WATER TRANSPORT AND OXYGEN CONSUMPTION IN *ASCIDIELLA ASPERSA* IN RELATION TO SIZE AND SALINITY

V. R. PRAKASAM AND JAYAPAUL AZARIAH*

Department of Zoology, F.M.N. College, Quilon-691 001
**Department of Zoology, University of Madras, Madras*-600 025

ABSTRACT

A size dependent oxygen consumption of *Ascidiella aspersa* was registered in sub and supra normal salinities of 30, 25, 20 and 40‰. Further a decrease in oxygen uptake occurred at both sub and supra normal salinities. It was found that the percentage oxygen uptake of small animal was higher than large animal. In subnormal salinities *A. aspersa* showed contraction of its body and siphons.

The amount of water transported by *Ascidiella aspersa* was a function of size in 34‰ and 30°C, the regression coefficient being 0.6975. The volume of water transported decreased with decrease or increase of salinity, but the size dependent pattern of water transport was noted in all salinities except 20‰ where there was no increase in transport with increase in size of animal. The pumping rate of larger animal was comparatively lower than that of small animal. The low water transport was associated with partial closure of siphons and shrinking of the body.

Calculation of the amount of water transported per ml of oxygen consumed by the animal showed decrease in sub and supra normal salinities, but the decrease was not proportionate to the corresponding decrease in salinities. The amount of oxygen uptake per litre of water transported was found to increase with change in salinities. The physiological stress due to reduction in salinity was observed to be compensated by the increase in the efficiency of oxygen extraction. However, it was found that such an increase in efficiency was not proportional to the magnitude of decrease in salinity. It is likely that decrease in water transport alone may not account for the low oxygen uptake by the animal in sub and supra normal salinities.

INTRODUCTION

IT IS KNOWN that variations in salinity may modify the rate of metabolism of aquatic invertebrates. Animal may show varied responses to changes in salinity like increase or decrease in oxygen uptake or may remain unaffected (Kinne, 1971). Intertidal sedentary organisms such as *Metridium marginatum* (Shoup, 1932), *Mytilus edulis* (Kinne, 1971). and *Balanus tintinnabulum* (Rao and Ganapati, 1952) show a decrease in metabolic rate with dilution of sea water medium. In the present study an attempt was made to investigate the effect of various sub and supra normal salinities on the metabolism of a sedentary, intertidal ascidian *Ascidiella aspersa* Müller.

For the process of feeding and respiration ascidians maintain a current of water through their body cavity (Das, 1957 ; Jorgensen, 1966 ; Goodbody, 1974). Feeding and water propulsion of aquatic animals are qualitatively and quantitatively influenced by sudden variations in salinity (Kinne, 1964, 1971). Salinity changes are also known to bring about contrac-

tion of body and partial closure of siphons of *Ascidiella aspersa* (Prakasam, 1978). Water transport by way of squirting was also reported to be negligible in the same species (Prakasam and Azariah, 1978). Although a number of studies have been carried out to estimate the rate of water transport in ascidians (Hecht) 1916 ; Jorgensen, 1949 ; Hoyle, 1953 ; Carlisle, 1966 ; Holmes,ʳ1973 ; Goodbody, 1974) no attempt has been made to study the influence of salinity changes on water transport. In the present study it was also investigated whether the morphological changes of *A. aspersa* at subnormal salinities are related to physiological functions like water transport. In doing so, the rate of water transport with reference to size was also attempted.

A number of studies have been carried out in filter feeders interpreting the relationship between water transport and the energy spent for the same. Hamwi and Haskins (1969), Verudin (1969) and Loveland and Chu (1969) related metabolism of *Mercenaria mercenaria* with the energy spent for transport of water through the gills of the animal. Newell and Pye (1970) suggested that greater part of oxygen consumption of *Mytilus edulis* could be used in work done by ciliary feeding. The reviews of Das (1957), Jorgensen (1966), Goodbody (1974) emphasize that the maintenance of a current of water through the body cavity of ascidians is mainly for purposes of feeding and respiration. In contrast to the observations of earlier workers, Jorgensen (1975) based on the results of his study on bivalves, found it hard to rationalise the relationship between water transport and energy requirements for creating water current. In view of this, it was also attempted to investigate the relationship between rate of water transport and rate of oxygen uptake in the ascidian *Ascidiella aspersa*.

