

Respiration, Pumping Activity and Heart Rate in *Ciona intestinalis* Exposed to Fluctuating Salinities*

S.E. Shumway

N.E.R.C. Unit of Marine Invertebrate Biology, Marine Science Laboratories; Menai Bridge, Gwynedd, N. Wales, UK

Abstract

Specimens of *Ciona intestinalis* L. were exposed to both gradual (sinusoidal) and abrupt (square-wave) salinity fluctuations and the changes in pumping activity and oxygen consumption monitored. Heart rate was monitored under steady state conditions using a new *in vivo* method, and pumping activity was monitored as spontaneous squirting by use of a pressure transducer. Oxygen consumption was also monitored under steady state conditions and under conditions of declining oxygen tension. It was found that during periods of regular beating the mean heart rate for 5 ascidians was 33.2 beats min^{-1} for the branchial pacemaker and 32.6 beats min^{-1} for the visceral pacemaker. Regular periods of beating lasted for approximately 1 min, followed by periods of irregular activity prior to heart beat reversal. The heart beat became irregular and reversal occurred sporadically when the ascidians were exposed to dilute seawater. The ascidians showed a pumping rate of 14 "squirts" per hour in full-strength seawater. This rate declined during decreasing salinity and ceased entirely when the external seawater concentration reached approximately 60% seawater (100% = 32‰ S). During periods of decreased salinity, the siphons were tightly closed and oxygen consumption was zero. The rate of oxygen consumption by *C. intestinalis* decreased during decreasing salinities and there was no evidence of an oxygen debt. Oxygen consumption under steady state conditions varied with body weight according to the following: oxygen consumption = $0.515 w^{0.831}$ (where w is body weight). The rate of oxygen consumption was found to be dependent on the external oxygen tension.

Introduction

Although ascidians are generally considered to be stenohaline osmotic conformers (Prosser, 1973), *Ciona intestinalis* is known to occur in waters of different salinities (Dybern, 1967). There is thus far no evidence for ionic or osmotic regulation in *C. intestinalis*, and it seems likely that specimens living in areas of constantly dilute seawater do not alter their osmotic or ionic concentration, but instead are physiologically adapted to life at low salinity from the time of settlement. On the other hand, *C. intestinalis* are also found in estuarine areas (MacGinitie and MacGinitie, 1968) and, lacking a physiological means of osmotic control, these ascidians must

have a behavioral adaptation if they are to survive in a changing environment. Such mechanisms have been demonstrated previously for marine bivalves and lugworms (Shumway, 1977a, b).

The obvious means of such a behavioral mechanism are the siphons. Hoyle (1952, 1953) studied spontaneous squirting in *Phallusia mammillata* Cuvier and the effect of mechanical stimulæ on these activities, but the effects of environmental factors such as salinity changes on this rhythm have not been investigated. Respiration in ascidians has been studied by Jørgensen (1952), Mangum and Burnett (1975) and Fisher (1976). Again, these studies were carried out under constant salinities.

The ascidian heart has been studied extensively by Kriebel (1967, 1968a, b, 1970) and Jones (1971). The heart of *Ciona* sp. is a long, V-shaped valveless tube, with a pacemaker at each end.

*Please address all requests for reprints to:
Dr. Sandra E. Shumway, 71 Clifford Street,
Taunton, Massachusetts 02780, USA.

Pumping action is produced by a peristaltic wave starting at one end and travelling to the other. The direction of the peristalsis reverses periodically (Kriebel, 1967). All the information to date has been collected from excised or exposed hearts and there is no information available concerning the function of the hearts of intact individuals.

The present study was undertaken to investigate the effect of salinity fluctuation on respiration, activity and heart rate in *Ciona intestinalis*.

Materials and Methods

Specimens of *Ciona intestinalis* L. were collected locally from the Anglesey (UK) coast and kept at 10°C in aquaria supplied with running seawater pumped from the Menai Strait (salinity approximately 32%). The ascidians were maintained in the laboratory for at least 1 week prior to use.

Oxygen consumption under conditions of constant salinity was measured with a radiometer oxygen electrode connected, via a radiometer PHM 71 meter, to a Smiths Servoscribe chart recorder (adjusted to 100 mV) as described by Davenport (1976). Results are expressed as ml O₂ consumed per hour per individual. Experiments ran until the ascidians had completely depleted the oxygen supply. Hourly readings were plotted at decreasing oxygen tensions created by the respiration of the ascidians in the sealed containers, as described by Sanders (1973). Changes in behavior when O₂ is reduced by the ascidian itself may be due to low pH or other metabolites excreted. Sassaman and Mangum (1973) monitored simultaneously the oxygen consumption of several species in closed containers and the pH of the water and found that the detectable increases in acidic metabolites were confined to oxygen levels below approximately 25 mm Hg; they concluded that the response of animals exposed to decreasing oxygen tension in sealed containers was due essentially to changes in the oxygen content.

