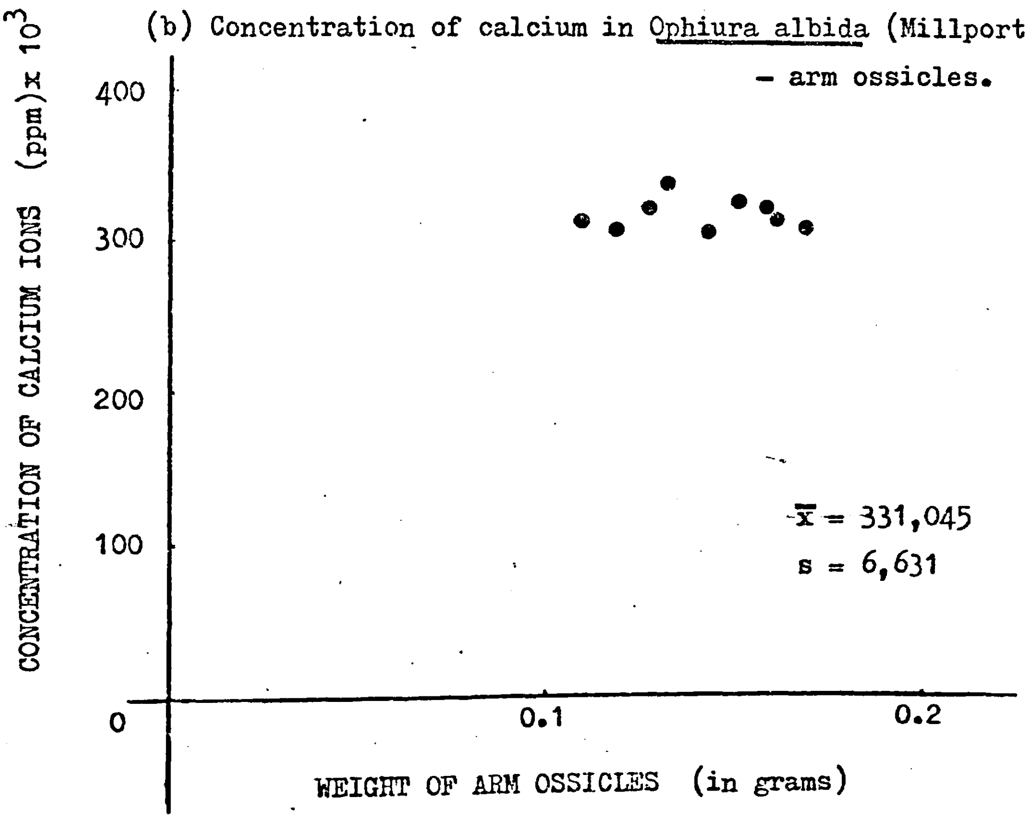
The Sr/Ca atom ratios of the arms of O.albida do not exhibit the same relationship to the dry weight (Graph 7.27) as do the arms of O.nigra. The calcium values of both O.nigra and O.albida arms are similar while the strontium content is much higher in O.albida (Graphs 7.28).

While the Sr/Ca atom ratio of the disc and arms of O.albida are uniform there appears to be a direct proportionality between Sr/Ca atom ratio and dry weight in the whole analyses. All the strontium and calcium concentrations in the disc, arms and whole animals are relatively uniform. The highest strontium values occur in the arms while the disc and whole animal have similar and lower values. The whole animal and the arms have similar calcium concentrations while the concentrations in the disc are somewhat lower.

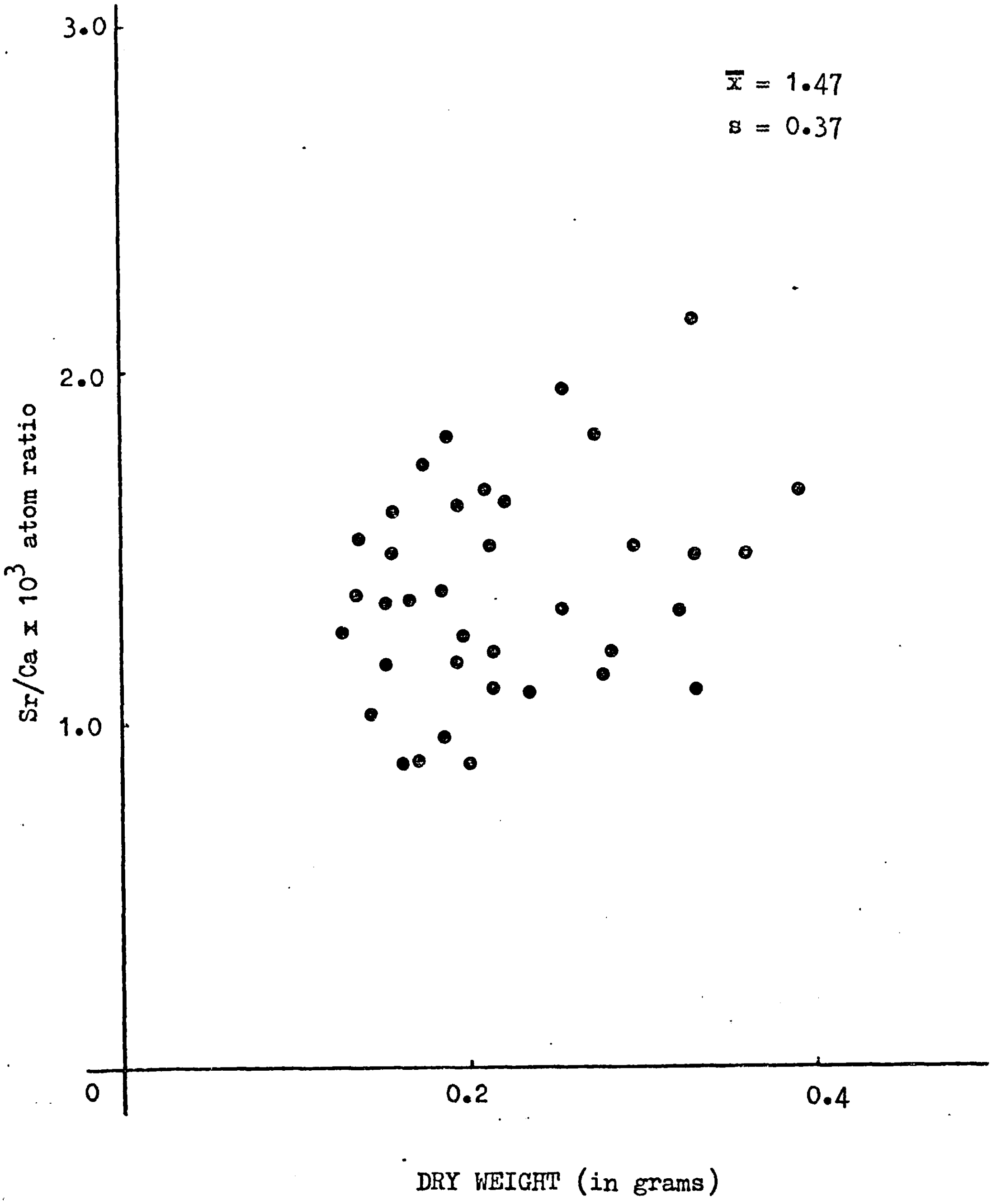
Graph 7.22 (a) Concentration of strontium in Ophiura albida (Millport) - arm ossicles.



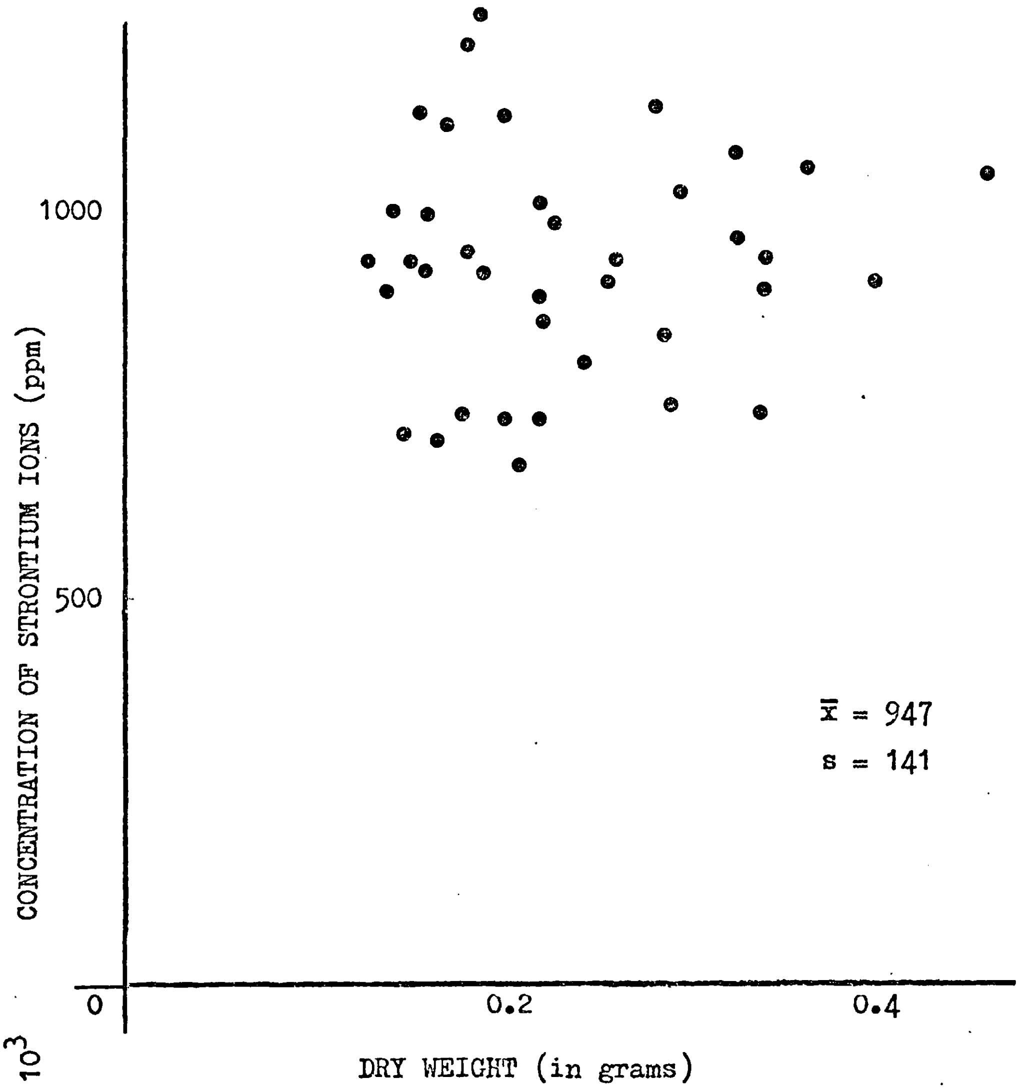
(b) Concentration of calcium in Ophiura albida (Millport) - arm ossicles.



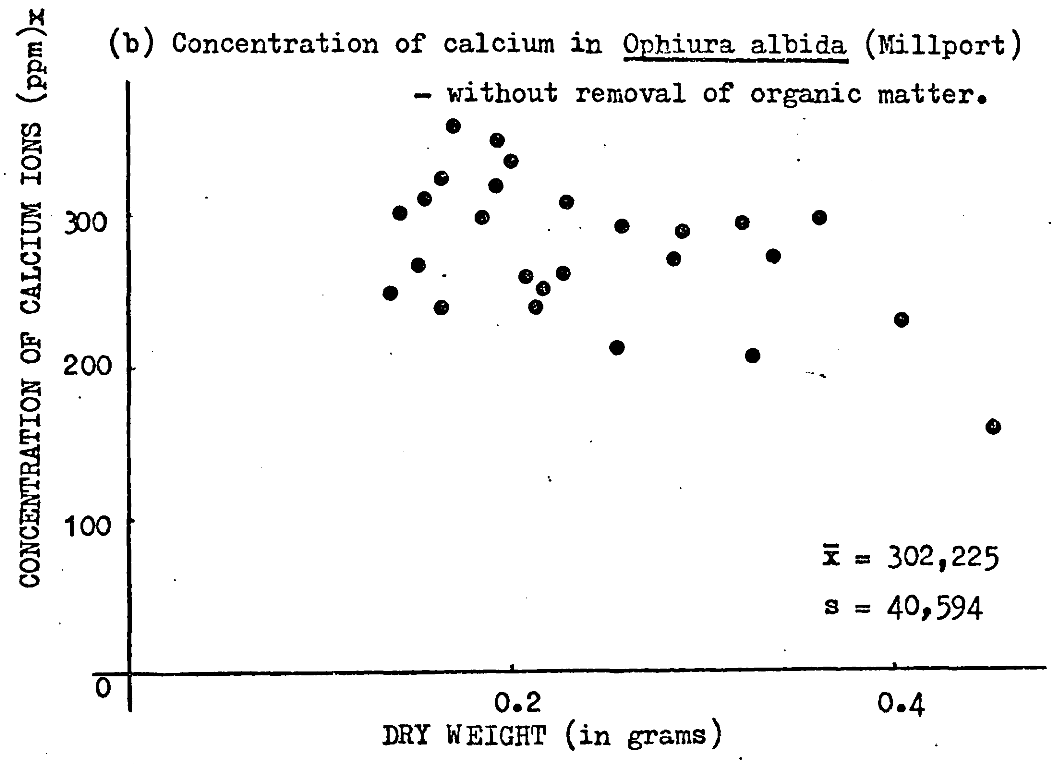
Graph 7.23 Sr/Ca x 10^3 atom ratio in <u>Ophiura albida</u> (Millport) - without removal of organic matter.



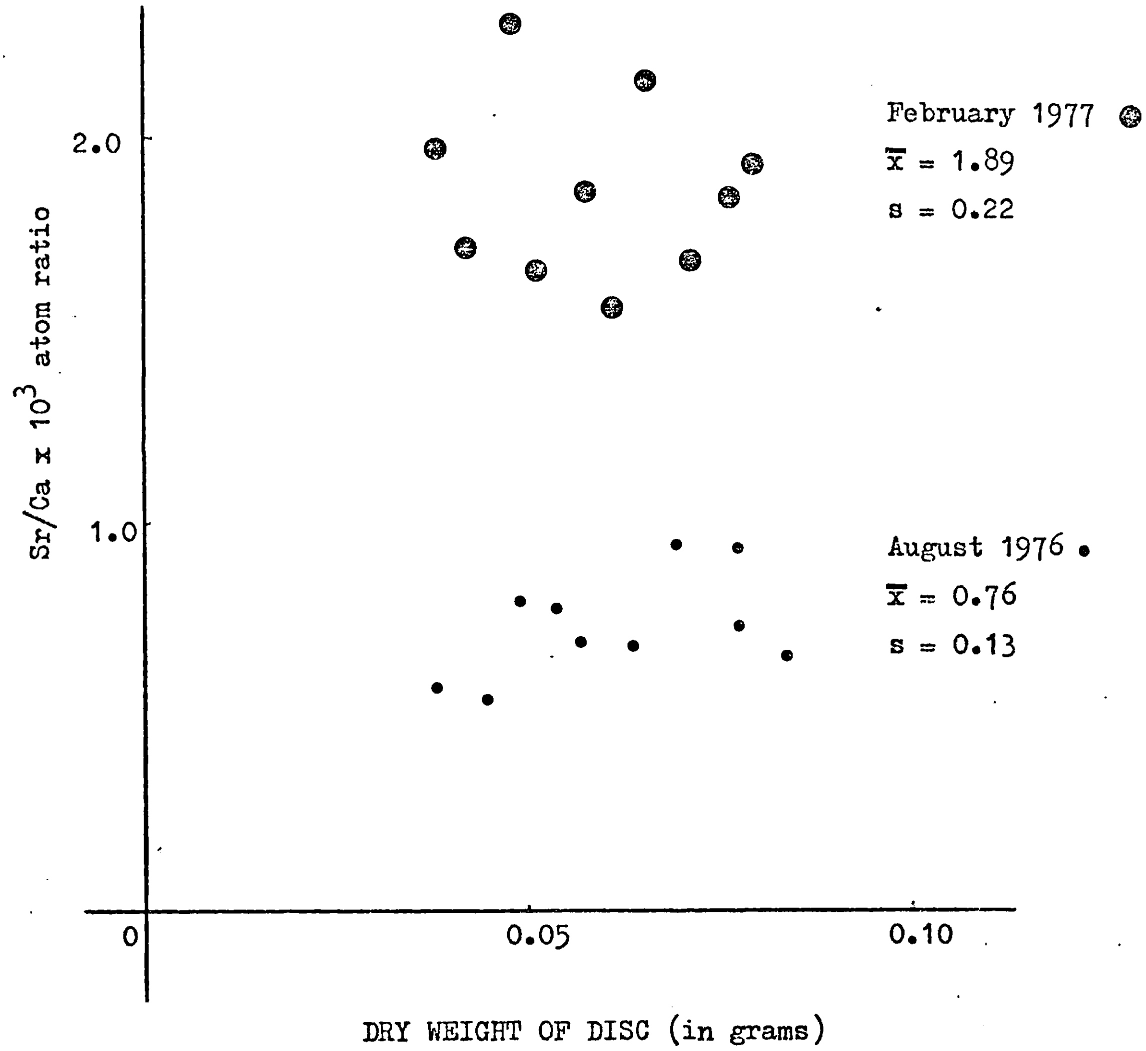
Graph 7.24 (a) Concentration of strontium in Ophiura albida (Millport) - without removal of organic matter .



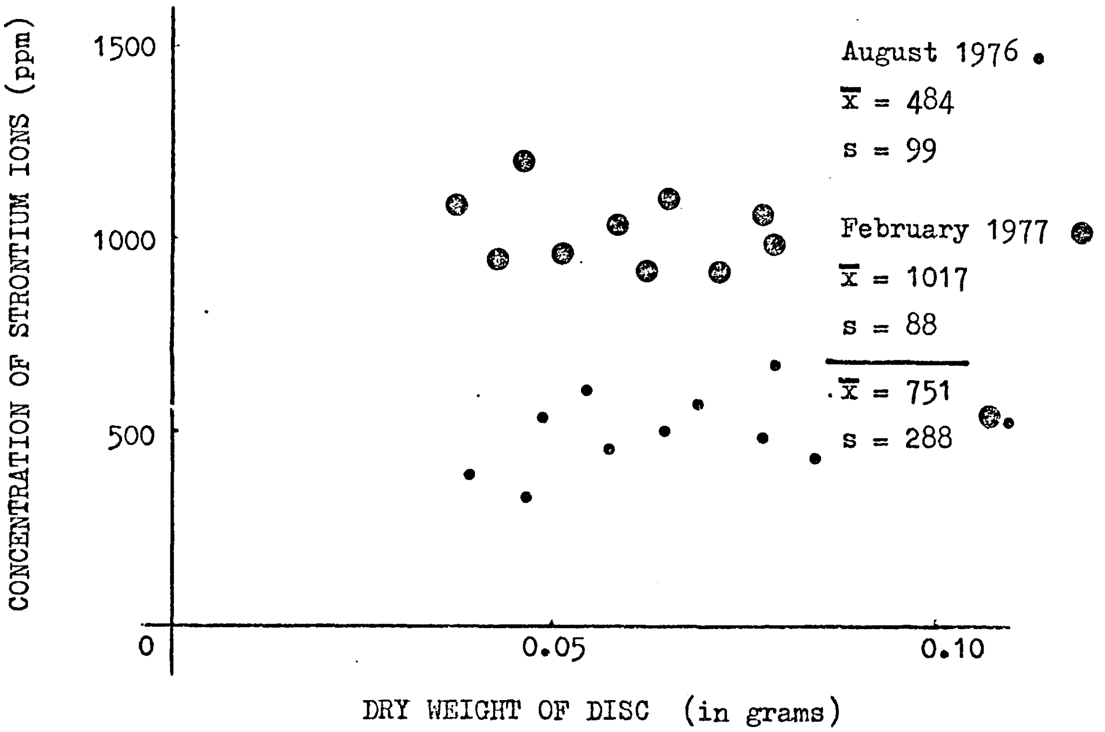
(b) Concentration of calcium in Ophiura albida (Millport) - without removal of organic matter.