MATERIALS AND METHODS

Specimens of *Ascidiella aspersa* Müller were obtained from the coast of Madras. Animals collected were maintained in glass troughs containing normal sea water ($34\%_0$) at room temperature ($30°C$). About 24 hrs after collection, animals were used in respiration experiments. The respiration was measured in a constant flow apparatus described by Fry and Hart (1948) and modified by Job (1955). The oxygen content was determined by Microwinkler's method (Welch and Smith, 1949).

Since the salinity tolerance of *Ascidiella* ranged from 20 to $40\%_0$, experiments for the determination of oxygen uptake were confined to salinities of 40, 34, 30, 25 and $20\%_0$. Animals of varying sizes (20.8 to 92 mg dry weight) were acclimated for 3 days in the respective salinity. Sea water was changed once in 24 hrs during the course of acclimation period. After 3 hrs of equilibration in the experimental chamber, 10 readings were taken in a period of 8 hrs and the average was calculated to representt he oxygen consumption at that salinity. In all cases the transfer of animals was abrupt from normal sea water medium except at $20\%_0$ where animals used were transferred to $20\%_0$ after 24 hours of acclimation to $25\%_0$. This was found necessary in view to heavy mortality due to direct transfer. The data were subject to statistical analysis to obtain regression coefficients and regression lines.

For comparison of oxygen uptake at various salinities, the oxygen uptake of the animal at normal salinity ($34\%_0$) and normal temperature ($30°C$) was taken as 100 and the values to be compared are represented as percentage of this basal value. The oxygen uptake rates of small and large animals were compared by arbitrarily choosing 2.0 mg and 70.0 mg dry weight, the former representing a small animal and the latter a large one. The oxygen uptake rates of these animals were calculated from regression formula.

Active specimens of *Ascidiella aspersa* Müller, ranging in size from 6.1 mg to 60.5 mg dry

weight were used in the study of water transport. The water transport was measured following the colloidal graphite method described by Jorgensen (1943). The graphite suspensions were prepared in clean beakers containing 200 ml of sea water of desired salinity and one animal was introduced into each experimental beaker. Control was also set up without the animal. The concentration of the suspension was measured, after one to three hours, using an Erma photoelectric colorimeter. The percentage clearances were read out from a standard graph and the rate of propulsion was calculated.

The transport was measured at normal salinity (34‰) and at subnormal salinities of 30, 25 and 20‰ and at a supranormal salinity of 40‰, after 3 days of acclimation of the animals in the respective salinity. Except in the case of 20‰, where animals were transferred after a given period of acclimation in 25‰, in all the other instances transfer of animals to varying salinities was abrupt.

Comparison of water transport rate at various salinities was made by taking the water transport at normal salinity (34‰) and normal temperature to (30°C) at 100 (basal value) and the other values are expressed as percentage of the basal value. The transport rate of small and large animals was compared by arbitrarily choosing 20.0 mg and 70.0 mg dry weight, the former representing a small animal and the latter a large ascidian. The water transport rates of 20.0 mg and 70.0 mg animals were obtained by calculation from regression graph.

From the values of oxygen uptake and water transport, calculations were made to obtain oxygen consumed per litre of water transported, water transported per ml of oxygen consumed and extraction efficiency (Thompson and Bayne, 1972).

