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Switching of metabolic-rate scaling between allometry and isometry in colonial ascidians

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The metabolic rate and its scaling relationship to colony size were studied in the colonial ascidian *Botrylloides simodensis*. The colonial metabolic rate, measured by the oxygen consumption rate (V_{O_2} in millilitres of O_2 per hour) and the colony mass (wet weight M_w in grams) showed the allometric relationship ($V_{O_2} = 0.0412\ M_w^{0.799}$. The power coefficient was statistically not different from 0.75, the value for unitary organisms. The size of the zooids and the tunic volume fraction in a colony were kept constant irrespective of the colonial size. These results, together with the two-dimensional colonial shape, excluded shape factors and colonial composition as possible causes of allometry. Botryllid ascidians show a takeover state in which all the zooids of the parent generation in a colony degenerate and zooids of a new generation develop in unison. The media for connection between zooids such as a common drainage system and connecting vessels to the common vascular system experienced reconstruction. The metabolic rate during the takeover state was halved and was directly proportional to the colonial mass. The scaling thus changed from being allometric to isometric. The alteration in the scaling that was associated with the loss of the connection between the zooids strongly support the hypothesis that the allometry was derived from mutual interaction among the zooids. The applicability of this hypothesis to unitary organisms is discussed.

Keywords: allometry; body size; colonial organism; criticality; metabolic rate; tunicates

1. INTRODUCTION

The relationship between two variables in an organism is often expressed using the power function $Y = aX^b$, where a is a normalization constant and b is the power exponent. When $b = 1$ the relationship is isometric and when $b \neq 1$ the relationship is allometric. The most famous and thus most studied allometric relationship is the basal metabolic rate of animals. This increases in proportion to body mass according to a power of 0.75 (Kleiber 1961). The mechanism that produces this exponent of 0.75 has been researched for decades and various hypotheses have been proposed (Peters 1983; Calder 1984; Schmidt-Nielsen 1984). The oldest proposal is the surface law (Rubner 1883) that correlates the metabolic rate with the body surface. It predicts the exponent to be 2/3. Whether this measured exponent value of 0.75 is different from this proposed value of 2/3 is yet to be settled (Dodds *et al.* 2001); although various hypotheses explaining the 0.75 exponent have been put forward (Patterson 1992; West *et al.* 1997; Darveau *et al.* 2002), there is still much debate about which hypothesis is the most likely one.

The 0.75 power relationship of the metabolic rate has been established in unitary organisms, but little attention has been paid to the metabolic scaling rate in colonial organisms. The pioneering work of Hughes & Hughes (1986) on colonial bryozoans showed that the metabolic rate was proportional to the colony mass. It has been believed since that the metabolic rate of colonial organisms is isometric. The recent work on colonies of stony corals, however, showed an allometric relationship in which the metabolic rate hardly increased with colony size (Vollmer & Edmunds 2000). Colonial animals have modular construction—a colony is made from the iteration of the same modules. When there is little interaction between the modules, the metabolic rate of a colony is expected to be a simple sum of the modules and thus isometric scaling will appear. This is the conclusion Hughes & Hughes (1986) drew based on the morphology of bryozoans where the zooid is encased in a rigid box, although they showed some integrative behaviour between zooids (Thorpe *et al.* 1975; Harvell 1984). In the case of stony corals, the growth of a colony is associated with changes in the colonial shape and in the volume fraction of inactive materials such as bony skeletons. Allometric scaling has often been attributed to such physical and morphological changes both in colonial organisms (Sebens 1987; Vollmer & Edmunds 2000) and in unitary organisms (Hemmingsen 1960; Schmidt-Nielsen 1984). The morphological factor that is regarded as having the most influence on the metabolic rate is the surface area to volume ratio (Gould 1966; Schmidt-Nielsen 1984). In geometrically proportional growth, the volume is proportional to the mass; however, the surface area increases in proportion to the mass by a power of 2/3.

Here, we report the relationship between the metabolic rate and colony size in the ascidian *Botrylloides simodensis* (Saito *et al.* 1981). The colony is composed of zooids that do not differentiate in size and shape (see § 3), and thus it could be regarded as a simple system made of identical modules. It differs from bryozoans in that the modules have rich interconnections. The colony of this ascidian has a common vascular network to which each zooid is connected through vessels. Another possible source of interaction is a drainage system. In *B. simodensis* several zooids

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form a common drainage system to which the exhalent current of each zooid is expelled. This drainage system possibly causes mechanical interactions between the zooids. If the independence and lack of mutual interactions of modules lead to isometry then the ascidian colony is expected to be allometric rather than isometric.

B. simodensis forms a thin and flat colony. All the zooids are arranged without overlapping in a single layer under which runs a common vascular network. Because the colony grows only two-dimensionally, the surface area to volume ratio (S/V ratio) of the colony is kept constant as it grows. The colony does not harbour any symbiotic algae and does not produce any bony skeletons. The volume fraction of the tunic in which the zooids are embedded is kept constant (see § 3). If the allometric scaling derives either from the changes in the S/V ratio or from the changes in the volume fraction of inactive materials, this ascidian colony is expected to be isometric rather than allometric.

Here, we report the result that the metabolic rate of the colonial ascidians *B. simodensis* followed an allometric relationship corresponding to a power of 0.75. We further compared the metabolic rates of this colonial animal in different phases. *B. simodensis* shows a phase transition called 'takeover', which is characteristic of the Botryllidae family (Watanabe 1953; Milkman 1967). All zooids give rise to the simultaneous, weekly generation of daughters. During takeover the zooids of the parent generation close both the inlets and the outlets of the feeding currents and stop all pumping activities and, thus, their bodies gradually degenerate. The small filial zooids that have been produced by budding from parent zooids in the preceding ordinary phase then start growing. They grow to full size and reconstruct the common drainage system. The feeding current restarts and this is the beginning of the next ordinary phase. We measured the metabolic rate of *B. simodensis* in this ordinary state and the takeover state. The reason we compared the two states is not just because there are no reports on the metabolic rate during takeover but also because the mutual interaction between zooids is possibly lost during takeover. If the allometric relationship found in the ordinary state is derived from the mutual interaction between zooids, then the metabolic scaling in the takeover state may well change to being isometric.

