QUANTITATIVE ASPECTS OF THE FOOD OF **LITTORINA LITTOREA** (LINNAEUS)

FRANCIS E. MURPHY


CENTRE FOR NFLD. STUDIES
APR 7 1978
MEMORIAL UNIVERSITY
OF NEWFOUNDLAND

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS REÇUE

NL-339 (3/77)

QUANTITATIVE ASPECTS OF THE FOOD OF *LITTORINA LITTOREA* (LINNAEUS)

by

© Francis E. Murphy B.A.

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

Department of Biology

Memorial University of Newfoundland

July 1974

St. John's Newfoundland

ABSTRACT

Two series of feeding experiments were carried out on Littorina littorea, each using three groups of 33 snails. At Bonne Bay, each group was fed one of Ascophyllum nodosum, Fucus vesiculosus, and Laminaria longicruris. Algae used at Logy Bay were the same, except that the third was Laminaria digitata. Controls were of two kinds. An initial group of 33 was measured and weighed, then killed to obtain dry weights, caloric content and ash content. The second control was a group of 100 marked snails, weighed and measured, then released upon the shore. At the end of six weeks, 15 of these were recovered alive. Data and discussion are provided for the 13 identifiable snails.

The experimental animals were marked, weighed and measured. During six weeks, record was kept of the amount of food ingested and the amount of feces produced. At the end of the experiment, animals were again measured and weighed; they were then killed to obtain dry weights, caloric content and ash content.

The results generally show a decrease in all measurements. Shell length remained unchanged in four groups, increased slightly (but significantly) in two. Wet weights decreased in four groups, significantly in one, and increased

in two groups, significantly in one. Dry weight decreased in all groups, significantly in two. Highly significant decreases in caloric content occurred in all six groups. With other factors considered, the Bonne Bay Laminaria group showed a gain in caloric content. Factors causing this gain are discussed.

Lack of growth is considered to be due to conditions of captivity, natural periods of decrease in growth, and especially to the probable unsuitability of the algae used as food.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the part played by the following people in the preparation of this thesis, and to express my sincere gratitude to them. My wife, Jean, who first encouraged me to return to studies, and my children, have been understanding and very patient, though at times it was difficult. Dr. F. A. Aldrich, my initial contact at Memorial, was a constant source of encouragement. Dr. M. Laird (then head of the Department of Biology) and Dr. D. M. Steven (Department of Zoology, McGill University), director of the IBP Gulf of St. Lawrence Project, together made it possible for me to undertake the studies culminating in this thesis. The members of my supervisory committee, Drs. G. C. Davis, G. R. South, and D. M. Steele, collectively and individually gave advice, comment, and criticism. Dr. Y. A. Emara made himself freely available for suggestions and advice related to the statistical aspects of this study, and gave me many hours of his time; his student, Mr. Gerald Freake, was no less generous in his help with programs and the operation of the Olivetti 101 computer. Mr. Robert Hooper was my constant source of expert advice on all matters related to algae. Many members of the technical staffs of both the Department of Biology and the Marine Sciences

Research Laboratory proved especially helpful in solving many problems which only they could solve.

I wish to say a special word of thanks to my supervisor, Dr. Davis. He must have wondered at times whether this thesis would ever be written, but he was always patient, understanding, and sympathetic. Throughout the study, he gave freely of his time, his literature, his equipment. I am most grateful to him, though, for the friendship he and his wife bestowed on me and my family.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Littorina littorea (L.), the common periwinkle, occurs most commonly in that part of the intertidal zone characterized by rocks made slippery by a mat of microphytes and encrusting algae. That periwinkles graze on this algal film is generally accepted as a matter of observation. I have seen on a cobble beach a band of Littorina littorea, seven or eight individuals wide and several metres long, slowly advancing up the beach. Before the snails, the rocks were covered with a layer of green algae (Chlorophyta: Urospora penicilliformis and Ulothrix flacca); behind, the rocks were bare as a result of the grazing activities of the snails. While many workers have apparently made the same observation (Haseman, 1911; Newell, 1958; Lewis, 1964; Southward, 1964), and at least one study (Newell et al., 1971) has made use of an algal film to study the feeding rate of L. littorea, several workers have indicated that the winkle can, and does (at times) feed on larger algae. Tattersall (1920) attributes to Blevgad (XXIV Rep. Danish Biol. Station, 1915, p. 66) the observation that L. littorea is "able to bite off the tips of large fresh algae." Bell (1927) reports that fronds of such algae as Scytosiphon lomentarius (sic) and Halosaccion ramentaceum were "mutilated and eaten by the periwinkle."

Wright (1936) observed a group of large winkles devouring a frond of Laminaria sp. Graham (1971), referring to the family Littorinidae, says that while they feed mainly on the film of detritus on rocks, "they also rasp weeds." Thus, there seemed to be sufficient reason to think that macrophytes could be used with some measure of success to carry out quantitative feeding experiments on L. littorea.

Probably because this particular gastropod occurs abundantly in most places where it occurs at all, a great variety of research has been carried out using it as an experimental animal. Williams (1964) gives a review of the main work up to that time. Subsequent work includes a key to the species of the family Littorinidae and of their digenean parasites (James, 1968). Williams (op. cit.) studied the growth and distribution of L. littorea populations. Fish (1972) compared growth and breeding of populations living on the open coast and in estuaries. Newell et al. (1971) investigated some factors influencing rate of feeding. Arnold (1972) reported on salinity tolerances, Cornelius (1972) on thermal acclimation, and Sandison (1966) on oxygen consumption in relation to zonation. The relationship between temperature and respiration was also studied by Sandison (1967). A series of investigations by Newell and Pye (1970a and b; 1971a and b) was carried out to examine the interdependence of temperature, rate of metabolism, body size, and oxygen consumption in both intact L. littorea and

cell-free homogenates of the soft tissues.

Many workers have investigated some aspect of energy utilization by gastropods; the scope of such work may be illustrated by the following examples. Paine (1965) made a field study of the carnivorous opisthobranch, Navanax inermis. Population density in the field, and quantity of food in the gut of individual Navanax were known; the investigator calculated caloric value of the prey, oxygen consumption of the predator and length of time food remained in the gut. He was then able to determine the amount of energy available for growth in the field.

In another study, this one involving the intertidal herbivorous gastropod Tegula funebralis, Paine (1971a) was able to estimate energy flow at the population level, based on his study of two subpopulations of the species. The subpopulations were distinguished on the basis of their position on the shore, and exhibited differences in ecological efficiencies, as well as in several other characteristics. A similar study was carried out by Sutherland (1972), who compared growth and biomass of two populations of Acmaea scabra (Gould), one at a high level on the shore, the other at a low level.

Odum and Smalley (1959) went a step further in the study of population energy flow, when they compared two species of salt marsh invertebrates, one a herbivorous

grasshopper, Orchelimum fidicinium, the other a detritus-feeding gastropod, Littorina irrorata. They concluded that populations which are very different in life history, age structure and metabolic rate cannot be compared on the basis of numbers and biomass; studies of energy flow are necessary to understand the true role of populations in a community.

Carefoot (1967a) did a comparative study of nutrition and growth in three species of molluscs, based on total food energy, and the total energy of growth, metabolism, excretion and secretion. In another study, Carefoot (1967b) investigated a single species, Aplysia punctata, which he fed a variety of marine algae. Wide variations in the results were directly attributable to differences in the diets of the animals.

The present study was an attempt to investigate some aspects of the energetics of Littorina littorea, based on a series of feeding experiments using macrophytes so that quantitative food intake during a given period could be estimated with reasonable accuracy. It was realized at the outset that this was a wholly contrived study, performed in a laboratory under conditions which could not be designated 'natural' or 'normal.' Nevertheless, in view of the many difficulties which would be encountered were one to attempt quantitave feeding of the 'normal' fare of diatoms, detritus,

and filamentous microphytes, the use of macrophytes seemed justified, especially since preliminary experiments had shown that the snails did eat such food.

MATERIALS AND METHODS

The initial experiments were carried out at Norris Point, where the author was primarily engaged in research for an IBP project sponsored by McGill University. Several factors in these experiments were, directly or indirectly, related to or dependent upon, the IBP work. For example, a simple running seawater system, adequate for IBP work, did not permit any form of temperature control. That the seawater was changed at irregular intervals was due to scheduled IBP duties. A drying temperature of 60°C was used because the only available oven was required to be kept at that temperature for the IBP work. However, this temperature appears to be used fairly commonly for such drying, and was considered suitable for the present experiments.

Briefly, each experiment consisted of feeding individual snails (whose size and weight were known) a given quantity of food over a specified period. During that time, feces were collected from each individual. At the end of the experiment, each animal was again measured and weighed; it was then killed so that dry weights could be obtained. Subsequently, caloric content of the dry matter was determined,

as was the ash content. Similar determinations were made on the food algae. The resulting data were intended to be used to make some estimation of the use of energy by L. littorea.

Two series of experiments were performed, one at the Bonne Bay Biological Station (Norris Point, Bonne Bay, Nfld.; 49°31'N, 57°06'W) from 3 August to 15 September, 1971, and the other at the Marine Sciences Research Laboratory (Logy Bay, Nfld.; 47°34'N, 52°41'W) from 8 November to 19 December, 1971. In each series, 99 snails were used, divided into three groups of 33. Each group was fed a different alga, as follows: at Bonne Bay, Ascophyllum nodosum, Fucus vesiculosus, and Laminaria longicruris; at Logy Bay, A. nodosum, F. vesiculosus, and L. digitata. Controls were of two kinds: a group of 100 snails, representing a sample similar to the experimental groups, was released on the shore from which the entire experimental population had been collected. The snails in this 'shore group' were marked, weighed and measured prior to being released. They remained on the shore for the duration of the experiment (six weeks), after which an attempt was made to recover them.

The second control used in each series of experiments was an additional group of 33 snails, similar to the experimental groups. The snails in this 'initial group' were marked, weighed and measured; they were then killed

and removed from the shells and opercula, so that 'original' dry weights and caloric values could be obtained for later comparison with the corresponding 'final' values obtained from the experimental animals.

Of the 100 snails released on the shore, 15 were recovered alive at the end of the six-week period; data for these are provided. A similar group of 100 was released at the beginning of the Logy Bay experiments; of these, none was recovered, due to ice cover in the intertidal zone at the time the experiments were completed.

A. Collection of snails and selection of experimental groups

In late May, 1971, several hundred periwinkles were collected during low tide, throughout the lower intertidal zone at Burnt Point, Bonne Bay. The snails were placed into a large fibreglass tank supplied with running seawater; several pieces of shale, as well as a few large bunches of intertidal macrophytes, were placed into the tank. At irregular intervals, the tank was drained for periods of several hours, exposing the snails to drying conditions; no attempt was made to imitate tidal rhythm. The animals were maintained thus for several weeks while preliminary experiments were performed. Dead snails were removed when discovered.

Prior to establishing the experimental groups, a few hundred randomly selected snails were removed from the tank and divided into four arbitrary size groups. The distribution is summarized in Table 1.

Table 1. Size distribution of original groups of snails (Bonne Bay experiments)

Size (mm)	No. of Snails	%
<10	7	1.8
10-15	62	15.5
16-20	277	69.4
21-25	53	13.3
TOTALS	399	100.0

Because of the small number of individuals less than 10 mm, this group was omitted from the study. The experimental animals were selected so that they approximated the percentages given in Table 1; it is assumed these proportions represented the size distribution in the natural population. An experimental group of 99 snails was selected, using random numbers, from the 392 snails in the 10-25mm groups of Table 1. The size distribution is given in Table 2.

A fourth group of 33 snails was selected in the same way, to be used as a control group. A final group of 100 was selected, approximating the natural population. Its size distribution was: 10mm, 2; 10-15mm, 16; 16-20mm, 69; 21-25mm, 13. Members of this group were individually marked, measured and weighed, and then released on the shore as a 'natural' control group.

Table 2. Size distribution of experimental group of snails (Bonne Bay experiments)

Size (mm)	No. of Snails	%
10-15	15	15.15
16-20	69	69.70
21-25	15	15.15
TOTALS	99	100.00

B. Marking and measuring

When the snails had been out of the water 24 hours, the shells were marked. With fine sandpaper, a small area on the uppermost surface of the shell was lightly sanded to remove irregularities and attached organisms. A very small drop of acetone was applied to remove traces of moisture,

and a small patch of plastic model enamel was painted on. At the same time, a thin line of enamel was applied to the edge of the lip, to be a visual indicator of shell growth. When the paint was dry, each snail was numbered using India ink on the paint spot. This method of marking proved very successful in the laboratory, no labels having been lost or obliterated during six weeks.

Measurements were made with a vernier caliper accurate to 0.1mm; the customary length, obtained by measuring parallel to the shell axis from the apex to the most distant point on the lip, was recorded.

