# The ecological energetics of *Parartemia zietziana* Sayce (Crustacea:Anostraca) in two saline lakes in western Victoria

by

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## ABSTRACT

*P. zietziana* (brine shrimp) was studied for two years in two shallow ($\leq$1 m), saline ($>$60 ‰) lakes, Lake Cundare and Pink Lake, 26 km north of Colac, Victoria. There were marked fluctuations in salinity in both lakes during this study, the highest salinities occurring in summer.

Quantitative samples of *P. zietziana* were taken monthly. These showed that on most occasions the shrimp were contagiously distributed, most probably due to wind generated currents. Despite stratification of the samples, the confidence limits of the average population density were 40-50%. However, sampling was representative because stable trends emerged and variability was not so great that significant differences could not be detected.

Cohorts of the shrimp were distinguishable and a regression established between length and dry weight. Thus growth could be calculated and then production by combining density and growth data in Allen curves. Generally, there were two or three generations each year, but time of recruitment was not predictable. In all cohorts there was more or less continuous mortality which was not due to salinity or temperature stress except in summer. Production was largely due to the death of small individuals and was about ten times higher in Pink Lake (11.3 g $m^{-2}$ $year^{-1}$) than in Lake Cundare (1.0 g $m^{-2}$ $year^{-1}$), as was the population density.

Respiratory rate was measured by incubating *P. zietziana* *in situ* in B.O.D. bottles. Tests in which the oxygen decline was monitored continuously showed there was no handling effect and that respiratory rate was constant down to 1.8-1.9 mg $O_2$ $l^{-1}$, about 30% of the usual initial concentration. Incubations over twenty-four hours demonstrated

there was no diurnal variation in oxygen consumption. A multiple regression analysis of the data indicated that 90% of the variance in respiratory rate was accounted for by changes in salinity (3%), temperature (7%) and dry weight (80%). From the regression equation and data on population density, population respiration was calculated: 91864.5 mg $O_2$ $m^{-2}$ $year^{-1}$ in Pink and 12367.5 mg $O_2$ $m^{-2}$ $year^{-1}$ in Cundare.

Primary production was known to be very low and was shown to be insufficient for the observed assimilation (production + respiration). Usually the shrimp ate sediment. The caloric content of mud samples taken over six months in Pink was measured by wet oxidation giving an average value of 211.1 cal $g^{-1}$ dry washed mud or 4% organic matter. Ingestion rate was measured <u>in situ</u> by following the uptake of $^{14}C$ by shrimp feeding on labelled mud in the lake. Faecal pellet production was also measured <u>in situ</u>. Variation in dry weight appeared to be the only factor affecting feeding or defaecation rates. By combining these data, assimilation efficiencies of 30-60% were calculated. Comparison of assimilation rates with respiratory rates (from regression equation) showed that in most cases shrimp were not assimilating enough energy although they always ingested sufficient; comparison of ingestion rate with respiratory rate over a range of dry weights showed that small shrimp ($\leq$0.2 mg) could not even ingest enough sediment. It was argued that the poor assimilation rate caused the observed mortality in each cohort and the unpredictability in the time and extent of recruitment.

## DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and to the best of my knowledge contains no material published or written by another person except where reference is made in the text.

R. MARCHANT

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# CHAPTER 1 - Introduction

Energy flow through animal populations has been studied by those interested in quantifying energy relations of various trophic levels. Sometimes energy flow is measured through every trophic level but more often a population of one species or a community of closely related species is studied and data collected on at least three important aspects:

1. the rate of energy intake (food);
2. the rate of energy elimination (faeces);
3. the rates at which assimilated energy is metabolised for maintenance and growth.

For a given period, usually a year, the sum of 2 and 3 should equal 1 and represents an energy budget. This is one way, no less reliable than others, of quantifying ecosystem structure.

Unfortunately budgets can give the impression of a static ecosystem in which energy fluxes are invariable, thus missing a significant ecological question. What controls rates of energy flow and what are their significance for the distribution and abundance of animals? If some attempt is made to discover the dynamics of the system by showing how and why the partitioning of energy described above varies with external conditions, e.g. temperature or food supply, and internal conditions, e.g. size of animal, or whether, in fact, energy demand is always satisfied by the input, then it may be possible to answer these questions. In my view the study of the energy relations of a species must not only show how much energy flows, but what physiological and ecological features control its flux once likely physical and chemical factors have been accounted for.

The Western District of Victoria, an agricultural area of cleared volcanic plains west of Melbourne, contains a number of shallow salt lakes of various salinities and depths. The shallow and more saline ones ($< 2$ m, $\geq 60‰$)

possess an invertebrate community of few species in which the anostracan crustacean Parartemia zietziana, the brine shrimp, is usually prominent. My aim has been to measure the energy flow in two populations of this species and determine the major factors controlling it.

Over the last decade various aspects of the ecology of these lakes and biology of the animals inhabiting them have been studied. Most of this work has been summarised by Bayly and Williams (1973). The shallow lakes, particularly the most highly saline (>60‰), are described as simple homogeneous ecosystems of low species diversity which Williams (1972) has suggested would be ideal for studies of trophic relations and energy flow. So far a number of studies on primary production have been completed (Walker, 1973; Hammer, Walker and Williams, 1973), on the less saline lakes (<40‰) at Red Rock, 13 km NW of Colac, Victoria and on Lake Corangamite (25‰), 13 km W of Colac. Some of the more highly saline lakes in the region have also been studied but the results have yet to be published. The indications [Hammer, 1970 (abstract) and Williams (personal communication)] are that primary production in these is very low because of high turbidity. In these lakes allochthonous inputs which are broken down by bacteria may be the major source of energy (Williams, 1972).

Much less is known about secondary production in saline lakes of this or any other region. Paterson and Walker (1974) estimated the annual net production of Tanytarus barbitarsis, a benthic chironomid, in Lake Werowrap (13 km north west of Colac, 36-56‰). In addition, Walker (1973) investigated the population dynamics of the rotifer Brachionus plicatis in the lake but did not estimate its production. There have been no further quantitative studies in this region of the life cycles of zooplankton or other aquatic invertebrates. The only seasonal study (one year) of the lakes containing P. zietziana was by Geddes (1976). He sampled qualitatively a series of lakes of increasing salinity (26 km N of Colac) and was able to show that the brine shrimp withstood wider fluctuations in salinity than any

of the other invertebrates present and that cohorts of this animal were distinguishable lasting for three to nine months.

Geddes' study was part of a larger work concerned with the taxonomy of the genus in Australia and physiological investigations of its osmoregulatory abilities (Geddes 1975 a,b,c). Parartemia is endemic to Australia, P. zietziana being the only species in south east Australia and Tasmania. There are at least seven other species in northern and western Australia. All have been found in astatic saline waters, and P. zietziana in lakes in which the salinity varies seasonally between 41.5-300‰. They survive drought by laying eggs which resist both drying and salinities high enough to kill the shrimp. When the salinity is subsequently lowered or the lake refills, the eggs hatch (Geddes, 1976). They also produce eggs which hatch inside the egg sac (subitaneous). According to Geddes, as the salinity rises they switch from producing subitaneous to resistant eggs.

Parartemia is not closely related to the brine shrimp of the northern hemisphere Artemia salina, another halobiont, that has been known for at least one hundred years (Littlepage and McGinley, 1965): Artemia belongs to the monogeneric Artemiidae, Parartemia to the Branchipodidae. Both, however, have the same general form and characteristically swim on their backs while filter feeding with the setae on their legs. Food is transferred down the legs to a ventral groove which transports the particles to the mouth (Reeve, 1963 a). In the wild, A. salina usually feeds on algae, although Eardley (1938) reports them feeding on sediments in the Great Salt Lake, Utah. In the laboratory they have been successfully cultured reaching maturity in twenty to forty days (Gilchrist, 1960; Reeve, 1963 d; Mason, 1963) with algae, yeast and bacteria as food. Both are strong hypo-osmotic regulators, the mechanisms of which have been elucidated by Croghan (1958 a,b,c) for A. salina and Geddes (1975 a,b,c) for P. zietziana. A major difference between the two is that A. salina is parthenogenetic with few males occurring in wild populations (Flowers and Evans, 1965; Carpelan, 1957); Parartemia

is always dioecious.

Surprisingly, there has been little quantitative work on the ecological dynamics of A. salina. In general the shrimp overwinter as resistant eggs that hatch in spring, giving rise quickly to juveniles and adults. Generation time must be close to that in culture i.e. thirty days because they breed at least twice in the summer producing two types of egg: thin walled that hatch immediately and thick walled or resistant eggs that overwinter after the remaining adults die in autumn. Occasionally juveniles also overwinter. There have been no field studies in which a reliable sampling scheme has been used to quantify their life history; mortality and natality rates have not been measured in the field. Carpelan (1957) estimated a minimal value for biomass production of A. salina in a commercial salt field in California based upon the probable number of generations per year multiplied by the average size of his samples. Mason (1967) in a study of Mono Lake, California, quantitatively sampled A. salina, but did not use his data to estimate production or clarify its life history. There appears to have been sporadic work on the Great Salt Lake, Utah, summarised by Flowers and Evans (1966) from which the life history outlined above is known, but little else. There is thus scant information in the literature which bears on energy flow through natural populations of brine shrimp and no clues as to what may be the most important factors controlling this.

A. salina, however, is readily cultured in the laboratory (many references in Littlepage and McGinley, 1965) and here its feeding, respiration and growth have been quite thoroughly studied (Gilchrist, 1954, 1956, 1958, 1960; Kuenen, 1937; Eliassen, 1952; Reeve, 1963 a,b,c,d; Mason, 1963; von Hertig, 1971). These investigators were mainly concerned with the influence of salinity, temperature and animal size on these processes. Reeve and Mason particularly discussed the influence of food (algae) concentration on feeding rates and growth efficiencies. No one has measured the rates of any of these processes for wild populations of A. salina to estimate

their contribution to the metabolism of a salt lake, let alone to determine which, if any, control survival of the shrimp or how they compare with such influences as competition or predation. Suschenya (1962) and Klekowski (1970) have combined laboratory rates into energy budgets but these tell us little about what controls energy flow in wild populations.

To decide the influence of these physiological considerations on the bioenergetics of the brine shrimp in the simple communities of salt lakes, field estimates of their feeding, respiration and growth rates plus a programme of quantitative sampling are necessary and possible. One of the major considerations of this thesis is to show that field rather than laboratory data are essential for understanding the flow of energy through natural populations of P. zietziana. Such single estimates are often difficult to relate to the total energy expenditure of a community. In my case this is less true because there are no tertiary producers and energy from primary producers will be consumed mainly by P. zietziana.

There are some data on the ecology of other anostracans (Hartland-Rowe, 1972) many of which live in temporary, but less saline waters than brine shrimp. This mainly describes various life histories and investigates hatching stimuli. The only work in which population dynamics and energetics have been studied in the field is Daborn's work (1975) on the large predator, Branchinecta gigas, in a shallow (about 1 m), turbid fresh water lake in eastern Alberta, Canada.

# CHAPTER 2 - Study area

## Physical and Chemical features

The two lakes in which P. zietziana were studied, Lake Cundare (38° 09' S, 143° 37' E) and Pink Lake (38° 06' S, 143° 40' E), lie approximately 26 km north of Colac, Victoria (Fig. 1). Lake Cundare is approximately 3 km long and 1 km wide, with an area of about 300 ha. and a mean depth of 50 cm; Pink Lake is roughly square with a side of approximately 360 m, an area of 13 ha. and a mean depth of 1 m. Both lakes have flat mud bottoms composed at least partly of faecal pellets from P. zietziana. Cundare's sediments are firm and clayey while Pink's are looser and more silty.

The regional climate (summarised in Table 1) is cool temperate. Rain occurs mainly during winter and early spring, and was generally higher than average during my study. The excess of evaporation over rainfall and the absence of any rivers draining the region north of Colac explain the predominance of saline lakes.

I visited the lakes approximately monthly. Salinity, temperature and water level were always measured. The field station of the Zoology Department, Monash University at Alvie (12 km NW of Colac) was used as a base.

## Methods

Total dissolved solids (T.D.S.) were taken as the best measure of salinity. They were calculated from conductivity measurements using the regression equation of Williams (1966) relating T.D.S. to conductivity. Samples with a T.D.S. greater than 150‰ were diluted because the regression is not accurate at such high salinities (Williams, 1966). Water temperature was measured with a mercury in glass thermometer and a maximum/minimum thermometer located about 30 cm below the surface. Water level was gauged from a datum marked on a pole in each lake.

FIGURE 1

The study area. The lakes not named are all saline. (C = Colac)

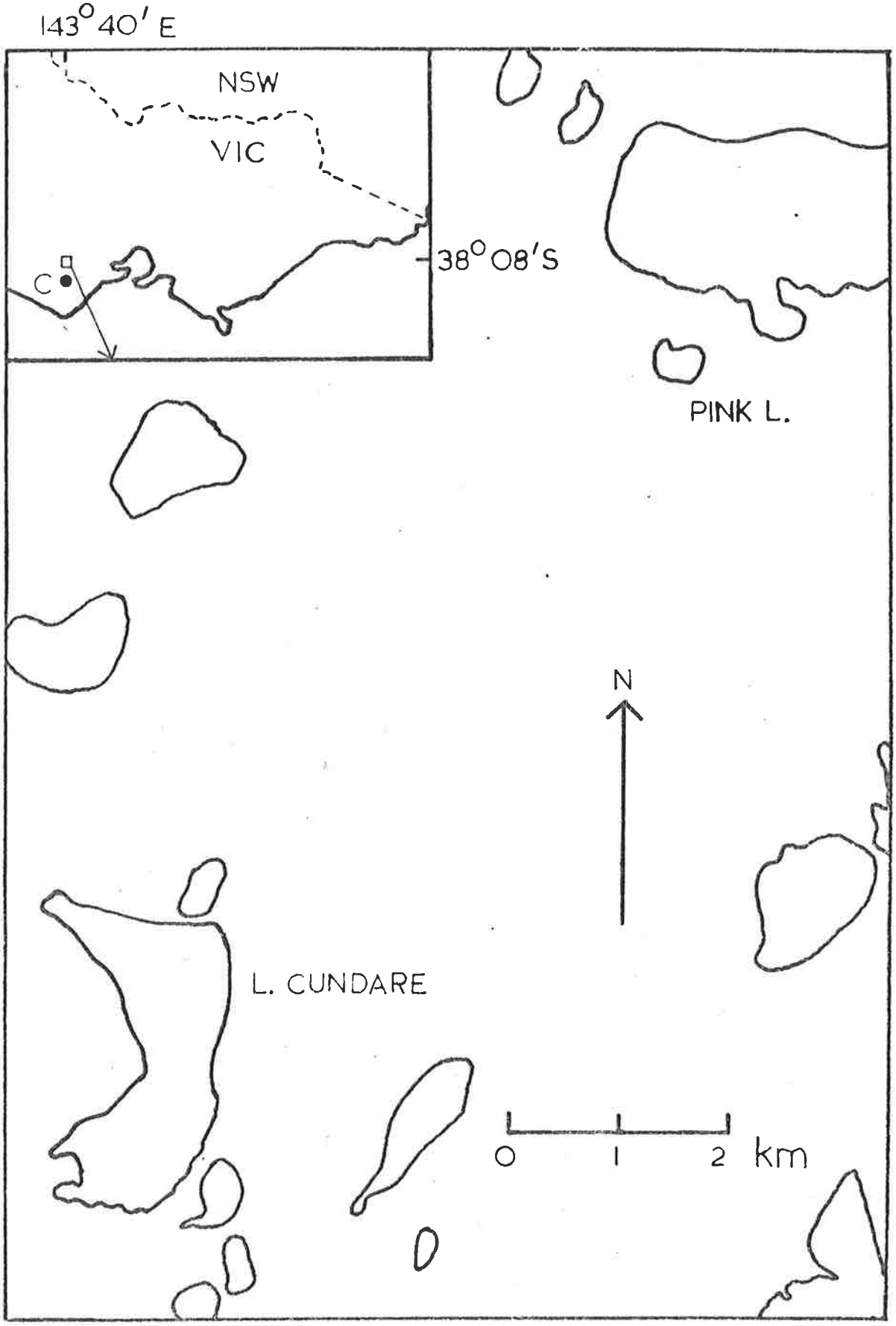

143°40′ E
NSW
VIC
38°08′S
C
PINK L.
N
L. CUNDARE
0
1
2
km

TABLE 1

Meteorological data recorded at Colac and at Alvie, 11 km north west

| | Jan. | Feb. | Mar. | Apr. | May | June | July | Aug. | Sep. | Oct. | Nov. | Dec. | Year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean daily[a] Max temp C° | 26.2 | 25.4 | 23.4 | 19.4 | 15.3 | 13.6 | 12.0 | 13.5 | 15.4 | 17.8 | 20.1 | 22.8 | 18.7 |
| Mean daily[a] Min temp C° | 9.0 | 9.6 | 9.0 | 6.8 | 5.1 | 4.0 | 3.2 | 3.9 | 4.8 | 6.0 | 7.2 | 8.5 | 6.4 |
| Mean[a] Rainfall (mm) | 32 | 37 | 44 | 56 | 70 | 76 | 76 | 86 | 74 | 68 | 57 | 45 | 721 |
| Rainfall (mm)[b] 1973 | 28 | 136 | 61 | 131 | 106 | 67 | 34 | 73 | 84 | 97 | 49 | 20 | 886 |
| Rainfall (mm)[b] 1974 | 44 | 26 | 24 | 108 | 40 | 28 | 153 | 138 | 125 | 87 | 40 | 64 | 877 |
| Rainfall (mm)[b] 1975 | 28 | 5 | 84 | 35 | 73 | 51 | 117 | 130 | 146 | 212 | - | - | - |
| Evaporation (mm)[c] | 180 | 169 | 120 | 83 | 49 | 33 | 43 | 49 | 59 | 91 | 116 | 157 | 1149 |

[a] From Commonwealth Bureau of Meteorology 1975, Colac Station, Victoria

[b] From Monash University field station, Alvie, Victoria

[c] Mean values for 1969 and 1970 at field station from Walker (1973)

Results and Discussion

T.D.S. (Figs. 2 and 3) shows the typically wide annual fluctuations which have been recorded in many of the lakes in this region (Williams and Buckney, 1976), being lowest after the winter rain when rainfall exceeds evaporation. As expected, movement of the water level follows the salinity cycle.

The chemistry of both lakes is dominated by the Na and Cl ions. Williams and Buckney (1976) found their ionic proportions remained very constant over four years. They concluded that, chemically, these lakes are very homogeneous. The average pH is 8.4 and stable in both, and the mean turbidity (secchi disk) ranges from 9 cm in Cundare to 49 cm in Pink (Williams, personal communication).

The water temperatures are presented in Figs. 4 and 5. The mean temperature at each visit is based on either a single reading within two hours of midday or four to five readings spaced throughout twenty four hours. The readings were somewhat erratic more so in summer and in Cundare, as expected in shallow lakes. Mean monthly water temperatures (important in calculating annual respiration rates) were obtained either directly from the mean temperature on each visit or by averaging this with the adjacent maxima and minima. In this manner the available data were fully used and gave results (Tables 12 and 13, Chapter 5) which are close to the mean monthly water temperatures Walker (1973) recorded in Lake Werowrap (13 km NW of Colac; mean depth 1.4 m) with a continuous temperature recorder. There is no temperature stratification because of wind and the shallowness of the lakes (Hussainy, 1969).

Biological features

Both lakes are surrounded by fields used for sheep and cattle or crops. They are quite exposed to wind. Consequently allochthonous organic material readily enters the lakes and is probably their major source of energy.

FIGURE 2

Fluctuation in the salinity (● ) and water level ( o ) of Pink Lake

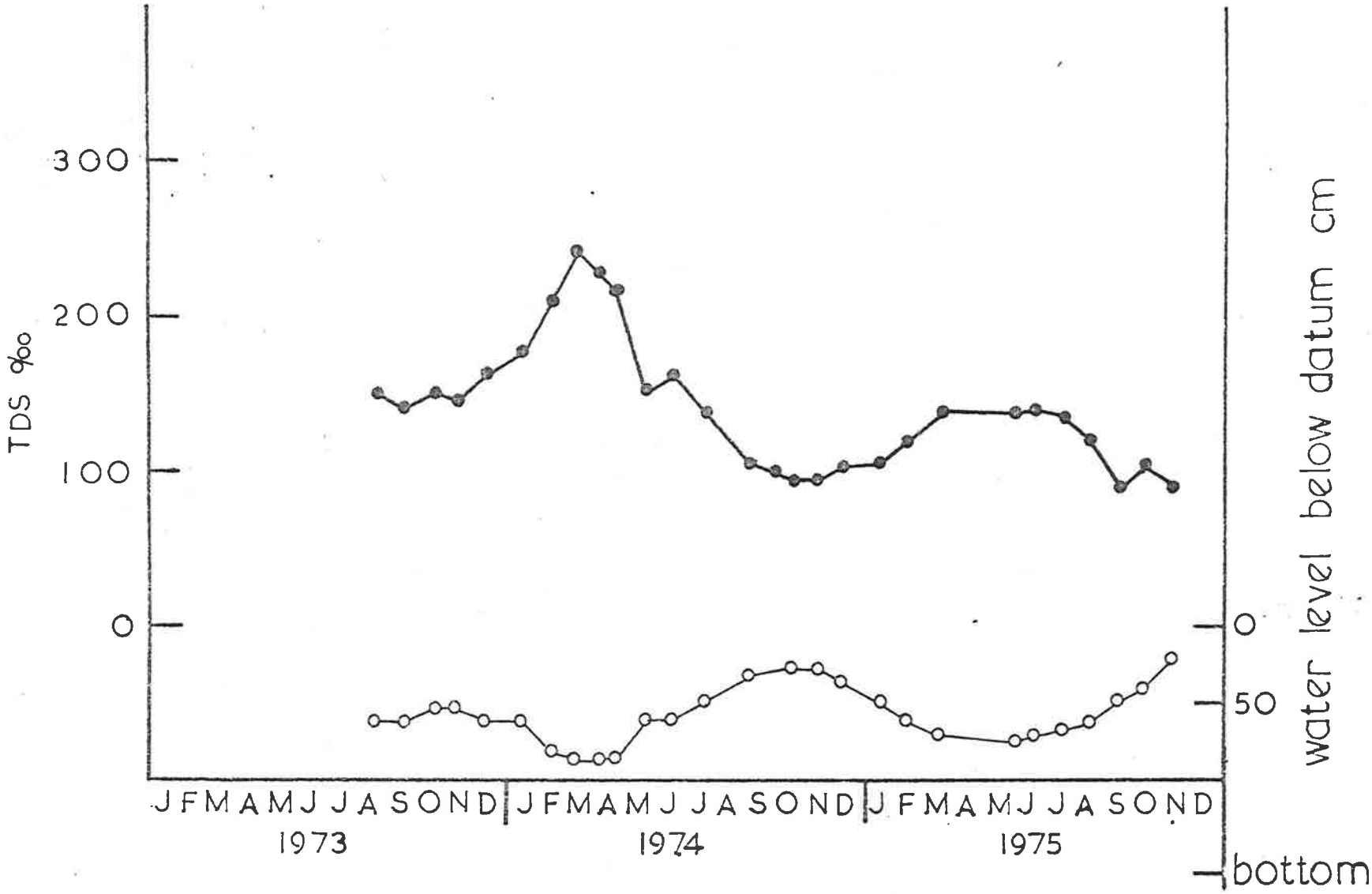

300
200
100
0
TDS ‰
0
50
water level below datum cm
bottom
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1973
1974
1975

FIGURE 3

Fluctuation in the salinity ( ● ) and water level ( o ) of Lake Cundare.

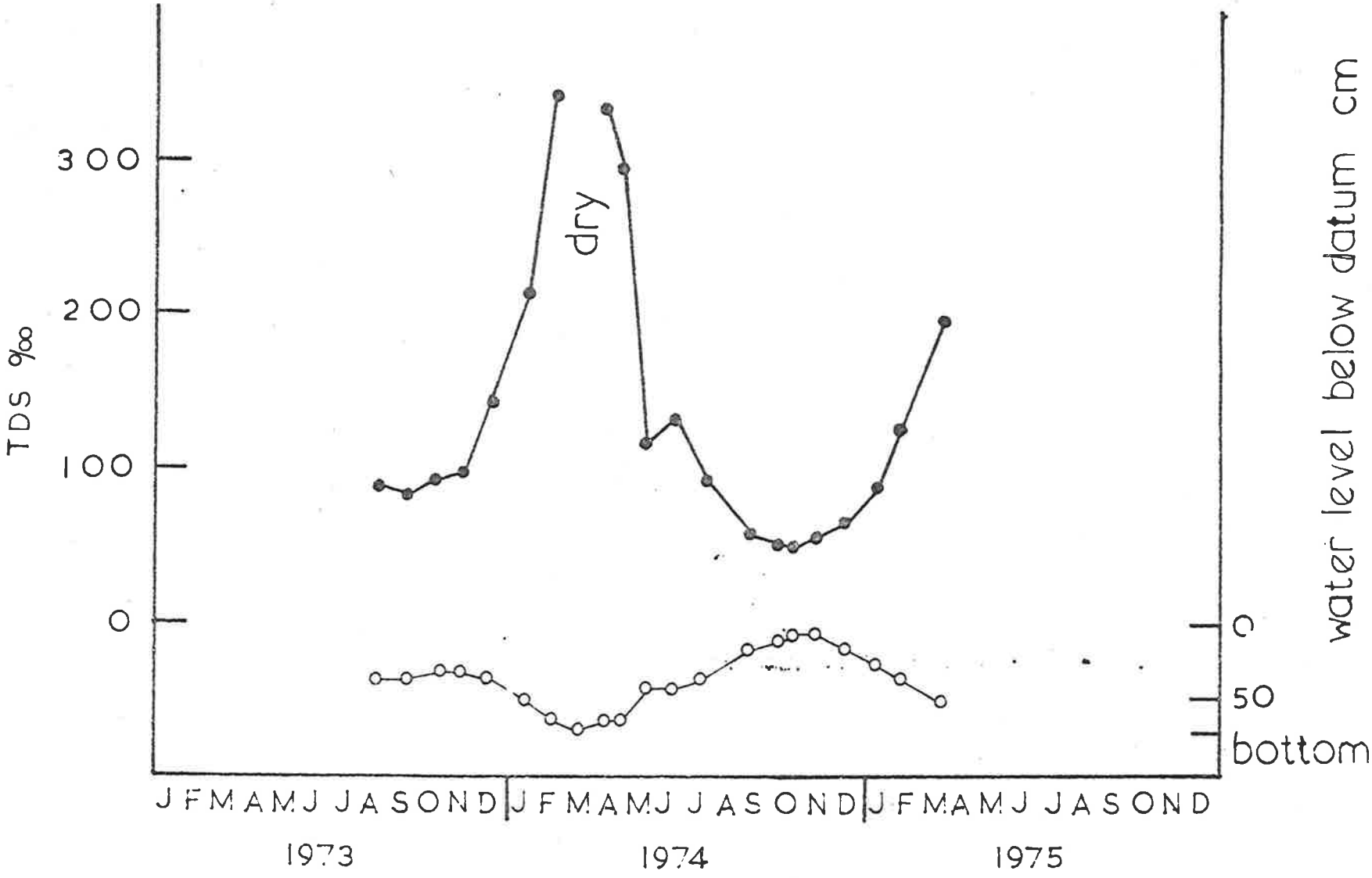

TDS ‰
300
200
100
0
dry
water level below datum cm
0
50
bottom
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1973
1974
1975

FIGURE 4

Mean temperature ( ● ) of Pink Lake on each visit and maxima and minima between visits. Gaps indicate no records were taken.