The switching of scaling without any changes in either the colonial shape or the colonial biomass and the presence of the 0.75 power law in a simple two-dimensional system whose vascular network shows no hierarchical branching required reconsideration of the current approach to the 0.75 power law. A novel concept dealing with biological allometric scaling that is related to the criticality of the statistical dynamics will be discussed.

2. MATERIAL AND METHODS

Colonies of *B. simodensis* were collected in the vicinity of Nabeta bay, Shimoda, Shizuoka Prefecture, Japan. A small colony was put on a slide glass, which was then set in a container hung from a raft in the bay. The colonies spread out on the slide glass increasing their size. They were reared for at least two weeks before being brought into the laboratory. The slide glass was cleaned of any fouling organisms and detritus.

On a slide glass *B. simodensis* made a simple-shaped colony as it would in the field. The zooids, *ca.* 2.0 mm in length and 1.0 mm in width, were arranged in the horizontal plane without overlapping to make a mono-layered sheet-like structure (Saito *et al.* 1981). The colony surface was smooth and flat. The thickness of the colony was, however, *ca.* 2.5 mm irrespective of its size. The zooids were embedded in a tunic of an organic matrix that included several types of cell (Hirose *et al.* 1991). The tunic and zooids did not harbour any symbiotic organisms and did not bear a bony skeleton. Several tens of zooids made up a ladder system in which the exhalent currents from the zooids shared a common drain that led to a common cloacal aperture. A vascular network ran underneath the tunic, spreading out evenly below the zooid plane. Each zooid was linked to the common vascular network by connecting vessels (Mukai *et al.* 1978). Every colony experienced the weekly takeover phase shift. In the takeover phase the zooids of the parent generation degenerate and those of the filial generation develop equally and synchronously from a bud to being fully grown. All zooids in a colony were thus identical not only in their morphology but also in age (Mukai 1974). We determined the phase of the colony by microscopic observations.

We used the artificial seawater, My sea (Jamarine, Japan), dissolved in distilled water. The colonies were not fed during the measurement period. To measure the metabolic rate, the colony was acclimatized to experimental conditions in air-saturated artificial sea water (ASW; salinity of 33‰; temperature of 20 °C) for 3 h. The slide glass to which the colony was attached was placed in an experimental chamber filled with air-saturated ASW. The chamber was placed in a water bath that was maintained at a temperature of 20 ± 0.03 °C (CL-80L, Taitec, Japan). The metabolic rate was measured via the rate of oxygen consumption using polarographic oxygen electrodes (Model 5300, Yellow Spring Instrument Co., USA). The polarographic oxygen electrodes consumed oxygen at a steady, gentle rate. A magnetic stirrer ball, set in front of the electrode, supplied the fresh seawater to the electrode and also stirred all the water in the chamber. The electrodes were calibrated using a zero solution (0.01 M sodium tetraborate and sodium sulphite) and air-saturated ASW (Vollmer & Edmunds 2000). The measurements were finished at an oxygen concentration level that was not less than 80%. Data were collected through an A/D board with a PC and were converted into absolute values. The rate of change, V_{O_2}, in the dissolved O_2 of the experiments and the controls was calculated using a simple linear regression ($r^2 > 0.9$). The control values were measured every five respiration measurements using the chamber filled with just ASW. The respiration rate per colony was calculated by deducting the control value.

The metabolic rate was measured throughout the transition of the states in three colonies (wet weight M_w of 0.954, 2.31 and 2.41 g, respectively). Their cycles of state transition were recorded beforehand during culture in the bay. They were brought to the laboratory 1 day before the predicted date of takeover and the metabolic rate was measured every 6 hours throughout the transition cycles, i.e. from the ordinary state to the takeover state and back to the ordinary state. The colonies were microscopically observed to monitor their states and then photographed.

The metabolic rate scaling was studied using 60 colonies in the ordinary state with M_w values ranging from 2.86×10^{-2} to 4.1 g and 28 colonies in the takeover state with M_w values ranging from 6.73×10^{-2} to 1.32 g. Towards the end of the takeover state, the branchial apertures of the filial generation opened first

and were then followed by the atrial apertures opening. Exhalent currents coming through atrial apertures were the sign of the beginning of the ordinary state. Because the respiration rates were constant throughout in the respective states except at the very beginning of the takeover state (see § 3), the colonies in the middle of the state were used in the scaling studies: the colonies that were at least 1 day away from the start of their ordinary state were used for ordinary state samples; for takeover samples, we used colonies in which both the branchial and atrial apertures of the filial generation were yet to open. The states of all the samples were carefully checked and any obscure ones were not used.

Logarithmic linear regression was obtained by a least-squares method, giving the following equation:

$$\log V_{O_2} = \log a + b \log M_w,$$

where V_{O_2} is the rate of oxygen consumption, M_w is the colonial wet weight, b is the slope of the regression and a is the proportionality coefficient with the intercept at unity mass. This relationship gives the power function $V_{O_2} = a M_w^b$. The coefficient a at 1 g gives the weight-specific metabolic rate that was used to compare the metabolic activities between species (Schmidt-Nielsen 1984). It should be noted that the specific metabolic rate does not mean the metabolic rate per gram, but the metabolic rate of the animal whose body size is 1 g. The 95% confidence interval of the population regression α and β was estimated to test for the regression intercept a and slope b. The following equations were used:

$$\alpha = a \pm t\ (\text{d.f.} = n - 2;\ p = 0.05) \times \text{s.e.}_a$$

and

$$\beta = b \pm t\ (\text{d.f.} = n - 2;\ p = 0.05) \times \text{s.e.}_b$$

where d.f. is the degrees of freedom, p is the critical value and s.e.$_a$ and s.e.$_b$ are the standard errors of a and b, respectively. The deviation of a constant value from the interval indicates the significant difference ($p < 0.05$). It was also examined whether each power coefficient (b) was different from a constant value (b_{const}). The null hypothesis that each power coefficient (b) equalled a constant value (b_{const}) was tested using a t-test (H_0: $b - b_{const} = 0$), where the level of significance was 0.05.

The parameters (a_t, b_t) of the regressions in the takeover state were statistically compared against those (a_o, b_o) in the ordinary state. The null hypothesis that the parameter in the takeover state (a_t, b_t) equalled the parameter in the ordinary state (a_o, b_o) was tested using a t-test (H_0: $a_t - a_o = 0$, H_0: $b_t - b_o = 0$), where the level of significance was 0.05.