C. Weighing

The five groups of labelled and measured snails were placed into tanks containing only filtered seawater. (Seawater was vacuum-pumped through a Whatman G F/c filter at a pressure of 30-40cm of mercury.) Snails were weighed at the end of a three-day period, during which food was withheld to permit clearing of the gut. In preparation for weighing, one entire group was placed into a finger bowl completely filled with filtered seawater. The bowl was then covered, forcing the snails to remain submerged, thus minimizing differences in contained water. To weigh an individual, the animal was disturbed enough to make it withdraw; the operculum was given a light, sharp blow, causing

complete withdrawal and closure. The snail was then transferred to the balance, where it was weighed under water. The weights so obtained were converted to wet weights using a correction factor, derivation of which is described below.

Reference is frequently made in the literature to the difficulties inherent in obtaining wet weights, particularly of aquatic organisms. Contained water, adhering surface water, and evaporation, all of which can vary considerably, contribute to the problem. A very casual survey of the literature reveals several trends among workers in the ways they treat the wet-weight problem.

Many simply acknowledge the difficulties: "Wet weights were considered somewhat unreliable" (Davis, 1968); "... weight measurements of fresh Halichondria were totally unreliable ..." (Carefoot, 1967a); "... it is not possible to get accurate wet weights of a medusa ..." (Fraser, 1969).

While some workers refer to an undefined 'damp-dry' weight (Paine, 1965; Carefoot, 1967a, b; 1970), others attempt to standardize their procedure to varying degrees. Hyman (1938) tried to wet-weigh medusae which "were simply drained for a few minutes." Barnes et al. (1963) worked with the soft parts of barnacles which "were dried quickly on filter paper, transferred to a small tube and weighed." Pantelow (1939) weighed small trout "dried gently with a towel to remove as much surface water as possible." Clearly, it is difficult to determine how much water is 'excess',

how long to blot, what is meant by 'damp-dry', or to standardize or define these and related approaches.

Lowndes (1942) described an elaborate method, involving density and volume determinations, for weighing aquatic organisms. He had hoped to show that, when dealing with larger animals, the error in direct weighing would be small and relatively constant. Instead, he found the contrary: an error of 74% in the case of a prawn in berry. He concluded that "direct weighing forms a useful check, but for accurate work it is useless."

In the present study on animals with maximum wet weights in the order of three grams, whose weight increase was to be measured after a relatively short period, it was essential that wet weights be as accurate as possible. Thus, a method of indirect weighing was used, closely resembling that of Henderson (1963), itself a modification of the method of Jacobs (1941). A preliminary experiment was performed, to determine the ratio between 'direct' weights and 'indirect' weights. Details of the experiment follow.

Two similar groups of snails were randomly selected in the same manner as the experimental groups. Snails in group A were numbered 1 to 36, those in group B, 37 to 72. Both groups were placed into two finger bowls completely filled with filtered seawater and covered to prevent snails from leaving the water. Weighing was then carried out as described earlier (p. 10), using first a snail from group A,

then one from group B, until 72 'water weights' were obtained. From this point, the two groups were treated somewhat differently.

The group A snails were kept submerged, and weighed directly. Having been agitated enough to cause withdrawal and closure, the snail was removed from the water by forceps, shaken vigorously to remove excess water, then weighed. When all 36 snails were thus weighed directly, the procedure was repeated three times, providing four 'air weights' per snail; an 'average' air weight was then obtained. Finally the ratio of each 'average air weight' to the corresponding 'water weight' was calculated for each of the group A snails. The ratio ranged from 2.08 to 2.44, with mean 2.25. All weights and ratios are presented in Table A 1, Appendix A.

Group B snails 37 to 72 were then weighed under water to obtain their 'water weights.' Each of these weights was then multiplied by the ratio 2.25 to obtain a set of 'calculated air weights.' The snails were then weighed directly, in the same way as those in group A, providing four 'air weights' and an 'average air weight' for each snail. The weights are summarized in Table A 2, Appendix A.

Finally, the 'average air weights' and the 'calculated air weights' for group B snails were compared statistically, using the standard t-test. The results show an absolute difference in the means of 11.3 mg; with $t = 0.064$, there is no significant difference between the means. Therefore,

the ratio 2.25 was considered a reliable 'correction factor,' and all subsequent weighing of snails was done indirectly; the resulting weights were converted to the 'wet weights' shown in all tables throughout this study.

Weighing necessitated some modification to the Mettler H 15 balance used. A plastic 'bench' was made to fit inside the balance chamber, so that it could straddle the balance pan without interfering with its movement. On the bench was placed a beaker of filtered seawater, into which was suspended a small glass pan, attached by stainless steel wire to the balance pan support (see Figure 1 p. 15). The glass pan was entirely submerged; its weight was recorded as pan weight.

D. Experimental tanks

Throughout the experiments, each snail was kept in an individual 1-liter polythene container, numbered the same as the snail, and covered with the upper or lower half of a disposable Petri dish of suitable size. Filtered seawater was added, removed and changed at irregular intervals, without attempting to imitate tidal movements. Fresh filtered seawater was usually added after feeding, and always after collecting feces. The containers were kept in a wet bench supplied with a constant flow of seawater.

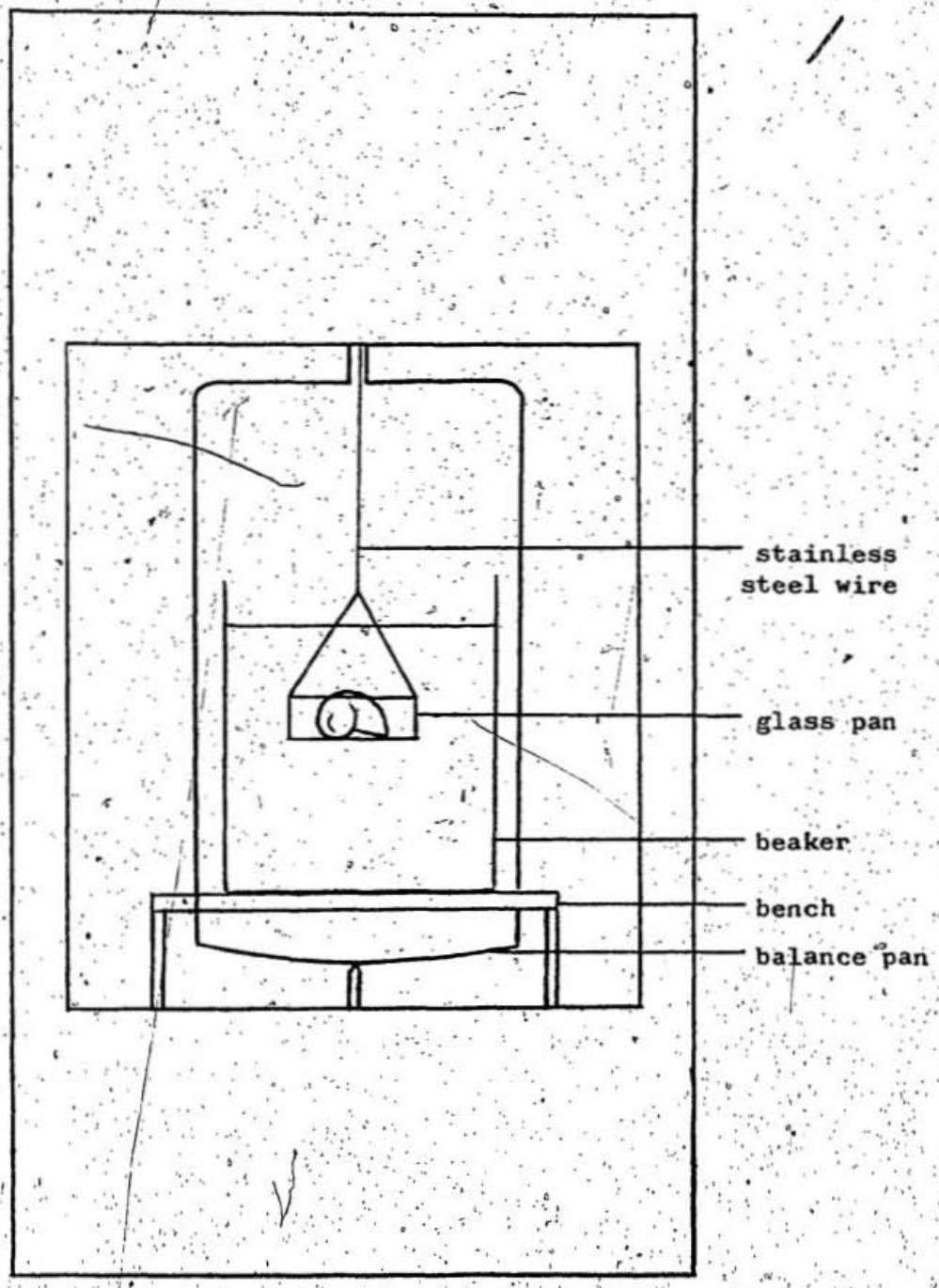

Fig. 1. Modifications to Mettler H15 balance.

E. Temperature

Temperature could not be regulated, and since it was influenced by both room temperature and the temperature of the running seawater, it fluctuated over a wide range. A record was kept of readings taken at average intervals of three days. These intervals ranged from one to seven days at Bonne Bay, and one to six days at Logy Bay. The recorded temperature was the result of eight readings, taken at the inlet and outlet of the wet bench, and in six tanks: two near the inlet end of the bench, two in the middle, and two near the outlet end. Temperatures are given for Bonne Bay in Table 5 (p. 25) and for Logy Bay in Table 6 (p. 26).

F. Food and feeding

Feeding was carried out four times during the experimental period, at average intervals of approximately ten days. During the ten days, no visible deterioration of algae occurred (i.e. other than that caused by the snails), and the pieces of algae were considered 'fresh' for that period.

Algae used as food were collected while still submerged, and until required were kept in a large tank, the water of which was frequently changed. For feeding, the algae were cut into small pieces. In the case of Fucus and

Ascophyllum, some selection was used to avoid air bladders, as it was desirable to have the pieces sink. Pieces of frond 2-3cm were used, except that Laminaria was cut into quadrangles 2-3cm on a side. The pieces were carefully cleaned free of debris and attached organisms, and thoroughly rinsed in filtered seawater before being weighed.

Wet weights of fresh algae were difficult to obtain with reliability, wide variations occurring when a given piece of plant was reweighed even after a short interval of several minutes. Since the outcome of the experiments depended to a great extent on being able to obtain reliable 'before' and 'after' weights of the algae, an attempt was made to reduce the error. Through a series of trial-and-error experiments, the following method was chosen. While variability was considerably reduced, it was by no means eliminated. Ascophyllum and Fucus were blotted by being placed on paper towelling and covered with a second piece of towelling. The pieces of plant were then placed into a drying oven at 40° C for fifteen minutes, after which they were weighed. Laminaria was simply blotted to remove surface water, and weighed without drying. Algae pieces were weighed in aluminum weighing pans numbered to correspond with the snails and their containers.

The dry weight equivalent of the food-algae was calculated as follows. Typical pieces of algae, similar to

those fed to the snails, were weighed and placed into containers of filtered seawater. After ten days, the pieces were weighed again. The two sets of weights were then compared statistically. No difference was found in the weights of Ascophyllum nodosum and Laminaria digitata. A difference significant at the 95% level was found in the weights of Fucus vesiculosus, and one significant at the 99.9% level in those of Laminaria longicruris. Because of the differences in weights of Fucus and L. longicruris, it was considered justifiable to average the 'before' and 'after' wet weights to arrive at a 'mean wet weight' value; to have consistent results, weights of all four algae were averaged in this way. The pieces of algae were then dried, and the ratio mean dry weight/mean wet weight was calculated. This ratio was used to convert the wet weight of food intake to the equivalent dry weight. Algae wet weight-dry weight relationships are summarized in Tables 7, 8, 9, 10 (pp. 27-30).

G. Collection of feces

Feces were collected from each animal nine times during the experimental period, at intervals averaging 4.5 days. These intervals ranged from 2 to 9 days at Bonne Bay, with a mean of 5.1 days; and from 2 to 6 days at Logy Bay, with a mean of 4.5 days. To collect the feces most of the water in each tank was poured off, care being taken not to

lose any feces. The snail and alga were rinsed with filtered seawater from a plastic wash-bottle, and put aside. The remaining water in the tank was then poured through a piece of fine (#8) plankton netting; the tank was thoroughly rinsed to ensure that all fecal particles were removed into the netting. Distilled water was then used to flush the feces from the netting into small glass dishes. Each dish, containing feces of an individual snail, was placed under a stereo microscope. It was thus possible to watch as a fine-bore pipette was used to draw off the distilled water, presumably containing dissolved salts. It was also possible to observe that no feces were drawn into the pipette. Finally, the feces were flushed with distilled water into an aluminum weighing pan and dried to constant weight at 60°C.

H. Dry weights of snails

At the end of the experiment, snails were kept in their containers for an additional three days, with no food. These were then measured and weighed as at the beginning of the experiment. Snails were then killed by immersion in hot (approximately 55°C) water for 45 seconds, when they were easily removed from the shell and operculum. The soft parts were then oven-dried at 60°C to constant weight, which required two days of drying. Dry matter was weighed after cooling in a dessicator for 15 minutes.

I. Calorimetry

Caloric values for snails, algae and feces were obtained using a Parr 1411 oxygen-bomb calorimeter. The instrument was calibrated by performing ten runs over a 14 day period, using standard samples of benzoic acid, supplied by the Parr Instrument Company.