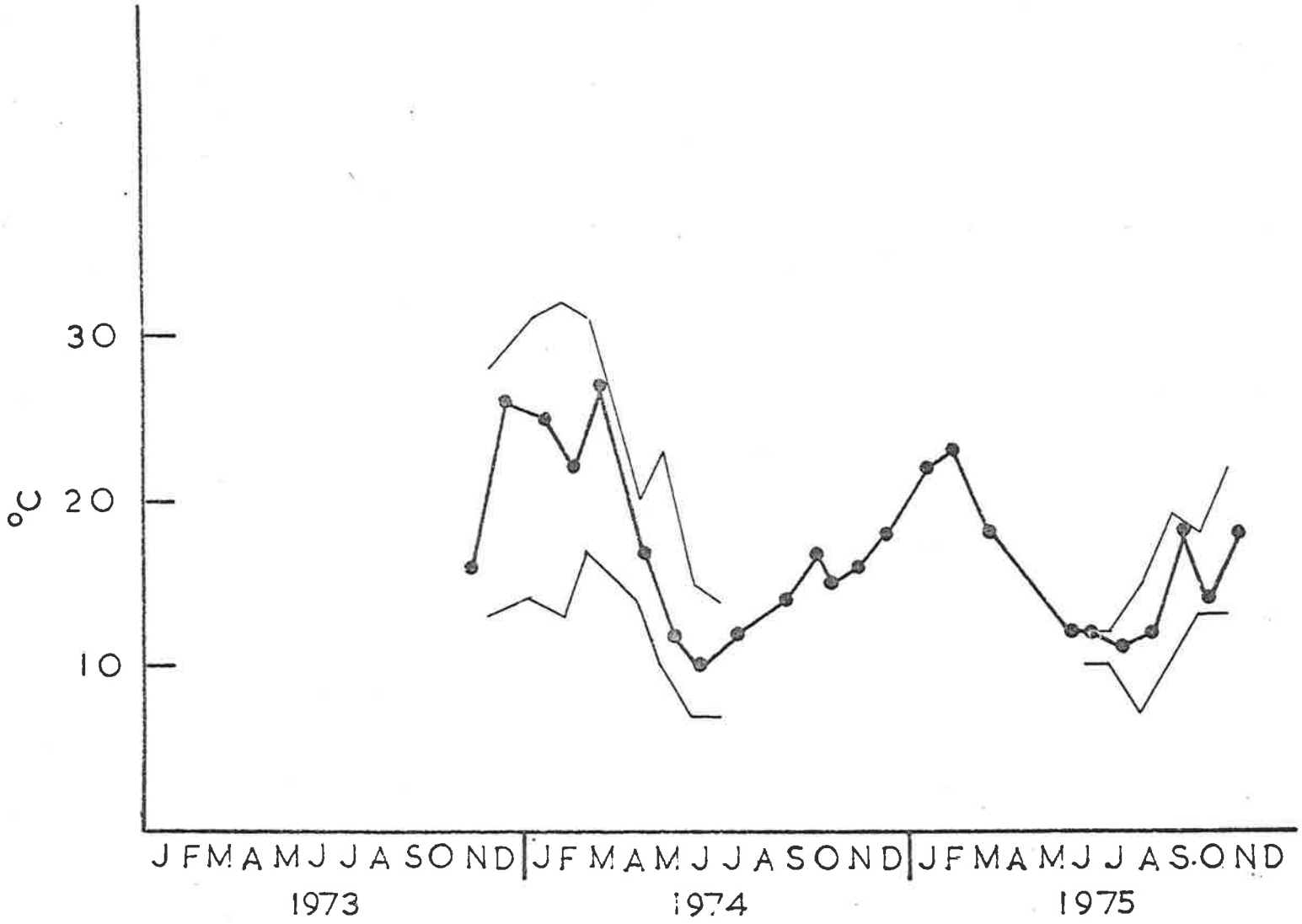
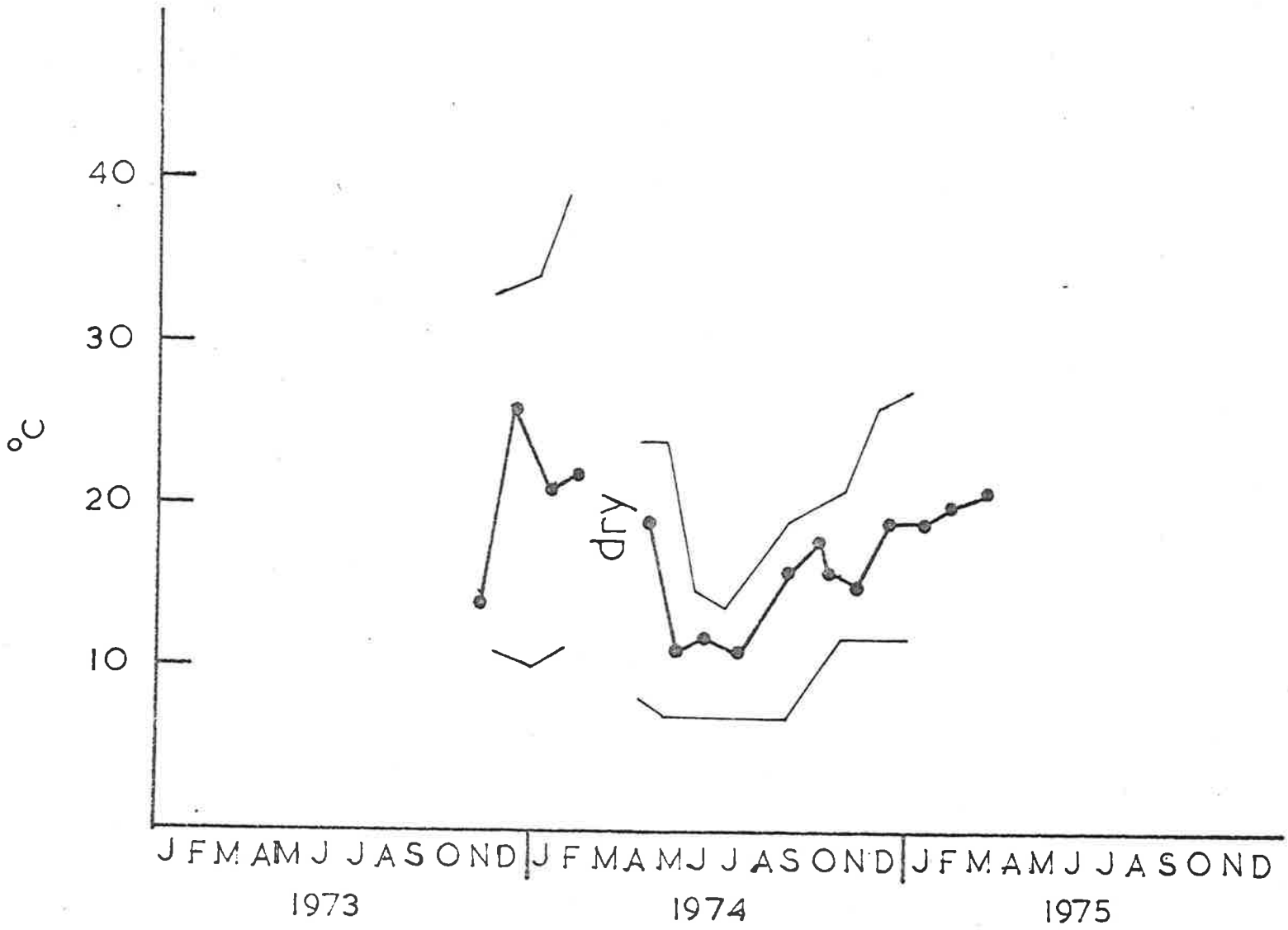

30
°C 20
10
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1973
1974
1975

FIGURE 5

Mean temperature ( ● ) of Lake Cundare on each visit and maxima and minima between visits. Gaps indicate no records were taken.


40
30
20
10
°C
dry
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1973
1974
1975

Primary production is, of course, the other energy source but turbidity limits it. The only algae so far recorded in both lakes is the halobiont dinoflagellate Dunaliella salina (Hussainy 1969). Williams (personal communication) has completed a four year study of the primary production of four shallow saline lakes in this region, including Pink and Cundare, and only found very low values. Hammer (1970) in a brief study of Pink Lake measured 20-60 mgC $m^{-2}$ $day^{-1}$, equivalent to an annual rate of 15gC $m^{-2}$ - also a low figure.

The only information on the fauna other than P. zietziana comes from Geddes' (1976) seasonal study. In Cundare he found three species of ostracod: Diacypris sp., Platycypris sp. and Australocypris robusta; and two species of copepod: Calamoecia salina and Microcyclops arnaudi. In general, these only occurred during the period of lowest salinity ($\leq$100‰), although Platycypris was present up to 150‰; in Pink Lake he only found the brine shrimp. My observations were similar, except Platycypris was also present in Pink perhaps because the average salinity was lower. In addition, I occasionally collected larvae of the brine fly Ephydrella and sometimes saw large emerging swarms around the edge of Pink in summer.

How these other members of the invertebrate community interact with the brine shrimp is not known. They all share the same food so possibly competition for this occurs. A few times I collected Ephydrella larvae attached to P. zietziana, but whether they were eating or harming the shrimp was not clear.

The only known predators of P. zietziana are birds. A record was kept of the species and numbers seen during each visit, Table 2. The Silver Gull (Larus novaehollandiae) was the most common, and was the only one that regularly attempted to catch brine shrimp. The other birds used the lakes more as a refuge than a food source.

TABLE 2

Birds seen on Lake Cundare (C) and Pink Lake (P) from November 1973 to November 1975

| Species | Lake | 1973 N | D | J | F | M | 1974 A | M | J | J | A | S | O | N | D | 1975 J | F | M | A | M | J | J | A | S | O | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Silver Gull | P | | 20 | | | | 200 | 50 | | | | | 4 | 3 | 100 | 220 | 30 | | | | 90 | 200 | 14 | 5 | 80 | 2 |
| Larus novaehollandiae | C | | 50 | | | | + | 500 | 600 | 120 | | | 180 | 100 | 100 | 150 | 50 | 130 | | | | | | | | |
| Red-Capped Dotterel | P | 4 | 2 | 2 | | | | 2 | | | | | 2 | | | 2 | | | | | 10 | 3 | 4 | | | |
| Charadrius alexandrinus | C | | | | | | 2 | | | | | | + | | | | | | | | | | | | | |
| Sharp Tailed Sandpiper | P | 4 | | | | | + | | + | | | | | | | | | | | | | | 1 | | | |
| Calidris acuminata | C | | | | | | total 50 | | total 80 | | | | | | | | | | | | | | | | | |
| Red-Necked Stint | P | | | | | | + | | + | | | | 12 | | | 1 | | | | | | | | | | |
| Calidris ruficollis | C | | | | | | | | | | | | 70 | | | | | | | | | | | | | |
| Red-Necked Avocet | P | | | | | | | | | | | | | | | | | | | | | | | | | |
| Recurvirostra novaehollandiae | C | | 2 | | | | | | | | No observations | | | | | 10 | | | No observations | | | | | | | |
| Grey Teal | P | | | | | | | | | | | | | | | | 16 | 1 | | | 100 | 11 | 40 | | 15 | |
| Anas gibberifrons | C | | | | | | | | | | | | | | | | | | | | | | | | | |
| Australian Little Grebe | P | | | | | | | | | | | | | | | 12 | | | | | 13 | 11 | 15 | 15 | 10 | 10 |
| Podiceps novaehollandiae | C | | | | | | | | | | | | | | | 30 | | | | | | | | | | |
| Musk Duck | P | | | | | | | | | | | | | | | 8 | 6 | 1 | | | 3 | 2 | | | | 1 |
| Biziura lobata | C | | | | | | | | | | | | | | | | | | | | | | | | | |
| Black Swan | P | | | | | | | | | 1 | | | | 4 | | 2 | | | | | 7 | | | | | |
| Cygnus atratus | C | | | | | | | | | | | | | | | 13 | | 1 | | | | | | | | |
| Mountain Duck | P | | | | | | | | | 30 | | | | | | 9 | 6 | + | | | | | | | | |
| Padorna radornoides | C | | | | | | | | 200 | | | | | | | 200 | 3 | | | | | | | | | |

+ = species present but not counted

## CHAPTER 3 - Population density of P. zietziana

Reliable estimates of population density are the basis of all quantitative field work. In constructing an energy budget for a population they are essential in all calculations and make it possible to relate energy usage to its availability. It is usually easier to see why we need precise measures of population size than it is to obtain them.

Plankton populations are rarely distributed randomly in lakes (George, 1974; Cassie, 1971). Horizontal and vertical currents or upwellings of nutrients combined with behavioural traits such as diurnal vertical migration or phototaxis cause zooplankton to become aggregated or clumped. This results in the variance of a series of samples being much larger than their mean, whereas they would be equal if a random distribution prevailed. Consequently for a given effort the sampling error increases.

### Methods

Samples were taken at approximately monthly intervals by pushing an open galvanised iron cylinder (0.11 $m^2$ in cross section and 60 cm high) into the sediments. The column of water so isolated was bailed out with a bucket through a 200$\mu$ zooplankton net attached to the side of the cylinder in a frame. P. zietziana nauplii were $>$ 600$\mu$ long; their eggs were 250$\mu$ in diameter. Occasionally in Pink Lake, the loose bottom gave way when bailing because of the surrounding water pressure. In such cases the shrimp were collected by repeatedly dipping a small zooplankton net (250$\mu$) into the cylinder until no more were caught.

The cylinder could only be used in water shallower than 60 cm. Cundare was always shallower than this, while the shore region sampled in Pink was usually so except for the months October 1974 to March 1975 and August to November 1975. When the water was too deep a standard zooplankton net (250$\mu$) was towed vertically. The area of the mouth of this net was 0.07 $m^2$. Therefore the catches were multiplied by 1.56 (0.11/0.07). These

vertical hauls were calibrated by taking four series of paired samples with the net and the cylinder, one in Cundare and three in Pink (Table 3). Significant statistical differences could only be shown twice using the Wilcoxon signed-ranks test for paired samples (Sokal and Rohlf, 1969), although the corrected net catches were always smaller than those of the cylinder. If all the data are combined then the net caught significantly fewer ($p < 0.005$, one tailed test; $n = 31$) shrimp than the cylinder, the average efficiency being 65%. This factor was used to correct all samples taken with the net.

Preliminary samples taken before November 1973 indicated a contagious distribution of P. zietziana in both lakes, but more so in Pink, and thus a high sampling error. Precision was improved in two ways. First, the number of samples taken on each visit was increased to sixteen in Pink and twelve in Cundare. This occupied a whole day in each case. To reduce the error to 10% with the degree of contagion encountered would have required approximately one hundred and fifty samples from both lakes.

Second, variance was decreased by stratifying the samples. To accomplish this the habitat is split into sub-areas or strata where it appears numbers per sample will be similar. I had observed that wind generated currents caused the shrimp to accumulate against one or two shores of the lake. Therefore the four shores of each lake were considered as strata. The benefit of this method is that when calculating the total standard error a weighted mean of the variances from each stratum is obtained thus ignoring heterogeneity between them due to the wind. Sampling effort was proportional to the relative area of a stratum. In Pink Lake four samples were taken on each shore because the lake is roughly square. On the other hand Cundare is rectangular with the long side (four samples) being about twice the short (two samples). In both lakes samples were spaced along the shores not clumped.

The samples were preserved on the day of collection in 10% formalin.

TABLE 3

Calibration of the zooplankton net (250$\mu$) against the 0.1$m^2$ cylinder. Catches with the net have been multiplied by 1.56 before expressing them as percentage efficiency.

| Lake | Net efficiency (%) | signed ranks test for n pairs (one tailed) |
|---|---|---|
| Cundare 16 Oct. 74 | 79 | not significant, n = 12 |
| Pink 1 June 75 | 49 | $p < 0.05$ , n = 8 |
| Pink 22 June 75 | 83 | not significant, n = 8 |
| Pink 19 Jul. 75 | 47 | $p < 0.05$ , n = 8 |
| Mean | 65 | $p < 0.005$, n = 31 |

Each sample was counted within three months, the various cohorts present being treated separately. Occasionally subsampling of individual samples was necessary. Animals were selected from a randomised mixture of the sample with a wide mouthed 50 ml bulb pipette until at least four hundred had been counted. The distribution of these subsamples was found to be random just bordering on an even distribution; no size appeared to be favoured.

Before calculating the 95% confidence limits of the stratified samples the data were converted to logarithms as suggested by Elliott (1971) for contagiously distributed populations. The resulting logarithmic confidence limits were converted to the arithmetic scale and combined with the arithmetic mean to give the actual confidence limits. This is a hybrid method because strictly speaking the log method provides confidence limits for the geometric mean. However, according to Elliott (personal communication) the arithmetic mean is the best unbiassed estimate of the mean density of a finite population even if contagion prevails.

Results

Population densities in both lakes are shown in Figs. 6 and 7. There are at least two distinct generations per year and in Pink usually three. These will be described in detail in the next chapter with the data on life history.

The densities are given per 0.1 $m^2$ instead of 0.11 $m^2$. The figures were not decreased to allow for this because no significant over-estimation would result. Preliminary analysis had shown at most 5% of a sample could be lost in the debris and mud that was inevitably collected along with the shrimp. Another 5% or less could be lost in the actual sampling procedure. The 10% increase to 0.11 $m^2$ could thus reasonably be balanced by these losses; the confidence limits of the mean were always greater than 10%. Sampling per unit area was chosen instead of per unit volume for ease of calculating production (see next chapter), which is

FIGURE 6

Fluctuations in population density of *P. zietziana* in Pink Lake; bars indicate 95% confidence limits.

- ● - samples taken with 0.11 $m^2$ cylinder
- Δ - only 12 samples taken
- o - samples taken with zooplankton net (250$\mu$)
- X - samples taken during calibration of net against cylinder

Numbers refer to the various generations discussed in the next Chapter

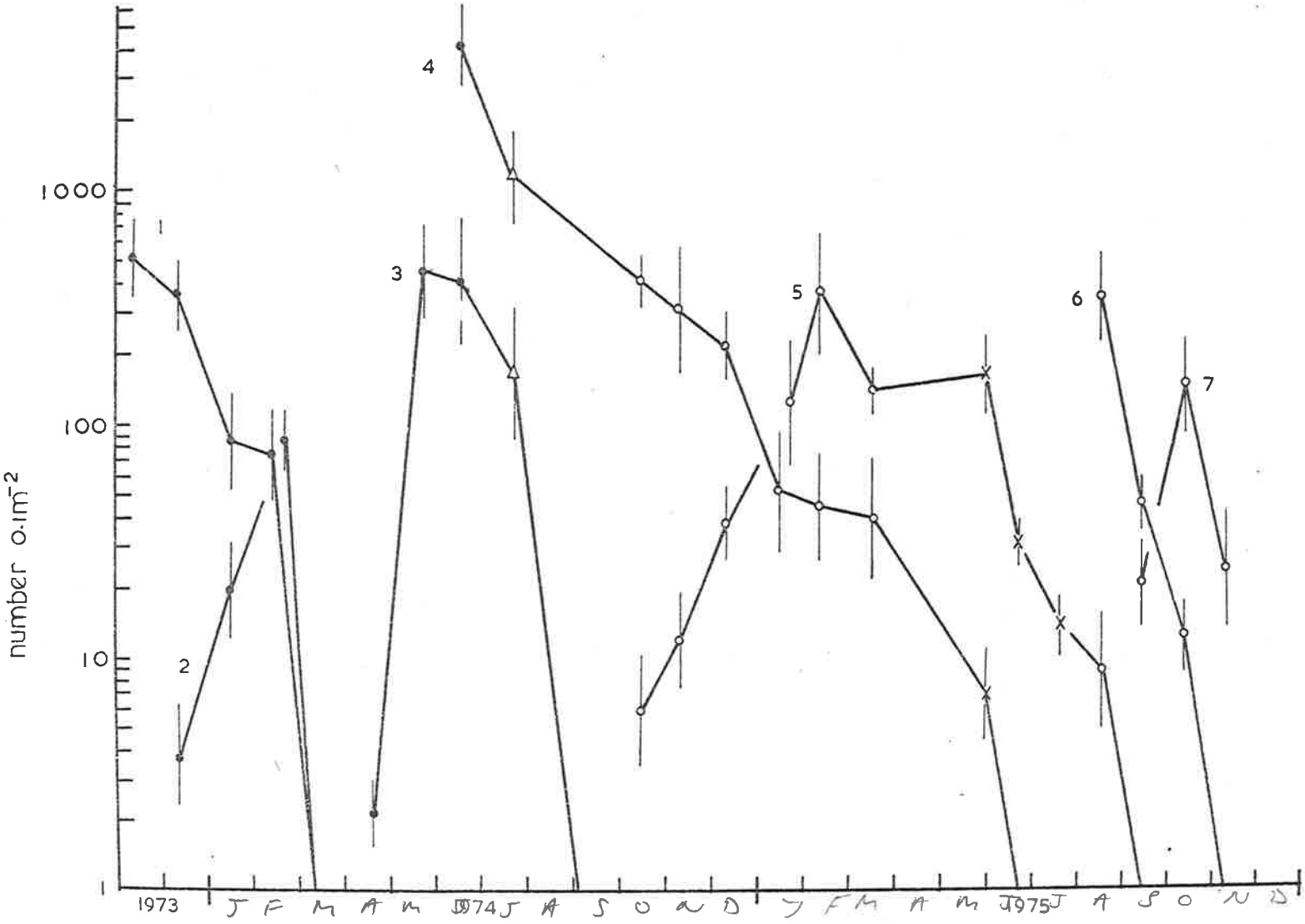

1000
100
10
1
number 0.1m⁻²
1
2
3
4
5
6
7
1973
J F M A M J 1974 J A S O N D J F M A M J 1975 J A S O N D

FIGURE 7

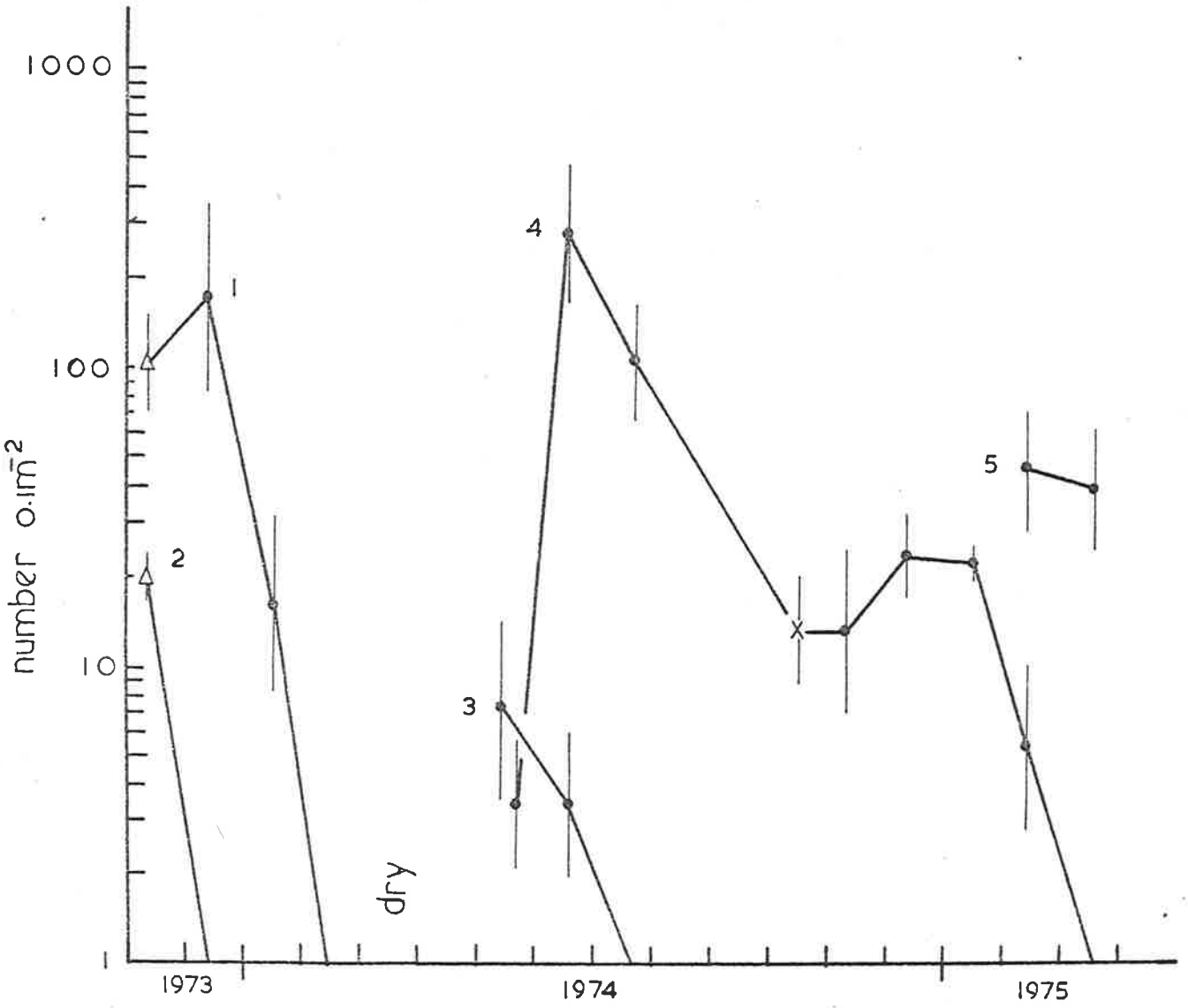
Fluctuations in population density of P. zietziana in Lake Cundare; bars indicate 95% confidence limits

● - samples taken with 0.11 $m^2$ cylinder

Δ - samples taken on only three shores

X - samples taken during calibration of net against cylinder

Numbers refer to the various generations discussed in the next Chapter.


1000
100
10
1
number o.im⁻²
1
2
3
4
5
dry
1973
1974
1975

always based on area. Preliminary sampling had shown that both units produced just as variable data.

The width of confidence limits (40-50% of mean) implies that the 35% underestimation by the vertical hauls (Table 3) was usually not significant. However, the paired samples, which largely ignore horizontal heterogeneity, showed that this underestimation was significant. Therefore, the correction should still hold even if it is relatively unimportant in the final estimate of the mean.

To separate the individuals in a sample into cohorts I used analyses of size frequency. The data for this will be given in the next chapter. In most cases it was easy to decide to which cohort an individual belonged. Inevitably, the size class at which the division occurred was more or less arbitrary, but the proportion of the population this represented was insignificant.

Discussion

In general, I believe, the sampling was adequate because stable trends emerged and the variability was not so great that significant differences could not be detected even between months. In other words the samples were at least representative.

No significant bias was introduced by taking samples near the shore in both lakes. Pink Lake was almost twice as deep in the middle (1 m approximately) as it was near the sides. However, there was no correlation between volume or depth of water in one sample and the size of the catch. Nor was there any irregular variability in mean shrimp density as would be expected from such a correlation in a lake fairly constantly stirred by wind. Furthermore, P. zietziana often appeared to congregate near the bottom. Thus the shrimp would tend to be distributed by area rather than volume making any influence of the water column on population density even less likely; later work shows they are largely bottom feeders. Finally, if

they had been evenly spaced through the water column (50-70 cm) then no significant differences would have been expected when calibrating the net against the cylinder; the net would have filtered completely a column this length.

Water depth could not influence sampling in Cundare because depth was the same ($\leq$50 cm) virtually to the edges of the lake. However, there was once a significant increase in shrimp density (October to December 1974) that could not be explained by recruitment. This largely resulted from a number of blank samples in October and November, possibly due to irregular currents.

Wind is the other important factor in the distribution of P. zietziana in these lakes. The circulation pattern of wind induced currents in shallow lakes has not been well studied. However according to Smith (1975) in a shallow circular lake longshore currents would result from water blown towards the lee shore rather than the vertical circulation typical of deeper lakes. This would account for large numbers of shrimp often found clumped along only one or two shores of Pink. In Cundare this also occurred, but not to the same extent indicating perhaps that longshore currents are less well developed. In fact, the only random distribution occurring was recorded from this lake. The detection of clumps depends at least partly on their persistence. Behavioural responses of the shrimp to the strength and direction of currents would undoubtedly influence this.

To some extent the probability of longshore currents justifies using the shore as a stratum. However, the variances within each stratum were often large, sometimes of the same order of magnitude as the total variance without stratification. This was expected because it was impossible to redefine the strata on each occasion to suit the wind direction. It seems that in these shallow lakes the main advantage of stratified sampling, compared with random, is its greater efficiency. By chance a series of random samples could well have missed large clumps of shrimp blown onto one

shore. In contrast stratification ensured that a more complete range of shrimp densities was represented.

Samples in each strata were not picked at random but spaced apart along the shoreline, more or less evenly. Provided they do not coincide with any repeated pattern in the population then according to Tidmarsh and Havunga (1955) the samples can be treated as an equivalent number of random points to which the usual techniques for measuring dispersion can be applied. However, there is no simple method of calculating the confidence limits (Elliott, personal communication) for the arithmetic mean of small samples from clumped distributions. The method of converting the data to logarithms before calculating the dispersion and combining the reconverted results with the arithmetic mean provides confidence limits which are probably too wide.

CHAPTER 4 - Life history, growth and production of P. zietziana

The data on population density can be used to estimate mortality. From additional data on life history and growth production can be determined. This is conveniently calculated, for an animal such as P. zietziana with distinguishable cohorts, by integrating Allen curves - graphs of mean number versus mean individual weight.

Biomass produced during reproduction is not included in the above estimation, but must also be considered. By measuring female clutch size this contribution can be assessed, if the survival of ovigerous females, the number of clutches produced and the weight of their reproductive products is known. Finally, for crustaceans, the weight of moulted exoskeleton should be added because this represents biomass gained, but subsequently lost. In practice it was impossible to collect the fragile exuviae. However, their contribution is likely to be negligible compared with sampling errors.

Winberg (1971) and Edmondson and Winberg (1971) reviewed the various numerical methods used to calculate production of aquatic invertebrates from the preceding type of data. Some of these assume fixed patterns of growth and mortality. Allen curves, which are plotted from empirical data, do not.

Methods

From each series of monthly samples at least one, unpreserved, was examined on the day of collection under a dissecting microscope. The shrimp were sorted into arbitrary length classes, washed with freshwater and placed in vials in a desiccator. Within at most one week they were properly dried in a vacuum desiccator at 60°C and then stored over silica gel until weighed. Rarely was there any bacterial contamination before this was done. All lengths were measured in arbitrary units with a micrometer eyepiece at a magnification of 6.3, the length being the distance from the join of the second antennae with the head to the tip of the telson,

between the two cercopods. The remaining samples from each visit, preserved in 10% formalin, were amalgamated after counting and at least one hundred shrimp subsampled to determine the length frequency of the population; when the catch was small all were measured. Any shrinkage from preservation in formalin had a negligible effect because the mean length of preserved individuals was never significantly different from that of unpreserved. The number of ovigerous females in each length class was always noted. They were considered a separate class when sorting the sample used for dry weights.

All dry weights were measured to a precision of $\pm$ 0.05 mg. A linear regression between the logarithms of mid-class length and dry weight of the arbitrary size classes (Fig. 8) enabled the mean individual weight to be calculated at each visit from the length-frequency data. Weight due to the egg sacs of pregnant females was not included in these calculations.

The clutch size of the ovigerous females was determined by dissecting the egg sacs of preserved animals. The frequency of clutches was noted by raising P. zietziana in aquaria at 18°C to 20°C, the temperature range over which ovigerous females were usually found in the lakes. Shrimp for this were collected at the ICI salt works, Dry Creek, Adelaide and brought to the laboratory within an hour. The dry weight of the reproductive products was calculated by subtracting the dry weight of a non-ovigerous female of a particular length class (from the regression equation) from its dry weight when carrying a full egg sac.

## Results

### (a) Life history

This is best understood from the length-frequency histograms (Figs. 9 and 10) together with the population density curves (Figs. 6 and 7) from the last chapter. Figs. 11 and 12 summarise the growth of individuals in the

FIGURE 8

Mid-class length of *P. zietziana* versus mean dry weight. Numbers refer to the size classes in arbitrary units. The regression equation relating length (mm) to dry weight (mg) is:

$$w = 1.44 \times 10^{-3} \, l^{2.63} \quad (n = 119, \; r^2 = 0.99)$$

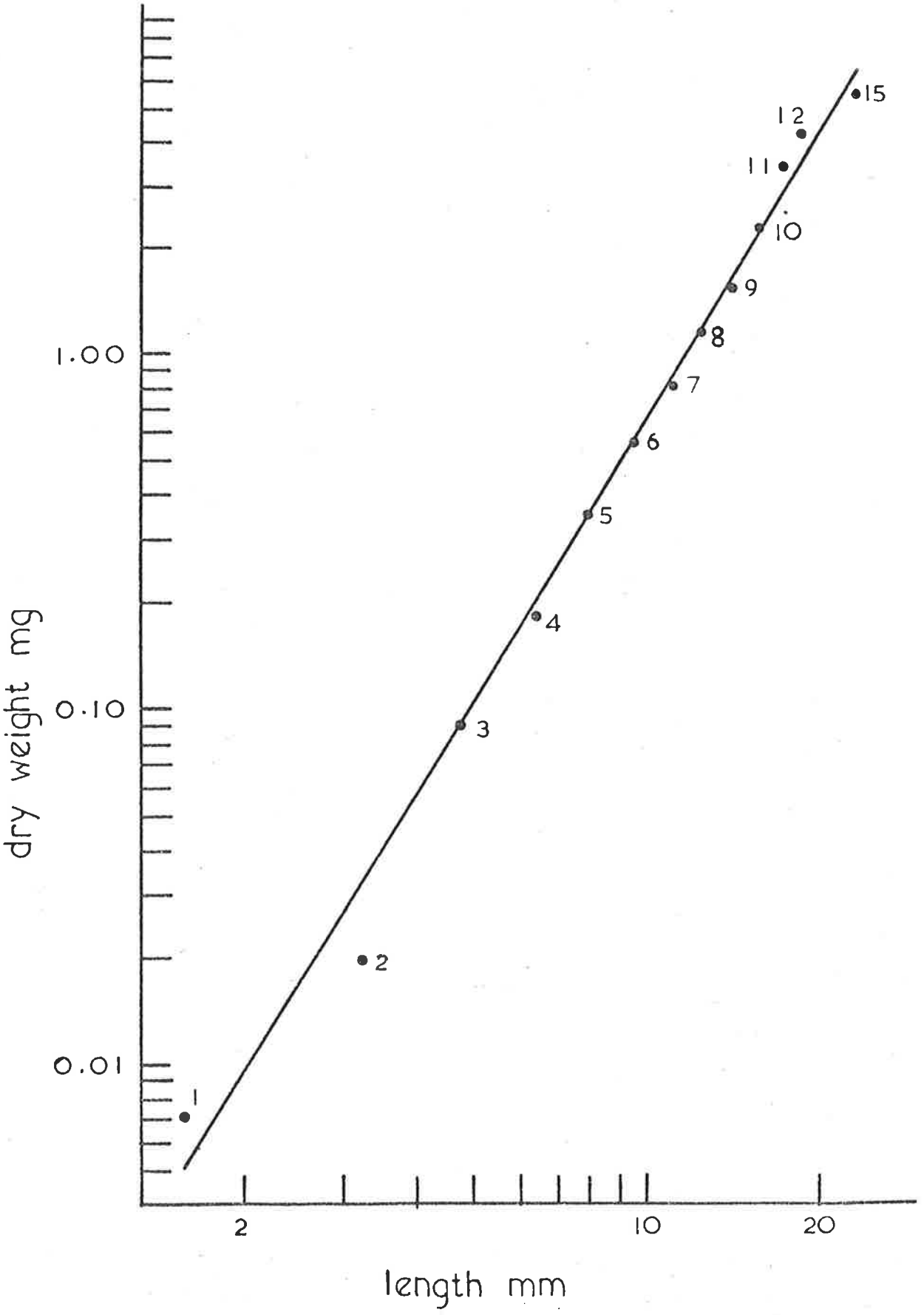

dry weight mg
1.00
0.10
0.01
1
2
3
4
5
6
7
8
9
10
11
12
15
2
10
20
length mm

FIGURE 9

Length-frequency of P. zietziana in samples from Pink Lake based on sixteen samples per visit. Open histograms represent ovigerous females; a vertical dash indicates the separation between two cohorts.

