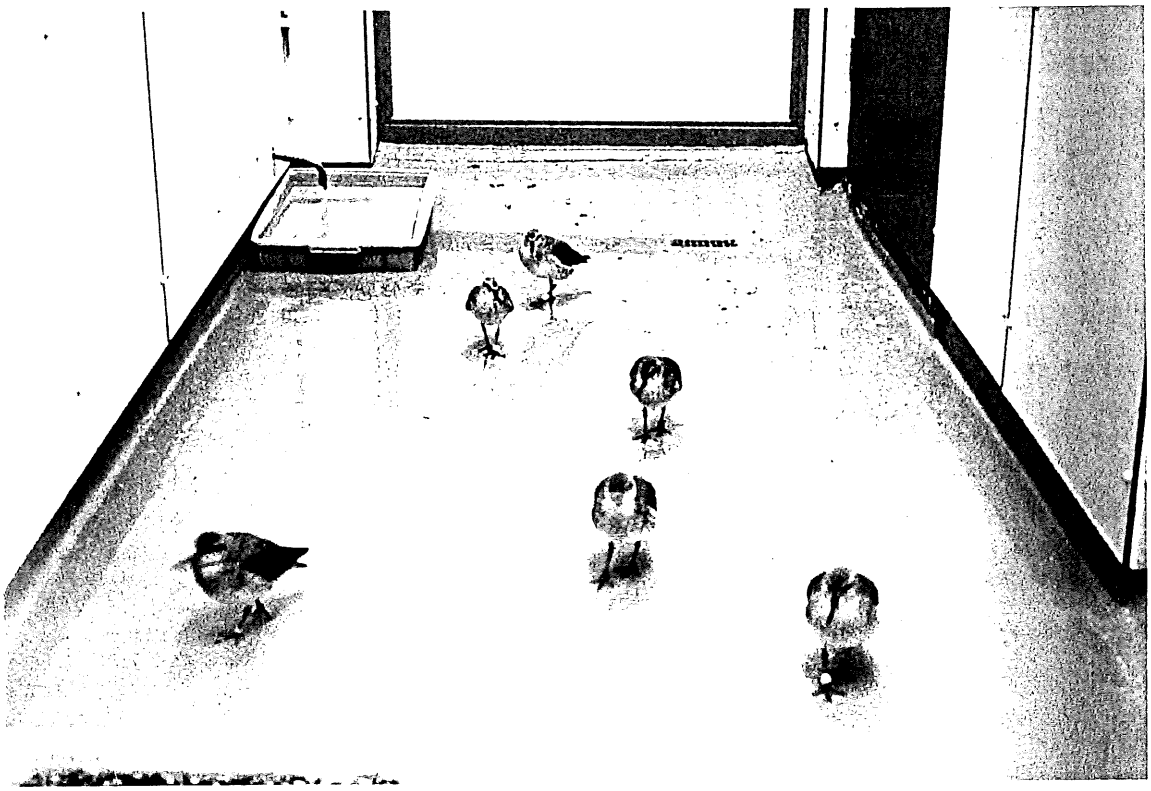
Costs of Foraging and Food Processing in a Mollusk Eating Shorebird, the Knot (*Calidris canutus*).



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1 Introduction

Energy is a very useful currency in ecology, because energy can link very different aspects of the biology of a species or ecosystem. Each Joule can be expended only once, and energy is often limiting the options available to an organism (Verhoef and Daan 1995; Cuthill and Houston, 1997). The energy budget of an animal consists of intake and expenditure which must be a balanced on a long-term basis, and the putting on and using of bodily stores, which enable short term unbalances between intake and expenditure. In birds, the energetic consequences of eating a certain food type or being on a certain spot are very important in habitat- and food choice. In migrating birds such as the Red Knot (*Calidris canutus)*, wintering sites are chosen on account of energetics, while the other important factors influencing these choices are mainly risks such as the risk of being predated (Piersma, 1994).

In the Red Knot intake and storage have already been studied intensively (Piersma, 1994) but with respect to energy expenditure (EE), many aspects remain unknown. A better understanding of these aspects would help to solve important questions like why subspecies *islandica* Knots overwinter in the Wadden Sea but *canutus* Knots in Western Africa, and on a smaller scale unveil the secrets of site selection and prey choice. In this frame the present study was conducted.

1.1 Composition of an avian energy expenditure budget

Generally four components are distinguished:

1. Basal metabolism. This is the minimum amount of energy used, when the post-absorptive animal is asleep in a thermoneutral environment.
2. Thermoregulation. The amount of energy needed to keep body temperature at 41°C.
3. Heat Increment of Feeding (HIF). Digestion and absorption of food produces heat. If there is no thermoregulation, this energy is lost. If there is, HIF can substitute for thermoregulatory costs.
4. Costs of activity. These costs can also substitute thermoregulatory cost.

For Knots wintering in the Wadden Sea feeding on a water and salt rich diet, these components are not all you want when investigating the important expenditures. Several additional components could be important that do not fit nicely with the categories above, namely the cost of upwarming ingested matter, maintaining salt balance and mechanical processing of the ingested food.

1.2 Concentration on foraging costs

Aspects of the total energy expenditure that are easiest or most logical to measure are tackled already: Thermoregulation is studied intensively in the past ten years and BMR is well known (Piersma 1994, papers 6 & 18). Costs associated with flying or with foraging are unknown up to now. The costs of flight are being investigated at the moment;
this research report dives into the energetic costs associated with foraging. Here, we asked two questions:

1) What is the total EE due to foraging?
2) What is the relative importance of different aspects in this foraging cost?

The aspects that we distinguished were chosen on account of importance for prey and habitat choice. This study was one of the first where foraging costs were split up.

Wintering *islandica* Knots roughly keep the same body mass during their stay in the Wadden Sea so energy budgets should be balanced. For much of the low tide, Knots are on the intertidal mudflats, foraging for their molluscan prey which they ingest whole and crack in the stomach. (Piersma, 1994, general discussion)
With this kept in mind, we investigated the importance of foraging costs for the energy expenditure of Knots wintering in the Dutch Wadden Sea. We aimed at subdividing total foraging costs into:
-the muscle activity during search and capture;
-the crushing of the bivalves in the stomach;
-the HIF; together with heating and transport of the ingested prey (water, shell, meat) ; the excretion of ingested salt and water.

2 Materials and Methods

2.1 measuring energy expenditure

Rate of energy expenditure can be measured via various ways. Rate of CO2 production or O2 uptake can be measured directly but this is only convenient in small closed boxes. Energy expenditure can also be measured with the doubly labeled water method, which was originally designed by Lifson and McClintock and published in 1966. This is an indirect way of estimating CO2 turnover. Blood samples need to be taken before and after the behavior under study, so this method is impractical with wild animals unless they can be efficiently captured twice. Capturing wild Knots in the Wadden Sea twice is impossible so an indoor intertidal aviary, was used in this study.

2.2 design

The components of foraging costs were measured by offering 8 different circumstances, were food type and location were varied, to a group of six Knots. Temperature was kept as constant as possible and above the lower boundary of the thermoneutral zone. The eight treatments were: Experiment 1: feeding in the intertidal arena on Cockles (MUDCOCK1, 6 january), Exp 2: feeding on the roost on Cockles (ROOSTCOCK, 22 january), Exp 3: feeding in the arena on dead and dying Cockles (MUDDEAD, 26 january), Exp 4: similar to Exp 1 (MUDCOCK2, 28 january), Exp 5: NOT feeding, on the roost (ROOSTFAST, 30 january), Exp 6: feeding on trouvit pellets on the roost (ROOSTTROUV, 2 february), Exp 7: feeding on pieces of Cockle-flesh on the roost (ROOSTFLESH, 4 february), Exp 8: Not feeding, but foraging on the mudflat in the intertidal arena (MUDFAST, 13 february). Six of the circumstances were designed beforehand, Muddead was a failure of Mudcock, and Mudcock was done twice because this was a very important treatment and the first time the Knots were in a poorer condition than we would like. This design with the expected energy expenditures is visualized on the last page of this report and in figure 1.
This design alone should be enough to answer the study's questions, but also a more detailed and explicit approach was taken: a number of independent variables, determining EE, were monitored during the experiment. These are introduced from §2.4 onwards.

SUMMARY DESIGN		treatment:		cost factors present:		
title	date	location	food type	activity	crushing	digesting
MUDCOCK1	06/01/98	intertidal flat	Cockles	+	+	+
MUDCOCK2	28/01/98	intertidal flat	Cockles	+	+	+
MUDDEAD	26/01/98	intertidal flat	dying Cockles	+	?	+
ROOSTCOCK	22/01/98	roost	Cockles	-	+	+
ROOSTTROUV	02/02/98	roost	trout pellets	-	-	+
ROOSTFLESH	04/02/98	roost	Cockle flesh	-	-	+
MUDFAST	13/02/98	intertidal flat	none	+	-	-
ROOSTFAST	30/01/98	roost	none	-	-	-

Fig. 1. Summary of the design as it was actually carried out.

2.3 the animal subjects

During experiments we used six adult Knots (*Calidris canutus islandica*) The Knots were captured in de Wadden Sea one year before the experiment, and had become accustomed to a diet of soft trout pellets (Trouvit, Produits Trouw, Vervins, France). Such Knots have problems with eating shellfish because their muscular stomach is very much reduced in size (Piersma et al 1993, paper 14). Before the experiment the birds were made fully capable of living on shells again by abruptly denying them the trout pellets, offering only shells. In the meantime, the animals had also to be used to the indoor mudflat (l x w x h: 7.3 x 8 x 3) and had to learn to fly to the roost (4.7 x 1.1 x 2.5 m) when flood came in (see figure 3). The mudflat was quite readily used: even the first time, the Knots soon jumped from their roost to the mud when the door was opened. By contrast, the way back when the water level rises to uncomfortable depths was harder to learn. After a few days training, six Knots easily took this hurdle but a seventh individual bumped into every wall, even when it should be able to see his cage mates at the roost border. It seemed to have a very bad eyesight and was returned to a non-experimental Knot cage.

Climatized Intertidal Aviary

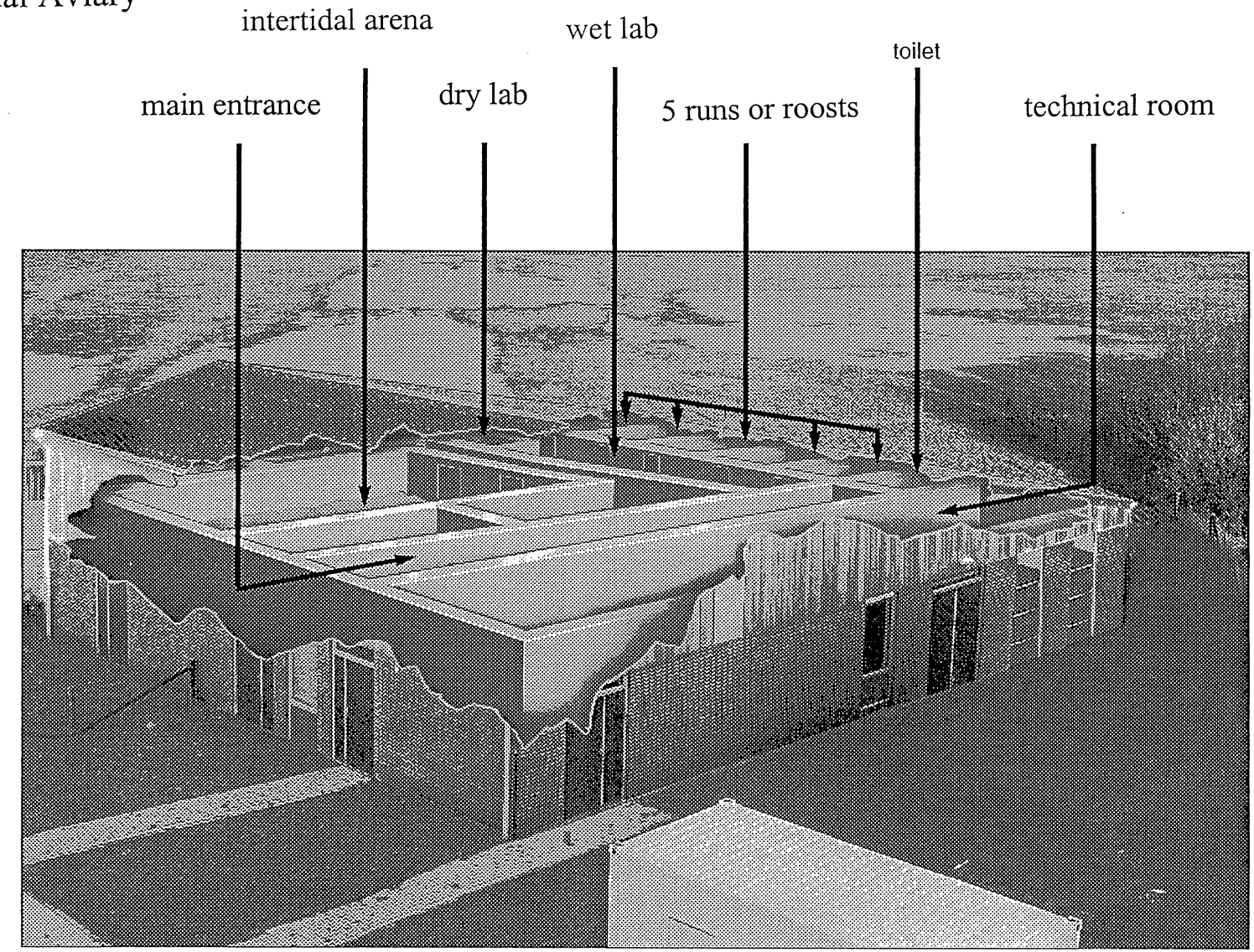


Fig. 2. (Other page) Overview of the Climatized Intertidal Aviary at the NIOZ. The roost that was actually used is between the main entrance and the arena. Measurements and blood or feces samples were taken in the dry lab.

2.4 Routine during experimental period.

Every morning, the animals were weighed to the nearest gram and the roost was cleaned. The body mass of the animals was carefully watched during the whole period as a crude measure of well being. Two birds losing too much mass in the accustoming period were fed trout pellets for two days to regain strength. Normally, the birds were fed ad libitum with weighed amounts of shellfish and there was always access to fresh water to drink and bath.

2.5 Behavioral observations

On experimental days, behavior of the birds was observed almost continuously. About every five minutes an activity scan was performed for all birds, distinguishing sleeping (bill tucked under wing, eyes closed), resting, preening, walking, foraging and flight. Drinking was not noted separately but incorporated in resting. After the scan, attention switched to a particular Knot which was followed for the next three minutes to get a reliable estimate of intake rate and in case of Cockles, prey size choice. Ingested cockles were estimated to the nearest mm with help of a series of measured cockles glued to the wall of the experimental unit. Sometimes a dead, open cockle was encountered. This as noted to as flesh but we were unable to estimate the size of the bite. The same individual was followed for another minute when the number of steps was counted. The observation proceeded with a new activity scan and another Knot in the spotlight.
On the two days when no food was (planned to be) available, intake rate was likewise not measured so activity scans and step counts were made in rapid progression. When no single cockle should have been present on the mudflat, the Knots were very keen on finding the last ones. In the morning they were quite successful, but after three hours the mudflat was virtually emptied. During an hour in the morning, prey catching was noted during step counting. Cockle length was not estimated.
For all days, individual intake rates per size class were extrapolated to the period of presence on the mudflat. This yields an estimate of how many cockles of each length a Knot has eaten during that day. Note that the periods on the mudflat are a little shorter than the periods between initial and final blood samples. The time in between the end of the treatment and the final sample will be called "waiting time".

2.6 Cost of Walking.

Distance covered per unit time, the parameter of interest, could not be measured directly. Step rates estimated by direct observation are extrapolated to the period of presence in the experimental arena. The number of steps per second multiplied by step length gives the distance covered per second.
Step length measurements were done on several days after the study period, i.e. 12 march and 2/ 3 April 1998. One Knot was released in the intertidal aviary for a few minutes. It was observed where the animal foraged and where it just walked. Then the Knot was caught and clearly visible trails (length 2 to 12 footprints) were chosen, preferably where behavior was known. The distances between footprints were measured and averages computed per trail to avoid pseudo-replication. There were so few data per bird per behavior that only the average of the six birds per behavior (walking or foraging) was used.
Now behavior specific walking rate (ms^{-1})= step rate (s^{-1})* behavior specific step length (m)
As time devoted to foraging and walking is known, the walking rates can be converted to the distance covered during the whole experimental period. This should then be divided by total duration to compute the distance covered per second, ready for use in the model as the average walking rate.

Energy expenditure per covered meter was kindly provided by Leo Bruinzeel, for which we are grateful. It can be assumed that energy expenditure is constant across a wide range of walking rates. This value is for a Knot of 109 gram, but is assumed to be proportionally to body mass so individual costs per meter were computed. Now costs of walking can be quite accurately estimated, as average walking rate is computed in the previous paragraph.

Cost (js^{-1})= walking rate (ms^{-1}) * body mass specific factor (jm^{-1})

This should be a check on our own estimate of walking costs. (See discussion)

2.7 Food peculiarities

In this period Mussel *Mytilus edulis*, Edible Cockle *Cerastoderma edule* and *Spisula subtruncata* were used. Mussel (occasionally) and Edible Cockle belong to the usual diet of Knots in the Wadden Sea; *Spisula* is a sublitoral bivalve which Knots just never encounter under natural conditions. They are able to process *Spisula* but blood repeatedly found in the feces suggests that sharp *Spisula* fragments are a problem. Another problem with *Spisula* is their habit of coming up to the surface at low tide, probably an escape behavior from unsuitable, supralitoral habitat. Therefore, during training as well as on experimental days the indoor mudflat was stocked with high densities of Edible Cockle of sizes ingestable. These were found in enormous numbers on the mudflats near De Cocksdorp, Texel. The Knots' favorite prey species, *Macoma balthica*, was no alternative because densities in the Wadden Sea were too low to collect sufficient numbers efficiently.