A new technique was employed for the *in vivo* measurement of heart rate in *Ciona intestinalis*. The ascidians were placed under a binocular microscope, where the heart was clearly visible. Double-stranded bathythermograph wire (the Sippican Corp., Massachusetts, USA), used as the electrode, was threaded, one strand each, into small hand-sewing needles. The needles were then inserted one on each side of the beating heart. After ensuring that the electrodes were in position (Fig. 1A), the needles were

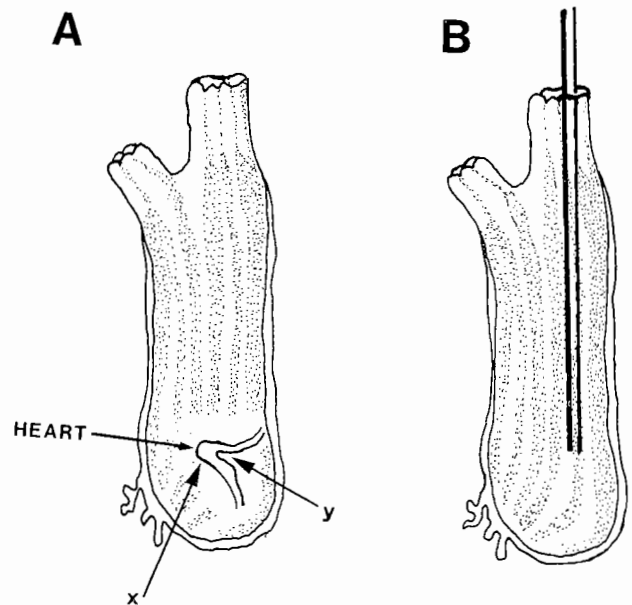


Fig. 1. *Ciona intestinalis*. (A) Position of electrodes for measurement of heart rates; (B) position of glass capillary tube for measurement of squirting rates

removed from the ascidians by pushing them through and cutting the wires; the opposite ends of the wires were then connected to an Oscillograph 400 MD/2 (George Washington Ltd., Sheerness, Kent, England). A sensitivity setting of 0.1 mV with a chart speed of 1.2 mm/sec gave the best recordings. Approximately 3 out of every 5 preparations were successful.

Squirting activity was monitored by inserting a thin glass capillary tube into the inhalent siphon (Fig. 1B). The capillary tube was then connected to an SE Laboratories SEM 4-82 pressure transducer and the changes in pressure recorded on a Servoscribe twin-channel recorder as used previously for recording activity in *Arenicola marina* (Shumway, 1977b). A recorder setting of 0.05 mV was used.

The apparatus used to produce fluctuating salinity regimes has been described by Davenport *et al.* (1975). Ascidians were subjected both to gradual (sinusoidal) and abrupt (square-wave) salinity changes. To simulate idealized near-tidal fluctuations, the programs ran for 24 h. The maximum seawater concentration was always 100% seawater (32‰ S); the minimum seawater concentration was 30% seawater (9.6‰ S) (see Figs. 6 and 8).

Fig. 2 (after Bettison and Davenport, *in preparation*) shows the apparatus used to monitor O₂ consumption during fluctuating salinity regimes. A single *Ciona*

