[Jap. J. Parasit., Vol. 19, No. 1, 35-53, 1970]

The effect of diet on the fecundity of *Oncomelania hupensis nosophora**

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(Received for publication; December 11, 1969)

Introduction

Currently there is no suitable therapy for schistosomiasis and by necessity, increased emphasis has been placed on research of taxa of *Schistosoma*, the intermediate hosts of the parasite, and drug screening operations. Davis & Iwamoto (1969) discussed reasons why studies dealing with *S. japonicum* have lagged far behind those on *S. mansoni*. In summary, it has not been possible to provide numerous laboratory reared *S. japonicum* with the same space and manpower with which an abundance of *S. mansoni* is produced.

Part of the problem is due to the fact that a great deal has yet to be learned about 1) factors which will assure a high, predictable year-round production of young *Oncomelania* in the laboratory and 2) methodology for maintaining a high uniform growth and maturation rate of young snails isolated from parental cultures.

The basic parameters necessary for a successful culture program are reviewed by van der Schalie & Davis (1968). In recent studies (Davis & Iwamoto, 1969) it was found that when culture conditions used by van der Schalie and Davis in Michigan, U.S.A. were used at the 406 Medical Laboratory, Japan, production of young per female per month (y/f/m) dropped to a comparatively low level (4.8 y/f/m at Michigan contrasted with 0.2 y/f/m for the first 12 months at the 406 Medical Laboratory using medium clay pot cultures in room level light). The hypothesis was tested that the level of food energy in various soils used for culturing *Oncomelania* was a major factor relating to the production of young (no food additives other than soil and filter-paper had been used). It was found (Davis & Iwamoto, 1969) that additions of rice powder cereal had a profound effect on productivity. This had been suggested previously by Komiya *et al.* (1959, 1960) and Moose *et al.* (1962). Subsequently, culture environments were refined so that *O. h. nosophora* produced large numbers or y/f/m when adults were maintained in 9 cm Petri dishes at a density of 5 individuals (3 ♀, 2 ♂) and with a rice cereal food additive (production jumped to 7.8 y/f/m with light cycled on from 8 a.m. to 4 p.m.).

A problem still exists that, while the greatest productivity in one culture in one month may reach 44 y/f/m, the highest average production attained for a culture type over a 12 month period has been 7.8 y/f/m. Further, once a culture has been initiated, the greatest percentage of young have been produced in 1 or 2 months in a short burst of productivity (25 to 50 %) and production in ensuing months has been low. Methods are needed to encourage a higher monthly out-put per female and a more evenly regulated production each month.

The purpose of this paper is to test the hypothesis that a more enriched and readily assimilated diet is provided by the use of diatoms so that a heightened and prolonged production of young results. Accordingly,

* This study was supported by a research and development grant provided by the Office of the Surgeon General, U.S. Army, Washington, D.C.

the effects of diet on fecundity, oogenesis, spermatogenesis, and reproductive organ morphology and histology are here recorded.

Materials and Methods

Source of Snails: Field collected *Oncomelania hupensis nosophora* were used throughout the experiment. The estimated age of the snails was 8 to 9 months based upon shell condition and presence of a varix (see p. 120, Davis, 1967).

Culture Conditions: Methods are those of Davis & Iwamoto (1969) using 9 cm Petri dishes, alternating fluorescent light, and 3 ♀, 2 ♂ per culture. Seventeen dishes were used for each of 3 experimental diets. In condition I (diet a_1), filter-paper and soil served as the basic source of food and constituted the "control" condition. Condition II (diet a_2) was derived by adding 25 mg rice powder on a small square of filter paper to each culture each week. Condition III (diet a_3) was created by pipetting 3 ml of a 4 to 5% suspension of the diatom *Navicula luzonensis* into each culture each week.

Isolation and Culture of Diatoms: A diatom culture solution was constituted as shown in Table 1. Diatoms were isolated by mixing 1 mg soil[1)] per 50 cc sterile distilled water; 1 cc of this mixture was pipetted into 15 cc of a cooled agar plate (1% agar in diatom culture solution). The plates were placed under light and observed for colony development. Single colonies of diatoms were isolated and sub-cultured in agar and subsequently in 15 cm Petri dishes with liquid culture solution. Each week diatoms were collected from the dishes, centrifuged and the water volume adjusted to give a 4 to 5% suspension.

Data Gathered: The effects of the experimental conditions were measured in 3 ways; 1) fecundity in terms of y/f/m produced, 2) dimensional changes in selected reproductive organs, 3) histological examinations of the selected reproductive organs.

Although there were 17 cultures for each diet at the beginning of the experiment, 2 to 3 were withdrawn from each diet group each month to provide snails for steps 2 and 3 above. Accordingly, data on fecundity were analyzed in 2 ways; 1) an analysis of variance of young produced per culture type in the 4 cultures of each condition which were maintained for 7 months and 2) calculations of y/f/m for each culture throughout the entire period even though the number of cultures was decreasing. Data were recorded starting 1 month after cultures were established because it takes from 20 to 30 days for eggs to hatch after being deposited (Otori *et al.*, 1956); therefore, data were collected over 6 consecutive months.

Each month (October to March) the greatest length and width of the ovary, bursa copulatrix, posterior pallial oviduct, anterior pallial oviduct (width only), testis, prostate, and verge (width at the base) were measured from 4 to 5 snails of each diet group. Measurements were made by the same person (J.K.W.) using a standard ocular micrometer mounted in a Nikon SM dissecting microscope while the snails were pinned out on a wax surface in a Petri dish, under water, with the ventral (or columellar) side up. Measurements of the verge were made by pinning out the verge without removing it from the neck of the animal. The data for month 1 (September) were recorded from 5 males and 5 females taken from the population of approximately 800 snails from which the cultures were established and these served to represent the average condition for the population.

Of these snails, 2 females and 1 male were fixed in Baker's formaldehyde-calcium solution, embedded in gelatin, frozen, cut at 8 micra and stained with Sudan B and carmalum. The remaining individuals were fixed in Bouin's, sectioned at 8 to 10 micra and stained with standard haemotoxylin and eosin.

The condition of the testes was scored 1 to 5 based on the following: 1) no primordia; 2) primordia, few or no spermatocytes; 3)

1) Source of Soil: Yamanashi Prefecture, Nakakoma Gun, Shirane Machi, Iinoshinden Swamp, habitat of *O. h. nosophora*.

spermatocytes, few or no spermatid ; 4) spermatid, few or no spermatozoa ; 5) numerous spermatozoa. The condition of the ovaries was scored as follows : 1) no primordia ; 2) primordia, no oocytes ; 3) small oocytes budding from the ovarian wall ; 4) oocytes large, numerous, the cytoplasm being unevenly stained with H & E, and no fatty yolk droplets formed ; 5) oocytes free in the lumen, the cytoplasm evenly stained and fatty yolk droplets present as detected by staining with Sudan B. These stages are shown in Figures 1-3. Stages 1 and 2 are shown (gross longitudinal section) in van der Schalie & Davis, 1965, Fig. 3 A, B.

