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Influence of environmental factors on burrow irrigation and oxygen consumption in the mudflat invertebrate *Urechis caupo*

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Abstract We examined burrow irrigation activity by the mudflat worm *Urechis caupo* in response to suspended food, ambient hypoxia (down to 3.3 kPa PO_2), hydrogen sulfide exposure (up to 100 $\mu\text{mol l}^{-1}$), and short-term temperature change (range 10–22°C). In normoxic, nutrient-free water at 14°C, O_2 consumption ($\dot{M}O_2$) was 45 $\text{nmol min}^{-1} \text{g}^{-1}$, water flow rate (\dot{V}_W) was 27 ml min^{-1} (0.66 $\text{ml min}^{-1} \text{g}^{-1}$), frequency of peristaltic waves (F_P) was 2.6 contractions min^{-1} , stroke volume (SV) was 11 ml, and O_2 extraction coefficient (EO_2) was 0.27. Adding suspended food to the burrow water occasionally elicited stereotypical feeding behavior but had no effect on any measured variables during nonfeeding periods. Hypoxia greatly decreased $\dot{M}O_2$ (75% reduction at 3.3 kPa PO_2) but did not affect \dot{V}_W , F_P , SV, or EO_2 . Sulfide at 50 $\mu\text{mol l}^{-1}$ or less had no effect on burrow irrigation activity, whereas 100 $\mu\text{mol l}^{-1}$ sulfide decreased \dot{V}_W by 58% and F_P by 50% but had no effect on SV. Temperature strongly affected \dot{V}_W (Q_{10} of 1.9 from 10°C to 22°C). We propose that *U. caupo*'s ability to live in the hypoxic, sulfidic mud of productive mudflat environments, combined with its very efficient mucous net, allows it to process much less water for feeding than other suspension-feeding invertebrates. This, in turn, necessitates an efficient O_2 extraction mechanism, which is provided by the water lung activity of *U. caupo*'s unique hindgut.

Introduction

The mudflat invertebrate *Urechis caupo* (Fisher and MacGinitie 1928a) is a large (up to 250 g, average ~40 g), burrowing echiuran worm found in tidal mudflats along the coast of California from Humboldt Bay at the north to Tijuana Slough at the south. *U. caupo* inhabits U-shaped burrows that reach an average depth of 36 cm in the sediment (Fisher and MacGinitie 1928b; Arp et al. 1992), which is well into the sulfide-rich reducing layers. *U. caupo* actively pumps water through its burrow with rhythmic, anterior-to-posterior peristaltic contractions of the body wall. This water current brings in fresh, oxygenated water and suspended food and expels waste products, gametes, and any hydrogen sulfide that has leached into the burrow water from the surrounding sediment.

U. caupo is a nonselective filter feeder that captures its food in a mucous net (Fisher and MacGinitie 1928b). This funnel-shaped net, which is secreted from mucous glands located near the anterior end of the body wall, is attached to the burrow at one end and to the integument of the anterior body wall at the other. *U. caupo* irrigates water through the burrow and the net and then swallows the net, including all captured particles, which can be as small as 4 nm in diameter (MacGinitie 1945). *U. caupo*'s use of a mucous net for feeding is unique among the echiura and is unusual among invertebrates, resembling most closely the mucous nets of *Nereis diversicolor* and the chaetopterids.

Many *U. caupo* burrows are located in the intertidal zone and during low tides the water in these burrows can become severely hypoxic (PO_2 less than 3 kPa) and can accumulate up to 65 $\mu\text{mol l}^{-1}$ hydrogen sulfide (Arp et al. 1992). Hydrogen sulfide (referred to henceforth as "sulfide") is a potent metabolic toxin, and many adaptations to sulfide and hypoxia have been discovered in *U. caupo* (Arp et al. 1995, for review; Menon and Arp 1998; Julian et al. 1999). Although the effect of sulfide and hypoxia on *U. caupo* body wall peristaltic activity in vitro has been

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studied (Julian et al. 1998), the effect of these factors on burrow irrigation activity and behavior has not yet been reported. Maintenance of irrigation and ventilation during sulfide exposure is likely to be important for sulfide-adapted, burrow-dwelling invertebrates, since sulfide detoxification is generally O₂-dependent (Grieshaber and Völkel 1998; Julian et al. 1999). Nonetheless, only two publications have described sulfide's effect on burrow irrigation activity (Miron and Kristensen 1993, for Nereid polychaetes; Wohlgemuth et al. 2000, for the lugworm *Arenicola marina*).