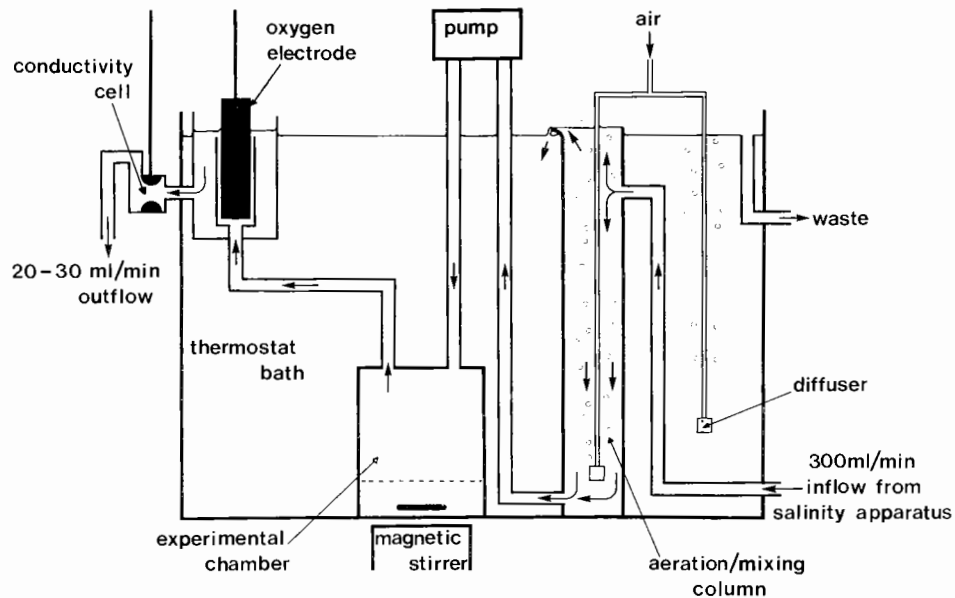


Fig. 2. Apparatus used for monitoring oxygen consumption during salinity fluctuations (from Bettison and Davenport, in preparation)

intestinalis of approximately 0.2 g dry weight (1.5 g wet weight) was placed in the experimental chamber and the oxygen consumption and salinity changes monitored simultaneously on a twin-channel recorder. Sample size for both sinusoidal and abrupt salinity changes was 5. The results were expressed as ml oxygen consumed per hour per gram dry weight of tissue.

All experiments were carried out at 10°C.

Results

Oxygen Consumption in Declining Oxygen Tension

A total of 30 experiments, with *Ciona intestinalis* over a size range of 0.01 to 1 g dry weight, were carried out, and the results are presented on logarithmic scales in Fig. 3. The relationship between oxygen uptake in virtually air-saturated water and the tissue dry weight in grams may be derived from the regression in Fig. 3 and expressed as: oxygen consumption = $0.515 \times \text{dry weight}^{0.831}$ (correlation coefficient = 0.923 for 29 degrees of freedom).

Fig. 4 shows the results of 6 individual experiments in which oxygen consumption was related to oxygen tension. In all of the individuals studied, the rate of oxygen consumption was directly dependent on the environmental oxygen tension.

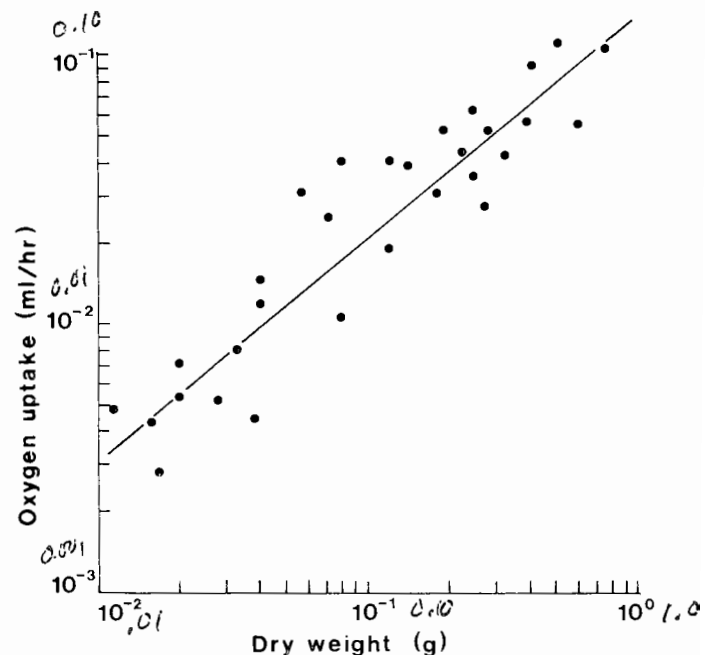


Fig. 3. *Ciona intestinalis*. Oxygen consumption in 100% seawater (= 32‰ S) at 10°C

Heart Rate

A typical heart rate trace is shown in Fig. 5A and an expanded trace in Fig. 5B. During periods of regular beating, the mean heart rate for 5 ascidians was $33.2 \text{ beats min}^{-1} \pm 0.8$ for the pacemaker