Analysis of Data : The effects of diet and time and their possible interactions relative to fecundity and organ dimension were analyzed by an analysis of variance for factoral experiments. Where diets differed significantly, comparisons between means were conducted using Duncan's New Multiple Range Test (fide Steel & Torrie, 1960).

Results

Fecundity

As is seen in Table 2, the greatest production of young occurred in cultures provided with diatoms. Mortality of young in the 3 conditions was low and no adult deaths occurred among the females. When only those 4 cultures of each condition which were maintained throughout the 7 months were considered (Tables 3, 4) it was evident that there were significant differences (F ; .05 [also at .01 level which was not tabulated]) involving time, diet and interaction of the two. As shown in Table 5, in this experimental series the production in cultures with rice powder did not differ significantly from the control condition while production in cultures provided with diatoms was significantly different compared with the other two.

There was a significant decline in pro-

Table 1 The composition of diatom rearing media as modified from Chu (1942)

Components added to distilled H_2O to make 1 liter	Amount
$Ca(NO_3)_2$	160 mg
K_2HPO_4	100 mg
$MgSO_4 \cdot 7H_2O$	25 mg
Na_2CO_3	20 mg
$NaSiO_3$	25 mg
Ferric citrate	0.4 mg
Minor elements	1.0 ml
Soil extract	25.0 ml
(pH adjusted with HCl to 8.0)	
Minor elements : add to 1 liter of distilled H_2O	
H_3BO_3	2.68 gm
$MnCl_2 \cdot H_2O$	1.81 gm
$ZnSO_4 \cdot 7H_2O$	220 mg
$CuSO_4 \cdot 5H_2O$	80 mg
$H_2MoO_4 \cdot H_2O$	90 mg
KI	30 mg
KBr	30 mg
$LiCO_3$	30 mg
Soil extract-autoclave 500 gm garden soil in 1 liter distilled H_2O, 15 lbs., 2 hours ; settle, decant, discard soil.	

Table 2 Reproductive rates of field-collected *Oncomelania hupensis nosophora* under varying conditions of diet

Food used	Production of y/f/m			% of Culture months that are productive†	% of young dead in culture	No. of female deaths per 100
	Average for productive months only	For months 1-6*	Greatest for 1 culture in 1 month			
Control	2.7	2.5	14.3	89.4	1.06	0
Rice	5.0	4.6	23.3	92.4	3.83	0
Diatoms	14.4	13.7	41.0	97.0	2.12	0

* Productive and nonproductive cultures

† Culture-months are defined in Davis & Iwamoto, 1969

Table 3 The influence of diet and time on the production of young per culture; a summary of data for treatment groups

FACTOR A† (young per culture)	FACTOR B(Time)						Total
	b_1	b_2	b_3	b_4	b_5	b_6	
a_1	94	56	41	15	8	6	220
a_2	102	121	44	19	5	9	300
a_3	184	210	110	265	101	28	898
Total	380	387	195	299	114	43	1418

Σx^2, 62, 718

Each value is the total of 4 replicates of 1 culture each.

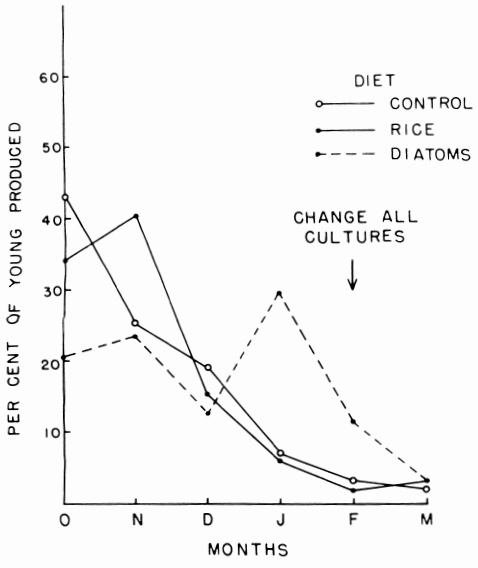

Fig. 4 The percentage of young produced each month in cultures provided with 3 different diets. The cultures were established in September.

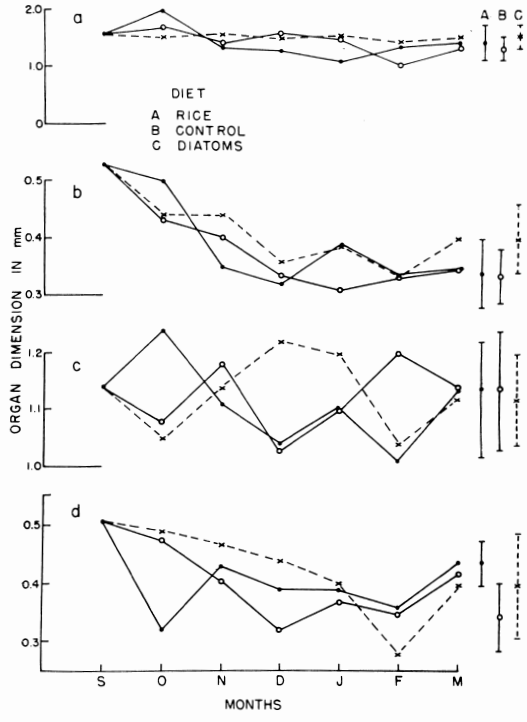

Fig. 5 The average change in organ dimension each month when snails were maintained on different diets. a, length of ovary; b, width of ovary; c, length of bursa copulatrix; d, width of the bursa copulatrix. One standard deviation is given for data from the last month.

ductivity through time in cultures with rice powder or soil only and this is seen graphically in Figure 4. In these two cases peak productivity occurred in October and November (20 to 43%), 2 and 3 months after the cultures were set, and decreased continually thereafter. The pattern of productivity through time is significantly different in cultures provided with diatoms. Production of young fluctuated until the 6th month (b_5) after which there was a sharp drop; peak production occurred in the 5th month (b_4). This trend for a more uniform production through time is reflected in the significant interaction between the 3 diets and time in Table 3 (significant differences for AB, Table 4).