Although we investigate energy expenditure, it is good to check whether this expenditure is mirrored in the energy intake corrected for increase in body mass. Much more important, the processing of food itself costs an amount of energy, which is unknown up to now, and which we want to investigate.
The number of eaten cockles per length class multiplied by the class specific fresh mass, ash free dry mass, water content and shell mass yields the total intake for these relevant parameters.

A random sample of the cockles was taken from the intertidal aviary, from the food given to the Knots, or from the storage boxes (see appendix 1). The Cockles from sizes ingestible by Knots (up to 17 mm) were measured and the numbers per length class were counted. The flesh was removed, put in small crucibles and dried to constant mass in three days at 60 °C. Then the crucibles were weighed, incinerated in a oven with a temperature of 600 degrees C and weighed again to obtain the ash mass. The difference between these two measurements is known as the "ash free dry mass" AFDM, and it is assumed to be equivalent to the contents that can be digested. However when shells are incinerated, one of the components, colchine, is also combusted and in that case digestible content is overestimated. Therefore, only the flesh was taken into consideration when computing AFDM. Small amounts of flesh, and some digestible detritus remain attached to the shell when removing the flesh. These were neglected.

Theory predicts that the relation between length and AFDM should be doubly logarithmic. (Zwarts, 1991) AFDM values per length class and day were analyzed with multiple regression analysis/anova in systat 7.1 Only data were the AFDM figure was composed of data from three or more individuals were used as this was recommended by Zwarts (1991) The model was:

"ln(afdm) = intercept + batchfactor1 + [ln(length)*(coeff+batchfactor2]"

The relation of the three other intake variables fresh mass, water mass and calcareous shell mass with length was estimated only from data of the 7 january batch, because normally wet mass was not weighed. A regression line was fitted trough these data with systat 7.1, the models were:
"ln(variable X) = intercept + [ln(length) * coeff_variable X]

These data were used for MUDCOCK1&2, ROOSTCOCK and ROOSTFAST

For MUDFAST all Cockles were assumed to belong to the 10-mm class.
For ROOSTCOCK, a second method to estimate intake parameters was also used. A sample was taken from the food trays before and a second after the foraging period; the average afdm, water, and shell mass determined as described above. The first method will be used in modeling because it is similar to the other Cockle days.

MUDDEAD: Nothing was measured on the exact content of these dead and dying cockles. Therefore we assumed that each piece of flesh was equivalent to the flesh of an average Cockle of 9 mm length, and we used the formula of batch 4 (12 January) because these Cockles were also in a very bad condition (nearly dead). Water content is assumed to be half that of a living Cockle, because a large part of the water is normally enclosed between the valves of the Cockle.

ROOSTFLESH: Before and after the Knots foraged on it, a sample of the flesh pieces of the large Cockles was taken and treated as before with the whole Cockles. Also wet mass was measured.

ROOSTTROUV: Information from the manufacturer was used. Trouvit contains 48% protein, 12% fat, 9.3% non-digestible fiber and ash and 30.7 % water. Strangely, these figures differ enormously from those in Piersma et. al. 1994, paper 15; and from Bruinzeel and Piersma 1998: 45%, 8%, 15% and 11%, respectively, but this does not sum up to 100%. Protein and fat were assumed to be comparable to AFDM.

As the condition of the Cockles (see results) was so bad that the amount of fat is negligible, AFDM is roughly equivalent to protein mass.

2.8 Temperature

Temperature was measured hourly during the experiments, in case of the Mud-days with five thermometers and on the roost with only one. The 11 hourly values per treatment were averaged for statistical use.

2.9 Doubly Labeled Water considerations

2.9.1 Isotope principles

The heavy isotopes in doubly labeled water, ^{2}H and ^{18}O, function as markers that make it possible to measure turnover rates of both H and O atoms. The doubly labeled water method uses the fact that whereas hydrogen (H) leaves the body only as water, oxygen (O) leaves the body also as carbon dioxide (CO2). Carbon dioxide production is directly linked to aerobic metabolism, which is by far the most important part of metabolism. Anaerobic metabolism results first in the production of metabolites that are subsequently also oxidized, resulting in the same ratio of energy/CO2. Our treatment period of more than ten hours makes the possible impact of anaerobic metabolism very insignificant. (Henk Visser pers. comm.)

2.9.2 Experimental procedure

On experimental days, animals were caught at 7.15 AM. From three animals, a blood sample was immediately taken from the brachial vein (located in the wing) to estimate isotope background values. Then all six individuals were injected a precisely known amount (range in doses 0.4-0.9 g) of isotope enriched water subcutaneously in the belly, using an insulin syringe which was weighed to the nearest 0.1 mg on a Mettler AE160 balance before and after administration. The animals were thereafter put in a small, dark box for an hour, to let the injected water equilibrate with the body water. In small, dark boxes Knots tend to fall asleep and therefore expend only a little energy.
Then a blood sample "initial" was taken of all Knots and they were released in the experimental arena at approximately 9 AM. At 8 PM, the birds were caught again and "final" blood samples taken from the wing vein. If possible, individual feces samples were also taken. All samples consist of three to six 15 µm aliquots stored in glass capillaries that were flame-sealed immediately with a propane torch. The capillaries were stored at 4°C until analysis.

2.9.3 Isotope analysis

These analyses were performed in triplicate by the Groningen Centre for Isotope Research.
For ^{2}H analysis the water from the blood sample was obtained by distillation in a vacuum line and was subsequently reduced to hydrogen gas. Then the Isotope Ratio Mass Spectrometer (type SIRA9) determines ^{2}H /^{1}H ratios, in delta units. During analyses internal gas standards were used in the form of enriched water that covered the expected enrichment range of samples to test the effect of uranium oven for each batch. To correct for cross-contamination between reference and sample channels of the IRMS, to internal gas standards were used, one at the background level and another with a 2H enrichment of about 0.15 atom percent. To increase the discriminative power of the comparison of the 2H enrichment in body water pool and fecal water in each animal, both types of samples were prepared and analyzed in the same batch, so possible errors between batches were avoided. Deuterium isotope concentrations are computed with:

Concentration in Sample = ((ratiosample-ratiostandard)/ratiostandard)*1000 ; this is in atom percent.
Background, Dose, Initial, and Final concentrations will be called Cb, Cd, Ci and Cf respectively.

Fractional turnover rate (Kd) is obtained from initial divided by final, corrected for background isotope values:

Kd = 1/t * ln[(Ci-Cb)/(Cf-Cb)] where t is the time elapsed between initial and final

For ^{18}O, fractional turnover rate (Ko) is determined similarly although the first steps are of course different.

2.9.4 Total Body Water estimates

During equilibration it is assumed that there is no isotope turnover; isotopes from the injection are diluted over the whole body water pool (TBW). This water pool can be calculated with quantity of the dose (Qd, moles) , concentration of the dose (Cd, atom percent) and the concentration in the body water pool before (Cb) and after (Ci) administration. This is the known as the "plateau method" (Visser et al 1999).

TBW= 18.02 * Qdose*(Cd-Ci)/(Ci-Cb)

Sometimes a correction for isotope loss during equilibration is recommended in the literature (Speakman 1997). This "extrapolation method" incorrectly assumes that turnover rates during equilibration are the same as during the experiments. This is obviously not true as we were planning to induce a wide range in turnover rates. Therefore the TBW calculated with the plateau method will be used in calculating water flux rates and energy expenditure.

2.9.5 Water Flux Rates

Water efflux = TBW * Kd
There is a possible problem, as heavy isotopes have less tendency to enter the gas phase and are accordingly underrepresented in the water vapor breathed out by the lungs. This is called the physical fractionation effects. Therefore you have to know what part of the water leaves the body via vapor (lungs) and liquid (feces, salt gland) pathways. The literature recommends a fixed value for fraction evaporation, e.g. 0.5 (Lifson and McClintock 1966) or 0.25 (Speakman 1997) but in view of the high expected water fluxes these values seemed unrealistic. Also the expected range in water fluxes discourages the use of a fixed fraction of evaporation. We used the value of 10 g evaporation/day (Verboven and Piersma, 1995) which results in a very wide range in fraction evaporating.

Water fluxes were expected to be very high due to the high water content of the diet of the Knots. Together with the high passage rates, this could be a problem if the equilibration between ingested water and body water was not complete. Therefore we compared isotope enrichments in the blood with those measured in the feces.

2.9.6 Energy Expenditure

First the turnover rate of CO2 is calculated, in principal from Ko – Kd . This is multiplied bij TBW to transform the fractional turnover rate to an absolute one in gram per second. Computation of carbon dioxide turnover rate is as follows:

rCO2 = (TBW/2.08) * (Ko – Kd) – (fraction evaporation * C* H2Oflux rate)

Then a few conversions are needed to arrive at energy values: zie uit bruinzeel en piersma

1 moll CO2 has a volume of 22.4 liter under standard conditions.

Assuming that the Knots mainly eat lean, fat-free Cockles, a respiration quotient (RQ) of 0.73 for protein can be used:
1 liter CO2 is produced together with 27.33 kJ.

2.10 Statistics

A General Linear Model (GLM) with repeated measures will be performed in spss 8.0 to test for differences in energy expenditure between treatments. The design with six birds enables comparing six days or treatments. The absence of bird Y on one occasion due to illness reduces this further to five treatments, so data of two days had to be pooled and for two other days neglected. (see results) So 30 of the 47 obtained EE values are used in the model. Days were ordered on account of decreasing expected energy expenditure, polynomial contrasts were chosen to check this expectancy.

One type of predictive models was tested. This predictive GLM, that was constructed in statgraphics 2.1 , indicates which of the factors under study --average Body Mass, change in Body Mass, temperature, distance covered, shell mass eaten, AFDM eaten, mass of the bulk eaten, water flux rate, number items eaten and measure of activity-- explain energy budgets. Variables with the highest p-values above 0.05 were dropped from the model, which was then performed again. At the same time this procedure determines the coefficients of the remaining independent factors, which can be used to predict EE in foraging Knots. 47 EE values were used for this latter model.

A Correlation matrix of the independent variables was made in spss 9.0 to evaluate the impact of multicollinearity on the conclusions.

3 Results

3.1 Was the plan successful?

Eigth DLW days were performed on six Knots, except for ROOSTFAST were Knot Y was not used due to illness. It showed leg cramp, which indicates stress, e.g. leg cramp often occurs in mist-netted Knots of the *canutus* subspecies (Piersma 1994, general discussion).
MUDDEAD was an attempt to repeat the MUDCOCK treatment, which failed due to Cockle mortality. This mortality was presumably caused by the high temperatures in the intertidal aviary, which induced the shallow occurrence of a black sulfide zone due to a lack of oxygen. Dead or dying Cockles have their shells opened, and to our astonishment the Knots started pulling out the flesh instead of swallowing the prey whole. This means that there are less crushing costs. Also, the amount of ingested water will be lower as the two valves normally enclose part of the water.
Backgrounds, Initials and Finals from the blood could always be taken without trouble. Finals from the feces were not always obtained: They were only taken on mudcock1, roostcock, roostfast, roosttrouv and mudfast.

3.2 Body Mass

Body Mass is a very crude measure of overall condition, which is useful as a guide. As we weighed the Knots once or twice a day during the whole period, the body mass graph tells the story of the investigation. The six Knots that we were able to train sufficiently, lost body tissue at a rate about 6 gram a day during the first three days adapting to the novel –shellfish instead of trout pellets- food. (Fig. 3). After six days, Body Mass increased again and after another two weeks original body masses were more or less restored. Then the training to the experimental regime started; the Knots had to learn to eat so much in twelve hours on the mudflat, that they would be able to live on the reserves for another 36 hours, until the next mudflat period begins. Body Mass was rapidly lost in these days with a characteristic indented pattern. The first doubly labeled water experiment was performed and it turned out that maybe we had asked too much of the Knots: haematocrit values were low, body masses were low and in some birds blood clotting was severely depressed.
After that day we changed the regime so the Knots would be in better shape, which is visible again in the graph. The body mass is more or less steady from then on and small fluctuations are caused by days with easy digestable food i.e. cockle-flesh and trouvit pellets, or days with limited food gift to keep the Knots' motivation high enough.
Figure 4 depicts the changes in Body Mass that occurred during the eight treatments.

Fig. 3. Development of Body Masses (g) during the whole experimental period. Shown are the 10^{th} , 90^{th} and 25^{th}, 75^{th} percentiles. Outliers are not shown. Experimental days were 67, 83, 87, 89, 91, 94, 96 and 105.

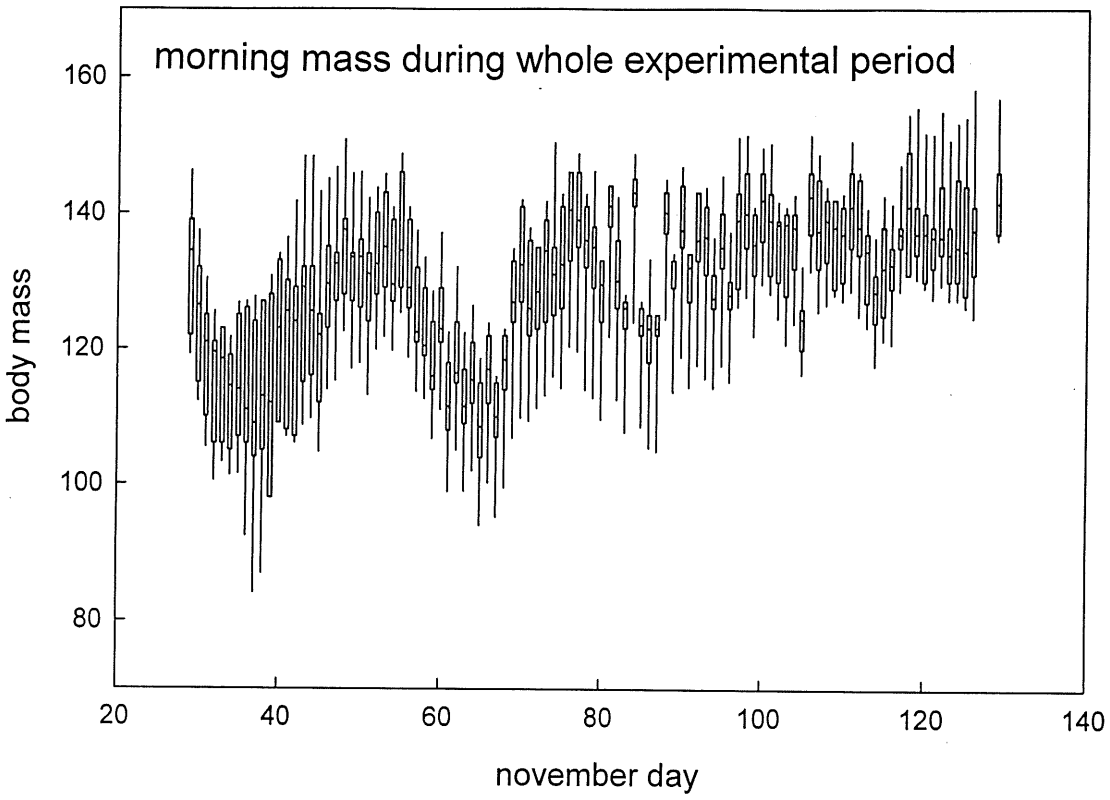
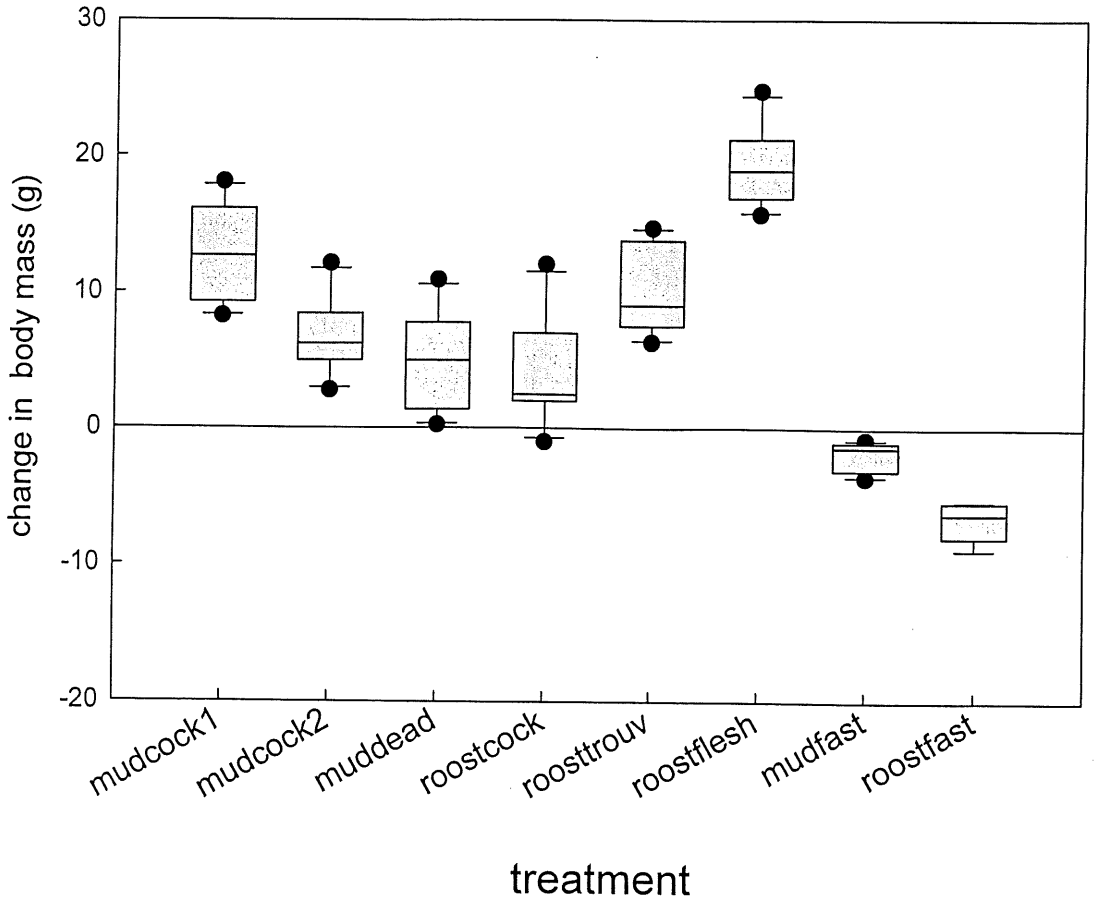


Fig. 4. Changes in Body Mass in g. Days are in order of decreasing expected Energy Expenditure. 10^{th} , 90^{th} and 25^{th}, 75^{th} percentiles are shown and outliers beyond 10^{th} and 90^{th} percentile.