The colonial wet weight was measured as follows: the samples were drained off and sandwiched between two sheets of tissue paper (KIMWIPE S-2000, Crecia, Japan) whose untouched parts were folded back to wrap around the sample. The samples were thus blotted for 180 s and then weighed with an electrical balance. The glass slide, used as a substrate, was weighed before culturing. The wet weight of a colony was calculated by deducting the weight of the substrate. The dry weight refers to samples after they were dried in an oven at 80 °C for 24 hours. The actual number of zooids was counted with a Macintosh PC using the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

The ratio of wet weight to dry weight (M_d) in the tunic was obtained as follows. In summer, *B. simodensis* shows seasonal recession in which zooids degenerate leaving a tissue made only of tunic. The method for measuring the wet and dry weights of the tunic tissue was the same as that for the colonies. The ratio M_w/M_d in tunic was then estimated from these materials.

3. RESULTS

(a) *Scaling of colony size*

The scaling between the number of full-grown zooids (N_z) in a colony in the ordinary state and the colony size is shown in figure 1. The colony size was measured in wet weight (M_w). From 39 colonies with M_w values ranging from 8.48×10^{-2} to 3.25 g and 39−1614 zooids the following relationship was obtained:

$$N_z = 418 M_w^{1.01}.$$

The 95% confidence interval of the power coefficient ranged from 0.962 to 1.05. The power coefficient was not significantly different from 1 ($t = 0.314$, d.f. = 37, $p = 0.755$). The coefficient of determination (r^2) was 0.952 and the scattering of the plots from the regression was quite small. The number of zooids was directly proportion to the colonial mass and thus the mass per zooid was independent of colony size.

The wet weight (M_w) of colonies in the ordinary state was directly proportional to the dry weight (M_d). The relationship $M_w = 13.1 M_d^{1.02}$ was obtained from 12 colonies with M_w values ranging from 3.61×10^{-1} to 2.27 g and M_d values ranging from 2.96×10^{-2} to 1.90×10^{-1} g (figure 2). The power coefficient was quite close to 1 and the coefficient of determination (r^2) was 0.990. The 95% confidence interval of the power coefficient ranged from 0.964 to 1.07. The power coefficient was not significantly different from 1 ($t = 0.702$, d.f. = 10, $p = 0.499$). The M_w/M_d ratio was thus constant irrespective of the colony size. The ratio M_w/M_d for a whole colony was 12.6 ± 0.155 (mean ± s.e., $n = 12$) and that of the tunic was 16.9 ± 0.280 (mean ± s.e., $n = 3$). The latter result was significantly different from the former result (Mann–Whitney U-test, $U = 36$, $n_1 = 12$, $n_2 = 3$, $p < 0.01$). We conclude from these results that the volume fraction occupied by the tunic did not change with colonial size. On average, each zooid always contributed the same amount of tunic to the colony.

From these results it is appropriate to regard the colony of *B. simodensis* as a geometrically simple system: it is made of units that are identical in size and shape; and the units are placed without overlapping to make a two-dimensional flat sheet. The morphology of the unit remained constant as the colony grew, with the S/V ratio of the colony also remaining constant irrespective of colony size.

(b) *State changes and metabolic rate*

B. simodensis followed a transition pattern that was similar to those described for other members of the Botryllidae family (Watanabe 1953). Figure 3 is a series of photographs of a colony that underwent successive state transitions from the ordinary state to the takeover state and then back to the ordinary phase. The takeover onset was taken to be time 0. At −8 hours the colony was in the ordinary state with active feeding currents. The onset of the takeover state was recognized by the start of the degeneration of the parent generation zooids (0 hours in

Table 1. Changes in the metabolic rate of a colony (mean ± s.e.) during the takeover state.
(Each value is the average of successive measurements, with a 6-hour interval.)

		colonial metabolic rate ($mlO_2\,h^{-1}$)		
colony no.	M_w (g)	before	takeover	after
1	2.41	0.178 ± 0.016 ($n = 4$)	0.0960 ± 0.0025 ($n = 3$)	0.135 ± 0.0086 ($n = 3$)
2	2.31	0.0795 ± 0.012 ($n = 2$)	0.0587 ± 0.0011 ($n = 5$)	0.0936 ± 0.0016 ($n = 4$)
3	0.954	0.0688 ± 0.0051 ($n = 2$)	0.0300 ± 0.0035 ($n = 5$)	0.0684 ± 0.0015 ($n = 4$)

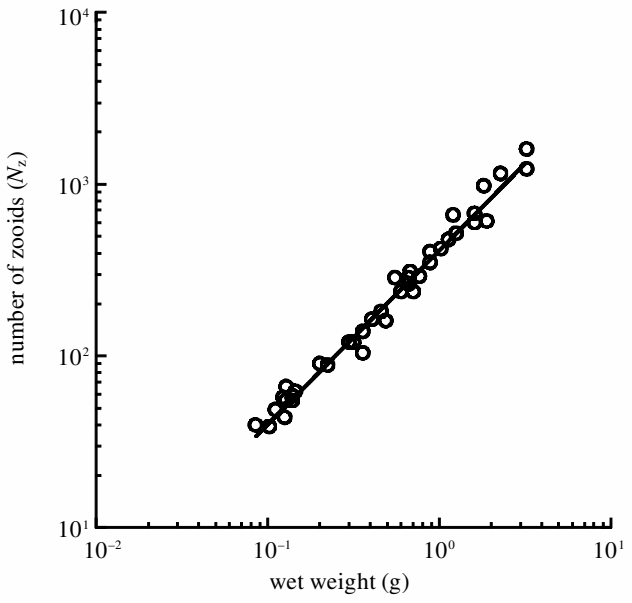

Figure 1. Relationship between the number of zooids (N_z) in a colony and colonial wet weight (M_w). The regression equation fitted to data is $N_z = 418M_w^{1.01}$ ($r^2 = 0.952$, $n = 39$; 95% confidence interval of the power coefficient, 0.962–1.05).