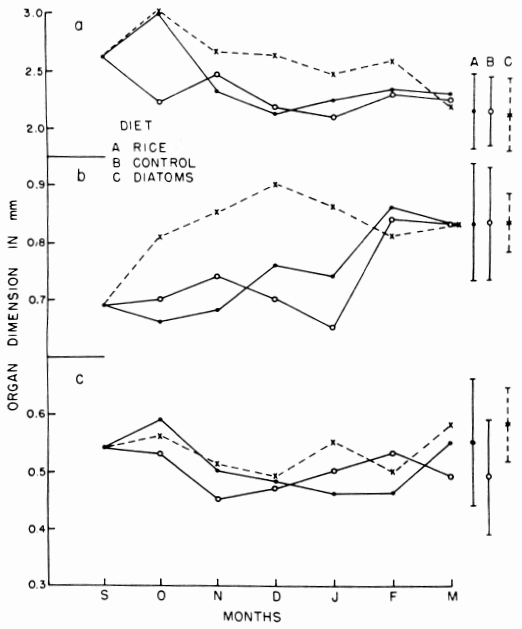

Fig. 6 The average change in organ dimension each month when snails were maintained on different diets. a) length of posterior pallial oviduct, b) greatest width of posterior pallial oviduct, c) greatest width of the anterior pallial oviduct. One standard deviation is given for data from the last month.

Morphological changes

The average values for organ dimension are plotted in Figures 5–8. While it was evident that there were no significant changes in organ dimension through time and between diets in the cases of ovary length (Fig. 5a), bursa copulatrix length, (Fig. 5c), width of the anterior pallial oviduct (Fig. 6c), testis length (Fig. 7a), etc., it was not so evident in other cases, or there were obvious changes. Therefore, results of analysis of variance are summarized in Table 6. Data are given in Tables 7 to 18 for cases where there were significant differences (F, .05 level). While there were 6 cases of change in dimension through time, in only 3 cases did diet have an effect on an organ's dimension and in these 3 cases there were no interactions with time (the length and width of the posterior pallial oviduct ; the width of the verge).

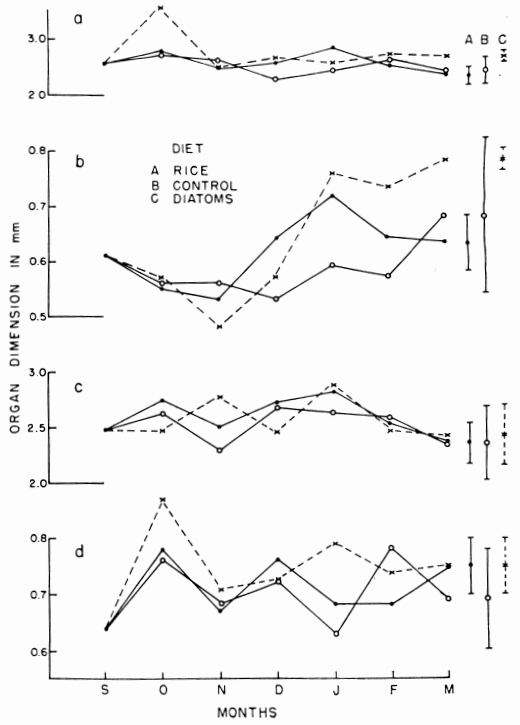

Fig. 7 The average change in organ dimension each month when snails were maintained on different diets, a, length of testis ; b, width of testis ; c, length of prostate ; d, width of prostate. One standard deviation is given for data from the last month.

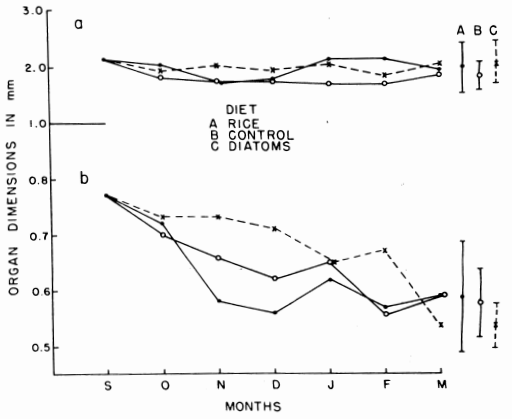

Fig. 8 The average change in organ dimension each month when snails were maintained on different diets, a, length of verge ; b, width of verge at the base. One standard deviation is given for data from the last month.

Table 4 Analysis of variance of the effect of diet and time on the number of young produced per culture

Source	df	SS	MS	Fcal.	F .05 tab.
Treatment	17	34,791.28			
A	2	11,440.11	5,720.055	32.36	3.1*
B	5	8,443.28	1,688.656	9.55	2.2*
AB	10	5,362.89	536.289	3.03	2.0*
Error	54	9,545.00	176.759		

* significant difference
† all symbols explained under Table 5

Table 5 Comparison of diets using Duncan's New Multiple Range Test relative to the significant effect of diet on the production of young per culture

	Range	
Value of p	2	3
SSR	2.82	2.95
LSR	10.67	11.24

difference in means	comparison to LSR	significance
a_3-a_1	28.25>11.24	SD
a_3-a_2	24.92>10.67	SD
a_2-a_1	3.33<10.67	—

$S_{\bar{x}}$ =3.783
SD =Significant difference, .05 level, error df-69
Σx^2 =sum of each datum squared
* =significant difference
$\bar{X}$ =mean value
A =diet
a_1 =control diet
a_2 =rice diet
a_3 =diatom diet
B =time
b_1 =time 1
b_2 =time 2, etc.
df =degrees of freedom
Fcal. =calculated value of F
Ftab. =tabulated value of F
MS =mean square
P =number of means for the range being tested
LSR =least significant range (=SSRx$S_{\bar{x}}$)
SD =significant difference
SS =sum of squares
SSR =significant range value (tabulated)
$S_{\bar{x}}$ =standard deveation of the mean

Table 6 The effects of diet and time or their interaction on the dimensions of selected reproductive organs

	Diet	Time	Interaction
Ovary Length	—	—	—
Width	—	SD(d)	—
Bursa copulatrix Length	—	—	—
Width	—	SD(d)	—
Posterior pallial oviduct			
Length	SD	SD)d)	—
Width	SD	SD(i)	—
Anterior pallial oviduct			
Width	—	—	—
Testis Length	—	—	—
Width	—	SD(i)	—
Prostate Length	—	—	—
Width	—	—	—
Verge Length	—	—	—
Width	SD	SD(d)	—

SD =significant difference at .05 level
(d) =decrease
(i) =increase

The width of the posterior pallial oviduct increased significantly during the experiment, As shown in Table 19 this is attributed to the diatom diet as the results with snails on the rice and control diets did not differ significantly. Concerning the length of the pallial oviduct, snails maintained on diatoms differed significantly only from those of the control diet, not from those maintained on rice cereal (Table 20). The diatom diet and rich powder diet differed significantly relative to the decreasing width of the verge (Table 21).

