RESPIRATORY AND OSMOREGULATORY PHYSIOLOGY OF A MEIOBENTHIC MARINE GASTROTRICH, *TURBANELLA OCELLATA* HUMMON I974.

by

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Résumé

Les mesures respiratoires montrent que l'adulte du Macrodasyidé Gastrotriche *Turbanella ocellata* consomme 4 à 8,5 nl O_2/animal h (5,7 à 12 nl O_2/µg sec wt h) à une température de 14 à 27 °C et à une salinité de 32 p. 1000. Ces valeurs sont supérieures à celles qu'on rencontre chez la plupart des autres animaux méiobenthiques marins. La consommation d'oxygène décroît aux salinités inférieures ou supérieures à celles auxquelles sont normalement soumis les animaux.

Les limites somatiques létales après 24 h dépassent 10 à 60 p. 1000 pour des températures de l'eau saisonnière comprises entre 14 et 27°. L'ajustement à la température et à la salinité aux changements de conditions thermiques est démontré pour cette espèce euryhaline.

T. ocellata est un régulateur osmotique de volume. L'augmentation ou la diminution rapide du volume du corps répond aux gradients anisosmotiques de salinité entre les milieux liquides interne et externe. Le phénomène est suivi d'un retour graduel au volume normal, sans doute par action d'un flux isosmotique solution-solvant. Les valeurs du Log» du flux net sont de 15 à 50 X 10^{-3} µm^3 / µm^2 osm sec pour les simples changements anisosmotiques de l'eau de mer et 1 à 1,5, pour l'ajustement consécutif isosmotique. Il est évident que le flux entrant sous des conditions hypo-osmotiques est la résultante de trois flux distincts : un flux entrant passif de la solution, un flux actif sortant de la solution et un flux passif entrant du solvant.

Introduction

Little is known of the respiratory or osmoregulatory biology of marine, brackish-water or fresh-water gastrotrichs. Aside from field observations on the occurrence of various species under measured conditions of temperature, salinity or oxygen, the only data reported are those of Hummon (1972, 1974 a and 1974 b) on lethal limits of several marine and brackish-water forms.

The present paper is based on laboratory studies of *Turbanella ocellata* Hummon, 1974. This species of Turbanellidae was obtained regularly from the lower intertidal zone on Pivers Island, Beaufort, North Carolina, U.S.A., and represents a more or less typical elongate, strap-shaped macrodasyid gastrotrich of the marine habitat. Adults of the species average 600 µm in total length and 58 µm in maximal trunk width. For a brief ecological description of the biotope see Hummon, 1974 b. Laboratory data, gathered during the summer of

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Tome XVI - 1975 - pp. 255-268

1966, consist of three parts: a) respiratory rates, b) osmotic lethal limits, and c) osmoregulatory mechanisms. Finally, a note appended provides preliminary information on respiratory rates and osmotic lethal limits in two members of the phylum Kinorhyncha.

RESPIRATORY RATES

Respiratory rates were determined by means of a Kirk differential microrespirometer, having a sensitivity of 0.1 µ.l/h (Grunbaum *et al*, 1955). The respirometer was immersed in a water bath, maintained within ± 0.5°C of a desired temperature. Acclimatization in a controlled temperature (BOD) box was for at least 48 hours at 27°C, 32 p. 1000, which approximates the summer ambient water conditions. Duplicate runs were made on 4-6 adult *Turbanella ocellata* for 0.5 to 2.0 hour periods of constant O_2 uptake, under test conditions.

Introduction of animals to test conditions occurred in two steps. Animals were selected and transferred by means of a mouth pipette to a 60 mm diam. Petri dish in < 0.1 ml water of acclimatization salinity. This was followed by addition of 30 ml water of test salinity, gentle stirring and a pause of 5-10 minutes. Animals were then transferred to a second Petri dish in < 0.1 ml mixed water, after which another 30 ml water of test conditions was added. Finally, animals were transferred to a respirometry flask under test conditions and a control flask was prepared as its mate.

Respiration was calculated in nl O_2/animal h and was converted to nl O_2/µg dry wt h after the mean dry weight of adult animals was determined. In no case did the standard deviation of duplicate measures exceed 15 p. 100 of their mean value.

Dry weights were determined on two groups of four adult *Turbanella ocellata* by means of a micro-torsion balance, having a sensitivity of ± 2 µg. Animals were rinsed in two separate dishes of distilled water and were quickly transferred to 1 cm^2 aluminium foil. They were then dried overnight at 50°C. Aggregate weights for the two groups were 2.7 and 2.9 µ*g*, giving a mean weight of 0.7 µg for each of the eight animals.

Results for various test conditions are given in Fig. I, 1. It shows an increase in respiratory rate with increasing temperature, for a

FIG. I

Turbanella ocellata

1 : Variations in respiratory rate with temperature for a particular salinity. 2: Incipient somatic lethal limit after 24 h; connected circles represent adults acclimatized at 27 °C, 32 p. 1000 salinity; connected triangles represent adults acclimatized at 14 °C, 32 p. 1000 salinity.