Samples to be combusted were dried at 60°C, ground to a fine powder with mortar and pestle, and the powder stored at 60°C until required. Snails were treated individually, each one providing sufficient dry matter for both combusting and ashing. Algal caloric values were determined as averages of 11 to 15 combustions per species. Feces had to be treated collectively, since there was such a small amount. Thus, the feces of all the snails in one group were combined, providing enough dry matter for two or three combustions, and a small portion for ashing.

J. Ashing

All samples were ashed the same way. Powdered dry matter was weighed in a numbered, pre-weighed porcelain crucible. The crucibles were put into a muffle furnace which was heated to 600°C over a 2 to 2½ hour period. At the end of six hours, the furnace was turned off, and the crucibles were slowly cooled. Final cooling took place in a dessicator, prior to weighing the ash.

K. Differences in the Logy Bay experiments

The experiments at Logy Bay were essentially an attempt to duplicate the Bonne Bay work. As far as possible, conditions were similar to those in effect at Bonne Bay, the obvious difference being one of temperature, as the work at Logy Bay was carried out during the beginning of winter. The most important difference, however, was in the algae used as food. At Bonne Bay, Ascophyllum nodosum, Fucus vesiculosus, and Laminaria longicruris were used. It had been intended to use local species at Logy Bay, but L. longicruris does not occur there, and A. nodosum is not abundant. It was possible to transport Ascophyllum from Bonne Bay, but not Laminaria, which would deteriorate during the long journey. Consequently, the algae used at Logy Bay were local Fucus vesiculosus, local Laminaria digitata (substituting for L. longicruris) and Bonne Bay Ascophyllum nodosum.

Snails used at Logy Bay were collected at Bonne Bay. Size distribution was slightly different at the time of these experiments, and is presented in Table 3. (Compare with Table 1). Consequently, the size composition of the experimental groups was changed, as in Table 4. (Compare with Table 2). As at Bonne Bay, a fourth (control) group of 33 was similarly selected, as well as a 'shore' control group, with size distribution: <10mm, 2; 10-15mm, 16; 16-20mm, 69; 21-25mm, 13.

Table 3. Size distribution of original groups of snails (Logy Bay experiments)

Size (mm)	No. of Snails	%
<10	12	2.3
10-15	118	22.5
16-20	368	70.2
21-25	25	4.8
>25	1	0.2
TOTALS	524	100.0

Table 4. Size distribution of experimental groups of snails (Logy Bay experiments)

Size (mm)	No. of Snails	%
10-15	21	21.2
16-20	75	75.8
21-25	3	3.0
TOTALS	99	100.0

RESULTS

Of the 100 snails returned to the shore at the beginning of the Bonne Bay experiments, 15 were recovered alive after six weeks. Of these, all but two were readily identifiable; the two had completely indiscernible labels, and were not included in the results. It was immediately evident that shell growth had occurred in most snails, and to a considerable extent in some of the smaller ones. One snail exhibited no shell growth, two showed a reduction in shell length; the remaining ten showed increases ranging from 0.1 to 1.1mm. In the latter case, the line of paint originally added to the edge of the lip was about 4mm posterior to the edge, indicating an extensive addition to the rim of the shell.

The mean of wet weight increase was 127.1mg (6.8%), with a range from 43.2mg to 227.9mg. One snail showed a loss of weight, 62.1mg. At the end of the experimental period, the mean dry weight was 78.3mg and the mean caloric value of the soft tissues was 4488.1cal/g. No ash determinations were made. Table 11 summarizes the results for the shore group.

No data are available for the 100 snails returned to the shore at the beginning of the Logy Bay experiments, as none of these animals were recovered.

For the three experimental groups, data were obtained at the beginning and the end of the experiments in two different ways according to the characteristic being measured. Shell lengths and wet weights were obtained by straightforward 'before' and 'after' measurements. Dry weights and caloric content for a particular group could be obtained only at the end of the experiment. The resulting data were compared with the corresponding data for the initial group obtained at the beginning of the experiment. Data on food intake and feces production were compiled at the end of the experiment. All 'before' and 'after' data were subjected to a t-test to determine whether there was a real difference in a given set of measurements at the end of the experiment.

Data for the three Bonne Bay experimental groups are summarized in Tables 12, 13, 14. The Logy Bay experimental groups were treated the same way as the Bonne Bay groups, and the resulting data similarly analyzed. The results are summarized in Tables 15, 16, 17.

Shell length remained unchanged in three of the six groups. Of the remainder, one group showed a decrease of 0.1mm; the other two groups each showed an increase of 0.1mm. Among the Bonne Bay groups, the Ascophyllum group remained unchanged; the other two groups both showed increases, significant at the 99.5% level for the Laminaria group, and at the 99.95% level for the Fucus group. Among the Logy Bay

Table 5. Water Temperatures at Bonne Bay (°C)

Date 1971	Inlet	Outlet	Tank No. 1	Tank No. 6	Tank No. 33	Tank No. 66	Tank No. 96	Tank No. 99	Average
14 Aug	15.2	15.8	15.5	15.7	15.8	15.8	15.8	16.0	15.8
18 Aug	16.3	16.1			15.8	15.8	15.8	15.7	15.8
20 Aug	16.0	16.2					16.2		16.2
23 Aug	15.1	15.1					15.2		15.2
25 Aug	15.3	15.3	15.3	15.3	15.2	15.3	15.3		15.3
31 Aug	14.7	14.2	14.4	13.7	14.2	14.1	14.3	14.2	14.4
2 Sept	15.1	15.4	15.3	15.4	15.3	15.3	15.3	15.4	15.4
4 Sept	14.8	14.7	14.7	14.7	14.7	14.7	14.8	14.7	14.7
5 Sept	14.1	13.9	13.9	13.8	13.8	13.8	13.8	13.8	13.8
8 Sept	14.7	14.7	14.8	15.2	15.0	14.8	14.9	15.2	14.9
15 Sept	14.3	14.2	14.3	14.4	14.3	14.3	14.3	14.4	14.4
16 Sept	14.9	14.9	14.9	15.1	15.1	15.1	15.0	15.1	15.1
19 Sept	13.6	13.6	13.5	13.5			13.6	13.6	13.6
20 Sept	12.8	12.7	12.8	12.5	12.5	12.6	12.5	12.4	12.6

Table 6. Water temperatures at Logy Bay (°C)

Date 1971	Inlet	Outlet	Tank No. 1	Tank No. 5	Tank No. 46	Tank No. 50	Tank No. 95	Tank No. 99	Average
9 Nov	6.3	8.2	8.6	8.8	8.8	7.9	9.0	8.3	8.5
11 Nov	5.8	7.8	6.8	6.3	6.5	6.5	7.5	6.8	6.7
16 Nov	5.7	7.2	7.4	8.0	7.7	7.5	8.1	7.7	7.7
22 Nov	5.8	7.7	9.8	9.8	9.4	9.2	11.3	10.0	9.9
26 Nov	5.7	6.8	6.3	6.3	6.8	6.7	6.9	6.8	6.6
1 Dec	5.8	9.0	10.8	10.2	12.2	12.8	15.7	13.7	12.5
2 Dec	5.2	6.2	7.6	8.5	8.2	8.2	8.8	8.8	8.3
7 Dec	5.0	7.1	8.8	8.8	8.7	8.7	9.8	9.7	9.0
9 Dec	4.1	5.3	6.5	7.0	7.7	7.7	8.3	7.8	7.5
13 Dec	3.5	5.1	4.8	5.1	5.2	5.3	5.8	6.8	5.5
14 Dec	4.2	5.6	6.6	6.8	7.2	7.2	7.8	7.3	7.1
16 Dec	3.8	6.5	6.8	7.0	7.5	7.7	8.9	7.0	7.4
19 Dec	3.3	4.8	5.9	6.0	6.1	6.2	6.8	6.3	6.2
22 Dec	2.8	3.3	6.2	6.2	5.5	5.7	6.1	6.0	5.9

Table 7. *Ascophyllum nodosum*: wet weight - dry weight relationships (Significance: N. S. not significant).

Sample No.	Initial wet weight (mg)	Final wet weight (mg)	Average wet weight (mg)	Dry weight (mg)
1	125.9	112.9	119.4	47.1
2	136.2	135.1	135.6	51.0
3	358.1	340.6	349.3	125.6
4	22.1	21.0	21.5	12.5
5	80.8	155.5	118.1	61.3
6	96.7	118.2	107.4	56.9
7	180.0	174.7	177.3	71.9
8	14.0	32.1	23.0	17.3
9	298.6	282.8	290.7	111.5
10	252.7	241.4	247.0	97.3
11	196.8	186.6	191.7	79.6
12	233.1	219.9	226.5	95.3
13	34.1	29.8	31.9	13.2
14	87.2	75.1	81.1	36.5
15	450.6	462.1	456.3	155.2
16	117.2	104.1	110.6	50.0
17	32.8	78.0	55.4	34.7
18	288.1	264.7	276.4	110.0
19	34.1	30.4	32.2	12.0
20	46.9	38.3	42.6	19.4
$\bar{x}$	154.3	155.1	154.7	62.9
$t(\bar{x}_1 - \bar{x}_2)$			.17 N. S.	
$\frac{\text{dry } (\bar{x})}{\text{avg. wet } (\bar{x})}$				.407

Table 8. Fucus vesiculosus: wet weight - dry weight relationships (Significance: * significant at 95%)

Sample No.	Initial wet weight (mg)	Final wet weight (mg)	Average wet weight (mg)	Dry weight (mg)
1	101.4	91.2	96.3	32.2
2	171.6	177.4	174.5	56.4
3	87.7	104.4	96.0	22.7
4	99.7	108.4	104.0	43.7
5	82.6	89.3	85.9	29.9
6	55.6	51.6	53.6	25.3
7	56.0	89.3	72.6	30.0
8	117.6	109.2	113.4	36.4
9	50.2	46.5	48.3	17.8
10	84.0	105.6	94.8	35.9
11	115.9	121.0	118.4	38.6
12	113.8	111.3	112.5	35.5
13	107.7	113.2	110.4	37.8
14	102.9	123.8	113.3	37.9
15	50.5	49.4	49.9	23.4
16	130.6	143.5	137.0	48.4
17	89.7	75.6	82.6	35.9
18	121.4	123.4	122.4	43.5
19	103.0	105.4	104.2	37.1
20	50.6	62.1	56.3	31.7
$\bar{X}$	94.6	100.0	97.3	35.0
$t(\bar{x}_1-\bar{x}_2)$			2.06*	
$\frac{\text{dry } (\bar{x})}{\text{avg. wet } (\bar{x})}$				.360

Table 9. *Laminaria digitata*: wet weight - dry weight relationships (Significance: N. S. not significant)

Sample No.	Initial wet weight (mg)	Final wet weight (mg)	Average wet weight (mg)	Dry weight (mg)
1	804.9	631.4	718.1	90.4
2	861.1	888.8	874.9	115.4
3	954.3	1003.7	979.0	135.4
4	612.6	657.5	635.0	85.1
5	843.9	793.9	818.9	107.5
6	753.3	735.4	744.3	111.2
7	862.9	932.1	897.5	123.6
8	697.5	738.4	717.9	98.9
9	714.7			
10	823.1	812.9	818.0	117.7
11	1099.2	1029.3	1069.2	133.8
12	998.3	956.1	977.2	127.6
13	836.9	837.9	837.4	118.1
14	973.6	803.7	888.6	97.3
15	916.5	896.8	906.6	121.7
16	939.5	910.3	924.9	119.3
17	750.0			
18	690.0	563.1	626.5	84.2
19	757.2	552.5	654.8	60.6
20	779.5	781.6	780.5	104.5
$\bar{X}$	844.6	807.5	826.0	108.4
$t(\bar{x}_1-\bar{x}_2)$			1.93 N.S.	
$\frac{\text{dry } (\bar{x})}{\text{avg. wet } (\bar{x})}$				.131

Table 10: Laminaria longicruris: wet weight - dry weight relationships (Significance: ** significant at 99%)

Sample No.	Initial wet weight (mg)	Final wet weight (mg)	Average wet weight (mg)	Dry weight (mg)
1	813.9	705.8	759.8	102.1
2	758.7	420.9	589.8	56.8
3	1065.3	1020.5	1042.9	155.3
4	952.4	710.3	831.3	108.9
5	897.0	747.7	822.3	110.7
6	921.0	845.4	883.2	126.2
7	898.0	811.8	854.9	127.7
8	800.7	737.5	769.1	115.8
9	867.5	855.9	861.7	124.1
10	778.6	603.7	691.1	70.0
$\bar{x}$	875.3	746.0	810.6	109.8
$t(\bar{x}_1-\bar{x}_2)$			4.11**	
$\frac{\text{dry } (\bar{x})}{\text{avg. wet } (\bar{x})}$				.135

Table 11. Bonne Bay shore group, recovered snails. Shell length, wet weight, dry weight, caloric content. (Significance: ** significant at 98.5% level; *** significant at 99.95% level).