RESULTS AND DISCUSSION

It was found that a size dependent oxygen uptake took place at all sub and supra normal salinities (Fig. 1). The values obtained were
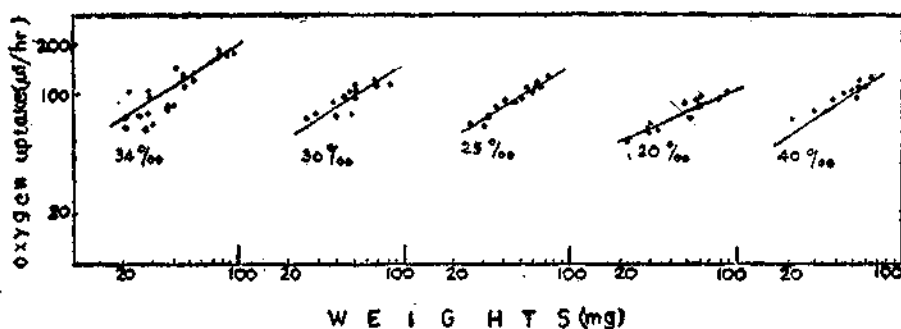

FIG. 1. Oxygen uptake of *Ascidiella aspersa* as a function of size in various salinities (40, 34, 30, 25 and 20‰).

The term ' water transport ' used in the text refers to the total amount of water transported per animal and ' water transport rate ' denotes the water transported per unit weight. In order to assess the relationship between water transport and body size, the data obtained with various sized animals were subjected to regression analysis.

0.6267, 0.5865, 0.5738, 0.5143 and 0.5728 respectively for salinities of 34, 30, 25, 20 and 40‰. A decrease in oxygen uptake was noticed with a corresponding decrease or increase in salinity from normal of 34‰. However, statistical analysis of coefficients at various salinities showed no significant difference at 5% level. The oxygen uptake rates

2

of animals, weighing 20.0 mg and 70.0 mg acclimated to varying salinities are given in Table 1 and Fig. 2. It can be seen that the percentage oxygen uptake of small animals was higher than large animals in all sub and supra normal salinities.

TABLE 1. *Oxygen uptake (μl/hr) of* Ascidiella aspersa *weighing 20.0 mg and 70.0 mg in normal salinity (34‰) and acclimated to various salinities (30, 25, 20 and 40‰). The values are calculated from regression formula*

Salinity (‰)	Dry weight of animal	Oxygen uptake		
		μl/hr	μl/hr/mg	% of the basal value
Normal				
34	20	68.31	3.41	100.00
	70	149.80	2.14	100.00
Subnormal				
30	20	58.55	2.93	85.71
	70	122.10	1.74	81.50
25	20	58.42	2.92	85.52
	70	119.20	1.71	80.04
20	20	52.34	2.62	76.62
	70	99.68	1.42	66.54
Supra normal				
40	20	63.27	3.16	92.62
	70	129.60	1.85	86.51

It is known that variations in ambient salinity may alter the rate functions of marine invertebrates. According to Kinne (1964), the metabolic rates of animals may decrease or increase or remain unchanged in new salinities. The pattern of oxygen uptake observed in *A. aspersa* appears to fit into the type IV of Kinne (1964) in that the oxygen uptake rates decrease whenever the animals are moved to a new salinity. Shoup (1932) reported a decrease in oxygen uptake in sea anemone *Metridium marginatum*, with a decline in salinity gradient