Echinoderes bookhouti

Trachydemus langi

2: Incipient somatic lethal limit after 24 h; connected E's represent adult *Echinoderes;* e represents juvenile *Echinoderes;* T represents adult *Trachydemus;* Kinorhyncha were acclimatized at 27 °C, 32 p. 1000 salinity.

particular salinity. The Q_{10} per animal from 26 to 30°C at 32 p. 1000 was 3.1; that from 30 to 34°C at 26 p. 1000 was 5.9, at 32 p. 1000 was 5.5, and at 38 p. 1000 was 6.2. Using the 26 to 30°C at 32 p. 1000

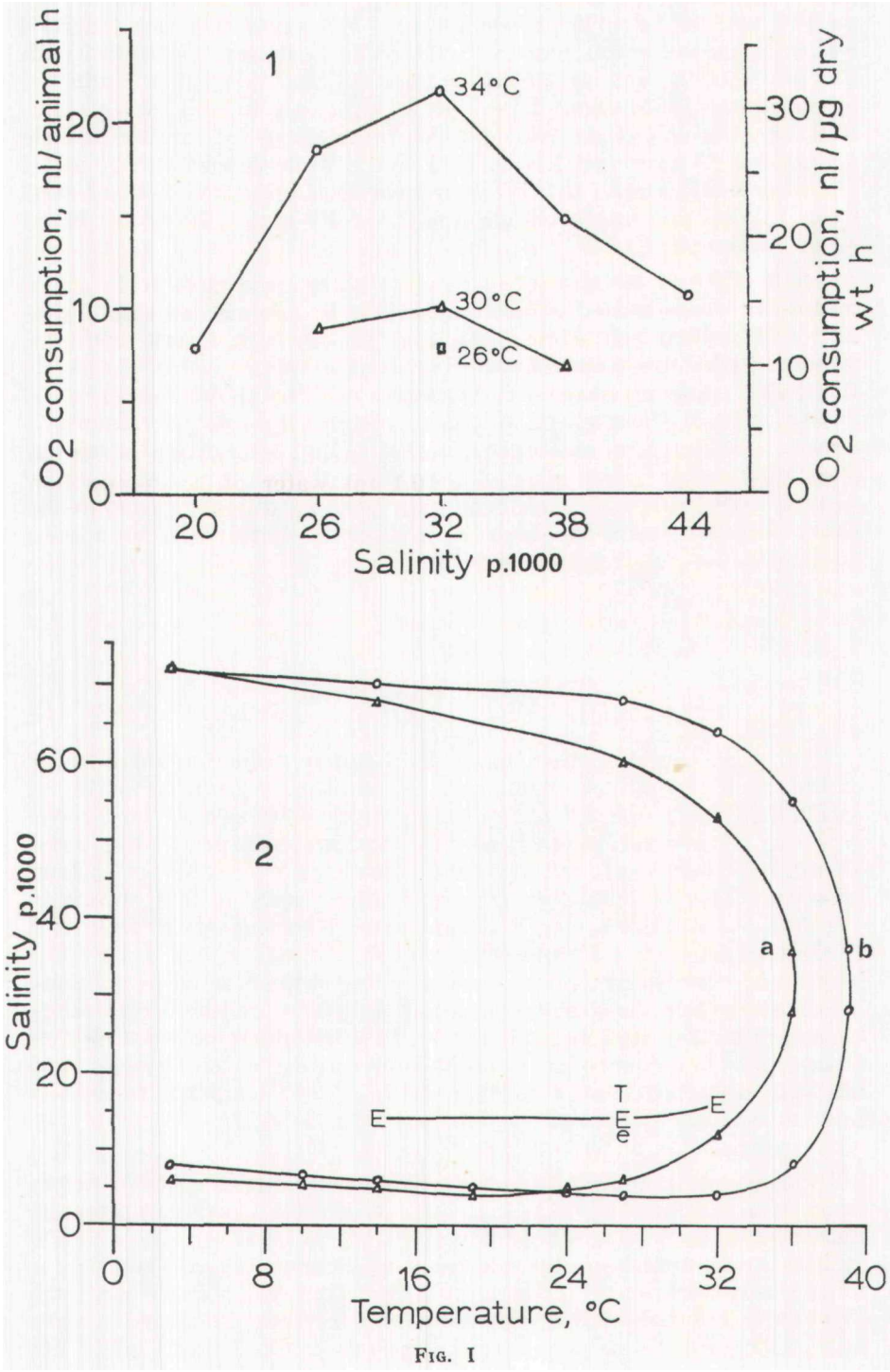

FIG. I

rate of respiratory increase ($\log_n$ = 0.056/°C) to predict respiratory rates under winter to summer ambient water conditions, 14 and 27°C respectively, values are obtained of 4 to 8.5 nl O_2/animal h (5,7 to 12 nl O_2/µg dry wt h or, assuming dry weight is 10 p. 100 of wet weight, 0.57 to 1.2 nl O_2/µg wet wt h). Values in this range accord well with those for soil nematodes (Overgaard-Nielsen, 1949) and with the interspecific comparison of metabolism and body size conducted by Zeuthen (1953), both based on 0_2 uptake per unit wet weight. However, the values are two or more times those for marine benthic nematodes (Wieser and Kanwisher, 1961), kinorhynchs (this paper), oligochaetes (Lasserre, 1971), mystacocarids (Lasserre et Renaud-Mornant, 1971a), and harpacticoids (Coull and Vernberg, 1970), all based on 0_2 uptake per unit dry weight.