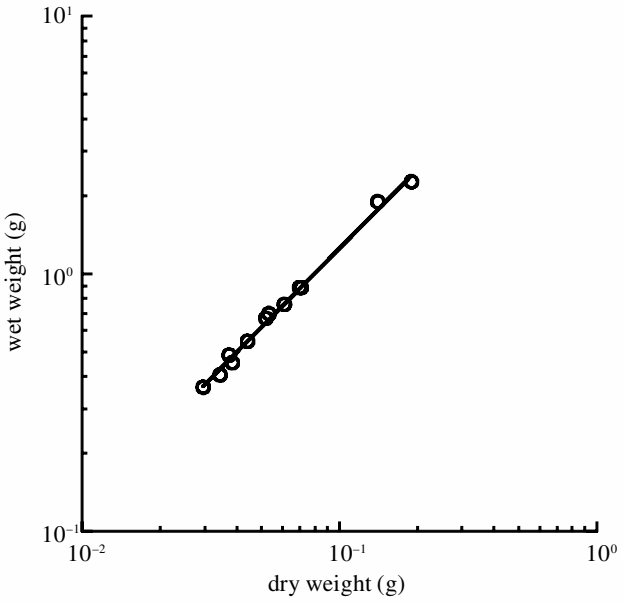

Figure 2. Relationship between colonial wet weight (M_w) and dry weight (M_d). The regression equation is $M_w = 13.1M_d^{1.02}$ ($r^2 = 0.990$, $n = 12$; 95% confidence interval of the power coefficient, 0.964–1.07).

figure 3). The small particles in figure 3*a*–*e* are the degenerating zooids that were *ca*. 25% of the parent zooids when this photograph was taken. The degeneration proceeded gradually and was completed in a few hours. The common vascular network underlying the zooids remained while the blood vessel connecting each zooid to the network degenerated. Figure 3*c*–*g* shows the growth phase of the filial generation. After all zooids of the parent generation had degenerated, all the zooids of the filial generation started growing synchronously. At the end of the takeover state the branchial apertures opened followed by the atrial apertures. Figure 3*h* shows the ordinary state: the growth of zooids had finished by this time; both apertures of all the zooids were opened and the feeding currents had appeared; and the vessels connecting the zooids to the common vascular network were restructured again.

Figure 4 shows the metabolic rate measured in the same colony that is shown in figure 3 over the same timeframes. As the degeneration proceeded, the metabolic rate decreased in the takeover state. When the degeneration of all the parental generation zooids was finished, the metabolic rate decreased to about half that of the ordinary state and stayed at that level for the rest of the takeover state. When the takeover was complete, the rate increased back to a similar level as for the previous ordinary state. The increased level was then kept constant for 18 hours. Table 1 gives the results of three colonies measured across these transitions. Each measurement was repeated about every 6 hours. All colonies showed lower metabolic rates in the takeover state and the rates for the ordinary states before and after takeover were similar. We concluded that the metabolic rate was higher in the ordinary state and that each state showed a constant metabolic rate throughout the respective states except at the very beginning of the takeover.

(c) *Scaling of the metabolic rate*

The metabolic rate of 60 colonies in the ordinary state with M_w values ranging from $2.86 \times 10^{-2} - 4.10$ g was measured. The rate was $2.39 \times 10^{-2} - 1.56 \times 10^{-1}$ $mlO_2\,h^{-1}$ and the allometric relationship $V_{O_2} = 0.0412M_w^{0.799}$ was obtained (figure 5). The coefficient of determination (r^2) was 0.952. The 95% confidence interval of the power coefficient ranged from 0.740 to 0.859. The power coefficient was not significantly different from 0.75 ($t = 1.65$, d.f. = 58, $p = 0.104$) but different from 1 ($t = 6.72$, d.f. = 58, $p < 0.001$) and from 0.67 ($t = 4.432$, d.f. = 58, $p < 0.01$), which implies that the scaling of this colonial organism in the ordinary state followed allometric scaling