♀ - ovigerous females present, but at a frequency 2%

* - histograms based on only one or two of the sixteen samples

† - histograms based on only one sample taken qualitatively

n - number of shrimp measured

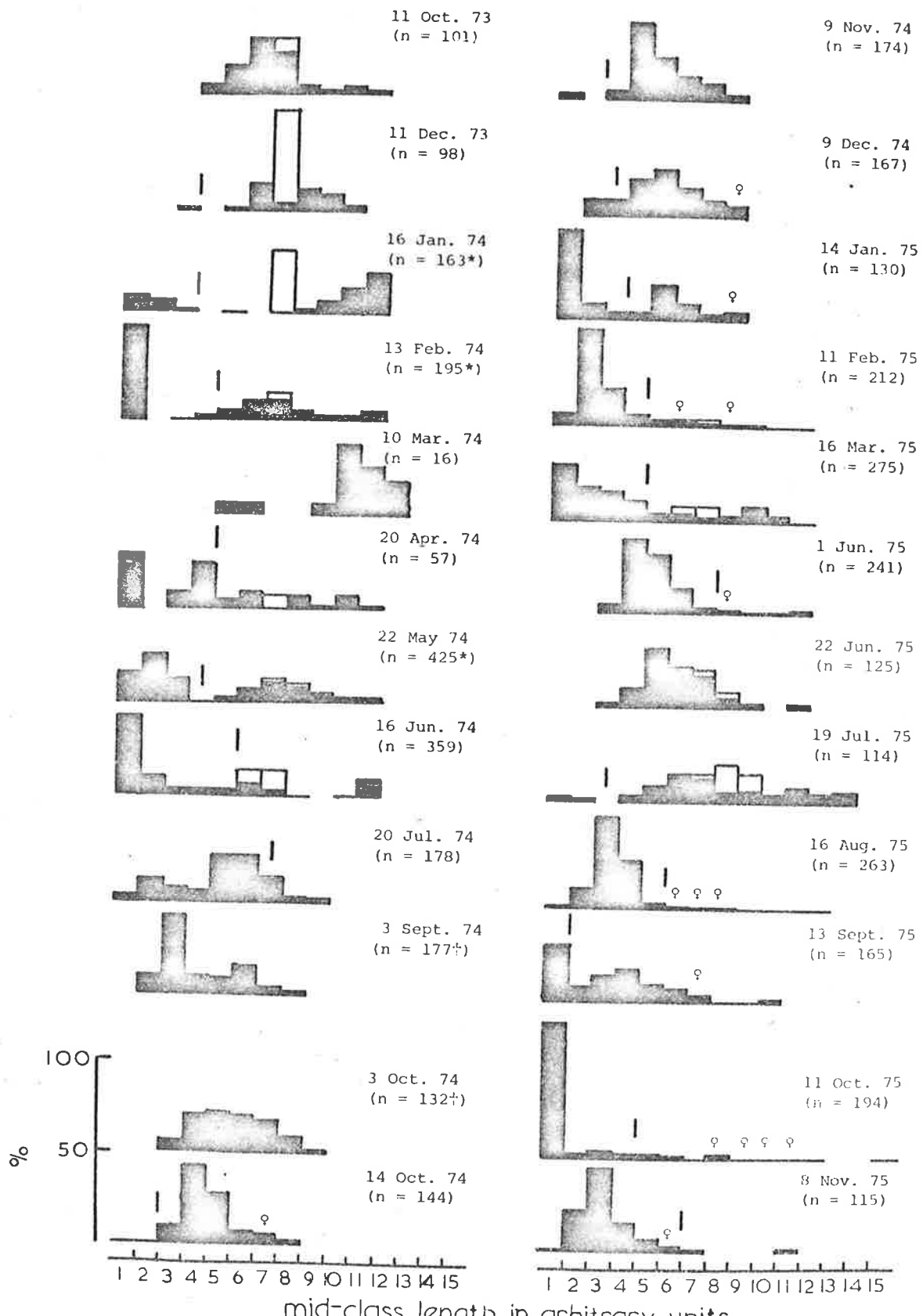

11 Oct. 73
(n = 101)
11 Dec. 73
(n = 98)
16 Jan. 74
(n = 163*)
13 Feb. 74
(n = 195*)
10 Mar. 74
(n = 16)
20 Apr. 74
(n = 57)
22 May 74
(n = 425*)
16 Jun. 74
(n = 359)
20 Jul. 74
(n = 178)
3 Sept. 74
(n = 177+)
3 Oct. 74
(n = 132+)
14 Oct. 74
(n = 144)
9 Nov. 74
(n = 174)
9 Dec. 74
(n = 167)
14 Jan. 75
(n = 130)
11 Feb. 75
(n = 212)
16 Mar. 75
(n = 275)
1 Jun. 75
(n = 241)
22 Jun. 75
(n = 125)
19 Jul. 75
(n = 114)
16 Aug. 75
(n = 263)
13 Sept. 75
(n = 165)
11 Oct. 75
(n = 194)
8 Nov. 75
(n = 115)
100
50
%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
mid-class length in arbitrary units

FIGURE 10

Length-frequency of *P. zietziana* in samples from Lake Cundare based on twelve samples per visit. Open histograms represent ovigerous females; a vertical dash indicates the separation between two cohorts. (Symbols as in Figure 9).

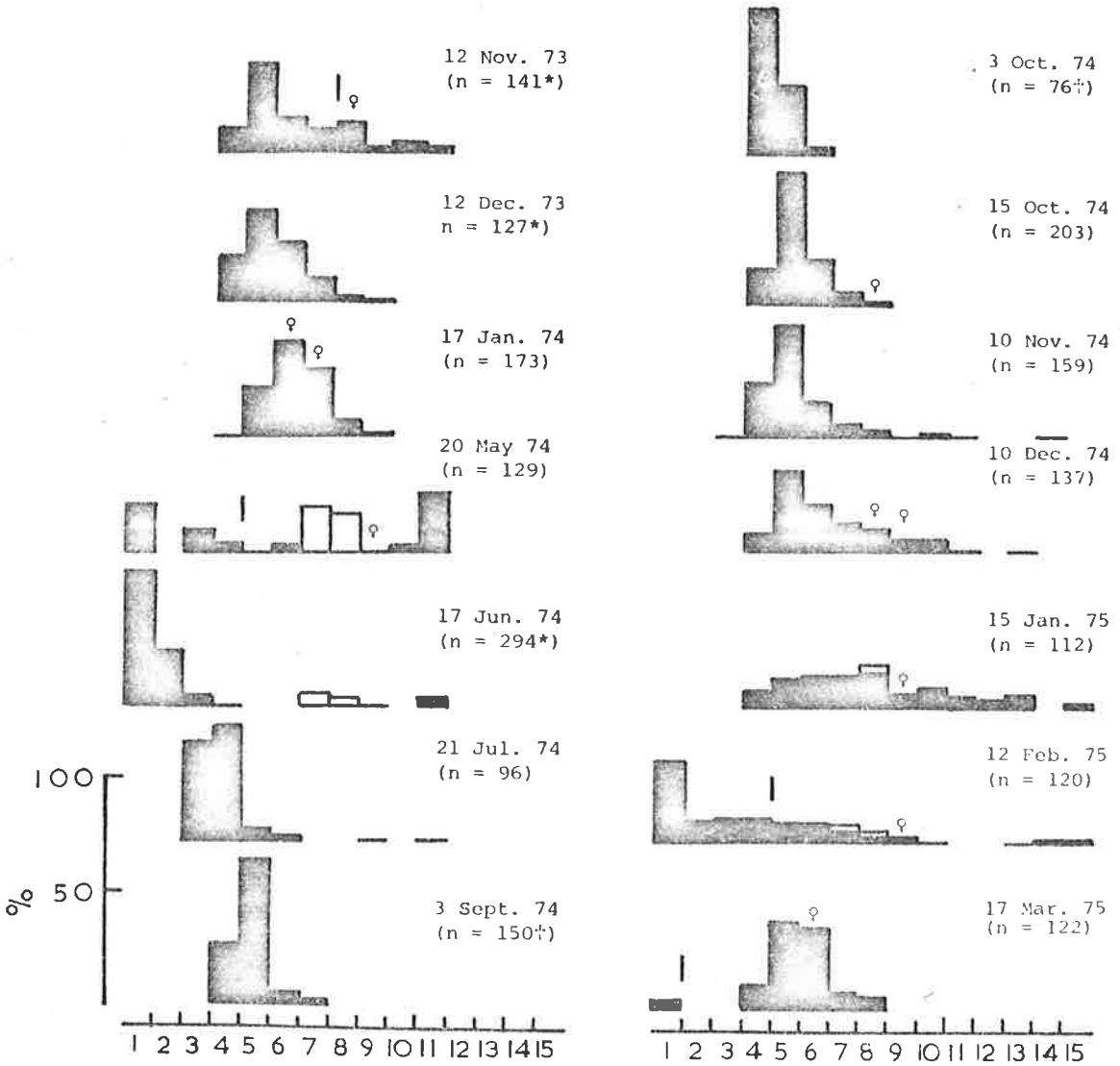

12 Nov. 73
(n = 141*)
12 Dec. 73
n = 127*)
17 Jan. 74
(n = 173)
20 May 74
(n = 129)
17 Jun. 74
(n = 294*)
21 Jul. 74
(n = 96)
3 Sept. 74
(n = 150+)
3 Oct. 74
(n = 76+)
15 Oct. 74
(n = 203)
10 Nov. 74
(n = 159)
10 Dec. 74
(n = 137)
15 Jan. 75
(n = 112)
12 Feb. 75
(n = 120)
17 Mar. 75
(n = 122)
100
% 50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
mid-class length in arbitrary units

FIGURE 11

Growth of P. zietziana in Pink Lake. The 95% confidence limits are shown by the bars except where they are too narrow.

1 - Overwintering generation, 1973

2 - Early summer generation, 1973

3 - Autumn generation, 1974

4 - Overwintering generation, 1974

5 - Mid-summer generation, 1975

6 - Mid-winter generation, 1975

7 - Spring generation, 1975

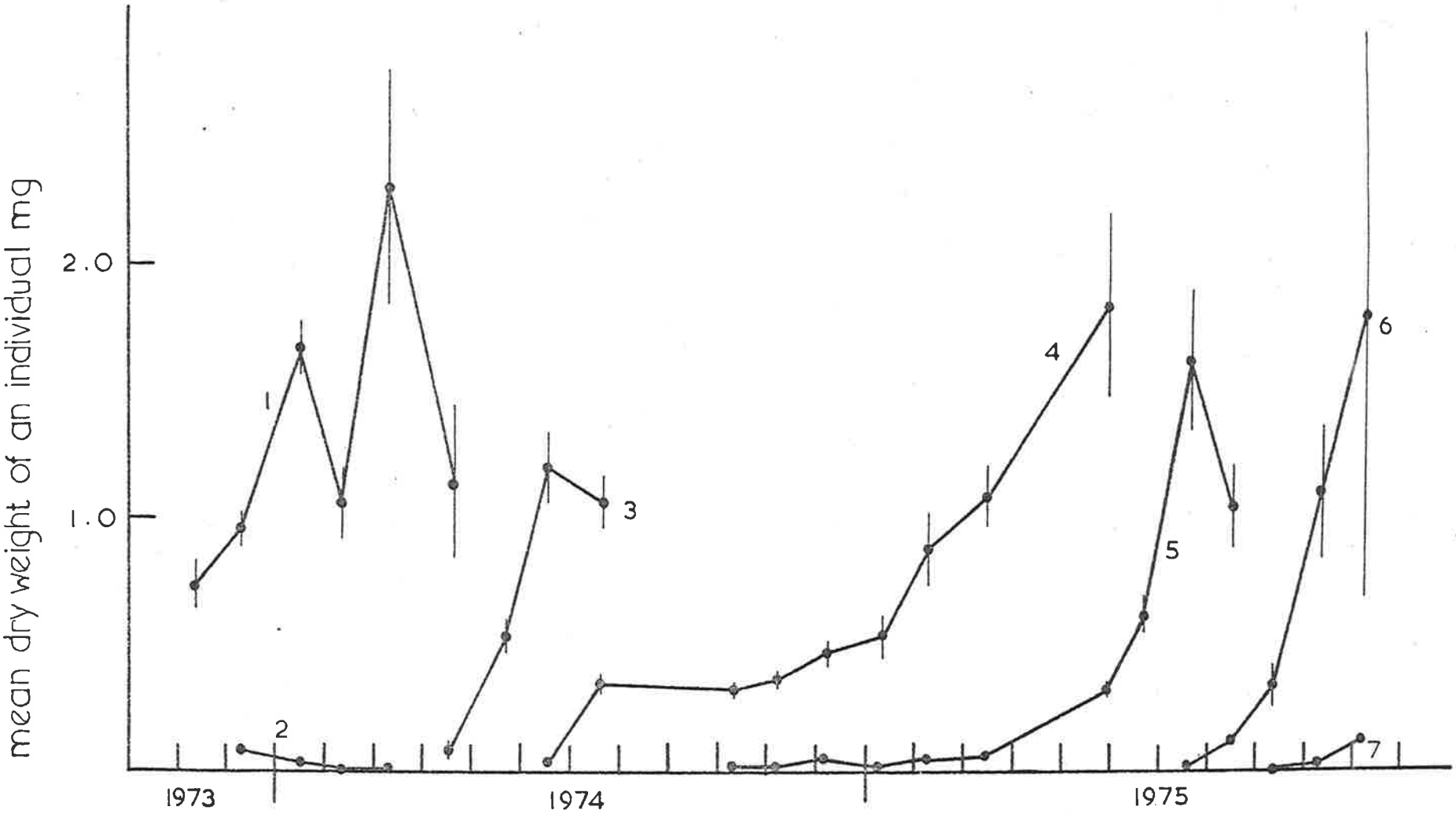

mean dry weight of an individual mg
2.0
1.0
1
2
3
4
5
6
7
1973
1974
1975

FIGURE 12

Growth of P. zietziana in Lake Cundare. The 95% confidence limits are shown by the bars except where they are too narrow.

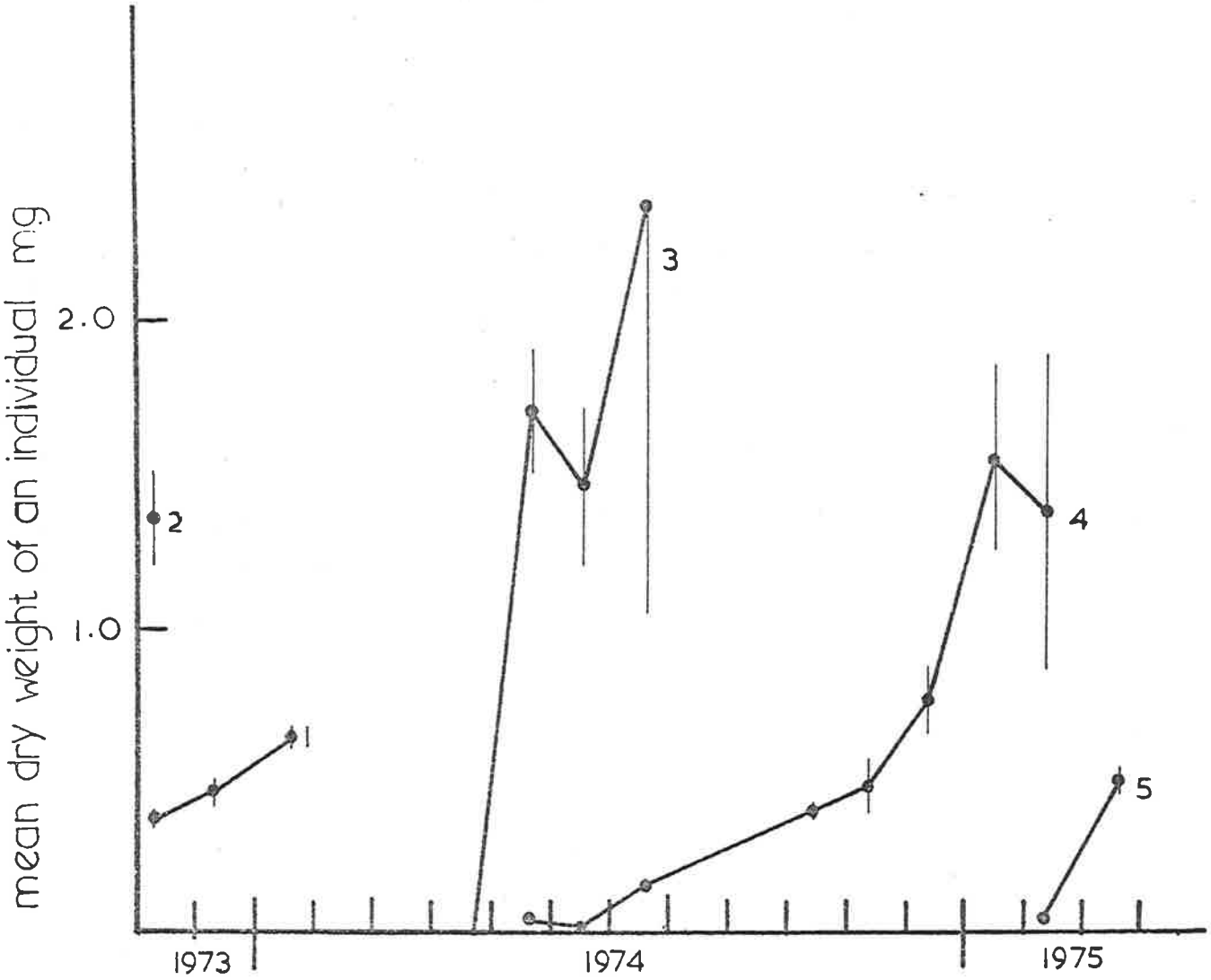
1 - Mid-winter generation, 1973

2 - Overwintering generation, 1973

3 - Autumn generation, 1974

4 - Overwintering generation, 1974

5 - Late Summer generation, 1975


mean dry weight of an individual mg
2.0
1.0
1
2
3
4
5
1973
1974
1975

two lakes.

Shrimp from the overwintering generation, the only one present in Pink Lake in late 1973, grew steadily reaching maximum size in autumn 1974, but at the same time most died because of salinity increasing over summer (Fig. 2) to levels at which significant mortality occurs (Geddes, 1975 a). A small early summer generation hatched as females became ovigerous but this died out quickly, also from increasing salinity. The size frequency distributions of the overwintering generation from January to April 1974 are unsteady and this is reflected in the growth curve. In fact, it seems at one stage (February) that recruitment occurred to the larger sized cohort. However, the January and February histograms were based on only one or two samples rather than on the sixteen usually taken while the March and April samples were very small. Thus sampling error is the probable explanation for this erratic growth.

In Lake Cundare during this period the mid-winter generation present also died out, but sooner and without any females becoming ovigerous to produce a summer generation. From preliminary sampling I think the larger size cohort which disappeared after the first sample was an overwintering generation from which the smaller sized one hatched in mid-winter.

After the above average rain in April and the consequent rapid drop in salinity eggs hatched in both lakes and grew quickly, some to become ovigerous within three weeks. These eggs must have been resistant or resting because Cundare was dry in March and there were very few ovigerous females in Pink during April. In May there were two well defined cohorts in Pink, but by June these had amalgamated. The population density of the larger sized cohort in May was significantly less than that in June ($0.05 \geq p \geq 0.02$; $t = 2.27$, $df = 24$), indicating that there must have been recruitment between visits, most likely from the smaller sized cohort present in May. Therefore considering the two cohorts in May as one

generation, its numbers remained constant at first and then declined as the ovigerous females died leaving only immatures and some large males. These disappeared by September. The growth of an individual in this generation was rapid reaching a fairly constant maximum size. Because of good survival of females to maturity the overwintering generation produced was large. Starting at about 4000 $m^{-2}$, the shrimp died at a more or less constant relative rate throughout the duration of this generation (one year). Individual growth was rapid to start, but stopped for about four months until December. Then the weight significantly increased and kept on doing so until all had died by June 1975.

In Cundare the autumn generation grew even more quickly than in Pink. The overwintering generation which it produced lasted ten months. The growth of individuals was steady during this period but their mortality rate varied. After initial rapid death numbers stabilised through spring into summer when increasing salinities again caused some mortality. By the time sampling stopped in this lake in March 1975 a late summer generation of stable density, but high growth rate, had arisen.

Salinity did not reach high enough levels over summer in Pink to cause any death and the overwintering generation produced eggs at first slowly and then more rapidly from October to February 1975. These formed a mid-summer generation that lasted the autumn apparently without loss, but died rapidly during winter. However, individuals had matured by early winter and produced a mid-winter cohort. These in turn matured quickly and despite high mortality produced a spring cohort before the generation ended in November.

In all generations in both lakes males always reached a larger maximum size than females. Such dimorphism explains the increasing confidence limits around the mean weight during growth. It was difficult to tell precisely when both sexes reached maturity, but they were distinguishable by the fifth size class (8.0 mm). Geddes (1973) determined

fifteen instars in P. zietziana before he considered it adult at 8-10 mm and three to four instars after this mainly representing growth of the males. Many of the smaller instars have been amalgamated by my choice of length classes. Only females larger than or equal to the sixth size class ($\geq$9.5 mm) became ovigerous. In fact females rarely grew larger than the eighth size class (12.7 mm) as Geddes (1973) also found and females were only seen copulating with larger males. How much larger a male had to be was not known and so a male the same size as an ovigerous female may not have been mature. Thus a meaningful sex ratio is impossible to calculate. However, males were not rare and P. zietziana is certainly dioecious not parthenogenetic.

Except after the summer drought in early 1974 when the autumn cohorts in both lakes must have hatched from resting eggs (probably stimulated by falling salinity), ovigerous females were always present when nauplii were caught. Evidently, these cohorts emerged from recently produced (subitaneous) eggs. Significant rainfall sometimes preceded such occasions. Geddes (1973, 1976) recorded this quite often and suggested the subsequent salinity drop triggered the process. However, it may not be the only factor. I found a period of heavy rain and falling salinity, July-September 1974, during which there was no recruitment; Geddes noticed several. Recruitment from subitaneous eggs also occurred when salinity was increasing, although Geddes claims P. zietziana switches to the production of resting eggs at such times. Thus hatching stimuli are not well characterised for the wetter parts of the year.

(b) Breeding biology

Calculation of the absolute number of females, classed as ovigerous, reveals that they rarely survived much more than one month. Most that were caught had either empty egg sacs or only developing eggs; very rarely did females grow larger than the maximum size at which they were

observed ovigerous (length class 9). My interpretation of this is that females only produce one clutch and that they die soon after releasing it. If they have more than one clutch, by producing eggs continuously, then females with full egg sacs would have been common in my samples. Attempts to culture P. zietziana support this conclusion. Those that laid eggs took between eleven and twenty one days to mature at about 20°C before copulating. The females produced one clutch which they bore for a week and then invariably died either before or within one or two days of shedding their eggs.

The clutch size of all groups of ovigerous females that developed was measured except those in Pink during the 1973 to 1974 summer (Table 4). They are within the range Geddes (1976) recorded during his study (1970-71) of these lakes. There was never a significant regression, i.e. slope different from zero, between clutch size and length of shrimp except perhaps in the October 1975 sample from Pink. In this case the amount of variation in clutch size explained by length of shrimp was approximately one third ($r^2 = 0.336$). In subsequent calculations of egg production I have considered that females at one period of recruitment had the same clutch regardless of length; they mostly occupied only one or two length classes at these times.

The dry weight of the reproductive products is shown in Table 5. Not only do the eggs account for this biomass, but so does the weight of the accompanying egg sac. Thus the values given are weight of an egg plus unit of egg sac. The increase in weight of males due to sperm production was considered negligible. With the value for mean weight per egg it is possible to calculate the clutch size of females during the 1973 to 1974 summer recruitment using the mean dry weight of ovigerous females collected in this period (1.18 mg; only length class 7 ovigerous). The clutch size was 21.4 ($\pm$ 2.5).

TABLE 4

Clutch size of ovigerous females for months when present and the average clutch size for the periods of recruitment. Only females with full egg sacs were dissected. The bracketed values are not significantly different. For Cundare the grand average is the best estimate of clutch size because of the small number of females dissected

| Month | number of ovigerous females dissected | clutch size (±95% confidence limits) | average clutch size (± confidence limits) for period of recruitment | | length classes (arbitrary) with ovigerous females |
|---|---|---|---|---|---|
| | | PINK | | | |
| April 1974 | 9 | 75.1 (±11.3) | 79.6 (± 7.5) | | 6-7 |
| May 1974 | 35 | 83.0 (± 8.2) | | | |
| December 1974 | 4 | 44.5 (±18.6) | 39.3 (± 2.8) | | 6-8 |
| February 1975 | 11 | 42.6 (± 7.2) | | | |
| March 1975 | 81 | 38.7 (± 3.1) | | | |
| July 1975 | 19 | 85.9 (±10.6) | 85.1 (± 9.6) | | 7-8 |
| August 1975 | 4 | 80.0 (±38.2) | | | |
| October 1975 | 44 | 105.0 (±10.5) | 105.0 (±10.5) | | 7-9 |
| | | CUNDARE | | | |
| May 1974 | 7 | 128.0 (±25.7) | 123.9 (±17.4) | 119.5(±14.5) | 7 |
| June 1974 | 14 | 121.9 (±26.5) | | | |
| January 1975 | 3 | 102.0 (±69.7) | 108.0 (±25.8) | | 6-8 |
| February 1975 | 5 | 111.6 (±54.2) | | | |

TABLE 5

Dry weight of an egg plus associated egg sac. When length classes were lumped a weighted mean was taken to calculate the weight of the non-ovigerous female

| dry weight (mg) of ovigerous female with full sacs | month | number weighed | length class | clutch size | dry weight (mg x $10^{-2}$) per egg | Lake |
|---|---|---|---|---|---|---|
| 2.10 | April 1974 | 4 | 7 | 79.6 | 1.59 | Pink |
| 2.24 | May 1974 | 18 | 7 | 95.1[a] | 1.48 | Pink and Cundare |
| 2.59 | May 1974 | 18 | 8 | 95.1[a] | 1.47 | Pink and Cundare |
| 2.30 | June 1974 | 2 | 7 | 119.5 | 1.23 | Cundare |
| 3.38 | June 1974 | 7 | 8 | 119.5 | 1.83 | Cundare |
| 5.45 | January 1975 | 1 | 10 | 119.5 | 2.77 | Cundare |
| 1.86 | January 1975 | 4 | 7-8 | 119.5 | 0.71 | Cundare |
| 2.72 | July 1975 | 29 | 7-8 | 85.9 | 1.83 | Pink |
| | | | | | $\bar{x}$ = 1.61 | |
| | | | | | ± 0.03 (95% confidence limits) | |

[a] = weighted mean of clutch size in Pink and Cundare

Discussion and Production estimates

The data on life history and population density show that there is no regular period of recruitment to the P. zietziana populations in either lake. In general there was a population increase after a drought through hatching of resting eggs. During wetter parts of a year the stimuli causing hatching of subitaneous eggs are unclear, and the number of generations likely to arise are not predictable. Recruitment was usually swift except over summer in Pink when it could be slow at first as only small numbers of females were maturing.

The life span of the cohorts varied from three months to a year depending partly on whether they survived periods of rising salinity. Geddes (1976) found the same variation during his survey. As already noted there is continuous though variable mortality of these cohorts even though the salinity is well within the tolerance limits of P. zietziana (6.2 and 267‰ at 18°C, Geddes, 1975). In fact mortality of the shrimp can only be ascribed to salinity during summer drought. In January and February 1974 lake temperatures reached 25°C and salinity was above 200‰. Geddes (1975) experimentally found a 60% mortality rate in seventy two hours with this combination. The next summer temperatures were lower (about 20°C) and salinity in Pink did not rise to levels at which he found significant mortality although it did in Cundare. For the rest of the study salinities were between 50 and 150‰ and temperatures between 10 and 20°C, conditions in which Geddes found less than 10% mortality after seventy two hours.

The problem now is to explain shrimp mortality during times of favourable salinity and temperature. There were no predators of any significance. Observations on seagulls indicated that they pecked at the water surface, presumably at shrimp, between thirty and sixty times a minute. It was impossible to determine how successful this was, but assuming (generously, I think) that a gull could capture one shrimp a minute and that there was an average of fifty gulls present each day on Pink from June 1974

to June 1975 (Table 2), feeding continuously, then they would have only eaten 0.5% of the overwintering generation. This is insignificant and probably an overestimate.

The obvious remaining cause of mortality is food or energy shortage either through an inability to assimilate sufficient because of the nature of the food or through competition for a scarce resource. So far there is no direct evidence for either and solution of this problem will require data on the nutritional balance of P. zietziana. Growth of the shrimp will obviously be affected by food shortage and also perhaps by temperature and salinity.

Growth was not sigmoidal (Figs. 11 and 12). Semilogarithmic plots gave straight lines, indicating exponential growth with the animals reaching a more or less stable maximum weight. In other words, there was no inflexion in the growth curve and consequent decrease in the growth rate as maximum size was approached. This is contrary to the sigmoidal growth pattern Reeve (1963 d) and Mason (1963) found for A. salina in culture and Daborn (1975) for the giant fairy shrimp B. gigas in the wild. Further comment cannot be made until the influence of the nutritional status of P. zietziana has been assessed.

The only exception to the exponential pattern (the lack of growth in winter and spring 1974, Pink) largely results from the frequency distribution for July being too skewed towards the larger lengths probably through sampling error. Low temperature had little influence because there was rapid growth during the following winter and spring. In Cundare over the same winter there was significant growth. In fact, the shrimp appeared to be able to develop and grow quickly in any season no matter what the temperature or salinity. In contrast Geddes (1976) from his seasonal study suggested that salinity controlled final development to maturity. He found that while salinity was stable during winter and spring P. zietziana remained largely in immature stages. It was only when salinity increased

during summer that most became mature, laying resistant eggs to ensure survival over the drought. This may have been an adaptation to the higher average salinity during his study; such an effect was not a strong influence during mine. For instance, shrimp in Cundare died before the drought at the beginning of 1974 without becoming mature.