The effect of salinity on respiratory rates appears to be in maximazing O_2 consumption at acclimatization (= summer ambient) salinity and reducing O_2 consumption at greater and lesser salinities. Similar peaks are found in marine benthic oligochaetes (Lasserre, 1969; 1971) and mystacocarids (Lasserre et Renaud-Mornant, 1971b), but in both of these cases, the peak respiratory rate was found to occur at greater than ambient salinities. The phenomenon is worthy of further study. Indeed, my data suggest that the peak may be more closely associated with acclimatization conditions which, in the above instance, were identical with ambient conditions, than with ambient conditions themselves.

OSMOTIC LETHAL LIMITS

Incipient somatic lethal limits (ISLL) were defined as those upper or lower salinity values which, over 24 hours at a given temperature, resulted in more than 50 p. 100 deaths in a population of *Turbanella ocellata.* If several populations are acclimatized under the same conditions, their ISLL salinity values, graphed over a series of temperatures, form a partial to complete ellipse such as that shown in Fig. I, 2a. If animals are acclimatized under a second set of conditions, ISLL data generate a second ellipse, as that shown in Fig. I, 2b. The length and breadth of tolerance zones included in these ellipses should indicate whether the species possesses eurytopic or stenotopic adaptations to temperature and salinity. The relationship between ellipses should privide evidence as to whether or not the species responds to differing acclimatization conditions with altered temperature-salinity tolerances.

Incipient lethal limiths refer to the beginnings of death in both individuals and populations, implying that increased severity of conditions over a similar time period or that similar conditions over an increased time period, would result in more rapid demise of individuals or extinction of the population. *Somatic* lethal limits refer to the cessation of all integrated body functions. There are also reproductive lethal limits, which were not directly measured. However, upper and lower values for reproductive lethal limits could not

exceed and would generally lie well within values for the somatic lethal limits. The zone between reproductive and somatic lethal limits in any case represents eventual extinction of populations resulting from lack of recruitment of juveniles into the population. A somatic *lethal limit* was defined for this work as the absence of voluntary locomotion and whole-body response to tactile stimuli, which approximately describes the point of irreversible damage to life processes in gastrotrichs. Since lethal concentrations (LC) causing 10 p. 100 death of a population (LC_{10}) did not usually differ by more than 1 p. 1000 from that causing 90 p. 100 death of the same population (LC_{90}), death of more than 50 p. 100 a population (LC_{50}) was a sufficiently accurate level from which to derive lethal limits for these animals.

This artificial format was chosen over one that attempts to duplicate natural phenomena for two reasons. First, its conditions easily can be duplicated from one location to another, and second, such a comparison can be made regardless of the variability in levels and periodicities of natural phenomena from location to location.

Populations consisted of at least 20 adult individuals. A series of controlled temperature (BOD) boxes, set at 3, 10, 14, 19, 24, 27, 32, 36, 39 and 41°C, provided temperature stability for the experiments. Temperatures were checked regularly to within ± 0.5°C by means of thermister or thermometer. A series of controlled salinities was established in sealable liter jars, using filtered sea water diluted with distilled water or concentrated by freezing. Salinities included 2, 4, 8, 16, 32, 40, 48, 56, 64, 72 and 80 p. 1000, and were checked regularly to within ± 0.5 p. 1000 by means of salinometer or temperature corrected hydrometer. Two acclimatization conditions used were 27°C, 32 p. 1000, representing summer ambient water condition and 14° C, 32 p. 1000, representing winter ambient water conditions. Test salinities were made up to the nearest 1 p. 1000 directly from controlled salinity solutions or by appropriate volumetric dilutions of that pair of solutions which most closely bounded a desired salinity value. Procedures for transfer to test conditions were as described in the previous section.

Two ellipses resulting from ISLL analysis are shown in Fig. I, 2. The zone of tolerance encompassed by ellipse 2a includes 8 to 72 p. 1000 at 3°C, 4 to 64 p. 1000 at 32°C and 28 to 36 p. 1000 at 39°C. Following acclimatization for 48 hours at 14°C, 32 p. 1000, the zone of tolerance encompassed by ellipse 2b is reduced somewhat, including 6 to 72 p. 1000 at 3°C, 12 to 53 p. 1000 at 32°C and 28 to 36 p. 1000 at 36°C. Unfortunately, data were not obtained at temperatures below 3°C for the extension of these ellipses.

Turbanella ocellata is clearly both eurythermal and euryhaline. The species shows both temperature and salinity adjustment to changed thermal conditions. Thus, acclimatization to winter ambient water temperatures restricts both temperature and salinity tolerances, when compared with acclimatization to summer ambient water temperatures. Acclimatization to winter temperatures also restricts upper salinity tolerances somewhat, while enhancing lower salinity tolerances slightly. In both cases, the greatest low salinity tolerance

occurs with temperatures at or just above those to which the species has been acclimatized, and in both seasons storms which are most likely to cause significantly reduced salinities are of tropical origin.