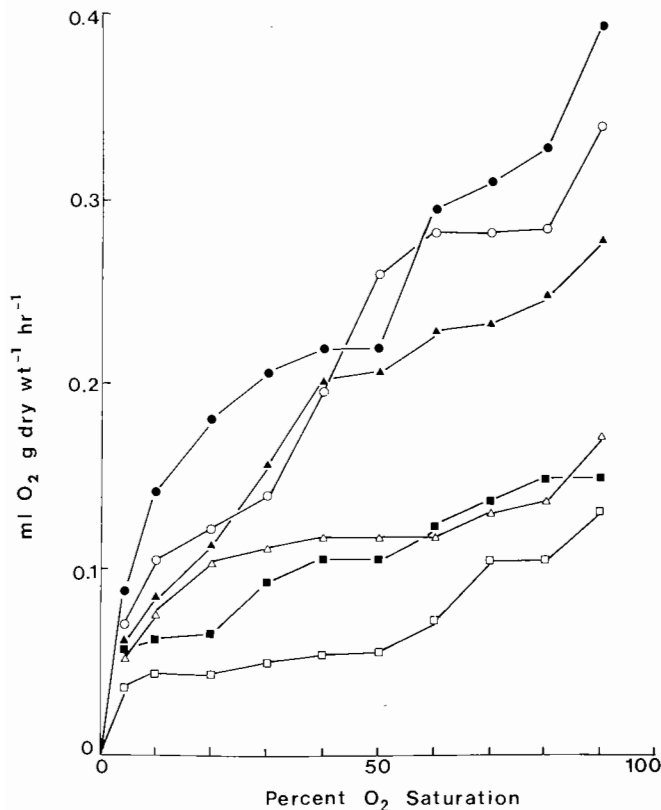


Fig. 4. *Ciona intestinalis*. Oxygen consumption (as ml O₂ per g dry weight per h) in declining oxygen tension; results of 6 individual experiments

closer to the pharyngeal basket (branchial), and 32.6 ± 0.4 beats min^{-1} for the pacemaker closer to the viscera (visceral). Regular periods of beating lasted for approximately 1 min, followed by periods of irregular activity (also of approximately 1 min duration) prior to heart beat reversal. Reversal occurred regularly at 1 min intervals while the ascidians were in full-strength seawater. Preliminary experiments with dilute seawater showed that heart beat became irregular and reversal occurred sporadically and sometimes not at all. It was decided, therefore, not to monitor heart rate during fluctuating salinity regimes.

Activity

Activity was monitored in terms of spontaneous squirts/hour, and the results are shown in Fig. 6. The ascidians showed a rate of 14 squirts per hour in full-strength seawater. In individuals exposed to a 30% seawater minimum sinusoidal salinity regime, the number of squirts/hour declined slowly at first, but stopped completely when the external seawater concentration reached approximately 60%. During these periods of exposure to low salinities the siphons were tightly closed. As the salinity