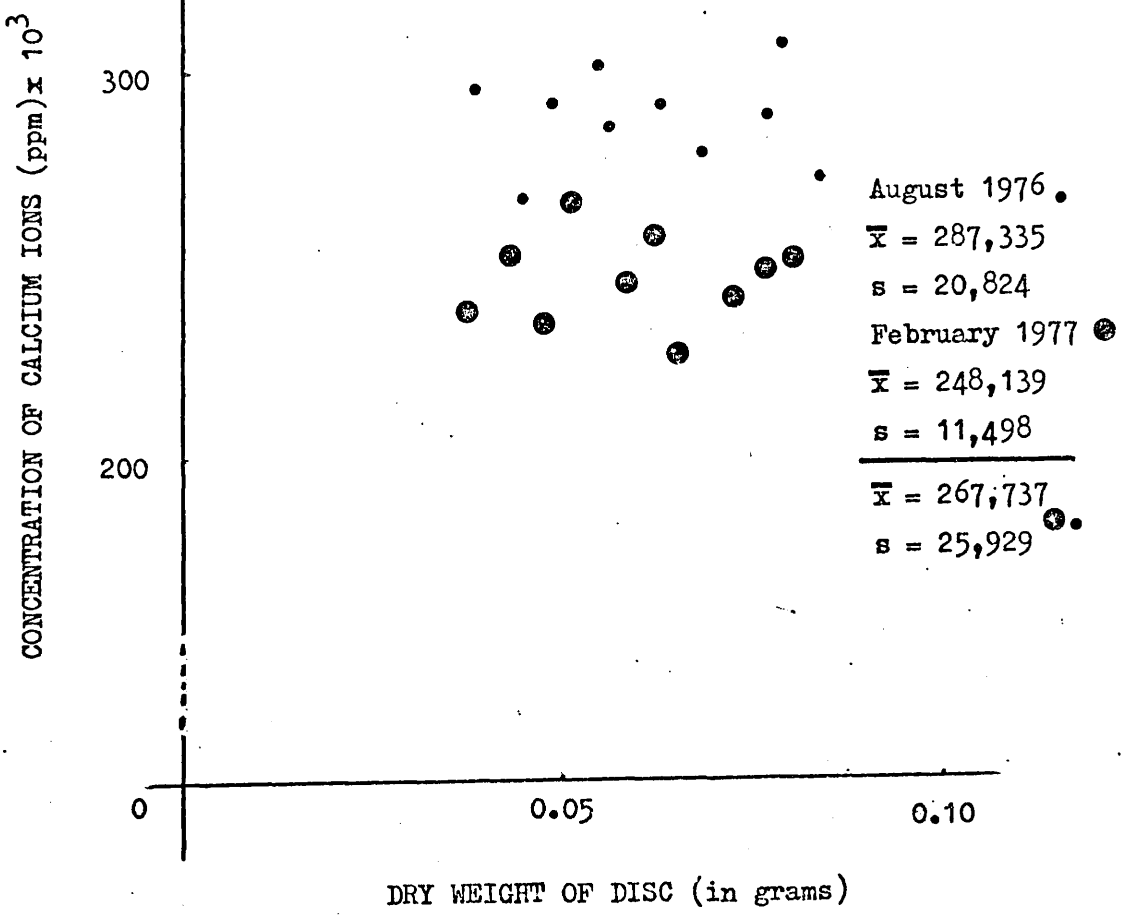
Graph 7.25 Sr/Ca x 10^3 atom ratio in Ophiura albida (Millport) - disc (without removal of organic matter) .



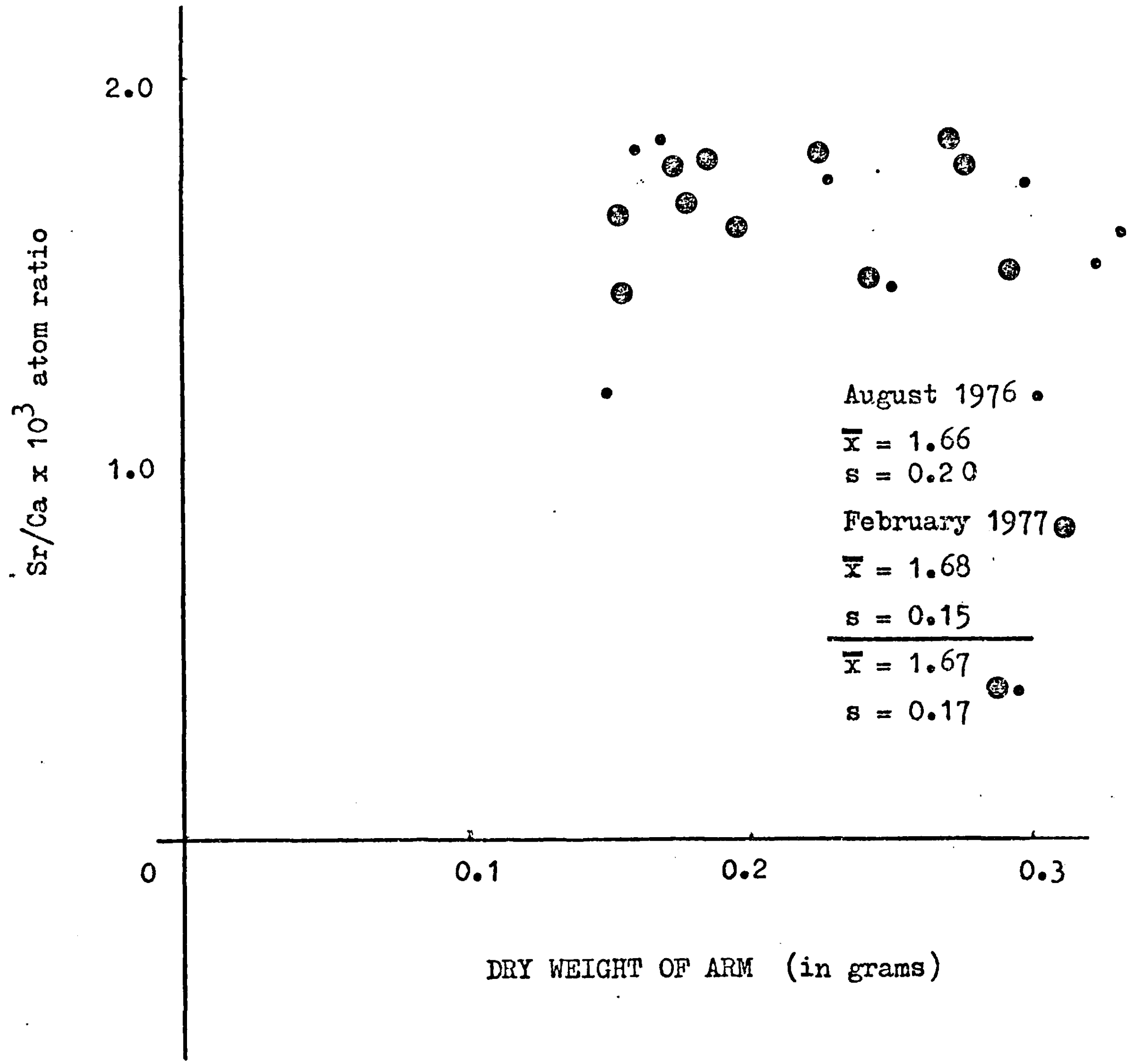
Graph 7.26 (a) Concentration of strontium in Ophiura albida (Millport) - disc (without removal of organic matter).



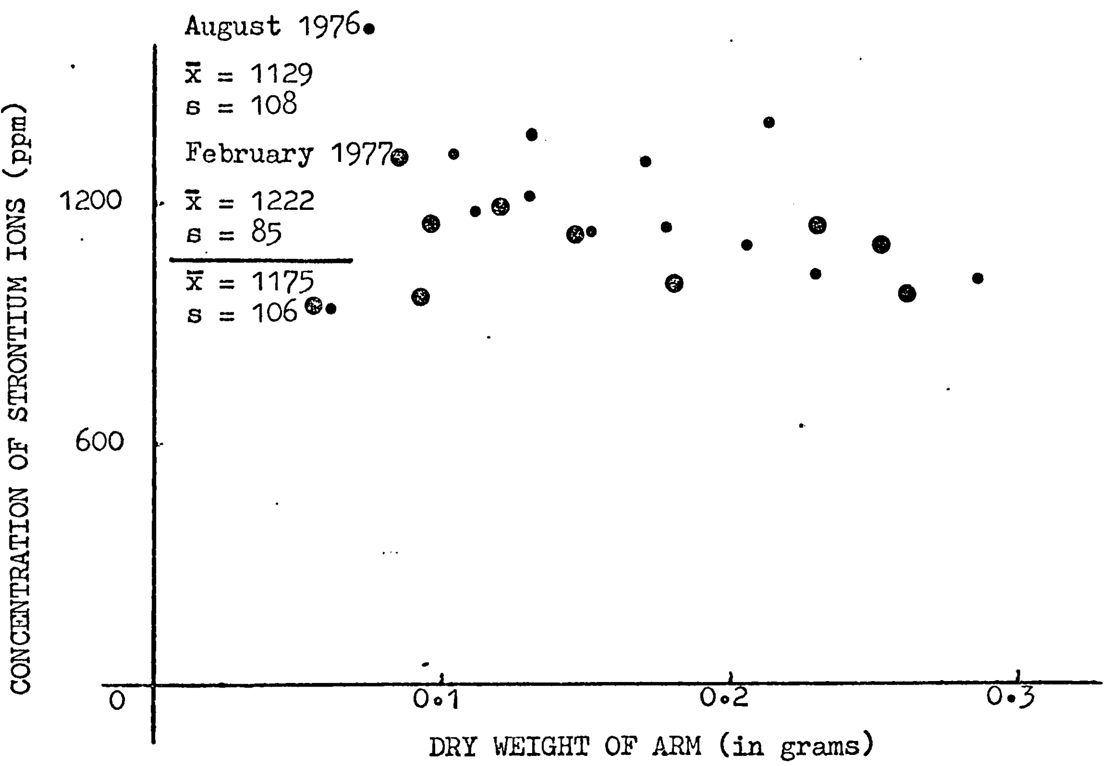
(b) Concentration of calcium in Ophiura albida (Millport) - disc (without removal of organic matter).



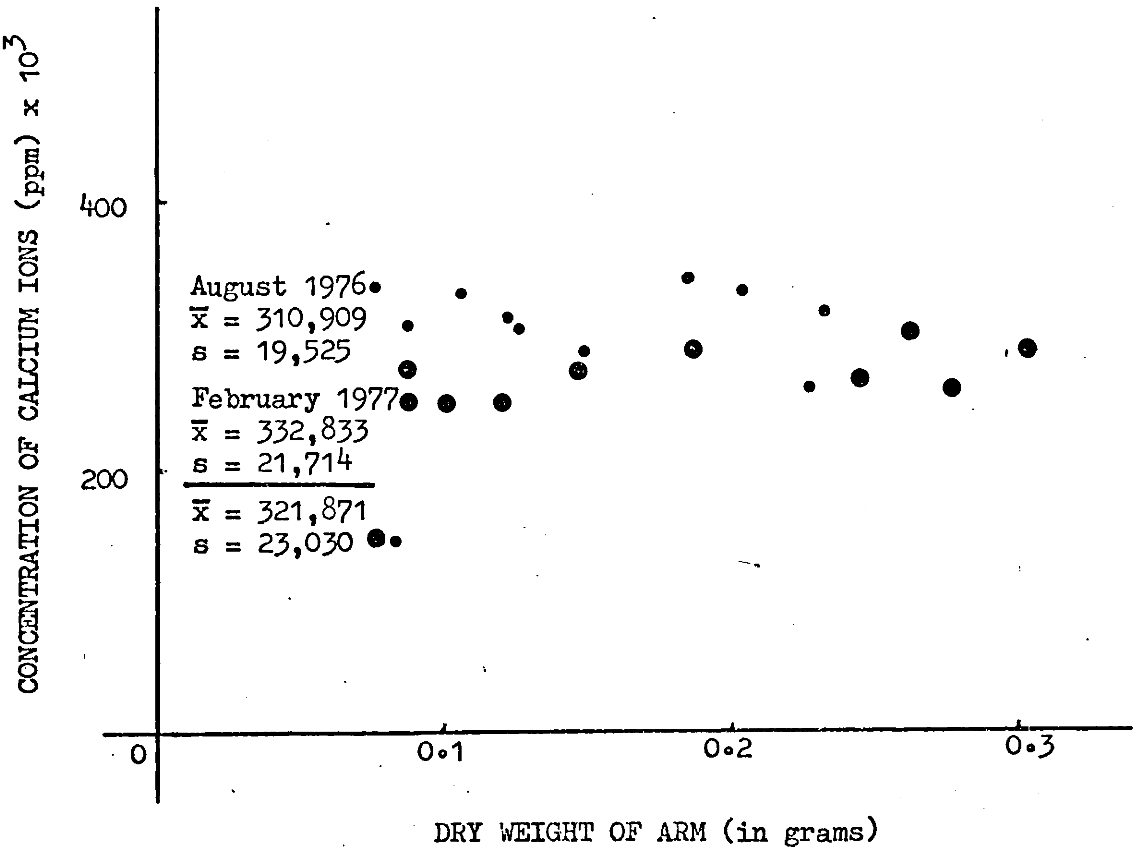
Graph 7.27 Sr/Ca x 10^3 atom ratio in *Ophiura albida* (Millport) - arm (without removal of organic matter).



Graph 7·28 (a) Concentration of strontium in <u>Ophiura albida</u> (Millport) - arm (without removal of organic matter) .



(b) Concentration of calcium in <u>Ophiura albida</u> (Millport) - arm (without removal of organic matter) .



With reference to Graph 7.29, Ophiura albida from Loch Etive appears to have a fairly varied Sr/Ca atom ratio. This is probably due to the slight variation in Sr/Ca atom ratio of the waters from the various sampling stations (see Table 7.1). The Sr/Ca atom ratio of whole analyses of O.albida from Loch Etive is slightly greater than that of O.albida from Millport. The calcium concentrations and the strontium concentrations in those individuals from Loch Etive are slightly higher than in those from Millport (Graphs 7.30 and 7.31).

Since the waters of Loch Etive have lower strontium concentrations than fully saline waters (see Table 7.30), this would suggest the need for an improvement in the strontium selection mechanism in Ophiura albida. Possible natures of such a process are discussed later.

Amphiura chiajei from Loch Etive has slightly higher Sr/Ca atom ratio (Graph 7.32) than does O.albdia from Loch Etive. With reference to Graphs 7.33 and 7.34, this appears to be due to the slightly lower calcium concentration to be found in A.chiajei.

Ophiura albida from the Öresund have slightly lower Sr/Ca atom ratios than those from Loch Etive and thus are more similar to those from Millport, (Graphs 7.35 and 7.36).

7.8 Discussion:

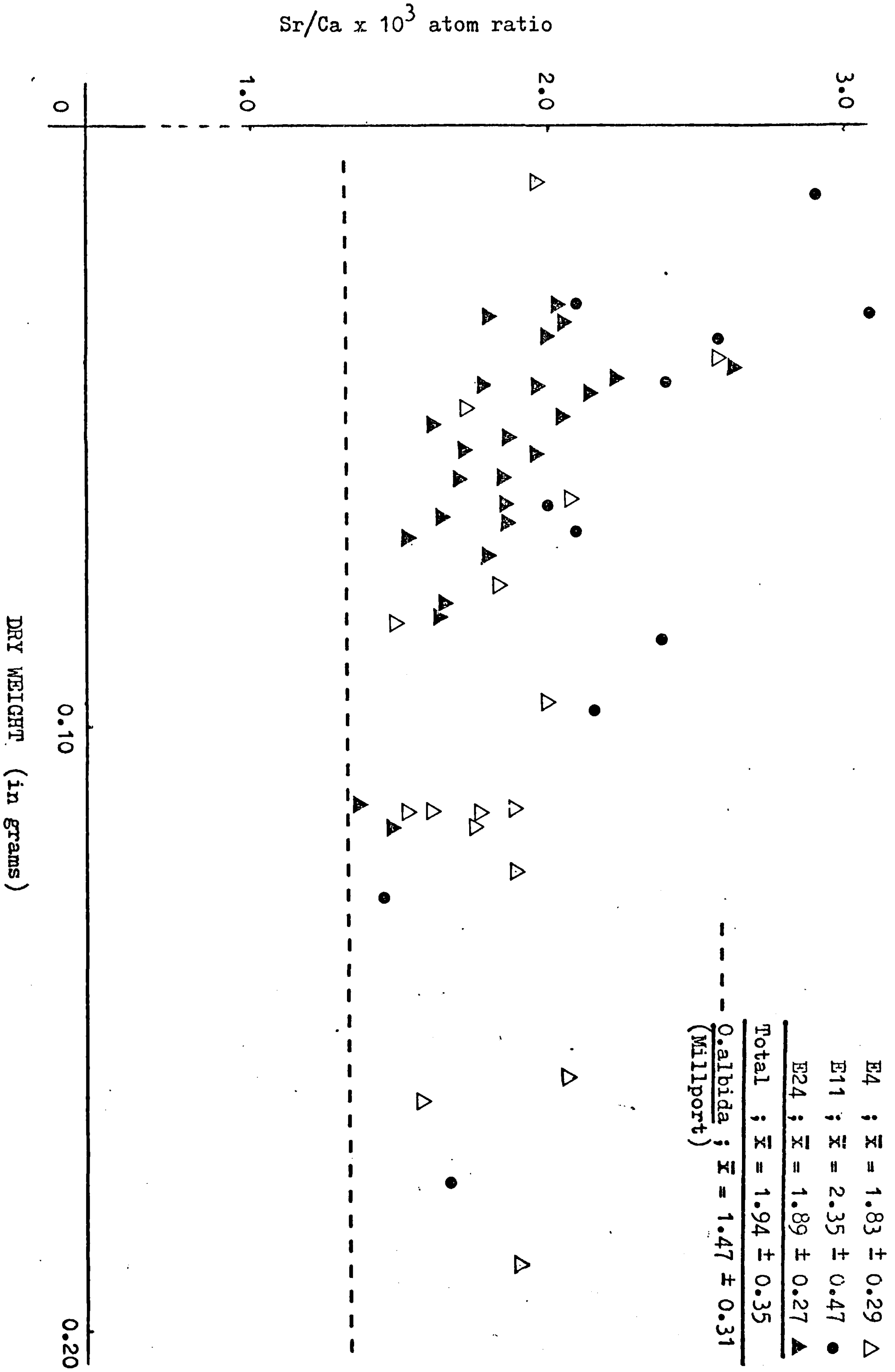
Previous work on the Sr/Ca atom ratios of the calcareous components of various members of the Echinodermata has indicated that the ratio for a given species can be stated as a single value. Indeed, the remarkable constancy of echinoderm calcareous Sr/Ca ratios has been widely reported (Noll, 1934; Webb, 1937; Odum, 1950, 1951a; Thompson and Chow, 1955; Kulp et al, 1952; Bowen, 1956).

The work of Odum (1957a) indicates a variable Sr/Ca atom ratio for the phylum as a whole and also between the classes. Mauchline and Templeton (1966) give values for several species ranging from 2.3 to 2.8. Banbury et al (1950) analysing one species, Echinus esculentus, report a ratio of 3.5. Calculating

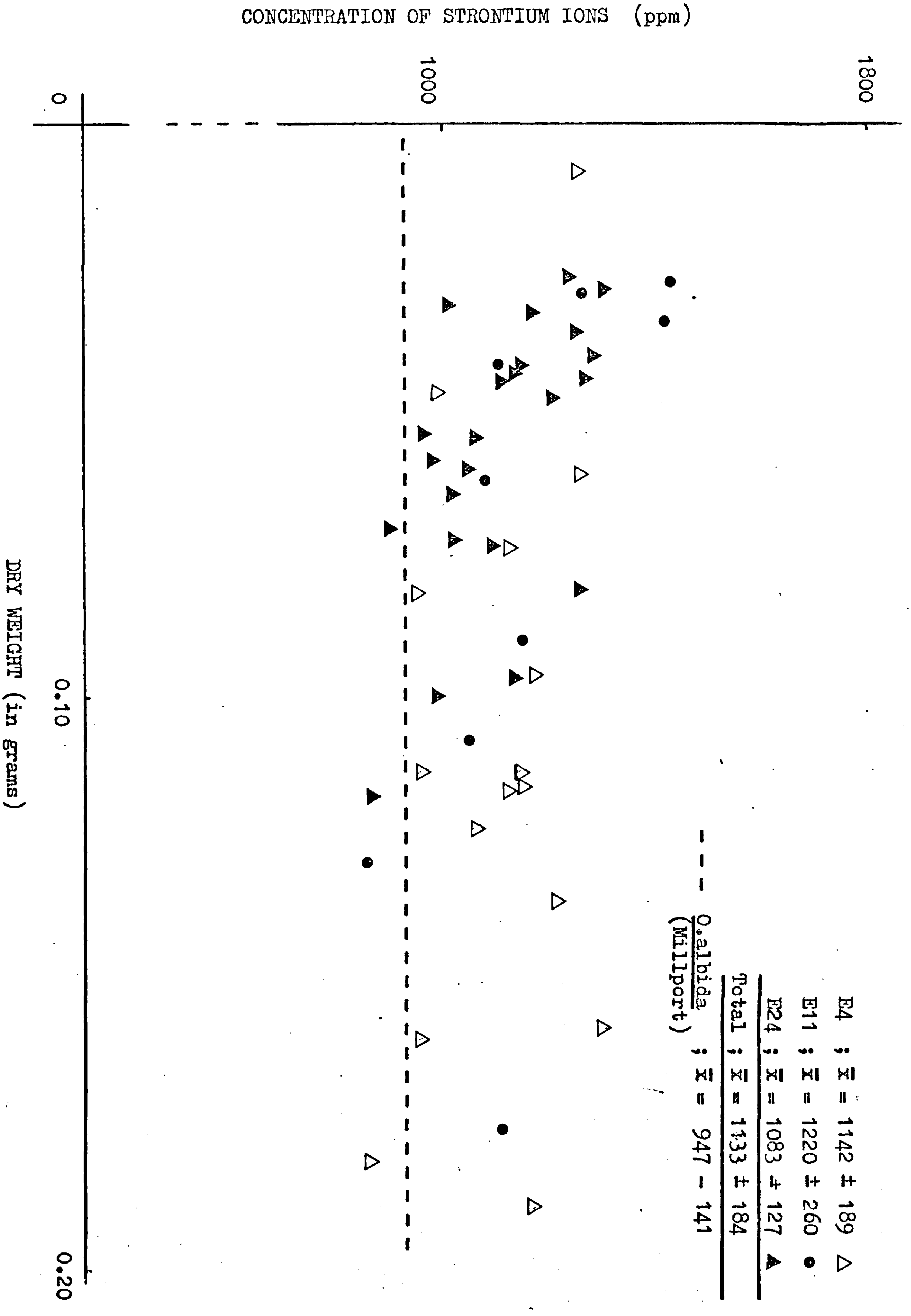
Table 7•1 Strontium, calcium and Sr/Ca x 10^3 atom ratio values for waters from various stations within Loch Etive •