Table 7 The influence of diet and time on the width (mm) of the ovary; a summary of data for treatment groups

FACTOR A† (Diet)	FACTOR B (Time)							Total
	b_1	b_2	b_3	b_4	b_5	b_6	b_7	
a_1	2.66	2.16	2.01	1.66	1.56	1.64	1.74	13.43
a_2	2.66	2.49	1.76	1.58	1.94	1.69	1.76	13.88
a_3	2.66	2.19	2.20	1.81	1.97	1.63	2.00	14.46
Total	7.98	6.84	5.97	5.05	5.47	4.96	5.50	41.77

Σx^2, 17.7072
Each value is a total of 5 replicates of 1 snail each
† symbols explained under Table 5

Table 8 Analysis of variance of the effect of diet and time on the width of the ovary

Sourch	df†	SS	MS	Fcal.	F. 05 tab.
Treatments	20	.5505			
A	2	.0152	.0079	1.2344	3.1
B	6	.4756	.0793	12.3906	2.3*
AB	12	.0597	.0050	0.7813	1.8
Error	84	.5402	.0064		

† symbols explained under Table 5

Histological Analysis

Females-Two conditions were noted in the histological sections of the female reproductive system as shown in Table 22; 1) the presence or absence of sperm in the bursa copulatrix and seminal receptacle, and 2) the developmental stages of oogenesis. With the exception of 1 female in the November control group and 1 female in the February control group, all females had mature sperm in the bursa and seminal receptacle throughout the experiment.

There was direct correlation between developmental stages of the oocytes and the food source. After 1 month the oocytes of all females except 1 in the control group were at stage 5. After 2 months, however, and throughout the remainder of the experiment, oocyte development was retarded in individuals from both control and rice powder groups. Monthly averages from the control group showed only 33 to 50% having stage 5 oocytes; those on rice powder ranged from 33 to 66%, while those fed diatoms ranged from 83 to 100%.

Males-Mature sperm was present in the testes of all males throughout the experiment (Table 22). No significant cell degeneration or delay in spermatogenesis was observed in the testes of any individual,

Discussion

Historical Lack of Quantification

Izumi (1951) noted that *Oncomelania hupensis nosophora* inhabited areas "richly fertilized with some vegetable decay, especially diatomaceous mould and where more freshwater algae is abundant..." He further stated that soil from snail areas in Yamanashi Prefecture had greater amounts of these organic substances than soil from the Tokyo suburbs.

Ritchie (1955) stated: "It appears that decaying matter, high in cellulose content and poor in protein, and living unicellular organisms of the soil constitute primary foods."

Mao (1958) reported that diatoms were a constituent of material ingested by *Oncome-*

Table 9 The influence of diet and time on the width (mm) of the bursa copulatrix; a summary of data for treatment groups

FACTOR A† (Diet)	FACTOR B (Time)							Total
	b_1	b_2	b_3	b_4	b_5	b_6	b_7	
a_1	2.54	2.38	2.03	1.61	1.87	1.76	2.10	14.29
a_2	2.54	2.59	2.13	1.96	1.94	1.78	2.22	15.16
a_3	2.54	2.46	2.35	2.20	1.98	1.41	2.01	14.95
Total	7.62	7.43	6.51	5.77	5.79	4.95	6.33	44.40

$\Sigma x^2 = 19.7851$

Each value is a total of 5 replicates of 1 snail each

† symbols explained under Table 5

Table 10 Analysis of variance of the effect of diet and time on the width of the bursa copulatrix

Source	df†	SS	MS	Fcal.	F .05 tab.
Treatments	20	0.4344			
A	2	0.0117	.0058	.87	3.1
B	6	0.3610	.0602	8.95	2.3*
AB	12	0.0617	.0051	0.77	1.8
Error	84	0.5647	.0067		

† symbols explained under Table 5

lania. Komiya *et al.* (1959) advocated feeding *Oncomelania* diatoms cultured with Knop's solution, and fine rice powder. Komiya *et al.* (1960) made an extensive study of the habitat of *Oncomelania* and found that *Navicula* abounded on a soil of pH 5.8 (650,000 living, 6,850,000 dead; maximum in one environment for 1 gram soil). In the laboratory, where they added diatoms to one culture but not another, it was found after a period out seecopy of time, that both cultures had about 350,000 living *Navicula* per gram of soil. Over a 6 month period, optimal survival of snails was in a culture provided with soil and rice powder, next best was with soil alone. However, their results both with mortality (54% in 6 months in the best condition) and growth (about 0.32 mm per week in the best condition) indicated that culture conditions other than food were poor as mortality should be no more than 20% for field snails over 6 months and growth rates should be in excess of 0.60 mm per week, (see Davis & Iwamoto, 1969, tables 3, 4; Van der Schalie & Davis, 1968, Fig. 18, Table 15).

Dazo & Moreno (1962) stated that *O. h. quadrasi* "appears to be a herbivore, its diet consists mainly of green algae and diatoms." H. van der Schalie & Davis (1965) considered "soil and its accompanying microflora" to be the crucial variable in supporting rapid growth and development of taxa of *Oncomelania*; they (1968) obtained satisfactory growth rates and fecundity (7.32 y/f/m for 12 m) with laboratory reared *O. h. nosophora* maintained on soil with a rich natural microflora of diatoms (300,000 to 450,000 living per gr. dried soil). In none of these reports are the effects of diatoms on fecundity quantified.

Unexpceted Poor Results With Rice Powder

Davis & Iwamoto (1969) showed that significantly greater production occurred with snails fed rice powder contrasted with soil-filter paper. The results with rice powder throughout this experiment were similar to those for control conditions current (Tables 2-5) and previous (Davis & Iwamoto, 1969).

Table 11 The influence of diet and time on the length (mm) of the posterior pallial oviduct. A summary of data for treatment groups

FACTOR A† (Diet)	FACTOR B (Time)							Total
	b_1	b_2	b_3	b_4	b_5	b_6	b_7	
a_1	13.11	11.10	12.29	10.58	10.46	11.40	11.19	80.13
a_2	13.11	15.00	11.61	10.65	11.22	11.53	11.34	84.46
a_3	13.11	15.00	13.30	13.03	12.36	12.83	10.84	90.47
Total	39.33	41.10	37.20	34.26	34.04	35.76	33.37	255.06

Σx^2, 640.049
Each value is a total of 5 replicates of 1 snail each
† symbols explained under Table 5

Table 12 Analysis of variance of the effect of diet and time on the length of the posterior pallial oviduct

Source	df†	SS	MS	Fcal.	F .05 tab.
Treatments	20	7.1417			
A	2	1.5408	0.7704	4.85	3.1*
B	6	3.4030	0.5671	3.57	2.3*
AB	12	2.1979	0.1831	1.15	1.8
Error	84	13.3314	0.1587		

† symbols explained under Table 5

It is possible that poor fecundity with rice powder in this study is correlated with seasonal effects on the reproductive cycle of these snails.

As shown before, using field snails, there was peak production in July-August with relatively few young produced from October to March, even after culture dishes were changed in January. It is possible that the food value of rice powder is sufficient to boost output of young during the period of seasonal influence on reproduction (July-August) but is not sufficient to maintain development and deposition of eggs throughout the year (i.e. October to March of both studies).