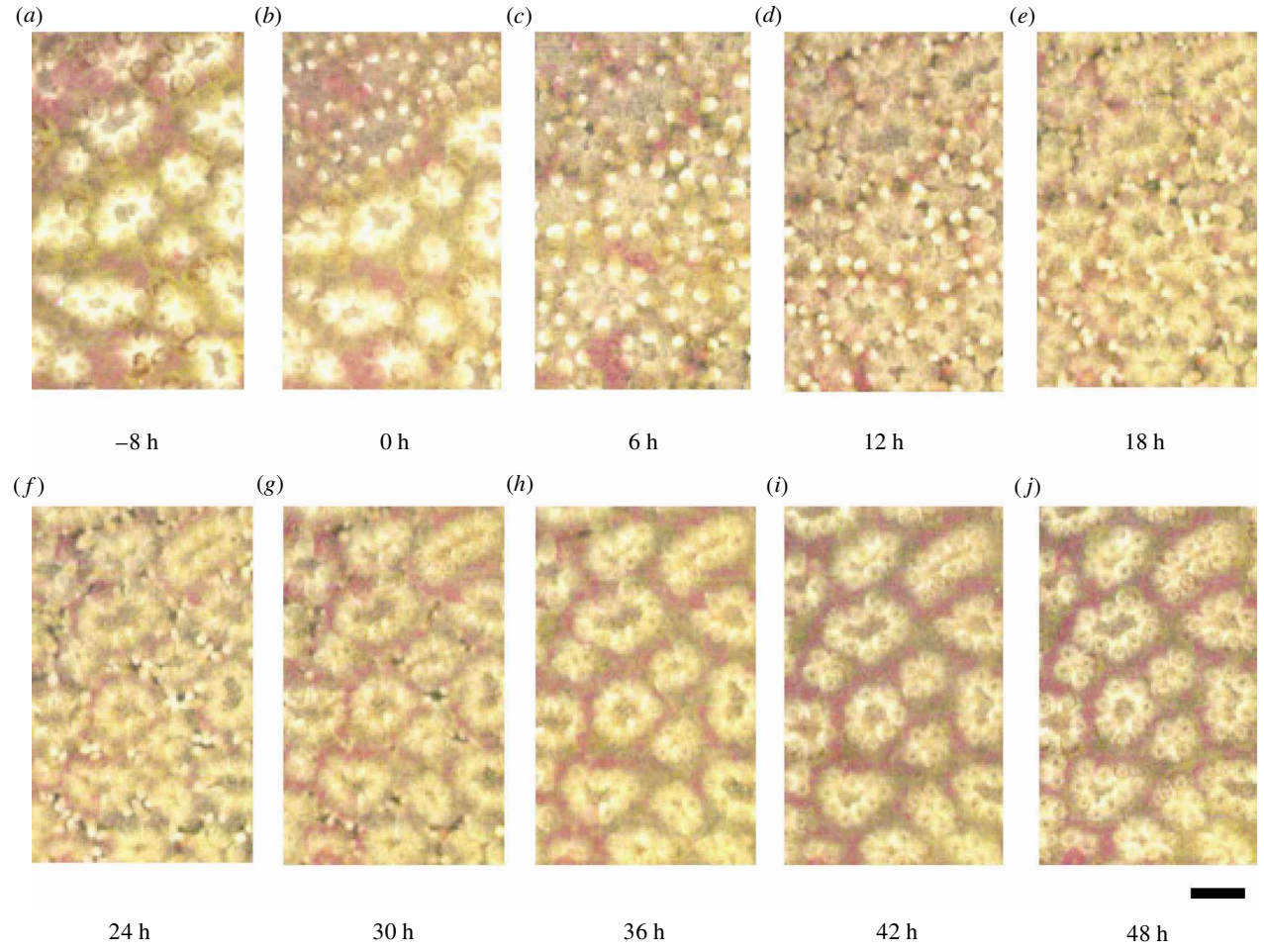

Figure 3. Successive photographs showing phase transition in a colony. The colony in the ordinary phase (*a*) was photographed 8 hours before the start of the takeover state. (*b*) The beginning of takeover, in which degeneration of the parental generation is apparent in the upper quarter of the figure. (*c–g*) The middle of takeover. The colony finished takeover 36 hours after the start of takeover and resumed the ordinary state as shown in (*h–j*). Scale bar, 1 mm.

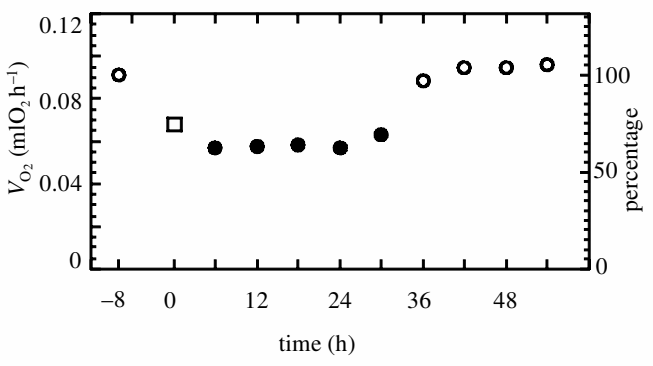

Figure 4. Changes in the rate of oxygen consumption (V_{O_2}) associated with the phase transition of the colony whose microscopic images, taken at the same time as the measurements, are given in figure 3. At −8 hours the colony was in the ordinary state (white circle). Measurement at 0 hours corresponds to the state just after the degeneration of the parent generation became apparent (white square). From 6 to 30 hours the colony was in the takeover state (black circles) and the metabolic rate remained reduced. After 36 hours the colony resumed the ordinary state and the metabolic rate recovered to the level before the generation changed.

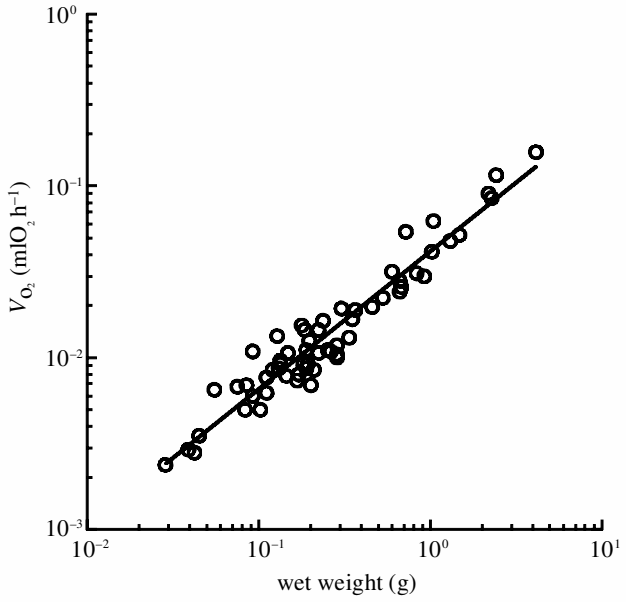

Figure 5. The relationship between the rate of oxygen consumption (V_{O_2}) and colonial mass in the ordinary state. The regression line gives the equation $V_{O_2} = 0.0412 M_w^{0.799}$ ($r^2 = 0.952$, $n = 60$; 95% confidence interval of the power coefficient, 0.740–0.859).

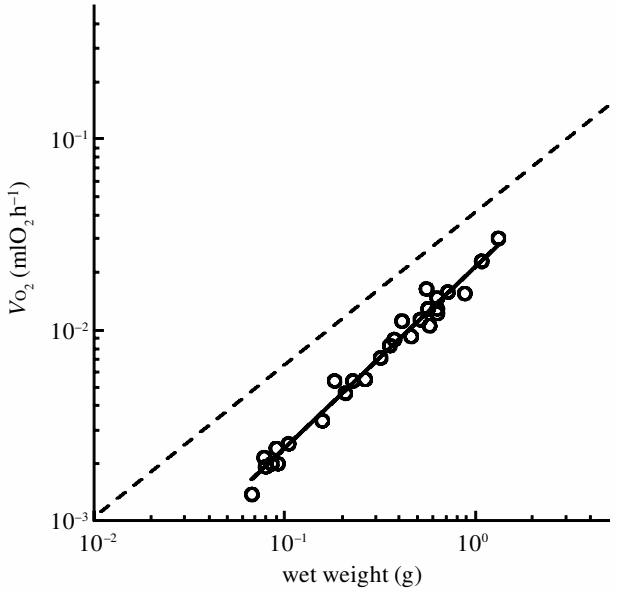

Figure 6. The relationship between the rate of oxygen consumption and colonial mass in the takeover state. Regression fitted to data is $V_{O_2} = 0.0214 M_w^{0.950}$ ($r^2 = 0.960$, $n = 28$; 95% confidence interval of the power coefficient, 0.894–1.01). The broken line is the regression line in the ordinary state.

that was not different from that of the organisms as individuals.