3.3 Behavioral observations

The percentages of time devoted to the six investigated behaviors are tabulated in figure 5. For convenience averages are given but individual values are shown in appendix 1. Unfortunately one behavior, drinking, was not scored separately; this was however not a very common behavior, and it was almost totally absent on mudflat days. When it occurred it was scored as resting. The active behaviors walking and foraging were performed much more on mudflat days than on roost days. Fortunately, very little time was devoted to flying. The enormous variation in time devoted to active behaviors, foraging and walking, enables estimating the effect of activity on energy expenditure.

	Mudcock1	Mudcock2	Muddead	Roostcock
Flight	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Foraging	85.1 (67.0-95.5)	72.8 (48.0-87.8)	88.9 (76.7-95.6)	23.5 (7.9-49.1)
Walking	3.0 (0.0-4.5)	4.9 (1.0-11.2)	5.6 (2.2-7.8)	7.3 (5.3-12.3)
Preening	0.8 (0.0-1.1)	4.1 (1.0-7.1)	2.2 (1.1-4.4)	5.3 (0.9-10.5)
Resting	10.7 (1.1-25.0)	17.3 (3.1-46.9)	3.1 (0.0-12.2	36.1 (16.7-57.9)
Sleep	0.4 (0.0-2.3)	0.9 (0.0-4.1)	0.2 (0.0-1.1)	27.8 (14.9-50.0)

	Roosttrouv	Roostflesh	Mudfast	Roostfast
Flight	0.0 0.0	0.3 (0.0-0.95)	0.3 (0.0-0.98)	0.1 (0.0-0.35)
Foraging	3.6 (1.7-5.0)	27.3 (11.5-51.9)	44.6 (36.5-51.5)	0.0 (0-0)
Walking	5.7 (1.7-10.0)	13.1 (7.7-18.3)	29.2 (19.0-40.4)	3.1 (2.1-9.6)
Preening	20.1 (4.2-27.5)	16.3 (4.7-26.9)	10.1 (5.2-13.0	7.4 (0.0-11.0)
Resting	47.9 (29.2-75.8)	40.9 (12.5-66.0)	8.2 (3.6-11.4	30.0 (23.8-67.0)
Sleep	22.6 (5.0-39.2)	2.2 (0.0-4.8)	7.8 (2.3-13.4	42.7 (11.7-67.7)

Fig. 5. Time budgets of the six Knots during the treatments as percentage of treatment duration; average and range.

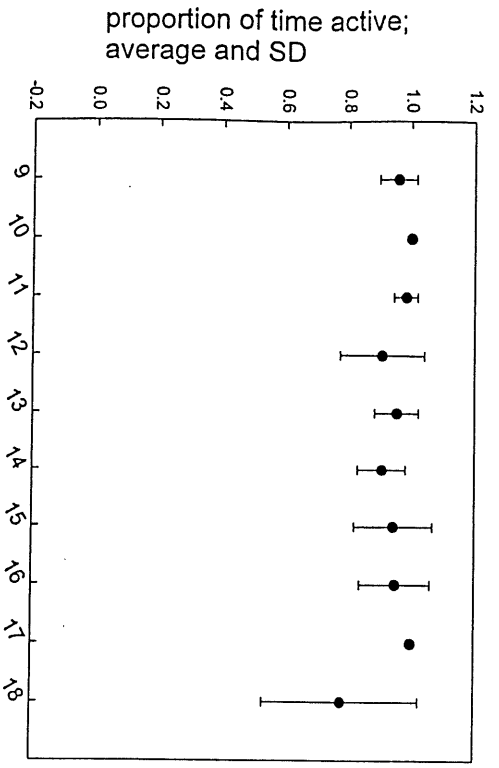
This kind of data is problematic, as the percentages of one behavior are dependent on the value for the other types: the total always equals 100 %! Therefore an "activity measure" was calculated: behaviors were divided in an "active" group (foraging, walking and flying) and an "inactive" group (preening, resting and sleeping), and the sum of "active" divided by total "inactive". To enable use in the intended model, the activity values have to be from a normal distribution and were therefore log transformed. These logarithmically transformed values are shown in figure 6.

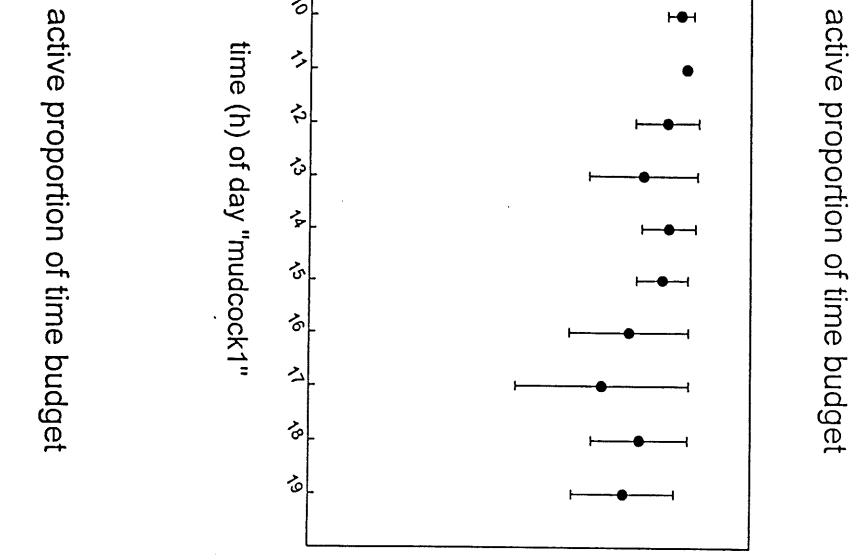
	Mudcock1	Mudcock2	Muddead	Roostcock	Roosttrouv	Roostflesh	Mudfast	Roostfast
B	0.6	0.0	1.1	-0.4	-1.1	-0.5	0.5	-1.6
LG	1.9	1.2	1.3	-0.8	-1.0	0.2	0.3	-1.6
O	1.9	0.8	1.5	-0.7	-0.8	-0.1	0.6	-1.6
RY	0.7	0.6	1.9	0.1	-1.1	0.0	0.5	-1.0
W	1.3	1.2	1.9	-0.1	-1.3	-0.2	0.5	-1.7
Y	0.4	0.2	0.7	-0.6	-0.8	-0.4	0.4	

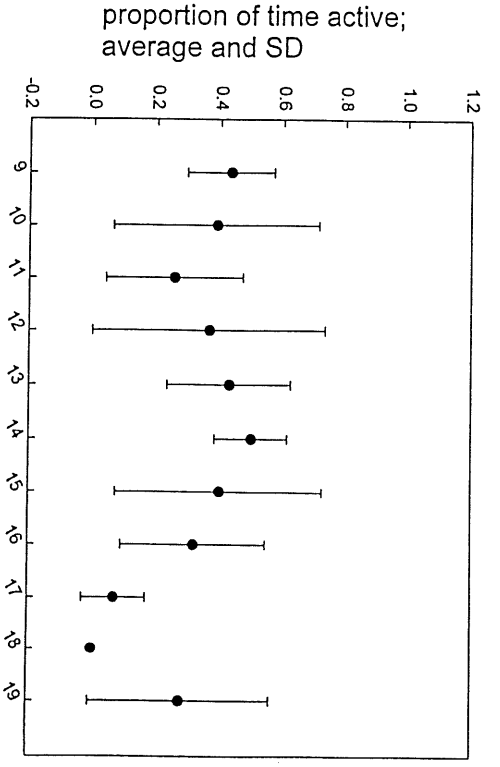
Fig. 6. Logarithmically transformed activity measures: ^{10}log (%active/%rest)

For the eight days, proportion of total time in the experimental arena devoted to active behaviors are computed per hour per bird and plotted against time. (fig 7a to h) The graphs indicate no important degree of periodicity in the behavior of the Knots. Therefore, this was not even tested.

Fig. 7 a / d. Activity patterns over the experimental days mudcock1, mudcock2, muddead and roostcock. The proportion of time a knot is active during the scans of one hour is calculated. These figures depict the average and SD of the flock of six birds.







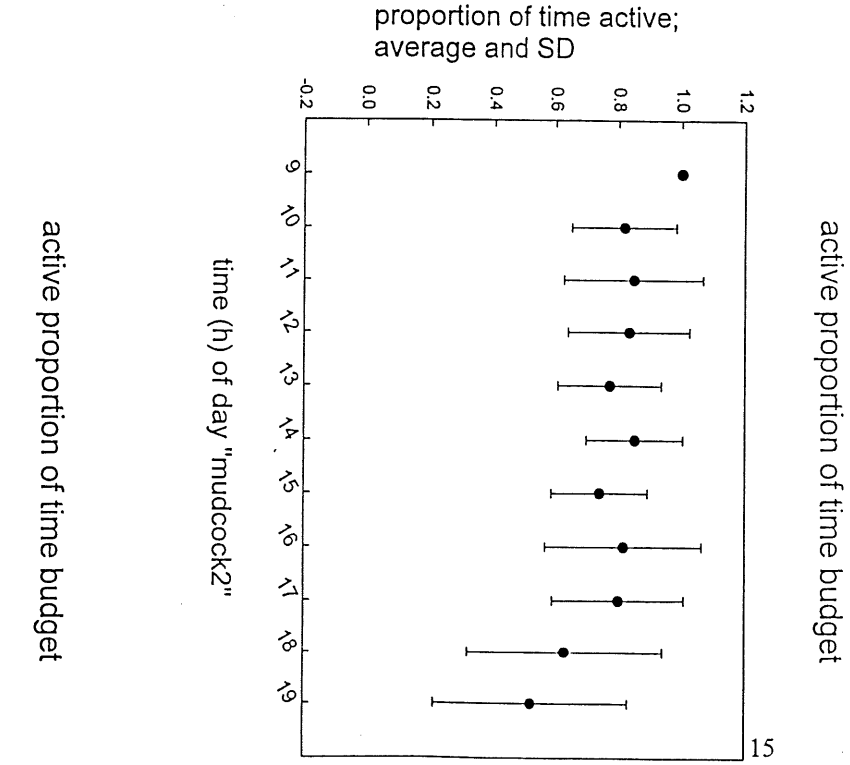
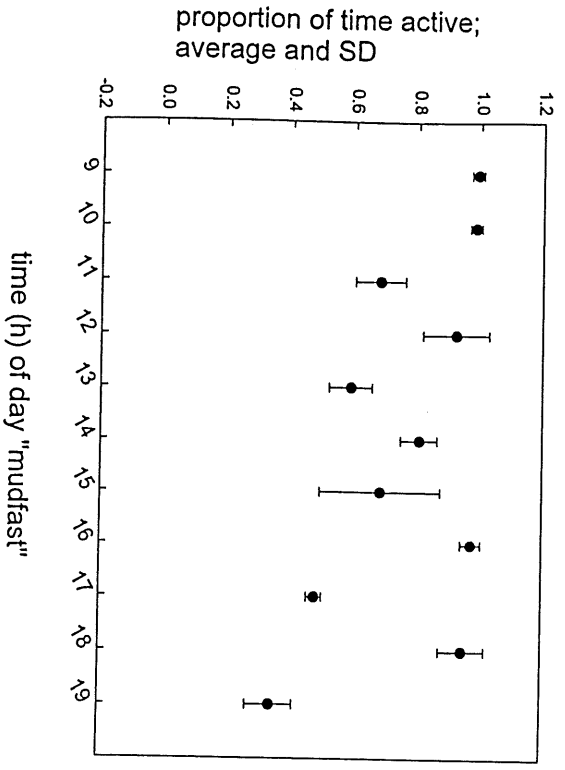
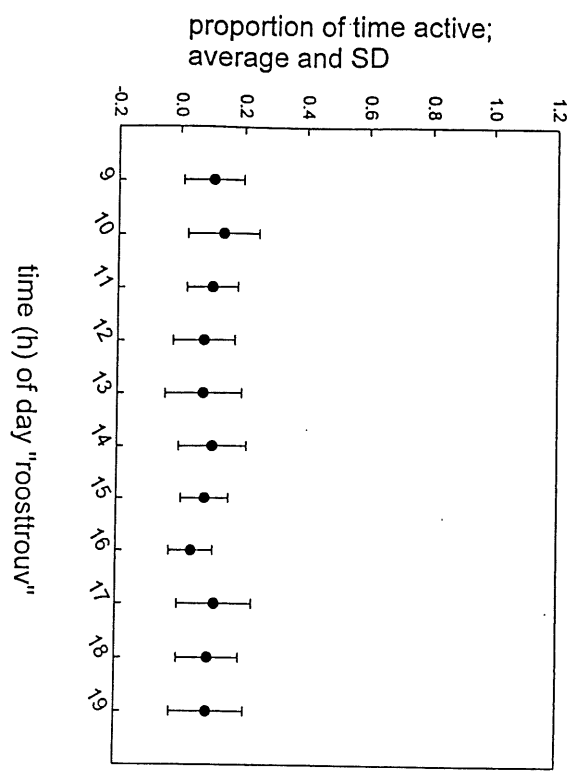
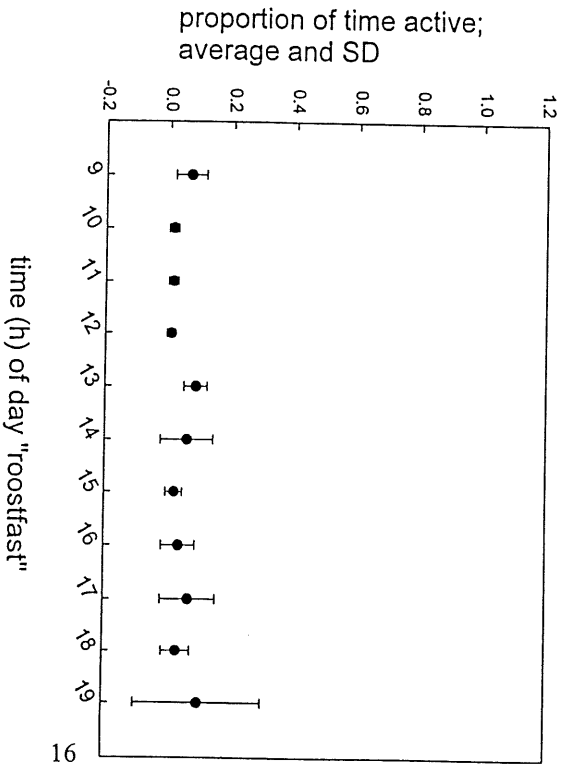
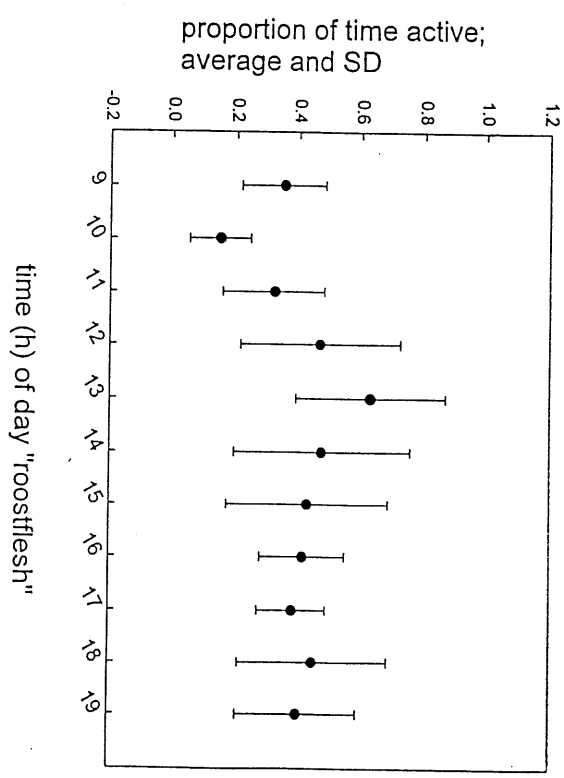


Fig. 7 e / h. Activity patterns over the experimental days roosttrouv, roostflesh, mudfast and roostfast. The proportion of time a knot is active during the scans of one hour is calculated. These figures depict the average and SD of the flock of six birds.









3.4 Distance

In figure 8, the average step rates are tabulated. Appendix 2 contains the step lengths of the 27 trails that were measured. The resulting covered distances are graphed for the different days in figure 9. On roostdays, there was still some walking, especially on ROOSTFLESH, which involved a novel food type. However, on the roost the Knots walked generally much less than on the mudflat days. These distances, covered in the experimental aviary (7 * 8 m) are quite remarkable.

steps/min	MUDCOCK1	MUDCOCK2	MUDDEAD	ROOSTCOCK	ROOSTFAST	ROOSTFLESH	MUDFAST	ROOSTTROUV
B	31.8	26.9	66.5	11.5	1.9	6.1	104	4
LG	80.1	71.9	76.5	13.4	6.2	38.5	70.6	2
O	52.6	43.9	76.6	9.6	8.8	31.9	78.5	3.7
RY	33.7	39.3	82.4	23.5	7	15.2	88.5	13
W	51.9	38.1	64.6	8.5	4.9	19.3	81.8	6.1
Y	29.8	27.9	53.9	14.3	7.2	16.9	62.1	

Fig. 8. Average number of steps per minute during the experimental period.