STATION	CONCENTRATION (ppm)		Sr/Ca x 10^3
	Strontium	Calcium	
E2	4•5	310,000	6•64
E4	4•5	280,000	7•35
E6	4•0	285,000	6•42
E11	4•7	325,000	6•61
E14	4•7	315,000	6•82
E24	4•0	252,000	7•26

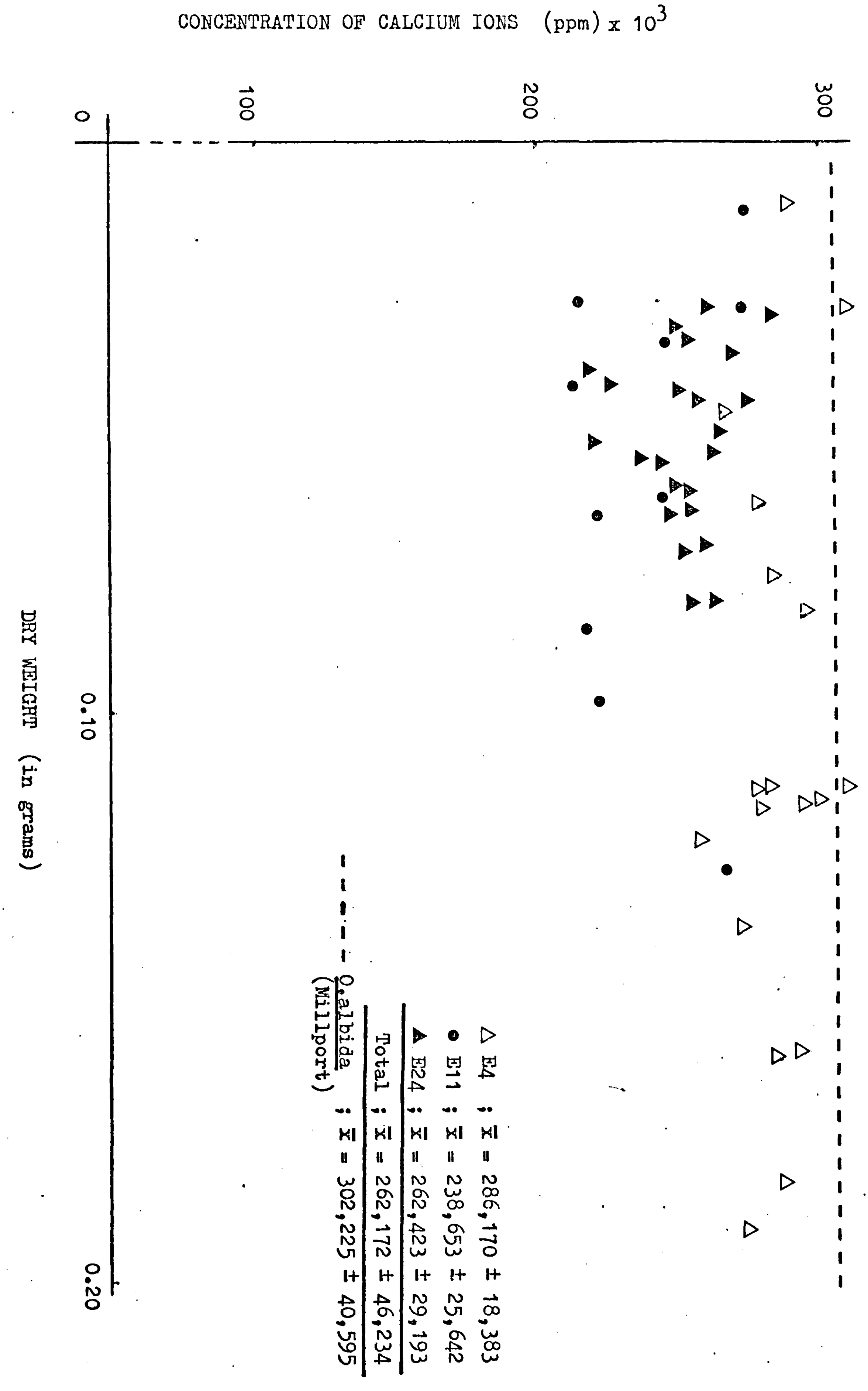
Graph 7.29 Sr/Ca x 10^3 atom ratio in <u>Ophiura albida</u> from different stations within Loch Etive.



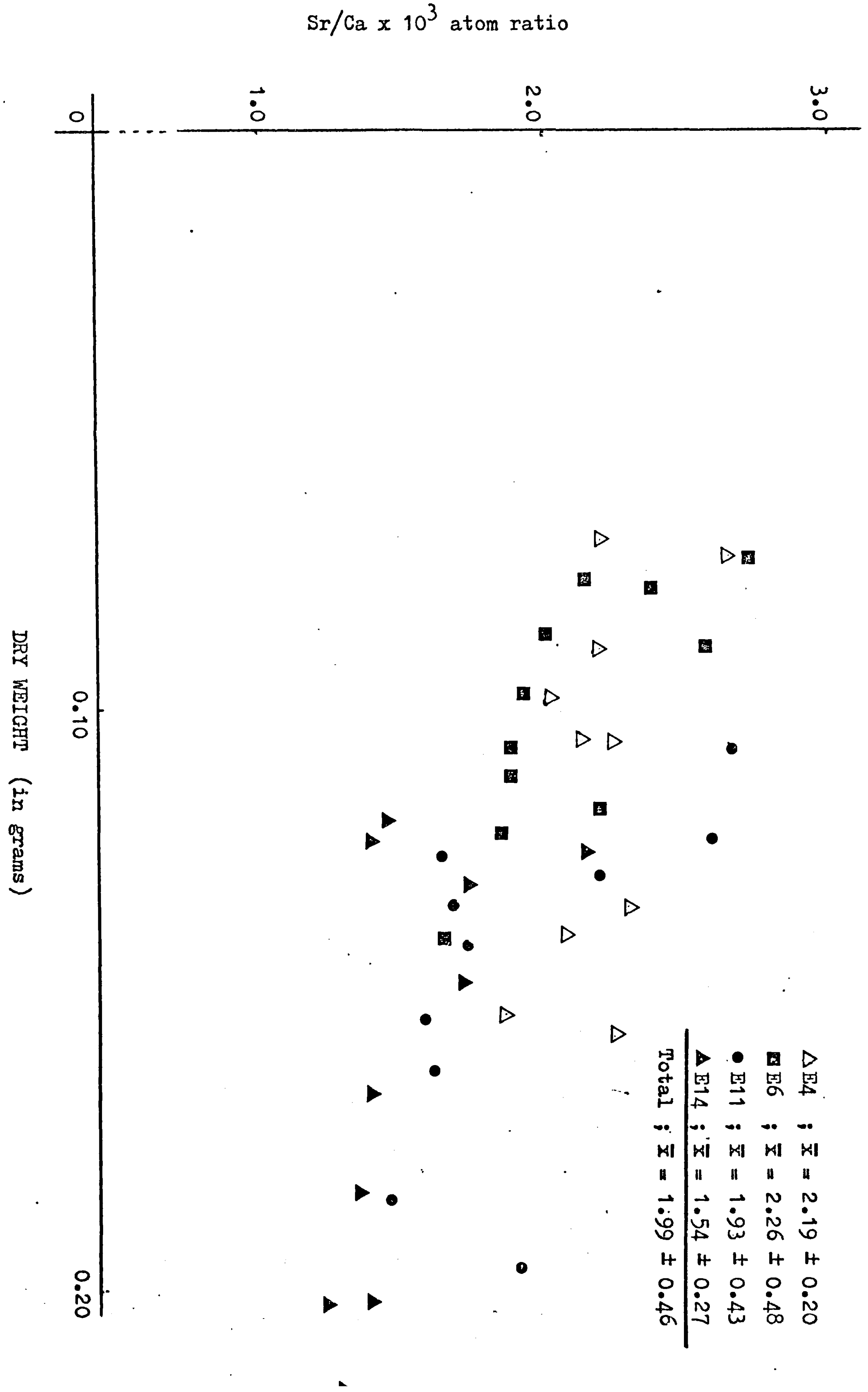
Graph 7.30 Concentration of strontium in *Ophiura albida* from different stations within Loch Etive.



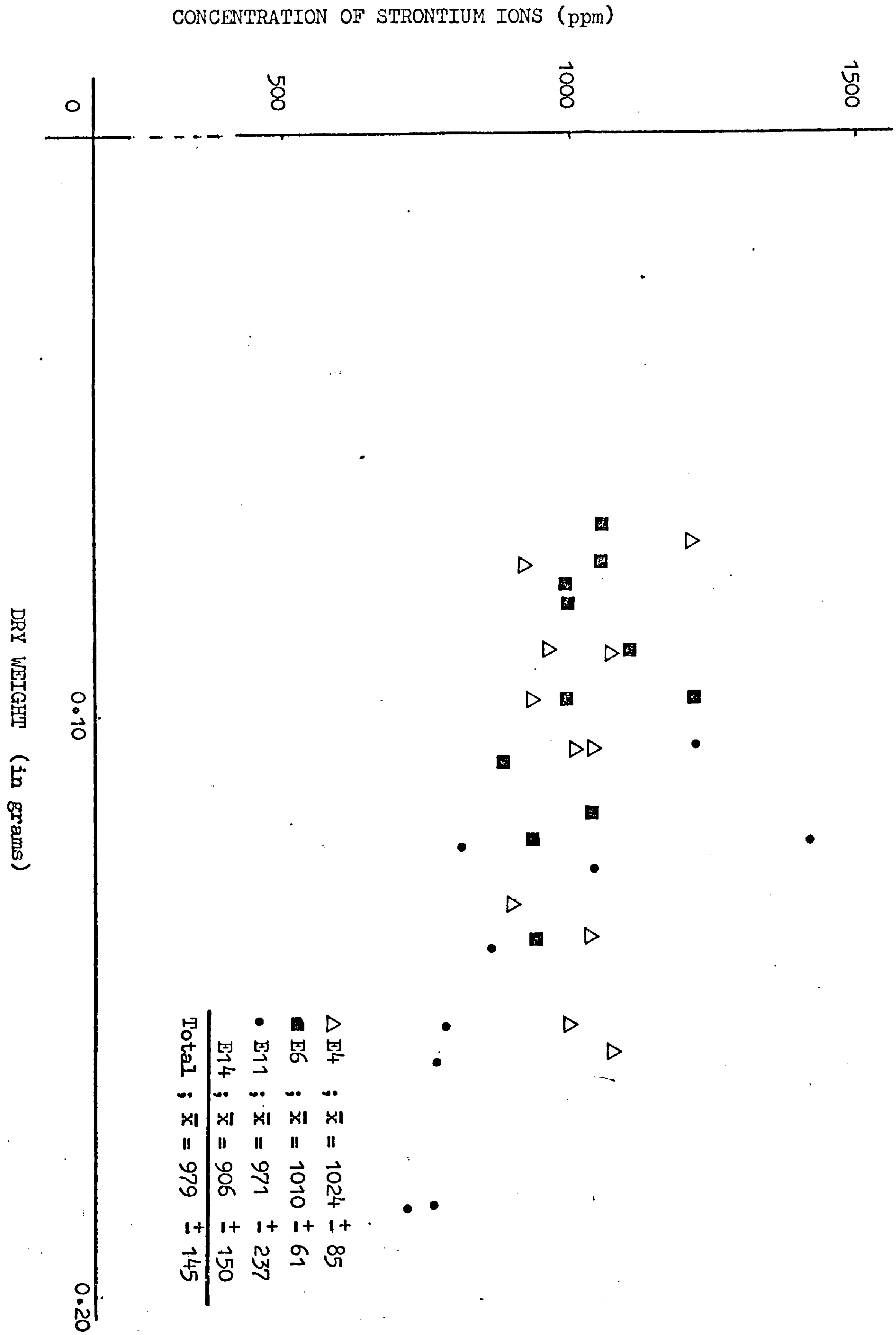
Graph 7.31 Concentration of calcium in Ophiura albida from different stations within Loch Etive .



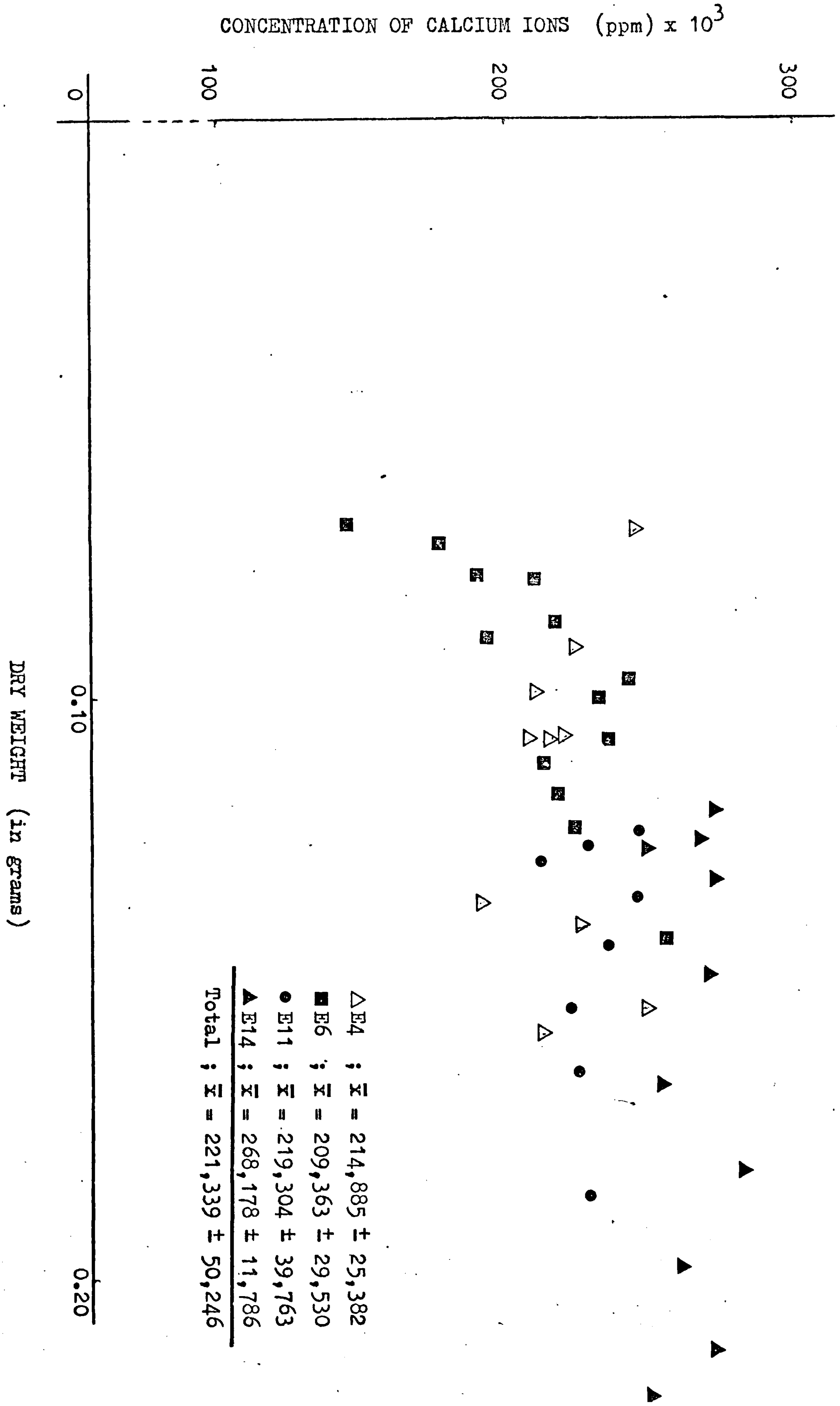
Graph 7.32 Sr/Ca x 10^3 atom ratio in <u>Amphiura chiajei</u> from different stations within Loch Etive.



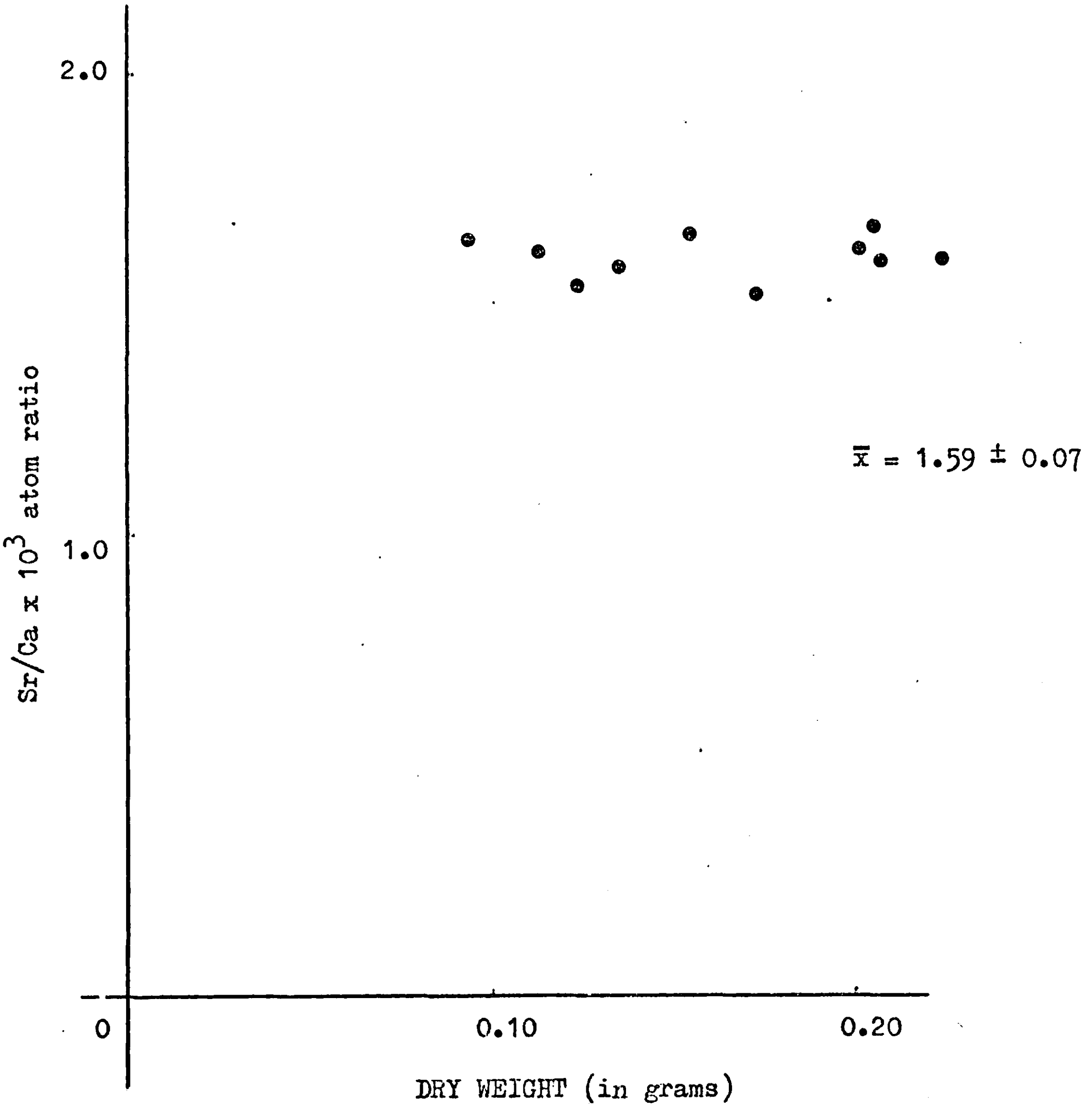
Graph 7·33 Concentration of strontium in <u>Amphiura chiajei</u> from different stations within Loch Etive.



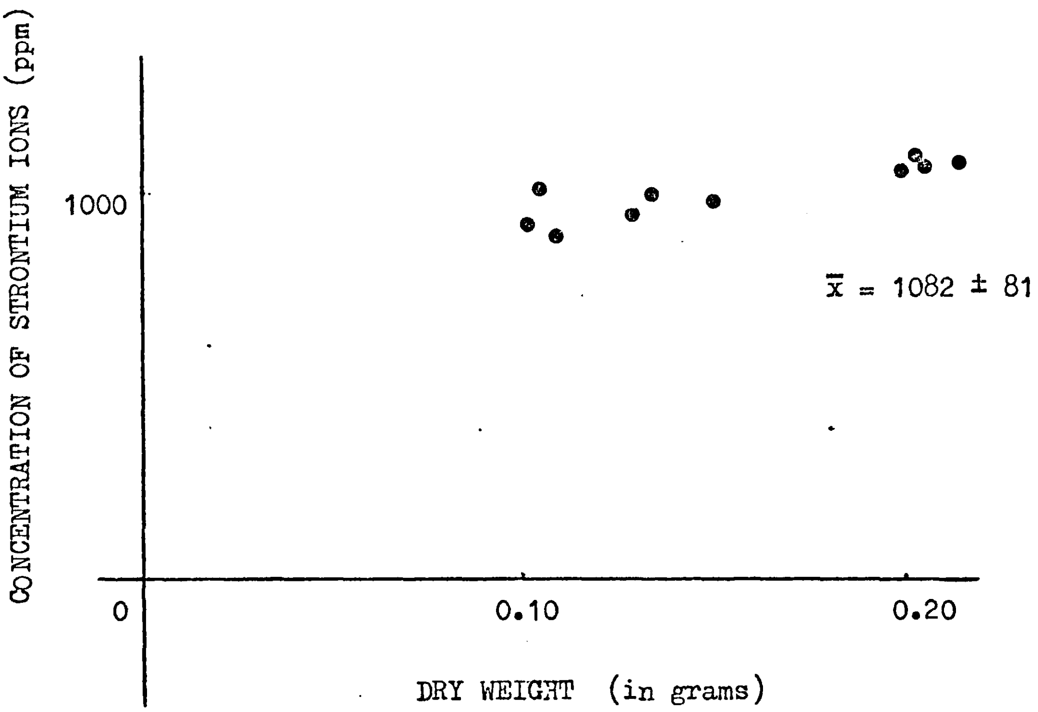
Graph 7.34 Concentration of calcium in _Amphiura chiajei_ from different stations within Loch Etive .