Another factor must also be considered. Peak production in this study occurred during October and November in cultures with rice powder or the controls. Thereafter fecundity decreased continually. As seen in Table 3, the number involved (a_1, a_2) were small even during these peaks relative to the production in cultures with diatoms (Table 3, a_3) or production in 1968 with rice powder (Davis & Iwamoto, 1969). It is possible that once a culture is constituted by selecting individuals from a field population and placing them in a new environment, there is a burst of reproduction which then subsides with only occasional subsequent spurts, even if culture containers are changed for the same individuals. The hypothesis presented here is that, in the case of field collected snails, the primary response is a burst of reproductive activity once a culture has been constituted which will not be equaled again throughout the remaining reproductive life of that group of snails. A secondary (and weaker) response is the seasonal effect influencing increased reproductive activity in July and August. Experiments are in progress to test this hypothesis.

Diatoms, A Superior Food for Oncomelania

As stated above, van der Schalie & Davis (1968) reared *Oncomelania* on soil naturally rich in living diatoms. They showed that

Table 13 The influence of diet and time on the width (mm) of the posterior pallial oviduct; a summary of data for treatment groups

FACTOR A† (Diet)	FACTOR B (Time)							Total
	b_1	b_2	b_3	b_4	b_5	b_6	b_7	
a_1	3.47	3.48	3.70	3.51	3.24	4.19	4.14	25.73
a_2	3.47	3.28	3.41	3.79	3.70	4.28	4.13	26.06
a_3	3.47	4.08	4.23	4.49	4.31	4.06	4.15	28.79
Total	10.41	10.84	11.34	11.79	11.25	12.53	12.42	80.58

Σx^2, 312.234
Each value is a total of 5 replicates of 1 snail each
† symbols explained under Table 5

Table 14 Analysis of variance of the effect of diet and time on the width of the posterior pallial oviduct

Source	df†	SS	MS	Fcal.	F .05 tab.
Treatments	20	0.6075			
A	2	0.1613	0.0806	5.798	3.1*
B	6	0.2470	0.0411	2.956	2.3*
AB	12	0.1992	0.0166	1.194	1.8
Error	84	1.1742	0.0139		

† symbols explained under Table 5

Table 15 The influence of diet and time on the width (mm) of the testis; a summary of data for treatment groups

FACTOR A† (Diet)	FACTOR B (Time)							Total
	b_1	b_2	b_3	b_4	b_5	b_6	b_7	
a_1	2.47	2.22	2.23	2.13	2.36	2.26	2.72	16.39
a_2	2.47	2.18	2.11	2.55	2.89	2.56	2.53	17.29
a_3	2.47	2.29	1.91	2.28	3.03	2.93	3.11	18.02
Total	7.41	6.69	6.25	6.96	8.28	7.75	8.36	51.70

Σx^2, 33.054
Each value is a total of 4 replicates of 1 snail each
† symbols explained under Table 5

laboratory reared *Oncomelania hupensis nosophora* would produce continunously over an 18 month period with an average production of 6.5 y/f/m for one experiment and 5.4 for another. Throughout these 18 months production fluctuated and there was no pronounced pattern of decreasing production through that time. Over a two year period the average y/f/m was 6.2 and 5.1 respectively reflecting the slight decreased production in the second year.

In the case of snails provided with diatoms in this study, there was a pronounced trend towards a more uniform production through time. Peak production occurred in January and a marked decrease did not occur until March. The implication is that a proper diet can sustain a long period of egg producing

Table 16 Analysis of variance of the effect of diet and time on the width of the testis

Source	df†	SS	MS	Fcal.	F .05 tab.
Treatments	20	0.516			
A	2	0.047	0.024	2.18	3.1
B	6	0.319	0.053	4.82	2.3*
AB	12	0.150	0.012	1.09	1.8
Error	63	0.718	0.011		

† symbols explained under Table 5

Table 17 The influence of diet and time on the width (mm) of the verge; a summary of data for treatment groups

FACTOR A† (Diet)	FACTOR B (Time)							Total
	b_1	b_2	b_3	b_4	b_5	b_6	b_7	
a_1	3.11	2.81	2.50	2.46	2.61	2.33	2.36	18.08
a_2	3.11	2.89	2.31	2.23	2.48	2.29	2.39	17.70
a_3	3.11	2.93	2.93	2.83	2.61	2.66	2.18	19.25
Total	9.33	8.63	7.74	7.52	7.70	7.18	6.93	55.03

Σx^2, 35.8299
Each value is a total of 4 replicates of 1 snail each
† symbols explained under Table 5

Table 18 Analysis of variance of the effect of diet and time on the width of the verge

Source	df†	SS	MS	Fcal.	F .05 tab.
Treatment	20	0.4875			
A	2	0.0466	0.0233	3.6984	3.1*
B	6	0.3530	0.0588	9.333	2.3*
AB	12	0.0879	0.0073	1.1587	1.8
Error	63	0.4028	0.0063		

† symbols explained under Table 5

and deposition as well as heightened productivity, off-setting the established trend for a burst of production and subsequent declining fecundity.

It is apparent that natural soil and its microflora are the most important factors to be considered in rearing *Oncomelania* when employing the van der Schalie-Davis model for rearing *Oncomelania*, whether in medium clay pots or 9 cm Petri dishes. These factors have the most profound influence on fecundity of the snails. Simple enrichment of the cultures with diatoms may not be sufficient; the micro-chemical composition of the soil must be an important factor. The ability of a soil to sustain a proliferation of diatoms is dependent on the presence of these micro-chemical constituents.

The y/f/m produced over 6 months by snails maintained on a diatom diet in this study were nearly double those reported previously when rice powder was used (Davis & Iwamoto, 1969). The maximum reproductive potential for *O. h. nosophora* is 44 y/f/m as recorded by Davis & Iwamoto (1969). In our experimental breeding programs we have realized

Table 19 Comparison of diets using Duncan's New Multiple Range Test to determine which diet(s) had an effect on the width of the posterior pallial oviduct

	Range	
Value of p†	2	3
SSR	2.80	2.95
LSR	0.58	.061

Difference in means		Comparison to LSR	Significance
a_3-a_1	=	0.087>0.061	SD
a_3-a_2	=	0.077>0.058	SD
a_2-a_1	=	0.010<0.058	—

$S\bar{x}=.021$
SD=significant difference, .05 level
error df=102
† symbols explained under Table 5

Table 20 Comparison of diets using Duncan's New Multiple Range Test to determine which diet(s) had an effect on the length of the posterior pallial oviduct

	Range	
Value of p†	2	3
SSR	2.80	2.95
LSR	0.202	0.215

Difference in means		Comparison to LSR	Significance
a_3-a_1	=	0.295>0.215	SD
a_3-a_2	=	0.171<0.202	—
a_2-a_1	=	0.124<0.202	—

$S\bar{x}=0.073$
SD=significant difference, .05 level
error df=102
† symbols explained under Table 5

only 1/3 of this potential. Further experimentation is necessary to determine if efficient procedures can be instituted to gain full reproductive potential or production at least at a level of 30 y/f/m each month.