Snail No.	Shell length (mm)			Wet weight (mg)			Dry weight (mg)	Caloric value (cal/g)
	orig.	final	diff.	orig.	final	diff.		
7	14.7	15.3	+0.6	1349.3	1520.3	+171.0	67.4	4634.2
12	13.1	14.0	+0.9	744.5	952.9	+208.4	40.1	4339.3
13	14.7	15.4	+0.7	1094.4	1272.8	+178.4	28.7	3970.4
14	11.1	12.2	+1.1	518.9	686.7	+167.8	29.6	4368.6
17	14.9	15.4	+0.5	1247.0	1474.9	+227.9	59.4	4547.3
35	16.8	16.5	-0.3	1819.6	1757.5	- 62.1	87.9	4647.8
39	15.1	15.3	+0.2	1346.0	1394.1	+ 48.1	64.8	4491.3
51	16.8	17.0	+0.2	1682.8	1791.9	+109.1	36.4	4574.3
54	20.0	20.0	0	2444.6	2487.8	+ 43.2	123.3	4429.9
58	18.1	18.3	+0.2	2127.6	2239.2	+111.6	86.0	4428.6
67	17.9	17.2	-0.7	1544.0	1745.3	+201.3	81.8	4729.7
88	22.4	22.5	+0.1	3688.4	3819.8	+131.4	154.4	4572.4
100	23.9	24.0	+0.1	4609.8	4725.9	+116.1	158.1	4612.1
$\bar{X}$	16.9	17.2		1862.8	1989.9		78.3	4488.1
S	3.6	3.3		1154.0	1133.8		42.1	186.0
$S_{\bar{x}}$	1.0	0.9		319.7	314.1			
t			2.05**			5.67***		

groups, both the Ascophyllum group and the Fucus group remained unchanged; the Laminaria group showed a decrease of 0.1mm, but with t=0.67, the difference is not significant.

Mean wet weight increased in two groups, decreased in four. The increases were recorded in the Bonne Bay Ascophyllum and Laminaria groups. The difference was not significant for the Ascophyllum group, but was significant at 99.95% for the Laminaria group. The decrease in the Fucus was not significant. All three Logy Bay groups had wet weight losses. The loss in the Ascophyllum group was significant at 99.5%; that in the Laminaria group, at 99.95%. The loss of only 1.3mg in the Fucus group was not significant.

In all three Bonne Bay groups, there was a decrease in dry weight, ranging from 12% in the Laminaria group to 35% in the Ascophyllum group. The loss in the Ascophyllum group was significant at the 99.95% level. The loss in the Fucus group was significant at the 99% level, while the decrease in the Laminaria group was not significant. Dry weight decreases occurred also among all three Logy Bay groups, but the decreases were considerably lower, ranging from 3% in the Laminaria group to 14% in the Ascophyllum group, and were not significant in any group.

Decreases in caloric content, ranging from 199.8cal/g to 381.5cal/g, were recorded in all groups; in all cases, the loss was significant at the 99.95% level. Tables 18

and 19 summarize the various caloric values on both total dry weight and ash free dry weight bases. Compared to a value of 4521.9cal/g for the initial group at the beginning of the experiment, the Bonne Bay experimental animals had final caloric values ranging from 4131.2cal/g to 4204.5 cal/g. On an ash free basis, the range was from 4854.5 cal/g to 4907.5cal/g, compared to an initial value of 5282.6cal/g. The Logy Bay initial value, on a total dry weight basis, was 4470.6cal/g; values for the experimental animals ranged from 4089.1 to 4270.8cal/g. Using ash free data, the initial value was 5192.3cal/g; values for the experimental animals ranged from 4788.2 to 4989.3cal/g. Comparable caloric data for other gastropods are 4497cal/g (total dry weight) for the nudibranch Hermissendra crassicornis (Paine 1965); 4666cal/g for Aplysia dactylomela and 4811 cal/g for A. juliana, both measured on an ash free basis (Carefoot 1970).

Food intake varied over a wide range among the six groups, from a low of 19.8mg (wet weight) in the Logy Bay Ascophyllum group to a high of 1636.2mg in the Bonne Bay Laminaria group; the dry weight equivalents corresponding to these values are 8.1mg and 220.9mg respectively. Highest food intake occurred among the three Bonne Bay groups. The caloric value of the algae ranged from 2200.4cal/g for Laminaria longicruris to 3378.7cal/g for Ascophyllum nodosum.

Table 12A. Bonne Bay Ascophyllum group. Shell length, wet weight, dry weight, caloric content, ash content. (Significance: N. S., not significant, ***, significant at 99.95% level).

	Shell length (mm)	Wet weight (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Original $\bar{X}$	17.2	1839.2	105.2	4521.9	
S	2.3	704.5	64.3	159.4	
$S_{\bar{X}}$	0.4	122.5	6.0	28.2	
Final $\bar{X}$	17.2	1847.2	68.4	4204.5	13.5
S	2.4	691.1	29.1	165.5	
$S_{\bar{x}}$	0.4	120.2	5.5	31.2	
Orig.-final	0	+ 8.0	- 36.8	- 317.4	
t	0.27N.S.	1.25N.S.	4.47***	7.48***	

Table 12B. Bonne Bay *Ascophyllum* group.

Food intake, feces production ($\bar{X}$, mean)

	Wet weight (mg)	Dry weight equiv. (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Food intake $\bar{X}$	50.6	20.6		3378.7	16.4
Feces production $\bar{X}$			10.3	2321.0	39.4

Table 13A. Bonne Bay Fucus group. Shell length, wet weight, dry weight, caloric content, ash content. (Significance: * significant at 98.5% level; ** significant at 99.9% level; *** significant at 99.95% level).

	Shell length (mm)	Wet weight (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Original $\bar{X}$	17.3	1864.7	105.2	4521.9	
S	2.1	622.3	34.3	159.4	
$S_{\bar{X}}$	0.4	110.0	6.0	28.2	
Final $\bar{X}$	17.4	1853.0	80.9	4131.2	14.9
S	2.8	626.0	38.3	263.7	
$S_{\bar{X}}$	0.5	110.6	6.9	47.3	
Orig.-final	+ 0.1	- 11.7	-24.3	- 340.7	
t	4.92***	1.86*	2.65**	7.02***	

Table 13B. Bonne Bay *Fucus* group.

Food intake, feces production ($\bar{X}$, mean)

	Wet weight (mg)	Dry weight equiv. (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Food intake $\bar{X}$	123.3	44.4		3206.6	19.5
Feces production $\bar{X}$			13.1	2326.7	37.0

Table 14A. Bonne Bay Laminaria group. Shell length, wet weight, dry weight, caloric content, ash content. (Significance: N. S., not significant; ** significant at 99.5% level; *** significant at 99.95% level).

	Shell length (mm)	Wet weight (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Original $\bar{X}$	17.1	1842.3	105.2	4521.9	
S	2.7	737.8	34.3	159.4	
$S_{\bar{x}}$	0.5	130.4	6.0	28.2	
Final $\bar{X}$	17.2	1883.4	92.5	4181.2	14.8
S	2.6	753.1	34.2	213.5	
$S_{\bar{x}}$	0.5	129.9	6.1	38.3	
Orig.-final	+ 0.1	+ 41.1	-12.7	-340.7	
t	3.01**	7.15***	1.47N.S.	7.11***	

Table 14B. Bonne Bay *Laminaria* group.

Food intake, feces production. ($\bar{X}$, mean)

	Wet weight (mg)	Dry weight equiv. (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Food intake $\bar{X}$	1636.2	220.9		2200.4	31.7
Feces production $\bar{X}$			37.5	3094.9	20.9

Table 15A. Logy Bay Ascophyllum group. Shell length, wet weight, dry weight, caloric content, ash content. (Significance: N. S., not significant; ** significant at 99.5% level; *** significant at 99.95% level).

	Shell length (mm)	Wet weight (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Original $\bar{X}$	15.8	1553.8	72.7	4470.6	
S	1.7	444.1	31.9	173.8	
$S_{\bar{X}}$	0.3	77.2	5.5	30.7	
Final $\bar{X}$	15.8	1547.2	62.8	4243.6	12.7
S	1.7	445.4	20.6	247.4	
$S_{\bar{X}}$	0.3	77.5	3.6	44.4	
Orig.-final	0	- 6.6	- 9.9	- 227.0	
t	0.23N.S.	3.31**	1.46N.S	4.22***	

Table 15B. Logy Bay *Ascophyllum* group.

Food intake, feces production ($\bar{X}$, mean)

	Wet weight (mg)	Dry weight equiv. (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Food intake $\bar{X}$	19.8	8.1		3378.7	16.4
Feces production $\bar{X}$			3.6	1720.9	

Table 16A. Logy Bay *Fucus* group. Shell length, wet weight, dry weight, caloric content, ash content. (Significance: N. S., not significant; *** significant at 99.95% level).

	Shell length (mm)	Wet weight (mg)	Dry weight (mg)	Caloric content (cal.g)	Ash (% of dry weight)
Original $\bar{X}$	15.9	1639.9	72.7	4470.6	
S	2.3	664.8	31.9	173.8	
$S_{\bar{x}}$	0.4	117.5	5.5	30.7	
Final $\bar{X}$	15.9	1638.6	64.4	4089.1	14.6
S	2.3	673.4	28.6	332.4	
$S_{\bar{x}}$	0.4	119.0	5.1	53.2	
Orig.-final	0[1]	- 1.3	- 8.3	- 381.5	
t	3.40***	.15N.S.	1.08N.S.	5.51***	

1. The t-value of 3.40 was obtained, despite the lack of difference in the means, because the statistical test compared the difference in the shell length of each individual, on a 'before' and 'after' basis.

Table 16B. Logy Bay Fucus group.

Food intake, feces production. ($\bar{X}$, mean)

	Wet weight (mg)	Dry weight equiv. (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Food intake $\bar{X}$	34.2	12.3		2817.1	30.8
Feces production $\bar{X}$			5.6	2160.3	23.1

Table 17A. Logy Bay *Laminaria* group. Shell length, wet weight, dry weight, caloric content, ash content. (Significance: N. S., not significant; *** significant at 99.95% level).

	Shell length (mm)	Wet weight (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Original $\bar{X}$	15.9	1612.7	72.7	4470.6	
S	2.0	597.6	31.9	173.8	
$S_{\bar{x}}$	0.3	103.9	5.5	30.7	
Final $\bar{X}$	15.8	1579.9	70.6	4270.8	
S	2.1	622.2	26.5	222.9	14.4
$S_{\bar{x}}$	0.4	108.2	4.6	38.8	
Orig.-final	- 0.1	- 32.8	- 2.1	- 199.8	
t	0.67N.S.	3.68***	0.32N.S.	4.08***	

Table 17B. Logy Bay Laminaria group.

Food intake, feces production. ($\bar{X}$, mean)

	Wet weight (mg)	Dry weight equiv. (mg)	Dry weight (mg)	Caloric content (cal/g)	Ash (% of dry weight)
Food intake $\bar{X}$	143.4	18.8		3287.3	18.5
Feces production $\bar{X}$			13.9	2375.6	17.9

Table 18. Bonne Bay Ascophyllum, Fucus, and Laminaria groups.

Summary of caloric and ash contents of snails, algae, and feces.

	Ascophyllum	Fucus	Laminaria
Snails			
Original cal/g	4521.9	4521.9	4521.9
ash%	14.4	14.4	14.4
cal/g, ash free	5282.6	5282.6	5282.6
Final cal/g	4204.5	4131.2	4181.2
ash%	13.5	14.9	14.8
cal/g, ash free	4860.7	4854.5	4907.5
Total calories in group	287.8	334.0	386.7
Algae			
cal/g	3378.7	3206.6	2200.4
ash%	16.4	19.5	31.7
cal/g, ash free	4041.5	3983.4	3221.7
Total calories ingested	69.5	142.2	711.7
Feces			
cal/g	2321.0	2326.7	3094.9
ash%	39.4	37.0	20.9
cal/g, ash free	3830.0	3693.2	3913.6
Total calories egested	23.7	30.7	116.2

Table 19. Logy Bay *Ascophyllum*, *Fucus*, and *Laminaria* groups.
Summary of caloric and ash contents of snails, algae, and feces.

	Ascophyllum	*Fucus*	*Laminaria*
Snails			
Original cal/g	4470.6	4470.6	4470.6
ash%	13.9	13.9	13.9
cal/g, ash free	5192.3	5192.3	5192.3
Final cal/g	4243.6	4089.1	4270.8
ash%	12.7	14.6	14.8
cal/g, ash free	4860.9	4788.2	4989.3
Total calories in group	266.4	263.4	301.4
Algae			
cal/g	3378.7	2817.1	3287.3
ash%	16.4	30.8	18.5
cal/g, ash free	4041.5	4071.0	4033.5
Total calories ingested	27.1	34.6	61.7
Feces			
cal/g	1720.9	2160.3	2375.6
ash%		23.1	17.9
cal/g, ash free	2294.6[1]	2809.2	2893.5
Total calories egested	6.2[1]	12.1	33.0

1. See text

Ash content of the algal dry matter varied between 16.4% for Ascophyllum nodosum and 31.7% for Laminaria longicruris.