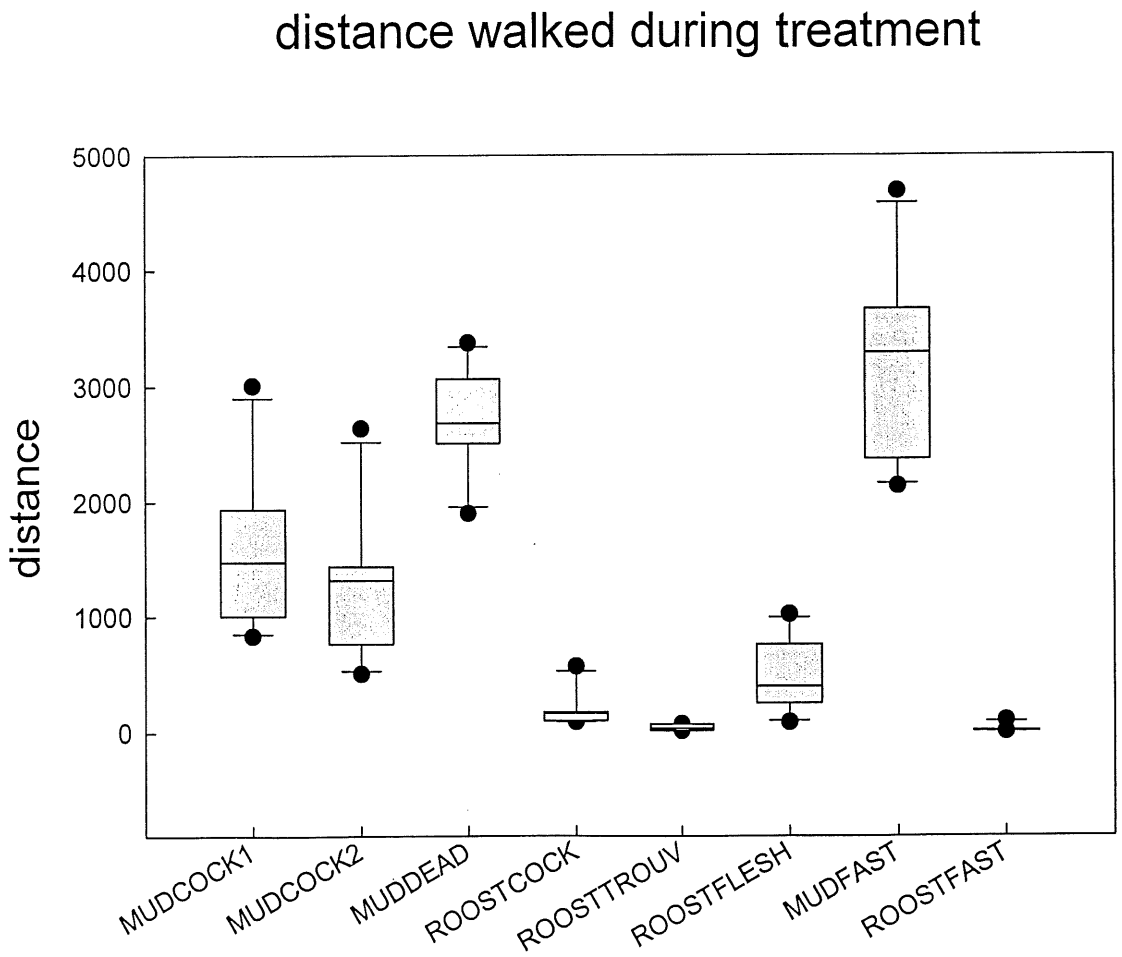


Fig. 9. Distances in meters, walked during the experimental period of the day (which differs a bit between days). Boxes depict 25th and 75th percentiles, the median and 10th and 90th percentiles are also shown. Black dots are values beyond 10th and 90th percentiles.

3.5 Food intake

3.5.1 Quantities

The six Knots ate an almost unbelievable amount of shellfish. During the two month period when the doubly labeled water treatments were performed, daily (24h) intake per individual was on average 852 ± 294 (SD) g fresh Cockles, which contains 67.7% water (577g) and 1.7% AFDM (14.5 g).
This was similar during the doubly labeled water treatments. Figure 10 tabulates the number of Cockles and items of other food kinds and the average length of the Cockles that were eaten. Sizes of other food types were not estimated.

treatment	#/ min	SD	length	SD
mudcock1	3.7 (2.4-5.8)	1.2	10.3 (7.7-12.9)	2.2
mudcock2	3.5 (1.9-5.9)	1.3	9.9 (7.2-12.0)	1.7
muddead: whole	0.9 (0.5-1.3)	0.3	10.3 (8.4-11.9)	1.2
muddead: flesh	3.7 (2.4-5.5)	1.1	n.a.	
roostcock	1.3 (0.7-2.1)	0.5	8.9 (7.6-9.9)	1.0
roosttrouv	0.3 (0.0-1.0)	0.4	n.a.	
roostflesh	0.4 (0.2-0.6)	0.2	n.a.	
mudfast	0.1	n.a.	n.a.	
roostfast	0	n.a.	n.a.	

Fig. 10. Average, range, and SD of the number of ingested food items per minute and their size (length in mm) in case the food consisted of Cockles. N.a. means "not available". (For an overview of food types see fig. 1.)

3.5.2 Contents of Cockles

In seven Cockle batches, AFDM was weighed per length class. The results are shown in appendix 3. These values were used to estimate a formula for the relationship between AFDM and length. There was a strong correlation between ln(length) and ln(afdm); r2=0.9842 . The multiple regression analysis indicated no significant interaction between batch and length, which means that the dependence of AFDM on length is the same for all batches. Intercept differed significantly between batches and therefore batch specific formulas are given, see figure 11. These formulas are used to compute the AFDMasses per length class, see examples (also figure 11)
A formula for the three other intake variables fresh mass, water mass and calcareous shell mass, was derived from data of only 7 January, but the methodology was the same. (fig 11) About one third of the mass of a living Cockle is made up by the calcareous shell, and about two thirds is water. The amount of useful ingredients is almost negligible, see figure 12!

The contents for the food items other than whole Cockles are shown in § 2.7.

ROOSTFLESH: The flesh of large Cockles contained 15.1 % afdm and 82.3 % water. In total, 1.46 * 10^3 items were eaten that weighed 808 g together. Thus one item contains 0.08 g afdm and 0.45 g water.

3.5.3 Total Intake

Number of items eaten, multiplied by their contents as described in the previous paragraph give the total daily intake, see figure 13. The individual values (not depicted) will be used in the predictive model.

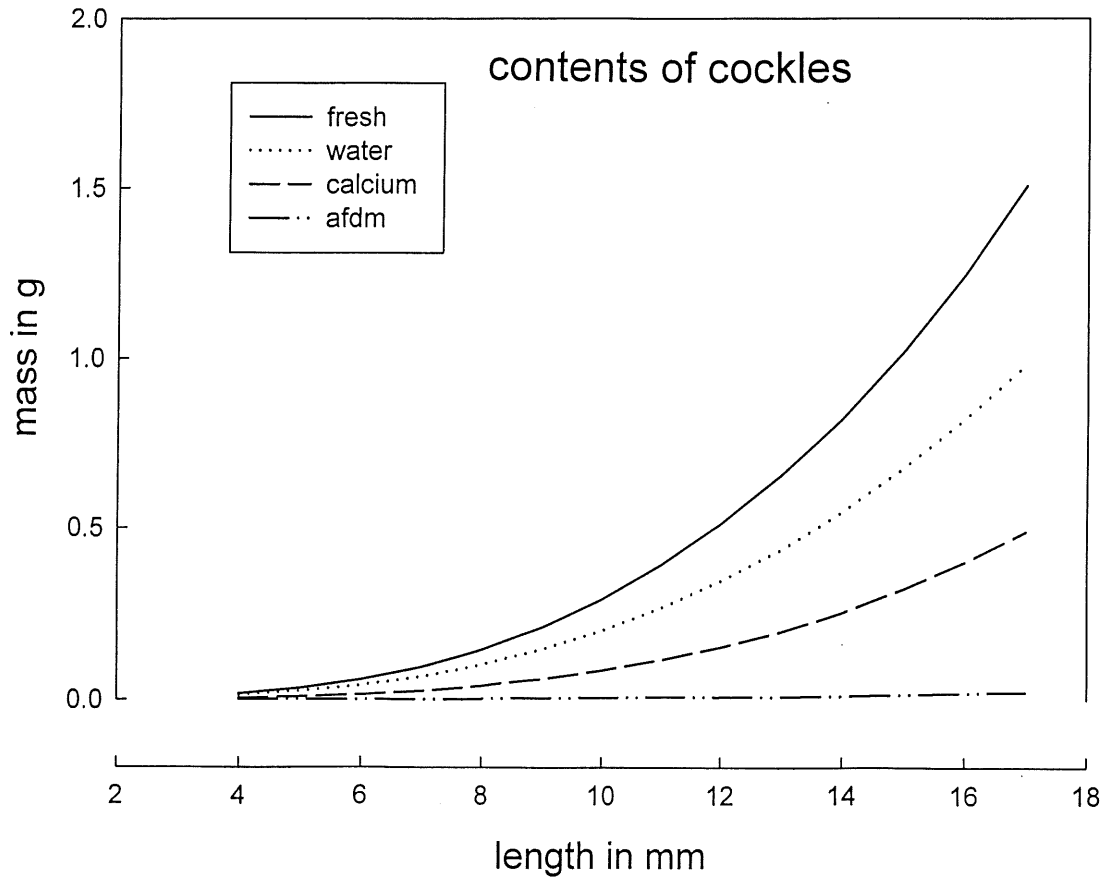
Fig. 11. Batch specific formulas for AFDM and formulas for bulk, calcareous and water mass, all dependent on length of the Cockles.

AFDM

Model: ln(afdm)= intercept +batch correction +ln(length)*coeff_length

sampling			formula:			examples (in g)		
	sampling date	used for:	intercept	correction	length coefficient	8mm	10mm	12mm
batch1	12/16/97		-12.615	0.0179	3.1087	0.0022	0.0043	0.0077
batch2	1/7/98	MUDCOCK1	-12.615	0.0996	3.1087	0.0024	0.0047	0.0083
batch3	1/9/98		-12.615	-0.0417	3.1087	0.0020	0.0041	0.0072
batch4	1/12/98	MUDDEAD	-12.615	-0.3044	3.1087	0.0016	0.0031	0.0055
batch5	1/16/98		-12.615	-0.145	3.1087	0.0018	0.0037	0.0065
batch6	1/27/98	MUDCOCK2	-12.615	0.2238	3.1087	0.0027	0.0053	0.0094
batch7	2/12/98		-12.615	0.1498	3.1087	0.0025	0.0050	0.0087
overall		ROOSTCOCK &MUDFAST	-12.615	0	3.1087	0.0021	0.0043	0.0075
bulk								
batch2	1/7/98	all if relevant	-8.401		3.1100	0.14	0.29	0.51
calcareous								
batch2	1/7/98	all if relevant	-10.145		3.3270	0.04	0.08	0.15
water								
batch2	1/7/98	all if relevant	-8.546		3.0100	0.10	0.20	0.34

Fig. 12. The components of the average Cockle per length class. This is to show clearly that the amounts of AFDM are indeed very low, but unsuitable for comparing relative amounts of AFDM. (but see fig discussion)



variable:	Mudcock1	Mudcock2	Muddead
afdm (g)	12.0 (7.6-18.5)	11.9 (8.4-14.5)	7.7 (6.3-9.2)
shell (g)	218.9 (131.0-348.5)	191.9 (125.4-236.5)	64.5 (29.5-136.8)
water ingested (g)	500.4 (325.1-760.1)	440.0 (317.3-531.6)	315.6 (235.7-408.4)
bulk: from observations	737.6 (467.1- 1137.6)	647.9 (452.9-788.0)	397.3 (279.3-564.1)
	Roosttrouv	Roostflesh	Mudfast
afdm (g)	11.9 (0-40.2)	20.4 (10.3-34.4)	0.3 n.a
shell (g)	0.0 0.0	0.0 0	6 n.a
water ingested (g)	6.1 (0-20.6)	110.8 (55.8-187.0)	14 n.a
bulk: from observations	19.8 (0-67.0)	134.7 (67.8-227.2)	20 n.a

	Roostcock
afdm (g)	2.6 (1.6-3.4)
shell (g)	50.1 (30.7-66.6)
water ingested (g)	119.6 (74.4-158.4)
bulk: from observations	173.9 (107.7-230.7)
	Roostfast
afdm (g)	0
shell (g)	0
water ingested (g)	0
bulk: from observations	0

Daily intake (g) during ROOSTCOCK;
second method; all values are for an average bird

average ingested: (g)	Roostcock
afdm	4.9
water influx	168.2
bulk	248.3

Fig. 13. Daily (24h) intake: average and range for ingested AFDM, shell mass, water and total mass ("bulk" or "fresh") Values are derived from observed number of ingested items, see fig. 10. Values per day=24h are given for convenience, but in the model intake per second was used.

3.5.4 Water flux rates

The values are given in figure 14 per second, for use in the model.

The highest values (0.008 g/s) correspond to 700 g /day (24h). Water flux rates were also estimated via the ingested food. There are strong reasons however to prefer DLW derived values:

1) The amount of water the Knots drank is unknown.
2) The amount of water ingested with food is difficult to estimate, because it depends on how many Cockles remains closed when being eaten and therefore contain much more water.

3.6 Temperature

Higher temperatures were measured on the Mudflat than on the Roost (see figure 15 a-h, next pages). Although all values are about the lower border of the thermoneutral zone, some values are certainly below.

Fig. 15a-d . Hourly averaged air temperatures in °C. a) Mudcock1 b) Mudcock2 c) Muddead d) Roostcock

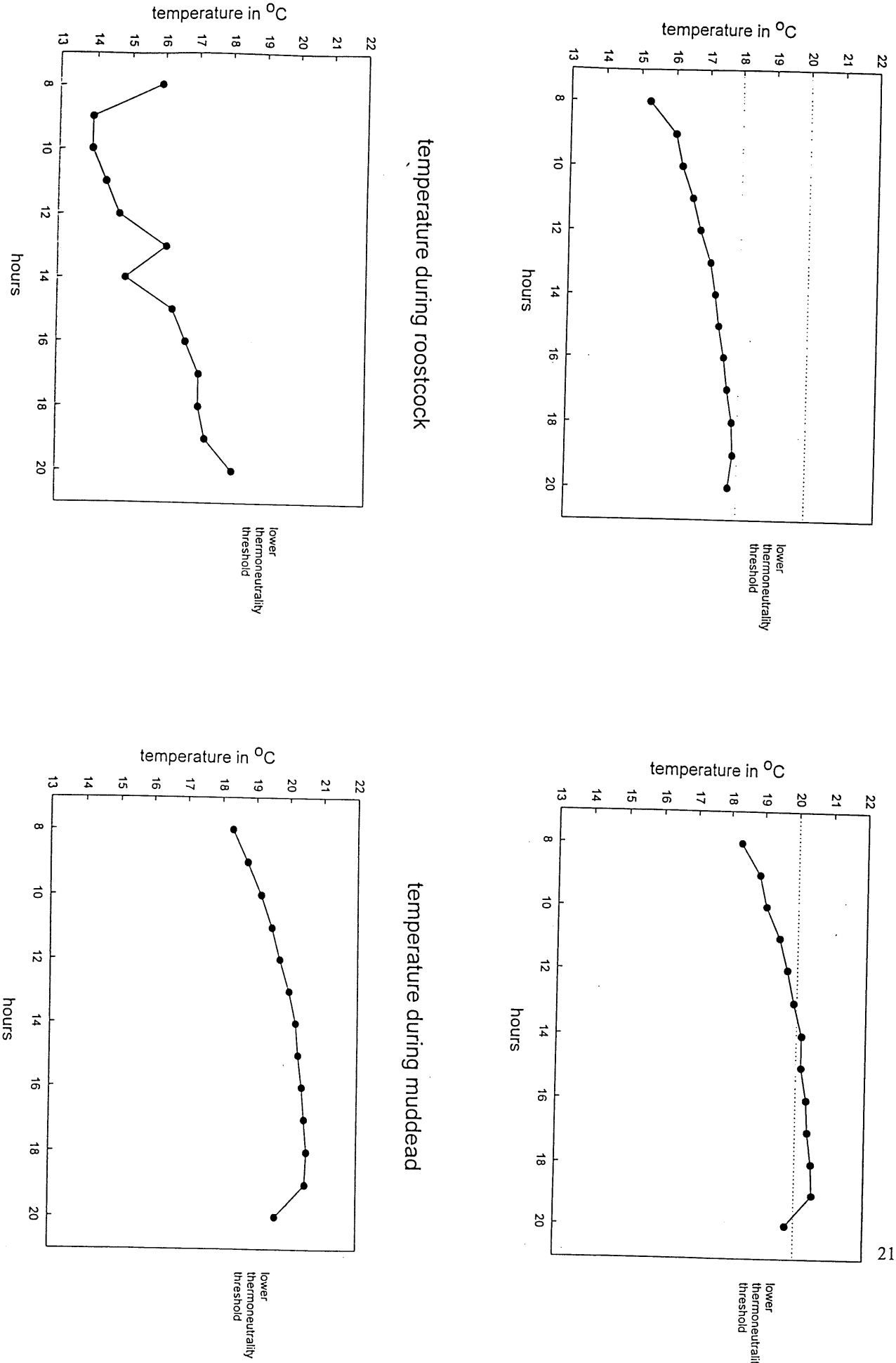
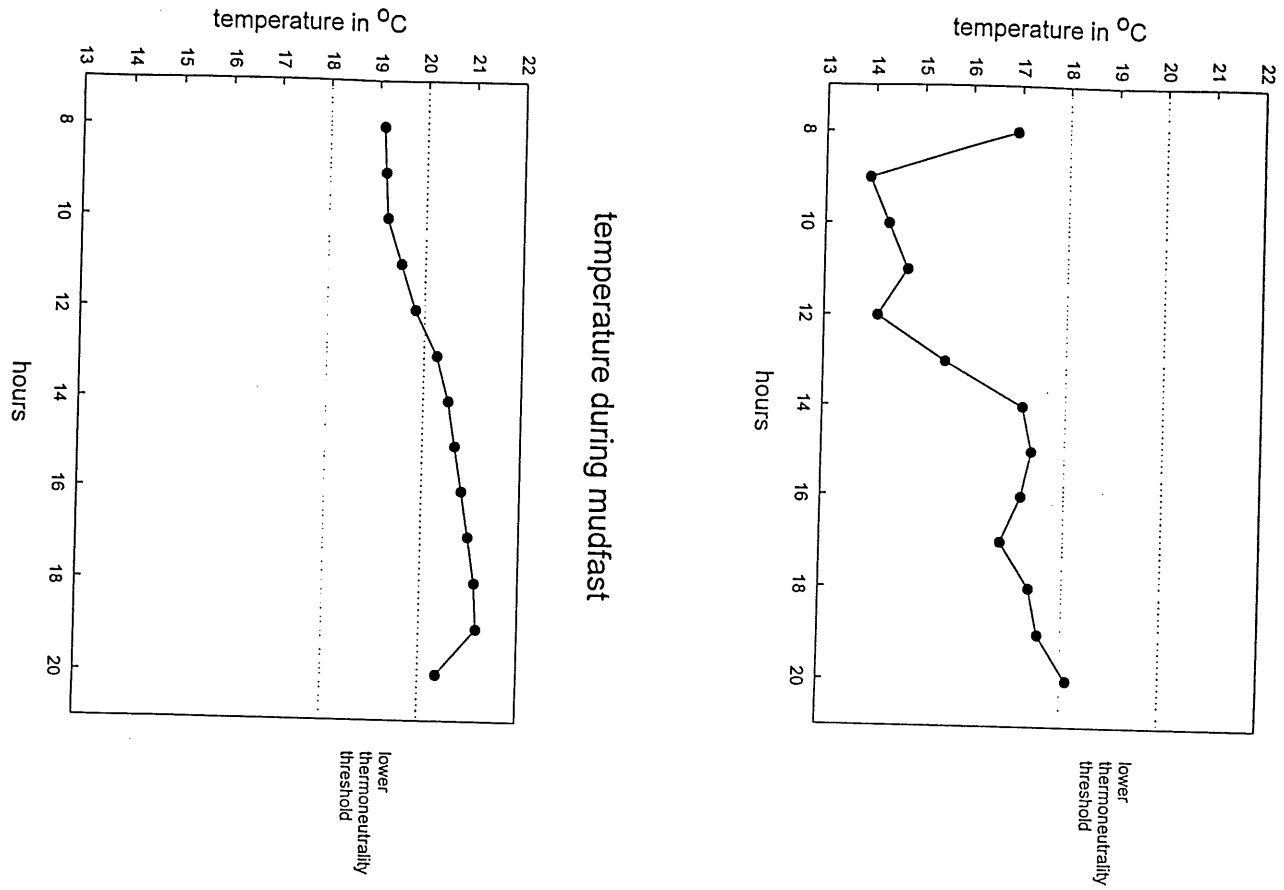