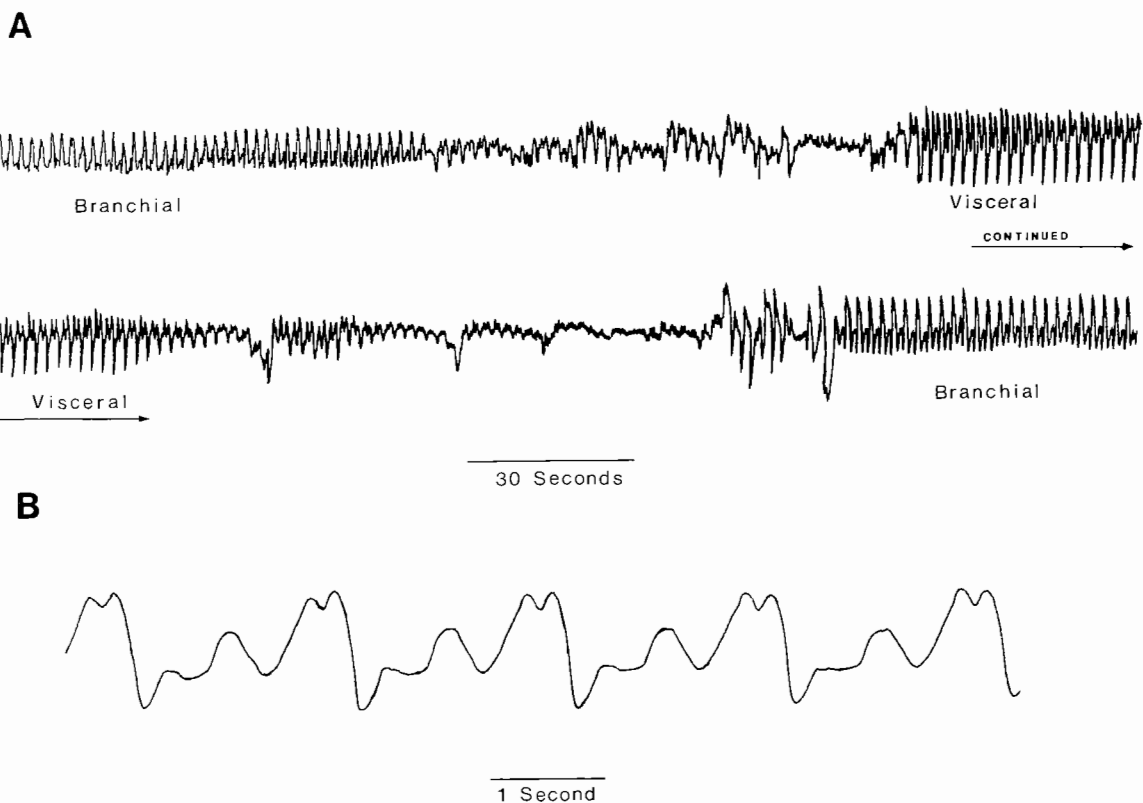


Fig. 5. *Ciona intestinalis*. (A) Actual trace of heart rate measured *in vivo*; (B) expanded heart rate trace

began to rise again there was a slow and steady increase in the number of squirts, which again ceased during the second cycle when the external seawater concentration decreased to approximately 60%. The cessation of squirting was much more pronounced in the abrupt salinity profile. Squirting ceased almost immediately after dilution of the seawater, but took 3 h to return to the control value.

Fig. 7 shows an actual trace of squirting activity in ascidians exposed to a 30% seawater minimum abrupt-salinity profile in which the cessation of squirting is clearly shown. All squirting activity ceased within approximately 3 min.

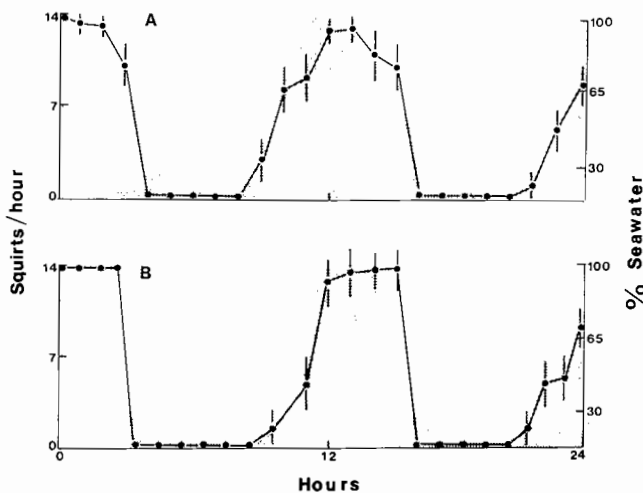


Fig. 6. *Ciona intestinalis*. Changes in rate of spontaneous squirting of ascidians exposed to (A) 30% seawater minimum sinusoidal salinity regime and (B) 30% seawater minimum square-wave regime. Stippled areas represent changes in external medium. Each point is mean of 5 individuals. Error bars represent 95% confidence limits

Oxygen Consumption During Fluctuating Salinities

The oxygen consumption of *Ciona intestinalis* exposed to gradual and abrupt salinity changes is shown in Fig. 8. Oxygen consumption decreases as the salinity decreases owing to a shorter period of active pumping, and it is interesting to note in the sinusoidal regime that oxygen consumption falls sharply at approximately the same seawater concentration as squirting cessation. There is no "overshoot" when the ascidians return to full-strength seawater to indicate any appreciable oxygen debt. The response is repeated in the second cycle of the salinity profile.

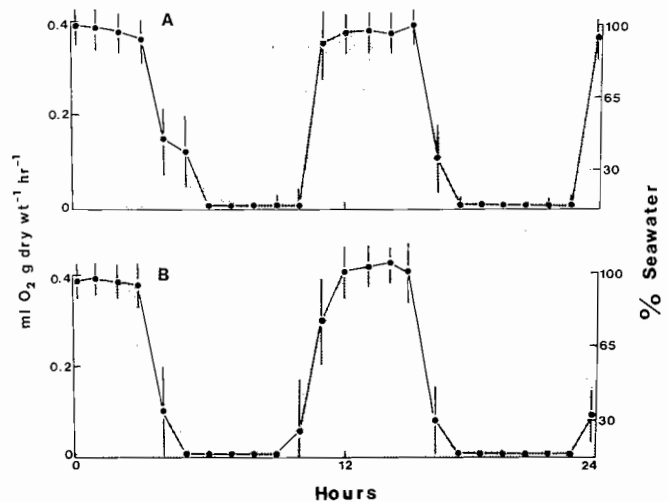


Fig. 8. *Ciona intestinalis*. Oxygen consumption (as ml O₂ per g dry weight per h) during exposure to (A) 30% seawater minimum sinusoidal salinity regime and (B) 30% seawater minimum square-wave regime. Stippled areas represent changes in external medium. Each point is mean of 5 individuals. Error bars represent 95% confidence limits