Feces production by the experimental animals was at a low of 3.6mg (dry weight) in the Logy Bay Ascophyllum group and a high of 37.5mg in the Bonne Bay Laminaria group. Ash content of the feces, expressed as a percentage of dry weight, was lowest in the Logy Bay Laminaria group, 17.9; and highest in the Bonne Bay Ascophyllum group, 39.4.

DISCUSSION

I. Shore Group

Because of the extremely different conditions to which the shore group snails were exposed, compared to the conditions of the experimental animals, no direct (i.e. statistical) comparisons were made between the shore group and any experimental group. Moreover, data obtained for the shore group are not as extensive as those for the experimental groups; ash content was not measured, and, obviously, food intake and feces production could not be determined. Nevertheless, although only 15 snails were recovered alive out of a total of 200 released (and two of the 15 could not be identified), data from the recovered snails were statistically compared with corresponding data from the initial (control) group. In some respects, the results from the shore group are sufficiently different from

those of the experimental groups that they merit some comment.

When the shore snails were recovered, it was immediately evident that growth, in terms of shell length, had occurred; in almost all cases, the band of paint originally applied to the lip was now some distance behind the lip. In a few cases, the distance was several millimeters. This shell growth occurred despite the many factors at work in the natural habitat of Littorina littorea which tend to erode the shell (thus affecting shell length measurements), especially through blunting of the apex and chipping of the edge of the shell.

It may be that significant erosion of the shell would not occur in the relatively short period of six weeks, especially at the time of the year (August, early September) when erosional factors are probably least effective. In any case, the shore group did show an increase in the mean shell length of 0.3mm, 0.2mm more than the increase in any laboratory group under conditions entirely free of erosional factors. Using the one-tail t-test of the difference between final and initial shell lengths of the recovered snails, the value of 2.05 was found to be significant at the 98.5% level. The 0.3mm increase corresponds well with Moore's (1936) average monthly shell growth rate of 0.3 to 0.4mm for natural populations of L. littorea with mean shell length 17mm.

Shell growth is only one measure of growth in snails, though some workers use only this characteristic as an indicator of growth (Williams, 1964; Fish, 1972). An increase in tissue weight to correspond with shell growth is expected, and Moore (1936) clearly demonstrated a close relationship between shell length and dry weight of tissues. In the present study, there was a significant (at 99.95%) increase in the wet weight of the recovered snails. The mean increase of 127.1mg was more than three times the greatest increase recorded among the experimental animals.

In contrast to shell length and wet weight, the dry weight of soft tissues does not appear to have increased. A comparison of the shore group dry weight and the initial group dry weight reveals the following. The initial (control) group mean dry weight was 105.2mg. That of the shore group, at the end of the experiment, was 78.2mg, considerably less. However, the statistical test yields a t-value of 1.98, indicating no significant difference between the two dry weights.

The lower dry weight of the shore group snails is probably related to the sizes of the snails recovered. In the initial group, 27% of the animals fell into the 10-15mm size category; of the recovered shore snails, 46% were in that class. Also, while 67% of the initial group were in the 16-20mm size class, only 38% of the recovered shore

snails were in this category. Therefore, the lower dry weights are likely related to the larger proportion of smaller-sized snails in the recovered group, as smaller snails have a smaller quantity of dry matter.

Consideration of another factor offers supporting evidence that there is no difference between the dry weights. If the ratio of dry weight to wet weight is calculated for the initial group, the result is 0.0516, or 5.2%. If the same ratio is calculated for the shore group, using the final wet weight, the value is 0.0393, or 3.9%. Thus, the shore group, with the smaller wet weight (1989,9mg), also has the smaller proportion of dry weight (3.9%). If the dry weight/wet weight ratio were constant, two alternatives are evident. One is that the initial group ratio would be 3.9%, the same as the shore group. In this case, the dry weight of the initial group would be 80.0mg (2034.9 x 3.9%), which is very close to the 78.3mg dry weight of the shore group. The other alternative is that the shore group ratio would be 5.2%, the same as that of the initial group. In this case, the dry weight of the shore group would be 102.7mg (1989.9 x 5.2%), corresponding well with the 105.2mg dry weight of the initial group. Reasoning in this manner confirms the results of the statistical test.

The increase in wet weight was only 7% of the original wet weight of the shore group. Because of the small

dry weight/wet weight ratio, the increase in dry matter was very small indeed. Since the weight of the shell is fairly constant at about 70% of the total wet weight, the increase of 127.1mg contains approximately 90mg of additional shell weight. The remaining 37mg contains 3.9%, or approximately 1.4mg, of dry matter.

Experimental error may contribute to some extent to the apparent lack of increase in dry weight. While precautions were taken to maintain uniformity in all procedures, it is very likely that varying proportions of water were present in the mantle cavities of individuals during weighing. However, while the dry weight/wet weight ratios of the recovered shore snails ranged from 2.0% to 5.0%, eight of the snails had such ratios between 4.0 and 4.9%. These fairly consistent ratios seem to indicate that error from this source would be small, and not likely to indicate a complete lack of increase in dry matter.

Perhaps the real reason for such a lack may be found in the natural history of the animals. In a study of L. littorea in Wales, Williams (1964) found that sexual maturity was reached when the snails were 11 or 12mm in shell length. Fish (1972) confirmed this finding in his study of the breeding of the winkle in estuaries and on the open coast. In the present study, of the 15 snails recovered from the shore, only one was as small as 11.1mm when first

released. The remaining snails were all larger than 13.0mm. At the end of the experiment, the smallest snail had a shell length of 12.2mm. Since the mean shell length of the recovered snails was 17.2mm, it is reasonable to assume that most of these snails, if not all, were sexually mature. Williams (1964) correlated sexual activity with seasonal changes in temperature. He found that maturity is reached during winter, when water temperatures are lowest; spawning follows, reaching a maximum when sea temperature begins to rise.

Available information indicates that L. littorea spawns at various times throughout the year, generally between January and September (Moore, 1936; Williams, 1964; Fish, 1972). The closest location to Bonne Bay for which information is available is St. Andrews, New Brunswick. There, according to Hayes (1929), spawning occurs from April to July. At Bonne Bay, it is not uncommon for winter ice to persist until late April or early May; therefore, sea temperatures do not begin to rise until about that time. Assuming that the spawning period for L. littorea is as long at Bonne Bay as it is at St. Andrews, and considering the delay in the warming of the water, it is likely that spawning occurs there from May to August. While evidence of spawning was not sought as part of this study, it is very probable that the snails in the shore group were mature individuals which spawned

during the period under consideration. Yonge (1966) found that growth in L. littorea was depressed during spawning, presumably because the food reserves were utilized in the production of gametes. It is suggested that in the case of the shore snails, the increase in shell size indicates a period of tissue growth (hence an increase in dry matter), when food was being stored. The subsequent spawning once again reduced the amount of tissues in the snails, so that, despite the increase in shell length, the amount of dry matter appeared to remain constant.

II. Bonne Bay experiments

(a) Ascophyllum group

Among the Ascophyllum group snails, there was no change in shell length, and the slight increase of 8.0mg in wet weight is not significant. Judging from these criteria, there was no growth in the Ascophyllum snails during the experiment. Dry weight was lower at the end of the period. Whereas the dry weight of the initial group was 105.2mg, the final dry weight was 68.4mg. The difference, 36.8mg, is significant at the 99.9% level.

Corresponding with this loss of dry weight is a decrease in caloric content. The mean caloric content of the initial group was 4521.9cal/g; that of the Ascophyllum

group was 4204.5; the difference, 317.4cal/g, is significant at the 99.95% level.

Ash content was not determined for the initial group. Therefore, the average ash content of the three experimental groups was used as the assumed ash value for the initial group. The relevant values are: Ascophyllum group, 13.5%; Fucus group, 14.9%; Laminaria group, 14.8%; mean, 14.4%. This gives a caloric value for the initial group of 5282.6cal/ash free gram.

The Ascophyllum group, with ash content 13.5%, had a mean caloric value equivalent of 4860.7cal/ash free gram. If the caloric content of the Ascophyllum group were proportional to that of the initial group, the value would be 312.7 calories, not 287.8. This lower value supports the result of the t-test, that the difference in values is significant. The loss in dry weight, combined with the loss of caloric content, appears to indicate that the Ascophyllum group experienced negative growth during the experiment.

The Ascophyllum snails ate 50.6mg (wet weight) of Ascophyllum nodosum; the dry weight equivalent is 20.6mg. Since the ash content was 16.4%, an ash free dry weight of 17.2mg was ingested. The caloric value of A. nodosum was 3378.7cal/g, or 4041.5cal/ash free gram. Therefore, the 17.2mg of A. nodosum contained a total of 69.5 calories, the amount of energy ingested by the snails.

Feces production was 10.3mg of dry matter. This is equivalent to 6.2mg, ash free, since the ash content was 39.4%. The caloric content of the feces was 2321.0 cal/g, or 3830.0cal/ash free gram. Thus, 23.7 calories were eliminated in 6.2mg of feces.

If the caloric value of the ingested food is added to the value for the initial group, a total of 545.5 calories is obtained; subtracting the 23.7 calories lost in feces leaves a net total of 521.8 calories. But the final value for the Ascophyllum group was 287.8 calories, a difference of 234 calories. Undoubtedly, metabolism accounts for part of the difference, but the conclusion seems to confirm negative growth for the group.

Oxygen consumption was not measured during these experiments, but by using data provided by other workers, some estimate may be obtained of the energy used in respiration by the experimental animals. Newell and Pye (1970a), investigating seasonal changes in the effect of temperature on oxygen consumption by L. littorea, present a standard rate of oxygen consumption at 15°C (the average temperature during the Bonne Bay experiments) of 0.3μl O_2/mg dry tissue per hour. Assuming a duration of 1008 hours (42 days) for the experiment, and using the average of the initial dry weight and the final dry weight as the weight of dry tissue, a volume of 26.2ml of O_2 is obtained, based on the standard rate. Sutherland (1972) gives an oxy-caloric

equivalent of 4.8cal/ml O_2; Hughes (1971) and Paine (1971a) give values of 4.83 and 4.86cal/ml O_2, respectively. Using the somewhat arbitrary value of 4.85 cal/ml O_2, a value of 127.1 calories is obtained from the 26.2ml. It must be noted that this is an estimate only, probably low, since the minimum rate of oxygen consumption was used in making the calculations. However, it does indicate that of the 234 calories in question, at least half were consumed in metabolism

(b) Fucus group

Shell length in this group increased by 0.1mm, a difference which tested significant at the 99.95% level. This indicates that this group may have fared better, generally, under laboratory conditions that did the Ascophyllum group. There was, however, a slight loss in wet weight of 11.7mg, not a significant loss; and a loss of 24.3mg dry weight, significant at 98.5%.

The caloric value of the Fucus group snails was 4131.2cal/g, which, with ash content 14.9%, is equivalent to 4854.5 cal/ash free gram. The dry weight of the snails, 80.9mg, is equivalent to 68.8mg ash free, with a caloric content of 334.0 calories. Thus, with the initial group value of 476.0 calories, there is a difference of 142 calories. If the caloric content of the Fucus group were

proportional to the value of the initial group, the 68.8 mg of dry matter would contain 427.4 calories, compared to the actual value of 334.00 calories. This lower value corresponds with the results of the t-test. With an initial value of 4521.9cal/g, and a final (i.e. Fucus group) value of 4131.2cal/g, the 390.7cal/g difference is significant at the 99.95% level.

The Fucus snails ingested a wet weight of 123.3mg of Fucus vesiculosus, having a dry weight equivalent of 44.4 mg; with 19.5% ash, that is equal to 35.7mg ash free alga. The caloric content of F. vesiculosus was 3206.6cal/g, or 3983.4cal/ash free gram. Thus, the snails ingested 142.2 calories in the 35.7mg of alga.

Feces production for the group was 13.1mg, dry weight, equivalent to 8.3mg ash free (37.0% ash). The feces had a caloric content of 2326.7cal/g, or 3683.2cal/ash free gram; thus, 30.7 calories were lost in feces.

Adding the number of ingested calories to the initial value, and deducting the calories lost in feces, gives a value of 587.5 calories. But the final value for the Fucus group is 334.0 calories, a difference of 253.5 calories. Assuming a volume of 28.2ml of oxygen used (a conservative estimate based on 93.1mg of dry tissue), 136.7 calories would have been consumed in respiration. This leaves approximately 117 calories unaccounted for; considered along with the decrease in dry weight, this loss of caloric

value seems to indicate negative growth for the Fucus group.

(c) Laminaria group

Like the Fucus group, the Laminaria group showed an increase of 0.1mm in shell length; the addition was significant at the 99.5% level. There was also a significant (at 99.95%) increase of 41.1mg in wet weight. There was a decrease in dry weight, but the 12.7mg difference was the smallest loss among the three experimental groups, and was not significant.

The caloric value of the Laminaria group was 4181.2 cal/g, 340.7cal/g lower than the initial group value (4521.9 cal/g); the difference is significant at 99.95%. The Laminaria group snails had an ash content of 14.8%; the caloric value was, therefore, equivalent to 4907.5cal/ash free gram. The 92.5mg of dry matter, converted to ash free dry weight, is equivalent to 78.8mg, and contains 386.7 calories.