Fig. 15 e-h . Hourly averaged air temperatures in °C. e) roosttrouv, f) roostflesh, g) mudfast, h) roostfast.



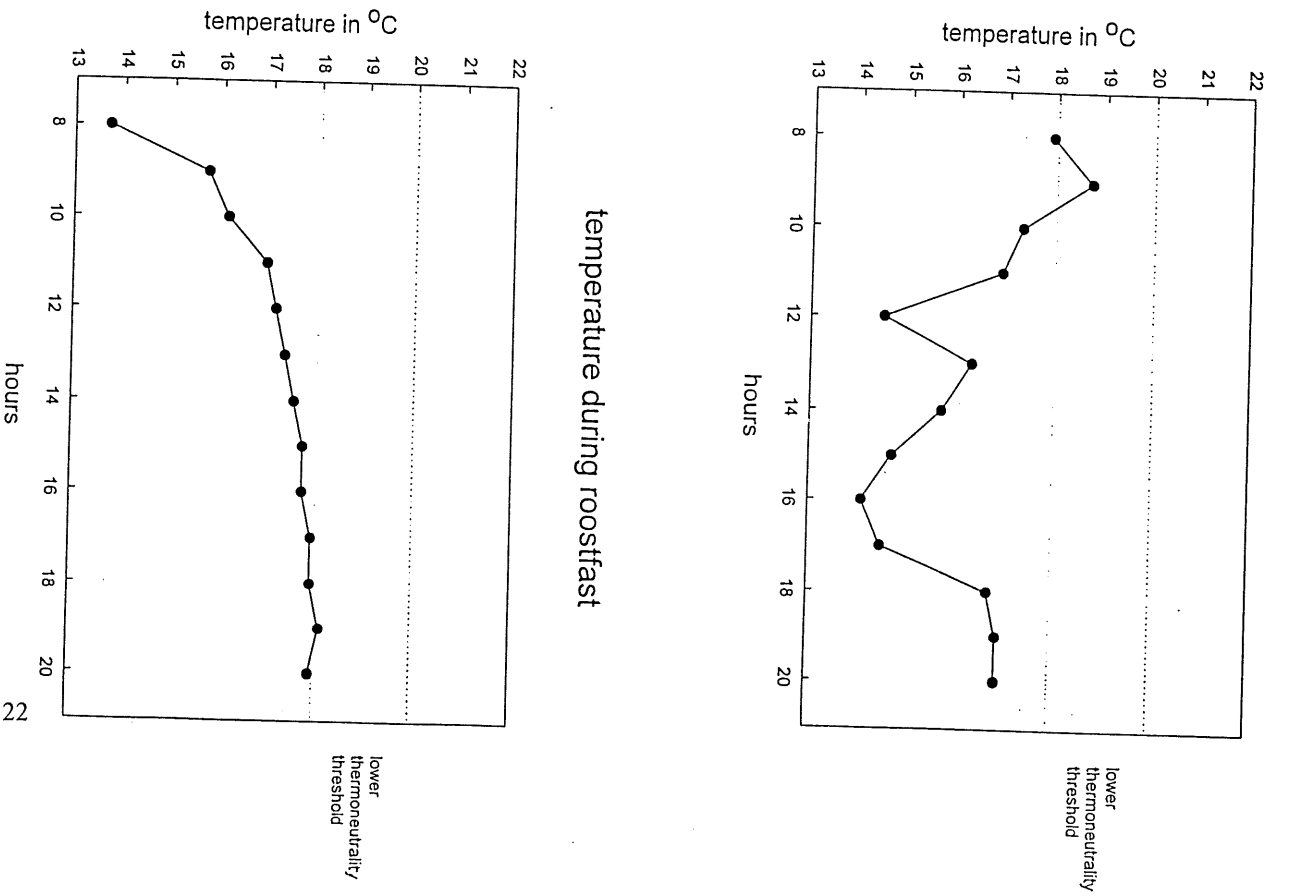


Fig. 14. Water flux rates measured with the DLW technique. Boxes depict 25th and 75th percentiles, the median and 10th and 90th percentiles are also shown. Black dots are values beyond 10th and 90th percentiles.

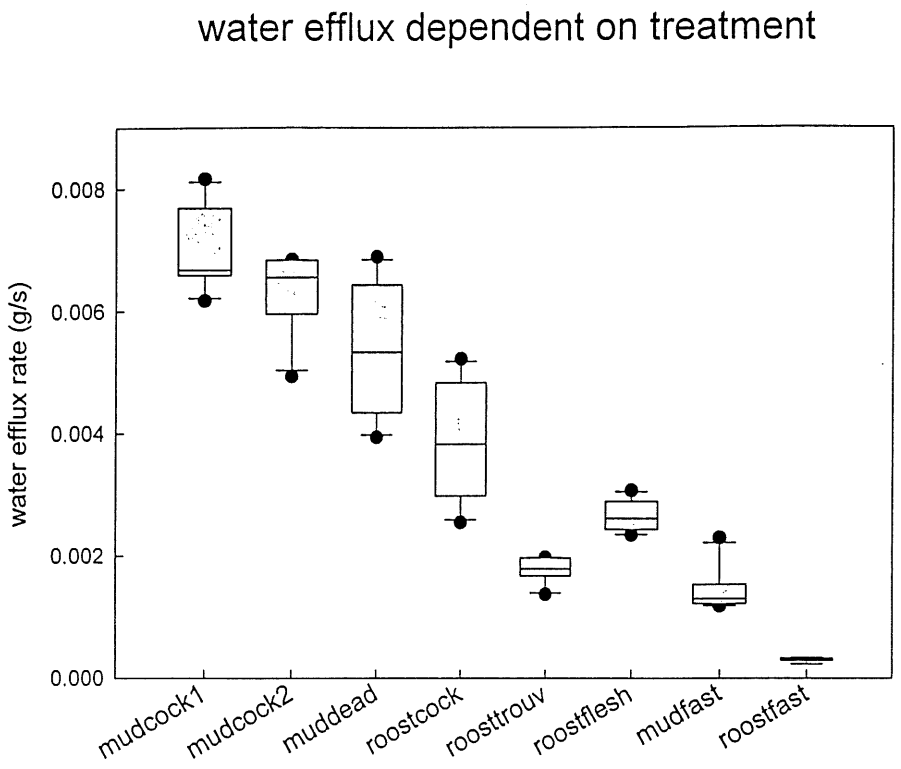
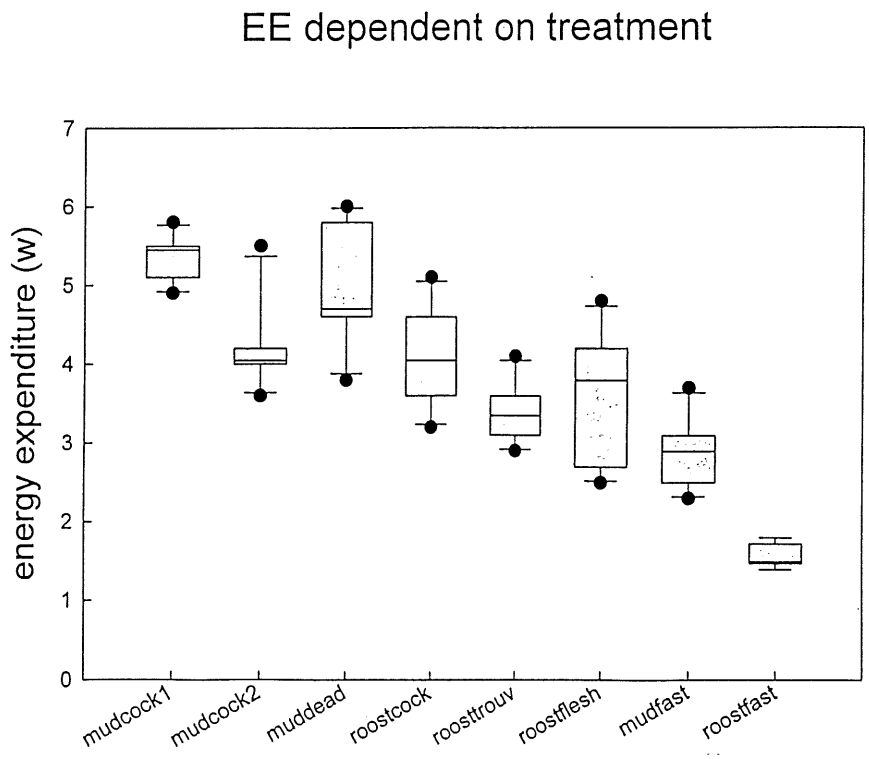


Fig. 16. Energy Expenditures (watt) of the six Knots in the 8 days. Boxes depict 25th and 75th percentiles, the median and 10th and 90th percentiles are also shown. Black dots are values beyond 10th and 90th percentiles.



3.7 energy expenditure (EE)

Isotope enrichments in the feces were not different from those in the blood (Visser et. al. subm.). The estimates of energy expenditure are shown in figure 16. An ANOVA procedure in spss 8.0 (GLM repeated measures) could be performed to test if there are significant differences in energy expenditure between days. However with five individuals, only five days can be tested, so some days have to be pooled or neglected.
In two cases pairs of days exist where we had the same expectation about EE. Two paired t-tests were performed to check whether the data from the pairs MUDCOCK1/ MUDCOCK2 and ROOSTTROUV/ROOSTFLESH can be considered as stemming from Knots experiencing the same energy expenditure, and hence can be pooled. MUDCOCK1 differs significantly from MUDCOCK2 (t=3.791, df=5, p=0.011) and these cannot be pooled. MUDCOCK1 was then discarded for this analysis, because the worse condition of the animals compared to all other days presumably caused the difference.
ROOSTTROUV and ROOSTFLESH do not differ significantly (t= -0.764, df=5: p=0.479, NS) and are therefore pooled for the following analysis. Still, one extra day should be dropped. For MUDDEAD the expectation for EE was least clear and it was also the least interesting treatment. Therefore, the EE values of MUDDEAD are skipped from this analysis.

The overall test indicates significant overall differences in EE (overall $F_{(4,1)} = 910.6$, p=0.025). As Mauchly's W was 0.001 and therefore a trend (p=0.097) exists that spherecity assumptions can't be made, Huynh-Feldts epsilon was used to correct the degrees of freedom in the test of within subjects effects. There are highly significant within subject effects which indicate differences in EE between days ($F_{(2.5\ ,\ 10.1)}= 19.9$, p<0.001). The tests of within-subjects contrasts show that whereas linear effect is highly significant ($F_{(1.4)}= 164.4$, p<0.001) the higher (2^{nd}, 3^{rd}, 4^{th}) order effects are not significant ($F_{(1.4)} = 2.3$, 0.0 and 0.7 respectively, corresponding p-values 0.205, 0.892, 0.448).

This does not necessarily mean that these differences in EE were caused by the treatments. Since every treatment was given only once and birds were not randomly assigned to a treatment, our design cannot separate the effects of treatment and day.
The six replicates we have per treatment are no real replicates. They only improve the estimate for EE.

By the way, there are also differences between subjects, birds in this case, these were highly significant. (F=1102, p<0.001)

Statistically founded is now the general pattern "days differ in EE" but we can't make the intended statements "searching/crushing/digesting costs amount to...watt". Neglecting this for a short moment, it is tempting to see in fig. 16. that the two days where the Knots were fasting have much lower results than the other days. The difference between mudfast and roostfast which reflects activity costs, is much higher than the difference between mudcock and roostcock should reflect the same activity cost; but it will be shown in fig. 8. that Roostcock had higher activity levels than roostfast. Also it is remarkable that the results for MUDDEAD and MUDCOCK1, both dropped from this analysis, are higher than those for MUDCOCK2. Maybe this figure is a bit too low?
These facts call for another way to interpret the data. Fortunately, it is still possible to construct a predictive model for energy expenditure, based on independent factors that were viewed in the previous paragraphs.

3.8 Predictive models

The general linear model (GLM) in principle estimates the effect of an independent factor on the dependent variable (Energy Expenditure = EE in watt) when other variables would be constant. The problem with such models is not so much how to come to a result-output is always given- but what factors to begin with, and how many interactions to build. Therefore a number of possible starting points is discussed: in this way the reader gets an idea of the robustness of the conclusions to different assumptions. I'm not entirely sure at the moment that it's statistically permissible to use the 47 bird-day combinations instead of the 8 average values. So also the average values will be analyzed statistically and the results compared. The independent factors in the analyses are Average Body Mass (avgBM), Change in Body Mass (dBMs), Temperature (temp), Activity Measure (logAR), Distance (dist_s), Nr Items (items_s), AFDM (afdmg_s), Shell (shellg_s), Fresh (freshg_s) and Water Flux Rate (fluxg_s). It must be remarked here that AFDM (normally protein from Cockles) does not mean the

same on ROOSTTROUV were it is the amount of fat and protein from the pellets, and that "number items" is another factor that has multiple meanings: pellets, pieces of flesh or wholeCockles.

3.8.1 Individuals

Not all factors were known to individual level for some days: This is the case for items and hence amounts eaten on Roosttrouv and Mudfast because there were not enough observations for a reliable estimate of individual intake rate. On Roostfast, all five individuals ate nothing, and of course also the room temperature was always the same for each individual.
Including all 10 independent variables and EE, a correlation matrix was produced and given in fig 17.

There are a lot of significant correlations, especially when using all birds separately. LogAR, dBM, items, afdm, shell and fresh have significant correlations with flux, and avgBM, temp and dist are significantly correlated with logAR. This means in fact that the "independent" variables are not really independent and will give multicollinearity problems when incorporated together in a predictive model. (Zar 1984, page 338)

3.8.2 Models (N=47):

A] First, all 10 independent variables were incorporated and also the expected interaction between avgBM*distance. This results in the following model:

Adjusted r^2=75.6 $p<0.01$ EE= 6.60 - 0.222* temp +0.562* logAR + 310* flux

LogAR correlates strongly with temp and also with flux. This raises the problem of multicollinearity. Maybe because the difference in artificial lighting, most variation in temperature is in fact between Mud days (high temp) and Roost days (low temp), and of course logAR has the same pattern. Within the location there is rather a negative relationship between logAR and temp. Therefore it's perhaps better to not incorporate temp. Other reason to drop temp from the variable list is that temperatures are almost in the thermoneutral zone, and heat produced by digestion, assimilation and activity can then supposedly compensate for the energy needed in thermoregulation (Bruinzeel and Piersma, 1998).

B) Without temp, other variables make up the model.