It also showed marked contractions of its body in 10% and hypertonic sea water. Similar was the case in *A. aspersa* at subnormal salinities. Organisms may also respond to changes in salinities by closure of shells and withdrawal of sensitive parts (Kinne, 1964). A decrease in metabolic rate associated with closure of shells due to changes in salinity was reported in *Mytilus edulis* (Potts and Parry, 1964 ; Bayne, 1973; Kinne, 1971 ; Bass, 1977). In the case of *A. aspersa* also siphonal contraction or closure was observed along with the decrease in oxygen uptake. Thus, it may be reasonable to correlate the siphonal contraction of *A. aspersa* with reduction in oxygen uptake under reduced salinity conditions. Siphonal contraction may also mean low transport of water through its body cavity.
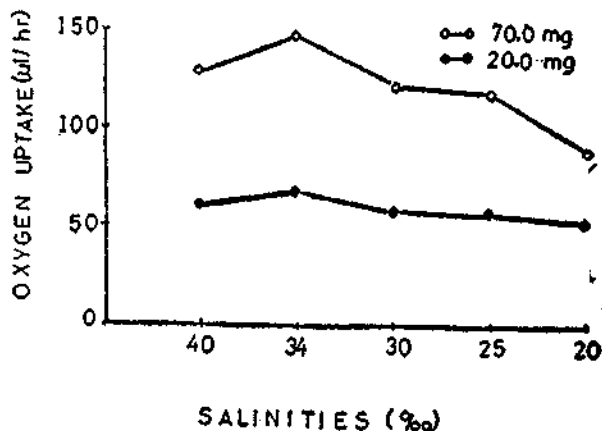

FIG. 2. Oxygen uptake rate of *A. aspersa* weighing 20.0 mg and 70.0 mg at a temperature of 30°C in different salinities (30, 25, 20 and 40‰), the rates being calculated from regression lines. Values obtained following three days of acclimation to the respective salinities are used in the construction of the graph.

At normal salinity and temperature, it was found that the amount to water transported (ml/hr) showed a corresponding increase with increse in size of the animals, indicating that water transport is a function of size and the rate of water transport (ml/hr/mg) showed a

decrease with increase in weight. On regression analysis b value of 0.6975 was obtained. Although not directly comparable, the present observation is similar to the findings of Holmes (1973) in the same species in that the rate of water transport is size dependent.

The rate of water transport of *A. aspersa* as a function of size in various salinities is illustrated in Fig. 3. The regression coefficients (b values) obtained were 0.6975, 0.5674, 0.3497, 0.1390 and 0.5537 for salinities 34, 30, 25, 20 and 40%₀ respectively. The rates of
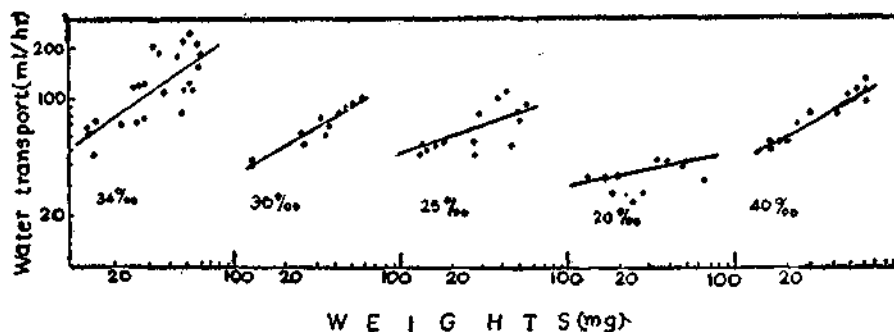

FIG. 3. Water transport of *A. aspersa* as a function of size in various salinit es (40, 34, 30, 25, 20%₀).

TABLE 2. *Water transport (ml/hr) of Ascidiella aspersa, weighing 20.0 mg and 70.0 mg in normal salinity (34%₀) and in various acclimation salinities (30, 25 20 and 40%₀). The values are calculated from regression formula*

Salinity (%₀)	Dry weight of animal (mg)	Water transport		
		ml/hr	ml/hr/mg	Basal value (%)
Normal				
34	20	84.45	4.22	100.00
	70	202.30	2.89	100.00
Subnormal				
30	20	55.64	2.78	65.88
	70	113.30	1.61	56.00
25	20	63.90	3.19	75.66
	70	99.04	1.41	48.95
20	20	37.96	1.89	44.94
	70	45.19	0.64	22.33
Supranormal				
40	20	63.56	3.17	72.26
	70	127.20	1.81	62.87

water transport of 20.0 mg and 70.0 mg animals acclimated for 3 days to different salinities are given in Table 2 and Fig. 4. It can be seen from the figures that water transport is a function of size in various sub and supra normas salinities excpet in 20%₀ where there was no proportionate increase in water transport corres, ponding to the increase in size of the animal. However, the volume of water transported decreased with decrease or increase of salinity. It was also found from the present study that in all sub and supra normal salinities, the water transport was dissimilar for small and large animls, the former showing a large percentage of water transport than the latter.