The evident progression of shrimp through the size classes (Figs. 9 and 10) especially during periods of little or no mortality e.g. cohort 5 in Pink from March to June/July 1975, indicates growth was generally not affected by size-selective death. Mean maximum weight is probably lower than shown, as death of ovigerous females may artificially cause an increase. This will not significantly alter estimates of production. Occasionally amongst mature individuals, mean weight decreases by the next visit.

Significant decreases e.g. July-August 1975 (Fig. 11) have been allowed for in the Allen curves (Figs. 13 and 14) as they provide the best estimate of the weight of those dying. Otherwise the curves were drawn averaging insignificant anomalies in growth or density. Production was calculated by measuring the area under each curve with a planimeter. The death of immature shrimp i.e. individual weight less than 0.6 mg or length less than 9.5 mm (sixth length class), accounted for the majority of this in most cases. There were two cohorts (those hatched after the drought in autumn 1974) in which this did not occur. In both there may have been significant mortality before the first samples were taken, implying production has been under-estimated. Because the dry weight of an egg is about three times that of an individual in the first size class, production equal to initial cohort density multiplied by dry weight of an egg was subtracted from the estimates for each cohort.

Weight of eggs produced was calculated separately. The density of ovigerous females per visit during a recruitment period was known from the size-frequency histograms. From this the number of females per 0.1 $m^2$

FIGURE 13

Allen curves for the cohorts of P. zietziana in Pink Lake. Numbers show the order in which the samples were taken.

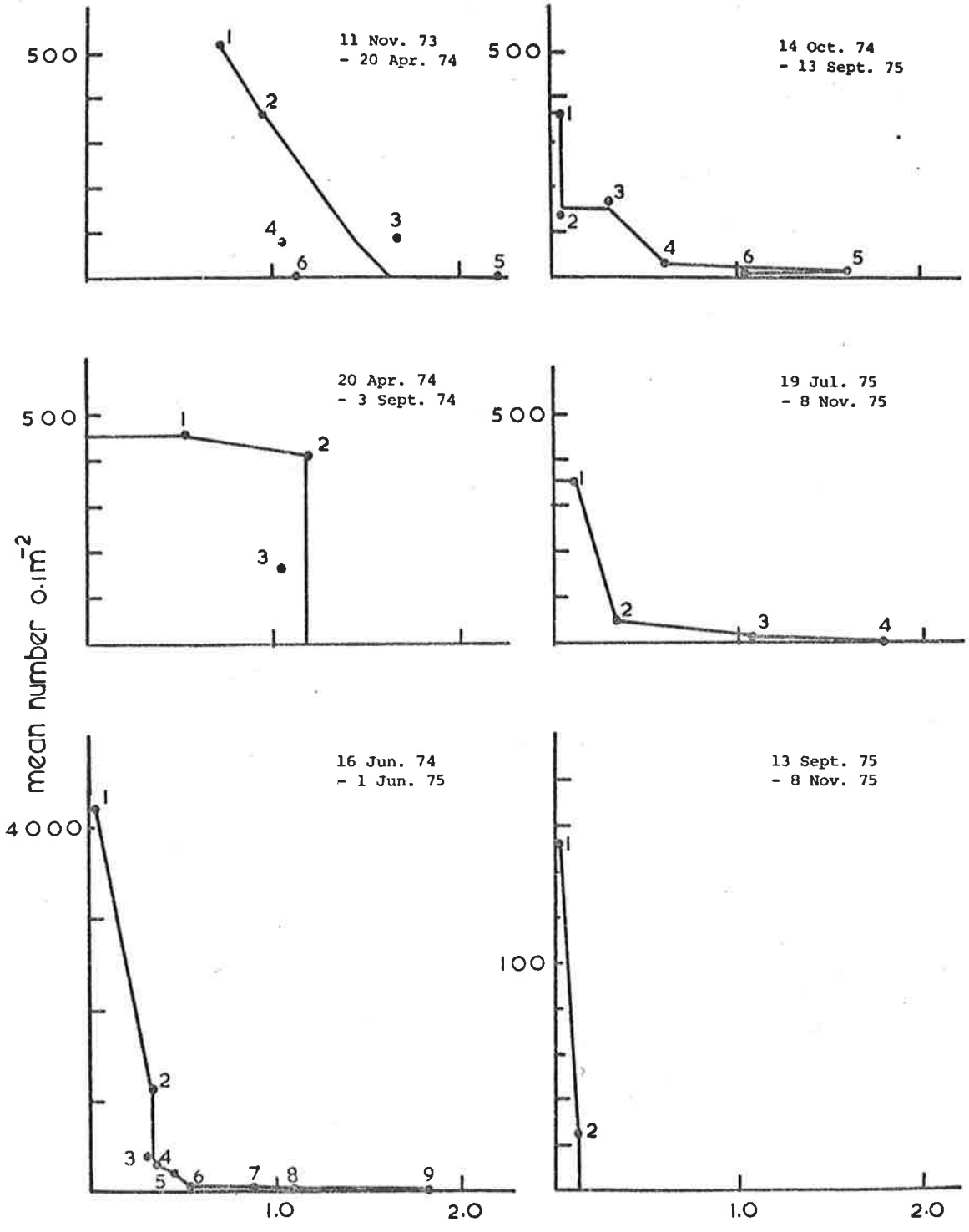
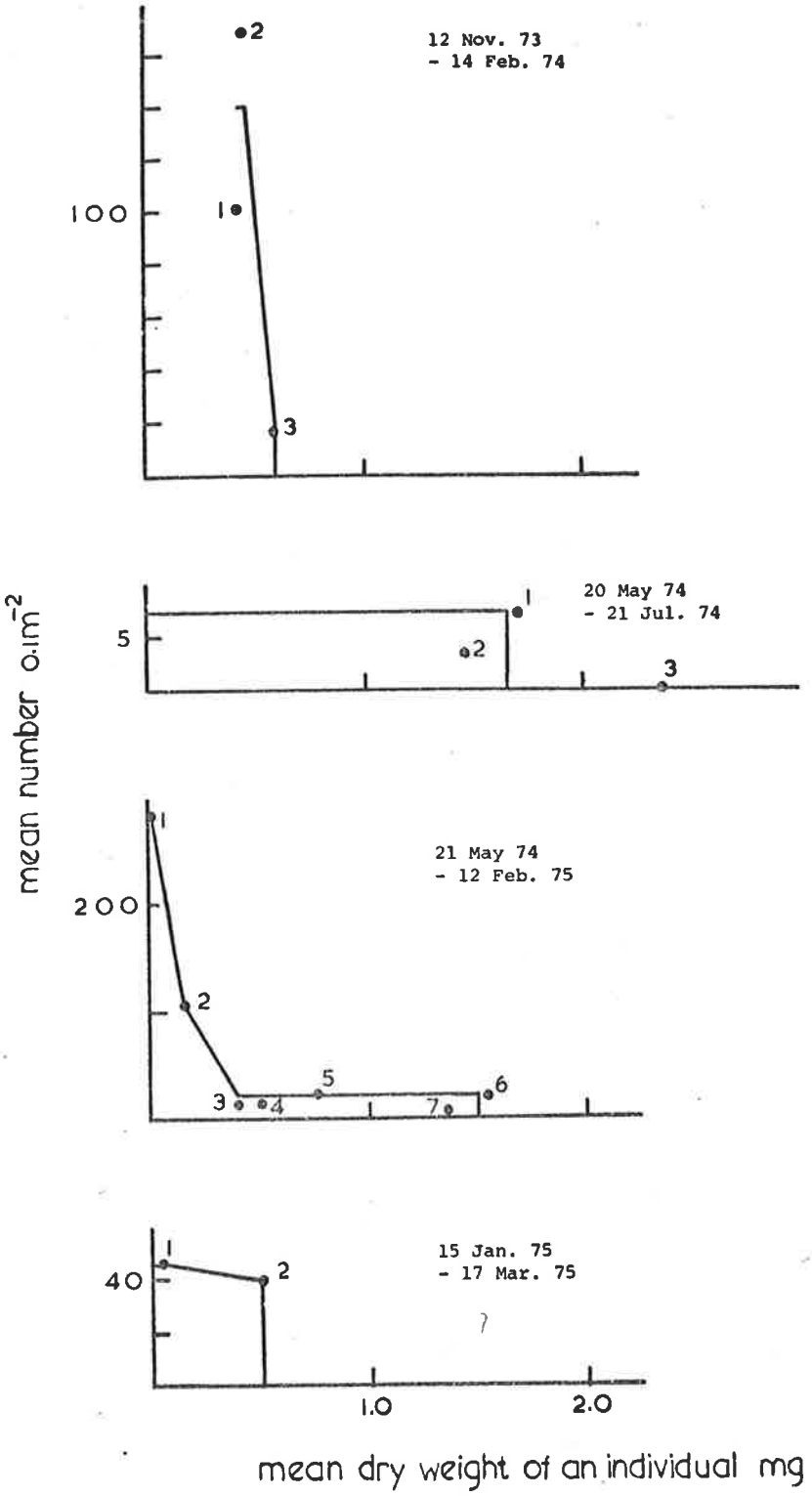

11 Nov. 73
- 20 Apr. 74
14 Oct. 74
- 13 Sept. 75
20 Apr. 74
- 3 Sept. 74
19 Jul. 75
- 8 Nov. 75
16 Jun. 74
- 1 Jun. 75
13 Sept. 75
- 8 Nov. 75
500
4000
100
1.0
2.0
mean number o.1m-2
mean dry weight of an individual mg

FIGURE 14

Allen curves for the cohorts of P. zietziana in Lake Cundare. Numbers show the order in which the samples were taken.


12 Nov. 73
- 14 Feb. 74
100
20 May 74
- 21 Jul. 74
5
21 May 74
- 12 Feb. 75
200
15 Jan. 75
- 17 Mar. 75
40
1.0
2.0
mean number o.m$^{-2}$
mean dry weight of an individual mg

shedding eggs could be determined by assuming that significant decreases in population densities between successive visits during recruitment were due in part to the death of females that had laid their clutch. Laboratory cultures had suggested that females died before or soon after laying. Summing their mortalities gave an estimate which could be multiplied by clutch size and then egg weight to give total dry weight of eggs produced per 0.1 $m^2$ (Table 6). One problem with this method was the difficulty of accounting for immature females. They may disappear from a population by death or by becoming ovigerous and then dying. When population density declined rapidly it was impossible to distinguish these. Therefore I assumed that immatures that did not reappear in rising densities of ovigerous females died before reaching maturity. Another point is that the size frequency data indicates that females carried eggs in the lake for longer periods than they did in the laboratory (one week approximately). However, any female with full or empty egg sacs was classed as ovigerous. Thus the period of actual egg bearing is exaggerated by these data; large numbers with full sacs were usually caught on only one visit during a period of recruitment.

Table 6 also gives the initial densities of each cohort for comparison with the estimated number of eggs produced by their parents. These are always less than the potential recruitment indicating either that a sizeable reservoir of resting eggs is maintained or that any mortality during the period before maximum density in a cohort is recorded is comfortably accounted for. It can be seen that an important factor in determining the size of the recruitment is the number of females surviving to become ovigerous.

The values for egg and biomass production derived as above are presented in Tables 7 and 8. On average Pink is approximately ten times more productive than Cundare. This reflects its larger population density. In fact the greatest production in both lakes is from the overwintering

TABLE 6

The production of eggs during periods of recruitment in numbers and dry weight (mg)

| recruitment period | estimated number of ♀ per 0.1 $m^2$ shedding eggs (95% confidence limits shown) | clutch size (±95% confidence limits) | number of eggs produced (a x b) 0.1 $m^{-2}$ | dry weight (mg) of eggs produced 0.1 $m^{-2}$ (c x 0.016 mg) | initial density of cohort resulting (numbers 0.1 $m^{-2}$) |
|---|---|---|---|---|---|
| | a | b | c | d | |
| | | PINK | | | |
| Nov. 1973 - Feb.1974 | 186 (130-266) | 21.4 (± 2.5) | 3,980 | 64.1 | 85 |
| May 1974 - July 1974 | 198 (107-367) | 79.6 (± 7.5) | 15,761 | 253.8 | 4214 |
| Oct. 1974 - Mar 1975 | 18 (11-31) | 39.3 (± 2.8) | 707 | 11.4 | 361 |
| June 1975 - Aug 1975 | 5.3 (4.0-7.0) | 85.1 (± 9.6) | 451 | 7.3 | 353 |
| Sep 1975 - Nov 1975 | 3.2 (2.2-4.4) | 105.0 (±10.5) | 336 | 5.4 | 152 |
| | | CUNDARE | | | |
| Nov 1973 | 1.3 (1.1-1.6) | 119.5 (±14.5) | 155 | 2.5 | – [e] |
| May 1974 - July 1974 | 3.8 (1.9-7.6) | | 454 | 7.3 | 284 |
| Dec 1974 - Feb 1975 | 2.4 (1.6-3.8) | | 287 | 4.6 | 46 |

[e] recruitment from this generation occurred before sampling began; no clutch size was measured, but it was assumed to equal the reasonably constant value during 1974-1975

TABLE 7

Production of P. zietziana in Pink Lake (mg dry weight 0.1 $m^{-2}$)

| generation | biomass production | egg production | total (P) | |
|---|---|---|---|---|
| 1. overwintering generation: Nov. 1973 - April 1974 | 210.8 | 64.1 | 274.9 | |
| 2. early summer generation: Dec. 1973 - Mar. 1974 | 0.5 | — | 0.5 | |
| 3. autumn generation: Apr. 1974 - Sep. 1974 | 511.7 | 253.8 (33%)[a] | 765.5 | Total = 2255.6 mg 0.1 $m^{-2}$ 2 $years^{-1}$ = 22.6 g $m^{-2}$ 2 $years^{-1}$ or 11.3 g $m^{-2}$ $year^{-1}$ |
| 4. overwintering generation: June 1974 - June 1975 | 982.2 | 11.4 ( 1%)[a] | 993.6 | |
| 5. mid-summer generation: Oct. 1974 - Sep. 1975 | 96.2 | 7.3 ( 7%)[a] | 103.5 | i.e. Nov. 1973 - Nov. 1974: 1923.1 $mg_2$ 0.1 $m^{-2}$ or 19.2 g $m^{-2}$ |
| 6. mid-winter generation: July 1975 - Nov. 1975 | 102.3 | 5.4 ( 5%)[a] | 107.7 | Nov. 1974 - Nov. 1975: 332.5 $mg_2$ 0.1 $m^{-2}$ or 3.3 g $m^{-2}$ |
| 7. spring generation: Sep. 1975 - Nov. 1975 | 9.9 | — | 9.9 | |

[a] egg production as percentage of total production (P) of cohort

TABLE 8

Production of *P. zietziana* in Lake Cundare (mg dry weight 0.1 $m^{-2}$)

| generation | biomass production | egg production | total (P) | |
|---|---|---|---|---|
| 2. Remains of overwintering generation: Nov. 1973 - Dec. 1973 | — | 2.5 | 2.5 | |
| 1. Mid-winter generation: Nov. 1973 - Feb. 1974 | 14.9 | — | 14.9 | Total = 130.3 mg 0.1 $m^{-2}$ 1.3 $years^{-1}$ = 1.3 g $m^{-2}$ 1.3 $years^{-1}$ |
| 3. Autumn generation: May 1974 - July 1974 | 11.8 | 7.3 (38%)[a] | 19.1 | or 1.0 g $m^{-2}$ $year^{-1}$ |
| 4. Overwintering generation: May 1974 - Feb. 1975 | 68.4 | 4.6 ( 6%)[a] | 73.0 | i.e. Nov. 1973 - Nov. 1974 = 83.7 mg 0.1 $m^{-2}$ or 0.8 g $m^{-2}$ |
| 5. late summer generation: Jan. 1975 - Mar. 1975 | 20.8 | —[b] | 20.8 | Nov. 1974 - Nov. 1975 = 46.6 mg 0.1 $m^{-2}$ or 0.5 g $m^{-2}$ |

[a] egg production as a percentage of total production (P) of cohort

[b] there was a small early autumn cohort present in the last sample of this generation, whose value for production was negligible

generation in 1974 which had the highest initial densities of any recorded. Egg production is only a significant proportion of the total in both lakes in the autumn cohort of this year when the shrimp populations were re-establishing after total or near total extinction during the summer drought. This is due to increased survival of females to maturity as much as anything else and perhaps to under-estimation of biomass production. (I will explain more in Chapter 8 of the significance of the varying clutch size and numbers recruited). At other times egg production is only an insignificant proportion of the total considering the 45-50% error in the production estimate, (Table 9).

The approximate 95% confidence limits of production (P) for each cohort were calculated from the mean 95% confidence limits of density and weight measurements. These two errors were combined using the formula suggested by Brown (Charles, East, Brown, Gray and Murray 1974) in which:

$$V(P) \doteq N^2 V(W) + W^2 V(N)$$

where $V$ = variance

$N$ = mean population density

$W$ = growth

Strictly speaking this formula should be applied to data from adjacent visits and the results summed throughout the life of a generation. Instead, I substituted mean population density of a cohort for N and production of a cohort divided by this $(\frac{P}{N})$ for W, estimating their variances from the prevailing mean 95% confidence limits for density and weight. Because the limits for density are asymmetrical (they were derived from logs) further refinement of the calculation of error was not worthwhile. The resulting 95% confidence limits of production $(2\sqrt{V(P)})$ are dominated by the errors in density, (the larger of the two) demonstrating that precision in sampling the population determines precision in estimating production. I have ignored any error in converting length to

TABLE 9

Estimates of error in the calculation of total production (P) expressed as percentages and the mean biomass ($\bar{B}$)[c] and turnover ratio ($P/\bar{B}$) per cohort

| cohort[a] | mean 95% confidence limits of density (%) | mean 95% confidence limits of weight (%) | approximate 95% confidence limits of total production P (%) | | $\bar{B}$ (mg dry weight $m^{-2}$) | $P/\bar{B}$ |
|---|---|---|---|---|---|---|
| | | PINK | | | | |
| 1 | 40 | 15 | 40 | $\bar{x} = 45$ | 157.3 | 1.8 |
| 2 | 38 | 28 | 46 | | 0.3 | 2.0 |
| 3 | 54 | 13 | 51 | | 227.5 | 3.4 |
| 4 | 50 | 16 | 49 | | 133.9 | 7.4 |
| 5 | 45 | 15 | 44 | | 24.1 | 4.3 |
| 6 | 33 | 14 | 33 | | 18.2 | 5.9 |
| 7 | 52 | 20 | 55 | | 3.8 | 2.6 |
| | | CUNDARE | | | | |
| 1 | 64 | 8 | 56 | $\bar{x} = 50$[b] | 32.5 | 0.5 |
| 2 | 20 | 12 | 21 | | 32.4 | 0.1 |
| 3 | 54 | 17 | 50 | | 6.2 | 3.1 |
| 4 | 52 | 18 | 49 | | 13.4 | 5.4 |
| 5 | 52 | 10 | 46 | | 11.2 | 1.9 |

[a] cohorts numbered as in Tables 7 and 8

[b] 21 excluded from mean

[c] calculated by averaging standing crop for each visit

weight, but it is not likely to be large because there is little variation in their regression (Fig. 8).

The values for annual production vary widely in Pink but appear to be more constant in Cundare. The turnover ratios P/B (Table 9) are equally variable in both lakes and perhaps indicate a lack of any endogenous regulation of cohort production if Waters' (1969) idea is correct that freshwater invertebrates will usually exhibit ratios between 2.5 and 5 with a mode at 3.5. Waters showed that for an animal with exponential growth, like P. zietziana, relative size of the final population compared with the initial has the greatest effect in altering turnover ratios. Thus if there is any regulation of production it would be through control of brine shrimp mortality. Shape of the Allen curve has virtually no influence provided initial weight is less than 1-2% of final weight, as it usually was.

The only aspect of production, neglected so far, is loss of biomass through moulting. Geddes (1973) estimated twenty instars for P. zietziana in Pink and Cundare. If the integument lost represents 5% of the dry weight at each moult (Daborn, 1975) then production would be increased by an average of 15%. This is insignificant compared with sampling error.

There are few values for secondary production in saline ponds or lakes with which my estimates can be compared. Carpelan (1957) computed a value of 6.3 g dry weight $m^{-2}$ $year^{-1}$ for A. salina in the Alviso salt works in San Francisco bay, but this was only a guess based on mean standing crop multiplied by the possible number (8) of generations in a year according to generation times in culture. Data for total zooplankton production of lakes in temperate regions of the Northern hemisphere e.g. Poland with 30-45 g dry weight $m^{-2}$ $year^{-1}$ are somewhat higher and less variable than my measurements and appear to depend on the degree of eutrophy of the lakes (Hillbricht-Ilkowska, Gliwicz and Spodniewska, 1966). The only other estimates of secondary production in a saline lake are those of Paterson and Walker (1974) for Tanytarsus barbitarsis, a benthic chironomid, in Lake

Werowrap (13 km northwest of Colac; salinity 36-56‰). Annual production (in dry weight) amounted to 64.6 g $m^{-2}$ exclusive of mortality and first and second instar biomass and so is an underestimate. This was only 7.9% of the prevailing primary production yet the lake had one of the highest rates of secondary production on record and T. barbitarsis was considered an efficient herbivore.

From the scant data for primary production in Pink Lake a similar calculation can be made. In this case annual secondary production is about 40% of the annual primary production. This value is not very accurate because the data on primary production were collected for six months only. According to Williams (personal communication) annual primary production is likely to be lower and so the percentage higher. This, I think, indicates that P. zietziana cannot be obtaining energy solely from phytoplankton because no efficiency as high as this has been recorded for aquatic invertebrates (Kozlovsky, 1968). The only other source of food is organic matter, probably bacteria, in the sediments.

Therefore the data in this chapter pose two questions: on what do the brine shrimp mainly feed and what causes their mortality? If they largely rely on sediment then poor assimilation of this probably dilute supply of energy could well result in death. To investigate both problems I have concentrated on measuring the rates of respiration and ingestion in the field.

CHAPTER 5 - Respiration of *P. zietziana*

Measurements of respiratory rate represent the minimum energy intake of an individual. Given data on animal numbers, the respiration of a population or cohort can be calculated. This, added to estimates of production, equals assimilation or the total intake of metabolisable energy.

Usually the respiratory rate of aquatic invertebrates is measured in the laboratory under controlled conditions. It is well known that weight of an individual and water temperature will affect metabolic rate. These can easily be controlled and by choosing a number of different combinations respiration can be specified accurately. The difficulty with such measurements is in extrapolating them reliably to field conditions. There is mounting evidence, at least for aquatic invertebrates, that other factors such as nutritional history are equally important and that field acclimation to various temperature regimes invalidates the relation in the laboratory of respiratory rate to temperature.

Blazka (1966) working with natural and cultured populations of *Daphnia* found this relation differed between field and laboratory animals, and in field animals at different seasons. In general, the respiratory rate of the natural population increased much less with rising temperature; from this he concluded that they had acclimated more thoroughly. He also studied the interaction of nutrition and respiratory rate. First, he showed that oxygen consumption in the field of *D. hyalina* was proportional to seston concentration in three consecutive winters. At their lowest rate of oxygen consumption they could last for six to seven weeks on body reserves. Second, he found by measuring ammonia excretion and comparing this with oxygen consumption measured simultaneously that animals with a food surplus (the laboratory culture) metabolised no protein while the field populations, which fed on lower concentrations of food,

obtained between 12 and 80% of their energy from protein catabolism. The percentage increased with temperature. Kersting (1973), who also worked with Daphnia (D. magna), suggested that respiratory rate should be correlated with filtering rate because the same appendages were used for both functions. By measuring respiration and filtering rate simultaneously, the latter with a coulter counter, he was able to show this. At high food concentrations, above the minimum level at which the filtering rate started to decline, respiratory rate decreased; at lower concentrations it was more or less constant.

To enable variations in respiratory rate due to the above factors to be included in estimates of assimilation, oxygen consumption can be measured directly in the field. Duncan, Cremer and Andrew (1970) used a field technique to study the annual fluctuations in respiratory rate of macro-zooplankton communities in two reservoirs of the lower Thames valley. They also measured the respiratory rate of Daphnia hyalina in the laboratory at 20°C. Using field estimates of density and water temperature they extrapolated and compared these results with field respiratory rates measured during a period when D. hyalina was predominant in the zooplankton. They found the pattern of changes in population respiratory rate given by the two methods was similar, but the field levels were always significantly higher perhaps because of increased activity of the daphniids during the field measurements. This, they suggested, was due either to disturbance from handling or to greater scope for active movement in the field respirometers (B.O.D. bottles) than in the laboratory ones (Cartesian divers). Lack of food in the divers, but its presence in the field bottles, though at concentrations lower than ambient, may have had an additional effect.

I have also used a field technique for measuring the respiration of P. zietziana. Previously, brine shrimp respiration has only been measured for laboratory cultures of A. salina. Usually the investigators

(Kuenen, 1939; Eliassen, 1952; Gilchrist, 1956,1959) were interested in the effects of salinity on respiration in addition to those of body size and temperature. A. salina was known to hypo-osmoregulate and it was thought that as salinity increased more energy via a higher respiratory rate would be needed. Kuenen found that respiratory rate increased one and a half times between 29‰ and 116‰. Eliassen, on the contrary, found a decrease between 10‰ and 50‰ which was most marked in the nauplii, but which faded in the adults. Finally, Gilchrist could show no difference in respiratory rate at 35‰ and 140‰. Gilchrist and Eliassen reared their shrimp at the salinity they were to be tested at while Kuenen transferred his from 58‰ to two other concentrations, measuring respiration after twenty four hours of acclimation. Such differences make it difficult to extrapolate these results to the natural habitat of either A. salina or P. zietziana where only gradual changes of salinity occur usually in a consistent direction. According to Styczynska-Jurewicz (1970), changes in respiratory rate due to salinity change are probably caused by physiological adaptation and disappear when this ends.

Methods

The respiration of P. zietziana was measured in both lakes on most visits. Shrimp were enclosed in 300 ml clear B.O.D. bottles whose volume had been checked and the oxygen concentration before and after an incubation measured using the azide modification of the Winkler method (Golterman, 1969), by titrating 50 ml subsamples with standardised 0.01 N sodium thiosulphate. Variation in sample titre was usually $\leq 5\%$. A similar procedure was used in the field by Daborn (1970) for measuring respiratory rates of B. gigas, a predatory anostracan.

Shrimp were collected with a 200$\mu$ zooplankton net. Immediately, the catch was filtered through a coarse (approximately 1 mm) mesh

which separated the brine shrimp from the detritus and small ostracods (Platycypris) inevitably collected. Larger organisms, that were present only occasionally and did not pass through the mesh, e.g. Ephydrella larvae or Australocypris, were picked out with tweezers. Jets of filtered (particles $<200\mu$) lake water from plastic squeeze bottles considerably quickened the filtering. The smallest brine shrimp (nauplii) passed through the mesh and it was only possible to measure their respiration when there was little detritus and no ostracods.

Filtered lake water was obtained from approximately 30 cm beneath the water surface with a Van Dorn bottle containing a $200\mu$ filter in the outlet hose. Four B.O.D. bottles (two with and two without shrimp) were filled for each experiment, the first bottle filled being an initial water sample and the last a control. To increase the speed of these operations, the B.O.D. bottles were flushed once only. This was justified because the water in these shallow lakes was nearly always fully saturated; there was rarely any significant difference between the oxygen concentration of the initial and control bottle.

Usually the above manipulations, from catching the shrimp to replacing them in the lake inside B.O.D. bottles, took less than ten minutes. When shrimp were scarce or there were many contaminating organisms handling time increased to a maximum of twenty minutes. The B.O.D. bottles were always placed upright on the bottom near the shore, usually inside the 0.1 $m^2$ galvanised iron cylinder to prevent disturbance by waves, at a depth $\geq 60$ cm in Pink and $<50$ cm in Cundare. The oxygen content of the initial sample was fixed immediately after starting the incubation.

The number of brine shrimp used in each experiment to avoid depleting the initial oxygen concentration by more than 50% depended on their size and the oxygen content of the water. This was a matter of judgement, but usually required twenty to two hundred animals. Obviously,

the period of the incubation also affected this. However, I preferred to alter numbers of shrimp rather than incubation times. Generally one experimental bottle was incubated for half an hour and the other with the control for one hour. A few times incubation lasted two to three hours. During all incubations the shrimp swam freely, but no more rapidly than seen in the lake. All short incubations were made within two hours of midday. However, to determine whether there were any diurnal fluctuations in respiratory rate I tried longer incubations of twenty four hours. For these only three to six animals were used. Unfortunately they always gave respiratory rates about half those of the shorter incubations. Probably this was caused by inhibition from the build up of metabolic wastes and the effects of starvation. Instead four to five one hour incubations spaced throughout twenty four hours were used to detect any diurnal fluctuations. Water temperature was read at the beginning and end of all incubations. If the shrimp were not swimming freely at the end, the experiment was discarded.

The oxygen in the experimental and control bottles was fixed beside the lake. The shrimp did not die immediately the reagents were added, but survived half to three quarters of an hour, presumably on oxygen within their tissues. Strickland and Parsons (1968) state that all the oxygen is precipitated rapidly once both reagents have been added and the bottle shaken. All bottles were taken to the field station for titration. This was always done within five hours of collection and immediately after adding the concentrated sulphuric acid. At the end of the titrations of the experimental bottles the shrimp were counted, rinsed in freshwater, and placed in vials in a desiccator. Within a week they were dried at 60$^{o}$C and stored over silica gel until weighed (see methods, Chapter 4). Occasionally a few shrimp were lost in the overflow after adding the reagents. These were included in the subsequent calculations of respiration per animal. Also a few animals sometimes died during an incubation. It

was impossible to allow for these because all the shrimp were dead when they were finally counted after a titration.

The filtered lake water used in these experiments would have contained any phytoplankton present because only a 200$\mu$ mesh was used. Dunaliella salina, an algae reported from Pink, has a maximum diameter of 20$\mu$. At first to avoid the possible influence of algal photosynthesis I used B.O.D. bottles darkened with layers of black tape. However, I found no difference in oxygen contents of the initial and control bottles during short incubation ($<$2 hours) and thereafter used clear bottles.

A possible source of error with B.O.D. bottle respirometers (Kamler, 1966) is an increased oxygen consumption at the start of an incubation from disturbance of the animals by handling. To check this the decrease in oxygen concentration was also monitored using a Yellow Springs Instrument B.O.D. oxygen electrode. Three experiments were made, one in the laboratory with P. zietziana collected from the Dry Creek Salt Works, Adelaide and two at the field station with shrimp collected a few hours before in Pink Lake. Shrimp and filtered lake water ($<$200$\mu$) were added to the B.O.D. bottle which was then submerged in a basin of water and temperature and oxygen concentration recorded every fifteen minutes until the animals died. The experiment in the laboratory was similar except the shrimp had been kept in aquaria at about 20$^{o}$C for a few days. At the end of all three experiments the animals were counted and prepared for weighing as before.