Adjusted r^2=75.0 $p<0.01$ EE= 2.73 + 0.314* logAR +267*10^1* dBMs – 199*10^1* afdmg_s + 287 * fluxg_s

LogAR correlates with flux; dBMs correlates with afdm en flux; afdm also correlates with flux. This again raises multicollinearity problems. Possible solutions: solving the most biggest multicollinearity problem (dBMs with afdm) by omitting dBMs causes first afdm and next logAR to be skipped and the model becomes:

Adjusted r^2=68.1 $p<0.01$ EE= 2.25 + 430 * flux

Omitting afdm results in yet another model:

Adjusted r^2=72.4 $p<0.01$ EE =2.53 + 0.317*logAR + 116*10^1* dBMs + 289* flux

Correlation, based on values per bird and per day (N=47), between all variables used in the predictive models

		AVGBM	TEMP	LOGAR	DBMS	DISTM_S	ITEMS_S	AFDMG_S	SHELLG_S	FRESHG_S	FLUXG_S	EEJ_S
AVGBM	Pearson Correlation			*	*	*		*				
TEMP	Pearson Correlation	-.132		**	*	**	**					
	Sig. (2-tailed)	.377										
LOGAR	Pearson Correlation	-.302*	.580**			**	**		**	**	**	**
	Sig. (2-tailed)	.039	.000									
DBMS	Pearson Correlation	.334*	-.360*	.183				**	*	**	**	**
	Sig. (2-tailed)	.022	.013	.217								
DISTM_S	Pearson Correlation	-.352*	.778**	.785**	-.177		**					
	Sig. (2-tailed)	.015	.000	.000	.233							
ITEMS_S	Pearson Correlation	-.264	.393**	.792**	.152	.438**			**	**	**	**
	Sig. (2-tailed)	.073	.006	.000	.307	.002						
AFDMG_S	Pearson Correlation	.349*	-.230	.136	.833**	-.202	.153		*	**	*	*
	Sig. (2-tailed)	.016	.120	.361	.000	.174	.304					
SHELLG_S	Pearson Correlation	-.230	.207	.454**	.296*	.123	.560**	.325*		**	**	**
	Sig. (2-tailed)	.120	.163	.001	.044	.411	.000	.026				
FRESHG_S	Pearson Correlation	-.210	.247	.563**	.375**	.188	.674**	.430**	.974**		**	**
	Sig. (2-tailed)	.156	.094	.000	.009	.205	.000	.003	.000			
FLUXG_S	Pearson Correlation	-.140	.220	.683**	.465**	.246	.798**	.369*	.845**	.900**		**
	Sig. (2-tailed)	.347	.137	.000	.001	.095	.000	.011	.000	.000		
EEJ_S	Pearson Correlation	-.131	.084	.680**	.507**	.261	.704**	.313*	.576**	.652**	.830**	
	Sig. (2-tailed)	.380	.576	.000	.000	.077	.000	.032	.000	.000	.000	

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Fig. 17. Correlation matrix of all 47 bird-day combinations, showing pearson correlation coefficients and the significance of their departure from zero.

Correlations, based on average values per day (N=8), between all variables used in the predictive models

		AVGBM	TEMP	LOGAR	DBMS	DISTM_S	ITEMS_S	AFDMG_S	SHELLG_S	FRESHG_S	FLUXG_S	EEJ_S
TEMP	Pearson Correlation	-.203				**						
	Sig. (2-tailed)	.629										
LOGAR	Pearson Correlation	-.432	.618			*	*			*	*	*
	Sig. (2-tailed)	.285	.103									
DBMS	Pearson Correlation	.336	-.362	.298				**				
	Sig. (2-tailed)	.415	.378	.473								
DISTM_S	Pearson Correlation	-.461	.851**	.807*	-.148							
	Sig. (2-tailed)	.250	.007	.015	.727							
ITEMS_S	Pearson Correlation	-.431	.426	.828*	.245	.461			*	**	**	*
	Sig. (2-tailed)	.286	.292	.011	.558	.250						
AFDMG_S	Pearson Correlation	.479	-.240	.243	.941**	-.176	.255					
	Sig. (2-tailed)	.230	.568	.561	.000	.676	.542					
SHELLG_S	Pearson Correlation	-.447	.230	.639	.297	.250	.777*	.301		**	**	
	Sig. (2-tailed)	.266	.584	.088	.476	.551	.023	.469				
FRESHG_S	Pearson Correlation	-.402	.270	.742*	.391	.312	.876**	.404	.974**		**	*
	Sig. (2-tailed)	.324	.518	.035	.338	.452	.004	.321	.000			
FLUXG_S	Pearson Correlation	-.356	.237	.804*	.469	.335	.916**	.421	.898**	.957**		**
	Sig. (2-tailed)	.387	.572	.016	.241	.417	.001	.299	.002	.000		
EEJ_S	Pearson Correlation	-.315	.108	.808*	.619	.354	.829*	.485	.700	.793*	.920**	
	Sig. (2-tailed)	.447	.800	.015	.102	.389	.011	.223	.053	.019	.001	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Fig.18. Correlation matrix of the variables when using only the 8 averages per treatment. Shown are pearsoncorrelation coefficient and the significance of the departure of the correlation coefficient from zero.

3.8.3 Residuals analysis (N=47)

The presented models are suffering from multicollinearity except of course the one with flux alone predicting EE. Is there nothing more in the data set? Another way to investigate this is by calculating the residuals from the regression of EE on flux, and then regressing those residuals on other independent variables. I regressed them on all nine remaining variables, and found the highest r^2 (of shell) to be 0.05, with p=0.13. The variation in residuals can therefore not be attributed to another independent factor.

3.8.4 Averages per day

The problem here is that there are more independent variables (10) than degrees of freedom (7) So the correlation matrix (**Fig. 18**) can be of help in choosing which variables are skipped from the analysis. Shell, fresh and items are strongly correlated with flux and can be omitted. Temperature correlates with distance and has other good reasons to be skipped (see previous paragraphs) Afdm is strongly correlated with dBMs.

A) With logAR , distance, avgBM, avgBM*distance, dBMs en flux the following model results:

Adjusted r^2=82.1 p<0.01 EE= 2.16 + 451 * flux

B) Alternatively the interaction is skipped from A, but afdm incorporated. Result: the same!
C) Alternatively logAR is omitted from A (correlates with flux) Result: idem!
D) If you omit the interaction avgBM*dist from A, and incorporate no new variables, then a model pops up without flux!
Adjusted r^2=87.0 p<0.01 EE= 4.64 + 1.75* logAR – 31.5* distm_s.
But of course there is significant correlation between logAR and dist.

To escape from multicollinearity, the residuals of EE on flux were regressed again on the independent variables, one by one. The biggest r^2 was 22.9 with an accompanying p=0.23. Again, like with the 47 bird-day combinations, the variation in the residuals can not be explained by the remaining independent factors.

3.8.5 Picture arising from the models

In all options but one, the model contains water flux rate and it becomes clear that the water flux rate is the factor that has the closest relationship with energy expenditure in foraging Knots, postponing the question whether this relationship is a causal one to the discussion.
The first presented option suffers from multicollinearity with an unknown impact. The result is not so strange, but I suspect that the high temperature on Mud-days versus low temperature on Roost-days can groundlessly assign this effect to temperature and therefore I skipped it in the next models.
The second option gives a positive relationship between Energy Expenditure and Body Mass Change which, is not expected because a High-EE-bird would in theory deposit less new body tissue than a Low-EE-bird, when eating the same amount. However for difference in Body Mass, it is the difference between intake and expenditure that matters and not expenditure alone. Also, the increase in Body Mass doesn't have to resemble newly deposited tissue, because it also incorporates increase in intestinal contents. The negative relationship of EE with afdm is unexpected because the simple regression (and common sense) yields a significant positive coefficient. I think the high and opposite coefficients in this results are caused by multicollinearity.
The third model, with only flux determining energy expenditure explains less variation, but has otherwise no problems. The fourth option has the same consideration about dBMs as the second, and still suffers from multicollinearity.

The residual analysis avoids multicollinearity. The results support model option three and the conclusion that water flux rate is what really matters in determining energy expenditure of foraging Knots. [According to the model with only water flux rate, the impact of flux on EE can be 3.4 between highest (mudcock1, Y) and lowest (roostfast, LG) recorded value.]

The analyses with the average values per day back up the same conclusion. In three of the four options, water flux rate comes out as the sole determinant of EE. In the other option, the flux is omitted but this is probably due to the correlation with logAR. The residual analysis again shows that no other variable can explain the

variance in residuals of EE on flux.

4 Conclusion and Discussion

4.1 Conclusion

This study focussed on energy expenditure of Knots undergoing a wide variety of foraging conditions, and data were collected on 8 days with 7 treatments (1 treatment was repeated). This should enable us not only to estimate total energy expenditure during foraging, but also to distinguish the importance of the three main aspects in which foraging costs are subdivided here: costs of gathering the bivalve prey, costs of crushing the their shells and the costs of digesting and processing the ingested food.
Energy expenditures ranging from 1.4 to 6.0 watt (flock averages: 1.6 to 5.4 watt) have been measured with the DLW technique. As BMR is slightly below 1 watt, this can be read as ranging from 1.6 to 5.4 times BMR. The latter value is high but still in the range of birds reaching their maximum sustainable level of energy expenditure (Bryant and Tatner, 1991; Obst and Nagy, 1992). However it must be born in mind that presented values only cover the foraging period, so Daily Energy Expenditure can in reality be much lower if the birds are at rest during the other 13 hours of the day.

Clear differences were found in energy expenditure between days. (see fig. 16 and also 1.) Alas, it can not be concluded that these differences are due to the treatment; there could also be day-effects. Most important of these is that the history that Knots underwent because of earlier treatments differed between days. Another point is that in the first treatment, MUDCOCK1, Knots were trained for another design and were therefore in more stressed condition than later on in the study. Although treatments were more or less planned randomly over the period, three of the four MUD days and all four Cockle days were among the first four days so effects of for example annual cycle are not totally excluded.

Despite these shortcomings of the design, a model was fitted through the data to construct a picture of what determines energy expenditure during foraging.
This modeling suffered from multicollinearity between the supposed independent variables, but it can be concluded that energy expenditure is mainly dependent on water flux rate.
Now first possible errors in estimating the results will be viewed. After that the focus will be on interpreting the energy expenditures.

4.2 Reliability of measurements

4.2.1 DLW data

There are two methods of estimating TBW, the "extrapolation" and the "plateau" method. There is of course at least some isotope loss during equilibration, but this turnover rate will in most cases be lower than during the treatment. Furthermore, loss during equilibration will be the same every day, but turnover rate during treatments was different. So the truth lies somewhere in between the results of the plateau and extrapolation method.
TBW is used in computation of water efflux rate as well as energy expenditure and errors in TBW will propagate in these estimates.
The plateau method results in a slight underestimation of initial enrichment and therefore in an overestimation of amount of body water. As the birds were always treated the same way every day, it would presumably be better to use the extrapolation method, but with the value of fasting animals for all days, instead of using the experimental turnover rate.
Because of the generally high water turnover, the differences in Ko and Kd were very small and small errors in estimating Cf can have quite dramatic effects on estimated EE (tens per cent). Although errors may be large, they will be random errors and will only effect the test for differences in EE between days, not the predictive models.
Some researchers recommend heart rate as a good predictor of energy expenditure (Nolet et. al. 1992) but this involves individual calibrating and is sensitive to stress influences that were certainly present in our study animals.
To correct for fractionation effects (see methods) a constant value of evaporative loss was used. This value is a very rough one because not all parameters (eg water vapor pressure) needed for calculation are known, but is the best estimate we have. (Verboven en Piersma, 1995).

Water flux rate estimates are much more reliable than EE estimates, because in the latter errors in two fractional turnover rates multiply.

4.2.2 Other data

4.2.2.1 Body mass

Body masses are normally consistently higher in the evening than in the morning. As the difference in mass of an empty and a filled gut is enough to account for the difference, it could easily be explained by a higher food intake during the day. However, Knots are known to be actively foraging at night, so this difference is still unexpected.

4.2.2.2 Behavior

The number of activity scans per bird per day varied between 89 and 307. The average period between scans varied between 2.1 min and 7.1 min. This should be enough to estimate reliably the time budgets.
It is important to know whether the time allocated to the different behaviors stays the same during the experimental period. If there would be clear active and inactive phases, or Knots would for example be more active in the beginning than at the end of the day, the measured EE would be less informative. However, at a glance (see 7) behavior seems fairly constant over the day.

4.2.2.3 Intake

The consumed number of food items is more problematic because there are roughly six times fewer values than with the scans. On some (mudflat) days Knots forage more or less constantly so problems are negligible, but on ROOSTROUV it happened that one animal was not observed eating at all, although it clearly increased in mass. For this treatment, the frequency of intake observations was too low for use of individual data in the model.
When Knots eat trouvit, they drink a lot of fresh water, so the estimate of water intake from the trouvit alone in **Fig. 13.** is in fact far too low but was given nevertheless here, because we didn't know how much water the birds drank. In the model, the values estimated with the DLW method were used.
The length of the eaten Cockles is fairly difficult to estimate. The reference Cockles glued to the wall were generally not visible in one view with the foraging Knot, which was observed from 0 to 8 meters away. Bill length was also used as a reference but not all birds have exactly the same bill length, which can result in errors. Length estimates could not be made when the bird turned its tail to the observer. The possibility of estimating the length depends on handling time, and the Cockles that could be estimated are therefore biased towards the larger ones. This would result in a systematic overestimation of the intake variables on the Cockle days, especially Mudcock1 where far less ingested Cockles could be estimated.
The Cockle contents, measured per length class (mm) could have been used directly to calculate intake of AFDM, shell, water and bulk. Instead, first doubly logarithmic formulas were derived from the Cockle masses dependent on length. The rationale for this was that theoretically, the masses should follow such a doubly logarithmic curve; we assessed that deviations from this curve are more likely caused by errors in estimating the mass parameter of Cockles of that specific length, than by real-life differences in dependence of mass on length. However, we don't know if this method really gives more accurate results.

4.2.2.4 distance

The covered distance had no significant effect on energy expenditure. However, step lengths were only estimated on the mudflat. On the roost where the food is easily accessible in one spot, step lengths were much shorter. This explains the high values on roostdays in **Fig. 8.** This possible underestimation of the difference between mud and roost days could prevent the finding of a significant effect of walking in our model.
When measuring step lengths, walking was really at speed, maybe the estimate is a bit to high because "walking" included all terrestrial locomotion without foraging, which was not always at top speed.
During the final stage of writing this report, I discovered an irritating error in the reasoning how to compute covered distance. Although step lengths are known for both walking and foraging, and time devoted to both is also known, I do not know the number of steps per unit time for both behaviors, for step numbers were counted separately from activity scans. The wrong values of covered distance underestimate the fraction of steps made during walking and will therefore also underestimate the covered distance. The overestimation of step length during walking decreases this effect. It is possible to take the next activity scan before or after the step counts, but the bird did not necessarily perform only one behavior during the "step count" minute. There was

unfortunately no time to calculate the covered distance again, but the general pattern of differences between days and birds will be more or less okay.

4.2.2.5 waiting time

When taking the final blood and feces samples, the birds were all caught at the same time, but the samples necessarily taken with intervals of a few minutes dependent on the visibility of the vein and how calm the bird is. As a result, there is a period at the end of the DLW day where the treatment is already stopped and EE is lower. The EE value for the whole day is then slightly underestimated. We assumed that these periods are as long every day, so this waiting time does not interfere with testing for differences between days. (See appendix 4)

4.3 Discussion

What can be learnt from the results of this study? Water flux rate is very important in determining the energy expenditure of Knots. This is reasonable, for the costs of water flux stand for a wide variety of costs; some of these costs are directly associated with water flux rate, such as water heating in the stomach and salt excretion in the kidneys and the salt glands. Other are less clear, for example the activity of smooth muscles during transport in the intestines.

Can now safely be concluded that the relationship between water flux rate and Energy Expenditure is a causal one? Maybe for part of the large positive coefficient, the explanation could still be a correlation between water flux rate and other factors, notably crushing and activity. However the outcome of the analysis of the residuals of EE on water flux rate does not point in this direction. If the relationship seems causal, is it possible to look in more detail to the energetic costs caused by water intake? Below, some costs directly caused by water flux are quantified via other ways.

Another important question is how this result can be related to the three components of foraging budget that were distinguished in the introduction. Can we estimate these costs via other ways?

4.3.1 COMPONENT 1: Search & Capture

Activity does of course cost energy, but although activity levels were correlated with EE, and log of time active divided by time at rest often remained in various predictive models, we did not prove a positive relationship with EE with certainty. The effect may be small and better analyzed via another way: relating the amount of time spent in different behaviors directly with EE without taking other factors into account. This approach was not taken here.

If we look in detail, foraging behavior implies various body movements: the Knot walks slowly, constantly probing the mud, which is done by quite violent movements of neck and head. Walking was estimated separately and its possible impact is treated below; there is no additional information about probing. But not only energy expenditure in muscle work is increased by foraging: from a comparison between Mudfast and Roostfast we conclude that even when no prey is ingested, foraging behavior leads to higher water flux rates and increase/ less decrease in body mass.

4.3.1.1 Walking

Fortunately, there is independent information on the cost of walking in Knots (Bruinzeel, pers. comm.) The cost of walking is independent of running speed (Fedak et al 1974 in Bruinzeel and Piersma, 1998), is linearly dependent on body mass and measures 1.48 J/m for a 109 g Knot. Unfortunately there are problems with calculating covered distance (see § 4.2).Therefore, I chose not to treat all cases here but to just pick out two. The best example to calculate is Knot Orange during Mudcock1. This bird was foraging every time in the 90 activity scans, except one instance where it was at rest. This is an example of what the impact of walking on the energetics could be for an actively foraging Knot. If our estimate of step length during foraging is correct, this Knot travels on average 0.053 m/s with a cost of 1.66 J/m and thus spends 0.088 watt on walking, which is 1.5 % of its estimated EE. This is of course unexpectedly low. Trying to exaggerate the highest value to be sure not to underestimate the impact of walking, the case of Knot B during Mudfast is the taken. This bird made the most steps of all cases: 104/min on average. Taking all steps as if made during running (although it foraged for

more than 30% of time!), the energetic cost of walking would be 0.20 m/s * 1.70 J/m = 0.34 watt, which is 11 % of estimated EE. It looks as if walking is really not a very important cost factor. With such a small cost, effect of walking is difficult to find back in EE, even though relative differences in amount of walking may be very high. Then it is also easily understandable that an interaction between body mass and distance covered was not found either.