Similar decreases in transport water have been reported in other animals with change in salinities. In *Martesia*, Nagabhushanam (1956) observed a reduction in trasport rate with decrease in salinity. Employing the neutral red technique, Nagabhushanam and Sarojinil (1965 a, b) reported that at 18%₀ and 26°C, *Branchidontes recurves* removes within one hour about 51% of neutral red, whereas at 6%₀ it removes only 8% in one hour and this

was similar to their results with the lamelli-branch *Mulinia lateralis.*

Thus the shrinking and partial closure of siphons of *A. aspersa* in sub-normal salinities are indicative of the low rate of water transport by the animals. The poor rates of water pumping may be due to salinity induced disturbances in metabolic rate as stated by Vernberg and Vernberg (1972).

consumed decreases in sub and supra normal salinities. Further the decrease in the water transport per ml of oxygen consumed is not proportionate to the corresponding decrease in salinities.

It may be noted that the oxygen uptake per litre of water transported increased in both sub and supra normal salinities
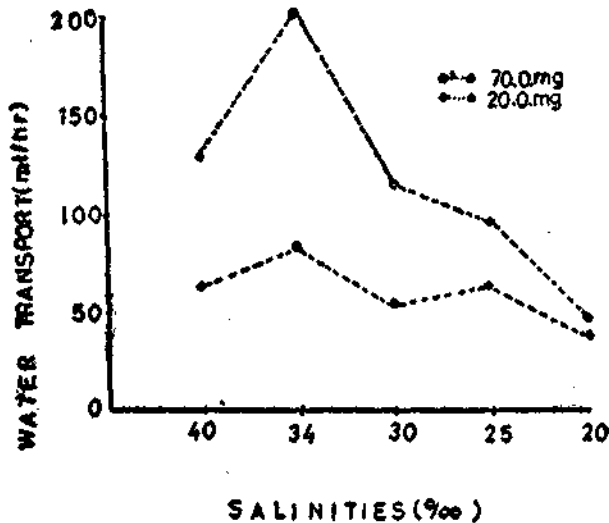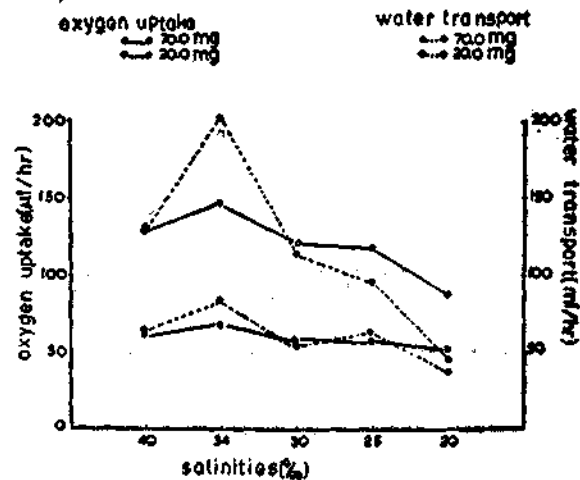

FIG. 4. Water transport rate of *A. aspersa* weighing 20.0 mg and 70.0 mg at a temperature of 30°C in different salinities (34, 30, 25, 20 and 40‰), the rates being calculated from regression lines. Values obtained following three days of acclimation to the respective salinities are used in the construction of the graph.

FIG. 5. Comparison of oxygen uptake rate and water transport rate of *A. aspersa,* weighing 20.0 mg and 70.0 mg, at a temperature of 30°C, in different salinities (30, 25, 20 and 40‰), the rates being calculated from regression lines. Values obtained following three days of acclimation to the respective salinities are used in the construction of the graph.