Results

The three experiments monitoring oxygen decline were made during July and August 1975. The results are in Fig. 15. Each point represents the respiratory rate during the fifteen minute interval between readings, expressed as a percentage of the maximum constant rate recorded. From the graph the respiratory rate appears more or less constant until an

FIGURE 15

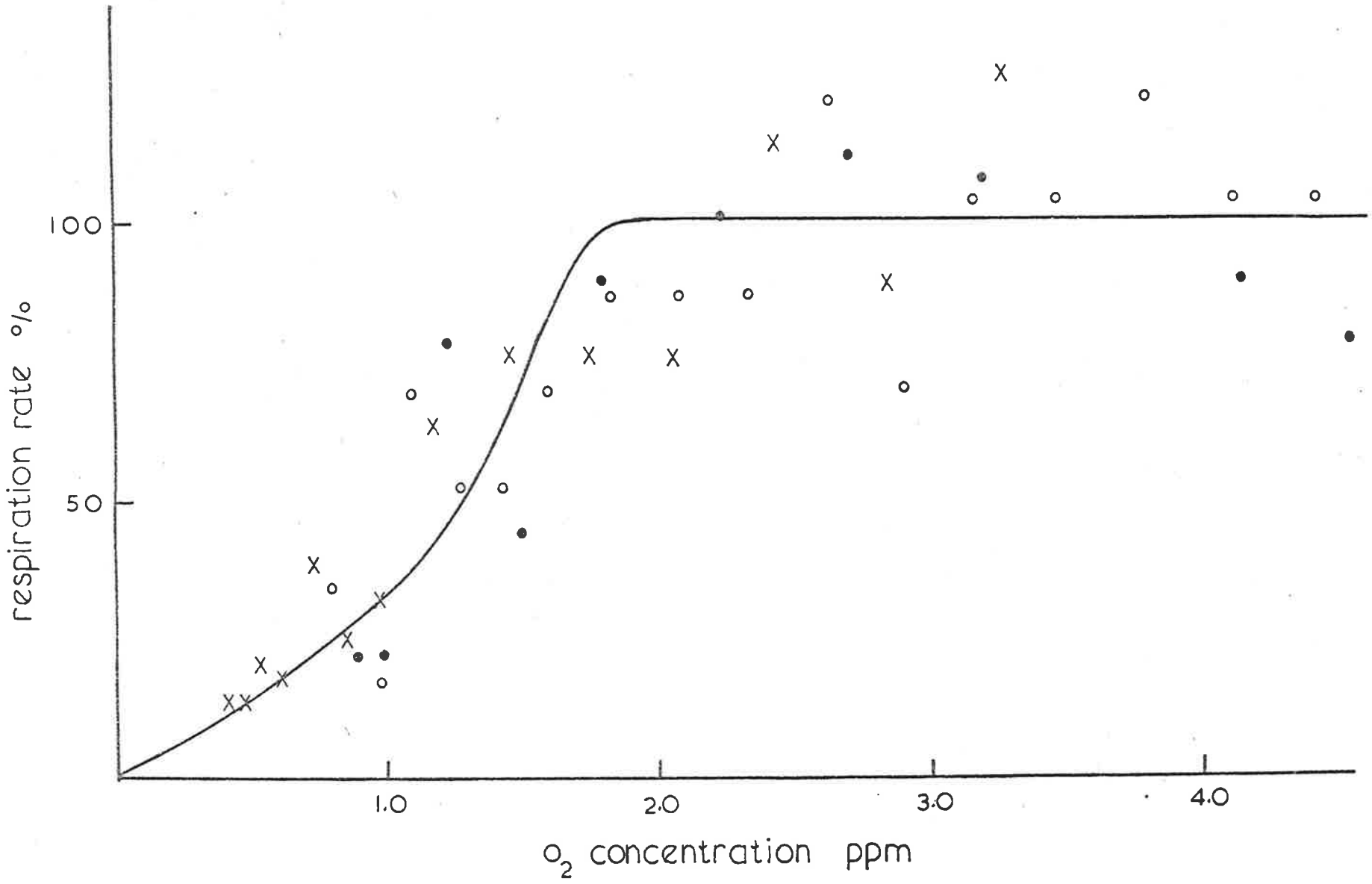
Respiratory rate of P. zietziana versus oxygen concentration. Respiration is shown as a percentage of the mean constant rate. Oxygen concentration for each value of respiratory rate is the mean of the two readings (15 min. apart) used to calculate this rate. The horizontal line is the average of the points to the right of 2 ppm; the curve was fitted by eye.

at field station

- ● - 305 shrimp (0.337 mg dry wt. individual$^{-1}$); 10-13°C; initial $O_2$ concentration 4.70 ppm, time to reach critical $O_2$ level 1.75 h; 120%.
- o - 54 shrimp (1.601 mg dry wt. individual $^{-1}$); 12-14°C; initial $O_2$ concentration 4.55 ppm, time to reach critical $O_2$ level 2.25 h; 135%.

laboratory:

- X - 84 shrimp (0.546 mg dry wt. individual$^{-1}$); 16-20°C; initial $O_2$ concentrations 3.50 ppm; time to reach critical $O_2$ level 1.00 h; 106%.


100
50
respiration rate %
1.0
2.0
3.0
4.0
$O_2$ concentration ppm

oxygen concentration of 1.8-1.9 mg $l^{-1}$ is reached. Shortly before this stage the shrimp were becoming noticeably less active and started to collect near the top of the bottle. Whenever this crowding of shrimp was noticed at the end of a field incubation the result was discarded, because the oxygen concentration subsequently measured was about 1.7-1.9 mg $l^{-1}$. Never was an initial oxygen concentration as low as this recorded.

These experiments confirm that there was no increase in respiratory rate at the beginning of an incubation due to disturbance. They also show that P.zietziana is a respiratory regulator. That is this animal can maintain a constant rate of respiration while oxygen concentration falls until a critical level is reached below which the rate drops rapidly. The respiratory rate when constant is not significantly different from that measured for the particular combination of weight, temperature and salinity in the field.

I analysed the results of the short-term field incubations with a step-wise multiple regression relating respiratory rate of an individual (logarithm) to its mean weight (logarithm), temperature and salinity. The first two independent variables are well known to affect respiration while the third is suspected of doing so. There were no significant differences between regression coefficients when the data from each lake (60 incubations in Pink, 19 in Cundare) were treated separately. Therefore the data were lumped and a single regression equation obtained:

$$\log R = -1.123 + 0.002\ S + 0.021\ T + 0.756 \log W$$

where R = respiration in $mgO_2 \times 10^{-4}\ hr^{-1}$ individual$^{-1}$

S = salinity in ‰

T = temperature in $^{o}C$

W = dry weight of an individual in mg $\times 10^{-3}$

The analysis of variance is given in Table 10 and the confidence limits of the regression coefficients in Table 11. Each of the three independent variables accounts for a significant part of the variance in respiration (weight alone contributing 80%) and together they explain about 90% of the total sum of squares. There were no correlations between the independent variables.

Temperature varied between 9 and 27°C and salinity between 49 and 240‰ for these experiments. The average $Q_{10}$ for this temperature range is 1.62 while for a salinity increase of 50‰ respiration increases by 1.26. All the arbitrary length classes were represented in these incubations, although only two were made in which the two smallest length classes were predominent.

For any particular incubation shrimp were not of all the same length or weight. This is perhaps the most serious error introduced into these experiments. However, only the larger of the two cohorts, usually present, was sampled except occasionally when both were of about equal strength. Thus the frequency distribution of shrimp weights in an experiment would have been the same as for a cohort i.e. roughly normal. This distribution is broader for the bigger shrimp because the variance of the mean weight of a cohort increases as the animals grow, especially once sexual dimorphism (♀ smaller than ♂) begins. Logarithmic transformations of weight and respiration largely correct this for the purposes of the regression calculations. The respiration of eggs was not measured but pregnant females were quite often present in the incubations. I assumed that the increase in weight from their eggs would add to respiration as equally as would a corresponding increase in weight due to growth.

Another problem is the absorption of iodine by the shrimps when this is released on acidification. In my experiments the amount so isolated would never have been large. The total dry weight of shrimp used varied between 13 and 350 mg averaging 100 mg. If dry weight is 10% of wet weight

TABLE 10

Analysis of variance for the multiple regression

| Source of variation | df | Sum of squares | | Mean Square | F | Significance |
|---|---|---|---|---|---|---|
| weight | 1 | 11.19927 | (79.5%)[a] | 11.19927 | 579.07 | $p \ll 0.001$ |
| temperate | 1 | 0.96416 | ( 6.8%)[a] | 0.96416 | 49.85 | |
| salinity | 1 | 0.47968 | ( 3.4%)[a] | 0.47968 | 24.80 | |
| regression | 3 | 12.64311 | (89.7%)[a] | 4.21437 | 217.95 | |
| residual | 75 | 1.45026 | | 0.01934 | | |

[a] Percentage of total sum of squares

TABLE 11

Confidence limits of the regression coefficients

| variable | b | standard error of b | 95% confidence limits |
|---|---|---|---|
| weight | 0.756 | 0.03519 | 0.686 - 0.826 |
| temperate | 0.021 | 0.00351 | 0.014 - 0.028 |
| salinity | 0.002 | 0.00041 | 0.001 - 0.003 |
| constant | -1.123 | 0.11096 | -1.344 - -0.902 |

then 1 g wet weight or approximately 1 ml of shrimp was present. Iodine was only seen on the legs of the shrimp so the volume absorbed would always have been less than 1 ml, an insignificant amount when titrating 50 ml aliquots from a total volume of 300 ml. Also if iodine absorption was large then the results of incubations done on the same day should have varied significantly, amongst other things, according to the number of shrimp used. Such a correlation was never observed.

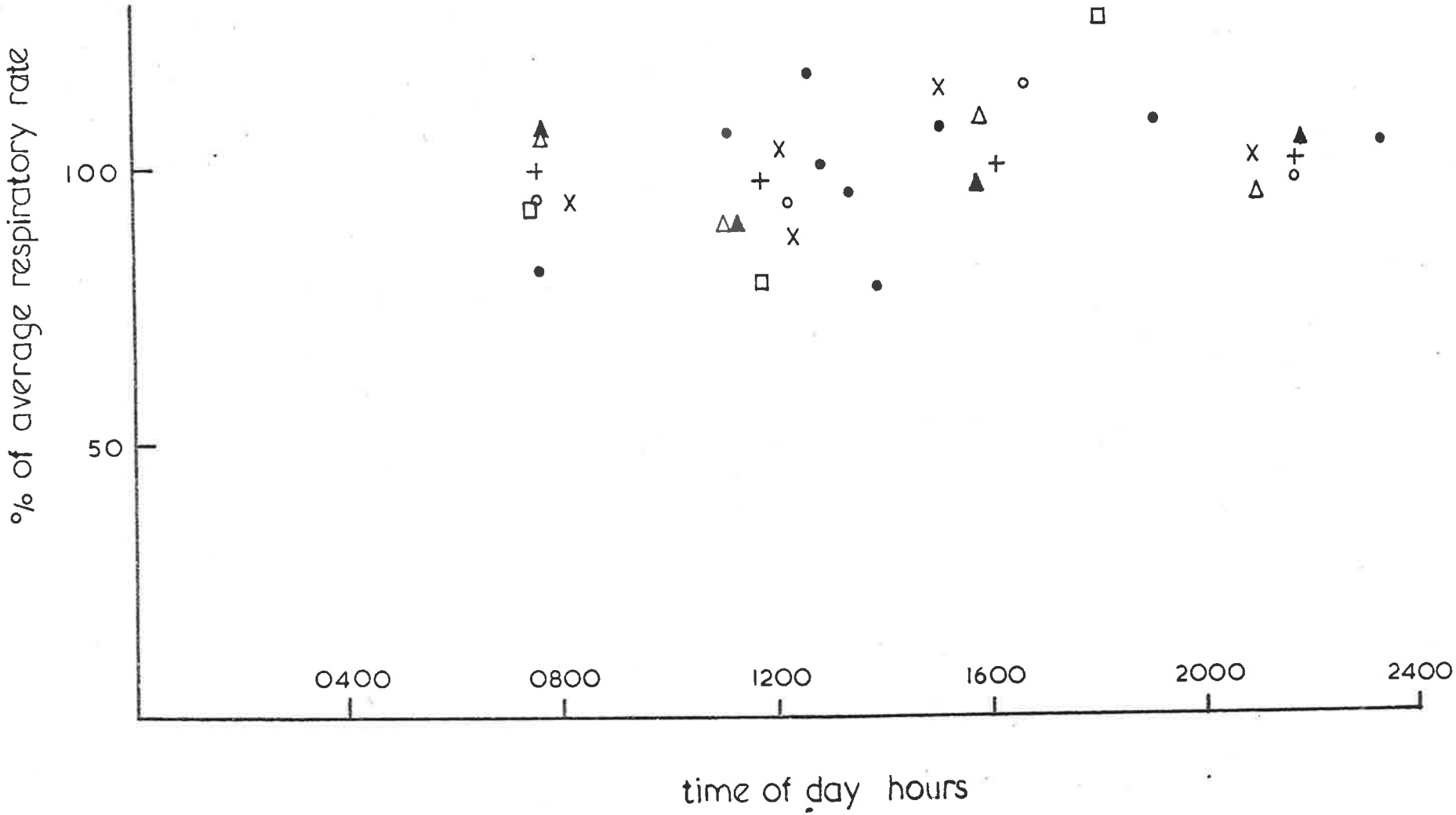
In fact, the success of the regression in explaining most of the variation in respiration indicates that this and any other effect, such as crowding, likely to be proportional to the number of shrimp used (twelve to two hundred) was not significant. This argument also applies to bacterial contamination of the experimental bottle. It is possible that bacteria adhered to the exoskeleton of the shrimp or were excreted in faeces during an incubation, thus increasing the amount of oxygen consumed. Such an effect was probably not detectable with short incubations especially because there was rarely any significant difference between the oxygen content of the initial and control bottles both of which would have contained bacteria, derived at least partly from faeces in the sediments. Even during the twenty four hour incubations oxygen only decreased in the control by a maximum of 8%. Sometimes a few very small ostracods or copepods contaminated the experimental bottles; their individual respiratory rate would have been very low.

Before reliable estimates of population respiration can be calculated the effect of possible diurnal fluctuations in respiratory rate associated with some endogenous rhythm of the shrimp must be assessed. In Fig. 16 I have plotted the results of seven sets of one hour incubations made at intervals throughout twenty four hours. These sets, each corrected for variations in weight and temperature, show that there were no significant diurnal fluctuations in respiratory rate.

FIGURE 16

Daily course of P. zietziana respiratory rate measured with 1 h incubations. Respiration is given as a percentage of the mean value measured over the twenty four hours. Each set of readings has been corrected for variations in mean individual weight and diurnal temperature change using the regression coefficients. The midpoint of an incubation was considered its time of day.

● - 2.6.75 : 12°C, 138‰
X - 23.6.75 : 12°C, 140‰
o - 20.7.75 : 11°C, 135‰
+ - 17.8.75 : 12°C, 120‰
Δ - 15.9.75 : 18°C, 89‰
□ - 13.10.75 : 14°C, 105‰
▲ - 10.11.75 : 18°C, 90‰


% of average respiratory rate
100
50
0400
0800
1200
1600
2000
2400
time of day hours

The respiration rate on each visit of an individual P. zietziana was calculated from the regression equation using previous estimates of mean temperature and salinity (Chapter 2) and mean weight (Chapter 4). Individual rates were converted to daily population rates, i.e. mg $O_2$ 0.1 $m^{-2}$ $day^{-1}$, with the data on population density (Chapter 3), each cohort being treated separately. Tables 12 and 13 show the data used and the results for each lake, which are also graphed in Figures 17 and 18. By measuring the area under each curve with a planimeter the total respiration per 0.1 $m^2$ per cohort was calculated.

Discussion

The estimates of total respiration per cohort are given in Table 14 for both lakes. In general respiration is more or less proportional to production with some exceptions, e.g. generation 1 in both lakes, resulting from rapidly rising salinity and temperature or improved survival of the shrimp to heavier weights. As with the values for cohort production the largest source of error in these respiration rates is the 40-50% error in measuring population density. The error in predicting respiratory rate per individual from the regression equation is much smaller (8-15%), and errors in the independent variables, particularly mean weight of an individual and mean temperature, are unlikely to increase it. Because the larger error will dominate any calculation of the combined error, as shown in Chapter 4, I have assumed that the probable error for values of cohort respiration is 40-50%. Any error from calculating total respiration by integrating a series of daily values will be small. Phillipson (1970) found that an estimate of annual respiration calculated by multiplying mean annual respiratory rate per unit weight by mean annual biomass was usually within 5% of that from a detailed analysis of the oxygen consumption of the various life stages.

It is possible to calculate the assimilation rates for each cohort

TABLE 12

Calculation of daily respiration of the P. zietziana population in Pink Lake, using the regression equation and population densities from Chapter 3

| date | salinity (‰) | mean[a] temperature (°C) | mean dry weight[b] of individual in cohorts present (mg) | mg $O_2 \times 10^{-3}$ $hr^{-1}$ $individual^{-1}$ | mg $O_2$ 0.1 $m^{-2}$ $day^{-1}$ |
|---|---|---|---|---|---|
| 13.11.73 | 146 | 18 | 0.758 | 5.41 | 67.13 |
| 13.12.73 | 162 | 23 | 1.133 | 10.07 | 86.28 |
| | | | 0.090 | 1.48 | 0.13 |
| 16.1.74 | 178 | 23 | 1.810 | 15.47 | 31.93 |
| | | | 0.024 | 0.59 | 0.28 |
| 15.2.74 | 209 | 22 | 1.088 | 11.59 | 20.58 |
| | | | 0.006 | 0.23 | 0.47 |
| 11.3.74 | 240 | 23 | 2.290 | 24.69 | 0.59 |
| 22.4.74 | 216 | 17 | 1.208 | 10.17 | 0.34 |
| | | | 0.092 | 1.45 | 0.08 |
| 23.5.74 | 151 | 13 | 0.590 | 3.59 | 39.04 |
| 16.6.74 | 160 | 10 | 1.821 | 7.60 | 74.37 |
| | | | 0.040 | 0.42 | 42.48 |
| 22.7.74 | 138 | 11 | 1.060 | 4.78 | 18.93 |
| | | | 0.349 | 2.06 | 56.66 |
| 18.10.74 | 94 | 15 | 0.323 | 1.92 | 18.85 |
| | | | 0.016 | 0.20 | 0.03 |
| 12.11.74 | 95 | 16 | 0.364 | 2.22 | 16.25 |
| | | | 0.007 | 0.11 | 0.03 |
| 11.12.74 | 102 | 18 | 0.461 | 3.02 | 16.67 |
| | | | 0.051 | 0.57 | 0.33 |
| 16.1.75 | 107 | 22 | 0.548 | 4.28 | 5.34 |
| | | | 0.014 | 0.27 | 0.80 |
| 11.2.75 | 120 | 23 | 1.039 | 7.75 | 8.37 |
| | | | 0.050 | 0.78 | 6.76 |
| 16.3.75 | 139 | 18 | 1.309 | 7.91 | 7.59 |
| | | | 0.054 | 0.71 | 2.37 |
| 1.6.75 | 138 | 12 | 1.875 | 7.72 | 1.29 |
| | | | 0.317 | 2.01 | 7.96 |
| 22.6.75 | 140 | 11 | 0.685 | 3.47 | 2.58 |
| 20.7.75 | 135 | 11 | 1.977 | 7.55 | 2.54 |
| | | | 0.014 | 0.18 | 0.00 |
| 17.8.75 | 120 | 13 | 1.213 | 5.36 | 1.14 |
| | | | 0.121 | 0.94 | 7.96 |
| 16.9.75 | 89 | 16 | 0.359 | 2.14 | 2.40 |
| | | | 0.005 | 0.08 | 0.04 |
| 13.10.75 | 105 | 16 | 1.463 | 6.66 | 2.01 |
| | | | 0.021 | 0.27 | 0.98 |
| 8.11.75 | 90 | 18 | 1.793 | 7.98 | 0.14 |
| | | | 0.142 | 1.17 | 0.70 |

[a] calculated from monthly readings averaged with adjacent maxima and minima

[b] includes weight of egg sacs of pregnant ♀

TABLE 13

Calculation of daily respiration of the P. zietziana population in lake Cundare, using the regression equation and population densities from Chapter 3

| date | salinity (‰) | mean temperature[a] (°C) | mean dry weight of individual[b] in cohorts present (mg) | mg $O_2$ x $10^{-3}$ $hr^{-1}$ individual | mg $O_2$ 0.1 $m^{-2}$ $day^{-1}$ |
|---|---|---|---|---|---|
| 14.11.73 | 98 | 18 | 0.438 | 2.86 | 7.07 |
| | | | 1.737 | 8.09 | 3.88 |
| 14.12.73 | 142 | 23 | 0.456 | 4.61 | 18.59 |
| 17.1.74 | 215 | 23 | 0.645 | 8.43 | 3.32 |
| 21.5.74 | 119 | 13 | 2.725 | 9.84 | 1.71 |
| | | | 0.052 | 0.49 | 0.04 |
| 17.6.74 | 131 | 11 | 2.602 | 9.12 | 0.77 |
| | | | 0.018 | 0.21 | 1.43 |
| 21.7.74 | 92 | 11 | 2.370 | 7.08 | 0.04 |
| | | | 0.164 | 0.94 | 2.39[c] |
| 15.10.74 | 49 | 15 | 0.416 | 1.89 | 1.04[c] |
| 13.11.74 | 54 | 17 | 0.483 | 2.38 | 1.31[c] |
| 13.12.74 | 66 | 19 | 0.795 | 4.05 | 2.31 |
| 15.1.75 | 89 | 20 | 1.636 | 8.17 | 4.47 |
| 12.2.75 | 133 | 20 | 1.683 | 10.25 | 2.16 |
| | | | 0.054 | 0.76 | 0.84 |
| 17.3.75 | 199 | 21 | 0.503 | 5.88 | 5.70 |
| | | | 0.005 | 0.18 | 0.01 |

[a] calculated from monthly readings averaged with adjacent maxima and minima

[b] includes weight of egg sacs of pregnant ♀

[c] actual population density too low because of sampling error; instead average of densities in November and December 1974 used for calculation

FIGURE 17

Monthly variation in the daily respiratory rate of the P. zietziana population in Pink Lake. Numbers refer to the generations described in Chapter 4. Area under each curve estimates total respiration. 0.1 $m^{-2}$ of a cohort.

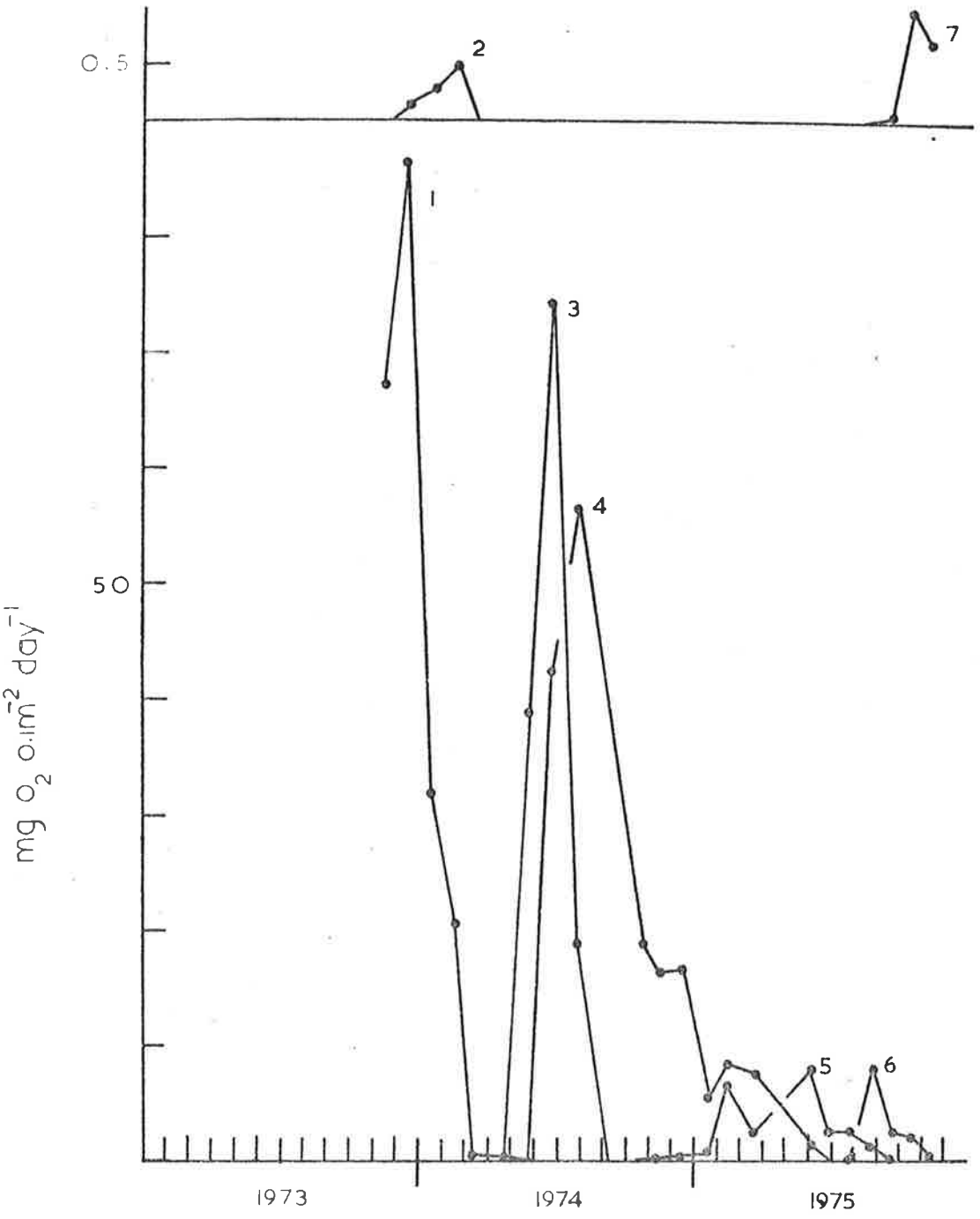

0.5
50
mg $O_2$ o.m$^{-2}$ day$^{-1}$
1
2
3
4
5
6
7
1973
1974
1975

FIGURE 18

Monthly variation in the daily respiratory rate of the *P. zietziana* population in Lake Cundare (Numbers as before)

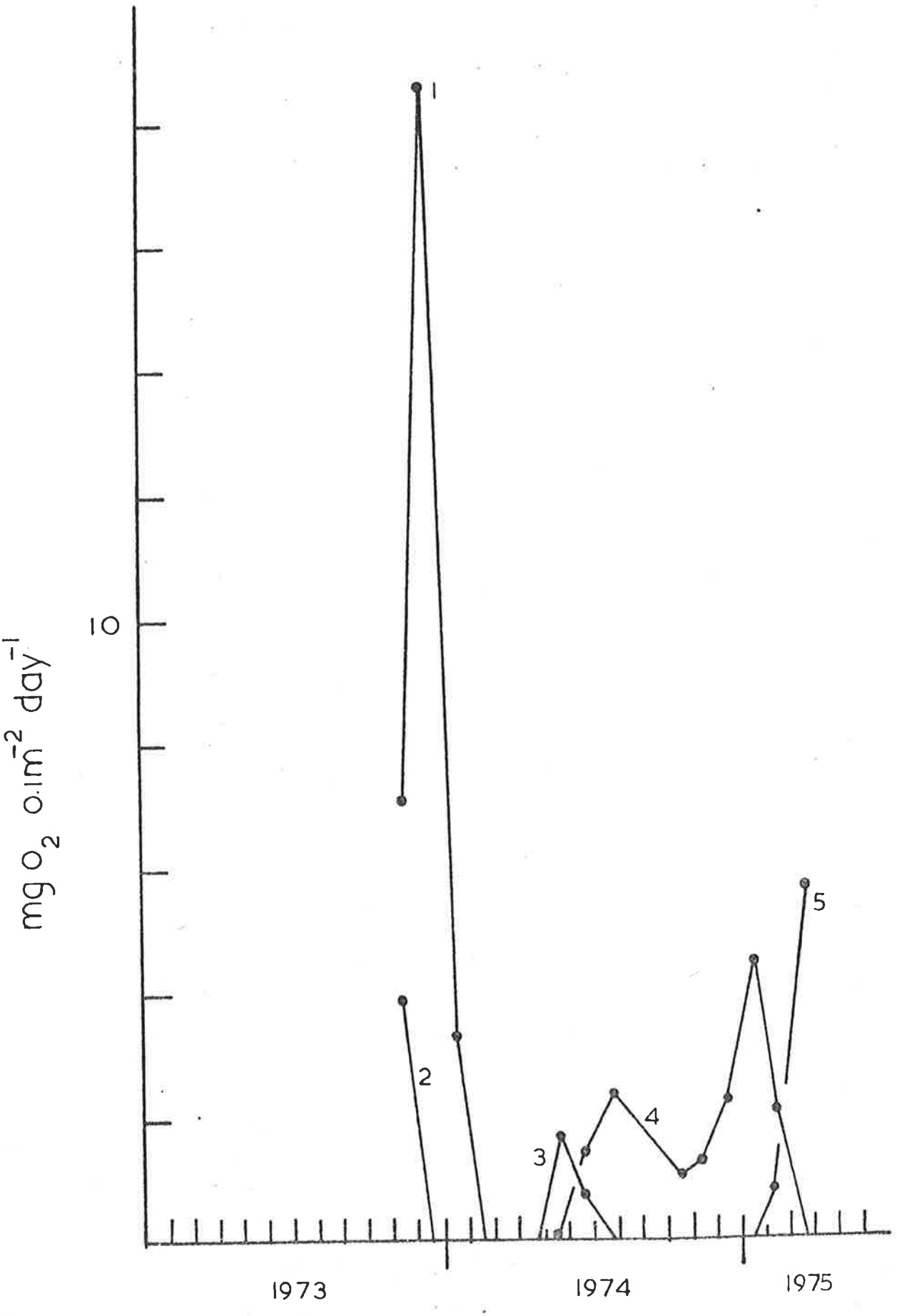

mg $O_2$ 0.1$m^{-2}$ $day^{-1}$
10
1
2
3
4
5
1973
1974
1975

TABLE 14

Total respiration of the various generations in Pink and Cundare

| generation | mg $O_2$ 0.1 $m^{-2}$ | |
|---|---|---|
| **PINK** | | |
| 1. Overwintering generation: Nov. 1973 - Apr. 1974 | 5209.7 | |
| 2. Early summer generation: Dec. 1973 - Mar. 1974 | 27.8 | |
| 3. Autumn generation: Apr. 1974 - Sep. 1974 | 4158.0 | Total = 18372.9 mg $O_2$ 0.1 $m^{-2}$ 2 $years^{-1}$ |
| 4. Overwintering generation: June 1974 - June 1975 | 7701.2 | = 183729.0 mg $O_2$ $m^{-2}$ 2 $years^{-1}$ |
| 5. Mid-summer generation: Oct. 1974 - Sept. 1975 | 906.0 | or 91864.5 mg $O_2$ $m^{-2}$ $year^{-1}$ |
| 6. Mid-winter generation: July 1975 - Nov. 1975 | 336.5 | |
| 7. Spring generation: Sept. 1975 - Nov. 1975 | 33.7 | |
| **CUNDARE** | | |
| 1. Mid-winter generation: Nov. 1973 - Feb. 1974 | 812.8 | |
| 2. Remains of overwintering generation: Nov. 1973 - Dec. 1973 | 71.2 | Total = 1649.0 mg $O_2$ 0.1 $m^{-2}$ 16 $months^{-1}$ |
| 3. Autumn generation: May 1974 - July 1974 | 60.8 | = 16490.0 mg $O_2$ $m^{-2}$ 16 $months^{-1}$ |
| 4. Overwintering generation: May 1974 - Feb. 1975 | 555.3 | or 12367.5 mg $O_2$ $m^{-2}$ $year^{-1}$ |
| 5. Late summer generation: Jan. 1975 - Mar. 1975 | 148.8 | |

by summing data from this and the last chapter. This will be postponed until Chapter 8 when all the data is converted to energy units. Here I will only discuss the possible ecological significance of some of respiratory characteristics of this animal.