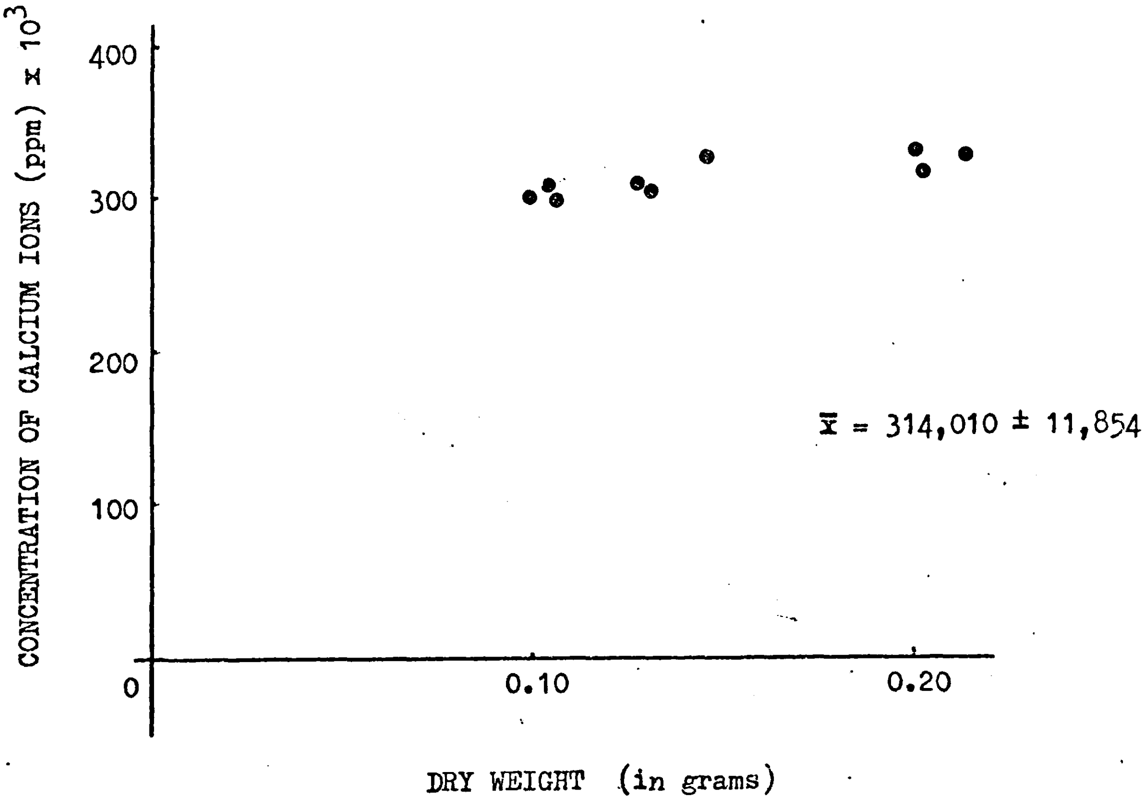
Graph 7.35 Sr/Ca x 10^3 atom ratio in *Ophiura albida* (Öresund).



Graph 7.36 (a) Concentration of strontium in Ophiura albida (Öresund).



(b) Concentration of calcium in Ophiura albida (Öresund).



Sr/Ca atom ratios from the data given in Riley and Segar (1970) it appears that a variable ratio is indicated for the five species assayed, .54 to 1.79. Hallam and Price (1968) found a wide range of concentrations in the outer shell of the lamellibranch mollusc, Cardium edule: 910 - 2660 ppm, and doubt the usefulness of quoting a single value for any given species, except for the most general of comparisons. The present work also suggests the unreliability of reporting just one Sr/Ca atom ratio for a species without some qualification.

There are few reports of Sr/Ca atom ratio determinations of ophiuroid material. Thompson and Chow (1955) quote figures of 2.63 - 2.79 for five species. Mauchline and Templeton (1966) report values of 2.7 and 2.8 for two species. Odum (1957a) mentions ratios of 3.02 and 3.85 for two species. Riley and Segar (1970) give a ratio of 1.06 for an unidentified ophiuroid. There appears to be a number of ratios ascribed to ophiuroids such that it is difficult to conclude that the Sr/Ca atom ratio is constant within this class of echinoderm. It is interesting to note that where one or more authors have analysed specimens of the same species a variety of atom ratios have been reported. For instance, the values for Ophiura sarsi quoted are 3.02 (Odum, 1957a) and 2.71 and 2.65 (Thompson and Chow, 1955); for Gorgoncephalus species - 3.85 (Odum, 1957a) and 2.72, (Thompson and Chow, 1955). Swan (1956) commenting upon Sr/Ca atom ratios in bivalve molluscs, points out that differences in Sr/Ca atom ratio between specimens of the same species may be real and significant.

In the present work, the calcareous parts of whole Ophiocomina nigra were analysed. It was found that the Sr/Ca atom ratio was inversely proportional to the weight of the ossicles. That is to say, smaller animals have higher values than larger animals over the size range, 0.2190 grams to 2.4325 grams. Surveying the individual concentrations of strontium and calcium it is clear that the calcium content remains fairly steady over the weight range while the strontium content is inversely proportional. Why the concentration of strontium should decrease with an increase in size is not immediately clear. One possible explanation can be sought in the following argument.

It is necessary to assume that an increase in size is commensurate with an increase in age. This does not always hold in echinoderms. Some echinoids, for example Strongylocentrotus purpuratus, have the ability to absorb some of the test plate material (Ebert, 1968). However, it is probably a fair assumption that younger ophiuroids are smaller than their older counterparts. If this is accepted, then the next step is to state that these younger animals have greater strontium concentrations than the older individuals.

A number of workers have commented upon ideas concerning the relationship between growth rate and the Sr/Ca atom ratio. Thompson and Chow (1955) state that the Sr/Ca atom ratio is independent of age. Dodd (1965) showed, using Mytilus californianus, that the concentration of strontium decreases with an increase in size. Since bivalve molluscs grow faster in warmer water they postulate that in Cerastoderma edule a high growth rate does not correlate inversely with the strontium content. They suggest that the growth rate is not a significant variable since there is no general tendency for strontium concentration to increase with size.

Swan (1956) proposes that differences in growth rate may be important in determining the Sr/Ca atom ratio in bivalves and suggests that thin-shelled individuals have lower ratios than do the thick-shelled specimens. He points out that, from the literature, the thin-shelled animals are characteristic of rapidly growing individuals, whereas heavier shells develop on more slowly growing individuals. Using the work of Hallam and Price (1968) and applying the considerations of Swan (1956) there is evidence that, in Cerastoderma, the thin-shelled animals (and by inference, the rapidly growing individuals) have greater concentrations of strontium.

Dodd (1967) notes that a rapid growth rate would result in a high Sr/Ca atom ratio in those forms which discriminate against strontium and lower ratios in those which select for strontium since a rapid growth rate would mean less time would be available for exclusion or selection of strontium.

If a value is taken from the Graph 7.8 comparing dry weight and Sr/Ca atom ratio in the whole analyses of Ophiocomina nigra, say 1.80 for an animal of 0.4 grams dry weight, this would give a discrimination ratio of 0.2. This figure is significantly less than one implying that the ossicles are discriminating against the strontium ions. So, if small animals had a rapid growth rate then this would result in a higher Sr/Ca atom ratio than in larger animals.

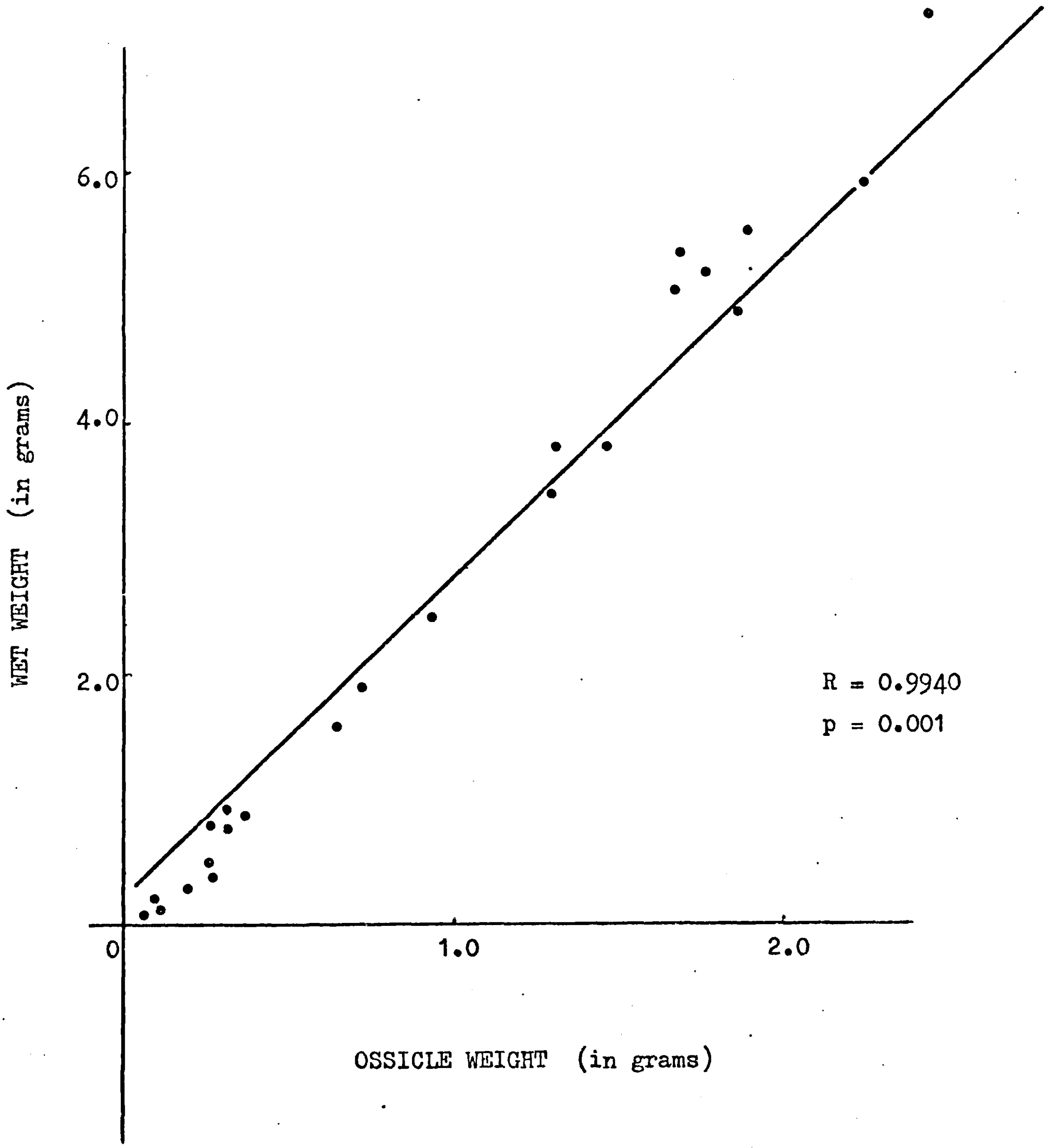
Orton (1926) has pointed out that there is a decline in growth rate with age. If this can be applied to ophiuroids then smaller animals would have faster growth rates, and since they discriminate against strontium ions this would explain the inverse relationship between Sr/Ca atom ratio and the size of the animal which has been demonstrated in the present work. Graph 7.37 shows ossicle weight and the wet weight of an individual are directly proportional, so that in the above argument it is acceptable to use the ossicle weight as a direct equivalent, in terms of size increments, of the wet weight, and thus the size, of the animal as a whole.

Further evidence that smaller animals are younger animals and thus have rapid growth rates, which are coupled with larger concentrations of strontium, is suggested by the analysis of the contribution of the regenerating portion to the whole arm in Ophiocomina nigra. It is reasonable to assume that the replacement of cast-off limbs or other parts, which are important, would warrant a fast growth rate, especially if these organs are used as part of a defence mechanism (such as escaping from predators).

If the above assumption is applied to the regenerating part of an arm then it would appear that there are two possibilities concerning young individuals and their larger strontium concentrations in comparison with larger animals. Firstly, strontium concentration is related to growth rate or, secondly, it is related to the physical size of the animal per se.

Inspecting Graphs 7.16 to assess the relative strontium and calcium contributions of the regenerating portion of the arm to the entire arm, it can be seen that the calcium contents of the regenerating part and of the older part of the arm are similar.

Graph 7.37 Relationship between wet weight of intact animal and ossicle weight in <u>Ophiocomina nigra</u>.



There is, however, a higher strontium content in the regenerating part than in the older section. This is consistent with the view that new growth occurs at a faster rate than growth in the more mature sections of the animal. That the Sr/Ca atom ratios of the regenerating sections lie within that found for young animals of equivalent dimensions also provides evidence that Sr/Ca atom ratio is related to the size of the animal and not only the growth rate.

Such work also has its analogy in the molluscs where the value of analysing animals in terms of structural components (Dodd, 1967) or mineralogy (Hallan and Price, 1968) has been stressed. Dodd (1965) demonstrated that at lower temperatures there is a greater strontium concentration in the nacreous layer than in the outer, prismatic layer of Mytilus californianus and suggests the separate treatment of individual layers.

Separate analysis of the whole animal in terms of the disc and arm ossicles indicates a fairly uniform and similar concentration of calcium and strontium if equivalently sized ossicles of the disc and arm are compared.

At first sight, the analyses of Ophiua albida, from Millport, provide contradictory data to the scheme of strontium distribution postulated for Ophiocomina nigra.

Taking the Sr/Ca atom ratios individually of the calcareous parts of O.albida it is seen that both strontium and calcium concentrations are uniform and have no inverse or direct relationship with dry weight as is found in O.nigra. Lacking small specimens of O.nigra it was not possible to directly compare such analyses with those of O.albida which is the smaller species. However, if the data for O.nigra is extrapolated so that animals of similar sizes can be compared then the Sr/Ca atom ratios appear similar and do not conflict with the proposed strontium distribution scheme.

Separate analyses of the discs and the arms of Ophiura albida indicate similarities with Ophiocomina nigra. Both strontium and calcium concentrations in the disc and in the arms are fairly uniform and similar in equivalently sized animals.

It is interesting to note that, despite the wide range of specimens sampled, the range of species and the varying Sr/Ca atom ratio of the different habitats, there is an approximate constancy of the discrimination ratio (see Table 7.2). The discrimination ratios reported in the present work are of a similar order of magnitude as those reported in the literature by other workers.

Since the Sr/Ca atom ratio of the Loch Etive O.albida is higher than in similarly sized animals from Millport this suggests that despite a lower ambient strontium concentration (252 - 325 ppm) in the bottom waters of the Loch, the animals maintain body levels of strontium equivalent to those of the same species from Millport where full salinity obtains and where there is a higher Sr/Ca atom ratio in the ambient sea water. Thus, there is evidence that the Sr/Ca atom ratio of the environment is not of absolute importance in determining the ratio in the animal as has been postulated by Kulp et al (1952).

It would also suggest that there is some concentrating mechanism in these ophiuroids so that the strontium level in the body of the animal can be maintained despite a lowering of the ambient concentration of strontium.

Data for the above work have been obtained by whole or skeletal analyses. It was not possible to separate and analyse, in isolation, the soft tissues of these species. It is likely (Binyon, 1978) that the soft tissues and skeletal material have different rates of strontium equilibration since incorporation into the skeleton would be most likely to occur (though perhaps not to the complete exclusion of ion exchange taking place) during growth of the individual. The passage of the strontium ions into the soft tissues and fluids is likely to be more rapid.

Table 7·2 Discrimination ratios·

SPECIES	LOCATION	DISCRIMINATION RATIO
Ophiocomina nigra	Plymouth	0·10 - 0·30
Ophiura albida	Millport	0·12 - 0·37
	Loch Etive	
	E24	0·19 - 0·35
	E11	0·22 - 0·47
	E4	0·18 - 0·35
Amphiura chiajei	Loch Etive E14	0·18 - 0·32
	E11	0·22 - 0·40
	E6	0·26 - 0·40
	E4	0·25 - 0·36

CHAPTER EIGHT

INVESTIGATION INTO THE EFFECT UPON THE STRONTIUM CONCENTRATIONS IN Ophiocomina nigra AND Ophiura albida WHEN EXPOSED TO ELEVATED LEVELS OF STRONTIUM.

8.1 Introduction:

If, as seems likely, there is no obvious relationship between the Sr/Ca atom ratio of the environment and the Sr/Ca atom ratio in the whole animal in its natural habitat then it would be instructive to examine the effects of elevating the strontium levels experimentally upon the discriminating ability of the organism.

Investigations involving the immersion of marine animals in an elevated level of strontium have been conducted only twice previously (Gibbs and Bryan, 1972; Binyon, 1978).

Gibbs and Bryan (1972) maintained the crustacean, Carcinus maenas, in strontium enriched sea water. They demonstrated that the Sr/Ca atom ratio of the exoskeleton could be increased by such treatment. These crabs, however, only absorbed strontium after ecdysis. It was noticed that the discarded exuviae showed a small increase in Sr/Ca atom ratio with time.

In echinoderms, however, it has been pointed out by Binyon (1978)that since moulting is unknown a relatively long exposure to raised strontium levels might be necessary in order to allow the growing calcarous ossicles to incorporate the available strontium ions.

Consequently, Binyon (1978) analysed the soft tissues of the asteroid, Asterias rubens, where incorporation of the ion might be expected to occur more rapidly. It was found that strontium in the soft tissues and coelomic fluids attained a new equilibrium rapidly and at a level commensurate with the strontium concentration in the medium.

8.2 Materials:

Two species were used for the investigation of the effects of raised concentrations of strontium upon ophiuroids:-

Ophiocomina nigra - Plymouth

Ophiura albida - Millport, Loch Etive

8.3 Method:

A 30 litre stock tank was set up with circulating sea water maintained at 12°C. The strontium concentration in the sea water was increased by the addition of 4·87 grams of strontium chloride ($SrCl_2.6H_2O$). The salinity was not found to have altered by the addition of this small amount of salt. Since the original concentration of strontium in the sea water was 6.2 mg/l this addition represents an increase of about 9 fold.

The Ophiocomina nigra were kept for one month without any morphological or deleterious effects upon the activity of the animals in this solution. After one month the animals were removed and treated as described in chapter 7 for the assay of strontium and calcium. A sea water sample was also taken for use in the determination of these two ions.

The procedure was then repeated for Ophiura albida from Millport and Loch Etive. Since the O.albida from the Loch are living in a lower ambient strontium concentration than those from Millport the experimental solution had proportionally less strontium chloride added.

8.4 Results:

The Sr/Ca atom ratio in whole specimens of Ophiocomina nigra from an elevated level of strontium was approximately 2.05 per 1000 atoms of calcium, whereas in normal sea water it was 1.0/1000 atoms of calcium for similarly sized animals of about two grams in dry weight (see Graph 8.1, 7.2).

Considering this increase in terms of strontium and calcium it is clear that the calcium concentration was unaffected by the increased level of strontium in O.nigra and remained similar at 240,000 ppm. The strontium concentration does, however, vary. The strontium concentration for a two gram specimen of O.nigra from elevated levels is 1130 ppm, which represents an increase of 105% over the concentration in an equivalent sized animal from normal strontium levels (see Graphs 8.2a, b).

While the calcium concentration in Ophiura albida from Millport when placed in elevated strontium levels also does not change, the strontium increases a little (Graphs 8.3a, b). It rises from 900 ppm to 1300 ppm which represents an increase of 44% .