The data comparing oxygen uptake and water transport of animals weighing 20.0 mg and 70.0 mg in various salinities are illustrated in Fig. 5. It can be seen that both oxygen uptake and water transport rates show a decrease in sub and supra normal salinities.

The amounts of water transported per ml of oxygen consumed by 20.0 mg and 70.0 mg *Ascidiella* in various salinities are given in Table 3 and illustrated in Fig. 6. It can be seen that the water transport per ml of oxygen

thereby suggesting that there was variation in the amount of oxygen uptake, eventhough there was a reduction in water transport following changes in salinity.

Since it was found that the amount of water transported per unit amount (1 ml) of oxygen consumed decreases in sub and supra normal salinities, it is likely that there may be alterations in the oxygen extraction efficiency of animals for survival in lower salinities On comparison of the extraction efficiency at normal salinity with various other

salinities, it can be seen that the efficiency showed an increase of 6.14%, 2.77% and 15% in the respective salinities of 30, 25 and 20%₀ for an animal weighing 20.0 mg (Table 3).

tively for a small (20.0 mg) and large (70.0 mg) animal. It could be inferred that although there was an increase in efficiency of removal of oxygen with the decrease or increase of

TABLE 3. *Water transport to oxygen uptake of* A. aspersa, *weighing* 20.0 *mg and* 70.0 *mg in various salinities at* 30°C

Salinity (%₀)	Dry. wt. of animal (mg)	Oxygen uptake (μl/hr)	Water transport (ml/hr)	Oxygen consumed per lit. of water transported $\frac{D}{C} \times 1000 (\mu l)$	Water transported per ml of O₂ consumed $\frac{D}{C} \times 1000$ (l)	Extraction efficiency O₂ cons. ———— × 100 O₂ avail.
Normal	20	68.31	84.45	809	1.23	21.28
34	70	149.90	202.30	740	1.35	19.49
Subnormal	20	58.55	55.64	1062	0.96	27.69
30	70	122.10	113.30	1078	0.93	28.35
	20	58.42	69.90	914	1.19	24.05
25	70	119.90	99.04	1211	0.83	31.85
	20	52.34	37.96	1379	0.72	36.28
20	70	99.68	45.19	2206	0.45	58.04
Supranormal	20	63.27	63.56	995	1.00	26.10
40	70	129.60	127.20	1019	0.98	26.81

Similarly the extraction efficiency of a 70.0 mg animal increased by 8.86, 12.36% and 38.55% from the normal in salinities of 30, 25 and 20%₀ respectively. Likewise, by an increase in salinity to 40%₀, the efficiency also increased from the normal by 4.91% and 7.32% respec-

salinity, such increase was not proportionate to the successive decrease or increase in salinity by a margin of 5%.

It could be derived from the foregoing sections that the oxygen uptake of *A. aspersa* in various

salinities is independent of the amount of water transported. Vernberg and Vernberg (1972)
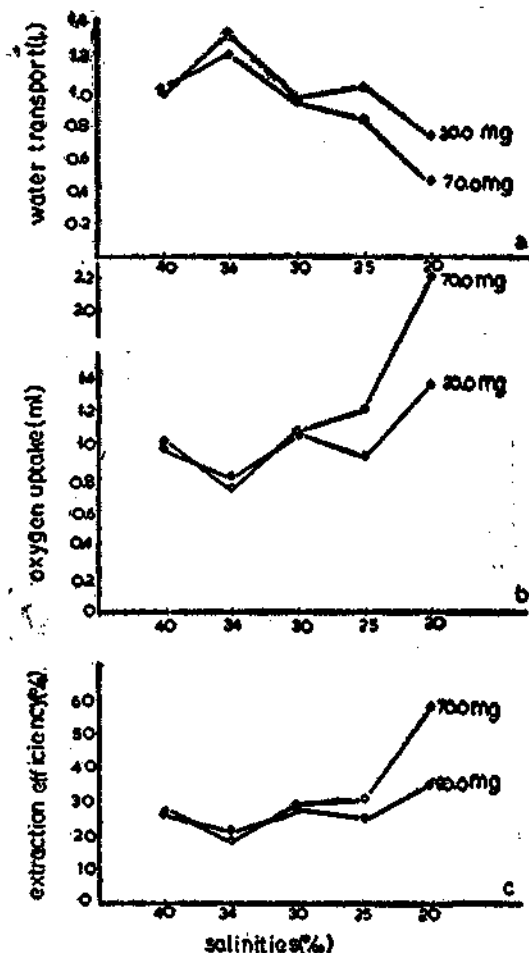