Long Term Experiments Are Necessary

As discussed previously (Davis & Iwamoto, 1969) evaluation of data of Chi & Wagner (1957) indicated a maximum possible production *of 3.88* eggs/f/m for *O. h. nosophora* over

Table 21 Comparison of diets using Duncan's New Multiple Range Test to determine which diet(s) had an effect on the decreasing width of the verge

	Range	
Value of p†	2	3
SSR	2.81	2.95
LSR	0.0486	0.0510

Difference in means		Comparison to LSR	Significance
a_3-a_2	=	0.0554>.0510	SD
a_3-a_1	=	0.0418<.0486	—
a_2-a_1	=	0.0136<.0486	—

$S\bar{x}=.0173$
SD=significant difference, .05 level
error df=82
† symbols explained under Table 5

a 6 month period (singly paired snails). Halstead & Wagner (1954) reported a fecundity rate of 10.20 eggs/f/m for *O. h. quadrasi* (time period not known). Pesigan et al. (1958) reported 11.70 eggs/f/m (for 13 recently collected field snails) over a 3 month period, and stated that 2 eggs were deposited every 5 days for each female. As not all eggs hatch, the y/f/m would be somewhat lower. In this study, as in past studies, if only the initial 3 months of data were gathered there would have been nearly double the y/f/m recorded. In this study 5.3 y/f/m for snails in control cultures and 7.5 y/f/m for those in cultures provided with rice powder were recorded during the first 3 months. Where culture programs are to be counted on for long term performance it is necessary to know the trend of culture performance over long periods of time. Six months are considered a minimum.

The Effect of Diet on Histology and Morphology of the Reproductive Organs

Because of the importance of understanding oogenesis in this experiment, a brief description is included only in so far as it pertains to these studies. We were interested in enumerating well defined stages as revealed by Haematoxylin and Eosin staining especially with regards to late oogenesis. Fahmy (1949)

Table 22 Histological condition of the ovary and testis under three different food conditions

Month	Histological Condition of Ovary			Sperm present in bursa copulatrix or/ and Sem. Receptacle			Histological Condition of Testis		
	Cont.	Rice	Diat.	Cont.	Rice	Diat.	Cont.	Rice	Diat.
Sept.	4*(2)			+(5)			3(0)		
	5(3)			−(0)			4(5)		
Oct.	4(1)	4(0)	4(0)	+(6)	+(5)	+(6)	3(1)	3(0)	3(0)
	5(5)	5(6)	5(6)	−(0)	−(0)	−(0)	4(3)	4(4)	4(4)
Nov.	4(3)	4(3)	4(1)	+(5)	+(5)	+(5)	3(0)	3(0)	3(0)
	5(2)	5(3)	5(5)	−(1)	−(0)	−(0)	4(4)	4(4)	4(4)
Dec.	4(3)	4(3)	4(1)	+(6)	+(6)	+(6)	3(0)	3(0)	3(0)
	5(3)	5(3)	5(5)	−(0)	−(0)	−(0)	4(4)	4(4)	4(4)
Jan.	4(5)	4(2)	4(1)	+(5)	+(6)	+(6)	3(0)	3(0)	3(0)
	5(1)	5(4)	5(5)	−(0)	−(0)	−(0)	4(4)	4(4)	4(4)
Feb.	4(5)	4(4)	4(1)	+(5)	+(6)	+(6)	3(0)	3(0)	3(0)
	5(1)	5(2)	5(5)	−(1)	−(0)	−(0)	4(4)	4(4)	4(4)
Mar.	4(5)	4(3)	4(0)	+(6)	+(6)	+(6)	3(0)	3(0)	3(0)
	5(1)	5(3)	5(6)	−(0)	−(0)	−(0)	4(4)	4(4)	4(4)

* Stage of development of ovary or testis
( ) Number of snails
Diat. = diatoms
+ = present
− = absent

and Crabb (1927) primarily used nuclear changes as an index to oogenesis in *Eremina desertorium*. The variability, however, in nucleolar budding and stainability precluded any use of these criteria in *Oncomelania*. Cytoplasmic changes provided us with a much easier tool to work with. Fahmy (1949), Hartung (1947) and Guraya (1957), all have categorically described cytoplasmic changes utilizing many specific fixatives and stains. The basic definition of stages and terminology here, follow closely that of Fahmy (1949). The first three stages of development, namely, 1) no primordia ; 2) primordia but no oocytes and 3) small oocytes budding from the ovarian wall never did appear as the most advanced stage in ovarian sections. All three stages, however, are easily recognized on the basis of oocyte presence, size and shape (Fig. 1). The budding oocyte is approximately 12μ in diameter and the cytoplasm stains uniformly basophilic. Growth of the budding oocyte involves a rapid increase in cytoplasmic and nuclear volumes. The nuclear diameter increases proportionately at a greater rate than the cell diameter. The cytoplasm soon displays a heterogeneous quality when stained with H & E. This is due to a heavy concentration of basophilic particles immediately around the nucleus while the remaining cytoplasm becomes slightly acidophilic. The same phenomena were described in the oogenesis of the desert snail by Fahmy.

The Aniline Acid Fuchsin, Methyl Green method as given by Lillie (1965) for mitochondria and DeFano's Cobalt Silver method as given by Galigher & Kozloff (1964) for Golgi bodies, indicated both mitochondria and Golgi bodies, constituted the mass of particles surrounding the nucleus. The diameter of the oocyte at this stage (our stage 4) ranges from 30–90μ (Fig. 1 e, f). The formation of yolk is generally regarded as the final developmental process in the maturing oocyte. Thus

the presence of fatty yolk droplets as detected by Sudan B constituted the basic criteria for our fifth stage (Fig. 2b, droplets as seen using H & E).

Correlated with yolk deposition, however, was the disappearance of the dark basophilic mass (mitochondria and Golgi bodies) surrounding the nucleus. This indicates that either or both organelles may play a role in yolk deposition. Such a theory has been suggested by several authors, among them Guraya (1957).

Oocytes in the ovary of females fed on diatoms were most frequently in the final stages of oogenesis than those of females fed on other food combinations. This is correlated with productivity which was more than tripled compared with fecundity of females fed on rice powder or soil. In the absence of diatoms, oogenesis is retarded after one month in culture. Reduction in fecundity may be correlated with a decrease in fatty metabolism within the developing oocyte and thus a decrease in rate of production of stage 5 oocytes. As noted in Komiya et al. (1960, Table 4), diatoms have a high gross lipid to protein ratio (1.08–1.27% lipid to 5.20–5.57% protein); it appears that the diatoms have a high energy content coupled with the ability of the snail to metabolize and utilize this energy source.