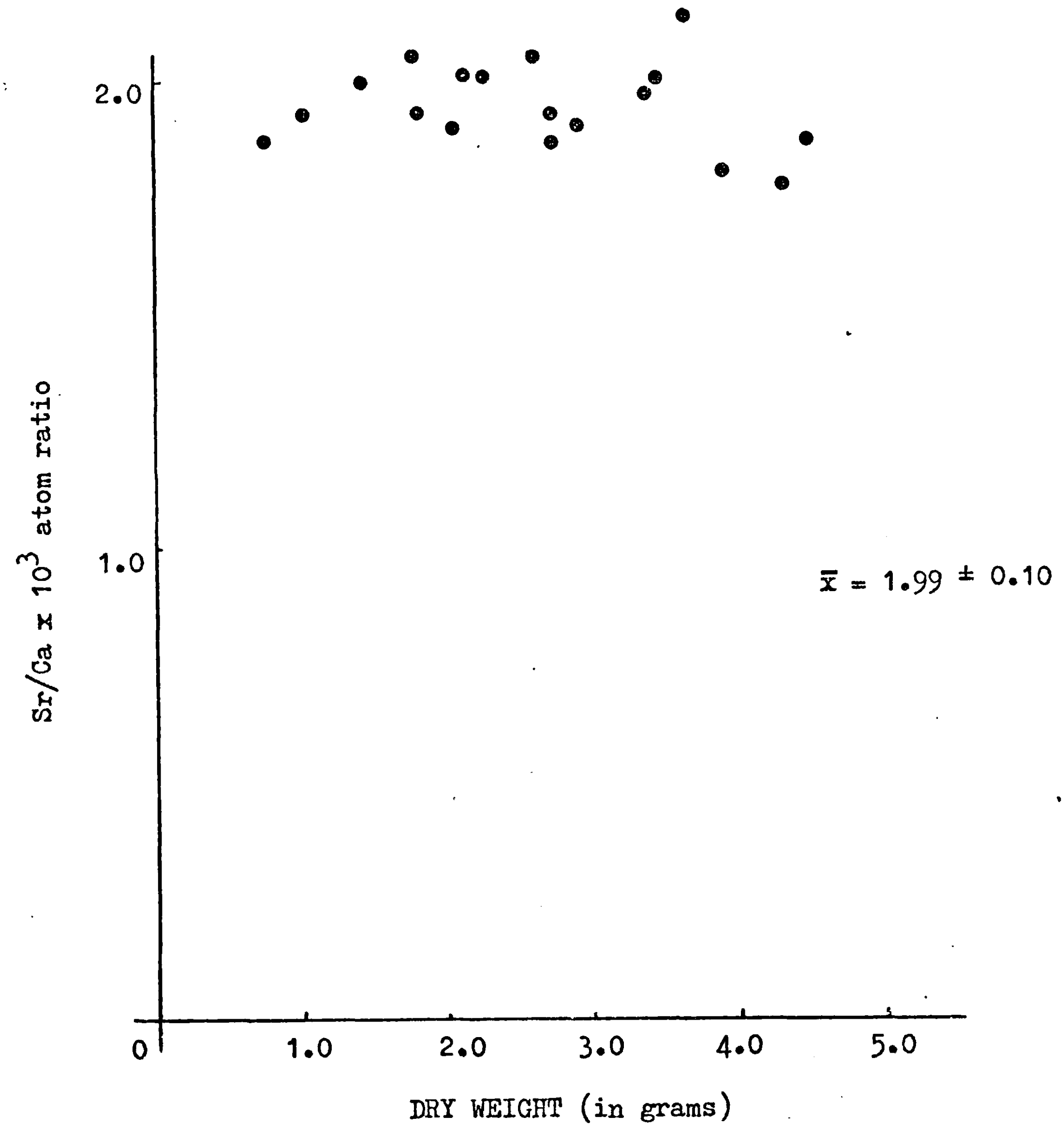
Although it was not possible to partition the animal so that the soft tissues could be assayed separately, it seems likely that the observed increases in strontium concentration of the animal were increases due to the uptake of this ion by the soft tissues rather than by the ossicles. During the length of the experiment it is thought improbable that sufficient growth could have occurred in order to account for the increases observed, assuming that incorporation followed a pattern similar to that in asteroids.

Ophiura albida from station E24 in Loch Etive do not show any notable increase in the strontium concentration of the whole animal when placed in elevated concentrations of strontium (see Graph 8.5a). However, since it was not possible to partition the individuals with respect to skeleton and soft tissues it is not known whether the above results are due to greater percentage of skeleton being present which would mean that there would be less soft tissue available to take up the 'extra' strontium available in the experimental medium. It has been demonstrated (Graphs 6.2, 6.4) that individuals from Millport and Loch Etive have similar ash contents in whole animal analyses.

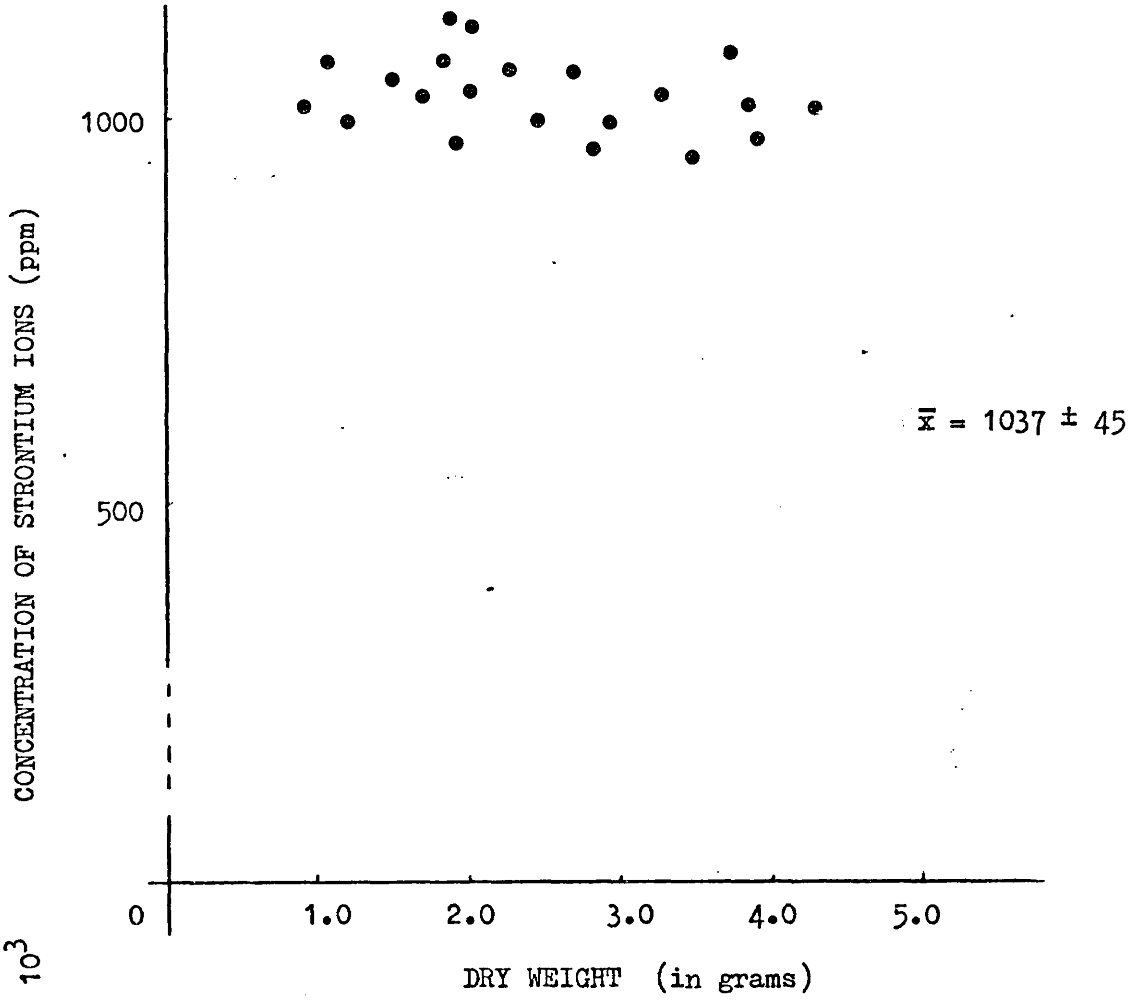
8.5 Discussion:

Though the ambient strontium concentration was increased

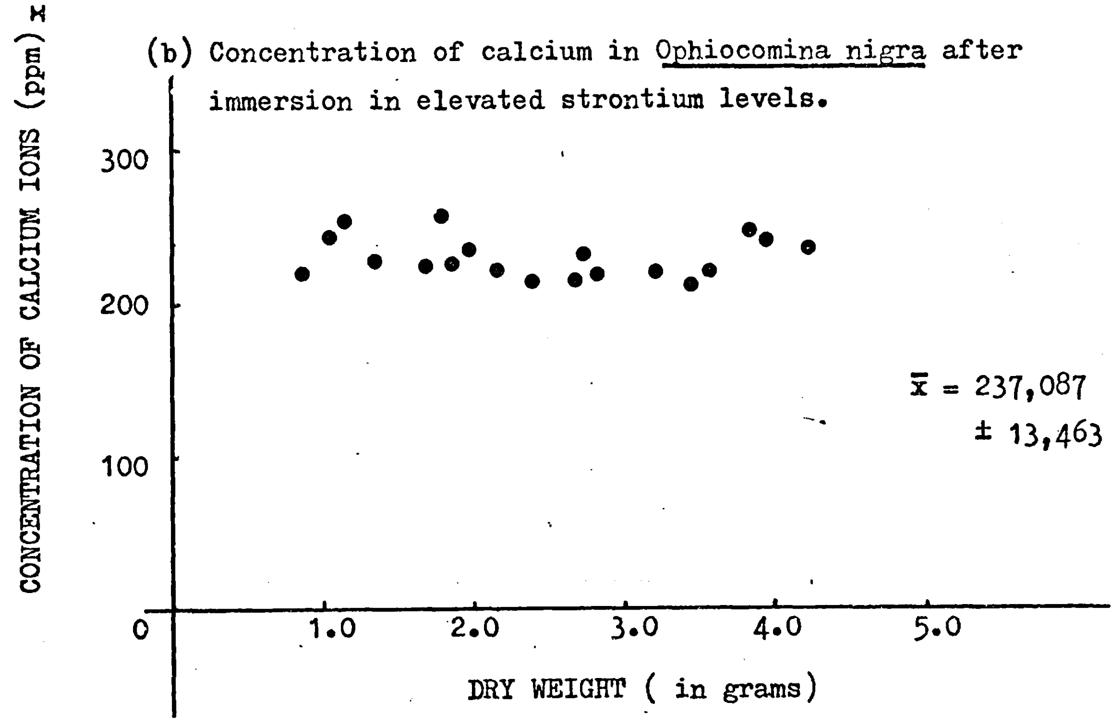
Graph 8.1 Sr/Ca x 10^3 atom ratio in <u>Ophiocomina nigra</u> after immersion in elevated strontium levels.



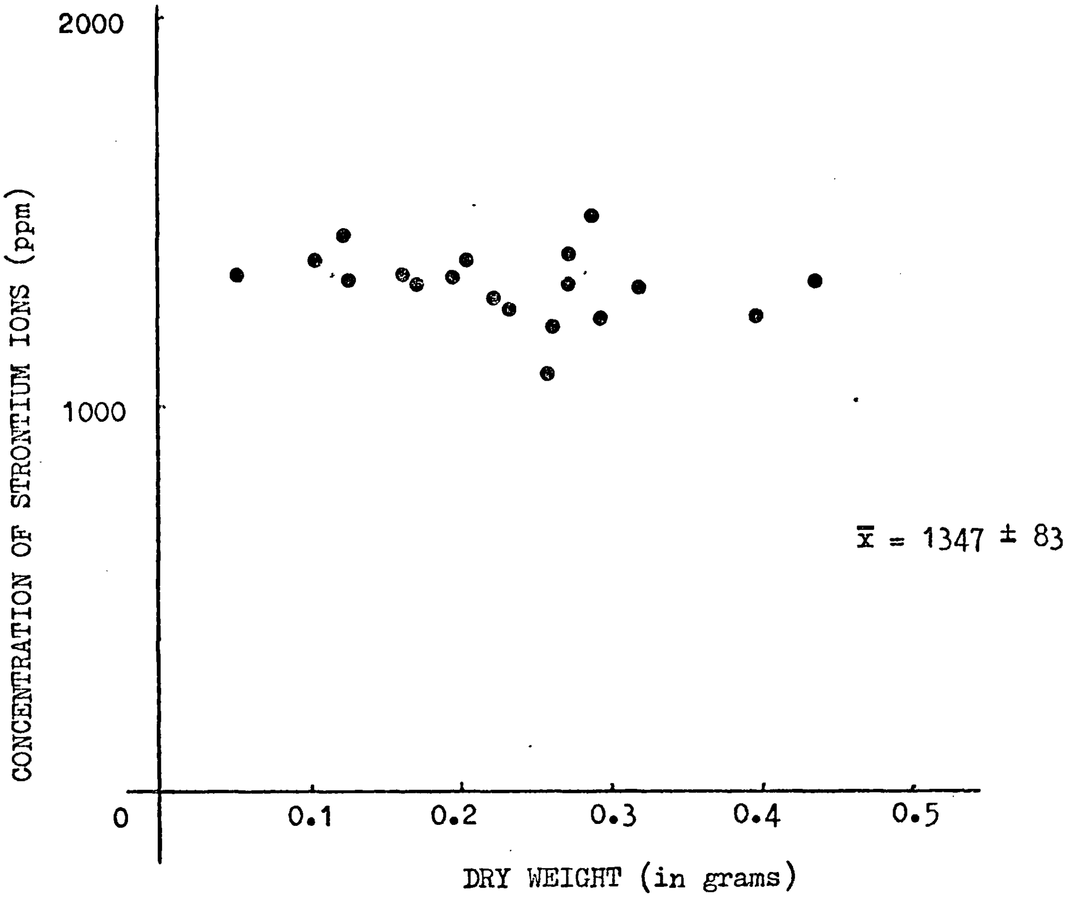
Graph 8.2 (a) Concentration of strontium in Ophiocomina nigra after immersion in elevated strontium levels.



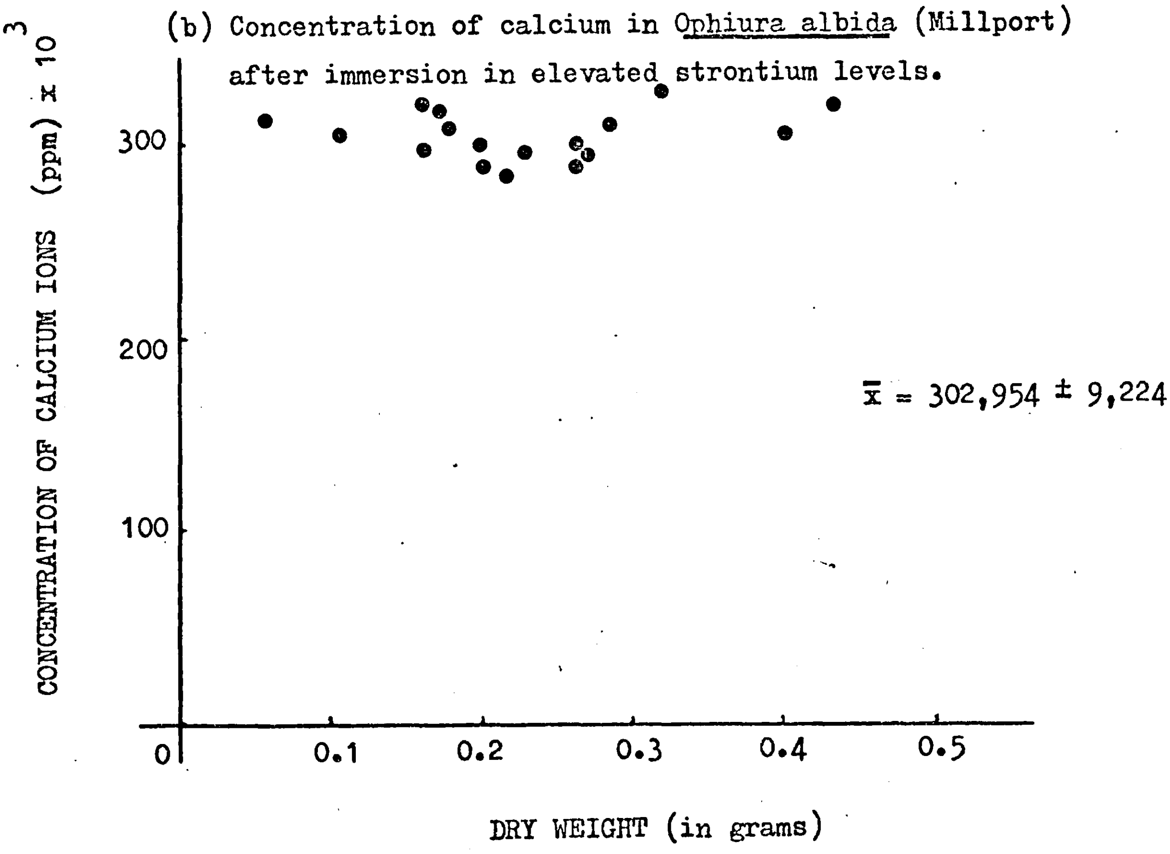
(b) Concentration of calcium in Ophiocomina nigra after immersion in elevated strontium levels.



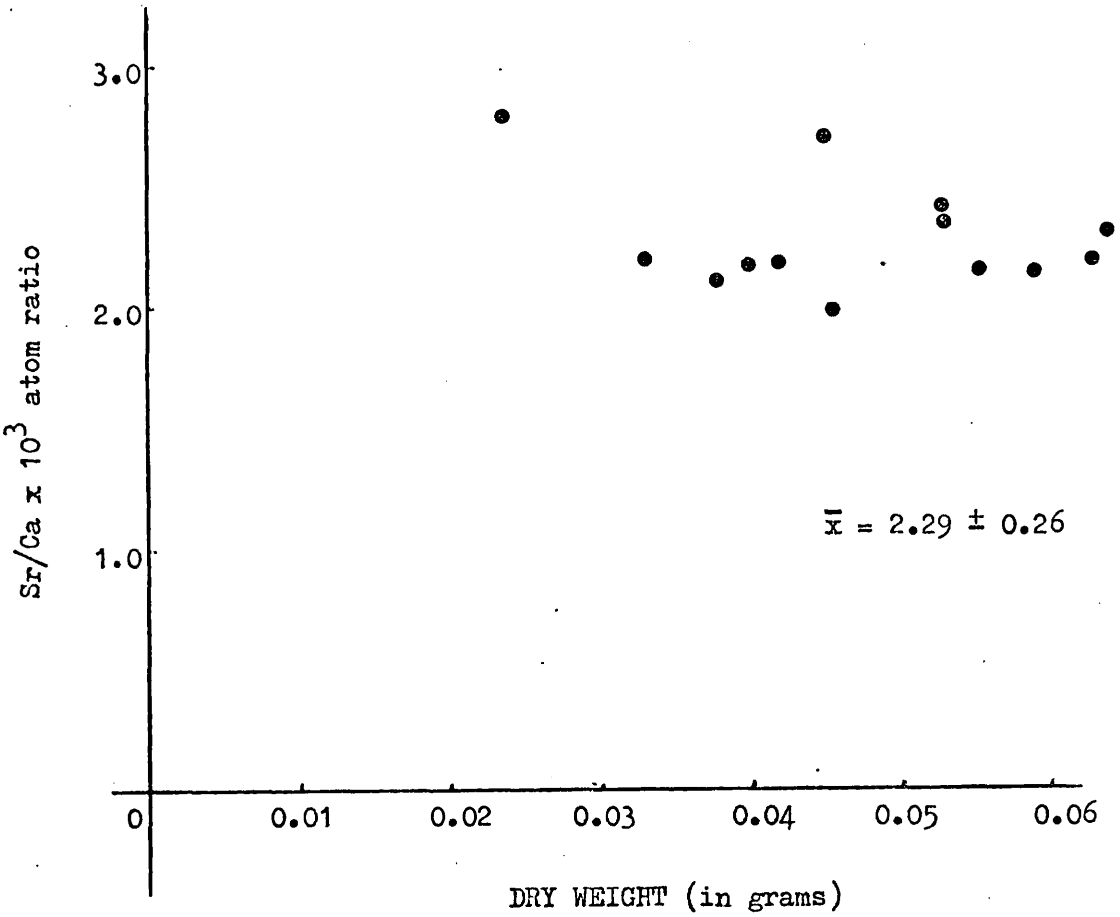
Graph 8.3 (a) Concentration of strontium in _Ophiura albida_ (Millport) after immersion in elevated strontium levels.