The respiratory rate of individual P. zietziana is the same order of magnitude as that predicted from Zeuthen's (1970) plot of log respiration against log body weight for poikilotherms. The range in values is very similar to that found by Gilchrist (1956, 1959) for A. salina: 0.9 to 4.0 mg $O_2$ $hr^{-1}$ $individual^{-1}$ for a change in dry weight from 0.05 to 0.4 mg at 25°C and 35 or 140‰. Over the same change in weight at 25°C and 140‰ the respiration of P. zietziana rises from 0.9 to 4.6 mg $O_2$ $hr^{-1}$ $individual^{-1}$; at 35‰ the range is only 0.6 to 2.8.

Gilchrist (1954) did not claim that A. salina was a respiratory regulator, but she did show that oxygen consumption decreased with oxygen concentration below 2.3 mg $O_2$ $l^{-1}$. This is higher than the critical level of 1.8-1.9 mg $O_2$ $l^{-1}$ found for P. zietziana, but Gilchrist was unable to monitor oxygen decline continuously and had to rely on a series of short incubations from various initial oxygen levels. Mitchell (1975) studied the respiration of both brine shrimps and by following oxygen decline with an $O_2$-electrode, as I did, showed that P. zietziana regulated its consumption down to 1.7-1.9 mg $O_2$ $l^{-1}$ and A. salina to 1.1 mg $O_2$ $l^{-1}$ at 20°C and at salinities between 75 and 260‰. The critical level for A. salina increased to about 1.9 mg $O_2$ $l^{-1}$ when their haemoglobin was removed. According to Geddes (1975 a) P. zietziana does not contain haemoglobin.

Neither Mitchell nor I (fig. 15) were able to show any significant variation in the critical level for P. zietziana with salinity, temperature or mean dry weight of an individual. By keeping the critical level constant at a low oxygen concentration P. zietziana maintains a steady supply of energy, an appropriate adaptation when oxygen levels fall due to rising

salinity or temperature. The lowest oxygen concentration recorded in either lake during this study was 2.2 mg $O_2$ $l^{-1}$ (27°C, 240‰) in Pink Lake when there were very few shrimp. Lower oxygen levels can only be reached in well mixed lakes by increasing the salinity beyond 240‰. This will kill the shrimp through salinity stress before oxygen shortage occurs (Geddes 1975 a). Thus oxygen concentrations lower than the critical level are not likely to affect the populations studied.

There is no evidence to suggest P. zietziana can respire anaerobically. As indicated, shrimp in the experimental bottles died within half an hour of the oxygen being fixed. When using the oxygen electrode the shrimp died at very low oxygen tensions. In general, acclimation to low oxygen concentrations favours regulation of consumption down to low concentrations in aquatic invertebrates (Prosser and Brown, 1961).

The regression coefficient of the independent variable weight has often been determined. It should lie between 1.00 (respiration proportional to weight) and 0.66 (respiration proportional to surface area). Zeuthen (1970) quotes a value of 0.80 for crustacea, which is not significantly different from my value of 0.76. Zeuthen points out that this value can change with the developmental stage of the animal and indeed Eliassen (1952) showed that A. salina has a value of 1.00 between body sizes containing 1 to 10 g N (about 0.01 to 0.1 mg dry weight), but a value of 0.75 below this range and 0.60 above it. Gilchrist (1956, 1959) over a size range of 0.05 to 0.4 mg dry weight found a coefficient of 0.66 for females at salinities of 35 and 140‰ and males at 140‰ but a significantly higher figure of 0.88 for males at 35‰. She showed that this was due to the more rapid increase in area of the second antennae of the males at 35‰ compared with 140‰. Similar variations may occur in the coefficient for P. zietziana, but by only fitting one line to the data, these are obscured. As already mentioned, this error and any error in basing my coefficient on the mean weight of the range of sizes in each incubation were insignificant in calculating population respiratory losses.

The same applies to the value for $Q_{10}$ of 1.62. The regression, which covered long term rather than short term changes, obscured any variations in this value with temperature regime or body size. Despite this because it is lower than $Q_{10}$s of 2.2 to 3.5 from Krogh's widely quoted curve for temperature correction of respiration (Winberg, 1971), it suggests that P. zietziana acclimates to gradually rising temperatures. This is obviously adaptative in maintaining a more steady respiration rate over the wide diurnal fluctuations in temperature sometimes encountered, (8°C or more in summer). The lack of any diurnal endogenous rhythm affecting respiration of P. zietziana indicates there were no respiratory or feeding cycles and that in the short term e.g. the duration of one of my visits, metabolic rate of the population could be considered constant.

Of the independent variables in the regression, variations in weight, because they contribute most to the explained sum of squares, cause the greatest change in respiratory rate. Salinity variations have the least effect. The same rate of increase with salinity is found in both lakes, although the average salinity is lower in Cundare. This rate of increase is also the same as that found by Kuenen (1939) when he compared respiratory rates of A. salina cultured at 58‰ with those twenty four hours after transferring the shrimp to 29‰ and 116‰. Styczynska-Jurewicz (1970) proposed that metabolic changes due to salinity variation are caused by physiological adaptation and disappear when this ends. This does not seem to apply in my case because significant increase in respiration occurred with only a slow rise in salinity. Such a slow rise presumably requires little energy for physiological adaptation. The simplest explanation is, of course, that more energy is needed for osmo-regulation as salinity increases. Gilchrist (1956) was unable to show an increase for A. salina, obtaining the same respiratory rate at 35‰ and 140‰. Perhaps this discrepancy

results from my measurements being taken on a wild population subject to varying salinity whereas Gilchrist's were made on cultured populations at constant salinity whose nutritional history and thus metabolic rate can be expected to be significantly different (Blazka, 1966). The regression, naturally, does not prove that salinity causes the change in respiration.

I showed at the end of the last chapter that it was unlikely that P. zietziana could obtain all its energy from primary production alone. Assimilation rates (to be given in the final chapter) prove this because they exceed primary production by about three times. Therefore the shrimp must depend on organic matter, e.g. bacteria, in the sediments for most of their energy. During the incubations the shrimp would have been able to ingest any phytoplankton or suspended mud particles enclosed in the bottles. Although the amounts of these appeared small, it is unlikely that food shortage had a significant effect during short incubations. The guts of the shrimp usually remained full of mud suggesting that the energy of digestion was included.

Decrease of respiration rate at high food concentration, as shown by Kersting (1973) for Daphnia, would not apply to these experiments because of the low particle concentration in the bottles. It could occur when P. zietziana feeds on the sediment surface of the lake. However, observation indicated no obvious decline in the rate at which shrimp on the bottom beat their legs.

# CHAPTER 6 - Energy content of lake sediments

## Introduction

To complete an energy budget for P. zietziana measurements are needed of its rate of energy intake. Sediments rather than phytoplankton provide most of this input (chapter 5). Therefore energy intake can be calculated from the dry weight of mud eaten per unit time and its energy or caloric content. This chapter deals with caloric content.

The organic composition of sediments of Australian saline lakes is poorly known. The only study is that of Timms (1976) who measured the organic content of sediments from various depths in lakes Gnotuk (salinity 59‰) and Bullenmerri (8‰) (approximately 35 km W of Colac), including estimates of percentage carbon and nitrogen. Nothing is known about the halophilic bacteria and other micro-organisms present in the organic fraction.

North American studies of particular note are those of Eardley (1938) and Bennett (1962). Eardley's work is especially relevant because it concerns the sediments of the Great Salt Lake, Utah, where A. salina is abundant. He distinguished three types of sediment: clays, oolites and calcareous algal sediments. Clays were commonest and ranged from fine black sulphurous clay to sandy clay loam, 45% of their dry weight being carbonate. Bacteria were found in all clays and Protozoa in a few. The black sulphurous surface clay had a higher organic content than underlying clay and generally had the highest content of all sediments. Artemiid faecal pellets were common. Eardley estimated that they constituted 31% of the total number of sediment particles and found they had the same organic content as the clays, but a higher carbonate content of 77%. He concluded that A. salina ingested much sediment and that faeces were a major source of sediment. This was contrary to earlier

suggestions that A. salina fed only on algae.

The sediments in Pink and Cundare are clays (according to Eardley's classification) more or less homogeneously distributed; oolites have not been noted and calcareous algal deposits are absent. Faecal pellets from P. zietziana are common and measure ~0.1 by 0.3-1.0 mm. The blackest and loosest sediments occur in Pink, those in Cundare are firmer and lighter in colour.

Methods

Mud samples were taken monthly from June to November 1975 in Pink Lake directly from the top 3 cm of the sediments by drawing a glass jar along the surface; only surface mud is available to the shrimp. Vertical variation in caloric content is not dealt with although this probably occurs (cf. Eardley, 1938). Generally, samples were taken away from the shore in places where there was no undecayed or partially decayed organic detritus overlying the sediments.

All the samples, except those taken in October and November, were washed with freshwater (x4) within a few hours of collection. They were then stored over silica gel until they were dried (within a week of collection) at 105°C for at least twenty four hours. Dried samples were ground to a powder and stored in desiccators for up to five months before analysis.

The caloric content of 200-300 mg from each sample was measured with the wet oxidation technique of Hughes (1970). This avoids endothermic decomposition of carbonate which occurs in bomb calorimetry (Paine 1964). Control samples (500 mg) of organic free sediment account for any interference by inorganic material. Caloric content is calculated by multiplying the difference between the titres of the control and experimental samples by the calories released when oxygen is used to burn organic matter, i.e. 3.38 cal $mg^{-1}$ $O_2$.

Organic free sediment and estimates of percent inorganic matter were obtained by burning 0.5 to 2.0 g of dry, washed or unwashed, sediment at 550°C in a muffle furnace for two to three hours. At this temperature some carbonate in addition to organic matter also decomposes. This was compensated for by decreasing weight losses by 3.7%, a value derived by comparing the percentage loss of paired samples, one of which had been treated with 2 N sulphuric acid to drive off the carbonate. Water of hydration, lost at 550°C, was reintroduced after an ignition by rewetting the samples and drying at 105°C for twenty four hours.

It was also necessary to account for organic material leached while washing the sediment. The extent of this was evaluated by comparing the caloric value of five samples (5 ml each) from washed and five from unwashed mud. To determine whether any organic material was lost from a sediment sample before it was dried e.g. by bacterial respiration, the caloric values of two halves of a sample were measured, one dried immediately after collection at 80°-90° for eighteen hours, the other stored over silica gel.

The caloric value of P. zietziana faeces, collected during experiments described in the next chapter, was also measured as were a few mud samples taken in Cundare during November 1975. Finally the organic nitrogen of samples taken in Pink from June to October was determined with the Kjeldahl method of Major, Dal Pont, Klye and Newell (1972).

Results

Table 15 gives estimates of the percentage inorganic material in samples. These are essential for correcting the titre of the control flask in the wet oxidation to that due to the smaller weight of inorganic material in the experimental flask.

There was no difference between the caloric content of the two halves of the mud sample, one dried immediately (117.8 cal $g^{-1}$) and the other dried later (118.8 cal $g^{-1}$), and thus no appreciable loss of organic

TABLE 15

The percentage of inorganic material in sediment samples from Pink Lake. The months of collection are in brackets. Faecal samples included some from Lake Cundare. The mean percentages were calculated from angular transformation of the original percentages

| | No. of samples burnt | mean percentage and range |
|---|---|---|
| Washed mud (June to October 1975) | 46 | 90.3 (88.4-91.8) |
| Unwashed mud (October and November 1975) | 13 | 88.2 (86.5-89.5) |
| Faeces (November 1974-January 1975; June-August 1975) | 16 | 92.6 (90.5-94.5) |

matter from samples before analysis. There was, however, a significant leaching of organics when washing the samples (Table 16). In both experiments to test this, an average of 26.4% of the organic matter in the unwashed sample was leached by washing. There was little variation between the experiments, although washing was apparently more effective in October because there is a significant difference ($0.05 \geq p \geq 0.02$) between the mean dry weights of the washed aliquots. These results indicate that soluble organic material in the sediment (the most readily leached) does not form a major fraction of total calores.

According to Hughes (1970) only 80% of the proteins are oxidised by this wet oxidation. Thus 7.1 cal $mg^{-1}$ Kjeldahl nitrogen should be added to wet oxidation values assuming that protein has a caloric value of 5.65 cal $mg^{-1}$ and that 16% of protein exists as Kjeldahl nitrogen. The concentration of nitrogen per sample and the corrections applied are given in Table 17; only washed samples were analysed. As the percentage of the total calories in a mud sample represented by the correction is fairly stable the mean value of 7.5% was used to correct the caloric value of those samples, washed or unwashed, whose nitrogen content was not measured. All corrections for unoxidised protein were applied before accounting for leaching.

The final corrected caloric values for washed mud are graphed in Fig. 19. They are shown as logarithms to emphasise relative changes in the concentration of organic matter, assuming this is proportional to the density of micro-organisms. The presence of bacteria was confirmed by culture of inocula from wet sediment samples. Some of the samples taken in October and November were unwashed, and thus needed correction for their larger inorganic content. This was calculated by subtracting from the weight of unwashed samples (Table 16) the weight of organic material, estimated using the mean caloric value for aquatic detritus: 5.17 cal $mg^{-1}$ ash-free dry weight. (Cummins and Wuycheck, 1971).

TABLE 16

The amount of organic material leached in two experiments to determine the effect of washing sediment samples with fresh water. The caloric content of the washed sediments used in the leaching experiment in October was not measured; the value used is that for small (250 l) washed mud samples taken during the same visit. All caloric contents have been corrected for unoxidised protein (see text).

| leaching experiment | | mean dry weight (mg) of 5 ml aliquots of wet sample | caloric content in calories $g^{-1}$ dry weight of sediment | total calories in 5 ml aliquot | % leached[b] |
|---|---|---|---|---|---|
| October 1975 | washed mud | 1420.10[a] | 174.8 | 248.2 | 27.2 |
| | unwashed mud | 1968.74 | 173.3 | 341.1 | |
| November 1975 | washed mud | 1620.83[a] | 163.4 | 264.8 | 25.5 |
| | unwashed mud | 1944.55 | 182.9 | 355.7 | |

[a] mean weight of washed mud significantly different ($p<0.001$) from that of unwashed mud

[b] average percent leached = 26.4

TABLE 17

Nitrogen contents of washed mud samples and the corrections applied to wet oxidation values for the 20% unoxidised protein. The mean percentage of the total calories in a mud sample represented by the corrections was calculated from angular transformation.

| date of sample collection | mg N $g^{-1}$ dry sediment (a)[c] | corrections applied (calories) (a) x 7.1 cal $mg^{-1}$ Kjeldahl nitrogen | calories from corrections as % of total calories in mud | |
|---|---|---|---|---|
| 1.6.75 | 2.5 | 17.6 | 7.7 | |
| 22.6.75 | 2.8 | 20.0 | 8.1 | |
| 21.7.75 | 1.7 | 12.1 | 6.0 | mean % = 7.5 $\pm$ 0.65 (95% confidence limits) |
| 18.8.75 | [b] 1.6 | 11.0 | 8.5 | |
| | 1.4 | 10.1 | 7.9 | |
| 13.9.75 | 0.9 | 6.1 | 6.8 | |
| 11.10.75 | [b] 2.0 | 14.1 | 7.5 | |
| | 1.8 | 12.1 | 7.8 | |

[b] analyses from two different samples in October; analyses from one sample in August

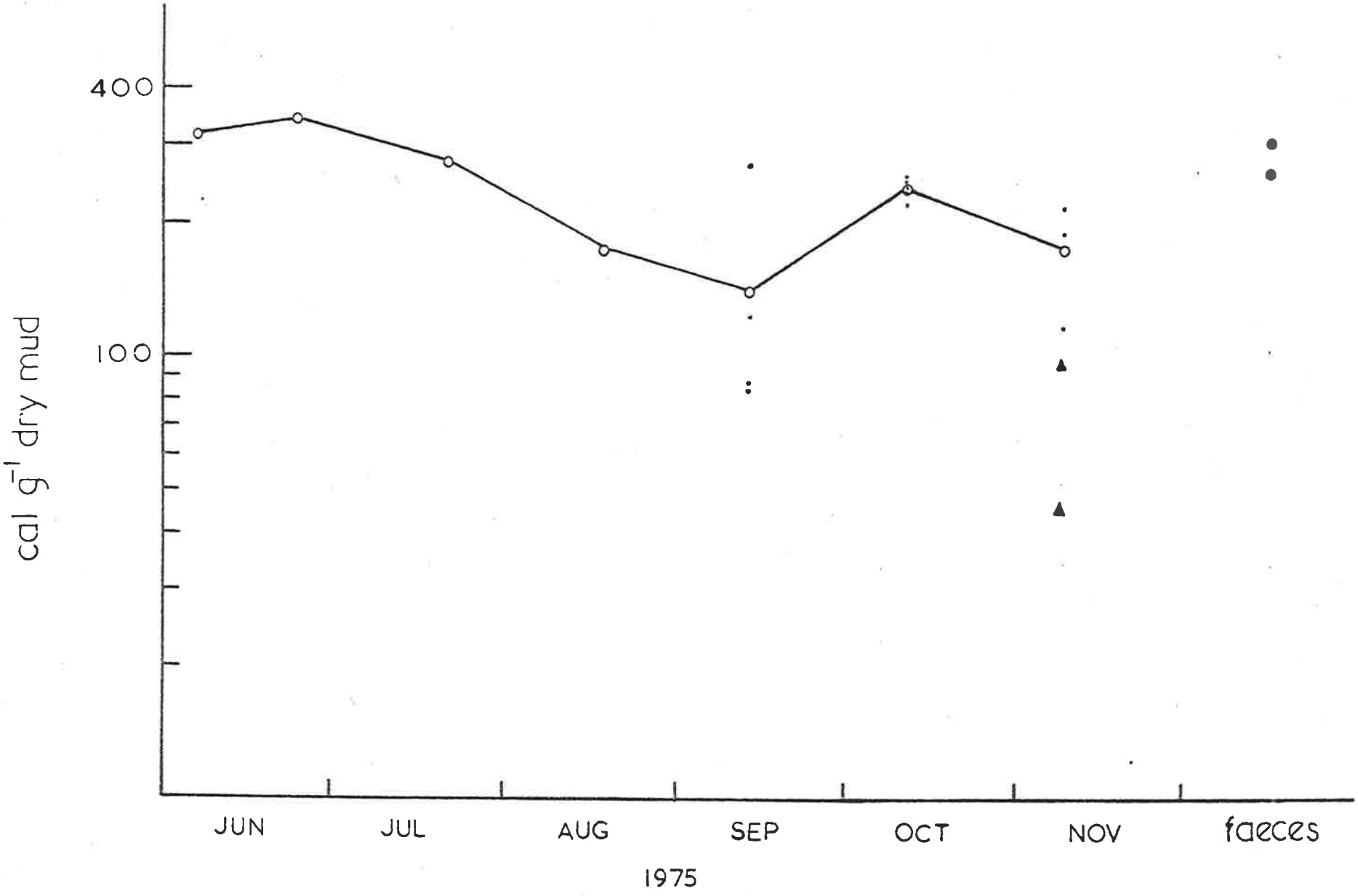
[c] mean = 1.8 mg N $g^{-1}$ dry washed mud (95% confidence limits: $\pm$ 0.5)

FIGURE 19

Monthly variation of the mean ( o ) caloric value of washed mud from Pink Lake. The first sample in June and those in October and November were taken off the west shore, the others off the east shore. Small dots represent individual samples

Δ - samples from Lake Cundare taken off the north and south shores, the higher value for the south sample.

● - P. zietziana faeces


400
100
cal g⁻¹ dry mud
JUN
JUL
AUG
SEP
OCT
NOV
faeces
1975

Included in Fig. 19 are some values for mud from Lake Cundare and faeces from both lakes. Only two samples of faeces were oxidised, one collected during the September visit to Pink, the other composed of samples collected in Pink and Cundare between November 1974 and January 1975 and in Pink between June and August 1975.

Discussion

Generally the caloric value of sediment from Pink Lake was stable; the fluctuations probably represent spatial heterogeneity. There were no significant differences between the means of samples from the last three months of collection tested with Sokal and Rohlf's (1969) modified t-test for use with unequal variances. The mean value of all samples is 211.1 $\pm$ 40.4 (95% confidence limits) cal $g^{-1}$ dry washed mud. The caloric value of mud from Cundare was less than this while that of the faeces was somewhat higher. Marine sediments analysed by Hughes (1970) contained about 64 cal $g^{-1}$ dry sediment; mud from Lake Ontario (Toronto harbour) contained 220-248 cal $g^{-1}$ (Brinkhurst, Chua, Kaushik, 1972).

The mean dry weight of organic matter in a sample can be estimated by converting the average caloric content to milligrams using, as before, the factor 5.17 cal $mg^{-1}$ dry weight. This gives 40.8 mg of organic matter per gram dry weight of washed mud, i.e. 4.1% organic matter, which reduces to 3.3% for unwashed mud because of the increase in inorganic content (Table 16). The average nitrogen content (Table 17) is equivalent to 0.18% of the dry washed sediment. Assuming that this is derived mainly from bacterial protein as Newell (1965) showed for marine sediment, and that nitrogen represents 15% of the dry weight of a bacterium (Altman and Dittmer, 1974) then there are 12.0 mg bacteria $g^{-1}$ dry mud constituting 29% of the total organics. The rest is possibly non-living organic material.

The organic content of the untreated mud is comparable to the levels of 0.5 to 2.2% Eardley (1938) found for the clay sediments in the Great Salt Lake, Utah and to those of 2 to 7% Bennett (1962) recorded from various saline lakes in Washington State. Timms' (1976) values for the muds of lakes Gnotuk and Bullenmerri, 24.1 and 20.1% respectively, are much higher as is his mean nitrogen content of 0.75%. In Rybak's (1969) attempt to classify the degree of eutrophy of Polish lakes according to the organic content of their muds, those with values of 11.2% to 20.0% were considered oligotrophic.

Usually the value of percentage organic matter is estimated directly from the weight loss of samples ignited in a muffle furnace. However, the values (Table 15) found by this method, 9.7 and 11.8% for washed and unwashed mud respectively, are higher than those derived from the wet oxidation measurements. The discrepancy perhaps arises from incomplete reconstitution of the water of hydration (Buckney, personal communication) after ignition or more decomposition of carbonate than estimated in tests of this. The latter is less likely because of the constant amount of decomposition from this source (see above). However, even if the error is in underestimating the percentage of inorganic material, there would have been no significant effect on calculations of caloric content; if 100% inorganic content had been assumed, the interference in the titre would have been small compared with the titre due to the oxidation of the organic fraction.

Because of the above difficulties, percentage organic matter is more reliably estimated from the wet oxidation. The only disadvantage with this method is that it oxidises all carbohydrates and fats, but only 80% of the proteins (Hughes, 1970). He found that the caloric value for bovine albumin (4.8 kcal $g^{-1}$) obtained by wet oxidation was lower than that (5.8 kcal $g^{-1}$) from standard bomb-calorimetry. However, Kersting (1972) has pointed out that the end products of protein oxidation in a

bomb-calorimeter are different from those of animal metabolism and, possibly, from those in a wet oxidation. He showed that $N_2$ was the main end product in a bomb whereas in aquatic invertebrates, at least, it was $NH_3$ and that a correction of -5.9 cal $mg^{-1}$ had to be applied for this non-biological oxidation of $NH_3$ to $N_2$. If the end product of wet oxidation of protein is also $NH_3$, a possibility because the oxidation procedure is similar to that of Kjeldahl digestion which converts proteins to ammonia, then applying Kersting's correction lowers Hughes' value of 5.8 kcal $g^{-1}$ by bomb-calorimetry to 4.9 kcal $g^{-1}$. This is very close to his value from wet-oxidation. Nevertheless, as the end products of this oxidation are still not clear, his correction has been retained; it was only an average increase of 8% for the samples.

Finally, it is significant that the caloric value of the faeces is about 36% higher than the mean value for the mud. It suggests that P. zietziana feeds on the sediments by selectively ingesting particles of high organic content. Brinkhurst, Chua and Kaushik (1972) showed the same for three benthic tubificid Oligochaetes from Toronto harbour. In their case the caloric value of the faeces was 25% higher than that of the mud, the Oligochaetes' only source of energy.

# CHAPTER 7 - Ingestion and egestion by *P. zietziana*

## Introduction

With data on sediment caloric values it is now possible to calculate the energy input to the population of *P. zietziana* from estimates of their rate of mud ingestion. This rate was measured directly in the field, again, to avoid extrapolation from laboratory data. Temperature, and body size will affect ingestion rate; for filter feeding animals food concentration and its size and chemical composition are also major influences (Haney, 1971). *P. zietziana* is a filter feeder, but when ingesting sediments rather than suspended particles food concentration is unlikely to be limiting.

Numerous methods have been used to measure ingestion rates and assimilation efficiencies (Klekowski and Duncan, 1975). I chose one in which food, in this case sediment, labelled with an isotope ($^{14}C$) is fed to the animals, and uptake monitored until significant loss of the isotope occurs through respiration or egestion. Rigler (1971) and Haney (1971) used a similar method for estimating the filtering rate of zooplankton feeding on algae. Also, I measured the rate of faecal pellet production by the shrimp in the field in order to calculate percentage assimilation of the ingested sediments and to check rate of assimilation obtained by summing data on metabolic and production rates. Conover's (1966) method of estimating percentage assimilation from differences in the percentage organic matter of the food and faeces could not be used because it assumes the animal ingests without selection; this was not so as shown in the last chapter.

Investigation of the feeding dynamics of brine shrimp (*A. salina*) has so far been confined to the laboratory (Reeve 1963a,b,c,d and Mason, 1963). The shrimp was considered a filter feeder of algae although

Eardley (1938) claimed a wild population in the Great Salt Lake, Utah, ingested sediments. Reeve determined the effect of algal concentration on the ingestion and filtration rates of the shrimp. He found, as have most others, a constant ingestion rate but decreasing filtration rate above a critical concentration and vice versa below. He also studied their production of faecal pellets in culture. Both Reeve and Mason were particularly interested in the efficiency with which food was converted to shrimp biomass and the effects on this of variations in temperature, salinity and food concentration. Mason made some estimates of this efficiency by feeding $^{14}C$ labelled algae and measuring the amount incorporated into the biomass of the shrimp, but usually both authors depended on measurements of the weight of algae consumed and the subsequent gain in weight of the shrimp.

Few estimates of the ingestion rates of aquatic invertebrates have been made directly in the field. Daborn (1975) in his study of the energetics of the large predatory anostracan B. gigas estimated feeding rate by counting the number of prey eaten in bottles submerged for twenty four hours. Another study employing field measurements is Haney's (1973) examination of the grazing of a zooplankton community. He designed a feeding chamber that first sampled the zooplankton and then released known amounts of bacteria labelled with $^{32}P$. By measuring body burden of isotope after a short incubation filtering rates of the community could be calculated and thus the percentage of the suspension in the lake that was filtered.

No one has estimated rates of ingestion of deposit feeders in the wild. However, Hargrave (1970) made a thorough laboratory study of the ingestion rates and assimilation efficiencies of the amphipod Hyalella azteca feeding on sediment. He seeded sterilised mud samples with various radioactive bacteria or algae (isolated originally from natural

sediment) and after measuring their uptake, showed that their assimilation was in general 50%. However, he found that the total organic matter in natural sediment was assimilated with an efficiency of only 7-15% (using Conover's method) and that non-living organic matter such as lignin or cellulose was not digested at all. He concluded that only a small fraction of the total organic material in the sediment was available for digestion. The sediments of my lakes have a much lower organic content than his (which were about 50% organic) and probably contain few algae or photosynthesising organisms because of the high turbidity.

Methods

(a) Isotope experiments

The ingestion rate of P. zietziana was measured six times in Pink lake between June and November 1975. On the evening of the day before an experiment a mud sample was taken (as described in the last chapter) and returned to the field station immediately. Any supernatant was decanted and after thoroughly mixing the sample with a glass rod 50-90 ml of wet mud were placed in a one litre beaker. To this approximately 5$\mu$Ci of D-$[U\text{-}^{14}C]$ glucose (284 mCi $mM^{-1}$, Radiochemical Centre, Amersham, U.K.) were added and well mixed into the mud. Fleischer (1975) and Wood and Chua (1973) showed that $^{14}C$-glucose was rapidly taken up by micro-organisms in sediment. The mud was incubated overnight for eleven to eighteen hours at room temperature (8-12°C). The next day, at the lake, approximately 100-200 ml of filtered (particles $<200\mu$) lake water collected immediately before, were introduced, and the contents of the beaker stirred and left to settle (5 minutes).

During this interval P. zietziana were sampled with a 200 zooplankton net. One hundred and fifty to two hundred shrimp were used in each experiment. These were placed in the beaker after removing those damaged. Their activity appeared unaltered by the handling which

took about five minutes. A diver's lead weight (1.5 kg) taped to the bottom of the beaker enabled it to sink; it was placed in shallow water at the edge of the lake, inside the 0.1 $m^2$ sampler (see chapter 3) to prevent disturbance. Water temperature was recorded.

The start of the experiment was considered to be the moment the shrimp were added. Just before this three 250 $\mu$l samples were taken, two of the sediment and one of the supernatant. An automatic pipette with removable tips was used. The orifice of the tips used for sampling the sediment had been widened to prevent clogging by removing the narrow end. Of the two sediment samples one was pipetted into a scintillation vial containing 10 ml of a scintillation cocktail, "Instagel" (Packard Instrument Co.), that absorbs large amounts of water; the other was placed in a glass vial and sealed. The modified pipette tip probably did not dispense 250$\mu$l, but it should have dispensed equal amounts of sediment into the two vials. The supernatant sample was also pipetted into 10 ml of "Instagel". By taking this set of three samples every hour for the three hours of the experiment any changes in the specific activity of the sediments or the supernatant could be determined. At the end of most experiments an additional six pairs of sediment samples were taken to check whether the variability in the previous four could be repeated.

For the first hour of the experiment the shrimp were sampled every fifteen minutes and thereafter usually every half an hour, but sometimes this varied. On each occasion usually more than fifteen shrimp were caught except towards the end of an experiment when sometimes numbers ran low. The shrimp were sampled using the top one third of a small plastic bottle (diameter 4 cm) whose screw cap had a hole with a disc of zooplankton mesh (200$\mu$) inserted. This was an efficient device for the confined space of the beaker. Shrimp were rinsed with lake water and sorted into arbitrary size classes (same as those in Chapter 4) by comparing

them with the relevant lengths incised on a strip of plastic, then counted and placed in scintillation vials containing 1-2 ml of "Soluene" (Packard Instrument Co.), a tissue digester. Mean individual length of shrimp in each sample was subsequently calculated and converted to weight.