Coastal mudflats and marshes contain a variety of burrow-dwelling, filter-feeding invertebrates, including *U. caupo*. These animals can encounter variable and challenging environmental conditions, such as decreased PO₂ (hypoxia), extremes of temperature, variations in food supply, and the presence of sulfide. Surprisingly, the effects of these conditions on burrow irrigation activity have been little studied. In this article, we examine the influence of food concentration, ambient hypoxia, temperature change, and hydrogen sulfide on burrow irrigation activity and oxygen consumption in *U. caupo*.

Materials and methods

Animal collection and maintenance

Urechis caupo (28–58 g wet weight) were collected from Pillar Point Harbor in San Mateo County, California (37°18'N, 123°03.3'W), during low tide. Collected worms were maintained unfed in aerated, recirculating seawater at 14–16°C and 32 ppt salinity. All worms in the irrigation and O₂ consumption studies were used within 3 weeks of collection, but we have been able to maintain *U. caupo* under these conditions for several months, so we assumed they were comparatively healthy. Laboratory experiments were conducted using artificial seawater (ASW; Instant Ocean, Aquarium Systems, Inc.) that was free of ammonium, nitrate, and phosphate at 32 ppt salinity (as measured by refractometer).

Feeding behavior

Each *U. caupo* was placed individually in a U-shaped artificial burrow, constructed of acrylic tubing (2.5 cm i.d., 119 cm length), that simulated the natural burrow. The openings of the tubing were attached to one of two water sources: a large capacity recirculating seawater system that also supplied the holding aquaria, or a small, closed circulation feeding system consisting of a 14.2-l acrylic particulate reservoir connected to a peristaltic pump. The water in the feeding system was continuously aerated and mechanically stirred, and water was pumped from the reservoir to the artificial burrows at a rate of 375 ml min⁻¹ and returned to the reservoir by gravity. The worms were fed by adding one of three commercial marine suspension-feeder food preparations to the reservoir (Marine Invertebrate Diet from Hawaiian Marine Imports, Inc.; Liquify Marine from Interpet Ltd.; and Invertebrate Smorgasbord from Coralife, Inc.). The commercial food preparation was added to the feeding reservoir at approximately 0.1 ml l⁻¹. Worms were fed continuously for 48–96 h each week, and feeding behavior was videotaped using a Sony CCD camera and photographed using a 35-mm camera equipped with a macro lens. The mucous feeding nets, which are transparent when created, were stained by introducing carmine particles suspended in seawater (5 g l⁻¹) into the nets.

Burrow irrigation activity (\dot{V}_W , F_P , and SV)

The artificial burrows were modified after Hall (1931) to allow measurement of burrow irrigation rate as the rate of water flow, \dot{V}_W (ml min⁻¹). Flexible vinyl tubing (51 cm length, 0.48 cm i.d.) connected the anterior burrow opening to an inlet reservoir and the posterior opening to an outlet reservoir. The burrow and reservoirs were placed in a 34-l recirculating water bath maintained at 14 ± 0.2°C. The inlet reservoir was continuously bubbled with air, and a peristaltic pump kept the inlet reservoir filled with ASW from a 6-l primary reservoir.

\dot{V}_W was measured in 10-min periods by placing a graduated cylinder at the outlet of the outflow reservoir. To minimize the hydrostatic pressure head, the height of the outlet was less than 1 cm above the water level in the inlet reservoir. The measurement was replicated six times for each treatment. If the worm reversed direction in the burrow during a measurement period, the inlet and outlet reservoir connections were switched and the measurement interval was restarted. The interval between replicate measurement periods was minimized, and the replicates were generally recorded successively. Therefore, the activity of each worm was measured for at least 1 h. During \dot{V}_W measurements, the peristaltic body wall contractions were observed visually and the total number of contractions was recorded. This was divided by the duration to give the frequency of peristaltic contractions (F_P). Stroke volume (SV; water pumped per one peristaltic contraction), was calculated as \dot{V}_W divided by F_P .