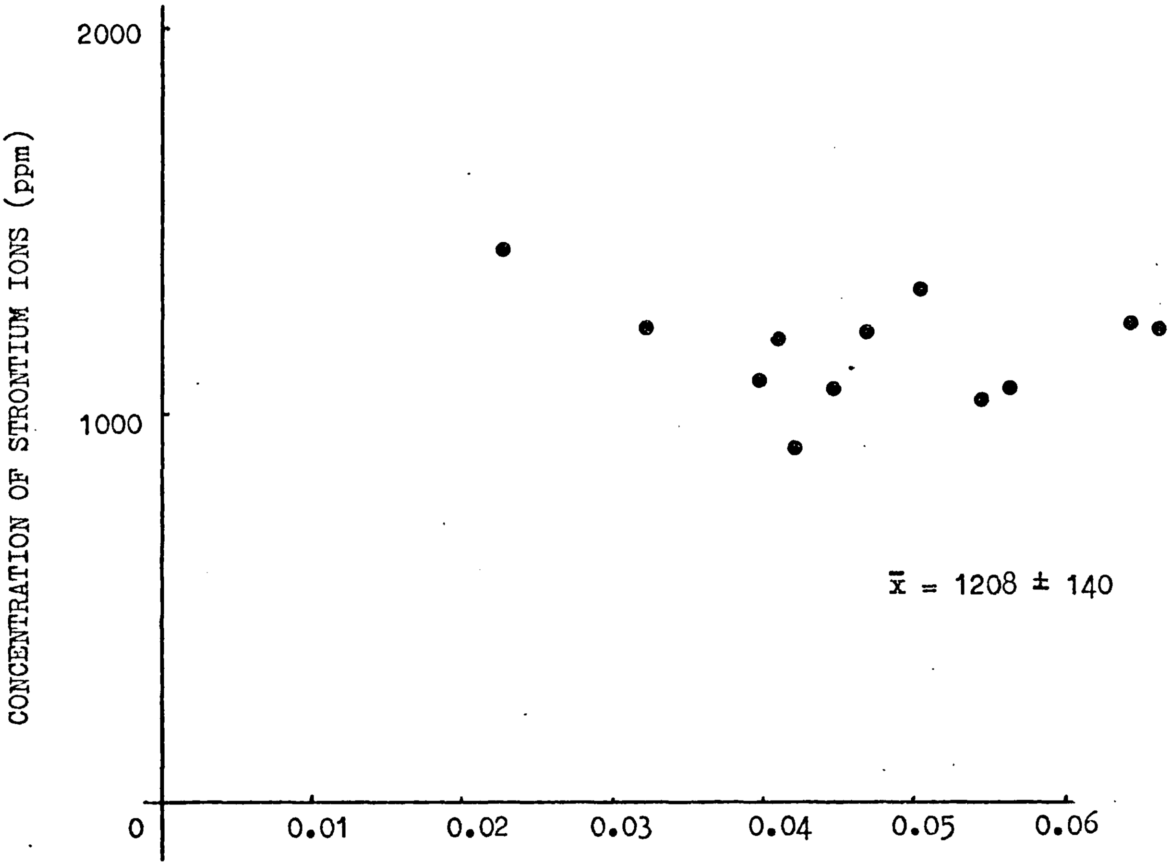
(b) Concentration of calcium in _Ophiura albida_ (Millport) after immersion in elevated strontium levels.



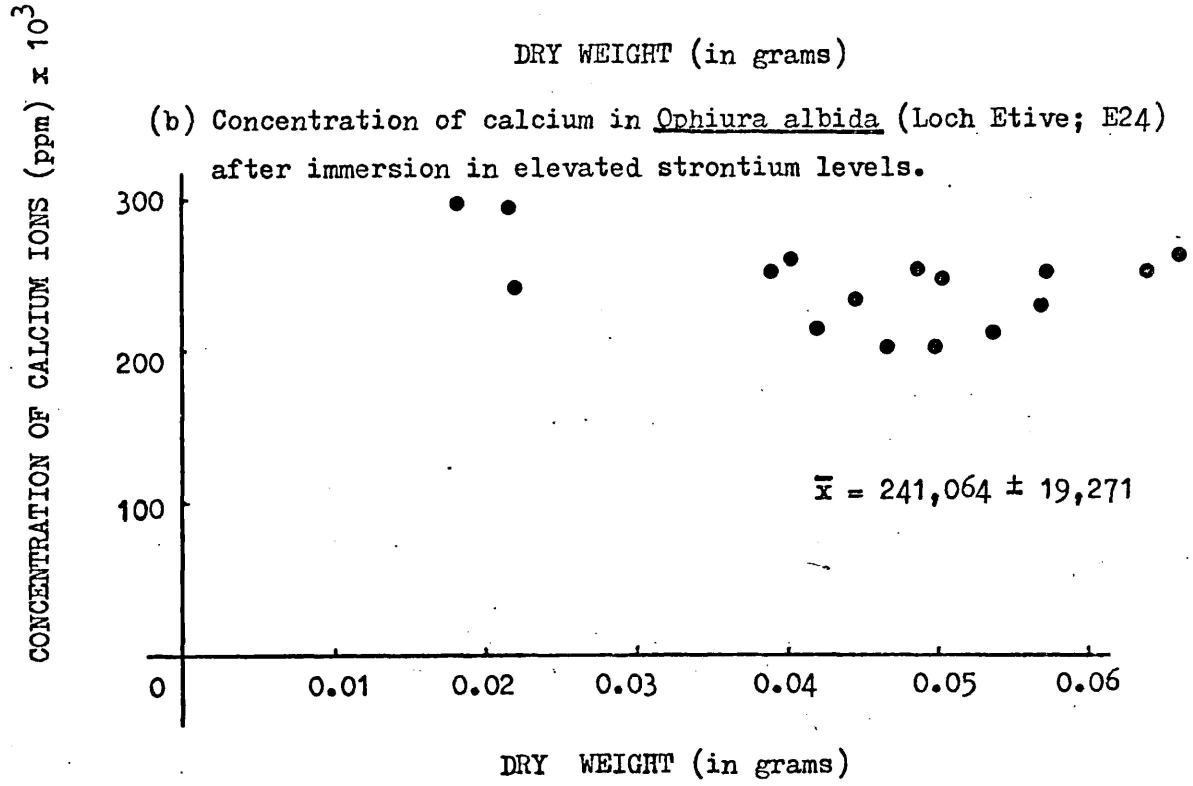
Graph 8.4 Sr/Ca x 10^3 atom ratio in *Ophiura albida* (Loch Etive; E24) after immersion in elevated strontium levels.



Graph 8.5 (a) Concentration of strontium in <u>Ophiura albida</u> (Loch Etive; E24) after immersion in elevated strontium levels.



(b) Concentration of calcium in <u>Ophiura albida</u> (Loch Etive; E24) after immersion in elevated strontium levels.



nine times, the concentration of this ion in the whole body analyses of O.nigra and O.albida from Millport do not reflect this increase to the same degree after one month's immersion in the experimental solution. O.nigra increases its strontium load two-fold while O.albida increases by 44%.

This compares with the work of Gibbs and Bryan (1972) and with that of Binyon (1978) where both authors report increases in the strontium concentrations of the species concerned after similar treatments.

The results for the individuals of Ophiura albida from Loch Etive are particularly interesting. These animals had similar Sr/Ca atom ratios as the specimens from Millport though they live in an environment which is consistently lower in strontium (see Table 7.1). The implication is that O.albida can maintain its Sr/Ca atom ratio despite a lowering of the ambient strontium concentration.

One can speculate as to how this maintenance is possible. There are at least two pathways by which strontium may enter the ophiuroid body. Firstly, via a food source or water imbibition into the stomach and thence into the perivisceral coelom, circulation of which could distribute strontium ions throughout the animal. Secondly, it may enter via general ion exchange between the body surface and the surrounding sea water.

Considering the visceral pathway first, if the food or the water were in equilibrium with the ambient medium, i.e. had similar strontium concentrations, then it is possible that any accumulatory or discriminatory mechanism could reside at the stomach wall/perivisceral coelom interface. Such a mechanism could be present at any organ/coelom interface. That is to say, accumulatory or discriminatory behaviour could be an expression of membrane activity.

Such a phenomenon could also utilise strontium entering via the second possible pathway, namely via exchange between the general body surface and the ambient sea water.

Incorporation of strontium ions into the ossicles of an ophiuroid may not rely upon membrane activity. The number of ions of strontium which are incorporated into the crystal lattice of calcite may reflect crystallographic constraints upon packing of calcium and strontium ions into the crystal.

It is interesting to note that the Loch Etive population of O.albida do not show an increase in strontium concentration when placed in elevated strontium levels. This might imply some sort of limit to the amount of strontium ions which can be handled by the ophiuroids. It has been shown that these individuals from Loch Etive maintain a strontium level similar to that of animals from Millport despite the lower strontium concentration in the waters of Loch Etive. The animals from Loch Etive appear not to take up the additional strontium present in the experimentally elevated sea water, perhaps due to discriminatory activity at the membrane level. It was not possible to analyse the body fluids for strontium or calcium ions because the quantities available of such fluids were not sufficient.

GENERAL DISCUSSION:

Hitherto, the Sr/Ca atom ratio of echinoderms have been presented as single values. It is considered that this may be misleading. The present work supports the contention that such ratios should be given with respect to some qualifying factor, such as dry weight of the animal.

The discrimination ratios reported in this study are of a similar order of magnitude as those reported in the literature by other workers. It is of interest that despite variations in Sr/Ca atom ratio of species and their environments (marine and brackish water) the discrimination ratios are broadly equivalent.

Work upon the ophiuroid, Ophiocomina nigra, has demonstrated that the Sr/Ca atom ratio of the calcareous parts is inversely proportional to the dry weight of the intact animal. It is argued that this inverse relationship is a resultant either of varying growth rates at different developmental stages during the life of the animal or of the physical size of the individual per se.

Work upon Ophiura albida from Loch Etive, where environmental levels of strontium are lower than those of Millport and Plymouth has shown that this species, irrespective of the ambient strontium concentrations, is able to maintain an overall body level of strontium equivalent to that of an individual from Millport. This raises the question of how much of this ion an ophiuroid is able to take up.

Studies with elevated levels of strontium have demonstrated that Ophiocomina nigra is able to take up strontium to reach a new equilibrium apparently consistent with the known variation in the dry weights of the experimental animals. This is in contrast to specimens of O.nigra from sea water carrying a normal strontium load where younger (smaller) animals contain more strontium than older (larger) animals. Although larger animals appear to have less strontium this is not thought to reflect some metabolic or physical constraint upon uptake since elevated studies have demonstrated that additional strontium can be taken up. Furthermore, it was shown that the final level of strontium which was reached appears to be constant despite size. It could be argued that this constancy is a consequence of the limited size spectrum which was sampled. It should be noted that the size range of the specimens used for the elevated studies was greater than that which was used for the initial studies.

Since the elevated studies were conducted over a period of time which is not considered sufficient for increases in growth to occur, this uptake in the face of an elevated concentration of ambient strontium reflects the ability of the body fluids and soft tissues to take up the available strontium. It would presumably require a much longer exposure time to elevated levels in order to allow the incorporation of the strontium ions into the growing ossicles. This incorporation would presumably represent a more permanent binding of the strontium ion than would result from the relatively rapid and potentially reversible uptake which would be expected in the soft tissues.

That Ophiocomina nigra and Ophiura albida (from Millport) can take up additional amounts of strontium when placed in elevated levels of this ion whilst O.albida from Loch Etive does not appear to do so poses an interesting problem. An explanation which goes some way towards solving this apparent anomaly may lie in possible differences between physical incorporation of strontium ions into growing ossicles and passage of ions into the soft tissues via, perhaps, selective membrane activity.

From the studies pursued so far, it is proposed that future work upon the Sr/Ca atom ratios of marine invertebrates, particularly those of echinoderms, should present these and concentrations of the ions, which contribute to the ratio, in relation to dry weight. That is to say, a range of sizes of a species should be analysed rather than a few specimens of a similar size spectrum. In this way a better view of the distribution of strontium within a species can be gained.

Furthermore, studies employing elevated concentrations of strontium could assess the ability for strontium ion selection or rejection. This could be of particular significance where an invertebrate is important in a food chain, in terms of the biomass consumed.

Studies of the effects of elevated strontium levels upon invertebrate tissue could, and should in future work, be augmented by assessments of the potential interactions between radioactive strontium and the endemic strontium in the seas. Should the world's currents not equally distribute the salt ions in the oceans then knowledge of potential environmental accumulations of strontium by marine invertebrates would be of use in predicting environmental and economic consequences of local elevations of radioactive strontium.

Although echinoderms are subject to relatively little predation in comparison to other invertebrates, such as some bivalves and polychaetes, the Ophiuroidea are potentially of some economic importance. Many species of flatfish and some species of pelagic fish crop the populations of brittlestars in the North Sea. Ophiura albida is a notable component of this food source and is readily eaten by immature plaice, Pleuronectes platessa.

It is hoped that the latter part of this thesis could act as a platform for such future research.

SUMMARY:

Further evidence is presented in support of the view that echinoderms are not the rigid, stenohaline group of invertebrates as has often been emphasised by opinion during the last fifty or sixty years.

Ophiura albida from marine waters (Millport) have some potential to tolerate some dilution of their ambient medium. This potential has been more fully realised by the population of Ophiura albida living in Loch Etive where they are subject to a brackish water salinity regime. This latter group of ophiuroids have a greater tolerance to reduced salinities than their counterparts from marine waters. It is suggested in this thesis that variations in the percentages of water and ash between Ophiocomina nigra, a stenohaline brittlestar from Plymouth and Ophiura albida, a euryhaline brittlestar from Millport, Loch Etive and the Baltic Sea, are related to these differences. Such relationships are drawn due to the likelihood that the skeletal material of an ophiuroid is osmotically inactive and that increases in this component would act as a 'buffer' in the face of a reduction in the environment of the salt content. An extension of this idea, that an increase in skeleton reflects a decrease in ambient salinity (in the context of the thesis), results in the possibility that the individuals of Ophiura albida from the Baltic Sea are at a more advanced stage in tolerance to a hypo-osmotic environment than those from Loch Etive. Cross-adaptation experiments between O.albida from Loch Etive and those from Millport to the salinity of the other suggest that the Loch Etive group of animals are fairly recently derived from typically marine Ophiura albida.

Comparisons between Ophiocomina nigra and various groups of Ophiura albida have indicated that with an increase in the degree of euryhalinity there is a decrease in the amount of water taken up by a species in similar dilutions of sea water.

No evidence was found which indicated that the production of mucus is of importance to ophiuroids in their penetration of brackish water.

The burrowing habit of Amphiura chiajei would seem to remove it from the effects of rapidly changing salinities.

Another important point to emerge from this thesis stems from the ionic studies carried out upon Ophiocomira nigra. These have indicated that the perivisceral fluid in this species is ionically and functionally analogous to the ambulacral fluids of echinoids and asteroids with respect to association with locomotor muscles.

There are a number of routes of experimentation which could be followed using this work as a proposed scheme concerning ophiuroids and penetration into brackish water. Firstly, there is the question of how does Ophiura albida tolerate reduced salinity. It is unlikely that increases in the percentage of skeleton are totally responsible. Physiologically, the presence and distribution of amino acids in stenohaline and euryhaline brittlestars would be worth investigating. Analogies with the 'cushioning' effect of amino acids in asteroids faced with salinity fluctuations are possible. Secondly, salinity tolerance and weight change experiments could be conducted upon Ophiura albida from the Öresund to identify the presence of any further variations between different geographical groups of this species. Thirdly, it would be instructive to establish conclusively whether or not O.albida can breed at station E24 in Loch Etive. If they cannot reproduce at this station then one might suppose that a reasonably elaborate and finely balanced mechanism would be required in order to maintain the population at the head of this loch. From what has already been discussed this would seem to be a less likely proposition.

As more physiological work is conducted upon ophiuroids it may be shown that they are indeed the most euryhaline of the echinoderm groups as has been surmised by the frequency of reports noting their presence in reduced salinity. In addition, there may be many more species of echinoderms to be recorded which have the ability to tolerate some reduction in the salt content of their environment. Should this be the case, then the term 'stenohaline' with regard to echinoderms will have to be applied with careful consideration.

With regard to the latter part of the thesis it has been demonstrated that the Sr/Ca atom ratio is not a constant value in ophiuroids - it appears to vary with size. This may be due to the effect of variation in the growth rates between young and older individuals or to the physical size of a specimen *per se* upon incorporation of strontium into the ossicles. It is suggested that future work, in which Sr/Ca atom ratios are quoted for echinoderms in general and ophiuroids in particular, should present such ratios with qualification by, for example, dry weight.

Data from *Ophiura albida* (Loch Etive) indicate that ophiuroids may have a constant strontium concentration despite ambient levels. However, it has been shown that body levels of strontium in some ophiuroids can be raised if the concentration of strontium in the environment is increased. This is true of *Ophiocomina nigra* and *Ophiura albida* from Millport. It is not the case, however, with *Ophiura albida* from Loch Etive.

Such increases are possibly a reflection of uptake of the strontium ion into the soft tissues and body fluids rather than into the skeleton since the duration of the experiments was not sufficient for an increase in the physical size of the animal to occur. Studies of strontium, both natural and artificially elevated, particularly in terms of uptake and/or discrimination may have much significance with respect to food chains.

It would be instructive to investigate the relationship between the endemic, stable strontium and radioactive strontium so that the effects upon the fauna of an increase in the oceanic burden of radioactive strontium could be assessed. At the moment, as a result of diluting processes, it seems unlikely that the overall strontium load in the world's oceans has increased substantially since the advent of nuclear fission. However, concentrating effects by local currents should not be dismissed especially near coasts and estuaries where differential dilution may occur. Thus it would seem that the next step in the study of strontium distribution in marine organisms should appraise radioactive and stable strontium interactions in the environment and in marine invertebrates. Those invertebrates which are of economic importance, particularly those which are of some import in the support of fisheries, are an obvious choice for investigation.

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APPENDICES

Appendix 1 : "EEL" Flame photometer Mk II operational details.

Air supply - Dry air at 16lbs per square inch from an "EEL" Air compressor.

Gas supply - Mains gas.

Filters - Sodium and potassium.

Standard solutions - These were prepared using " Analar " grade sodium chloride and potassium chloride. Stock solutions of sodium ions (1250 ppm) and of potassium ions (1000 ppm) were made up .

Range of sodium standard solutions :-

2.5 , 5.0 , 7.5 , 10.0 , 12.5 , 15.0 , 20.0 ppm

Range of potassium standard solutions :-

2.0 , 4.0 , 6.0 , 8.0 , 10.0 ppm

Appendix 2 : Theoretical possibilities concerning ophiuroid body fluids and potassium concentrations .

The volume of ambulacral fluids in the water vascular system of an ophiuroid (of the types characterised by *Ophiocomina nigra*) may be described by the expression,

$\pi r^2 h$ where,

π = 3.142

r = radius of water vascular canal

h = length of water vascular canal

(This expression is an approximation since Woodley (1967) has shown that the water vascular canal is not a tube of uniform dimensions) .

Measurements upon a medium sized ophiuroid, *Ophiocomina nigra*, give the following values for 'r' and 'h'.

r = 0.075 mm

h = 60 mm (300 mm for the five arms)

So, the volume of ambulacral fluids =

$$3.142 \times \left(\frac{0.15}{2}\right)^2 \times 300 = 4.7\mu\text{ls}$$

If the total volume of body fluids obtained by the centifuging technique are, say, 60μls then it could be assumed that the perivisceral fluid is of the order of 78% , by volume, of the " body fluids ".

In this example they are theoretically 55·3μls · This assumes that all the fluid is extracted which is unlikely. Consequently this means that the potential potassium concentrations of the ambulacral fluids have been conservatively estimated.

Assuming that the potassium concentration of the perivisceral fluids is equivalent to sea water (10·5 mM/l) then,

the potassium concentration in the ambulacral fluid is given by,

$$\frac{(60 \times 14{\cdot}5) - (53{\cdot}3 \times 10{\cdot}5)}{4{\cdot}7} = 66 \text{ mM}$$

Appendix 3 : Unicam SP90 Spectrophotometer operational details.

Gas supply - Acetylene supplied, via a pressure reducing regulator, at 5 lbs. per sq.in. and at a flow rate of 1.5 litres per minute.

Air supply - Supplied from a compressor at a pressure of 30 lbs/sq.in. and at a flow rate of 5 litres per minute.

Slit width, wavelength setting, and burner height were set according to the manufacturer's instructions.

Details of the preparation of the standard solutions and calibration curve variation are given on page 152 - 153.

Appendix 4 pH values of sea waters diluted by glass distilled water.

% SEA WATER	pH
100	7·72
90	7·78
80	7·81
70	7·84

DATA TABLES

Graph 1·1 Percentage weight fluctuation in 100% sea water.

Hours	Ophiocomina nigra	Ophiura albida (Millport)	Ophiura albida (Loch Etive)
1	8·53 ± 6·27	8·05 ± 4·97	3·87 ± 4·81
2	6·81 ± 5·57	9·12 ± 6·46	-2·54 ± 2·19
3	6·24 ± 4·98	10·22 ± 6·17	-1·85 ± 1·85
4	6·03 ± 6·13	9·25 ± 4·38	-1·61 ± 0·61
5	5·94 ± 6·27	7·56 ± 5·61	-2·67 ± 1·46
7	6·82 ± 5·27	9·71 ± 5·98	-3·09 ± 0·82

1·2 Comparison of relative fluctuation of wet weight.

Ophiocomina nigra	Ophiura albida (Millport)	Ophiura albida (Loch Etive)
1·72 ± 1·67	3·95 ± 1·38	-2·86 ± 1·68
2·05 ± 2·13	5·56 ± 1·94	-1·68 ± 6·76
3·84 ± 1·70	7·20 ± 2·19	-1·28 ± 1·89
6·82 ± 1·94	7·57 ± 3·37	-1·06 ± 1·95
9·40 ± 1·81	11·47 ± 3·60	-0·85 ± 1·63
16·22 ± 2·02	18·22 ± 1·49	-0·36 ± 4·85

1·3 Percentage weight change in 90% sea water.

Hours	Ophiocomina nigra	Ophiura albida (Millport)
1	9·19 ± 6·82	3·94 ± 3·01
2	8·09 ± 6·29	4·34 ± 4·49
3	8·85 ± 7·14	2·28 ± 3·56
4	6·12 ± 6·86	1·90 ± 3·59
5	7·63 ± 6·57	3·61 ± 3·87
7	6·62 ± 6·40	1·91 ± 2·81
15	0·88 ± 3·10	1·51 ± 5·31
20	0·52 ± 2·09	0·48 ± 4·29
25	0·45 ± 1·63	0·32 ± 4·10

Graph 1.4 Percentage weight change in 70% sea water.