Eurytopic tolerances of *Turbanella ocellata* compare favorably with those of the marine gastrotrichs *Turbanella cornuta* and *Chaetonotus testiculophorus* (Hummon, 1972; 1973), and are much broader than those of the brackish-water gastrotrich *Chaetonotus oligohalinus* (Hummon, 1974b). The latter species has a relatively stenotopic range of temperature-salinity tolerance compared to the others.

OSMOREGULATORY MECHANISMS

Osmoregulatory changes can be monitored in one of two ways, directly, measuring the altered concentrations of internal fluids, or indirectly, by measuring volume changes associated with altered concentrations of internal fluids. Attempts at direct measurement based on single individuals, with the aid of Dr. F. Kalber and his micro-cryosmometer, were unsuccessful. With difficulty, we were able to extract 4-5 µl of internal fluid per adult *Turbanella ocellata*, compared to the 20-30 µl needed to obtain readings of freezing point depression. Hence, data regarding osmoregulatory mechanisms were sought by indirect measurement.

The volume of an adult *Turbanella ocellata* can be approximated by $V = .4\pi \left[\left(\frac{.4W + W}{2}\right)/_2\right]^2 L = 0.154\ W^2L\ \mu m^3$, where W is maximal trunk width and L is total length in µm. The surface area of the same animal can be approximated by $A = .4\pi \left[\frac{.4W + W}{2}\right] L = 0.880\ WL\ \mu m^2$. Surface to volume ratio approximates $\frac{5.7\ \mu m^2}{W\ \mu m^2}$ or about 0.10 $\mu m^2/\mu m^3$ for a normal adult.

The procedure for making indirect measurements is as follows. A small drop of water of 32 p. 1000 salinity was placed on a microslide. After topping with a coverslip supported parallel to the microslide by means of plasticene-tipped corners, dimensions of the partially spread drop were measured and its surface area estimated. An amount of distilled water or other fluid was then added by means of a mouth pipette, resulting in an enlarged drop with greater surface area. Height of the drop remaining constant, volume is relative to surface area and the resultant salinity will have changed proportional to the change in surface area. This dilution process operates very efficiently to produce desired changes in external salinity, since mixing is both rapid and complete.

In practice, an animal was placed in such a drop and changes in trunk width were measured over time through a microscope by means of an ocular micrometer. Changes in body width are correlated with changes in internal volume and represent alterations in osmolarity of internal fluids.

Volume changes are shown on Fig. II as percent of original volume. Death as defined in this section was the cessation of ciliary activity, a point which occurs much further in the irreversible damage