At the field station, the sediment samples in ordinary vials were washed with fresh water in the same way as were those for caloric measurement (see chapter 6) and stored in desiccators for later drying (at 105°C) and weighing. Samples in scintillation vials were counted within about two weeks on a refrigerated Packard Scintillation Counter. Before the shrimp samples were counted, the digests were homogenised using a glass rod with a flattened tip until only a few specks of exoskeleton remained. The rod was washed with "Dimilune" (Packard Instrument Co.), a scintillation fluid compatible with "Soluene", and the contents of the vials made up to 10 ml. In this way self absorption was avoided. All vials were well shaken before being counted to resuspend any material that had settled. Rapid settling was only a problem with sediment samples, but these were sufficiently heavily labelled to obtain a precise count (error $\leq$5%) in one minute; counting for shorter periods did not alter the c.p.m. Generally all samples were counted to an error $\leq$5%. Any quenching was corrected using a calibration curve relating external standard counts to counting efficiency of internal standards. Unquenched samples had a counting efficiency of 90%. Most of my samples had efficiencies between 60 and 90%; rarely was there an efficiency less than 50%. Background counts were subtracted after measuring them with vials containing 10 mls of either "Dimilune" or "Instagel", for twenty four hours before counting a set of samples.

(b) Faecal pellet production

Provided the lakes were not too rough, the output of faecal pellets was measured on each visit between November 1974 and November 1975 to

either lake. As before, shrimp were sampled with a 200$\mu$ zooplankton net and the catch poured through a disc of coarse mesh (about 1 mm) to separate small ostracods. The remainder was sorted to remove any large ostracods and any dead or damaged shrimp. Then the 0.1 $m^2$ sampler was anchored in the lake by pressing it into the bottom mud as far as possible and a 200$\mu$ zooplankton net fixed to its side in the frame holder provided (see chapter 3) so that the mouth of the net was well above the water, but the bottom half or more was submerged. The net was carefully lowered into position so that water only entered through its sides thus being filtered. About two hundred shrimp were placed in the net (in which they swam as actively as in the lake) and left for twenty four hours. Any faeces produced fell into a collecting jar. At the end of each experiment shrimp were removed and any pellets clinging to the net shaken into the jar. Shrimp and faeces were washed in fresh water at the field station, the shrimp counted and both stored in a desiccator for later drying (the shrimp at 60°C, the faeces at 105°C) and weighing. Any debris falling to the bottom of the jar during the experiment was removed during the washing of the faeces. Such contaminants were few and always obvious. If there was significant mortality, the experiment was discarded.

Results

(a) Isotope experiments

Plots of the uptake of $^{14}C$ against time are shown in Fig. 20. Two regression lines can be calculated for most of the experiments representing two rates of uptake; a high initial rate that lasted approximately one hour followed by a slower or zero rate. In short term feeding experiments such as these it has been shown (Rigler, 1971; Haney, 1971) that the point, at which the initial rate of uptake declines, marks the beginning of defaecation of labelled material by the animal.

FIGURE 20

The uptake of $^{14}C$ by P. zietziana. Regression lines were calculated using all points except the open circles. In Graph 1 the high value for the first sample probably resulted from not rinsing the shrimp. In Graph 2 the points representing the 2 and 3h samples have been omitted; these are 109.6 and 174.1 DPM individual$^{-1}$ respectively. In Graph 5 the low points probably resulted from dead shrimp in the samples. The horizontal line in this graph and the less steep line in Graph 1 were fitted by eye. Equations of the regression lines are:

1 : DPM = 0.3 + 17.6 h; DPM = 14.8 + 5.1 h

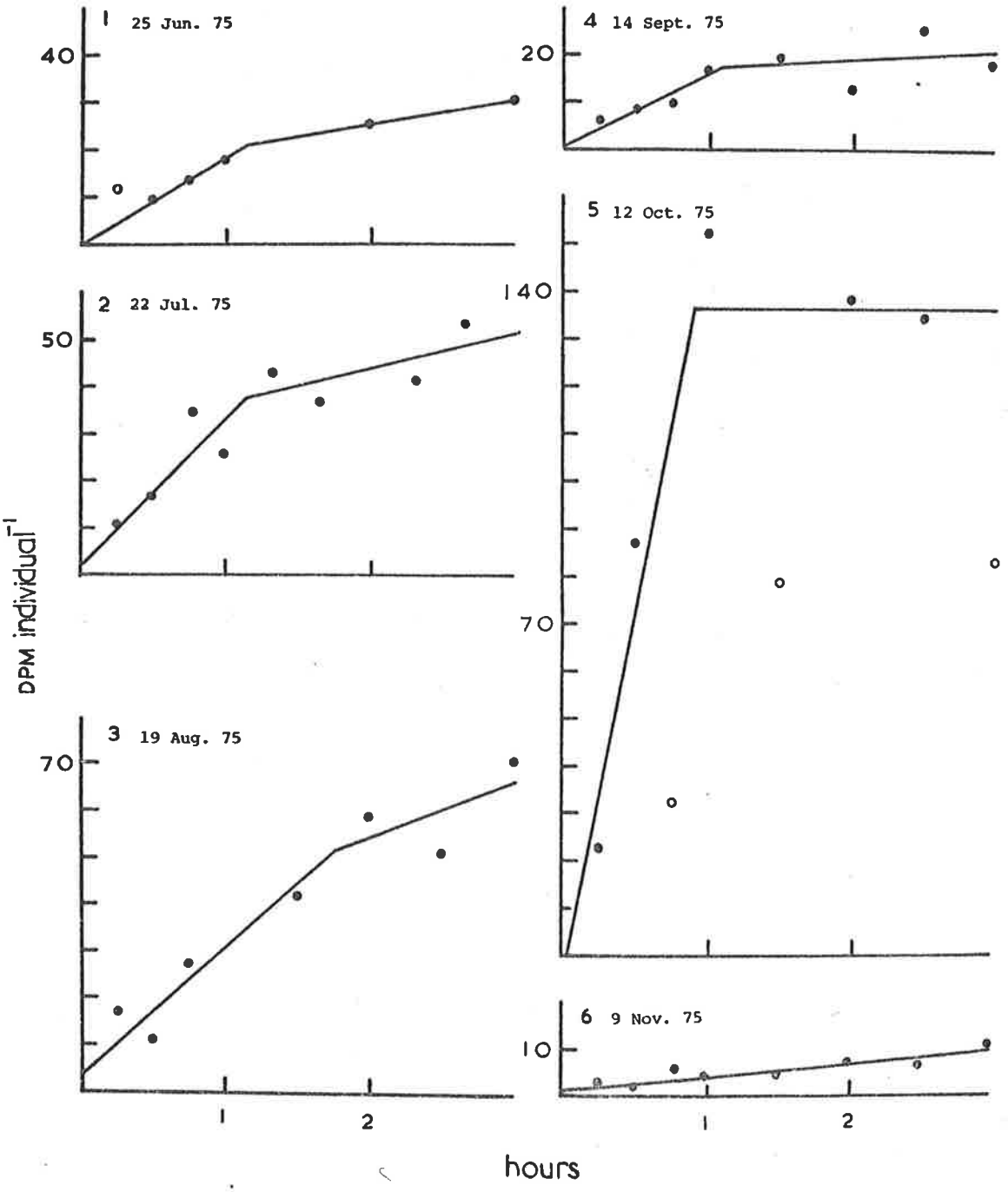
2 : DPM = 2.5 + 30.2 h; DPM = 28.5 + 7.4 h

3 : DPM = 3.4 + 27.4 h; DPM = 30.8 + 11.9 h

4 : DPM = 0.8 + 14.5 h; DPM = 15.0 + 1.6 h

5 : DPM = -3.8 + 158.6 h; DPM = 136.0

6 : DPM = 1.7 + 2.6 h


1 25 Jun. 75
40
2 22 Jul. 75
50
3 19 Aug. 75
70
4 14 Sept. 75
20
5 12 Oct. 75
140
70
6 9 Nov. 75
10
1
2
1
2
DPM individual$^{-1}$
hours

Therefore the high initial rate of uptake represents the rate of ingestion of sediment by the shrimp while the lower subsequent rate probably represents the rate of assimilation. The equations of the lines are given in the legend to Fig. 20 and the conditions under which each experiment was conducted in Table 18. The points in Fig. 20 have not been corrected for variation in mean individual dry weight during each experiment.

Before calculating actual rates of ingestion of the sediment it is essential to show that there is no change in the specific activity of the sediments or supernatant during an experiment and there is no respiration of isotope as $^{14}CO_2$ while the higher rate of uptake prevails. It is possible that P. zietziana has a metabolic pool that turns over incoming energy rapidly, as Lampert (1975) showed for D. pulex.

The first is demonstrated in Fig. 21 by the lack of significant variation in the DPM of mud and supernatant samples taken during each experiment. The DPM in the mud samples are the more variable, but such variation only occurred because it was impossible to sample a constant volume of the sediments with the automatic pipette. This is confirmed by the fact that variation during an experiment was usually repeated in the set of samples taken at the end.

The second of the above two assumptions cannot be demonstrated so directly as the first. However, if there was significant loss of $^{14}CO_2$ from the shrimp through respiration while the higher rate of uptake prevailed then the regression line representing this should not pass through the origin, but should intercept the Y-axis at a positive value. This would indicate that there was at the beginning of the experiment a short period with an even higher rate of intake during which there was no loss of isotope. Table 19 gives the standard errors and significance of the intercept and regression coefficient for this line. In no case

TABLE 18

Conditions during feeding experiments

| experiment | mean dry weight[a] of an individual shrimp in a sample for scintillation counting | total number of individuals sampled for scintillation counting | temperature (°C) | salinity (‰) |
|---|---|---|---|---|
| 1 | 0.79 (±0.10) | 99 | 13 | 140 |
| 2 | 1.19 (±0.22) | 72 | 13 | 135 |
| 3 | 0.66 | 363 | 13 | 120 |
| 4 | 0.76 (±0.14) | 192 | 19 (17-21)[b] | 89 |
| 5 | 2.32 (±0.47) | 106 | 15 | 105 |
| 6 | 0.20 (±0.07) | 159 | 19 (17-21)[b] | 90 |

[a] calculated from length to dry weight regression (Fig. 8, Chapter 4) except for experiment 3 where a large sample of the shrimp to be used was taken before the start of the experiment and dried and weighed

[b] range observed during the 3 hour experiment

FIGURE 21

Variation of the DPM in mud ( ● ) and supernatant ( o ) samples taken during the feeding experiments. At the end of experiments 3 to 6 a set of six or seven additional mud samples were taken.

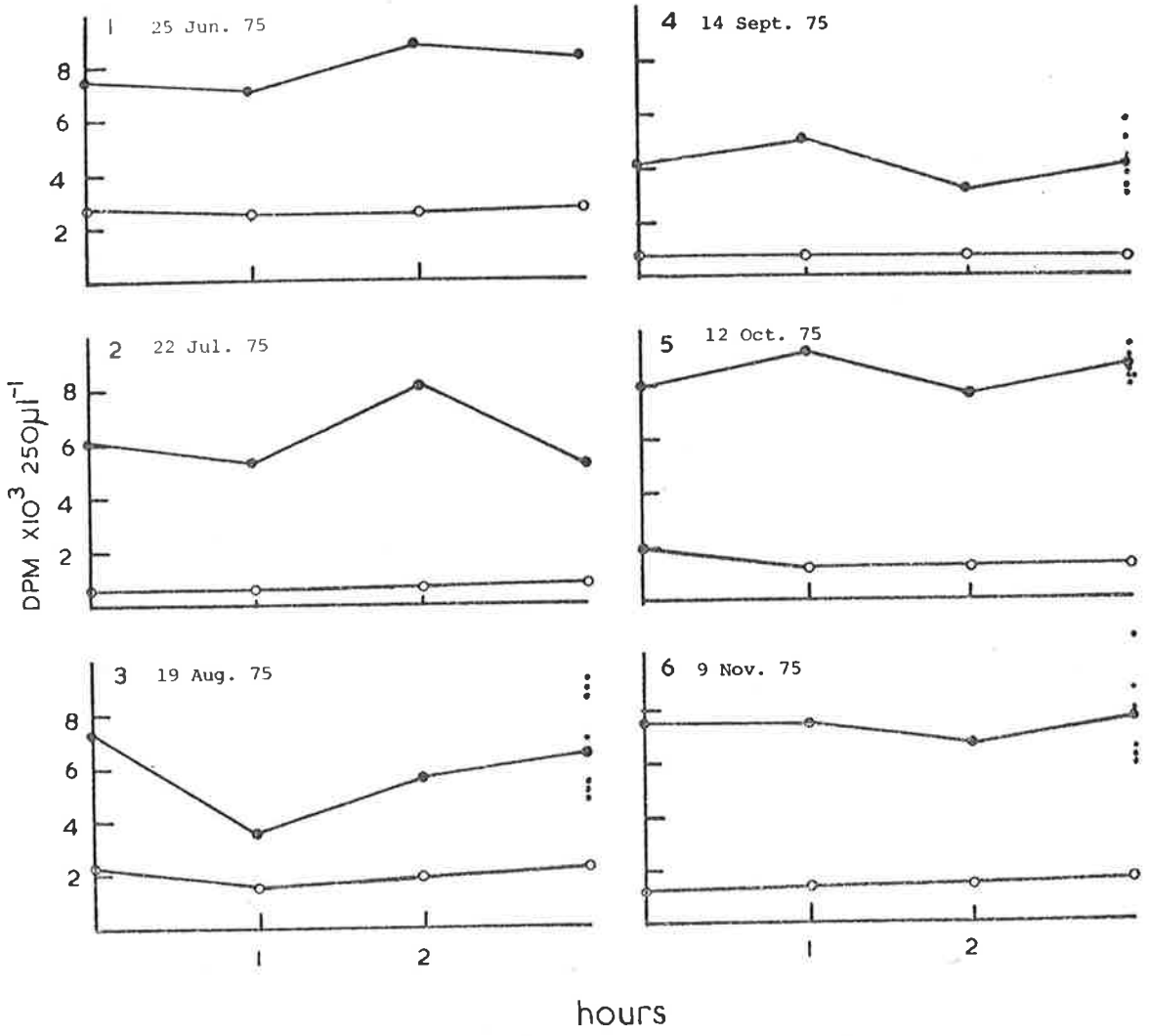

1 25 Jun. 75
2 22 Jul. 75
3 19 Aug. 75
4 14 Sept. 75
5 12 Oct. 75
6 9 Nov. 75
DPM X10$^3$ 250µl$^{-1}$
hours

TABLE 19

Standard errors and significance of regression coefficients and intercepts for regression lines representing the initial rate of uptake of DPM in the feeding experiments

| experiment | standard error of regression coefficient | probability of coefficient equalling zero | proportion of variation explained by regression ($r^2$) | standard error of intercept | significance of difference of intercept from zero |
|---|---|---|---|---|---|
| 1 | 0.60 | $0.01 > p > 0.001$ | 0.998 | 0.41 | n.s. |
| 2 | 5.47 | $0.01 > p > 0.001$ | 0.884 | 4.30 | n.s. |
| 3 | 3.10 | $p < 0.001$ | 0.950 | 3.40 | n.s. |
| 4 | 2.30 | $0.01 > p > 0.001$ | 0.929 | 1.42 | n.s. |
| 5 | 17.00 | $0.02 > p > 0.01$ | 0.978 | 9.70 | n.s. |
| 6 | 0.50 | $0.01 > p > 0.001$ | 0.799 | 0.80 | n.s. |

did the intercept differ significantly from zero thus making it unlikely that there was any short term loss of $^{14}C$. Haney (1971) was also unable to detect this.

The slopes of the lines, i.e. the rate of uptake of DPM, were highly significantly different from zero and the regression usually accounted for at least 90% of the variation. Undoubtedly some of the unexplained variation could be accounted for by error of the scintillation counter, but this is not shown on the graphs (Fig. 20) because it is insignificant compared with the biological variation. Table 20 gives the errors of the regression coefficients for the regression lines representing uptake after defaecation has begun. In no case where an error could be estimated was the coefficient significantly different from zero.

From the data on the dry weights of one member of each of the paired 250$\mu$l sediment samples the specific activity of the sediment could be estimated in mg $DPM^{-1}$. By multiplying the initial rates of $^{14}C$ uptake with these the rate of ingestion of mud is calculated as shown in Table 21. The 95% confidence limits of this rate are also given; again the errors of a product are shown to be dominated by the larger of the two constituent errors. The errors in specific activity are probably less than quoted in Table 21. When paired sediment samples were taken the percentage error in those weighed approximated that in those measured for DPM, indicating that the same variability in the operation of the pipette prevailed for both sets of samples. This is obscured in calculations of the errors in specific activity because the variation within a pair was often as great as that among one set.

Values for the dry weight of mud ingested are only accurate provided there is no selection of specific particles e.g. organic. As shown in the last chapter there is selection because the caloric value of the faeces is higher than the mean value for the sediments. Therefore the values

TABLE 20

Standard errors and significance of regression coefficients for regression lines representing the rate of uptake of DPM in the feeding experiments after defaecation has begun.

| experiment | standard error of regression coefficient | significance of difference of coefficient from zero | proportion of variation explained by regression |
|---|---|---|---|
| 1 | - [a] | - | - |
| 2 | 6.2 | n.s. | 0.420 |
| 3 | 15.6 | n.s. | 0.366 |
| 4 | 3.2 | n.s. | 0.077 |
| 5 | - [a] | n.s. | - |
| 6 | | no defaecation | |

[a] lines fitted by eye

TABLE 21

Rates of sediment ingestion and their 95% confidence limits estimated from initial rates of uptake of DPM and specific activities of the mud. The 95% confidence limits were calculated using the equation in Chapter 4 for estimating the variance of a product.

| experiment | initial rate of uptake (DPM $hr^{-1}$ individual $^{-1}$) (a) | 95% confidence limits of uptake rate (%) | specific activity of sediment (mg dry wt. x $10^{-2}$ $DPM^{-1}$) (b) | 95% confidence limits of specific activity (%) | rate of ingestion of mud (mg dry wt. x $10^{-1}$ $hr^{-1}$ individual$^{-1}$ (a x b) | 95% confidence limits of ingestion rate (%) |
|---|---|---|---|---|---|---|
| 1 | 17.6 | ± 14.8 | 0.54 | - [c] | 0.95 | - [d] |
| 2 | 30.2 | ± 50.3 | 0.75 | - [c] | 2.26 | - [d] |
| 3 | 27.4 | ± 31.8 | 0.52 | ± 58.2 | 1.43 | ± 56.1 |
| 4 | 14.5 | ± 51.0 | 1.63 | ± 39.0 | 2.36 | ± 46.9 |
| 5 | 158.6 | ± 46.1 | 0.82 | ± 16.6 | 13.01 | ± 21.5 |
| 6 | 2.6 | ± 45.0 | 1.32 | ± 22.2 | 0.35 | ± 42.6 |

[c] no estimate because paired samples (250 µl) of mud (one for weighing; one for measuring DPM) not taken

[d] cannot calculate, but probably about 50%

in Table 21 are probably over-estimates and are better considered as weights of mud searched, but not totally ingested by the shrimp.

Accepting that some selection of sediment particles exists the maximum dry weight of material in one full gut can be calculated by assuming that this is represented by the DPM ingested up to the point when the initial uptake rate declines. For the September experiment (graph 4, Fig. 20) this weight equals 0.28 mg which compares favourably with the volume or weight of 0.44-0.78 mg calculated from measurements of the length and width of the average sized shrimp (arbitrary size class 6 to 7) used in this experiment. Values calculated like this will of course be larger than values from the isotope experiments because the latter do not include the volume or weight of fluids. Despite this, because they are of the same order of magnitude, it is reasonable to assume that the point when the rate of the isotope uptake changes does indeed mark the start of defaecation and thus that the estimated rates of sediment ingestion are within the possible range. Only a rough correspondence can be expected when both calculations are subject to large errors.

(b) Faecal pellet production

The results of the experiments measuring faecal output are given in Table 22. On three occasions in Pink Lake (December 1974, late June 1975, November 1975) most or all of the shrimp died overnight in the net and so the accumulated pellets were not collected. Possibly the shrimp in these cases were starved by being separated too long from sediment. However, shrimp must have been able to replace their gut contents with suspended sediment particles during these experiments because they produced a greater weight of pellets in twenty four hours than could be provided by the weight of material in a full gut. Usually their guts appeared as full at the end of an experiment as at the start.

TABLE 22

Faecal pellet production of *P. zietziana* while swimming in the water column

| Month | Lake | faecal ouput mg x $10^{-2}$ dry wt. $hr^{-1}$ $individual^{-1}$ | mean dry weight of an individual (mg) | number of shrimp used | temperature range (°C) | salinity (‰) |
|---|---|---|---|---|---|---|
| Nov. 1974 | Cundare | 3.1 | 0.66 | 436 | 13-19 | 54 |
| Nov. 1974 | Pink | 2.3 | 0.32 | 1147 | 15-17 | 95 |
| Dec. 1974 | Pink | 10.5 | 0.86 | 280 | 15-21 | 102 |
| Jan. 1975 | Cundare | 20.3 | 2.62 | 94 | 15-23 | 89 |
| June 1975 | Pink | 1.1[b] | 0.47 | 123 | 10 | 138 |
| July 1975 | Pink | 3.5 | 1.60[a] | 99 | 10-12 | 135 |
| Aug. 1975 | Pink | 0.8 | 0.18 | 766 | 11-12 | 120 |
| Sep. 1975 | Pink | 5.3 | 0.60 | 524 | 18-19 | 89 |
| Oct. 1975 | Pink | 2.5 | 0.35 | 199 | 13-15 | 105 |

[a] weight too high because shrimp not washed thoroughly in fresh water.

[b] shrimp appeared to be mainly ingesting algae.

Such turnover can be demonstrated for the individuals used in the isotope experiments (Table 23). On average, the weight of pellets each would have produced was ten times their gut weight (calculated as above). These conclusions assume little contribution to faecal pellet production by phytoplankton ingestion; when P. zietziana seemed to be feeding largely on algae (June, 1975; Table 22) the weight of pellets produced was very low for the size of animal used.

Discussion

The accuracy of the rates of energy intake calculated from the feeding experiments depends on uniform incorporation of $^{14}C$-glucose into micro-organisms; these constitute a third of the calories (chapter 6) in the sediment. During the overnight incubations 70-80% of the isotope was lost (Table 24). Only the production of $^{14}CO_2$ from bacterial metabolism of the glucose could account for this. With such turnover it is likely that the remaining 20-30% had been uniformly distributed. Others (Sorokin, 1972; Wood and Chua, 1973; Fleischer, 1975) who have followed the uptake of $^{14}C$-glucose by microbial populations in sediment have also found rapid turnover rates. Fleisher demonstrated with chromatography that within thirty minutes of labelling most of the glucose had disappeared and labelled compounds of high molecular weight, probably polysaccharides, were being produced. It is most unlikely that the observed loss of isotope was caused by non-biological decomposition of the glucose. According to the manufacturers such decomposition occurs at a rate of about 1% a year at $-20^{\circ}C$.

Fleischer's demonstration that high molecular weight organics quickly became labelled is supported by the work of Nicholas and Viswanathan (1975) on the feeding of $^{14}C$ labelled Escherichia coli to the nematode Caenorhabditis briggsae. They found a much higher proportion of $^{14}C$ was incorporated into the biomass C. briggsae when E. coli were

TABLE 23

The estimated weight of a full gut for an individual used in the isotope experiments compared with the weight of faecal pellets it would have produced in twenty four hours isolated in the water column. Faecal output is calculated from the regression equation in Fig. 22 relating it to mean individual weight.

| experiment | weight (mg) of a full gut | weight (mg) of faecal pellets produced in twenty four hours |
|---|---|---|
| 1 | 0.11 | 2.14 |
| 2 | 0.28 | 4.10 |
| 3 | 0.27 | 1.62 |
| 4 | 0.28 | 2.03 |
| 5 | 1.07 | 11.66 |
| 6 | no defaecation | |

TABLE 24

Total amount of isotope in the sediment and supernatant fractions during an isotope experiment and percentage incorporation of amount (5 $\mu$Ci) added. Values are only approximate because the volumes of mud and supernatant could not be read accurately from the markings on a one liter beaker. Also the amount of label in the supernatant may have increased near the sediment surface

| experiment | mud (DPM x $10^6$) | supernatant (DPM x $10^6$) | total (DPM x $10^6$) | incorporation (%) | time of exposure of sediment to label (hrs) |
|---|---|---|---|---|---|
| 1 | 1.57 | 1.04 | 2.61 | 23 | 17.5 |
| 2 | 1.83 | 0.40[a] | 2.23 | 20 | 16.8 |
| 3 | 1.32 | 1.25 | 2.57 | 23 | 17.0[b] |
| 4 | 0.84 | 0.82 | 1.66 | 15 | 16.5 |
| 5 | 2.57 | 0.77 | 3.34 | 30 | 13.0 |
| 6 | 2.25 | 1.04 | 3.29 | 29 | 10.5 |

[a] mud not well stirred before start of experiment

[b] only approximate because starting time not recorded

pre-labelled with $^{14}C$-glucose rather than with $NaH^{14}CO_3$: 37% and 17-22% respectively. This, they suggested, was due to glucose being directly incorporated into structural compounds in the bacteria, e.g. polysaccharides, and subsequently being assimilated by the nematode without being broken down to labile metabolites. Thus $^{14}C$ left in the sediment after metabolism of the glucose was probably incorporated as structural compounds in the bacteria.

There is other organic matter in the sediment possibly non-living. Calculations of rates of energy intake assume that all the organic matter is ingested without selection. The ability of P. zietziana to produce faeces of similar caloric value to the sediment while filtering in the water column confirms this by indicating that they ingest material of more or less the same organic composition wherever they feed. Small discrepancies are probably not detectable considering the width of the confidence limits of the ingestion rate (Table 21). Selection of organic particles of sediment (as opposed to selection of particular organic matter from the range available in the sediment) cannot affect calculations of the rates of energy intake, although it will cause over-estimation of the weight of sediment ingested.

Two further possibilities exist which can invalidate the feeding experiments. First labelled bacteria could have adhered to the exoskeleton of the shrimp. This has not been shown to occur by others (Rigler, 1971; Haney, 1971) performing similar experiments and has thus been ignored; the shrimp were rinsed before being placed in scintillation vials. The second possibility is self absorption when measuring the DPM of the sediment samples. This was most likely negligible compared with the variation in counts between samples.

Rates of ingestion given in Table 21 in terms of mg dry weight can be converted to calories (Table 25) using the values for caloric content of the sediments from chapter 6. Also with values from chapter 6

TABLE 25

The rates of energy intake for P. zietziana ingesting sediment

| experiment | rate of ingestion of mud (mg dry wt. x $10^{-1}$ $hr^{-1}$ $individual^{-1}$) ±95% confidence limits | caloric content of sediments[a] (cal $g^{-1}$ dry mud) | rate of energy intake (cal x $10^{-1}$ $hr^{-1}$ individual) ±95% confidence limits |
|---|---|---|---|
| 1 | 0.95 | 334.6 | 0.32 |
| 2 | 2.26 | 271.9 | 0.62 |
| 3 | 1.43 (± 0.80) | 174.7 | 0.25 (± 0.14) |
| 4 | 2.36 (± 1.11) | 266.4 | 0.63 (± 0.30) |
| 5 | 13.01 (± 2.80) | 237.5 | 3.09 (± 0.67) |
| 6 | 0.35 (± 0.15) | 192.8 | 0.07 (± 0.03) |

[a] values for first three experiments are from mud samples collected near those subsequently used in the experiments; values for last three experiments are from the 250µl samples taken for weighing during an experiment. All values account for leaching (see chapter 6, table 16) which was assumed to equal that which occurred when washing the 250µl sediment samples

the rates of faecal output (Table 22) can be converted to calories and plotted against mean individual weight, Fig. 22. It is now possible to estimate in two ways the percentage of energy assimilated in each isotope experiment. First the ratio of the rates of uptake of DPM before and after defaecation can be calculated. Second the rate of energy loss through faecal production for shrimp of a specific mean individual weight can be interpolated from Fig. 22 and subtracted from the feeding rate to produce the rate of assimilation. The results of these two procedures are shown in Table 26.

There is some correspondence between the two sets of values. However, those derived from the isotope experiments are less accurate and more imprecise because of the large standard errors of the regression coefficients and the probability that once the rate of uptake of DPM changes the shrimp are not only defaecating but also respiring $^{14}CO_2$ (Lampert, 1975). As shown already the slopes of the regression lines representing the lower rates of uptake are not significantly different from zero. Therefore figures for percentage assimilation from the isotope experiments are not reliable. To have a low and variable rate of assimilation of the major source of food is, of course, one way in which an animal can starve.

Values for percentage assimilation based on rates of faecal pellet production are generally higher or equal to those from the isotope experiments. This is to be expected for three reasons. First during the defaecation experiments the shrimp must filter a solution with a lower concentration of sediment particles than they would encounter if feeding on the surface of the sediment. As shown they can still replace their gut contents under these conditions, but the amount of incoming sediment is probably less than during an isotope experiment and therefore assimilation efficiencies quoted in Table 6 are overestimated. Second,

TABLE 26

Percentage assimilation of P. zietziana ingesting sediment

| experiment | from isotope experiments | from rates of faecal pellet production |
|---|---|---|
| 1 | 29 | 26 |
| 2 | 25 | 27 |
| 3 | 43 | 28 |
| 4 | 11 | 64 |
| 5 | 0 | 58 |
| 6 | no defaecation | 61 |

it is possible during these experiments that the shrimp reingest deposited faecal pellets, thus increasing their percentage assimilation. Third, faecal pellets may have been leached to some extent while accumulating over the twenty four hours. This would increase still further the assimilation efficiencies.

Thus values calculated from rates of pellet production are probably maximum efficiencies. Nevertheless, nearly all the variation in their rate of production can be explained by regressing the log of this value against the log mean individual weight (Fig. 22). This indicates that the concentration of sediment particles in the water column and fluctuations in temperature and salinity (Table 22) had little or no effect. Perhaps particle concentration was always greater than the critical level, as discussed by Reeve (1963 a) for A. salina, above which ingestion rates remain constant. This would imply that particle concentration never limited the ingestion rate of P. zietziana when feeding on the sediment surface.

The slope of the regression line in Fig. 22 is 1.57. It seems to apply also to the few data from Lake Cundare and is not significantly different from that of the regression of log feeding rate against log mean individual weight, Fig. 23. There is more unexplained variation, however, in the values for feeding rate. It is unlikely that this is due to temperature or salinity fluctuations because these have no influence on faecal output. Ingestion and defaecation must be virtually continuous in P. zietziana as specimens with empty guts are rarely caught. Feeding rates and thus egestion rates probably vary little diurnally because respiration does not.

From my observations individual shrimp do not feed continuously on the bottom, but periodically ascend. By doing so they subject themselves alternatively to high and low rates of ingestion and thus high and low backward directed pressures which must regulate the length of time

FIGURE 22

Faecal output in calories versus dry weight of an individual for P. zietziana from Pink Lake ( • ) and Lake Cundare ( o ). A value of 265.8 cal $g^{-1}$ dry faeces (Chapter 6) was used to convert the rate of pellet production from mg $h^{-1}$ $individual^{-1}$ (Table 22). Two values recorded in Pink Lake have been omitted (see footnotes to Table 22). The regression line is: log (faecal output) = 0.54 + 1.57 log (mg) ($r^2$= 0.994; S.E. of slope = 0.068).