The metabolic rate of the colonies during the takeover state with M_w values ranging from 6.73×10^{-2} to 1.32 g was $1.37 \times 10^{-3} - 3.00 \times 10^{-2}$ mlO_2 h^{-1} ($n = 28$), from which the allometric relationship $V_{O_2} = 0.0214 M_w^{0.95}$ was obtained (figure 6). The coefficient of determination (r^2) was 0.960. The 95% confidence interval of the power coefficient ranged from 0.894 to 1.01. The power coefficient was not significantly different from 1 ($t = 1.88$, d.f. = 26, $p = 0.07$), which implies that the metabolic rate during takeover showed isometry, not allometry. Comparison of the two regression slopes revealed that the coefficient of 0.950 was statistically different from that of the ordinary-state value of 0.799 ($t = 3.46$, d.f. = 84, $p < 0.001$).

The weight-specific metabolic rate was obtained from figures 5 and 6. It was 0.0412 mlO_2 g^{-1} h^{-1} in the ordinary state and 0.0214 mlO_2 g^{-1} h^{-1} in the takeover state. When converted to the dry-weight-specific rate it was 0.323 mlO_2 g^{-1} h^{-1} in the ordinary state and 0.247 mlO_2 g^{-1} h^{-1} in the takeover state. The 95% confidence intervals for the wet-weight-specific metabolic rate in the ordinary and takeover states were 0.0371–0.0457 and 0.0197–0.0232, respectively. The weight-specific metabolic rate (=intercept) of the regression in the takeover state was statistically compared with that in the ordinary state. The weight-specific metabolic rate of the takeover state was significantly different from that of the ordinary state ($t = 21.7$, d.f. = 84, $p < 0.001$).

4. DISCUSSION

The present work is, to the authors' knowledge, the first report on the scaling of metabolic rate of colonial ascidians. The specific metabolic rate of the *B. simodensis* colony was 0.0214 (takeover state)−0.0412 mlO_2 g^{-1} h^{-1} (ordinary state), which was comparable to the value of 0.0348 mlO_2 g^{-1} h^{-1} of the unitary ascidian *Styela plicata* (Fisher 1976). The metabolic rate of the *B. simodensis* colonies in the ordinary state showed an allometric relationship with a power coefficient of 0.75. The metabolic rate of *S. plicata* also shows an allometric relationship with a power coefficient of 0.769. The weight-specific metabolic rate that was derived from the regression of the data of various poikilothermic animals was 0.144 mlO_2 g^{-1} h^{-1} and the allometric power coefficient was 0.751 (Hemmingsen 1960). Thus, in ascidians, irrespective of being unitary or colonial in character, the power coefficient did not differ from that of standard unitary poikilotherms, whereas the weight-specific rate was lower than the rate for standard poikilotherms.

The *B. simodensis* colony has two states—an ordinary state and a takeover state. The metabolic rates of the two states differ in their extent and scaling. The colonial metabolic rate was high for the ordinary state and showed an allometric relationship to the colonial mass. The metabolic rate was low for the takeover state and was directly proportional to the mass. This colony thus showed changes in its mode of metabolic scaling by changing from being allometric to isometric in character.

The present allometric study provided new morphological information. It has been known that the sizes of botryllloid zooids are identical in a colony (Ryland & Warner 1986), but there has been no report on whether the zooid size is the same or not in different colonies. The present study showed that the colonial mass was proportional to the number of zooids, and that the tunic volume fraction was kept constant. We conclude from these results that the size of zooids is the same not only within a colony but also in different colonies of this ascidian.

The identical property of the module is just one of the characters that make this system simple. The colony contains neither symbiotic algae nor a massive bony skeleton. Zooids are arranged without overlapping in a single layer to make a two-dimensional sheet-like structure. We do not need, therefore, to pay attention to the changes in the S/V ratio with colony size. The *B. simodensis* colonies were thus shown to be quite convenient for such a scaling study owing to their simple composition and shape. The colony can be regarded as a simple mono-layered structure made of identical modules.

The colony with a very small number of zooids may have a greater S/V ratio, and this may affect the regression slope. We simulated how the surface area for a zooid changes with colony growth. According to our preliminary observations zooids in this colonial ascidian seem to be closely packed together. With this in mind, we simulated colonial formation using a hexagonal close-packed lattice system. The ratio of length to thickness of each zooid was calculated as 2 : 1, according to actual measurements. With colonial growth the surface area per zooid converges into a constant value. The colonies with less than 19 zooids were estimated to have 10% more converged surface area per zooid. The colony with 19 zooids weighed less than 0.45 g. The power function that excluded data that were less than 0.45 g was $V_{O_2} = 0.041 M^{0.796}$ (cf. $V_{O_2} = 0.0412 M^{0.799}$ for the original). Thus, the bigger relative surface areas of the very small colonies are unlikely to

have affected the present result of the 0.8 power scaling of *B. simodensis*.

Ever since the work of Hughes & Hughes (1986) it has been believed that modular organisms are basically isometric as concerns their metabolic scaling. Any deviations from this have been attributed to the changes in morphological factors such as changes in the S/V ratio and in the volume fraction of inactive materials as the body size increases (Sebens 1987; Vollmer & Edmunds 2000).

B. simodensis in the takeover state showed isometry that agreed with the conventional view of metabolic scaling of colonial organisms. In the ordinary state, however, allometry was observed. This deviation from isometry is not attributable either to the change in the S/V ratio or to the change in the volume fraction of inactive materials. The S/V ratio was kept constant as the colony size increased owing to the two-dimensional shape of the colony, and our measurements strongly suggested constancy in the tunic volume fraction.

What caused the shift in the mode of scaling at state transitions? Here, we should critically review the morphological differences between the two states. The external shape of the colony remained unchanged during the state transitions. The total colonial biomass is probably unchanged because the colonial weight showed no changes before and after the state transition. All the materials of the degenerated zooids were absorbed into the common vascular network and were used in the growth of the new zooid generation (Mukai 1974; Lauzon *et al.* 1993). The tunic also looked unchanged and thus its volume fraction probably remained unchanged during state transition.