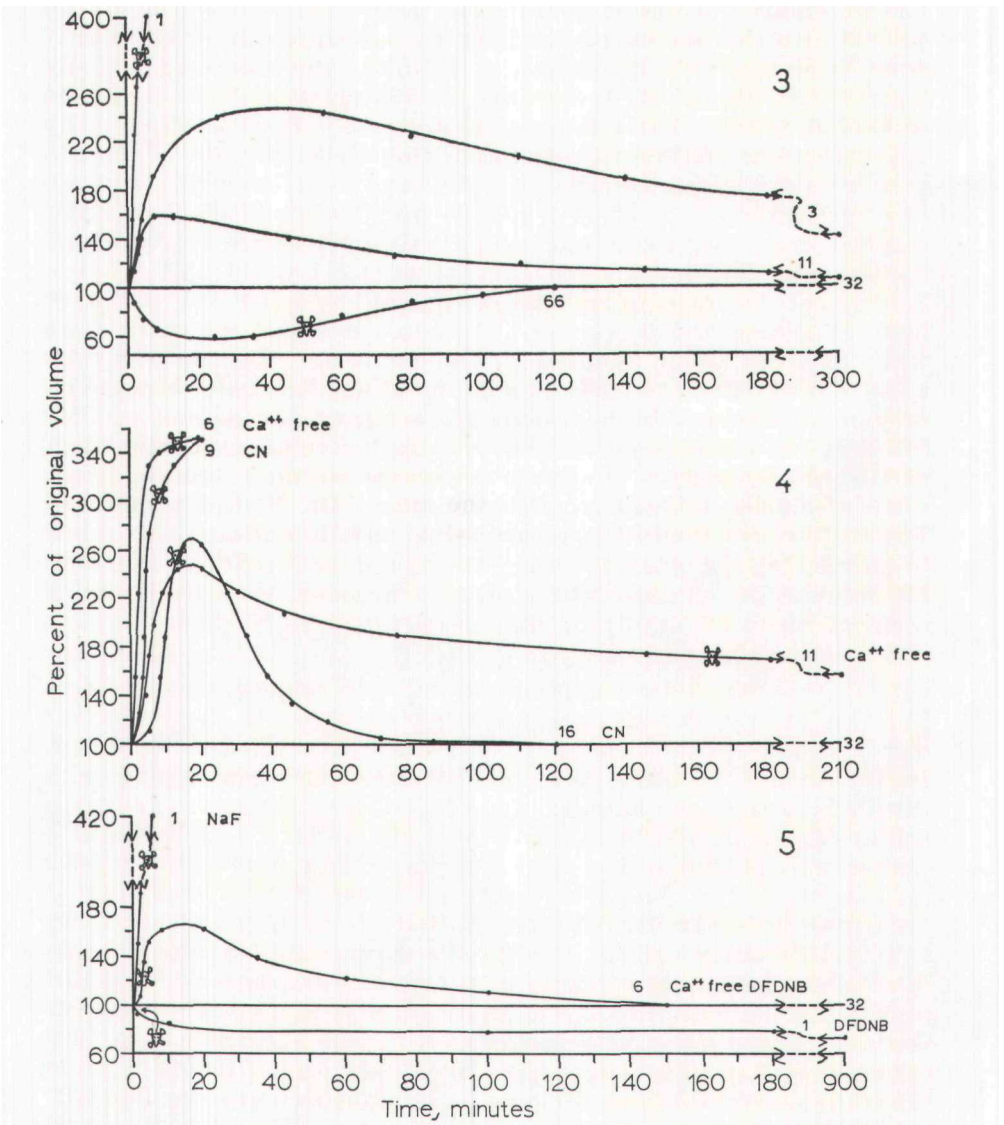

FIG. II

Turbanella ocellata

Volumes changes with salinity.

3: simple changes in salinity p. 1000; 4: reductions in salinity p. 1000 along with reductions of Ca^{++} or additions of CN; 5: reductions in salinity p. 1000 along with reduction of Ca^{++} or additions of NaF or DFDNB (difluoro-dinitrobenzene).

to life processes than absence of voluntary locomotion and whole-body response to tactile stimuli. Time of death, when it occurred, is symbolized by a skull and crossbones. Work was done at 27 to 30°C on animals which had been acclimatized 48 hours at 27°C, 32 p. 1000.

Duplicate runs of a given set of conditions gave nearly identical results.

Fig. II,3 illustrates the results of simple changes in salinity. Control animals, receiving drop volume increase of the same 32 p. 1000 salinity, showed no change in volume. Decrease in drop salinity from 32 to 11 p. 1000 by addition of distilled water resulted in rapidly increased volume of the test animal, to 176 p. 100 of original volume in 9-10 minutes. The following decrease in volume asymptotically approached the original volume and had reached 106 p. 100 of original volume in 300 minutes, with no visible harm to the animal. Decrease in drop salinity from 32 to 3 p. 1000 by addition of distilled water resulted in a more rapid and more persistent increase in volume of the test animal, which attained a maximum of 247 p. 100 in 34 minutes. Subsequent decrease in volume was slow, reaching 141 p. 100 of original volume in 300 minutes. Obvious damage to internal tissues was noted but death, as defined, did not occur. Decrease in drop salinity from 32 to 1. p. 1000 by addition of distilled water brought on very rapid increase in body volume, death of the animal at 330-340 p. 100 of the original volume and nearly explosive disintegration of tissues at about 4x the original volume within 6 minutes time. Finally, increase in drop salinity from 32 to 66 p. 1000 by addition of 72 p. 1000 water resulted in a decrease to 60 p. 100 of original volume in 20 minutes. A gradual return to original volume followed within 120 minutes of the time the change was initiated, with death having occurred about 50 minutes into the sequence.

Effects of reduced levels of calcium and of cyanide poisoning on volume changes under conditions of lowered salinity are shown in Fig. II,4. In the first instance, animals were placed in 32 p. 1000 Ca^{++} free seawater for 30 minutes prior to dilution with distilled water. In the second, animals in 32 p. 1000 seawater were diluted with distilled water containing 2 mM NaCN. Decrease in drop salinity from 32 p. 1000 Ca^{++} free seawater to 11 p. 1000 initiated a pattern not unlike that resulting from simple dilution of 32 to 3 p. 1000 in Fig. II, 3, but having a somewhat sharper peak occurring at an earlier time. Decrease in drop salinity from 32 p. 1000 seawater to 16 p. 1000 water with CN resulted in a sharp-peaked volume increase after a short lag period. Return to original volume was completed within 120 minutes from the beginning. Decreases in drop salinity from 32 p. 1000 Ca^{++} free seawater to 6 p. 1000 and from 32 p. 1000 seawater to 9 p. 1000 water with CN brought on patterns similar to those which resulted from decrease of 32 p. 1000 seawater to 1 p. 1000 in Fig. I,3. Animals in 9 p. 1000 water with CN received nearly 33 p. 100 more cyanide than did those in 16 p. 1000 water with CN.

Fig. II,5 demonstrates the effect of difluorodinitrobenzene (DFDNB), alone and combined with reduced levels of calcium, on volume changes under conditions of lowered salinity. In the first instance, animals were placed in 32 p. 1000 seawater containing 2.8 mM DFDNB for 60 minutes prior to dilution with distilled water. In the second, animals were placed in 32 p. 1000 Ca^{++} free seawater for 30 minutes before dilution with distilled water containing 2.8 mM DFDNB. In a third case, serving as control for the effect of the fluoride ion, when not present in an aromatic compound, animals

were placed in 32 p. 1000 seawater containing 5 mM NaF for 60 minutes prior to dilution with distilled water. Decrease in drop salinity from 32 p. 1000 seawater with DFDNB to 1 p. 1000 resulted in decreased volume, rapid at first but slower and more sustained thereafter. Decrease in drop salinity, from 32 p. 1000 Ca^{++} free seawater to 6 p. 1000 with DFDNB, resulted in a curve of increased volume. The curve rose quickly under the influence of reduced levels of calcium, soon leveled off as the difluorodinitrobenzene began to take effect, and then returned to the original volume in a total of 150 minutes. Finally, decrease in drop salinity from 32 p. 1000 seawater with F to 1 p. 1000 resulted in a curve nearly identical with that produced by simple dilution of 32 to 1 p. 1000 in Fig. II, 3.