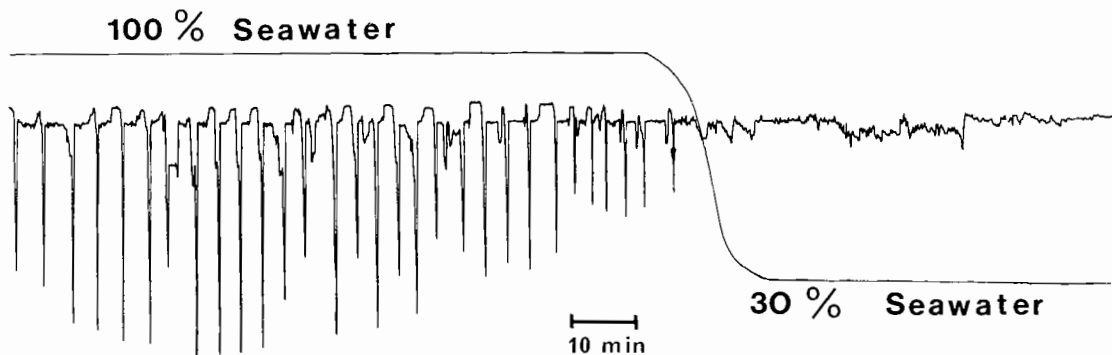


Fig. 7. *Ciona intestinalis*. Trace recorded during exposure to 30% seawater minimum square-wave salinity change

Discussion

The rate of oxygen consumption (v_{O_2}) is proportional to a fractional power (b) of the body weight (W) such that:

$$v_{O_2} = aW^b,$$

where a is the proportionality factor.

The weight-specific oxygen consumption rate (Q_{O_2}) is given by:

$$Q_{O_2} = aW^{b-1}.$$

The b value for *Ciona intestinalis* in this study was found to be 0.831 ($r = 0.8753$; $P < 0.001$) and $b-1$ is therefore -0.169.

As expected, oxygen consumption increases with increasing size, but smaller individuals respire at a faster rate per unit weight (Q_{O_2}) than do larger individuals. Fisher (1976) reported a b value of 0.7 (standard deviation = 0.1) for the solitary tunicate *Styela plicata*. The b value of 0.831 reported here is comparable to the interspecific values for the respiration constants reviewed by Zeuthen (1947) and Hemmingsen (1960).

The effect of reduced oxygen tension on the respiratory rate of *Ciona intestinalis* is shown in Fig. 4. The slope of the lines for small ascidians is greater than that for larger individuals, indicating that while the species is a metabolic conformer, smaller individuals are more dependent on the external oxygen tension than are larger ones. This supports the statement made by Prosser (1955) that, in general, animals living in well-aerated water have a high critical pressure and are oxygen-dependent. It is unlikely that *C. intestinalis* would normally encounter conditions of serious oxygen shortage for any length of time in their natural environment and therefore need no means of regulating their metabolic rate.

One of the most interesting features of tunicates is the periodic reversal in the direction of the heart beat. There is a wealth of information available concerning the anatomy and physiology of the ascidian heart (Millar, 1953; Krijgsman, 1956; Kriebel, 1967, 1968a, b, 1970; Jones, 1971). However, all of the information collected to date has come from exposed or excised hearts. In the present study, the heart was left intact and the results differ in several respects from those previously published. Kriebel (1968a) found that the average maximal frequency of 5 *in situ* hearts of *Ciona intestinalis* was 21.3 beats min^{-1} for the branchial pacemaker and 20.0 min^{-1} for the visceral pacemaker at 15°C. Reversal occurred about every 12 min after the ascidians were relaxed and siphoning normally, and the duration of the rever-

sal pause was short - only 5 sec in large individuals. The heart rate recorded in this study was reasonably close to that reported by Kriebel (1968a), but the time recorded for the period between heart beat reversals (1 min) was very different. Jones (1971) found that the pause before reversal varied from 0.5 sec to more than 15 sec in *Ecteinascida turbinata*, and that the mean avisceral rate was 66.63 in 93 ± 5 sec and the mean abvisceral rate was 62.3 ± 4.4 beats in 87.5 ± 5 sec. This is approximately equal to 43 beats min^{-1} for the avisceral and abvisceral pacemakers.