Food intake was highest in the Laminaria group: 1636.2mg (wet weight) of L. longicruris was ingested, having a dry weight equivalent of 220.9mg. The caloric value was 2200.4cal/g, or 3221.7cal/ash free gram (ash content 31.79%). Thus, a total of 711.7 calories was ingested by the snails.

Feces production was also highest in this group, with 37.5mg dry feces being produced. With 20.9% ash, this is equivalent to 29.7mg ash free. The feces had a caloric value of 3094.9cal/g, or 3912.6cal/ash free gram; thus, 116.2 calories were lost in feces.

Combining the original (initial group) value of 476.0 calories with the 711.7 calories ingested gives a total of 1187.7 calories; deducting the 116.2 calories lost in feces leaves a net total of 1071.5 calories. This represents an increase of 595.5 calories (about 125%) over the initial value. Assuming an oxygen consumption of 29.9 ml, having an oxy-caloric value of 145.0 calories, there is still a surplus of 450 calories, making this the only group showing such a gain.

III. Logy Bay experiments

(a) Ascophyllum group

Shell length of the Ascophyllum group did not change; there was a slight, but significant (at 99.5%) decrease of 6.6mg in wet weight, and a small, non-significant decrease of 9.9mg in dry weight.

A decrease of 227.0cal/g occurred, significant at 99.95%, as the initial value was 4470.6cal/g, and that of the Ascophyllum group was 4243.6cal/g. An assumed ash content of 13.9%, the average of the three experimental

groups (Ascophyllum 12.7%, Fucus 14.6%, Laminaria 14.4%) was applied to the initial group, giving it an ash free value of 5192.3cal/g; the corresponding value for the Ascophyllum group is 4860.9, based on 12.7% ash.

The dry weight of the initial group snails was 72.7mg, or 62.6mg ash free. The total caloric value was 325.0 calories in 62.6mg dry matter. For the Ascophyllum group, the value was 266.4 calories in 54.8mg ash free dry matter, based on a total dry weight of 62.8mg having ash content 12.7%.

The Ascophyllum snails ate 19.8mg (wet weight) of A. nodosum, having a dry weight equivalent of 8.1mg. With ash content 16.4%, this is equal to 6.7mg, ash free. The Ascophyllum was found to have a caloric value of 3378.7 cal/g, or 4041.5cal/ash free gram. Thus, a total of 27.1 calories were ingested in 6.7mg of algal dry matter.

Because a very small amount of feces was produced by this group (3.6mg dry), it was not possible to determine the ash content. However, for purposes of discussion, an arbitrary value of 25% has been assumed. The ash contents of the Bonne Bay feces were 39.4%, 37.0%, and 20.9% respectively, for the Ascophyllum, Fucus, and Laminaria groups. At Logy Bay, the corresponding values are (blank), 23.1% and 17.9%. Thus, 25% seems to be a reasonable, if subjective, estimate. The caloric value of the feces was 1720.9cal/g, equivalent to an ash free value of 2294.6cal/g (assumed). The

total (assumed) caloric content of the feces is 6.2 calories ingested by the Ascophyllum group, and subtracting the fecal calories (6.2), gives a net value of 345.9 calories. The final value for the Ascophyllum group was 266.4 calories, about 80 calories less. If the caloric value of the Ascophyllum group were proportional to that of the initial group, the 54.8mg of dry matter should contain 284.5 calories, not 266.4.

Using information from Newell and Pye (1970a), the standard rate of oxygen consumption at 7.7°C (the average temperature during the Logy Bay experiments) is 0.2 l O_2/mg dry tissue per hour. On the basis of 67.7mg dry weight (the average of the initial and Ascophyllum groups), the group utilized 13.6ml oxygen, having an oxy-caloric equivalent of 66.0 calories. Since this is a conservative estimate, it appears that the Ascophyllum group used all its available energy in respiration; that is, the snails merely maintained themselves.

(b) Fucus group

A glance at the tabulated data for this group (Table 16A, p. 42) reveals an apparent contradiction in the shell length values. While the three tabulated statistics are identical in both the Fucus group and the initial group, the t-test indicates a difference significant at the 99.95%

level. The test used in this instance compared the 'before' and 'after' measurements of shell length for each individual in the group. While some individuals showed an increase, others showed a decrease; the t-value indicates that significant differences occur within the same individuals. It is obvious, however, that the mean value for the group did not change, and the assumption here is that shell length did not change.

There was a slight, insignificant decrease of 1.3 mg in wet weight, and an insignificant decrease of 8.3mg in dry weight. While the caloric value of the initial group was 4470.6cal/g, that of the Fucus group was 4089.1cal/g; the difference, 381.5cal/g, is significant at 99.95%.

Ash content of the group was 14.6%. Therefore, the ash free caloric value was 4788.2cal/ash free gram. There were 263.4 calories in 55.0mg dry weight of the snails, based on a total dry weight of 64.4mg.

A wet weight of 34.2mg of Fucus vesiculosus was eaten, having a dry weight equivalent of 12.3mg. Since the ash content was 30.8%, the 12.3mg had an ash free equivalent of 8.5mg. With caloric value 2817.1cal/g, or 4071.0cal/g ash free gram, there were 34.6 calories in the 12.3mg ash free of alga taken in.

Feces produced by the group amounted to 5.6mg of dry matter, having an ash content of 23.1% and caloric content of 2160.3 cal/g. Corresponding values, on an ash

free basis, are 4.3mg dry matter with 2809.2cal/ash free gram; a total of 12.1 calories were lost in feces.

Combining the caloric value of the initial group with the results obtained above (325.0 + 34.6 - 12.1 calories), a net value of 347.5 calories is obtained. The actual value for the Fucus group is 263.4 calories, or about 80 calories less. Assuming a dry weight of 68.5mg for the group, 13.8 ml of oxygen consumed would have an oxy-caloric equivalent of 66.9 calories. As with the estimates for the other experimental groups, this is a minimal value. It seems then, that the Fucus group, like the Ascophyllum group, used all its energy simply to maintain itself; there was no growth in the group.

(c) Laminaria group

The Laminaria group was the only Logy Bay group showing a change in shell length; specifically, this was an insignificant decrease of 0.1mm. Thre was a decrease of 32.8mg in wet weight, significant at the 99.9% level, and an insignificant decrease of 2.1mg in dry weight.

The caloric value of the group was 4270.8cal/g, 199.8cal/g less than the value for the initial group, and a difference significant at the 99.95% level. With an ash content 14.4%, the Laminaria group snails had an ash free caloric value of 4989.3cal/g. The total dry matter for the

animals was 70.6mg, so the net value was 301.4 calories in 60.4mg of ash free dry matter.

Like the Bonne Bay Laminaria group, the Logy Bay Laminaria snails ingested the largest amount of food among the three groups. A wet weight total of 143.4mg of L. digitata was eaten, having a dry weight equivalent of 18.8 mg, or 15.3mg ash free (18.5% ash). The caloric value of the alga was 3287.3cal/g, or 4033.5cal/ash free gram. Thus, 61.7 calories were ingested in 15.3mg of algal dry matter.

A dry weight total of 13.9mg of feces was produced, with an ash content of 17.9% and an ash free equivalent of 114.mg. The caloric value of the feces was 2375.6cal/g, or 2893.5cal/ash free gram. A total of 33.0 calories were lost in feces.

With an original caloric content of 325.0 calories, an intake of 61.7 calories, and a loss of 33.0 calories, the Laminaria group came through the experiment with a gain of just under 29 calories. Assuming a dry weight of 71.6 mg for the group, the 14.4ml of oxygen used would have an oxy-caloric equivalent of 69.8 calories. It thus appears that the Laminaria group may have actually gained a little, at least in terms of energy content.

IV. Bonne Bay and Logy Bay Experiments

(a) Shell length

Little can be concluded concerning the outcome of the experiments from an examination of the shell lengths of the animals. Of the six groups, two showed an increase of 0.1mm, one showed a decrease of 0.1mm, and three remained unchanged. The only consistent point is that in both series of experiments, it was the Ascophyllum group which remained unchanged. The only groups which gave the expected results (namely an increase in shell length) were the Bonne Bay Fucus and Laminaria groups. On the basis of shell length alone, then, the conclusion seems to be that the two Ascophyllum groups, with the Logy Bay Fucus group, took in and used sufficient energy to maintain themselves in a state of equilibrium; the Logy Bay Laminaria group did not do so, and therefore "lost"; the Bonne Bay Fucus and Laminaria groups took in and utilized a surplus of energy, and thereby "gained" by the addition of shell material. Perhaps the correct conclusion, considering the shell length data in their entirety, is that there was no change in shell length.

Examination of the shell length measurements on an individual basis shows that, of the 'after' (or final) measurements, some were lower, some were unchanged, and some were higher, compared with the original measurements. Within a given group, if the difference between the lowest

and the highest is considered to constitute a range, the ranges vary form 0.5mm (-0.3mm to +0.2mm) for the Logy Bay Fucus group to 1.6mm (-0.5mm to +1.1mm) for the Bonne Bay Ascophyllum group. The majority of the measurements either did not change, or changed by only 0.1mm; with a maximum range of only 1.6mm, and with shell length being measured from apex to some 'most distant' point on the lip, it is not difficult to see how experimental error may have influenced the results, despite care being taken in measuring.

Perhaps a more useful alternative would have been to ignore shell length as such, and to use 'addition to shell,' or 'shell growth index,' or some other such term, instead. In this case, since all snails had a line of paint applied to the edge of the shell at the beginning of the experiment, the initial measurement would be zero. Final measurements would be obtained by simply measuring the perpendicular distance from edge of paint to edge of shell (the lip). This should eliminate negative results, as the values would be either zero (nothing added to edge of the shell) or plus some amount. Judging from the shore snails, such a method could provide an exaggerated measure of shell length; the degree of exaggeration could probably be determined, and from this, the actual change in shell length could be calculated.

That an increase in shell length was expected, at least in the Bonne Bay snails, is justified by a consideration

of the shore snails, which exhibited substantial shell growth during the same period. Nicol (1967) points out that shell growth in Littorina littorea is accelerated during spring and summer, and that, while shell formation does occur all year, it is slowed in winter. The Bonne Bay groups were subjected to a temperature range of 12.6 to 16°C as measured in the wet bench (see p. 16); the temperature of the incoming water was about the same, 12.8 to 16.3°C (Table 3). The Logy Bay snails, however, experienced a range of 5.5 to 12.4°C, but the temperature of the incoming water was considerably lower, 2.8 to 6.3°C (Table 4). Thus, the Logy Bay snails were in winter temperatures, and a slowing or cessation of growth is not unexpected.

Moore (1936) and Fish (1972), studying growth in natural populations of L. littorea, presented data indicating a decrease in shell length occurring about November and December; Fish had one population in which the decrease took place between September and November. Both these workers, however, were periodically sampling large natural populations, not necessarily measuring the same individuals on each occasion. Nevertheless, there does appear to be a definite downward trend with the onset of winter. Therefore, it does not seem too unreasonable to assume the laboratory animals were reflecting a natural phenomenon.

Newell (1958), studying the crawling rate of L. littorea at different temperatures, concluded that between

6°C and 8°C, winkles become inactive; thus, they are inactive during most of the winter. Inactivity, of course, implies a lack of feeding. In this regard it is interesting to speculate about possible absorption of the shell, especially in view of Paine's (1971a) considerations of the energy content of shells. The amount of energy involved, however, is very small, and as Fretter and Graham (1962) point out, no part of the shell of the periwinkle is lost during its lifetime. Presumably, this observation precludes shell absorption.

(b) Wet weight

Results obtained from measuring wet weights are inconclusive; except for the decreases in both Fucus groups, there are no clearly-defined patterns among the six groups. Among the Bonne Bay snails, there was no change in the wet weights of the Ascophyllum and Fucus groups, but the Laminaria group showed a significant increase. Among the Logy Bay snails, significant decreases occurred in the Ascophyllum and Laminaria groups, but there was no change in the Fucus group. The only correspondence between the two series of experiments was in the Fucus groups, both of which showed insignificant decreases in wet weight.

If shell lengths are considered with wet weights, the results complement each other, and are generally more consistent. Thus, the Bonne Bay Ascophyllum and Fucus groups,

and the Logy Bay Fucus group, all with no change in shell length, had no change in wet weight. The Logy Bay Laminaria group, with an insignificant decrease in shell length, had a significant decrease in wet weight. The Bonne Bay Laminaria group had a significant increase in both measurements. Somewhat inconsistent is the Logy Bay Ascophyllum group, with no change in shell length, but with a significant decrease in wet weight.

(c) Dry weight

A decrease in dry weight was observed in all six experimental groups. Insignificant in four groups, the loss was significant at the 99.9% level in the Bonne Bay Ascophyllum group, and at the 98% level in the Bonne Bay Fucus group. A summary of the relevant data is presented in Table 18, which illustrates several features of the data.