Using the formulae,

$$f = \frac{\log_n \left(\frac{V_t}{V}\right)}{t\,(\pi i - \pi o)\,\log_n \left(\frac{A_t}{A}\right)}$$

$$\text{and } f' = \frac{\log_n \left(\frac{V_t}{V}\right)}{t \log_n \left(\frac{A_t}{A}\right)},$$

$\log_n$ rates of net flux in 10^{-3} $\mu m^3/\mu m^2$ osm sec can be approximated from log phases of anisosmotic and isosmotic curves of volume change (where V is volume in μm^3 at beginning of log phase, V_t is volume in μm^3 at end of log phase, t is duration of log phase in seconds, $\pi i - \pi o$ is difference between inside and outside concentrations in osmoles, A is surface area in μm^2 at beginning of log phase and A_t is surface area in μm^2 at end of log phase).

Results of this analysis are shown in Table I. The term anisos-

TABLB I

Directions and rates of anisosmotic and isosmotic net fluxes resulting from the altering of conditions outside *Turbanella ocellata* relative to those inside.

	Outside conditions *	Direction of net flux	Log_n rates of net flux 10^{-3} $\mu m^3/\mu m^2$ osm sec
Anisosmotic	9, NaCN	In	68.5
	6, Ca^{++}free DFDNB	In	62.9
	6, Ca^{++}free	In	59.6
	1, NaF	In	50.8
	1	In	50.0
	16, NaCN	In	49.4
	11, Ca^{++}free	In	37.4
	3	In	27.0
	1, DFDNB	In	25.0
	11	In	18.5
	66	Out	15.4
Isosmotic	16, NaCN	Out	4.7
	11, Ca^{++}free	Out	2.4
	6, Ca^{++}free DFDNB	Out	1.6
	66	In	1.6
	11	Out	1.2
	3	Out	1.0

(*) Inside conditions, all 32 p. 1000; salinities p. 1000.

motic flux is used in cases of either hyper- or hypo-osmotic conditions, where solvent flux opposes solute flux. The term isosmotic flux is reserved for cases of isosmotic conditions where solvent flux parallels solute flux. As can be seen from the table, anisosmotic fluxes are considerably larger, though shorter lived, than isosmotic fluxes. Net anisosmotic fluxes decrease with increased salinity (1, 3, 11, 66 p. 1000), whereas isosmotic fluxes tend to decrease with decreased salinity (66, 11, 3 p. 1000). On the other hand, net anisosmotic fluxes increase with decreased salinity when dilutions involve cyanide or reduced levels of calcium; and larger fluxes, both anisosmotic and isosmotic, are associated with cyanide than with reduced levels of calcium for a given outside salinity.

It is clear from Fig. II and from Table I that *Turbanella ocellata* is a volume regulator, utilizing changes in body volume to accomodate for anisosmotic differences in salinity between internal and external fluids. Over time the fluids become isosmotic and body volume tends to return to normal, presumably by means of joint solute—solvent flux. It is contended here that solute flux is not restricted to isosmotic conditions but begins as soon as an anisosmotic gradient appears between inside and outside conditions. Further, in cases of hypo-osmotic conditions, I believe the net inward anisosmotic flux to be a resultant of three separate fluxes: a passive outward solute flux; an active outward solute flux (involving the utilization of energy) ; and a passive inward solvent flux (larger than the combined outward fluxes, accounting for the net inward flux and resulting increased body volume). Active transport along an electrochemical gradient, such as that postulated here, has been termed facilitated diffusion (Potts and Parry, 1964).

The recovery curves of animals under the hypo-osmotic conditions of Fig. II, 3 give evidence that solutes leave the animal as a result of pressure exerted by an expanded cuticle. And ironically, it is the recovery curve of dead animals under the hyper-osmotic conditions of Fig. II, 3 which gives evidence that solutes can also enter the body in the absence of this sort of pressure. If solutes can pass into or out of the animal isosmotically, they probably can also do so when supported by an anisosmotic gradient. Evidence for anisosmotic passage of solutes is derived from the DFDNB experiments. Difluoro-dinitrobenzene tends to bind tightly to amino acids by means of fluorine exchanged cross-linkages, spaced some 5 Å apart (Berg *et al*, 1965). This action, which appears to be similar on micrometazoan cuticle as on erythrocyte membrane, consists of sufficient overlapping cross-linkages to exclude the passage of water molecules (3 Å diam.), though allowing for the passage of potassium (1.5 Å diam.) and sodium (1 Å diam.) ions. Thus the 1 p. 1000 DFDNB anisosmotic flux can be taken as a measure of passive solute flux. Graphing a linear relationship (y p. 1000 = 32 p. 1000 — 2.5 x) between outside salinity and passive solute flux, allows estimates to be made of flux rates at intermediate salinities.