4.3.1.2 Flight

The Knots were not flying very much, only during three days (Roostflesh, Mudfast and Roostfast) and these flights seem to be correlated with unusual food circumstances (no food or very suitable food). But if flying is very costly, even 1% of time (the highest value reached, Y in Mudfast see appendix 2) devoted to this behavior could have a significant impact on EE. If we take the calculations and assumptions in paper 2 (Piersma, 1994) for granted where 32 kJ is expended in 40 minutes, then 1% of 660 min would yield an extra cost 5.25 kJ, expended in 660 min and hence 0.13 watt. [net cost of flying would be 13 watt]
This very very very rough calculation shows that, although the flying cost is maybe not negligible in all cases, the impact of flying has not obscured our estimation of foraging energy budgets.

4.3.2 COMPONENT 2: Crushing

Shell mass is the only factor that comes somewhat close to crushing cost, but it's also clear that it should be a dangerous estimate for crushing cost because shell mass and the force needed for crushing could have a different relationship with shell length. Crushing force has an exponential relationship with shell length with an exponent of three (Piersma et.al.1993), so increases may be only slightly faster with length than the increase in shell mass (exponent 3.3, this study). So shell mass could actually be quite a good estimate for crushing force. However, shell mass is also significantly positively correlated with water flux (r^2= 0.85, p=0.000) so multicollinearity problems will arise if both shell mass eaten and water flux rate are incorporated. The crushing cost is probably not very high, but remains elusive.

4.3.3 COMPONENT 3: Internal food processes other than crushing.

Under this heading we can identify Heat Increment of Feeding (HIF), heating up the food (dry matter and water), excretion of salt and water, and intestinal transport.

4.3.3.1 Heat increment of feeding

HIF is the heat generated by chemical processes during digestion and assimilation of food, for example in the liver. Cockles in poor condition contain almost no fat and the AFDM figures are completely accounted for by protein. In assimilating proteins, 30% (Kleiber, 1961) of the energetic content of 21kJ/g (references in Piersma and Morrison 1994 and in Piersma 1994 paper 7) is lost in HIF. When taking into account an assimilation coefficient of 0.7 (references also in Piersma and Morrison 1994 and in Piersma 1994 paper 7) the HIF can be estimated for the five days where the Knots ate protein:

average	afdm (g)	duration (s)	Afdm ($g*10^3/s$)	HIF (watt)
mudcock1	12.0	36580	0.327	1.44
mudcock2	11.9	36890	0.324	1.43
muddead	7.7	38120	0.201	0.89
roostcock	2.6	38150	0.067	0.30
roostflesh	20.4	39410	0.517	2.28

These values are unexpectedly high (range 7 to 63% of EE) and in case of roostflesh even BMR and HIF alone would sum up to more than the measured budget. If these values are correct, you would expect a clearer relationship with EE, but this was not found. It must be admitted though, that errors in our afdm estimates might be very high.
The value of assimilation efficiency is maybe too high for Roostflesh, because only on this day, the Knots were observed eating their own feces; but probably more important, the HIF factor could be lower than Kleiber suggests. For example in various carnivorous animals, values of around 15 have been estimated, which would halve the HIF compared with the values above. Still, the impact of HIF on EE seems fairly high. [Some values

from literature: 14.9 % of gross energy intake in harbour seals, Markussen et. al. 1994; 14.8 % of metabolizable energy intake in Adelie penguin chicks, Janes and Chappell, 1995; and 6.3 % in house wren chicks, Chappell et. al., 1997; 14.3-22.1% in Kestrels, Dietz et. al. 1992]
HIF is represented in the model by the amount of afdm ingested. Then why is such a positive relationship never a result in one of the predictive models?

4.3.3.2 Heating Up the Food

The energy involved in warming up the ingested water can be calculated from the warmth capacity of seawater: 3.93 J g^{-1} K^{-1}. For example, the highest water flux rate was measured in Knot Y during Mudcock1. The temperature difference was Tknot (41C) - Troom (17C)=24K, and water flux rate 8.16* 10^{-3} g/s. This would then amount to an expenditure of 0.77 watt for only warming up the ingested water. Also the dry part of the food has to be warmed up to body temperature. Using the warmth capacity 0.8 J $g^{-1}K^{-1}$ for mussel from De Leeuw, the highest recorded food intake (again Y on Mudcock1; fresh-water = 8.97*10^{-3} g/s) and the same temperature difference, this would lead to another energy expenditure of 24K* 0.8 J $g^{-1}K^{-1}$* 8.97*10^{-3} g/s=0.17 watt. The maximal cost of warming up the ingested food in this study is thus 0.94 watt, 17% of the energy budget of 5.5 watt.

4.3.3.3 Salt excretion

When foraging on isosmotic marine bivalves like in our study, Knots ingest a large amount of sodium chloride. Nehls' (1996) study revealed that salt excretion amounts to 2.0-2.4% of Metabolizable Energy Intake in Eiders *Somateria molissima*, which also have a diet of isosmotic marine bivalves, but that the maximum instanteneous rate of salt excretion could sometimes limit food intake. The Eiders had a daily mass specific salt intake of approximately 24 mg salt/g body. Assuming sea water has a salt content of 2% (Nehls 1996), our Knots, having water flux rates frequently exceeding 0.005 g/s (432 g/24h) and weighing roughly 130g, would have a mass specific salt intake of 66 mg salt/g body , this being a very conservative estimate!. It would therefore be logical to expect that cost of salt excretion makes up a bigger part of EE than 2 %. The fact that Knots have the highest relative salt gland mass of the 21 Charadriiform birds incorporated in a comparative study (Staaland, 1967) stimulates further experiments to get a grips of the impact of salt excretion.

4.3.3.4 Mechanical transport

I don't think the activity of the smooth muscles in the intestines will cost much energy, but have found no information about it.

4.3.4 Thermoregulatory cost and possible compensation

The costs of thermoregulation (TRC) was not the object of this study but we didn't always succeed in keeping the temperature above the lower critical temperature of 19.9 C (Wiersma and Piersma, 1994, fig 3). However, the TRC should have been very small , maybe up to 0.2 watt. This means that TRC is already difficult to find back in our EE values. Furthermore, such a small TRC can be easily substituted by heat unintentionally produced, due to activity or HIF. Walking has been found to partly substitute TRC in Knots (26-49% of TRC, Bruinzeel and Piersma 1998); walking produces heat, but at the same time increases heat loss because the air flow around the body and thermal conduction increase as well. HIF is probably more important in substituting TRC and possibly except from Roostfast, where the Knots neither moved much nor had any HIF, thermoregulatory costs were probably negligibly low in our study.
However if they were low thanks to substitution, then the estimate of total energy expenditure, the HIF and walking would on principle be too low.

4.3.5 Energy budget during foraging and food processing

Now we can calculate the energetic cost due to the variables discussed in the previous paragraphs and see if the total budget resembles our EE estimate of actively foraging Knots. (Situation as in MUDCOCK treatments)

1)	BMR	1 watt	
2)	TRC	0 watt	
3)	HIF	0.7 watt	
4)	Heating Up Food	0.9 watt	
5)	Salt Excretion	0.1 watt	
6)	Intestinal transport	0?	
7)	Crushing	?	
8)	Walking	0.088 watt	
9)	Probing	?	
10)	Flight	0	+
total budget expected		2.9 watt	

In fact, we measured 5.38 watt in Mudcock1 (2.5 watt higher) and 4.24 in Mudcock2 (1.7 watt higher). [Why do these mudcock days differ so much? Maybe a bad body condition causes body processes to be less efficient?]The high discrepancy between expected and and actually measured expenditure should be explained by erroneous assumptions, and by the costs of probing and crushing. Also some additional energy will be spend in tissue synthesis. The Roostfast budget is expected to be only composed of BMR and not much else (some activity), but we measured 1.6 watt, so subtracting BMR from EE during foraging would maybe give too high values for net cost of foraging.
Net cost of actively foraging on a mudflat for bivalve prey, can be estimated from the EE during Mudcock1, Mudcock2 and also Muddead because Crushing costs are unimportant; the averages for these treatments are 5.38, 4,24 and 4.93 watt respectively. It is reasonable to subtract 1.2 watt as an estimate of BMR and some thermoregulation (see 4.3.4), which yields a net cost of foraging of 3.65 watt. This value is slightly higher than the 3.1 watt estimated by Poot and Piersma (paper 7 Piersma 1994), but they assumed that probing during on the high tide roost without any prey intake is costly and therefore included the high tide in the time foraging. In view of the importance of water flux, this assumption might cause an underestimation of net cost of foraging. Their estimate of foraging cost during low tide only was 6.73 watt, much higher than our present result.

4.3.6 Limits to energy budgets and wintering Knots in the Wadden Sea

Although energy budget in birds can much higher than 5 times BMR during short periods, these levels can for some reason not be sustained. In some cases (Weiner, 1992; Kersten and Visser, 1996) the proximate cause for existence of metabolic ceiling is found to reside in the digestive tract, the limit being set by the maximum assimilation rate. Higher levels can't be sustained simply because there is a negative energy balance. Other studies propose a detrimental effect of higher energy expenditures on fitness, or even a maximum lifetime energy expenditure, but it is not known what the mechanism behind this may be.
What can be said about the energy expenditure of Knots wintering in the Wadden Sea? The birds are faced with low temperatures, and with an cost of flight between the roosts and the foraging areas. Following Poot and Piersma, maintenance metabolism is twice as high as indoors (2.5 watt) and the average flight cost is estimated at 0.3 watt. Assuming that the knots are able to forage during the whole low water period (half a day), we can add half the net cost of foraging 1.8 watt. This results in field metabolic rates of 4.6 watt (400 kJ/day), clearly more than 4 times BMR, which have to be sustained for months. This energy expenditure is high, but clearly not exceptional for birds (Bryant and Tatner, 1991).
But can Knots in the Wadden Sea ingest enough nutrients to balance their energy budgets? With an assimilation efficiency of 0.7, and an energetic density of the prey afdm of 21 kJ/g, Knots would have to reach intake rates of at least 0.75 mg afdm/s. In our two Mudcock treatments Knots seemed to work very hard, but reached intake rates of only 0.33 mg afdm/s on average. If Knots can't further increase prey capture rate, it is improbable that they could balance their budget while feeding on Cockles in such a bad condition as those from the high mudflats near De Cocksdorp.

4.3.7 *High cost of water turnover: consequences for optimal foraging*

Although the cause for a detrimental effect of high EE levels has been elusive, this need not bother us now: For some reason, everything else being equal, a lower energy expenditure seems valuable.
What are the options open to Knots? This is of course not a question to be answered in this study, but following the results, it seems reasonable to propose that what Knots should do is keep the water flux rate as low as possible by preferring prey with less water.

4.3.7.1 Cockle size

That prey size matters in optimal foraging has long been known: there are differences in handling time and reward. However, the costs associated with foraging have been largely overlooked in optimal foraging theory, concerning search time and handling time. The relative amount of water in a Cockle is dependent on length: see figure 17.

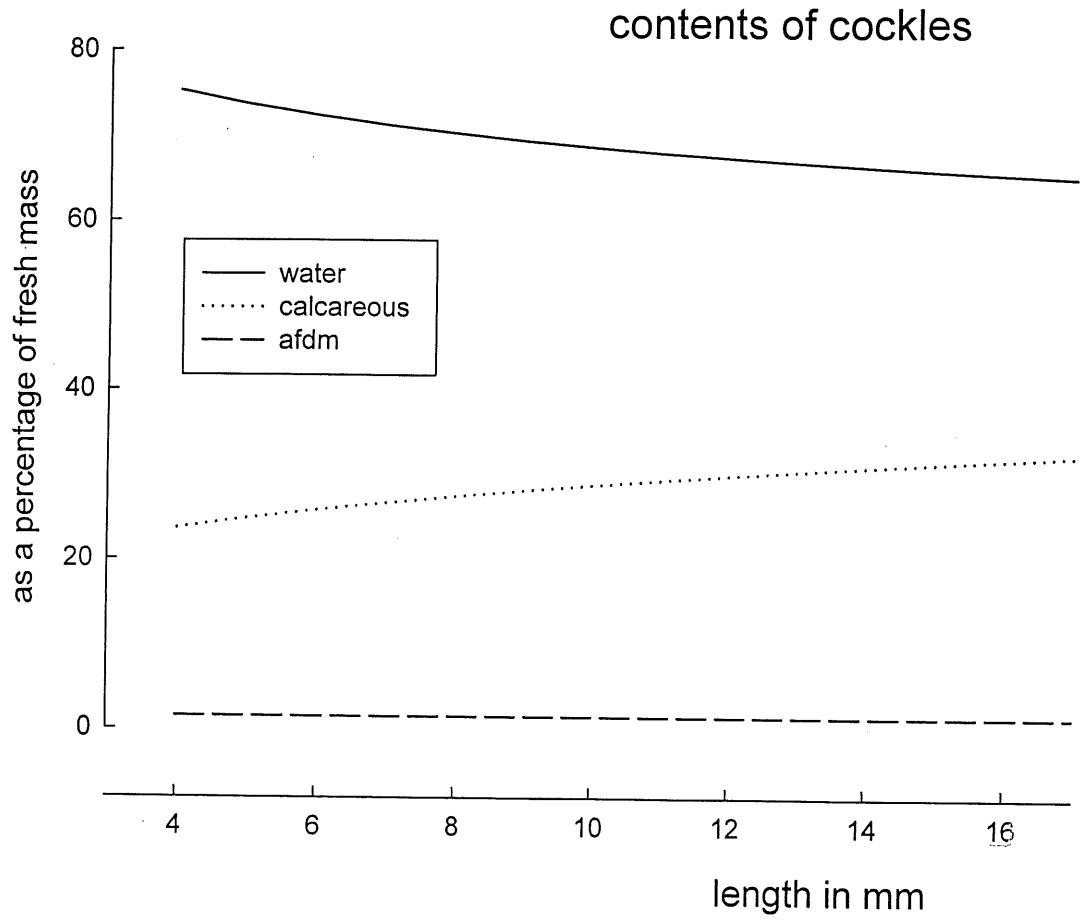


Fig. 17. The constituents (as a percentage of wet or "fresh" mass) of an average Cockle with variable length. AFDM is constant at 1.47% of total wet mass.

This graph has implications for Knots. Bigger prey have more calcareous shell per unit AFDM, but less water and water flux seems to be more important concerning energy expenditure than crushing cost. Therefore on energy expenditure grounds you would expect Knots to have a prevalence for larger Cockles.

4.3.7.2 Individual optimization?

In this study, where prey ingestion was noted per individual bird, we noticed that there were consistent individual differences in prey size selection. Assuming that each bird is an optimal forager, behaving optimal for its own phenotype, it would be very interesting to search for a functional explanation. The maximum size ingested is set by the throat diameter. Below this size, eating larger prey calls for a larger stomach, and maintaining more stomach tissue increases BMR. With larger prey, the needed amount of afdm can be collected faster because handling time per unit afdm seems to decrease (pers. obs., no literature search done). Obviously, expenditure is only one factor that determines optimal foraging in wintering Knots, but not one that can be neglected!

4.3.7.3 Prey species

Apart from the issue about the size of the prey, there is the broader issue concerning all available prey species for the Knots including other Bivalves, Mud-snails, Annelida and Crustacea. Maybe, the fact that Knots in the Wadden Sea only eat moderate amounts of Cockles can be attributed to their very low flesh/water ratio. This is especially the case with the Cockles we used, Cockles with such a bad condition should have been already dead according to the conventional theories about Cockle condition. However, Knots might have not much more to choose; if they're adapted to (or: especially good at) making a living on shellfish, they're simply faced with the fact that all shellfish contain much water relative to nutrients.

4.3.8 Final remarks

This study elucidated what high EE values can be reached due to foraging, and pointed to the importance of water flux. As is usual, more questions have been raised than answered. Impacts of search/capture and crushing on EE must still be accurately quantified in an experimental design. In fact, one could say that each of the 10 components summed up in 4.3.5, except BMR and thermoregulatory cost, need more detailed study. Our design where EE during different treatments is measured in a small flock of Knots is suitable for this, but next time more attention should be paid to repeating treatments and other demands of statistics. Sometimes, at least when no shellfish have to be used, it will be possible to assign random Knots at random to the different treatments.

5 Acknowledgements.

This study was my first "for real" study project and although it was very interesting, it was sometimes also very difficult for me to stay cool and go on. Fortunately, many people supported me during the adventure. First of all, there were my supervisors Henk Visser and Theunis Piersma, (and my unofficial-but-equally-important supervisor Anne Dekinga) who always kept incredibly rational and positive. Second, I thank the people of the research groups I was part of- Marine Ecology at the NIOZ and Animal Ecology in Haren- for all kinds of help and interesting discussions, especially Jan Drent, Pim Edelaar, Jan van Gils, Pieter Honkoop, Anita Koolhaas, Pieternella Luttikhuizen, Jaap van der Meer, Silke Nebel, Jeroen Reneerkens and Popko Wiersma. During the final stage of writing this report, the very generous back up of my student counsellor Joost Tinbergen has been extremely important for me. Finally I want to warmly thank my friends and family for hearing my stories, asking and answering questions and being there. Thank you!

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Appendix 1: Individual time budgets during treatment, percentage of treatment duration. Behaviors S- sleep, R- resting, P- preening, W-walking, Fo-foraging, and Fl-flying.