The dry weight results are consistent in several respects. In both series of experiments, it is the Ascophyllum group which showed the greatest loss in dry weight, and the Laminaria group which showed the least. This maintains whether the total dry weights or the ash free dry weights are considered.

Another obvious feature of the data is that the Logy Bay results are consistently lower than the Bonne Bay results. Not only are the values smaller on an individual group basis, but the ranges among the groups are smaller.

Among the Bonne Bay groups, the differences range from 12.7 to 36.8mg, a range of 24.1mg. Among the Logy Bay groups, the range from 2.1 to 9.9mg is only 7.8mg. Similarly, using the ash free dry weights, the range is 19.6mg for the Bonne Bay snails, but only 5.6mg for the Logy Bay snails.

If the difference between initial and final dry weights is expressed as a percentage of the initial value, the results show a similar pattern. For the Bonne Bay snails, the values are 34.9, 23.0, and 12.0% for the Ascophyllum, Fucus, and Laminaria groups, respectively. On an ash free basis, the corresponding values are 34.2, 23.6, and 12.5%. For the Logy Bay Ascophyllum, Fucus, and Laminaria groups, the total dry weight values are 13.6, 11.4, and 2.8%, respectively; the corresponding ash free values are 12.4, 12.1, and 3.5%.

While all groups lost dry weight, the loss is more evident among the Bonne Bay snails. This is probably a result of the greater activity of these snails in a higher temperature regime. Conversely, the low values for the Logy Bay snails may be another indication of their lack of activity at lower temperatures.

There also appears to be an inverse relationship between the quantity of dry matter ingested as algae, and the decrease in dry weight. The Bonne Bay snails, with the greater dry weight losses, ingested the greater amount of dry

Table 20. Six experimental groups. Dry weight data.
(BB, Bonne Bay; LB, Logy Bay; Asc, Ascophyllum;
Fuc, Fucus; Lam, Laminaria)

		Total dry weight (mg)		
		Initial	Final	Initial-final
BB	Asc	105.2	68.4	36.8
	Fuc	105.2	80.9	24.3
	Lam	105.2	92.5	12.7
LB	Asc	72.7	62.8	9.9
	Fuc	72.7	64.4	8.3
	Lam	72.7	70.6	2.1

		Ash free dry weight (mg)		
		Initial	Final	Initial-final
BB	Asc	90.1	59.2	30.9
	Fuc	90.1	68.8	21.3
	Lam	90.1	78.8	11.3
LB	Asc	62.6	54.8	7.8
	Fuc	62.6	55.0	7.6
	Lam	62.6	60.4	2.2

matter. The Logy Bay snails ingested the smaller amount of dry matter, and lost less dry weight. More specifically, in both series of experiments, the Ascophyllum snails ingested least dry matter, but lost most dry weight. On the other hand, both Laminaria groups ingested most dry matter, and showed the smallest loss in dry weight.

Carefoot (1967b) combined total dry matter ingested and lost as feces to calculate the percentage of food absorption: $\frac{\text{food consumed - feces}}{\text{food consumed}} \times 100$. For the Bonne Bay groups, these efficiencies are 50%, 70%, and 83% for the Ascophyllum, Fucus and Laminaria groups, respectively. The corresponding values for the Logy Bay groups are 81%, 54%, and 26%. Note that the order of efficiencies is reversed in the Logy Bay snails. That is, the Ascophyllum group has the highest efficiency among the Logy Bay snails, but the lowest among the Bonne Bay snails. Conversely, the Laminaria group has the highest efficiency among the Bonne Bay snails, but the lowest among the Logy Bay snails.

Carefoot's (1967b) results, based on a study of the sublittoral gastropod, Aplysia punctata, range from 45 to 71%. The range of values in the present study is considerably broader. Paine (1971a), referring to 'assimilation efficiencies' based on caloric value rather than dry weight, found a value of 70% for the herbivorous intertidal gastropod, Tegula funebralis, and indicated that "higher values are not

uncommon in the literature."

Differences in the efficiencies may be related to factors in the algae used in the experiments. Thus, the discrepancy between the two Laminaria values is probably due to factors in the two species. The different values for Fucus may be a result of environmental factors, since the alga was collected locally in each case. More difficult to explain is the 31% difference in the Ascophyllum groups, since not only was the same species used, but in both series of experiments, the Ascophyllum was collected at Bonne Bay. Possibly, seasonal variation of that magnitude can occur, though it does not seem likely.

Carefoot (1970), feeding two species of Aplysia on a wide variety of marine algae, observed loss of dry weight in both species when fed certain species of algae. He considered these algae to be "of less experimental value" than the others, and ceased using them in the experiments. In the present study, the dry weight results seem to indicate that the three species of algae used were unsuitable as food for Littorina littorea. It is interesting, perhaps, to speculate on the outcome were the experiments carried out over a much longer time. It seems likely the animals would slowly starve to death, despite an abundance of food.

(d) Caloric content

Date on calorimetry for marine benthic algae are provided by Paine and Vadas (1969). For Fucus distichus, they give values of 3.43kcal/g dry weight, 26% ash, and 4.64kcal/g ash free dry weight. Values given for Laminaria complanata are 2.65kcal/g dry weight, 39% ash, and 4.34 kcal/g ash free dry weight. For L. saccharina, the corresponding values are 2.84kcal/g, 35% ash, 4.37kcal/g; for L. setchelii, they are 3.28kcal/g, 26% ash, and 4.43 kcal/g ash free dry weight.

Values obtained in the present study are generally lower than these published data, in part because of different ash contents. Published data were not provided for Ascophyllum nodosum. However, it is one of the Fucales, as is Fucus distichus, to which it can be compared for discussion purposes. If the total dry weight caloric value of A. nodosum were converted to an ash free value, using the (published) ash content of F. distichus (26%), the result would be 4565.8cal/g, which is fairly close to 4.64kcal/g, the value for F. distichus.

Caloric values in the present study for both species of Laminaria are considerably lower than the data given above for three other species of Laminaria. However, by using the ash contents given for the three species to convert the present data to cal/ash free gram, a range of values is

obtained (2973.5 - 5389.0cal/g) which includes the published values.

Using the same procedure on Fucus vesiculosus still yields lower caloric values than those given for F. distichus, suggesting that other factors besides ash content are affecting the results. Paine and Vadas (1969) point out that there is some seasonal variation in caloric content of marine algae, and in some species, geographical variation of 7 - 14%. There is also that factor which Paine (1971b) calls "inate organismic variability." He cites the work on the bug Philaenus spumarius by Wiegert (1968), who found the ash free caloric values to be 6503cal/g for eggs, and 4976cal/g for first instar larvae. Wiegert also indicated that differences greater than 500 calories/g are "associated with sexual or nutritional differences or changes in state or season." The implication seems to be that differences as high as 500 calories are not necessarily related to any of these factors, nor are they necessarily abnormal.

Caloric values for feces had a range of about 700 calories/g dry weight for the Bonne Bay snails and about 650cal/g dry weight for the Logy Bay snails. Also, the Logy Bay values were lower than the Bonne Bay values by about 600 cal/g. Using ash free dry weights, the Bonne Bay range of values fell to about 320cal/g; at Logy Bay, the two known values differed by about 85cal/g.

Gross weight efficiencies for each group were considered in discussing dry weights. The same formula, $\frac{I - E}{I} \times 100$, may be used, substituting appropriate caloric values for the dry weights, to calculate the assimilation efficiencies of the experimental animals (Paine 1971a). Using ingested and egested calories/ash free gram, the assimilation effitiencies for the Bonne Bay snails are: Ascophyllum group, 65.8%; Fucus group, 78.4%; Laminaria group 83.6%. For the Logy Bay snails, the corresponding values are 77.1%, 65.0%, and 46.5%. The assimilation efficiencies followed the same pattern as the gross weight efficiencies, in that among the Bonne Bay groups, the value was lowest in the Ascophyllum group and highest in the Laminaria group, whereas among the Logy Bay animals, the value was highest in the Ascophyllum group and lowest in the Laminaria group. The values obtained in the present study, ranging from 46.5% to 83.6%, are comparable to a range of 50 to 70% for Navanax inermis (Paine 1965), and to Paine's (1971a) value of 70% for Tegula funebralis. Odum and Smalley (1959) calculated a value of 45% for Littorina irrorata.

The Logy Bay Ascophyllum group was the only one with a gross weight efficiency higher than assimilation efficiency. Since the gross weight efficiency is based on total dry weight, it is suggested that the higher value is related to ash contents of algae and feces. The fecal ash

content for this group was assumed to be 25%, the highest among the Logy Bay groups. At the same time, the ash content of the alga was 16.4%, the lowest value among all the algae.

The Bonne Bay Laminaria group had an assimilation efficiency of 83.6%, which almost coincides with the gross weight efficiency of 83%. In this case, the alga had an ash content of 31.7%, the highest of all the algae, while the fecal ash content was 20.9%, one of the lowest values. There appears to be an inverse relationship between the ash content and the assimilation efficiency.

The high value for the Bonne Bay Laminaria group may explain the fact that this group was the only one with a surplus of caloric content at the end of the experiment. This surplus resulted despite the low caloric value of L. longicruris, 2200.4cal/g, the lowest of all the algae.

The Bonne Bay Laminaria group had a final dry weight of 92.5mg; the snails ate 220.9mg of dry algal matter, or 238.8% of their final dry weight. The highest proportion ingested by a remaining group was 54.8% for the Bonne Bay Fucus group. The large quantity of alga ingested probably compensated, in part at least, for the low caloric content of the alga. The quantity of food ingested is evidently due to characteristics of the alga, and 'hardness' is likely the chief characteristic. Compared to Laminaria digitata, which

is tough and leathery, and resistant to damage, L. longicruris is soft and easily damaged. Thus, it is easier for a rasping organism like L. littorea to obtain a meal from L. longicruris. The combination of high temperatures (i.e. the summer temperatures prevailing at Bonne Bay) and 'soft' and abundant food was apparently a suitable one to permit the Bonne Bay Laminaria snails, and only these, to gain in terms of caloric content under experimental conditions.

V. Summary and Conclusions

Of six groups of snails fed on experimental diets of macrophytes, only the Bonne Bay Laminaria group showed any indication of growth at the end of the experiment. On the basis of the data obtained, it is obvious that, generally, there was no growth of the experimental animals. Considering all characteristics measured, there was either no change, or a decrease in a given measurement.

Lack of growth in the snails may have been due to one, or a combination, of many factors. The snails were considered mature, and growth is slower in mature snails. The times when the experiments were performed apparently coincided with natural periods of retardation or cessation of growth. At Bonne Bay, this is assumed to be the post-spawning period, when little growth occurs; at Logy Bay it is undoubtedly related to low temperatures severely limiting the activity of the snails.

The experimental conditions themselves were almost certainly limiting. Captivity removed many natural environmental factors, some of which act as stimuli, resulting in increased eating activity. For example, in nature it is the ebbing tide which acts as the stimulus for L. littorea to increase its browsing activity (Newell 1970). The diet of the snails was obviously restricted; in nature the winkles have access to a variety of algae. Such a variety may be necessary to satisfy the nutritional requirements of Littorina. The experimental algae may well have lacked certain amino acids, or the snails may have lacked the necessary enzymes to fully utilize a particular alga.

Considering all the data, the conclusion seems to be that the artificial diets provided in the experiments were unsuitable for Littorina littorea. Lack of growth in itself does not necessarily indicate unsuitability, but the decreases in almost all measurements, whether significant or not, confirm this conclusion.

REFERENCES

Arnold, D. C. 1972. Salinity tolerances of some common prosobranchs. J. mar. Biol. Ass. U. K. 52: 475-486.

Barnes, H., M. Barnes and D. M. Finlayson 1963. The seasonal changes in body weight, biochemical composition, and oxygen uptake of two common Boreo-Arctic cirripedes, Balanus balanoides and B. balanus. J. mar. Biol. Ass. U. K. 43: 185-211.

Bell, H. P. 1927. Observations on the seasonal disappearance of certain marine algae in the tidepools near the biological station, St. Andrews, New Brunswick. Nova Scotian Inst. Sci., Proc. and Trans. XVII: 1-5.

Carefoot, T. H. 1967a. Growth and nutrition of three species of opisthobranch molluscs. Comp. Biochem. Physiol. 21: 627-652.

Carefoot, T. H. 1967b. Growth and nutrition of Aplysia punctata feeding on a variety of marine algae. J. mar. Biol. Ass. U. K. 47: 565-589.

Carefoot, T. H. 1970. A comparison of absorption and utilization of food energy in two species of Aplysia. J. exp. mar. Biol. Ecol. 5: 47-62.

Cornelius, P. F. S. 1972. Thermal acclimation of some intertidal invertebrates. J. exp. mar. Biol. Ecol. 9: 43-53.

Davis, C. C. 1968. Quantitative feeding and weight changes in Poecilia reticulata. Trans. Amer. Fish. Soc. 97: 22-27.

Fish, J. D. 1972. The breeding cycle and growth of open coast and estuarine populations of Littorina littorea. J. mar. biol. Ass. U. K. 52: 1011-1019.

Fraser, J. H. 1969. Experimental feeding of some medusae and Chaetognatha. J. Fish. Res. Bd. Canada. 26: 1743-1762.