Fig. 6. The relationship between rate of oxygen uptake and rate of water transport in different salinites in *A. aspersa* weighing 20 and 70 mg : a. water transported (l/ml) of O_2 consumed, b. oxygen uptake (ml/l) of water transported and c. Extraction efficiency (%).

stated that at salinity induced disturbances in metabolic rate may lead to insufficient rates of water pumping. However, since it was found that the oxygen uptake per litre of water transported increased in subnormal salinities, a derivation of the relationship between the oxygen uptake and water transport in terms of energy requirements was found difficult. It can also be noted in the present study that the water transported per ml of oxygen consumed was nor proportionate to the corresponding decrease in salinities. Jorgensen (1975) reported that in bivalves, the energy expenditure due to transporting ciliated cells may constitute about 1% of the total oxygen uptake. Assuming similar condition in ascidian, it is unreasonable to relate the reduction in oxygen uptake as due to decrease in ciliary activity if any, resulting from reduction in salinity. The observation of Van Winkle (1972) in bivalves that with a decrease in ciliary activity, there was an increase in the oxygen consumption lend further support to the above contention. It may be added in this connection that the ciliary covering of the lateral edges of the pharyngeal ostia of *A. aspersa* is responsible for creating a water current in ascidian Jorgensen, (1966). It is likey that decrease in water transport alone may not account for the low oxygen uptake of the animal. However, the evidences presented in the study do not preclude the possibility that reduction in water transport might have been one of the factors for the low oxygen uptake of the animal in sub and supra normal salinities.

REFERENCES

BASS, E. L. 1977. Influence of temperature and salinity on oxygen consumption of tissues in the American Oyster (*Crassostrea virginica*). *Comp. Biochem. Physiol.*, 58 : 125-130.

BAYNE, B. L. 1973. Oxygen consumption by three species of lamellibranch molluscs in declining ambient oxygen tension. *Ibid.*, 40 A : 955-970.

CARLISLE, D. B. 1966. The cilliary current of *Phallusia* (Ascidiacea) and the squirting of sea squirts. *J. Mar. Biol. Ass. U. K.*, 46 : 125-127.

DAS, S. M. 1957. *Herdmania (The monascidian of the Indian seas)*. Indian Press (Publication) Private Ltd., Allahabad.

FRY, F. E. J. AND J. S. HART 1948. The relation of temperature to oxygen consumption in the gold fish. *Biol. Bull.*, 94 : 66-77.

GOODBODY, I. 1974. The physiology of Ascidians. *In :* F. S. Russell and C. M. Yonge (Ed.) *Advances in Marine Biology*, 12 : 1-149.

HAMWI, A. AND H. H. HASKINS 1969. Oxygen consumption and pumping rates in the hard clam *Mercenaria mercenaria* : A direct method. *Science*, 163 : 823-824.

HECHT, S. 1916. The water current produced by *Ascidia atra* Lesueur. *J. exp. Zool.*, 20 (3) : 429-434.

HOLMES, N. 1973. Water transport in the ascidians *Styela clava* Herdman and *Ascidiella aspersa* (Müller). *J. exp. Mar. Biol. Ecol.*, 11 : 1-13.