Cyanide is well known as a metabolic poison, which incapacitates the aerobic electron transport system. Active transport is evidenced in *Turbanella ocellata* by the proportional increase of net flux rates with decreasing outside salinity in the presence of cyanide. The effect

of cyanide in allowing increased net inward flux would be through the cessation of active outward transport of solutes. Hypo-osmotic net flux rates at 11 and 3 p. 1000 form a second linear relationship (y

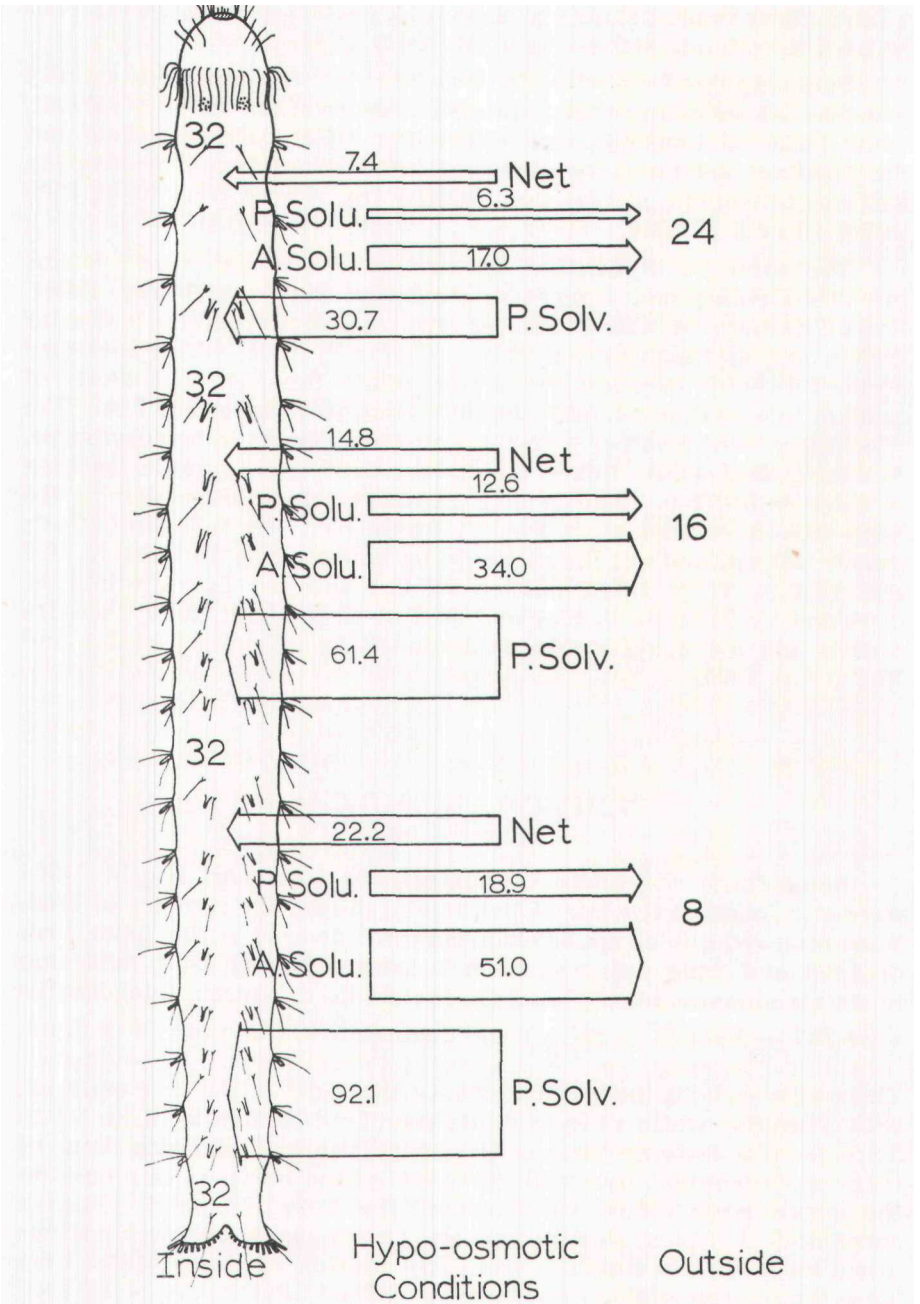

FIG. Ill

Turbanella ocellata

Component log» flux rates, directions and resultant net flux (10^{-3} $\mu m^3/\mu m^2$ osm sec) with hypo-osmotic dilutions from 32 to 24, 32 to 16 and 32 to 8 p. 1000 salinity P. *Solu* is passive solute; A. Solu is active solute; P. Solv is passive solvent

p. 1000 = 32 p. 1000 — 2.2 x) and can be used to estimate net flux rates at intermediate outside salinities. If net flux rates for 16 and 9 p. 1000 in the absence of cyanide are subtracted from net flux rates found for those salinities in the presence of cyanide, the results form a third linear relationship (y p. 1000 = 32 p. 1000 — 0.9 x), which can be used to estimate active solute flux rates.