Decrease in organ dimension through time for the ovary and bursa copulatrix is correlated with decreased oogenesis and reproductive activity. When the ovary is in peak activity the lobes are swollen with large oocytes in Stage 5 development. Likewise, the bursa copulatrix is swollen with decomposition products of sperm and fluid. In the case of the posterior pallial oviduct it is noted (Fig. 6b) that there is a significant and pronounced increase in width in November, December, January. Correlated with this is the peak production of young in January (Fig. 4). The posterior pallial oviduct is a highly glandular organ staining deeply with eosin. Products from the organ are essential for coating the oocyte with nutrient. The marked increase in width of the organ is correlated with the diatom diet. The significant decrease in length of the posterior pallial oviduct is not explicable.

Concerning males, there is no effect of a food additive on the histology or process of spermatogenesis. The fact that mature sperm was produced at all time indicates that the energy source derived from soil, rice powder, filter-paper or diatoms is sufficient for successful spermatogenesis. Reduction of fecundity is not related to the effects of diet and/or time on spermatogenesis or the ability of the female to store sperm in the seminal receptacle. The increase in testes width is correlated with increased numbers of spermatozoa swelling out the lobes of the testis. The decrease in width of the verge cannot be explained with the data available.

Abstract

The effect of a diatom diet on the fecundity of *Oncomelania* had not previously been quantitatively tested. We found, over a 6 month period, that an average production of 13.7 young per female per month (y/f/m) resulted when snails were maintained on a diatom diet. Results with rice powder and control soil were not significantly different over the same period (4.6 and 2.5 y/f/m). respectively). Poor results with rice powder during the October to March period are discussed. Fecundity correlated with a diatom diet was more regular throughout the period contrasted with continually decreasing fecundity by females maintained on other diets. Results of histological studies revealed that the diatom diet was correlated with continual development of fully mature oocytes (stage 5) throughout the experiment. Oocyte development did not reach full maturity after one month when the diets of rice powder or soil were used (33 to 66% reached stage 5 in the former, 33 to 50% reached stage 5 in the latter).

Significant changes in the dimensions of the ovary, bursa copulatrix, posterior and anterior pallial oviduct, testis and verge were recorded and discussed.

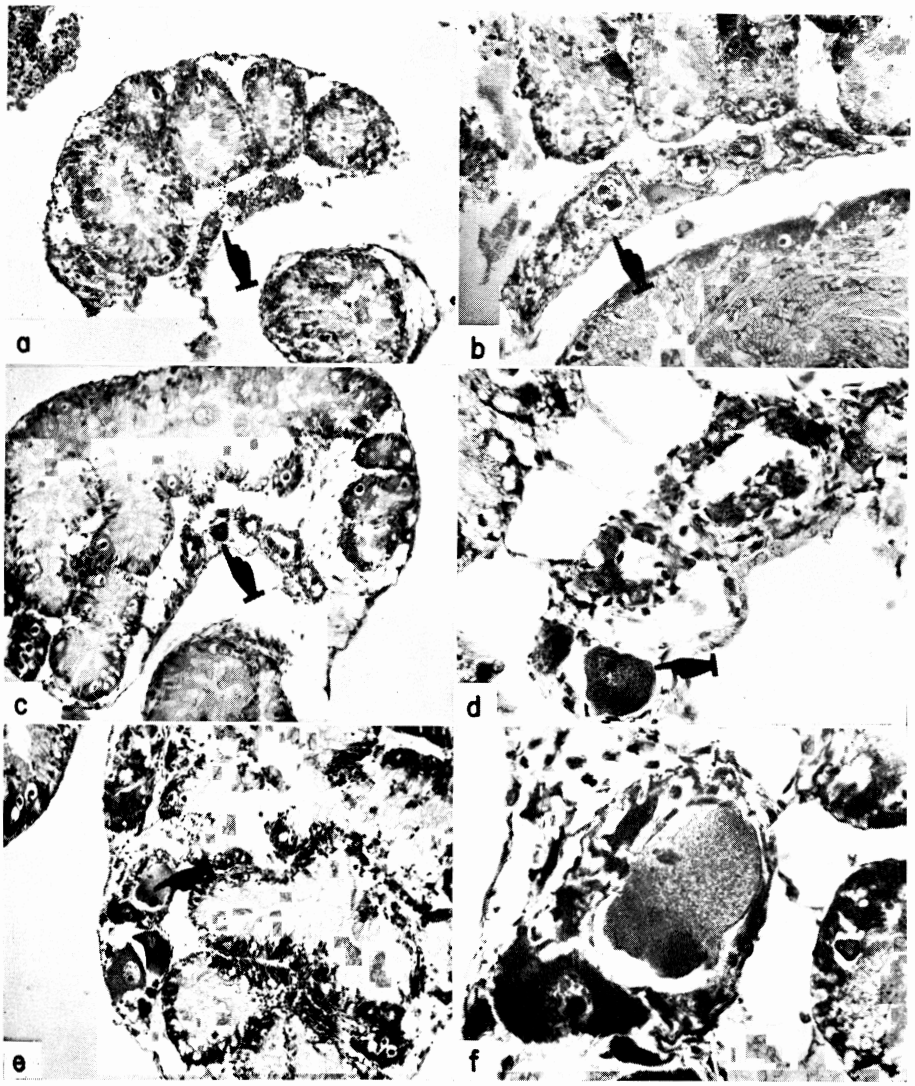

Fig. 1 Histology of the ovary showing developmental stages 2 to 4 as defined in the text. The photomicrographs were taken while viewing the slides at 100 and 400 X. a) The strip of ovarian promordium is pointed out and enlarged in b to show that no oocytes have yet developed. c) Stage 3 with a developing oocyte pointed out. The enlargement in d indicates the presence of oocytes just beginning to develop at the ovarian wall. e) Stage 4 with an oocyte having differentially stained cytoplasm pointed out (enlarged in f).