Hours	*Ophiocomina nigra*	*Ophiura albida* (Millport)	*Ophiura albida* (Loch Etive)
1	6.76 ± 1.19	4.82 ± 2.62	2.88 ± 5.58
2	8.05 ± 0.73	3.56 ± 2.41	-2.93 ± 1.96
3	11.66 ± 1.46	6.29 ± 2.39	-1.85 ± 1.85
4	17.04 ± 2.39	2.43 ± 3.18	-1.61 ± 3.40
5	17.42 ± 1.51	3.80 ± 4.21	-2.67 ± 1.46
7	18.36 ± 1.69	4.10 ± 6.34	-3.09 ± 0.82
15	3.61 ± 4.83	3.22 ± 5.15	-2.87 ± 5.32
20	2.26 ± 3.22	2.48 ± 5.02	-2.92 ± 4.12
25	2.80 ± 5.41	2.43 ± 4.99	-2.74 ± 3.46

1.5 Percentage weight change in 60% sea water.

Hours	*Ophiura albida* (Millport)	*Ophiura albida* (Loch Etive)
1	13.79 ± 6.80	5.55 ± 4.75
2	12.96 ± 8.08	2.73 ± 3.29
3	10.13 ± 3.55	2.90 ± 4.48
4	9.98 ± 10.66	6.56 ± 3.84
5	11.50 ± 9.82	6.82 ± 3.91
7	11.63 ± 8.75	5.10 ± 2,89
15	4.32 ± 5.62	-2.01 ± 5.12
20	4.31 ± 4.01	1.24 ± 4.87
25	4.86 ± 3.89	1.30 ± 4.44

1.6 Percentage weight change in 50% sea water.

Hours	*Ophiura albida* (Millport)	*Ophiura albida* (Loch Etive)
1	15.01 ± 6.31	5.55 ± 4.75
2	13.81 ± 8.35	2.73 ± 3.29
3	12.24 ± 6.10	2.90 ± 4.48
4	11.01 ± 8.21	1.56 ± 3.84
5	12.31 ± 4.87	-0.48 ± 4.92
7	14.43 ± 4.56	-0.83 ± 8.90
15	4.32 ± 2.86	-0.76 ± 3.72
20	2.22 ± 3.49	2.25 ± 3.81
25	2.45 ± 3.33	-0.92 ± 3.40

Graph 2·1 and 2,2 Distribution of potassium and sodium ions in the body fluids of *Ophiocomina nigra*.

	90% sea water		70% sea water	
HOURS	SODIUM	POTASSIUM	SODIUM	POTASSIUM
3	382	7·8	164	11·5
	218	6·9	218	10·2
	186	11·2	229	10·2
	240	8·7	218	11·2
	262	8·7	175	11·7
	273	9·2	196	9·7
	260 ± 67	8·7 ± 1·4	200 ± 26	10·8 ± 0·8
5	404	13·0	251	10·7
	327	15·3	218	7·4
	317	17·1	240	9·2
	404	13·5	251	9·7
	360	13·5	196	7·9
	284	13·5	262	10·2
	349 ± 48	14·3 ± 1·5	236 ± 25	8·6 ± 1·3
7	404	13·3	295	10·2
	393	12·0	284	10·2
	327	11·7	218	8·7
	371	8·4	284	11·2
	389	11·2	229	8·2
	349	10·5	262	7·6
	372 ± 29	11·2 ± 1·6	262 ± 32	9·4 ± 1·4
10	415	14·6	327	11·7
	295	12·8	284	11·5
	327	13·3	262	9·7
	360	15·3	305	11·2
	349	14·0	253	9·2
	371	14·0	215	8·7
	352 ± 40	14·0 ± 0·9	275 ± 39	10·3 ± 1·3

Graph 2.3 Relationship between chloride concentration in the body fluids of <u>Ophiocomina nigra</u> and the ambient sea water.

% SEA WATER	BODY FLUIDS	SEA WATER
100	451	442
	448	445
	448	436
	445	414
	448	448
	451	403
	448 ± 2.2	431 ± 18.4
90	405	405
	416	405
	405	391
	400	383
	402	391
	400	391
	404 ± 5.9	394 ± 8.8
80	389	349
	377	343
	374	343
	391	349
	389	352
	383	360
	384 ± 7.0	349 ± 6.3
70	324	287
	326	310
	324	326
	321	321
	310	326
	307	296
	319 ± 8.0	311 ± 16.4
60	267	262
	267	259
	267	265
	264	265
	270	262
	267	262
	267 ± 1.9	262 ± 2.2

Graphs 3·1 to 3·3 Comparison of salinity tolerances

SPECIES	DILUTION	EFFECTIVE TIMES FOR MORTALITY (in days)			's'	fET_{50}	CONFIDENCE LIMITS	
		84%	50%	16%			lower	upper
A·chiajei								
(E6)	40%	2·35	1·73	1·27	1·360	1·25	1·4	2·2
	50%	6·9	5·9	5·1	1·163	1·095	5·4	6·3
	60%	10·2	9·2	8·4	1·101	1·065	8·6	9·8
	70%	15·9	15·0	4·2	1·058	1·036	14·5	15·5
(E11)	40%	3·55	2·70	1·60	1·501	1·29	2·1	3·5
	50%	7·45	6·7	6·0	1·114	1·067	6·3	7·2
	60%	15·4	14·7	14·0	1·048	1·029	14·3	15·1
	70%	20·2	19·2	18·3	1·050	1·030	18·6	19·8
(E24)	40%	5·5	3·85	2·93	1·371	1·21	3·2	4·6
	50%	10·6	9·6	8·8	1·097	1·060	9·0	10·2
	60%	17·2	16·3	15·5	1·053	1·032	15·8	16·8
	70%	21·4	20·5	19·7	1·042	1·025	20·0	21·0
O·albida	40%	4·1	3·1	2·4	1·306	1·155	2·7	3·6
(E2)	50%	8·9	8·25	7·6	1·081	1·049	7·8	8·6
	60%	12·4	11·75	11·1	1·056	1·030	11·4	12·1
	70%	17·9	16·8	16·0	1·051	1·030	16·3	17·3
(E6)	40%	4·5	3·5	2·85	1·256	1·15	3·0	4·0
	50%	9·2	8·4	7·7	1·093	1·057	7·9	8·9
	60%	13·2	12·4	11·6	1·066	1·040	11·9	12·9
	70%	17·7	16·8	16·0	1·051	1·030	16·3	17·3
(E11)	40%	5·8	5·0	4·3	1·161	1·097	4·5	5·5
	50%	10·65	9·65	8·8	1·100	1·062	9·1	10·3
	60%	18·25	17·3	16·3	1·057	1·035	16·7	17·9
	70%	26·7	25·8	24·9	1·035	1·022	25·2	26·4

SPECIES	DILUTION	EFFECTIVE TIMES FOR MORTALITY (in days) 84%	50%	16%	's'	fET$_{50}$	CONFIDENCE LIMITS lower	upper
O.albida (E14)	40%	6.1	5.2	4.5	1.164	1.10	4.7	5.7
	50%	11.2	10.25	9.3	1.097	1.060	9.6	10.8
	60%	18.6	17.6	16.7	1.054	1.033	17.0	18.2
	70%	27.0	26.0	25.0	1.046	1.028	25.3	26.7
(E24)	40%	9.1	8.4	7.7	1.087	1.054	7.9	8.8
	50%	14.8	13.8	13.0	1.066	1.040	13.3	14.3
O.nigra	90%	8.4	7.8	7.0	1.094	1.055	7.4	8.2
	80%	6.7	6.0	5.3	1.12	1.057	5.6	6.3
	70%	4.0	3.7	3.2	1.118	1.070	3.45	3.9
O.albida (Millport)	90%	19.6	18.5	17.6	1.055	1.033	17.9	19.1
	80%	13.0	12.1	11.5	1.063	1.040	11.6	12.6
	70%	9.3	8.6	8.2	1.064	1.039	8.3	8.9
	60%	6.0	5.6	5.3	1.063	1.040	5.3	5.8

REACTION RATIOS

If 'RR' is greater than 'rr' then stations are significantly different.

	RR	rr	RR	rr
A.chiajei	E6 E11		E14	
40%	1.56	1.40	1.42	1.35
50%	1.13	< 1.0	1.43	1.00
60%	1.59	< 1.0	1.10	1.00
70%	1.28	1.0	1.06	1.00

	RR	rr
O.nigra	O.albida (Millport)	
90%	2.37	1.0
80%	2.02	1.1
70%	2.32	1.05

	RR	rr	RR	rr
O.albida	E2 \ / E6		E11 \ / E14	
40%	1.12	1.2	1.04	1.05
50%	1.02	1.05	1.06	1.10
60%	1.05	1.05	1.02	1.03
70%	1.01	1.02	1.00	1.01

	RR	rr	RR	rr
	E6 \ / E11		E14 \ / E24	
40%	1.42	1.1	1.61	1.1
50%	1.14	1.0	1,34	1.0
60%	1.39	1.0		
70%	1.53	1.1		

SPECIES	DILUTION	EFFECTIVE TIMES FOR MORTALITY (in days)			's'	fET_{50}	CONFIDENCE LIMITS	
		84%	50%	16%			lower	upper
A.chiajei	50%	3.7	2.9	2.5	1.217	1.125	2.5	3.2
	60%	6.0	5.1	4.4	1.167	1.090	4.6	5.9
(C12)	70%	8.4	7.7	7.0	1.095	1.075	7.1	8.2
	80%	13.0	11.0	10.0	1.140	1.085	10.1	11.9
O.albida	50%	5.3	4.4	3.7	1.184	1.12	3.9	4.9
	60%	7.2	6.0	5.1	1.188	1.15	5.2	6.9
(C12)	70%	10.4	9.2	8.3	1.119	1.070	8.5	9.8
	80%	13.1	12.0	10.8	1.101	1.060	11.3	12.7

Graph 6·1 and 6·2 *Ophiura albida* (Loch Etive) percentage water and ash contents·

	WET WEIGHT	% WATER	% ASH
E4	0·0480	40·83	21·45
	0·2435	43·08	26·16
	0·2139	45·44	27·16
	0·3617	46·77	26·81
	0·2111	44·67	25·58
	0·2772	42·17	27·41
	0·1179	46·31	23·40
	0·1410	41·06	26·73
	0·1985	41·81	25·13
	0·2711	39·72	32·38
	0·0836	43·42	23·44
	0·1916	37·89	30·12
	0·2075	39·51	32·67
	0·1993	41·59	27·99
	0·2832	35·20	31·39
	0·1554	37·25	30·37
	0·1269	36·17	33·80
	0·1984	41·38	27·11
	0·1932	39·13	30·12
		$\overline{x}$ = 41·22 ± 3·29	$\overline{x}$ = 27·89 ± 3·42
E11	0·5035	33·03	24·04
	0·3362	34·43	34·43
	0·3598	36·76	36·56
	0·2961	33·37	23·76
	0·5395	38·23	31·52
	0·4326	44·23	27·79
	0·5287	39·21	30·94
	0·7760	35·95	22·83
	0·5213	42·30	25·41
		$\overline{x}$ = 37·50 ± 3·89	$\overline{x}$ = 28·58 ± 4·99

Graph 6·1 and 6·2 continued·

	WET WEIGHT	% WATER	% ASH
E24	0·1420	47·95	19·78
	0·0502	39·04	31·14
	0·1584	48·29	20·51
	0·0565	39·11	22·22
	0·0495	42,62	25·64
	0·0927	43·47	25·05
	0·0502	35·83	25·78
	0·1212	44·47	35·45
	0·0704	38·06	25·33
	0·0948	40·92	20·17
	0·0680	43·52	22·67
	0·0819	39·31	28·67
	0·0594	38·55	22·95
	0·1088	47·70	28·28
	0·0775	40·90	17·00
	0·0665	39·84	28·12
	0·0769	40·53	26·46
	0·0477	41·22	28·23
	0·1107	45·07	31·44
	0·0769	36·63	25·47
	0·1122	43·26	33·77
	0·1151	42·39	26·06
	0·0769	45·86	17·03
	0·1122	41·70	22·72
		$\bar{x}$ = 41·74	$\bar{x}$ = 25·22
		± 3·73	± 4·63

Graph 6.3 and 6.4 Amphiura chiajei (Loch Etive) percentage water and ash contents.

	WET WEIGHT	% WATER	% ASH
E4	0.1457	48.18	24.15
	0.1892	53.01	20.98
	0.1399	48.67	23.65
	0.1978	53.18	20.87
	0.2293	52.98	22.11
	0.3007	53.07	20.91
	0.2258	52.17	21.92
	0.1915	47.41	25.01
	0.3057	55.21	20.15
	0.3291	52.56	21.05
		$\bar{x}$ = 51.64	$\bar{x}$ = 22.08
		± 2.59	± 1.63
E6	0.1751	54.54	21.35
	0.1926	54.04	19.41
	0.2609	54.54	20.58
	0.1557	51.89	24.27
	0.2535	57.45	19.37
	0.2125	53.31	20.75
	0.2993	58.30	18.37
	0.2533	55.94	19.77
	0.2698	47.36	27.42
	0.1479	53.00	26.91
	0.1834	55.56	18.15
	0.1895	52.45	18.73
		$\bar{x}$ = 54.03	$\bar{x}$ = 21.25
		± 2.85	± 3.20

Graph 6.3 and 6.4 Amphiura chiajei (Loch Etive) percentage water and ash contents.

	WET WEIGHT	% WATER	% ASH
E11	0.3295	49.71	26.10
	0.2362	48.85	25.95
	0.1804	49.50	25.99
	0.1560	50.89	27.56
	0.2921	51.04	25.57
	0.2472	45.22	31.43
	0.2427	47.46	28.71
	0.2246	44.70	30.63
	0.2959	56.23	24.36
		$\bar{x}$ = 49.19 ± 3.26	$\bar{x}$ = 27.36 ± 2.41
E14	0.2716	53.93	22.75
	0.2207	48.36	29.58
	0.2200	45.04	28.54
	0.4185	48.41	26.09
	0.3223	47.37	30.40
	0.3301	43.68	29.86
	0.4924	54.58	20.89
	0.2755	45.62	27.84
	0.2269	44.11	28.99
	0.3933	43.07	29.77
		$\bar{x}$ = 47.31 ± 4.29	$\bar{x}$ = 27.47 ± 3.26

Graph 7·1 (a) and (b) Calibration Curves·

Strontium

CONCENTRATION (ppm)	A·A·S· READING
2	96·2 ± 0·9
4	93·4 ± 1·3
6	90·4 ± 1·9
8	87·7 ± 2·4
10	84·4 ± 3·6
20	73·5 ± 4·2
30	64·7 ± 5·2

Calcium

10	75·5 ± 5·6
20	60·2 ± 6·7
30	49·0 ± 7·2
40	40·6 ± 7·3
50	33·7 ± 7·3

Graph 7·2 and 7·3 *Ophiocomina nigra* - ossicles

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x10^3 atom ratio
627	1·6552	302,078	0·94
762	1·9692	304,692	1·14
635	1·8118	324,263	0·89
726	1·5146	325,994	1·01
825	1·6356	359,159	1·05
720	1·1116	348,597	0·94
973	0·6297	277,910	1·60
671	1·6403	304,822	1·01
1174	0·3619	317,767	1·69
680	1·8390	316,068	0·98
1187	0·1790	296,788	1·83
1103	0·2492	321,027	1·57
925	0·6081	287,682	1·47
1027	0·2190	308,219	1·52
848	2·2124	290,705	1·33
1316	0·2967	299,402	2·01
1208	0·2695	296,403	1·86
835	1·8719	304,471	1·25
930	1·6672	320,115	1·33
949	1·7778	312,504	1·39
782	1·4378	325,117	1·10
872	1·2893	347,055	1·15
591	2·4325	316,515	0·85
1069	0·7050	361,418	1·35
1148	0·9230	261,089	2·01
932	1·2926	307,461	1·38
1160	0·2475	310,543	1·71

R = 0·7645 $\bar{x}$ = 312,744 R = 0·7730

p = 0·001 s = 23,379 p = 0·001

Graph 7·4 and 7·5 Ophiocomina nigra - arm ossicles

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
801	1·9346	284,296	1·29
1394	0·2511	298,686	2·13
1225	0·0510	306,372	1·83
797	1·6939	302,556	1·20
885	1·4693	323,283	1·25
917	1·5264	311,190	1·35
729	1·2690	328,014	1·02
818	1·1304	353,857	1·06
551	2·2009	318,052	0·79
1066	0·5773	387,968	1·26
1105	0·7067	246,403	2·05
1087	0·2022	303,785	1·64
877	1·1024	358,651	1·11

R = 0·8855 x̄ = 317,162 R = 0·7434
p = 0·001 s = 35,602 p = 0·005

Graph 7·6 and 7·7 Ophiocomina nigra - disc ossicles

1078	0·2778	321,724	1·53
1096	0·0456	301,535	1·66
1144	0·2185	257,437	2·03
1194	0·1780	323,034	1·69
1263	0·1979	296,867	1·94
1143	0·2514	320,704	1·63
1185	0·1688	303,614	1·78
1259	0·1589	298,930	1·93
972	0·2316	302,245	1·47
1077	0·1277	293,657	1·68
1271	0·2163	303,398	1·91
1117	0·1902	294,420	1·73
1380	0·0453	331,126	1·91
1194	0·0942	278,662	1·96
1136	0·0550	318,182	1·63
1044	0·2872	282,904	1·68
1166	0·2358	251,802	2·12

x̄ = 1159 x̄ = 300,098 x̄ = 1·78
s = 101 s = 22,017 s = 0·18

Ophiocomina nigra - without removal of organic matter

Graph 7·8 and 7·9 August 1976

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
615	1·8195	301,571	0·93
389	2·8612	243,657	0·73
571	1·7057	258,295	1·01
609	0·9404	256,078	1·08
468	2·5002	246,358	0·86
486	2·0492	305,532	0·72
403	2·6704	251,513	0·73
690	0·7507	278,368	1·13
679	1·9674	313,065	0·99
726	1·3262	353,229	0·94
771	0·8904	272,419	1·29
834	0·3517	273,419	1·39
800	0·3104	269,520	1·35
698	0·5072	247,691	1·28
605	1·2981	291,653	0·94
632	1·4105	302,981	0·95
591	1·5782	300,214	0·90
543	1·9245	263,720	0·94
587	1·9587	274,989	0·97
472	2·2934	288,641	0·74

R = 0·8568, p = 0·001 $\bar{x}$ = 261,972   R = 0·9084, p = 0·001

s = 65,339

Graph 7·8 and 7·9

September 1976

Sr^{2+}	DRY WEIGHT	Ca^{2+}	$Sr/Ca \times 10^3$ atom ratio
666	1·0493	262,080	1·16
517	2·5436	235,886	1·00
674	2·2243	244,457	1·26
672	2·1419	230,519	1·33
587	2·7189	220,676	1·22
477	2·0931	229,923	0·95
496	2·6450	233,933	0·97
661	2·1148	245,295	1·23
621	0·7243	198,467	1·43
730	1·4807	244,445	1·23
955	0·2878	230,195	1·43
918	0·3234	193,143	1·37
803	0·4386	190,187	1·90
826	0·7180	203,429	1·85
809	0·1099	212,551	1·74
658	1·4305	234,106	1·28
656	1·7282	230,098	1·30
609	2·4960	236,137	1·17
550	2·4200	237,481	1·05
508	3·3461	241,122	0·96

R = 0·8172
p = 0·001

$\bar{x}$ = 227,706
s = 19,050

R = 0·8602
p = 0·001

Ophiocomina nigra - disc (without removal of organic matter).

Graph 7·10 and 7·11

August 1976

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/C x10^3 atom ratio
499	0·4513	191,112	1·19
212	0·3554	79,135	1·22
748	0·2311	286,668	1·19
508	0·4618	216,538	1·07
939	0·3140	378,162	1·13
407	0·2426	154,574	1·20
875	0·5126	304,819	1·31
566	0·2850	214,904	1·20
724	0·3105	261,673	1·26
642	0·4607	244,190	1·20
$\bar{x}$ = 612		$\bar{x}$ = 233,177	$\bar{x}$ = 1·19
s = 220		s = 83,156	s = 0·06

September 1976

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/C x10^3 atom ratio
753	0·2818	224,006	1·54
748	0·3003	191,474	1·79
717	0·3832	208,768	1·57
311	0·3463	754,140	1·88
833	0·3140	216,957	1·76
769	0·2918	205,619	1·71
1003	0·1744	243,692	1·88
668	0·2617	191,058	1·60
665	0·3567	164,183	1·85
772	0·4680	218,462	1·61
$\bar{x}$ = 724		$\bar{x}$ = 261,836	$\bar{x}$ = 1·72
s = 174		s = 174,321	s = 0·13

Ophiocomina nigra - arm (without removal of organic matter).

Graph 7·12 and 7·13

August 1976

Sr^{2+}	DRY WEIGHT	Ca^{2+}	$Sr/Ca \times 10^3$ atom ratio
655	1·3682	338,036	0·88
495	2·2080	294,384	0·78
666	1·3503	305,486	0·99
562	0·7093	267,871	0·96
460	2·0384	253,137	0·83
417	1·4935	264,425	0·72
387	2·0175	274,080	0·64
825	0·5081	338,325	1·11
608	1·4548	316,011	0·88
769	1·0412	391,133	0·89
797	0·5799	277,853	1·31
644	1·3734	298,859	0·98

R = 0·7905 $\bar{x}$ = 301,633   R = 0·9084

p = 0·005   s = 39,501   p = 0·001

September 1976

452	2·0959	248,403	0·83
856	0·6906	249,386	1·57
724	1·1989	249,279	1·33
959	0·2472	241,574	1·74
911	0·4161	243,954	1·70
853	0·7934	240,817	1·62
734	0·8972	223,117	1·50
805	1·1918	224,666	1·63
547	1·4650	204,907	1·22
528	1·7313	200,019	1·20
433	1·7849	157,651	1·25
451	2·0901	217,691	0·94

R = 0·9626 $\bar{x}$ = 225,122   R = 0·8977

p = 0·001   s = 27,363   p = 0·001

Ophiocomina nigra - arm (without removal of organic matter).