Prominent changes at state transition occur in the systems of mutual interaction between the zooids. In ordinary states the *B. simodensis* colonies have at least two routes for mutual interaction (Mukai *et al.* 1978). One is the common vascular system to which each zooid has connections through anterior and peduncular connecting vessels. The other is the common drainage system to which the exhalent currents from each zooid are drained. The vascular system seems to be a major medium for interaction because the blood vessels of botryllids not only convey materials but also transmit action potentials through which the behaviour of zooids is coordinated (Mackie & Singla 1983). The drainage system may cause fluid dynamic advantages by which the cost of feeding the current production will be reduced. Mutual interactions among zooids are, no doubt, quite reduced during the takeover states. The degeneration of the zooid parent generation was accompanied by the disintegration of the interaction systems, while the small buds of the filial generation grew into full-sized zooids and constructed their vascular and drainage systems. The fluid dynamic effect with the common drainage system did not work during takeover because no feeding current was created in this period. The vascular system seems to be of little use in the mutual interaction between the zooids, because the vessels connecting the zooids to the network needed considerable reconstruction even though the common vascular network did not (Mukai *et al.* 1978).

In the classic work of Hughes & Hughes (1986) metabolic isometry was shown in the colonies of the bryozoan *Electra pilosa*. This bryozoan colony is made by the iteration of a zooid encased in a rigid box-like skeleton that seems to allow little mutual interactions among the zooids, although some integrative behaviour can be observed (Thorpe *et al.* 1975; Harvell 1984). Hughes & Hughes (1986) explained the isometry by the absence of mutual interaction among the zooids. This seems to be a reasonable explanation. If a system is made of isolated and identical modules, it is very probable that the metabolic rate of each module is unaffected by other modules. The colonial metabolic rate is simply proportional to the number of modules, and thus metabolic isometry is produced. The present result of metabolic isometry is very probably derived from the absence or the large reduction in mutual interactions among zooids during the takeover state.

Even though the absence of mutual interactions leads to metabolic isometry, logically, this not does mean that the presence of mutual interactions leads to metabolic allometry. In the case of modular systems, however, mutual interactions among modules may well affect the activities of each module in such a manner as to cause metabolic allometry. Because the obvious change observed during the isometric to allometric shift was the restoration of mutual interactions, we hypothesize that the mutual interaction among zooids is the cause of metabolic allometry in *B. simodensis*.

The allometric coefficient in the *B. simodensis* ordinary state did not differ from 0.75—the value of the basal metabolic rate of unitary organisms. The reason that we employed a new hypothesis to explain the existence of this power coefficient of 0.75 in unitary organisms was as follows. The current theoretical hypotheses predict a power coefficient of 0.75 in three-dimensional organisms. The predicted power coefficient takes different values when the hypotheses are applied to a system with a two-dimensional shape such as the present ascidian colonies. By the mass transfer theory, two-dimensional organisms have a power coefficient in the range of 1.10–1.25 and 0.75 (Patterson 1992). The fractal branching model predicts a power coefficient of 2/3 for two-dimensional organisms (West *et al.* 1997). Thus, the model is not applicable to the present colony in spite of being regarded as the most successful theoretical model proposed to date for the 0.75 power rule. This model is not applicable to the present colony for another reason. The model is based on the presence of a circulatory system with hierarchical branching. The common vascular network of the present colony, however, does not show a hierarchical branching pattern. The classical surface law says that the power coefficient is 2/3 for three-dimensional objects and 1 for two-dimensional ones. Therefore, no general models explain the power coefficient value for the present ascidian colony. The specific models developed for some specific animal groups are also not applicable to the present colony. The elastic similarity model (McMahon 1973) depends on the beam theory of the solid elastic skeletons of vertebrates. The foraging model (Witting 1995) is based on the foraging behaviour of mobile organisms. Thus, these two models are not applicable to sessile colonies without bony skeletons.

All the hypotheses that tried to explain the power coefficient with just a single reason do not explain the present results. A multiple-cause model was recently put forward (Darveau *et al.* 2002; Weibel 2002), which supposes that

the apparent 0.75 allometric relationship is derived from the sum of various metabolisms with various exponents. This idea seems to be applicable to the takeover state of the present ascidians.

During the takeover state the filial generation zooids probably concentrate their resources on growing their own bodies. In the context of the model of Darveau *et al.* (2002), cells of the growing zooids would show a strong metabolic demand and reach the maximal metabolic rate. Because the growing zooids are still smaller in size and the connection between the zooids is yet to be established, their metabolic rate may well be the maximum value that can be released from the constraint of the size effect. Thus, the exponent becomes higher, possibly even reaching 1; at the same time, the cells that constructed the parental generation zooids are degenerating, but their masses still remain in the colony. These remains would slightly consume energy and would exhibit no size effects as they are dismembered. The metabolic rate of the takeover state is the sum of two metabolic rates that both show isometry. Because the mass of the degenerating parent generation is large it causes the overall specific metabolic rate to decline and this, therefore, explains the reduction in the total specific metabolic rate with the higher power exponent in the takeover state.

We could safely apply the model of Darveau *et al.* (2002) to the results in the takeover state, because every contributor in that state had a common power exponent of $b = 1$. It should be remembered that the simple summation of the power functions does not become a monomial power function, except for the summation of the functions with a common power exponent.

The allometry of the ordinary state cannot be explained by the single-cause models that are available. The multiple-cause model also needs to explain why each contributor obeys the power law. The discrete organization of the ascidian colony has led us to explore the origin in the mutual interaction among the zooids. In our 'mutual interaction model' the system that is made of discrete modules is assumed to be the criticality level with respect to statistical mechanics. Statistical physics says that such mutual interactions may cause metabolic allometry. When a system is made of identical modules with local interaction, criticality can be produced spontaneously (Bak *et al.* 1987). Because power functions are associated with criticality, it is possible that allometric relations including metabolic scaling derive from criticality. Torres (2001) suggested that some biological phenomena are associated with criticality. We are now developing a theoretical model that produces metabolic allometry based on this idea. We need discrete mechanics rather than conventional continuum mechanics to handle such a model. The classic theoretical models proposed to date use continuum mechanics that treat the body of organisms as a continuous material. The theoretical problem in adopting continuum mechanics appears to be that the mechanics give a single fixed solution as a power exponent b, despite the fact that this value is, in actuality, not always fixed (Weibel 2002). One of the merits of adopting the discrete-mechanics models lies in the fact that one can derive the various power scalings by changing the mutual interaction between the units or the contributors. Another benefit is that these models can use classic theoretical models based on continuum mechanics, since discrete mechanics includes continuum mechanics as a special part of it. The mutual interaction model we are developing is a model based on colonial animals. This is, however, not a model that is specific to colonial organisms, but will be a general model that can be applied to unitary organisms. Unitary organisms could be regarded as a modular system composed of similarly sized modules, namely cells, with mutual interactions (Vogel 1988). To our knowledge, this methodology in dealing with unitary organisms has never been used before in theoretical scaling biology. We believe that it is worth pursuing.