	Behaviour								
bird		Mudcock1	Mudcock2	Muddead	Roostcock	Roosttrouv	Roostflesh	Mudfast	Roostfast
B	S	0.0	0.0	0.0	20.2	28.3	3.8	6.2	52.1
B	R	18.0	46.9	3.3	45.6	36.7	61.5	3.6	36.2
B	P	1.1	4.1	4.4	3.5	27.5	11.5	13.0	9.2
B	W	3.4	1.0	5.6	5.3	5.8	11.5	40.4	2.1
B	Fo	77.5	48.0	86.7	25.4	1.7	11.5	36.5	0.0
B	Fl	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.4
LG	S	0.0	1.0	1.1	24.6	22.5	1.0	13.4	66.3
LG	R	1.1	4.1	2.2	56.1	42.5	12.5	10.7	24.5
LG	P	0.0	1.0	1.1	5.3	26.7	26.9	8.1	6.7
LG	W	3.4	6.1	2.2	6.1	3.3	7.7	20.2	2.5
LG	Fo	95.5	87.8	93.3	7.9	5.0	51.9	47.6	0.0
LG	Fl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O	S	0.0	4.1	0.0	50.0	39.2	0.0	6.8	58.2
O	R	1.1	4.1	1.1	21.9	29.2	34.3	3.9	28.4
O	P	0.0	6.1	2.2	10.5	19.2	21.0	11.1	11.0
O	W	0.0	2.0	7.8	7.0	7.5	16.2	30.3	2.5
O	Fo	98.9	83.7	88.9	10.5	5.0	28.6	47.9	0.0
O	Fl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RY	S	0.0	0.0	0.0	22.8	10.8	1.9	2.3	11.7
RY	R	15.7	12.2	0.0	16.7	58.3	28.8	11.4	67.0
RY	P	1.1	7.1	1.1	5.3	23.3	19.2	12.1	11.3
RY	W	2.2	3.1	6.7	6.1	5.8	18.3	33.2	9.6
RY	Fo	80.9	77.6	92.2	49.1	1.7	31.7	40.7	0.0
RY	Fl	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.4
W	S	0.0	0.0	0.0	34.2	30.0	4.8	10.7	67.7
W	R	3.4	3.1	0.0	18.4	45.0	41.9	8.8	23.8
W	P	1.1	3.1	1.1	0.9	20.0	14.3	5.2	6.4
W	W	4.5	6.1	3.3	7.0	1.7	13.3	31.9	2.1
W	Fo	90.9	87.8	95.6	39.5	3.3	24.8	43.3	0.0
W	Fl	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
Y	S	2.3	0.0	0.0	14.9	5.0	1.9	7.2	0.0
Y	R	25.0	33.7	12.2	57.9	75.8	66.0	10.5	0.0
Y	P	1.1	3.1	3.3	6.1	4.2	4.7	10.8	0.0
Y	W	4.5	11.2	7.8	12.3	10.0	11.3	19.0	0.0
Y	Fo	67.0	52.0	76.7	8.8	5.0	15.1	51.5	0.0
Y	Fl	0.0	0.0	0.0	0.0	0.0	0.9	1.0	0.0

Appendix 2: Distance between two subsequent footprints in 27 trails. To correct for the fact that the prints are somewhat to the right or left of the straight line, half the distance between the two successive prints of the same foot should be used to calculate the covered distance with varying step rates. Distances between two prints of the same foot (which was not estimated for all trails) are given in the first part of the table. To the right are the corresponding distances between left and right footprints.

year	month	day	start	end	identity	behaviour	meth1(leftleft)	meth2 (per step)	2dist1	2dist2	2dist3	2dist4	2dist5	2dist6	<meth1
1998	april	2	11.51	12	B	f	8 out of 11	ja	14	15	14.5	14			
1998	april	2	11:10	11:14	LG	f	all	ja	15.5	16	14.5	15.5	12.5	11	meth2>
1998	april	2	11:10	11:14	LG	f	all	ja	13	13	14	15	10.5		
1998	april	2	11:10	11:14	LG	f	all	ja							
1998	april	2	10:32	10:43	O	f	no	ja							
1998	april	2	10:32	10:43	O	f	no	ja							
1998	april	2	10:32	10:43	O	f	all	ja							
1998	april	2	10:32	10:43	O	f	no	ja							
1998	april	2	10:32	10:43	O	f	no	ja							
1998	april	3	09:50	09:58	W	f	all	ja	12.5	9					
1998	april	3	09:50	09:58	W	f	no	ja							
1998	april	3	09:50	09:58	W	f	all	ja	13	14.5					
1998	april	3	09:50	09:58	W	f	all	ja	14.5	12.5	14.5	13.5	11.5		
1998	april	3	09:50	09:58	W	f	no	ja							
1998	april	2	11:10	11:14	LG	w	all	ja	25.5	20.5	23				
1998	april	2	11:10	11:14	LG	w	all	ja	18	21	21.5				
1998	april	2	10:32	10:43	O	w	no	ja							
1998	april	3	09:50	09:58	W	w	8 out of 10	ja	29.5	28.5	27.5	29.5			
1998	april	2	11.51	12	B	unknown	6 out of 7	ja	15	15.5	11.5				
1998	april	2	11.51	12	B	unknown	no	ja							
1998	april	2	11.51	12	B	unknown	no	ja							
1998	april	2	11.51	12	B	unknown	all	ja	14.5	14.5	15	15	16		
1998	april	2	11.51	12	B	unknown	all	ja	16.5	15.5	16.5	14.5	16		
1998	april	2	11.51	12	B	unknown	all	ja	17.5	15.5	15.5	15	15		
1998	april	2	11.51	12	B	unknown	all	ja	17	16.5	15.5	16.5			
1998	april	2	10:32	10:43	O	unknown	no	ja							
1998	april	2	10:32	10:43	O	unknown	no	ja							

identity	<meth1	length1	length2	length3	length4	length5	length6	length7	length8	length9	length10	length11	length12	length13	#steps
B		6	8.5	7	9	6.5	7.5	7.5	7	5.5	4	6			11
LG	meth2>	7.5	9	8	8.5	8	7.5	8	8.5	7	6.5	6.5	5		12
LG		7.5	5.5	7	6	7	7	8	7	4.5	6.5				10
LG		5.5	5	5.5											3
O		7.5	5.5	5.5	6.5										4
O		3.5	5	4											3
O		5	5	5	5	4.5	3.5								6
O		3.5	4.5	4.5											3
O		4.5	4	3.5	5										4
W		7	6	5.5	4.5										4
W		7.5	6.5	8	6.5										4
W		6.5	7	6	8										4
W		8.5	6.5	6.5	7	7	7.5	7	6.5	6	5.5				10
W		7	8	7	5.5	8.5	7.5	9	6	8					9
LG		12.5	14	10	11.5	12	11.5								6
LG		8.5	10	10.5	11	10.5	11								6
O		12	12	12.5	10.5	10	9.5	9							7
W		16	13.5	14.5	14	15	13.5	16	14	14.5	14.5				10
B		8.5	8	8	7.5	8	7.5	4.5							7
B		7.5	7.5	4.5	8	8.5	6	4.5							7
B		5.5	4.5	6.5	7	7.5									5
B		7.5	7.5	7	8	8	8.5	7.5	8	8	8.5				10
B		8.5	8.5	8.5	7.5	8.5	8.5	6	8	8.5	8				10
B		9.5	8.5	8.5	7.5	9.5	7	7.5	7.5	7	8				10
B		8.5	9	8.5	8.5	8.5	7	8	9						8
O		7	7	7.5	9	4	4.5	9							7
O		4	6.5	8	7.5	8.5	9.5	9	11	11.5					9

Appendix 3: Results of the weighed Cockle contents per length class in mm. Masses are in milligram.

batch	date	type	sample from:	length	number	mass	mass / individual
1	16/12/97	AFDM	de cocksdorp	5	1	0.0014	0.001
1	16/12/97	AFDM	de cocksdorp	6	10	0.0114	0.001
1	16/12/97	AFDM	de cocksdorp	7	19	0.0266	0.001
1	16/12/97	AFDM	de cocksdorp	8	42	0.0859	0.002
1	16/12/97	AFDM	de cocksdorp	9	31	0.0899	0.003
1	16/12/97	AFDM	de cocksdorp	10	20	0.0782	0.004
1	16/12/97	AFDM	de cocksdorp	11	10	0.0565	0.006
1	16/12/97	AFDM	de cocksdorp	12	10	0.0674	0.007
1	16/12/97	AFDM	de cocksdorp	13	1	0.0102	0.010
1	16/12/97	AFDM	de cocksdorp	14	3	0.0442	0.015
1	16/12/97	AFDM	de cocksdorp	15	1	0.0161	0.016
1	16/12/97	AFDM	de cocksdorp	16	2	0.0523	0.026
2	07/01/98	bulk	mudflat aviary	6	5	0.3054	0.061
2	07/01/98	bulk	mudflat aviary	7	5	0.4876	0.098
2	07/01/98	bulk	mudflat aviary	8	5	0.7055	0.141
2	07/01/98	bulk	mudflat aviary	9	5	0.9899	0.198
2	07/01/98	bulk	mudflat aviary	10	5	1.4265	0.285
2	07/01/98	bulk	mudflat aviary	11	5	1.9445	0.389
2	07/01/98	bulk	mudflat aviary	12	5	2.6513	0.530
2	07/01/98	bulk	mudflat aviary	13	5	3.0494	0.610
2	07/01/98	bulk	mudflat aviary	14	5	4.1935	0.839
2	07/01/98	bulk	mudflat aviary	15	4	4.282	1.071
2	07/01/98	bulk	mudflat aviary	16	1	1.2889	1.289
2	07/01/98	water	mudflat aviary	6	5	0.2164	0.043
2	07/01/98	water	mudflat aviary	7	5	0.3451	0.069
2	07/01/98	water	mudflat aviary	8	5	0.4961	0.099
2	07/01/98	water	mudflat aviary	9	5	0.7018	0.140
2	07/01/98	water	mudflat aviary	10	5	0.9756	0.195
2	07/01/98	water	mudflat aviary	11	5	1.3542	0.271
2	07/01/98	water	mudflat aviary	12	5	1.8114	0.362
2	07/01/98	water	mudflat aviary	13	5	2.0964	0.419
2	07/01/98	water	mudflat aviary	14	5	2.7173	0.543
2	07/01/98	water	mudflat aviary	15	4	2.7322	0.683
2	07/01/98	water	mudflat aviary	16	1	0.8087	0.809
2	07/01/98	calcareous	mudflat aviary	6	5	0.0835	0.017
2	07/01/98	calcareous	mudflat aviary	7	5	0.1303	0.026
2	07/01/98	calcareous	mudflat aviary	8	5	0.1911	0.038
2	07/01/98	calcareous	mudflat aviary	9	5	0.2644	0.053
2	07/01/98	calcareous	mudflat aviary	10	5	0.4159	0.083
2	07/01/98	calcareous	mudflat aviary	11	5	0.5387	0.108
2	07/01/98	calcareous	mudflat aviary	12	5	0.7797	0.156
2	07/01/98	calcareous	mudflat aviary	13	5	0.8809	0.176
2	07/01/98	calcareous	mudflat aviary	14	5	1.3667	0.273
2	07/01/98	calcareous	mudflat aviary	15	4	1.4445	0.361
2	07/01/98	calcareous	mudflat aviary	16	1	0.446	0.446
2	07/01/98	AFDM	mudflat aviary	6	5	0.0044	0.001
2	07/01/98	AFDM	mudflat aviary	7	5	0.0094	0.002
2	07/01/98	AFDM	mudflat aviary	8	5	0.0134	0.003
2	07/01/98	AFDM	mudflat aviary	9	5	0.0174	0.003
2	07/01/98	AFDM	mudflat aviary	10	5	0.024	0.005
2	07/01/98	AFDM	mudflat aviary	11	5	0.034	0.007
2	07/01/98	AFDM	mudflat aviary	12	5	0.0384	0.008
2	07/01/98	AFDM	mudflat aviary	13	5	0.0435	0.009
2	07/01/98	AFDM	mudflat aviary	14	5	0.0679	0.014
2	07/01/98	AFDM	mudflat aviary	15	4	0.0619	0.015
2	07/01/98	AFDM	mudflat aviary	16	1	0.0215	0.022
3	09/01/98	AFDM	mudflat aviary	5	1	0.0038	0.004
3	09/01/98	AFDM	mudflat aviary	6	3	0.0047	0.002
3	09/01/98	AFDM	mudflat aviary	7	15	0.0177	0.001
3	09/01/98	AFDM	mudflat aviary	8	15	0.0268	0.002
3	09/01/98	AFDM	mudflat aviary	9	15	0.0387	0.003
3	09/01/98	AFDM	mudflat aviary	10	15	0.0552	0.004
3	09/01/98	AFDM	mudflat aviary	11	9	0.0436	0.005
3	09/01/98	AFDM	mudflat aviary	12	4	0.0328	0.008
3	09/01/98	AFDM	mudflat aviary	13	3	0.0379	0.013
3	09/01/98	AFDM	mudflat aviary	14	4	0.0522	0.013
3	09/01/98	AFDM	mudflat aviary	15	3	0.0471	0.016
4	12/01/99	AFDM	mudflat aviary	5	1	0.0005	0.000
4	12/01/99	AFDM	mudflat aviary	6	4	0.0025	0.001
4	12/01/99	AFDM	mudflat aviary	7	15	0.0147	0.001
4	12/01/99	AFDM	mudflat aviary	8	14	0.0205	0.001
4	12/01/99	AFDM	mudflat aviary	9	15	0.0352	0.002
4	12/01/99	AFDM	mudflat aviary	10	15	0.0431	0.003
4	12/01/99	AFDM	mudflat aviary	11	8	0.0303	0.004
4	12/01/99	AFDM	mudflat aviary	12	3	0.0193	0.006
4	12/01/99	AFDM	mudflat aviary	13	3	0.0227	0.008
4	12/01/99	AFDM	mudflat aviary	14	2	0.0243	0.012
4	12/01/99	AFDM	mudflat aviary	15	5	0.0623	0.012
4	12/01/99	AFDM	mudflat aviary	16	1	0.0209	0.021
5	16/01/98	AFDM	cockle storage	8	10	0.0197	0.002
5	16/01/98	AFDM	cockle storage	9	10	0.0222	0.002
5	16/01/98	AFDM	cockle storage	10	10	0.0365	0.004
5	16/01/98	AFDM	cockle storage	11	10	0.0452	0.005
5	16/01/98	AFDM	cockle storage	12	10	0.0741	0.007
5	16/01/98	AFDM	cockle storage	13	10	0.0411	0.004
5	16/01/98	AFDM	cockle storage	14	4	0.0433	0.011
5	16/01/98	AFDM	cockle storage	15	5	0.0691	0.014
5	16/01/98	AFDM	cockle storage	16	1	0.0142	0.014
6	27/01/98	AFDM	mudflat aviary	6	10	0.0145	0.001
6	27/01/98	AFDM	mudflat aviary	7	10	0.0178	0.002
6	27/01/98	AFDM	mudflat aviary	8	10	0.0266	0.003
6	27/01/98	AFDM	mudflat aviary	9	10	0.0373	0.004
6	27/01/98	AFDM	mudflat aviary	10	10	0.0522	0.005
6	27/01/98	AFDM	mudflat aviary	11	10	0.0649	0.006
6	27/01/98	AFDM	mudflat aviary	12	10	0.0878	0.009
6	27/01/98	AFDM	mudflat aviary	13	10	0.1124	0.011
6	27/01/98	AFDM	mudflat aviary	14	10	0.1574	0.016
6	27/01/98	AFDM	mudflat aviary	15	4	0.0727	0.018
6	27/01/98	AFDM	mudflat aviary	16	2	0.0552	0.028
6	27/01/98	AFDM	mudflat aviary	17	1	0.0325	0.033
6	12/02/98	AFDM	de cocksdorp	6	9	0.0105	0.001
7	12/02/98	AFDM	de cocksdorp	7	15	0.0237	0.002
7	12/02/98	AFDM	de cocksdorp	8	15	0.037	0.002
7	12/02/98	AFDM	de cocksdorp	9	15	0.0551	0.004
7	12/02/98	AFDM	de cocksdorp	10	15	0.0719	0.005
7	12/02/98	AFDM	de cocksdorp	11	15	0.0924	0.006
7	12/02/98	AFDM	de cocksdorp	12	15	0.1167	0.008
7	12/02/98	AFDM	de cocksdorp	13	15	0.158	0.011
7	12/02/98	AFDM	de cocksdorp	14	15	0.222	0.015
7	12/02/98	AFDM	de cocksdorp	15	9	0.176	0.020

Appendix 4: Durations (min) exposed to treatment, the duration between initial and final blood sample (dlw), and the difference between the two durations.

DURATIONS

treatment	Mudcock1	Mudcock2	Muddead	Roostcock	Roosttrouv	Roostflesh	Mudfast	Roostfast
B	620	621	639	647	663	661	653	653
LG	600	608	631	626	652	653	642	648
O	614	617	636	640	660	658	648	651
RY	623	626	643	652	667	665	654	657
W	595	604	629	619	643	649	639	645
Y	606	613	634	631	655	655	645	

dlw	Mudcock1	Mudcock2	Muddead	Roostcock	Roosttrouv	Roostflesh	Mudfast	Roostfast
B	650	652	650	650	677	670	668	663
LG	644	654	661	650	680	676	668	664
O	650	652	654	655	680	673	667	664
RY	652	649	648	660	672	670	665	661
W	642	655	668	646	677	675	668	666
Y	646	654	659	650	678	675	667	

difference	Mudcock1	Mudcock2	Muddead	Roostcock	Roosttrouv	Roostflesh	Mudfast	Roostfast
B	30	31	11	3	14	9	15	10
LG	44	46	30	24	28	23	26	16
O	36	35	18	15	20	15	19	13
RY	29	23	5	8	5	5	11	4
W	47	51	39	27	34	26	29	21
Y	40	41	25	19	23	20	22	

SUMMARY DESIGN		treatment:		cost factors present:		
title	date	location	food type	activity	crushing	digesting
MUDCOCK1	06/01/98	intertidal flat	Cockles	+	+	+
MUDCOCK2	28/01/98	intertidal flat	Cockles	+	+	+
MUDDEAD	26/01/98	intertidal flat	dying Cockles	+	?	+
ROOSTCOCK	22/01/98	roost	Cockles	-	+	+
ROOSTTROUV	02/02/98	roost	trout pellets	-	-	+
ROOSTFLESH	04/02/98	roost	Cockle flesh	-	-	+
MUDFAST	13/02/98	intertidal flat	none	+	-	-
ROOSTFAST	30/01/98	roost	none	-	-	-

Fig. 1. Summary of the design as it was actually carried out.