Preliminary experiments showed that the heart rate of *Ciona intestinalis* exposed to reduced salinities was sporadic and that reversal did not always occur. In some cases the heart stopped beating altogether. Some of the ascidians were seen to be actively pumping, so it is unlikely that cessation of heart rate was due to aerobic shutdown. It is more likely to have been caused by changes in the osmotic concentration of the body fluids. While heart rate measurements from excised and/or exposed hearts may provide interesting information about the capabilities of the heart, it is suggested that *in vivo* measurements provide data more useful for the study of whole animal responses to environmental stresses.

Hoyle (1952, 1953) has studied in great detail the spontaneous squirting of the ascidian *Phallusia mammillata*. He found that there was a nearly constant rhythm of contraction, with spontaneous squirts at 6 to 9 min intervals (6.7 to 10 squirts h^{-1}). The only mention of such activity in *Ciona intestinalis* is by Polimanti (1911), who noted that the ascidians showed spontaneous siphon movement at high temperatures (30°C), but did not study the effect at lower temperatures. *C. intestinalis* exhibited a rhythm of 14 squirts h^{-1} at 10°C, and this rate decreased as the external salinity decreased. Siphon closure in ascidians during exposure to dilute seawater is probably analagous to the mechanisms of shell valve closure and/or siphon retraction in bivalves and quiescence in the burrow in lugworms, as described previously by Shumway (1977a, b). During periods of low-salinity stress, the ascidians simply close the siphons to avoid the noxious effects of the diluted seawater and keep them closed until the salinity of the external medium increases. This is clearly shown in Fig. 7.

Activity and metabolic rate are functionally correlated: changes in metabolism tend to alter the scope for activi-

ty and *vice versa* (Kinne, 1971). This is clearly illustrated by the present study, where it is shown that lowered salinity causes cessation of pumping and siphon activity which, in turn, causes a rapid decrease in the rate of oxygen consumption (Fig. 8). The metabolic rate of marine invertebrates varies in changing salinities, and Kinne (1971) has stated that there are 4 major types of respiratory response to temporary salinity changes: the oxygen consumption rate may (i) increase in subnormal salinities and/or decrease in supranormal salinities; (ii) increase in sub- and supranormal salinities; (iii) decrease in sub- and supranormal salinities; (iv) remain essentially unaffected. Types (i) and (ii) are characteristic of euryhaline invertebrates, Type (iii) of stenohaline animals, and Type (iv) of holoeuryhaline or extremely euryhaline forms. The results of this study place *Ciona intestinalis* in Type (iii), with a decreased metabolic rate at decreased salinities due to the behavioral response of siphon closure.

Das (1936) studied the test of *Herdmania pallida* in great detail, and found that ampullae occur very close to the outer surface of the ascidian, thus bringing the blood into close contact with the surrounding oxygenated water. He concluded that they form an apparatus for accessory respiration comparable with the cutaneous respiration in other animals. The test of *Ciona* sp. is also supplied with blood vessels (Barnes, 1974), but since in this study the rate of oxygen consumed is zero during exposure to low salinity it would appear that there is little or no gaseous exchange across the test of *C. intestinalis*. It is possible that the osmotic shock damaged the test resulting in swollen, impaired vessels. It is also possible that there was little gaseous exchange due to cessation of the heart beat.

There is thus far no evidence for ionic or osmotic regulation in *Ciona intestinalis* and, since they are found in waters of low salinities (Dybern, 1967) as well as in estuaries (MacGinitie and MacGinitie, 1968), it is not surprising that these ascidians have developed an effective behavioral mechanism for isolating themselves from noxious external environments.

Acknowledgements. The author would like to thank Dr. J. Davenport for the use of Fig. 2, and Professor D.J. Crisp, F.R.S., Dr. P.A. Gabbott and Dr. W.B. Stickle for critically reading the manuscript. The work was carried out under the tenure of a fellowship from the Marshall Aid Commemoration Commission.