The power coefficient of the present ascidian colonies did not differ from that of the basal metabolic rate of unitary organisms. If the 0.75 power rule is the intrinsic character of modular systems with interactions among modules, then the cellular organization of unitary organisms may well show the same power coefficient. If the organisms, whether colonial or unitary, share the same basis for metabolic allometry, the present colonial ascidians will provide an ideal model for the study of metabolic scaling. The system is simple. It is made of units that are identical in size and form and has a simple two-dimensional shape. In addition to its simplicity, the colony of the present ascidians provides a new tool for biological scaling. The study of scaling has, to date, depended only on the comparison between existing animals of different sizes. With the present colonial ascidians, however, we can cut a large colony into smaller healthy colonies and we can fuse several colonies into a larger one. The manipulation of the size of a system should open up entirely new experimental approaches to the study of biological scaling.

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REFERENCES

Bak, P., Tang, C. & Wiesenfeld, K. 1987 Self-organized criticality: an explanation for $1/f$ noise. *Phys. Rev. Lett.* **59**, 381–384.

Calder, W. A. 1984 *Size, function, and life history*. Cambridge, MA: Harvard University Press.

Darveau, C. A., Suarez, R. K., Andrews, R. D. & Hochachka, P. W. 2002 Allometric cascade as a unifying principle of body mass effects on metabolism. *Nature* **417**, 166–170.

Dodds, P. S., Rothman, D. H. & Weitz, J. S. 2001 Re-examination of the '3/4-power law' of metabolism. *J. Theor. Biol.* **209**, 9–27.

Fisher, R. T. 1976 Oxygen uptake of the solitary tunicate *Styela plicata*. *Biol. Bull.* **157**, 297–305.

Gould, S. J. 1966 Allometry and size in ontogeny and phylogeny. *Biol. Rev.* **41**, 587–640.

Harvell, C. D. 1984 Predator-induced defense in a marine bryozoan. *Nature* **224**, 1357–1359.

Hemmingsen, A. M. 1960 Energy metabolism as related to body size and respiratory surfaces, and its evolution. *Rep. Steno Meml Hosp. Nordisk Insulin Lab.* **9**, 1–110.

Hirose, E., Saito, Y. & Watanabe, H. 1991 Tunic cell morphology and classification in botryllid ascidians. *Zool. Sci.* **8**, 951–958.

Hughes, D. J. & Hughes, R. N. 1986 Metabolic implications of modularity: studies on the respiration and growth of *Electra pilosa*. *Phil. Trans. R. Soc. Lond.* B **313**, 23–29.

Kleiber, M. 1961 *The fire of life*. New York: Wiley.

Lauzon, R. J., Patton, C. W. & Weissman, I. L. 1993 A morphological and immunohistological study of programmed cell death in *Botryllus schlosseri*. *Cell Tissue Res.* **272**, 115–127.

Mackie, G. O. & Singla, C. L. 1983 Coordination of compound ascidians by epithelial conduction in the colonial blood vessels. *Biol. Bull.* **165**, 209–220.

McMahon, T. A. 1973 Size and shape in biology. *Science* **179**, 1201–1204.

Milkman, R. 1967 Genetic and developmental studies on *Botryllus schlosseri*. *Biol. Bull.* **132**, 229–243.

Mukai, H. 1974 A histological study on the degeneration of zooids in a compound ascidian, *Botryllus primigenus*. *Zool. Magazine* **83**, 18–23.

Mukai, H., Sugimoto, K. & Taneda, Y. 1978 Comparative studies on the circulatory system of the compound ascidians, *Botryllus*, *Botrylloides* and *Symplegma*. *J. Morph.* **157**, 49–78.

Patterson, M. R. 1992 A mass transfer explanation of metabolic scaling relations in some aquatic invertebrate and algae. *Science* **255**, 1421–1423.

Peters, R. H. 1983 *The ecological implications of body size*. New York: Cambridge University Press.

Rubner, M. 1883 Ueber den einfluss der Körpergrösse auf stoffund kraftwechsel. *Z. Biol.* **19**, 535–562.

Ryland, J. S. & Warner, G. F. 1986 Growth and form in modular animals: ideas on the size and arrangement of zooids. *Phil. Trans. R. Soc. Lond.* B **313**, 53–76.

Saito, Y., Mukai, H. & Watanabe, H. 1981 Studies on Japanese compound styelid ascidians II. A new species of the genus *Botrylloides* and redescription of *B. violaceus* Oka. *Publ. Seto Mar. Biol. Lab.* **XXVI**(4/6), 357–368.

Schmidt-Nielsen, K. 1984 *Scaling: why is animal size so important?* Cambridge University Press.

Sebens, K. P. 1987 Coelenterata. In *Animal energetics* (ed. T. P. Pandian & F. J. Vernberg), pp. 55–120. New York: Academic.

Thorpe, J. P., Shelton, G. A. & Laverack, M. S. 1975 Colonial nervous control of lophophore retraction in cheilostome Bryozoa. *Science* **189**, 60–61.

Torres, J. 2001 Biological power law and Darwin's principle. *J. Theor. Biol.* **209**, 223–232.

Vogel, S. 1988 *Life's devices*. Princeton University Press.

Vollmer, S. V. & Edmunds, P. J. 2000 Allometric scaling in small colonies of the scleractinian coral *Siderastrea sidereal* (Ellis and Solander). *Biol. Bull.* **199**, 21–28.

Watanabe, H. 1953 Studies on the regulation in fused colonies in *Botryllus primigenus* (Ascidiae Compositae). *Sci. Rep. Tokyo Bunrika Daigaku* B **10**, 253–284.

Weibel, E. R. 2002 Physiology: the pitfalls of power laws. *Nature* **417**, 131–132.

West, G. B., Brown, J. H. & Enquist, B. J. 1997 A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122–126.

Witting, L. 1995 The body mass allometries as evolutionarily determined by the foraging of mobile organisms. *J. Theor. Biol.* **177**, 129–137.

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