Graph 7.14, 7.15 and 7.16 Non-regenerating part of arm

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
457	2.0218	253,486	0.82
406	1.4729	265,587	0.69
515	1.0674	409,874	0.57
690	1.3768	535,661	0.58
396	1.9197	274,650	0.65
733	0.4441	351,836	0.95
606	1.4396	316,925	0.87
767	1.0365	391,942	0.89
647	0.4809	298,915	1.19
509	1.1900	304,622	0.88
R = 0.7139		$\bar{x}$ = 295,158	R = 0.8608
p = 0.001		s = 75,302	p = 0.001

Regenerating part of arm

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
961	0.0166	210,929	2.08
1184	0.0206	181,949	2.97
1321	0.0126	237,990	2.53
1286	0.0153	228,738	2.57
1368	0.0640	253,900	2.46
878	0.0152	230,200	1.74
998	0.0990	265,158	1.72
955	0.1834	265,805	1.64
1136	0.0704	250,355	2.08
1220	0.1051	249,762	1.74
838	0.0298	251,677	1.52
1076	0.0096	201,612	2.44
1007	0.1116	252,016	1.83
1074	0.0416	225,067	2.18
998	0.0667	236,541	1.93
959	0.0794	253,032	1.73
1100	0.1193	238,116	2.11
1107	0.1365	229,017	2.21
$\bar{x}$ = 1132		$\bar{x}$ = 235,491	$\bar{x}$ = 2.10
s = 266		s = 22,100	s = 0.27

Graph 7·17 and 7·18 *Ophiura albida* (Millport) - Ossicles

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca $x10^3$ atom ratio
1416	0·1908	339,567	1·91
1611	0·1653	313,390	2·35
1512	0·1226	340,747	2·03
1443	0·1573	334,599	1·97
1193	0·1537	354,672	1·54
1160	0·1401	401,499	1·32
1257	0·1918	353,411	1·62
1403	0·1158	377,806	1·69
1302	0·1824	411,184	1·44
1366	0·1830	375,683	1·66
1295	0·1351	416,358	1·42
1499	0·1894	363,007	1·88
1376	0·2058	350,301	1·79
$\bar{x}$ = 1371		$\bar{x}$ = 364,017	$\bar{x}$ = 1·74
s = 130		s = 31,161	s = 0·28

Graph 7·19 and 7·20 *Ophiura albida* (Millport) disc - ossicles

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^{3} atom ratio
1123	0·0445	308,988	1·66
1655	0·0302	372,516	2·03
2349	0·0266	281,955	3·81
1786	0·0238	367,647	2·22
1506	0·0332	338,855	2·03
1916	0·0261	383,142	2·28
2243	0·0181	408,052	2·51
1997	0·0199	415,119	2·20
2114	0·0219	376,000	2·57
1583	0·0381	352,490	2·05
1250	0·0383	331,711	1·80
1201	0·0178	322,117	1·70
1108	0·0191	333,629	1·52
1167	0·0223	335,331	1·59
1031	0·0249	325,245	1·45
1119	0·0251	346,561	1·48
1243	0·0259	330,818	1·72
1168	0·0274	317,804	1·68
1087	0·0282	339,921	1.46
1193	0·0293	333,220	1·64
1147	0·0298	325,033	1·61
1126	0·0323	329,110	1·56
1115	0·0340	323,007	1·58
$\bar{x}$ = 1445		$\bar{x}$ = 343,403	$\bar{x}$ = 1·92
s = 420		s = 31,218	s = 0·53

Graph 7·21 and 7·22 Ophiura albida (Millport) arm - ossicles·

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
1323	0·1606	326,899	1·85
1352	0·1387	324,441	1·90
1392	0·0988	328,947	1·93
1410	0·1241	332,393	1·94
1215	0·1276	342,868	1·62
1371	0·1500	333,333	1·88
1219	0·1083	327,404	1·70
1400	0·1516	332,356	1·95
1376	0·1543	321,724	1·92
1255	0·1510	340,091	1·69

$\bar{x}$ = 1331 $\bar{x}$ = 331,045 $\bar{x}$ = 1·83

s = 75 s = 6,631 s = 0·12

Graph 7·23 and 7·24 *Ophiura albida* (Millport) without removal of organic matter.

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
1082	0·3348	224,015	2·21
1059	0·4600	177,989	2·72
994	0·2314	270,094	1·68
1037	0·2170	273,617	1·73
1023	0·2249	269,564	1·73
968	0·2685	221,136	2·00
960	0·1821	305,463	1·44
947	0·1318	336,683	1·29
947	0·2029	360,397	1·22
916	0·1475	266,948	1·57
1007	0·1611	279,329	1·65
881	0·2213	259,828	1·55
745	0·1844	369,440	0·92
723	0·1724	362,528	0·91
688	0·2178	350,091	0·89
766	0·2935	293,865	1·19
738	0·2031	335,424	1·00
936	0·1602	355,025	1·20
933	0·2679	319,616	1·33
1130	0·1769	254,381	2·03
1003	0·1496	325,869	1·40
909	0·1647	295,992	1·40
909	0·2062	328,867	1·26
1011	0·1669	329,538	1·40
1070	0·3757	321,372	1·52
1028	0·3098	302,179	1·55
985	0·3340	325,156	1·38
945	0·1905	299,709	1·44
744	0·1544	314,068	1·08
1140	0·2823	276,829	1·88
1267	0·1995	310,864	1·86
1134	0·2038	311,432	1·66

Graph 7·23 and 7·24 (continued)

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
1142	0·1604	339,863	1·54
1227	0·1873	315,015	1·78
863	0·2269	323,142	1·22
951	0·2647	320,087	1·35
947	0·3418	283,439	1·52
912	0·4053	241,703	1·72
733	0·2218	291,807	1·14
810	0·2450	332,009	1·11
846	0·2905	313,119	1·23
741	0·3431	305,989	1·11
$\bar{x}$ = 947		$\bar{x}$ = 302,225	$\bar{x}$ = 1·47
s = 141		s = 40,594	s = 0·37

Ophiura albida (Millport) disc - without removal of organic matter·

Graph 7·25 and 7·26

August 1976

Sr^{2+}	DRY WEIGHT	Ca^{2+}	$Sr/Ca \times 10^3$ atom ratio
657	0·0796	307,786	0·97
473	0·0794	289,671	0·75
408	0·0855	274,855	0·68
334	0·0451	268,290	0·57
517	0·0498	288,715	0·81
382	0·0394	291,877	0·60
593	0·0548	333,111	0·81
430	0·0574	275,810	0·71
495	0·0649	282,004	0·80
552	0·0697	261,231	0·97
$\bar{x}$ = 484		$\bar{x}$ = 287,335	$\bar{x}$ = 0·76
s = 99		s = 20,824	s = 0·13

February 1977

1098	0·0656	228,658	2·19
982	0·0529	267,013	1·68
1058	0·0387	242,247	2·00
1202	0·0474	237,342	2·31
957	0·0435	251,036	1·74
1023	0·0583	247,112	1·89
918	0·0627	263,412	1·59
903	0·0727	242,001	1·71
1028	0·0778	250,129	1·88
1008	0·0794	252,447	1·95
$\bar{x}$ = 1017		$\bar{x}$ = 248,139	$\bar{x}$ = 1·89
s = 88		s = 11,498	s = 0·22

Ophiura albida (Millport) arm - without removal of organic matter·

Graph 7·27 and 7·28

August 1976

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
1181	0·2961	325,056	1·66
1220	0·2304	306,531	1·82
1181	0·2485	342,053	1·53
1135	0·1454	309,491	1·67
871	0·1147	322,579	1·23
1239	0·1715	313,414	1·81
1200	0·1300	288,226	1·90
1061	0·1911	328,370	1·48
1074	0·2556	293,918	1·67
1132	0·1134	279,456	1·85
$\bar{x}$ = 1129		$\bar{x}$ = 310,909	$\bar{x}$ = 1·66
s = 108		s = 19,525	s = 0·20

February 1977

1163	0·2107	284,764	1·87
1307	0·1339	336,072	1·78
1159	0·1509	318,920	1·66
1150	0·1217	359,491	1·46
1214	0·1399	330,593	1·68
1251	0·1173	343,859	1·66
1286	0·1732	321,261	1·83
1180	0·1881	359,117	1·50
1117	0·2240	333,244	1·53
1391	0·2119	341,009	1·86
$\bar{x}$ = 1222		$\bar{x}$ = 332,833	$\bar{x}$ = 1·68
s = 85		s = 21,714	s = 0·15

Graph 7·29, 7·30 and 7·31 *Ophiura albida*(Loch Etive).

STATION	Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
E4	1761	0·0284	308,099	2·61
	1226	0·1386	270,562	2·07
	1156	0·1167	299,914	1·76
	1169	0·1925	272,727	1·96
	1155	0·1168	278,252	1·90
	1309	0·1603	288,521	2·07
	1263	0·0633	276,461	2·09
	962	0·0831	293,321	1·50
	909	0·1151	308,441	1·35
	979	0·1634	283,047	1·58
	1004	0·0473	264,270	1·74
	1148	0·1176	340,135	1·54
	1135	0·1188	294,612	1·76
	1076	0·1255	258,963	1·90
	1159	0·1164	279,210	1·90
	872	0·1835	286,103	1·39
	1179	0·0975	269,231	2·00
	1263	0·0095	289,473	1·99
	1130	0·0774	282,621	1·83
	989	0·1163	279,449	1·62
	$\bar{x}$ = 1142 ± 189		$\bar{x}$ = 286,170 ± 18,383	$\bar{x}$ = 1·83 ± 0·29
E11	1259	0·0298	272,650	2·11
	1779	0·0118	275,424	2·95
	1431	0·0277	210,437	3·11
	1405	0·0356	245,787	2·61
	1085	0·0645	242,248	2·04
	1139	0·0439	213,553	2·44
	1154	0·0866	216,512	2·44
	1063	0·0987	221,630	2·19
	1031	0·0679	220,913	2·13
	860	0·1309	267,380	1·47
	$\bar{x}$ = 1220 ± 260		$\bar{x}$ = 238,653 ± 25,642	$\bar{x}$ = 2·35 ± 0·47

Graph 7·29, 7·30 and 7·31

STATION	Sr^{2+}	DRY WEIGHT	Ca^{2+}	$Sr/Ca \times 10^3$ atom ratio
E24	1269	0·0834	352,218	1·65
	887	0·1195	266,736	1·52
	1040	0·1019	343,474	1·38
	1014	0·0739	253,721	1·83
	1307	0·0306	285,948	2·09
	946	0·0819	259463	1·67
	1163	0·0344	254,360	2·09
	1232	0·0284	264,084	2·13
	953	0·524	262,405	1·66
	1009	0·0322	252,328	1·83
	929	0·0673	250,743	1·69
	1146	0·0436	229,357	2·29
	1072	0·0560	245,535	2·00
	1206	0·0497	264,084	2·08
	1232	0·0365	273,972	2·05
	966	0·0569	252,636	1·75
	1255	0·0458	259,449	2·21
	1250	0·0400	218,750	2·61
	1160	0·0452	276,549	1·83
	987	0·0608	256,990	1·75
	914	0·0711	263,713	1·58
	1072	0·0653	258,423	1·90
	1128	0·0443	254,950	2·03
	1021	0·0661	245,840	1·90
	941	0·0532	223,215	1·93
	1057	0·0615	254,064	1·90
	$\bar{x}$ = 1083 ± 127		$\bar{x}$ = 262,423 ± 29,193	$\bar{x}$ = 1·89 ± 0·27

Graph 7·32, 7·33 and 7·34 *Amphiura chiajei* (Loch Etive).

STATION	Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
E4	926	0·0755	158,940	2·66
	1218	0·0718	248,085	2·24
	1079	0·0926	224,157	2·21
	1044	0·1078	223,214	2·14
	1027	0·1411	228,118	2·06
	1018	0·1080	208,334	2·23
	934	0·1007	211,023	2·02
	956	0·1369	191,745	2·28
	980	0·1561	240,230	1·86
	1057	0·1595	215,006	2·25
	$\bar{x}$ = 1024 ±85		$\bar{x}$ = 214,885 ± 25,382	$\bar{x}$ = 2·19 ± 0·20
E6	1005	0·0796	211,996	2·17
	988	0·0885	218,926	2·06
	1054	0·1186	216,063	2·23
	1068	0·0749	175,234	2·79
	1011	0·1087	235,739	1·96
	1007	0·0992	233,114	1·97
	940	0·1248	225,360	1·90
	896	0·1116	212,815	1·93
	950	0·1420	253,280	1·70
	1079	0·0695	243,884	2·43
	1013	0·0815	191,717	2·41
	1108	0·0901	194,228	2·61
	$\bar{x}$ = 1010 ± 85		$\bar{x}$ = 209,363 ± 29,530	$\bar{x}$ = 2·26 ± 0·48
E11	799	0·1657	226,313	1·62
	886	0·1430	240,384	1·76
	924	0·1354	249,261	1·69
	843	0·1275	230,392	1·67
	1448	0·1242	251,610	2·63
	1062	0·1295	217,181	2·23
	810	0·2005	112,219	1·95
	1245	0·1064	211,465	2·69
	750	0·1875	230,115	1·49
	783	0·1543	224,099	1·60
	$\bar{x}$ = 971 ± 237		$\bar{x}$ = 219,304 ± 39,763	$\bar{x}$ = 1·93 ± 0·43

Graph 7·32, 7·33 and 7·34

STATION	Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
E14	838	0·1251	269,785	1·42
	1038	0·1325	273,585	1·73
	910	0·1209	279,156	1·49
	857	0·2159	277,906	1·41
	796	0·1696	257,960	1·41
	888	0·1859	285,772	1·42
	738	0·2236	251,564	1·34
	1052	0·1498	271,195	1·77
	1202	0·1268	251,379	2·19
	737	0·2065	263,479	1·28
	$\bar{x}$ = 906 ± 150		$\bar{x}$ = 268,178 ± 11,786	$\bar{x}$ = 1·54 ± 0·27

Graph 7·35 and 7·36 <u>Ophiura albida</u> (Öresund).

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
1170	0·2030	326,448	1·64
1168	0·2033	313,549	1·70
1162	0·2044	324,086	1·64
1087	0·1033	302,516	1·64
1014	0·1293	309,359	1·50
1029	0·1459	325,565	1·62
1131	0·2156	324,674	1·59
1035	0·1328	305,910	1·55
941	0·1063	293,979	1·46

$\bar{x}$ = 1082 ± 81

$\bar{x}$ = 314,010 ± 11,854

$\bar{x}$ = 1·59 ± 0·07

Graph 7·37 Relationship between wet weight of intact animal and ossicle weight in <u>Ophiocomina nigra</u>.

WET WEIGHT	OSSICLE WEIGHT
0·3328	0·0942
0·1893	0·0550
1·1539	0·2872
1·0412	0·2358
1·7983	0·6297
5·0079	1·6403
1·0523	0·3619
4·9444	1·8390
0·4876	0·1790
0·6795	0·2492
0·5867	0·2190
2·0527	0·7050
2·5408	0·9230
3·5200	1·2926
0·6636	0·2475
5·5400	1·8719
5·4595	1·6672
5·2363	1·7778
3·9541	1·4378
3·9442	1·2893
7·1560	2·4325
5·9247	2·2124
0·9962	0·2967

R = 0·9940

p = 0·001

Graph 8·1 and 8·2 *Ophiocomina nigra* in elevated strontium levels.

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
1144	1·7469	249,012	2·10
1002	2·3937	221,413	2·07
998	1·1013	227,004	2·01
953	2·7289	225,366	1·93
1077	2·1341	238,975	2·06
1060	2·6888	226,868	2·13
1047	1·5041	243,000	1·97
1084	1·6349	263,921	1·87
987	3·3101	213,116	2·11
1062	0·9599	251,457	1·93
1017	3·1249	223,649	2·07
1098	1·9489	243,192	2·06
993	3·7501	251,047	1·80
1053	2·688	237,409	2·02
1020	0·8057	241,063	1·93
1011	3·6021	247,003	1·87
1010	4·0610	237,912	1·94
1034	3·4980	217,401	2·17
1061	2·4864	245,849	1·94
$\bar{x}$ = 1037		$\bar{x}$ = 237,087	$\bar{x}$ = 1·99
± 45		± 13,463	± 0·10

Graph 8·3 *Ophiura albida* (Millport) in elevated strontium levels.

Sr^{2+}	DRY WEIGHT	Ca^{2+}	$Sr/Ca \times 10^3$ atom ratio
1408	0·1065	302,413	2·12
1323	0·3211	319,629	1·89
1325	0·1311	315,021	1·92
1305	0·2241	298,561	1·99
1556	0·2924	304,501	2·33
1479	0·1284	300,008	2·25
1217	0·2691	299,472	1·85
1264	0·2374	305,674	1·89
1346	0·4301	318,520	1·93
1220	0·3742	288,493	1·93
1367	0·2687	292,376	2·13
1340	0·1404	307,414	1·99
1298	0·1967	306,567	1·93
1401	0·2013	294,233	2·17
1350	0·1936	288,117	2·14
1349	0·1428	305,801	2·01
1420	0·2403	295,923	2·19
1278	0·2761	300,401	1·94
1352	0·0543	313,007	1·97

$\bar{x}$ = 1347 ± 83

$\bar{x}$ = 302,954 ± 9,224

$\bar{x}$ = 2·03 ± 0·14

Graph 8.4 and 8.5 *Ophiura albida* (Loch Etive) in elevated strontium levels.

Sr^{2+}	DRY WEIGHT	Ca^{2+}	Sr/Ca x 10^3 atom ratio
1467	0.0225	244,444	2.74
1286	0.0641	253,510	2.32
1386	0.0505	247,525	2.56
1245	0.0462	202,922	2.80
1239	0.0403	263,647	2.15
1147	0.0392	255,102	2.05
1114	0.0449	236,637	2.15
1212	0.0577	227,469	2.44
1096	0.0570	241,228	2.08
1285	0.0311	261,254	2.25
1057	0.0544	218,290	2.21
1239	0.0666	262,762	2.15
934	0.0428	219,041	1.95
$\bar{x}$ = 1208		$\bar{x}$ = 241,064	$\bar{x}$ = 2.29
± 140		± 19,271	± 0.26

Table 7·2 Discrimination Ratios·

From Graph Data 7·8 and 7·9

Ophiocomina nigra

August 1976		September 1976	
0·13	0·18	0·16	0·17
0·10	0·19	0·14	0·20
0·14	0·19	0·18	0·19
0·15	0·18	0·19	0·26
0·12	0·13	0·17	0·26
0·10	0·13	0·13	0·24
0·10	0·12	0·13	0·24
0·16	0·13	0·13	0·16
0·14	0·13	0·17	0·15
0·13	0·10	0·20	0·13

$\bar{x} = 0{\cdot}16 \pm {\cdot}029$

From Graph Data 7·23 and 7·24

Ophiura albida (Millport)

0·30	0·23	0·19	0·17
0·38	0·17	0·19	0·21
0·23	0·12	0·17	0·24
0·24	0·12	0·19	0·17
0·24	0·12	0·21	0·18
0·27	0·16	0·21	0·21
0·20	0·14	0·19	0·24
0·17	0·16	0·20	0·16
0·17	0·18	0·15	0·15
0·22	0·28	0·26	0·17
		0·26	0·15

$\bar{x} = 0{\cdot}20 \pm 0{\cdot}052$

Table 7·2 Discrimination Ratios (continued).

From Graph Data 7·29, 7·30 and 7·31

Ophiura albida (Loch Etive)

E4

0·35
0·28
0·24
0·26
0·26
0·28
0·28
0·20
0·18
0·21
0·24
0·21
0·24
0·26
0·26
0·19
0·27
0·27
0·25
0·22

$\bar{x} = 0{\cdot}25 \pm 0{\cdot}039$

E11

0·32
0·44
0·47
0·39
0·31
0·37
0·37
0·33
0·32
0·22

$\bar{x} = 0{\cdot}35 \pm 0{\cdot}071$

E24

0·23
0·21
0·19
0·25
0·28
0·23
0·28
0·29
0·23
0·25
0·31
0·27
0·28
0·28
0·24
0·30
0·36
0·25
0·24
0·21
0·26
0·28
0·26
0·26
0·26

$\bar{x} = 0{\cdot}25 \pm 0{\cdot}035$

Table 7•2 Discrimination Ratios (continued)•

From Graph Data 7•32, 7•33 and 7•34

<u>Amphiura chiajei</u> (Loch Etive)•

E4

0•36	0•30
0•31	0•27
0•30	0•31
0•29	0•25
0•29	0•31
0•28	

$\bar{x}$ = 0∘30 ± 0•028

E6

0•34	0•29
0•32	0•30
0∘34	0•26
0•43	0•37
0•30	0•37
0∘30	0∘40

$\bar{x}$ = 0•33 ± 0∘049

E11

0•24	0•39
0•26	0∘29
0•25	0∘40
0•25	0•22
0•39	0•24

$\bar{x}$ = 0•29 ± 0•071

E14

0•21	0•21
0∘25	0•20
0•22	0•26
0•21	0•32
0•21	0•18

$\bar{x}$ = 0•22 ± 0•040