Literature Cited

- Barnes, R.D.: Invertebrate zoology, 3rd ed. 870 pp. Philadelphia: W.B. Saunders Co. 1974
- Das, S.H.: On the structure and function of the ascidian test. *J. Morph.* 59, 539-560 (1936)
- Davenport, J.: A technique for the measurement of oxygen consumption in small aquatic organisms. *Lab. Pract.* 25, 693-695 (1976)
- , Ll.D. Gruffydd and A.R. Beaumont: An apparatus to supply water of fluctuating salinity and its use in a study of the salinity tolerances of larvae of the scallop *Pecten maximus* L. *J. mar. biol. Ass. U.K.* 55, 391-409 (1975)
- Dybern, B.I.: The distribution and salinity tolerance of *Ciona intestinalis* (L.) f. *typica* with special reference to the waters around Southern Scandinavia. *Ophelia* 4, 207-226 (1967)
- Fisher, T.R.: Oxygen uptake of the solitary tunicate *Styela plicata*. *Biol. Bull. mar. biol. Lab., Woods Hole* 151, 297-305 (1976)
- Hemmingsen, A.M.: Energy metabolism as related to body size and respiratory surfaces and its evaluation. *Rep. Steno meml Hosp.* 9, 7-110 (1960)
- Hoyle, G.: The response mechanism in ascidians. *J. mar. biol. Ass. U.K.* 31, 287-305 (1952)
- Spontaneous squirting of an ascidian, *Phallusia mammillata* Cuvier. *J. mar. biol. Ass. U.K.* 31, 541-562 (1953)
- Jones, J.C.: On the heart of the orange tunicate, *Ecteinoxidia turbinata* Herdman. *Biol. Bull. mar. biol. Lab., Woods Hole* 141, 130-145 (1971)
- Jørgensen, C.B.: On the relation between water transport and food requirements in some marine filter feeding invertebrates. *Biol. Bull. mar. biol. Lab., Woods Hole* 103, 356-363 (1952)
- Kinne, O.: Salinity: animals - invertebrates. *In: Marine ecology. Vol. 1. Environmental factors, Part 2.* pp 821-995. Ed. by O. Kinne. New York: Wiley Interscience 1971
- Kriebel, M.E.: Conduction velocity and intracellular action potentials of the tunicate heart. *J. gen. Physiol.* 50, 2097-2107 (1967)
- Studies on cardiovascular physiology of tunicates. *Biol. Bull. mar. biol. Lab., Woods Hole* 134, 434-455 (1968a)
- Pacemaker properties of tunicate heart cells. *Biol. Bull. mar. biol. Lab., Woods Hole* 135, 166-193 (1968b)
- Wave front analyses of impulses in a tunicate heart. *Am. J. Physiol., Lond.* 218, 1194-1200 (1970)
- Krijgsman, B.: Contractile and pacemaker mechanisms of the heart of tunicates. *Biol. Rev.* 31, 288-312 (1956)
- MacGinitie, G.E. and N. MacGinitie: Natural history of marine animals, 523 pp. New York: McGraw Hill Book Co. 1968
- Mangum, C.P. and L.E. Burnett: The extraction of oxygen by estuarine invertebrates. *In: Ecological physiology of estuarine organisms,*

- pp 147-164. Ed. by F.J. Vernberg. Columbia, South Carolina: University of South Carolina Press 1975
- Millar, R.H.: *Ciona*. L.M.B.C. Mem. typ. Br. mar. Pl. Anim. 35, 1-122 (1953)
- Polimanti, O.: Beiträge zur Physiologie des Nierensystems unter der Bewegung bei den niederen Tieren. II. *Ciona intestinalis* L. Arch. Anat. Physiol. (Suppl.) 59-152 (1911)
- Prosser, C.L.: Physiological variation in animals. Biol. Rev. 30, 229-262 (1955)
- Comparative animal physiology, 456 pp. Philadelphia: W.B. Saunders Co. 1973
- Sanders, F.: A comparative study of respiration in two tropical marine polychaetes. Comp. Biochem. Physiol. 46A, 311-323 (1973)
- Sassaman, C. and C.P. Mangum: Relationship between aerobic and anaerobic metabolism in estuarine anemones. Comp. Biochem. Physiol. 44A, 1313-1319 (1973)
- Shumway, S.E.: The effect of salinity fluctuation on the osmotic pressure and Na^+ , Ca^{2+} and Mg^{2+} ion concentrations in the hemolymph of bivalve molluscs. Mar. Biol. 41, 153-177 (1977a)
- Some aspects of the physiology of *Arenicola marina* L. exposed to fluctuating salinities. J. mar. biol. Ass. U.K. 57, 907-924 (1977b)
- Zeuthen, E.: Body size and metabolic rate in the animal kingdom with special regard to the marine microfauna. C. r. Trav. Lab. Carlsberg (Sér. chim.) 26, 20-165 (1947)

Dr. Sandra E. Shumway
Portobello Marine Laboratory
P.O. Box 8
Portobello
New Zealand

Date of final manuscript acceptance: June 2, 1978. Communicated by J. Mauchline, Oban