Fretter, V. and A. Graham. 1962. British Prosobranch Molluscs. London, Ray Society. 694 pp.

Graham, A. 1971. British Prosobranch and Other Operculate Gastropod Molluscs. London, Academic Press, 112 pp.

Haseman, J. D. 1911. The rhythmical movements of Litorina litorea synchronous with ocean tides. Biol. Bull. Woods Hole. 21: 113-121.

Hayes, F. R. 1929. Contributions to the study of marine gastropods. III. Development, growth, and behaviour of Littorina. Cont. Can. Biol. & Fisheries. N.S. 4: 413-430.

Henderson, A. E. 1973. On the underwater weights of fresh-water snails. Z. vergl. Physiol. 46: 467-490.

Hughes, R. N. 1971. Ecological energetics of the keyhole limpet Fissurella barbadensis Gmelin. J. exp. mar. Biol. Ecol. 6: 167-178.

Hyman, L. H. 1938. The water content of medusae. Science, 87: 166-167.

Jacobs, W. 1941. Der hydrostatische Bedeutung der Atmungsorgane von Wassertieren. S.-B. Ges. Morph. Physiol. München. 50: 45-52.

James, B. L. 1968. The distribution and keys of species in the family Littorinidae and of their digenean parasites, in the region of Dale, Pembrokeshire. Field Studies 2, 5: 615-650.

Lewis, J. R. 1964. The Ecology of Rocky Shores. London, The English Universities Press Ltd. 323 pp.

Lowndes, A. G. 1942. The displacement method of weighing living aquatic organisms. J. mar. Biol. Ass. U. K. 25, 3: 555-574.

Moore, H. B. 1936. The biology of Littorina littorea. Part I. Growth of the shell and tissue, spawning, length of life and mortality. J. mar. Biol. Ass. U.K. 21: 721-724.

Newell, G.E. 1958. The behaviour of Littorina littorea (L.) under natural conditions and its relation to position on the shore. J. mar. Biol. Ass. U.K. 37: 229-239.

Newell, R. C. 1970. Biology of Intertidal Animals. New York, American Elsevier Publishing Company, Inc. 555 pp.

Newell, R. C. and V. I. Pye. 1970a. Seasonal changes in the effect of temperature on the oxygen consumption of the winkle Littorina littorea (L.) and the mussell Mytilus edulis L. Comp. Biochem. Physiol. 34: 367-383.

Newell, R. C. and V. I. Pye. 1970b. The influence of thermal acclimation on the relation between oxygen consumption and temperature in Littorina littorea (L.) and Mytilus edulis L. Comp. Biochem. Physiol. 34: 385-397.

Newell, R. C. and V.I. Pye. 1971a. Quantitative aspects of the relationship between metabolism and temperature in the winkle Littorina littorea (L.). Comp. Biochem. Physiol. 38B: 635-650.

Newell, R. C. and V. I. Pye. 1971b. Variations in the relationship between oxygen consumption, body size and summated tissue metabolism in the winkle Littorina littorea. J. mar. Biol. Ass. U.K. 51: 315-338.

Newell, R. C., V. I. Pye and M. Ahsanullah. 1971. Factors affecting the feeding rate of the winkle Littorina littorea. Mar. Biol. 9; 2: 138-144.

Nicol, J. A. C. 1967. The Biology of Marine Animals (2nd edition). London, Pitman Paperbacks. 699 pp.

Odum, E. P. and A. E. Smalley. 1959. Comparison of population energy flow of a herbivorous and a deposit-feeding invertebrate in a salt marsh ecosystem. Proc. N. A. S. 45: 617-622.

Pantelow, F. T. K. 1939. The relation between growth and food consumption in the brown trout (Salmo trutta). J. exp. Biol. 16: 446-473.

Paine, R. T. 1965. Natural history, limiting factors and energetics of the opisthobranch Navanax inermis. Ecol. 46, 5; 603-619.

Paine, R. T. 1971a. Energy flow in a natural population of the herbivorous gastropod Tegula funebralis. Limnol. Oceanogr. 16: 86-98.

Paine, R. T. 1971b. The measurement and application of the calorie to ecological problems. In; Johnston, R. F., ed. Ann. Rev. Ecol. and System. 2: 145-162.

Paine, R. T. and R. L. Vadas. 1969. Calorific values of benthic marine algae and their postulated relation to invertebrate food preference. Mar. Biol. 4: 79-86.

Sandison, E. E. 1966. The oxygen consumption of some intertidal gastropods in relation to zonation. J. Zool. 149: 163-173.

Sandison, E. E. 1967. Respiratory response to temperature and temperature tolerance of some intertidal gastropods. J. exp. mar. Biol. Ecol. 1: 271-281.

Southward, A. J. 1964. Limpet grazing and the control of vegetation on rocky shores. In: Crisp, D. J., ed. Grazing in Terrestrial and Marine Environments. (Symposium of British Ecological Society.) 265-273.

Sutherland, J. P. 1972. Energetics of high and low populations of the limpet Acmaea scabra (Gould). Ecol. 53, 3: 430-437.

Tattersall, W. M. 1920. Notes on the breeding habits and life history of the periwinkle. Fisheries, Ireland, Sci. Invert. I: 1-12.

Wiegert, R. G. 1968. Thermodynamic considerations in animal nutrition. Am. Zool. 8: 71-81.

Williams, E. E. 1964. The growth and distribution of Littorina littorea (L.) on a rocky shore in Wales. J. Anim. Ecol. 33: 413-432.

Wright, F. S. 1936. Report on the Maldon (Essex) periwinkle fishery. Fishery Invesitgations II, XIV, 6: 1-37.

Yonge, C. M. 1966. The Sea Shore. London, Collins. 311 pp.

Table A1., Weight factor experiment. Snails 1-36; water weights, air weights, average air weight/water weight.

Snail No.	Water weight (mg)	Air weights (mg)				Average air weights (mg)	Average air weight/ water weights (mg)
		A	B	C	D		
1	804.6	1867.5	1817.4	1804.9	1826.8	1829.2	2.27
2	414.5	850.1	870.8	872.4	850.4	860.9	2.08
3	742.7	1611.6	1649.1	1635.9	1624.6	1630.3	2.20
4	465.2	1043.4	1059.0	1008.7	1000.3	1027.9	2.21
5	761.4	1910.5	1770.1	1780.5	1763.0	1806.0	2.37
6	975.4	2213.1	2179.3	2154.7	2204.3	2187.9	2.24
7	1225.3	2784.5	2784.4	2733.8	2634.4	2734.3	2.23
8	1008.5	2319.5	2304.0	2266.7	2266.0	2289.1	2.27
9	923.7	2265.0	2237.4	2218.2	2188.2	2227.2	2.41
10	948.1	2242.9	2244.8	2223.0	2233.6	2236.1	2.36
11	478.7	1028.6	1012.0	990.7	1028.5	1017.5	2.13
12	775.7	1899.9	1936.3	1826.7	1886.0	1896.2	2.44
13	652.4	1449.9	1474.7	1392.7	1416.4	1433.3	2.20
14	665.1	1508.8	1528.6	1463.3	1504.8	1501.4	2.28
15	495.8	1145.8	1194.2	1173.1	1140.7	1163.5	2.35
16	382.5	846.8	850.5	822.0	841.9	840.3	2.20
17	768.9	1735.7	1798.6	1707.9	1658.7	1725.2	2.24
18	1302.5	2847.5	2885.1	2733.5	2814.1	2820.1	2.17

APPENDIX A

The tables which follow, Tables A1 and A2, provide the raw data used in the weight factor experiment, in which a conversion factor was derived. This factor, 2.25, was used to convert the weights of snails weighed underwater to their 'wet weights'. See Materials and Methods for details.

Table A1. Weight factor experiment. Snails 1-36: water weights, air weights, average air weight/water weight.

Snail No.	Water weight (mg)	Air weights (mg)				Average air weights (mg)	Average air weight/ water weights (mg)
		A	B	C	D		
19	612.5	1471.5	1484.5	1433.6	1447.9	1459.4	2.37
20	815.1	1820.4	1820.8	1864.6	1819.6	1831.4	2.25
21	1264.1	2685.9	2678.3	2686.3	2683.1	2683.4	2.12
22	1406.3	3076.3	3055.5	2928.3	2941.8	3000.5	2.13
23	723.9	1654.1	1706.9	1606.2	1565.8	1633.3	2.26
24	675.3	1625.8	1635.0	1570.4	1525.8	1591.8	2.36
25	545.7	1170.3	1182.8	1181.7	1153.8	1172.2	2.15
26	213.7	499.5	462.7	466.5	441.2	467.5	2.19
27	1122.2	2740.9	2687.3	2656.3	2635.9	2680.1	2.39
28	738.3	1646.4	1630.0	1573.1	1621.4	1617.7	2.19
29	684.3	1514.5	1524.3	1501.0	1498.3	1509.5	2.21
30	752.3	1837.4	1793.7	1739.6	1697.4	1767.0	2.35
31	929.3	2053.0	2076.7	2003.0	2056.1	2047.2	2.20
32	538.5	1185.7	1207.2	1203.7	1175.5	1193.0	2.22
33	614.4	1453.8	1399.8	1343.2	1361.6	1389.6	2.26
34	624.8	1383.8	1417.6	1364.8	1388.5	1388.7	2.22
35	1785.7	3895.5	3381.3	3804.6	3836.4	3854.5	2.16
36	738.3	1619.6	1631.1	1613.0	1651.7	1628.9	2.21

Table A2. Weight factor experiment. Snails 37-72: water weights, calculated air weights (water weight x 2.25), air weights, average air weights.

Snail No.	Water Weights (mg)	Calculated Air Weights (mg)	Air weights				Average air weights (mg)
			A	B	C	D	
37	704.9	1586.0	1601.5	1591.7	1563.3	1533.8	1572.6
38	433.4	975.1	962.5	929.2	906.9	914.3	928.2
39	408.4	918.9	904.2	895.3	890.3	892.2	895.5
40	212.0	477.0	513.1	507.3	508.7	510.7	510.0
41	395.3	889.4	920.6	886.4	868.6	865.3	885.2
42	977.9	2200.2	2163.1	2167.3	2102.9	2155.1	2147.2
43	525.4	1182.1	1131.7	1147.2	1097.3	1117.1	2123.5
44	894.6	2012.8	2072.2	2066.0	1974.5	1957.1	2017.5
45	1317.8	2965.0	3278.0	3247.0	3238.6	3216.3	3245.0
46	550.0	1248.7	1187.3	1294.7	1229.1	1216.7	1232.0
47	275.4	619.6	672.1	683.1	638.9	638.9	660.8
48	1360.2	3060.4	3053.9	3119.7	2976.6	2969.5	3029.9
49	800.6	1801.3	1820.2	1856.3	1852.5	1844.5	1843.4
50	831.6	1871.1	1808.8	1873.9	1817.7	1850.6	1837.8
51	765.1	1721.4	1712.0	1682.2	1721.8	1689.6	1701.4
52	516.7	1162.5	1222.8	1170.7	1127.8	1109.4	1157.7
53	226.1	508.7	526.6	531.6	505.8	502.3	516.6
54	469.4	1056.1	1052.1	1039.7	1025.6	995.4	1028.2

Table A2. Weight factor experiment. Snails 37-72: water weights, calculated air weights (water weight x 2.25), air weights, average air weights.

Snail No.	Water Weights (mg)	Calculated Air Weights (mg)	Air weights A	B	C	D	Average air weights (mg)
55	822.1	1849.7	1892.0	1778.7	1782.6	1838.7	1807.3
56	738.7	1662.0	1671.8	1692.7	1658.6	1645.7	1667.2
57	935.5	2104.8	2313.2	2286.2	2199.6	2303.6	2275.7
58	442.6	995.8	1046.7	997.9	970.1	977.4	998.0
59	1000.6	2251.3	2449.6	2493.2	2318.7	2261.2	2380.7
60	532.9	1199.0	1239.8	1254.9	1173.5	1136.3	1201.1
61	485.9	1092.2	1341.2	1330.7	1300.5		1324.1
62	1241.1	[illegible]792.4	2860.0	2956.0	2920.3	2892.9	2907.3
63	1163.7	[illegible]618.3	2547.3	2610.9	2479.9	2479.8	2529.5
64	1230.2	2767.9	2626.2	2580.6	2607.8	2564.9	2594.9
65	722.7	1626.0	1582.2	1660.0	1641.0	1557.9	1610.3
66	898.0	2020.5	2004.0	1952.9	1908.7	1913.3	1944.7
67	933.8	2101.0	2286.6	2287.9	2222.3	2280.9	2269.4
68	1163.9	2618.7	2689.3	2720.3	2587.3	2594.4	2647.8
69	1367.3	3076.4	2973.1	3040.3	2984.5	2988.2	2996.5
70	1079.9	2429.7	2487.8	2465.1	2415.1	2470.2	2459.6
71	849.7	[illegible]1911.8	1876.7	1861.6	1808.6	1810.1	1838.3
72	807.4	1816.6	1842.9	1882.5	1790.4	1732.3	1812.0