Summing the three estimated flux rates for a given outside salinity provides an estimate of the inward flux rate for passive solvent. From these relationships, component flux rates, their directions and the resultant net flux rate were calculated, which would accompany anisosmotic volume increases in animals transferred from 32 p. 1000 to 24, 16 or 8 p. 1000. These are illustrated in Fig. III.

The presence of calcium in membrane and cuticular systems provides stability and reduces permeability (Potts and Parry, 1964). Use of Ca^{++}free seawater tends of leach calcium from the cuticular system and increases permeability. Unlike cyanide, which increases permeability by stopping the active solute flux, reduced levels of calcium increase permeability by increasing passive solvent flux. The effect of reduced levels of calcium, however, appears to be curvilinear. Assuming that solute fluxes remain constant, a 30 minute leaching of calcium in 32 p. 1000 Ca^{++}free seawater before immersion in the hypo-osmotic conditions of Fig. III would have the following effect: passive solvent and net fluxes would be increased by 10 units to 40.7 and 17.4 at 24 p. 1000; passive solvent and net fluxes would be increased by 24 units to 85.4 and 38.8 at 16 p. 1000; finally, passive solvent and net fluxes would be increased by 50 units to 142.1 and 72.2 at 8 p. 1000.

NOTES ON KINORHYNCHA

Some thirty specimens of *Echinoderes bookhouti* Higgins 1964 were also found in the interstitial habitat during the summer of 1966. They occurred 0 to 30 cm below mean low tide level in the upper 2 cm of sand and were obtained from substrata having both light and moderate amounts of detritus. Of five adults measured, total lengths were 307 ± 5 um ($\bar{X}$ ± SE) and maximum widths were 74 ± 2 um. These were ca 0.9 x the length and 0.8 x the width of the type material, with a length: width ratio of 4.1:1, compared to a ratio of ca 3.4 to 3.8:1 for the type material. The small size and elongate form of these specimens correlate with their interstitial habit, as compared to the larger, more robust specimens of the epibenthic mud. Such a correlation between substratum and morphology requires serious attention in future studies. Oven dry weights of three adults were 2.0 ± 0.1 ug; dry weight of one juvenile (140 um total length) was 0.5 ug. Tests with 2-3 adults per run in a Kirk differential microrespirometer showed a rise in oxygen consumption from 7 nl/animal h (3.5 nl O_2/ug dry wt h) in a 50 minute run at 26°C, 32 p. 1000, to 23 nl/animal h (11.5 nl O_2/ug dry wt h) in a 100 minute run at 34°C, 32 p. 1000.

Acclimatization conditions were 48 hours at 27°C, 32 p. 1000. Three 24-hour tests of ISLL (connected E's) are plotted in Fig. I, 2 for data from a series of twelve adults acclimatized 48 hours at 27°C, 32 p. 1000; a single 24-hour incipient lethal level (J) is also plotted for data from two juveniles subjected to the same acclimatization conditions as those used for the adults.

Three adult specimens of *Trachydemus langi* Higgins, 1964 were found in the same biotope. Measurements showed total lengths of 591 ± 21 um and maximum widths of 136 ± 4 um. These were of nearly the same length and about 0.8 x the width of the type material; they had a length: width ratio of 4.3:1 compared to a ratio of ca 3.6:1 for the type material. Oven dry weights of the three adults were 5.5 ± 0.1 ug. No respiratory data were obtained. A single test of ISLL (T) is plotted in Fig. I, 2 for data from the three adults, after 48-hour acclimatization conditions at 27°C, 32 p. 1000.

Acknowledgements

I would like to express appreciation to the Duke University Marine Laboratory and to Drs. John Vernberg, Winona Vernberg and Frederick Kalber, for material and technical assistance in this work.

Summary

Respirometry measurements show that the adult macrodasyid gastrotrich *Turbanella ocellata* uses 4 to 8.5 nl O_3/animal h (5.7 to 12 nl O_2/*ug* dry wt h) at 14 to 27 °C, 32 p. 1000. These values are higher than comparable values for most other marine meiobenthic animals. Oxygen consumption decreased with greater or lesser salinities than those to which animals were acclimatized.

Incipient somatic lethal limits over 24 hours exceed 10 to 60 p. 1000 over the range of seasonal ambient water temperatures, 14 to 27 °C. Both temperature and salinity adjustment to changed thermal conditions are demonstrated for this eurytopic species.

T. *ocellata* is a volume osmotic regulator. Rapidly increasing or decreasing body volume is in response to anisosmotic salinity gradients between internal and external fluids. This is followed by a gradual return of body volume to normal, presumably by means of isosmotic solute-solvent flux. Log» rates of net flux are 15 to 50 X lO^{-3} um/um^2 osm sec for simple anisosmotic changes from seawater and 1 to 15 for subsequent isosmotic adjustment. Evidence is presented that net inward flux under hypo-osmotic conditions is a resultant of three separate fluxes: passive outward solute flux, active outward solute flux and passive inward solvent flux.

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