HOYLE, G. 1953. Spontaneous squirting of an ascidian *Phallusia mammilata* (Cauvier). *J. Mar. Biol. Ass. U.K.*, 31 (3) : 541-562.

JOB, S. V. 1955. The oxygen consumption of *Salvelinus frontinalis*. *University of Toronto Biological Series*, 61 : 1-39.

JORGENSEN, C. B. 1943. On the water transport through the gills of bivalves. *Acta Physiol. Scand.*, 5 : 297-304.

———— 1949. Feeding rate of sponges, Lamellibranchs and ascidians. *Nature, London*, pp. 163-912.

———— 1966. *Biology of suspension feeding*. Pergamon Press, Oxford.

———— 1975. Comparative physiology of suspension feeding. *Ann. Rev. Physiol.*, 37 : 57-79.

KINNE, O. 1964. The effect of temperature and salinity on marine and brackishwater animals. 11 salinity and temperature — salinity combinations. *Oceanogr. Mar. Biol. Ann. Rev.*, 2 : 281-339.

———— 1971. Salinity. *In :* O. Kinne (Ed.) Marine Biology, 1 (2) : 821-995.

LOVELAND, R. E. AND D. S. K. CHU 1969. Oxygen consumption and water movement in *Mercenaria mercenaria*. *Comp. Biochem. Physiol.*, 29 : 173-184.

NAGABHUSHANAM, R. 1956. The rate of water filtration in the marine wood boring Mollusc *Martesia striata* Linn. *Proc. Indian acad. Sci.*, 43 : 223.

———— AND R. SAROJINI 1965 a. Rate of water propulsion of *Mulinia lateralis* (Say) in relation to salinity and temperature (Mollusca : Lamellibranchiata). *Sci. Cult.*, 31 : 438-439.

———— AND ———— 1965 b. The rate of water propulsion by the mussel *Branchidontes recurvus* (Mollusca : Lamellibranchiata). *Indian J. Physiol. All. Sci.*, 19 : 1-4.

NEWELL, R. C. AND V. J. PYE 1970. Seasonal changes in the effect of temperature on the oxygen consumption of the winkle *Littorina littorea* (L) and the mussel *Mytilus edulis* (L). *Comp. Biochem. Physiol.*, 34 : 367-383.

POTTS, W. T. M. AND G. PARRY 1964. *Osmotic and ionic regulation in animals.* Pergamon Press, London.

PRAKASAM, V. R. 1978. Studies on the respiratory physiology of an ascidian *Ascidiella aspersa* Müller (Urochordata) of Madras Coast. *Ph.D. Thesis. University of Madras.*

———— AND J. AZARIAH 1978. Squirting and water transport in an ascidian with reference to salinity and oxygen uptake. *Proceeding of the VII Annual Conference of Ethological Society of India, University of Madras.* Abstract.

RAO, D. G. V. PRASADA AND P. N. GANAPATI 1952. Respiration in relation to salinity variation in intertidal barnacles. *Proc. Ind. Nat. Sci. Acad. Part B. Biol. Sci.*, 38 : 425-429.

SHOUP, C. S. 1932. Salinity of the medium and its effect on respiration in the sea anemone. *Ecology*, 8 : 81-85.

THOMPSON, H. J. AND B. L. BAYNE 1972. Active metabolism associated with feeding in the mussel *Mytilus edulis* (L). *J. exp. mar. Biol. Ecol.*, 9 : 111-124.

VAN WINKLE, W. JR. 1972. Ciliary activity and oxygen consumption of excised bivalve gill tissues *Comp. Biochem. Physiol.*, 42 A : 473-485.

VERNBERG, F. J. AND W. B. VERNBERG 1972. *Environmental Physiology of Marine animals.* Springer Verlag, Berlin, New York.

VERUDIN, J. 1969. Hard clam pumping rates : Energy requirements. *Science*, 166 : 1309-1310.

WELCH, J. H. AND R. J. SMITH 1949. *Laboratory exercises in invertebrate physiology.* Burgers Publishing Company, Minneapolis.