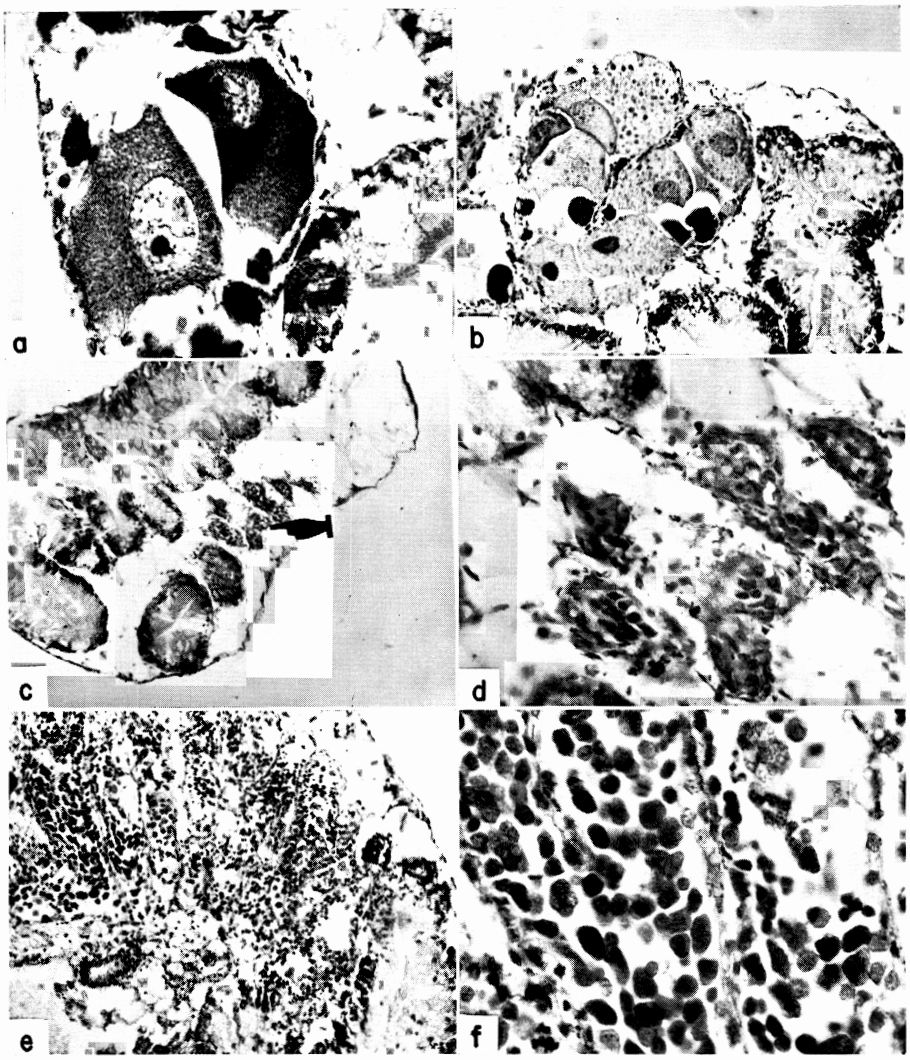

Fig. 2 Histology of the ovary and testis demonstrating developmental stages 2 to 5 as defined in the text. The photomicrographs were taken while viewing the slides at 100 or 400 X. Compare stage 4 oocytes (a) with those at stage 5 (b). c) Stage 2 testis with developing testicular lobes with few spermatocytes pointed out (enlarged in d). e) Stage 3 testis showing numerous spermatocytes in well developed testicular lobes (enlarged in f).

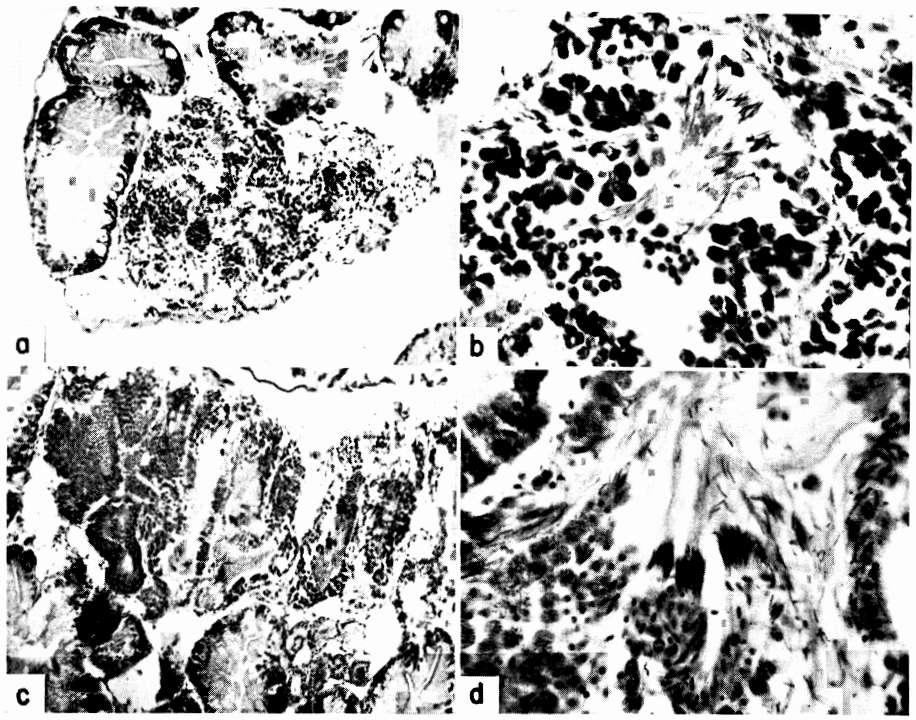

Fig. 3 Histology of the testis demonstrating stages 4 and 5. The photomicrographs were taken at 100 and 400 X. a) Stage 4 with spermatid and few sperm (enlarged in b). c) Stage 5 with numerous sperm (enlarged in d).

Acknowledgements

We wish to thank Miss's Yaeko Iwamoto and Chitose Hishiyama for their dedicated help in maintaining the snails used in this experiment. The histological aspects were conducted with the help of Takeshi Sakuma. Dr. C. Reimer of the Philadelphia Academy of Sciences is credited with identification of the diatom cultured in this study. Special thanks go to MAJ Jimmy Young of this laboratory for his help with the statistics. The continued interest and support of COL James McCarty, Commanding Officer of the 406 Medical Laboratory, is gratefully acknowledged.

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Oncomelania hupensis nosophora の繁殖に及ぼす珪藻食の影響

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珪藻食の投餌が，宮入貝の生殖力に与える効果についての定量的な実験は，従来行われていなかつた．われわれは，6カ月以上の実験において，宮入貝が珪藻食で飼育された場合に，一雌貝当り1カ月平均，13.7 の稚貝 (y/f/m) が得られることが判つた．同じ実験において，米粉投餌群および対照群（土壌のみ）の両者の間には，有意的な差がみられなかつた(4.6 および 2.5 y/f/m)．10月より3月にかけて，米粉投餌群には良好な結果が得られなかつた．珪藻食投餌群の生殖値は全期間を通じて規則的であつたのに対して，他の食餌群の生殖値には連続的な減少がみられた．組織学的にみて，珪藻食投餌群においては，全実験期間を通じて，充分に成熟した卵母細胞 (stage 5) の発達がみられたが，米粉および対照土壌群においては，卵母細胞の発達は1カ月を経過してもなお，充分な成熟に達しなかつた（米粉群では33—66%，土壌群では33—50%が stage 5 に達した）．

更に，卵巣，交接のう，前部および後部の外とう卵管，こう丸および陰茎の大きさでは，各群について有意的な変化がみられた．