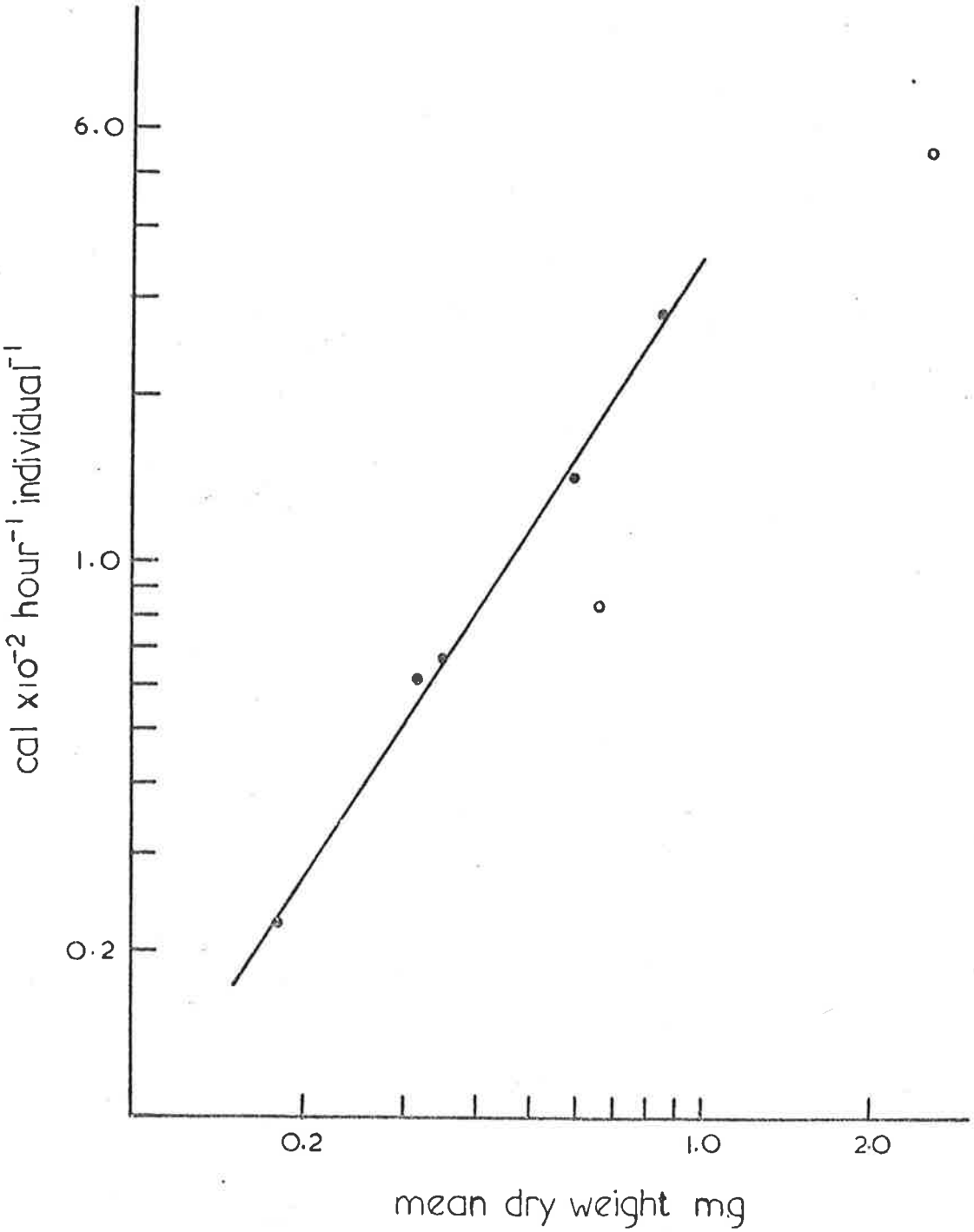
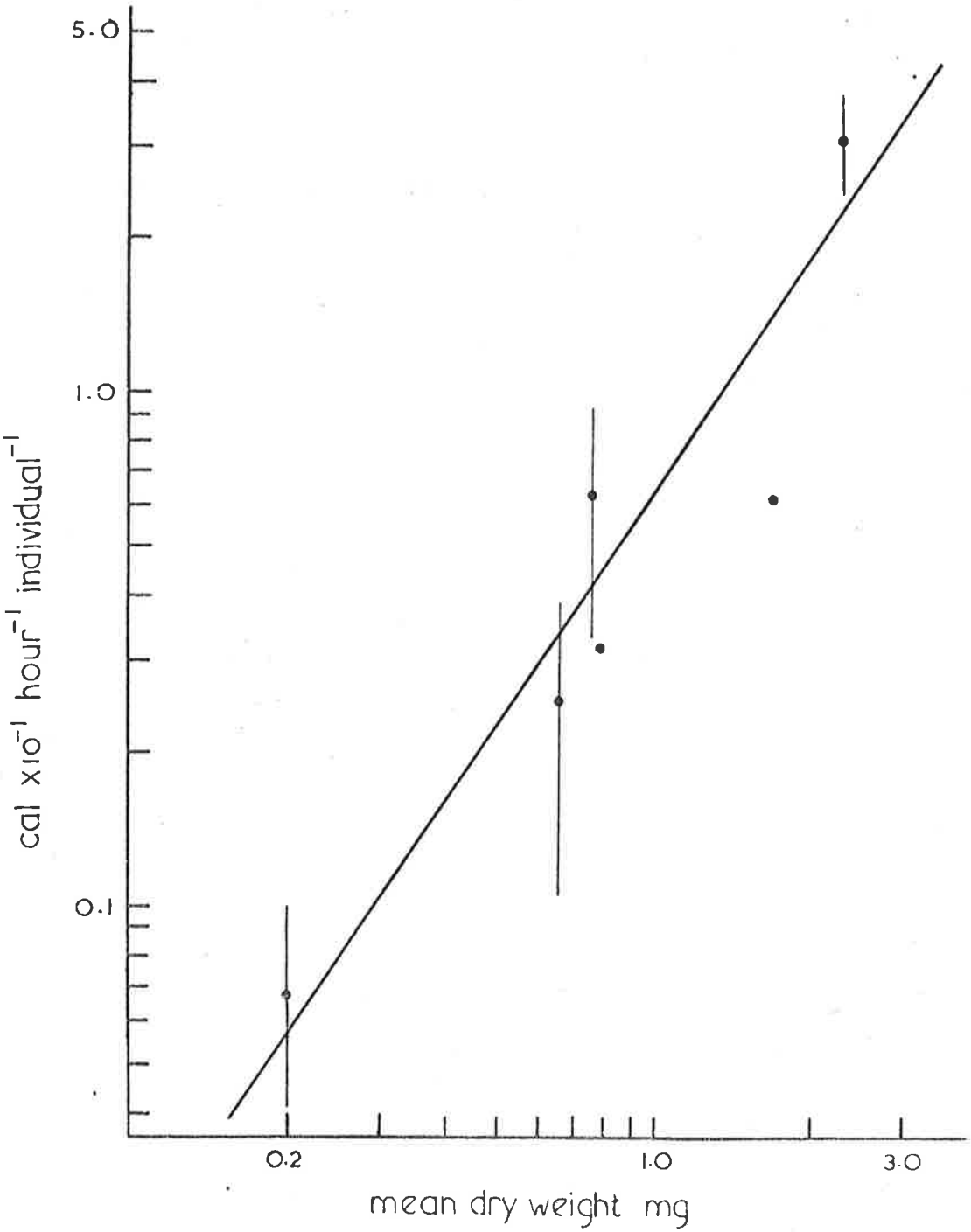

6.0
1.0
0.2
cal x10$^{-2}$ hour$^{-1}$ individual$^{-1}$
0.2
1.0
2.0
mean dry weight mg

FIGURE 23

The feeding rate of P. zietziana in calories versus the mean dry weight of an individual. The points have not been corrected for variations in temperature and salinity (Table 18). The regression line is: log (feeding rate) = -0.20 + 1.49 log (mg) ($r^2$ = 0.924; S.E. of slope = 0.214). The bars represent the 95% confidence limits.


5.0
1.0
0.1
cal x10⁻¹ hour⁻¹ individual⁻¹
0.2
1.0
3.0
mean dry weight mg

food remains in the gut. The longer it remains, the more, presumably, can be digested. This was suggested by Reeve (1963 d) to explain the higher growth efficiency of A. salina at low food concentrations. At such concentrations back pressure was least. He also found that A. salina enclosed each faecal pellet in a chitonous membrane and proposed that food was processed only as fast as this membrane was formed. Occasionally a membranous sheath, open at both ends, enclosed faecal pellets produced by P. zietziana, but generally they were absent.

Therefore assimilation percentages based on rates of faecal pellet production in the water column may not be as over-estimated as implied before. They are comparable with published values for other deposit feeders. Hargrave (1970) showed that the benthic amphipod H. azteca assimilated only 7-15% of the organic fraction which composed 50% of the sediment. On the other hand, Davies (1975) found that chironomid larvae ingesting sediment with a 3-4% organic content assimilated about 65%. Two marine gastropods studied by Newell (1965) would only digest bacteria in the organic fraction of their faeces.

If P. zietziana only digested bacteria then their maximum assimilation percentage would be 29%, the fraction bacteria compose of the total organics present (chapter 6). Assimilation during the first three experiments (Table 26), calculated by either method, is close to this; during the last three (considering only data based on pellet production) it has doubted. This suggests, if indeed only bacteria are digested, that the egestion rates for the last experiments are underestimated. There is some evidence for this: their rates of energy intake lie above the regression line (Fig. 23) while those of the first group lie below. Such could occur if the second group spent more time feeding on the sediment surface, indicating that its assimilation efficiency should not be based on egestion rates in the water column.

On the other hand there is also evidence that all the organic matter in the sediment is potentially useful to P. zietziana. From the caloric values for the sediment (chapter 6) C:N ratios can be calculated by assuming that all the $O_2$ used in the wet oxidation is converted to $CO_2$. This gives a ratio of approximately 13:1. Provasoli and D'Agostino (1969) showed that A. salina grew best in cultures with C:N ratios between 17:1 and 11:1. They also showed that it was an obligate phagotroph or particle ingester; nutrient solutions were incapable of sustaining growth even at concentrations approaching inhibitory levels. Vitamins, however, were ingested as solubles. The continuous growth of individuals in each generation of P. zietziana (chapter 4) suggests that food quality was not limiting.

# CHAPTER 8 - Energy budgets for P. zietziana and conclusions

## Introduction and Methods

Once the preceding data have been converted to energy units, energy budgets for the various cohorts in Pink Lake and Lake Cundare can be presented. Unfortunately the budgets cannot be completed for all generations of P. zietziana because there are only limited data on ingestion rates. However, rates of assimilation can be calculated for each cohort and therefore in those for which there are estimates of ingestion rate and assimilation efficiency I can determine whether energy input meets energy demand.

To convert production estimates from mg to calories eleven dried samples (approximately 200 mg each) of washed P. zietziana were burnt in a Galenkamp bomb calorimeter. Ash content of the shrimp was determined separately after ignition for two hours at 500°C; this gave an average value of 33.0($\pm$1.5)% ($\pm$95% confidence limits). The mean caloric value was 5.7 ($\pm$0.2) cal. $mg^{-1}$ ash free dry weight ($\pm$95% confidence limits), which is very close to the most probable value of 5.6 $cal.mg^{-1}$ ash free dry weight quoted by Winberg (1971) for aquatic organisms. However, it is possible that the caloric value has been slightly underestimated because the samples were kept for one to two years instead of a maximum of thirty days (recommended by Paine, 1971) before being burnt. In this time some of the organic material may have been partially oxidised.

The energy equivalents of oxygen consumption have been reviewed by Elliott and Davison (1975). They concluded a $Q_{ox}$ of 3.38 cal $mg^{-1}$ is suitable for herbivorous animals with a high proportion of carbohydrate in their diet, if RQ values are unavailable. The organic

fraction of the sediments that P. zietziana ingest contains 27.3% protein and thus 72.7% carbohydrate and fat. Assuming they are metabolised in these proportions, I have used the $Q_{ox}$ above.

Protein is not completely oxidised during catabolism and nitrogenous wastes are produced. By knowing their composition, a factor for energy loss ($Q_{ex}$) can be calculated from the rate of oxygen consumption. Parry (1960) claimed that Crustacea were ammonioteles and Bernice (1972) showed that 75% of the total nitrogen excreted by tne anostracan Streptocephalus dichotomus was ammonia. The $Q_{ex}$ calculated by Elliott and Davison (1975) for ammonioteles was 0.62 cal $mg^{-1}$. This was used in calculating energy loss for P. zietziana, assuming that 27.3% of the food they metabolise is protein.

Results

Tables 27 and 28 give the amounts of energy assimilated by each cohort in Pink Lake and Lake Cundare and the relative contributions of production and metabolism in joules (4.18 x calories). In all cases respiration accounts for most of the energy assimilated. Production contributes between 15 and 30% except when the final stages only of a generation were sampled e.g. cohorts 1 and 2 in Cundare and cohort 1 in Pink. The low percentages in these cases are to be expected because most of a cohort's production occurs through the death of the younger animals. High respiration rates due to high temperatures and salinity or mortality through salinity stress, e.g. cohort 2 in Pink, also increase this imbalance. Excretion of metabolic wastes is always less than 5% of the total and considering that there is an error of 40 to 50% in the production and respiration estimates this percentage is not significant. If the shrimp metabolised only protein then it would increase to 18%.

In Table 29 rates of assimilation derived from the feeding experiments are compared with rates of respiration predicted for shrimp of the same weight as used in these experiments. In each case except 4

TABLE 27

The amount of energy assimilated by the various cohorts in Pink Lake. All values are in joules 0.1 $m^{-2}$ (4.18 x calories). Excretion represents energy loss through nitrogenous wastes assuming 27.3% of the assimilable food is protein. Figures in brackets are percentages of the total assimilation

| cohorts[a] | production (P) | respiration (R) | excretion (E) | assimilation (A) (i.e. total) | |
|---|---|---|---|---|---|
| 1 | 6549.6 ( 7.8) | 73604.8 (87.8) | 3685.9 (4.4) | 83840.3 | |
| 2 | 11.9 ( 2.8) | 392.9 (92.6) | 19.6 (4.6) | 424.4 | |
| 3 | 18239.0 (22.8) | 58745.7 (73.5) | 2941.9 (3.7) | 79926.6 | Total = |
| 4 | 23673.4 (17.2) | 108805.8 (78.9) | 548.6 (3.9) | 137927.8 | 326320.9 j $0.1\ m^{-2}$ $2\ yr^{-1}$ i.e. 1,631,604.5 j $m^{-2}\ yr^{-1}$ |
| 5 | 2466.2 (15.5) | 12800.4 (80.5) | 640.8 (4.0) | 15907.4 | |
| 6 | 2566.1 (33.9) | 4754.3 (62.9) | 238.3 (3.2) | 7558.7 | |
| 7 | 235.8 (32.1) | 476.1 (64.7) | 23.8 (3.2) | 735.7 | |
| Total | 53742.0 (16.5) | 259580.0 (79.5) | 12998.9 (4.0) | 326320.9 | |

[a] as described in Chapter 4

TABLE 23

The amount of energy assimilated by the various cohorts in Lake Cundare. All values are in joules 0.1 $m^{-2}$. To calculate energy losses by excretion 27.3% of the assimilable food was assumed to be protein, the same as in Pink, although the caloric value of mud is lower in Cundare.

| cohorts[a] | production (P) | respiration (R) | excretion (E) | assimilation (i.e. total) (A) | |
|---|---|---|---|---|---|
| 1 | 354.9 ( 2.9) | 11483.7 (92.5) | 575.2 (4.6) | 12413.8 | |
| 2 | 59.8 ( 5.4) | 1006.1 (90.1) | 50.6 (4.5) | 1116.5 | Total = 27568.3 j 0.1 $m^{-2}$ 1.3 $yr^{-1}$ i.e. 212,063.8 j $m^{-2}$ $yr^{-1}$ |
| 3 | 455.2 (33.5) | 859.0 (63.3) | 43.1 (3.2) | 1357.3 | |
| 4 | 1739.3 (17.4) | 7845.4 (78.6) | 392.9 (4.0) | 9977.6 | |
| 5 | 495.7 (18.3) | 2102.1 (77.8) | 105.3 (3.9) | 2703.1 | |
| Total | 3104.9 (11.3) | 23296.3 (84.5) | 1167.1 (4.2) | 27568.3 | |

[a] as described in Chapter 4

TABLE 29

Rates of energy assimilation by P. zietziana ingesting sediment compared with its rates of energy consumption through respiration. Assimilation was calculated from the percentages based on rates of faecal pellet output in Table 26. Respiration was predicted from the regression equation in Chapter 5 using the values of temperature, salinity and mean individual weight given in Table 18 for each feeding experiment. The units are $j \times 10^{-1}$ $hr^{-1}$ $individual^{-1}$.

| experiment | energy intake (C) (±95% confidence limits) | energy assimilated (A) (95% confidence limits) | energy respired (R) | 95% confidence limits of predicted respiration |
|---|---|---|---|---|
| 1 | 1.34 | 0.35 | 0.59 | 0.54 - 0.64 |
| 2 | 2.59 | 0.70 | 0.79 | 0.72 - 0.87 |
| 3 | 1.05 (± 0.59) | 0.29 (0.13 - 0.46) | 0.47 | 0.43 - 0.51 |
| 4 | 2.63 (± 1.26) | 1.68 (0.88 - 2.49) | 0.61 | 0.55 - 0.67 |
| 5 | 12.92 (± 2.81) | 7.49 (5.86 - 9.12) | 1.25 | 1.12 - 1.40 |
| 6 | 0.29 (± 0.13) | 0.18 (0.10 - 0.26) | 0.22 | 0.19 - 0.25 |

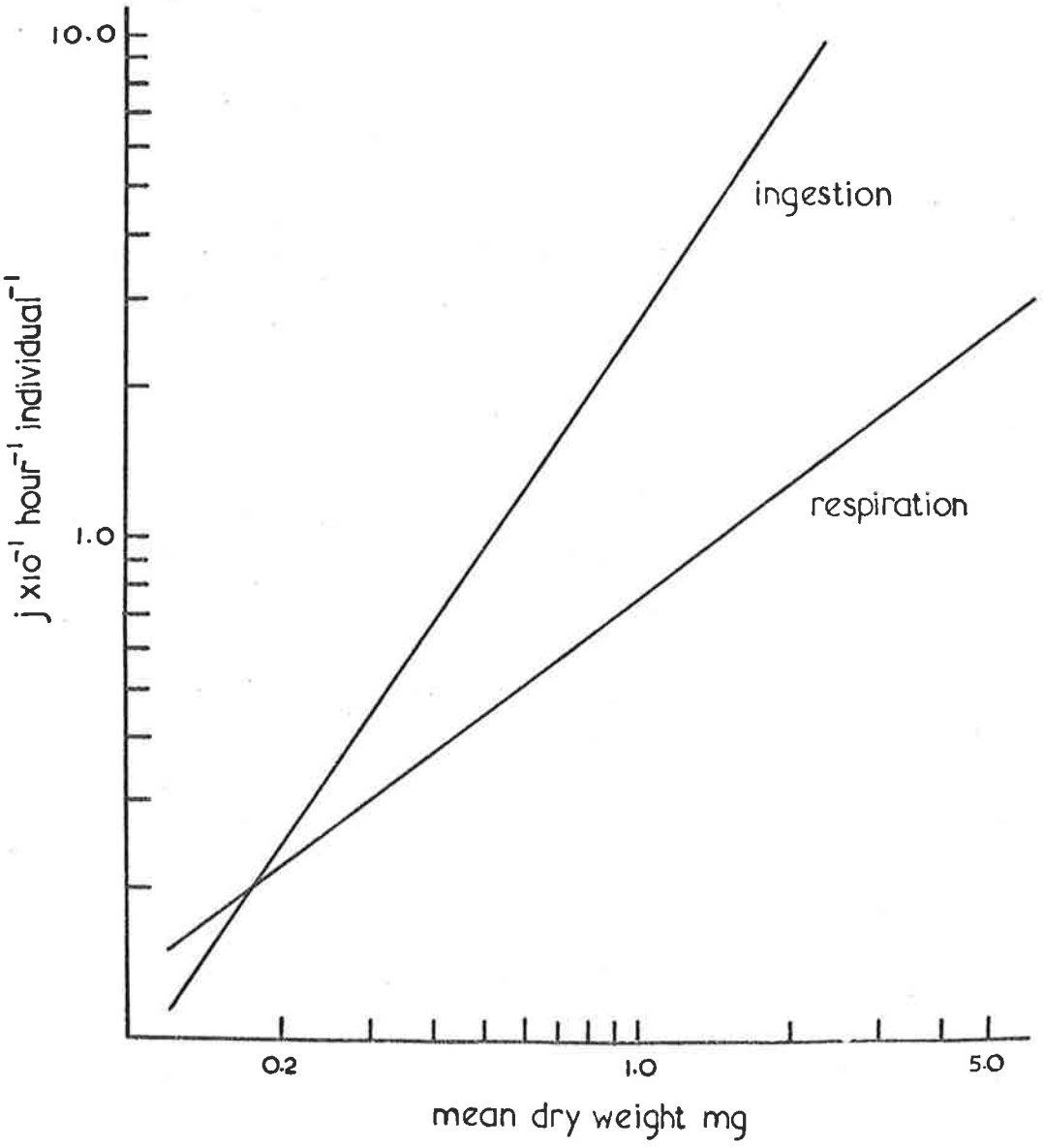
and 5 energy was consumed at a higher rate by respiration than it was supplied by assimilation. In fact, the shortage is larger than shown because the animals were also growing to some extent as well as excreting. In all cases, however, the rate of energy ingestion by P. zietziana was greater than its rate of consumption. Therefore assimilation efficiency must largely determine whether there will be a shortage. In these calculations assimilation is at a maximum, as previously discussed, because it is based on egestion rates measured in the water column rather than on the sediment surface. Thus only by increasing ingestion rate can shrimp survive in these conditions, but this is only advantageous provided it is not offset by a decline in assimilation efficiency (see chapter 7). It is, of course, possible that P. zietziana may be able to survive for some time on body reserves. However, its caloric value (5.7 cal $mg^{-1}$) suggests that large deposits of fat (9.5 cal $mg^{-1}$), the usual form of such reserves, are not present. Also at no stage do animals that survive lose weight.

Considering the wide confidence limits of the values for energy intake it is unwise to rely too heavily on the accuracy of their corresponding values for assimilation. Nevertheless, they suggest that P. zietziana is unable to meet its energy demand when feeding on sediment with a low organic content because of poor assimilation. This is a plausible explanation for the consistent mortality in each cohort.

It becomes more convincing if the relation between the feeding and respiration rates is followed throughout the life of an individual. This can be done by comparing their rates of increase with weight, Fig. 24. The slopes of the two lines (1.49, 0.76) are significantly different ($p<0.001$). Therefore small shrimp (<0.2 mg) will not be able to ingest, let alone assimilate, enough energy by feeding on sediment to meet their respiratory needs and cohorts will suffer a high initial rate of mortality. This is evident for generations 4 and 5 in Pink and Cundare (Figs. 6 and 7), provided

FIGURE 24

A comparison of the increase with weight of the rates of ingestion and respiration (both in joules) for P. zietziana. The line representing respiration was calculated for the average values of temperature (15°C) and salinity (133‰) in Pink Lake during the study; it will move up or down (but maintain the same slope) according to the values of these two parameters thus increasing or decreasing the weight at which the two rates balance.


10.0
1.0
j x10⁻¹ hour⁻¹ individual⁻¹
ingestion
respiration
0.2
1.0
5.0
mean dry weight mg

numbers recruited are also considered (Table 6). Periods of stability or with low rates of mortality follow; these also occur in other generations, but, with no data on recruitment or because of rapid death due to salinity stress, high initial mortality cannot be distinguished. In all generations mortality rate increases after these periods probably because of the energy balance becoming increasingly negative due to poor assimilation. The death of ovigerous females coincides with this increase suggesting that egg production taxes the energy supply. Sometimes, e.g. cohort 6 in Pink, mortality is virtually constant throughout the life of the generation implying that generally the environment of these saline lakes is not optimal for P. zietziana.

Of course, the switch from positive to negative energy balance is not as precise nor as invariable as shown in Fig. 24. It will depend on assimilation efficiency and the prevailing temperature and salinity. Changes in these last two will alter the intercept (but not the slope) of the respiration line in Fig. 24; as shown previously (chapter 7) they should have little effect on ingestion.

For recently hatched P. zietziana to survive it seems necessary for them to use another source of energy. Undoubtedly the nauplii can survive for some time on yolk reserves, but for them to grow past this stage they must eat. From observation their guts rarely contained sediment, but were often coloured red. Hussainy (1972) suggested that the orange pigment of the copepod Calamoecia clitellata from nearby Lake Gnotuk was derived from pink autotrophic sulphur bacteria. If these also exist in Pink and Cundare, P. zietziana nauplii may be able to ingest and assimilate them efficiently. They may also rely on primary production. However, this is low and unpredictable. If it is also heterogeneously distributed within a lake then survival of young shrimp may well be purely fortuitious.

A further possibility is that the slope of the feeding line in Fig. 24 decreases after 0.2 mg (the lowest weight at which ingestion was measured). If at the same point the slope of the respiration line increases then there would be a wider scope for positive energy balance. Eliassen (1952) found an increase from 0.60 to 1.00 below a dry weight of about 0.1 mg for A. salina. Without detailed measurements at these small sizes for P. zietziana it is impossible to comment further. However, a reasonable conclusion from the present data is that the larger the shrimp the more likely it is able to at least ingest sufficient energy from the organic matter in the sediment.

Discussion

There is no doubt about the importance of the sediment in the diet of P. zietziana. Primary production in Pink Lake as measured so far (15gC or approximately 564,000j $m^{-2}$ $yr^{-1}$) only equals 35% of annual assimilation; this is most probably an over-estimate. Therefore the shrimp must rely on the organic matter in the sediment as an energy source. Their ability to assimilate this governs their survival more than anything else.

As shown in chapter 4, clutch size and recruitment were very variable. Clutch size does not correlate with salinity, temperature, population density or size of animal within either lake and recruitment shows no inverse relation with the size of the parent generation; in fact, for the densities encountered it varies directly with the number of females becoming ovigerous. Growth rates, although variable, were not affected by temperature or salinity. Turnover ratios (P/B) were not constant; it would be impossible to predict production from an estimate of mean biomass. Therefore there appears to be no close internal regulation of these processes by the shrimp nor environmental control and the likely explanation of this variability is that it reflects variations

in the assimilation efficiency of P. zietziana. There does not appear to be an absolute lack of food leading to competition as the caloric value of mud samples was fairly constant implying microbial production could offset grazing.

The lack of inflexion in the growth curves probably results from an increased potential for growth as size increases rather than a relaxation of competition as population density decreases. Ingestion increases with weight at the same rate as egestion (Figs. 22 and 23), but faster than respiration (Fig. 24). Thus assimilation efficiency is constant within a generation while the relative availability of energy for growth instead of metabolism increases. The low variability of clutch size ($\pm$ 10% approximately) at one period of recruitment is perhaps indicative of a constant percentage assimilation.

As mentioned in chapter 4 growth is exponential and thus relative growth rate within a generation is constant. Energy shortage through poor assimilation must affect the relative growth rate of individuals, but this is not distinguishable from the growth curves of a cohort because these are derived from the mean size of survivors. However, between generations there are detectable differences in this rate (and in the mean clutch size) which reflect differences in assimilation efficiency and perhaps ultimately in the degree of microbial productivity.

Between the populations in the two lakes there are also differences in growth and more importantly in numbers of shrimp. Production and assimilation rates of P. zietziana are higher in Pink than in Cundare. The caloric value of the sediment in Cundare is smaller implying that the difference is caused by less abundant food not less efficient assimilation. This is not unexpected because animal numbers must ultimately be controlled by the productivity of their ecosystem; but it is improbable that this is the immediate influence regulating production within a population.

The rate of faecal pellet production is also less in Lake Cundare (Fig. 22). Accepting similar assimilation efficiencies this indicates that the rate of energy uptake is lower because it is more dilute. The higher average clutch size of P. zietziana and the presence of larger specimens in Lake Cundare can probably be explained by a lower average salinity rather than by an increase in assimilation efficiency. At a lower salinity less energy is required for respiration, as shown in chapter 5, and therefore of a fixed amount assimilated more is available for production of biomass either as individual growth or eggs. This correlation only seems to exist between the two populations, not within them, suggesting it is a long term response to a particular salinity range. Geddes (1973, 1976) however, noted an inverse correlation of P. zietziana length with salinity for specimens taken in different lakes or the same lake at different times. He also showed a low positive correlation between clutch size and length of ovigerous female; the largest females were found at the lowest salinity.

Whether or not such correlations are significant, differences in clutch size or maximum length have no detectable influence, at least in my lakes, on net growth efficiency (P/A). The same range of values 15-30% (Tables 27 and 28) prevails in both lakes, and there appears no correlation between efficiency and salinity within a population. Considering the large variability in the production and respiration estimates meaningful differences in efficiency probably do not exist. Daborn (1975) found values of 23 and 33% for male and female, respectively, of the predator B. gigas. This was based on energy budgets for individuals in the wild rather than populations, the higher value for females being due to production of eggs. Sushchenya (1962) with A. salina raised in the laboratory on algae recorded net growth percentages of 24 to 27% for a five fold increase in food concentration. Both these results agree well with my average efficiency (excluding the small values) of 23%.

Over the same change in food concentration Sushchenya found a gross growth efficiency (P/C) of 9 to 19%, the highest percentage being at the lowest concentration. Reeve (1963 d) also noticed this for A. salina, but his average efficiency was 25% and his maximum was 79%; efficiency varied with salinity, temperature and food concentration and generally was highest when the fastest growth occurred. Mason (1963) on the contrary only found values of 4 to 5%. Daborn's values were 17 and 31% for male and female B. gigas, respectively, and my values approximately estimated from Table 29, assuming the same ratio between respiration and production as in cohorts 5, 6 and 7 of Table 27, are 5-12%. These apply to the population as a whole; individual P. zictziana unable to meet their energy demands would not be growing. Both these efficiencies (P/A, P/C) are cumulative obscuring wide fluctuations during the life cycle. They probably reach a maximum with mature P. zietziana because of exponential growth.

Despite this its gross growth efficiency is low. Although the other values are largely derived from cultured populations with abundant supplies of food, the comparison is still useful. It probably reflects the difficulty P. zietziana experiences in meeting its energy demands from ingestion of the available sediment. Because of variable assimilation efficiencies individuals may quite often only be able to extract enough energy for respiration but not growth. This view is supported if the annual estimates of production and respiration in both lakes are compared with the regression line McNeill and Lawton (1970) calculated from a number of studies relating the logarithms of these two values for short lived poikilotherms ($\leq$2 years). Taking log P as the independent variable, in both cases the regression seriously under-estimates the amount of respiration. This implies that compared with other studies the amount of energy stored as production by P. zietziana is very low for the amount respired.

Therefore there is some independent confirmation for the conclusion that the survival of P. zietziana in these lakes depends largely on their ability to assimilate the organic matter present in the sediment. In a more complex ecosystem it would be difficult to be confident of such a conclusion because of the influence of predators and possible competitors. Their apparent absence in simple communities makes the analysis of energy flow through salt lakes particularly worthwhile.

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