Oxygen consumption rate ($\dot{M}O_2$) and extraction coefficient (EO_2)

For measurements of O₂ consumption, the artificial burrow was further modified by placing Clark-style oxygen electrodes in the closed inflow and outflow reservoirs. Each reservoir was kept well stirred. The difference between average inflow and outflow PO₂ during each 10-min \dot{V}_W measurement period was then used to calculate the O₂ consumption rate:

$$\dot{M}O_2 = \left(\frac{\Delta PO_2 \cdot \alpha \cdot \dot{V}_W}{G} \right) \quad (1)$$

where $\dot{M}O_2$ = weight-specific oxygen consumption rate (nmol min⁻¹ g⁻¹), ΔPO_2 = average difference in oxygen partial pressure (kPa), α = O₂ solubility coefficient at 14°C and 32 ppt salinity (12.7 μmol l⁻¹ kPa⁻¹, Boutilier et al. 1984), \dot{V}_W = flow rate (l min⁻¹), and G = mass of the worm (g). The O₂ extraction coefficient, EO_2 , was calculated as ΔPO_2 divided by the inlet PO₂.

Effect of suspended food on $\dot{M}O_2$, EO_2 , \dot{V}_W , F_P , and SV

The effects of suspended food on burrow irrigation behavior and $\dot{M}O_2$ were determined by adding commercial marine invertebrate food ("Invertebrate Smorgasbord" from Coralife, Inc.) to the inflowing water. Five worms were allowed to acclimate for at least 12 h in nutrient-free ASW. After the acclimation period, baseline measurements of $\dot{M}O_2$, \dot{V}_W , and F_P were recorded simultaneously. Then, the food suspension was added to the primary reservoir to achieve a final concentration of 0.36, 3.6, or 36 mg l⁻¹. Field studies of *U. caupo* have indicated that food concentrations in the summer peak average 3.6 mg l⁻¹ (Judd 1993). After 3 h in the presence of food, $\dot{M}O_2$, \dot{V}_W , and F_P were again measured. After replacing the reservoir water and allowing the worm to irrigate normally for 3 h (or overnight), the process was repeated in random order until each worm had been exposed to all food concentrations. The ASW in the inlet reservoir was kept normoxic throughout this experiment and the burrow was maintained at 14 ± 0.2°C.

Effect of hypoxia on $\dot{M}O_2$, EO_2 , \dot{V}_W , F_P , and SV

Seven worms were placed in the artificial burrow and allowed to acclimate for at least 12 h. During this period, the inlet reservoir ASW was kept normoxic (21 kPa PO₂) by bubbling with air.

After baseline measurements of $\dot{M}O_2$, \dot{V}_W , and F_P , as described above, the inflowing PO_2 was changed to 13 kPa, 10 kPa, or 3.3 kPa by vigorously bubbling the inlet reservoir ASW with premixed O_2 and N_2 . After 3-h acclimation to each oxygen level, $\dot{M}O_2$, \dot{V}_W , and F_P were measured as before. After the inflowing PO_2 was changed back to normoxia, and the worm was allowed 3 h (or overnight) to recover, the inflowing PO_2 was switched to one of the remaining hypoxic levels and the process was repeated. The order of hypoxic levels for each worm was randomized. The ASW in this experiment was nutrient-free and the burrow was maintained at $14 \pm 0.2^\circ\text{C}$.

Effect of temperature change on \dot{V}_W

Four worms were placed in the artificial burrow for 12 h at 17°C after which \dot{V}_W was measured. The temperature was then adjusted to either 10°C or 22°C for 3 h and \dot{V}_W was again measured. After the temperature was adjusted back to 17°C for 12 h, the experiment was repeated at the remaining temperature. The order of temperatures for each worm was randomized. The ASW in this experiment was normoxic and nutrient-free.

Effect of sulfide on \dot{V}_W , F_P , and SV

The artificial burrow was identical to that used above, except for an additional inlet to the inflowing reservoir, through which a stock solution of 100 mmol l^{-1} sulfide was added using a peristaltic pump. The pump rate was adjusted to achieve a steady-state, total sulfide (H_2S , HS^- , and S^{2-}) concentration of 25, 50, or $100 \mu\text{mol l}^{-1}$. The reservoir ASW was kept vigorously bubbled with air. The sulfide concentration in the inflowing ASW was measured at the start of each exposure and then again at the end using a standard spectrophotometric assay (Cline 1969).

Four worms were placed in the artificial burrow at normoxia for 12 h, after which \dot{V}_W and F_P were measured. After the pump was adjusted to achieve the desired sulfide concentration for 3 h, \dot{V}_W and F_P were again measured. The sulfide pump was stopped, and the worm was allowed to irrigate the burrow with fresh ASW for a recovery period of 3 h (or overnight). The sulfide was then adjusted to achieve one of the remaining sulfide concentrations, and the process was repeated. The order of sulfide concentrations for each worm was randomized. The ASW in this experiment was nutrient-free and the burrow was maintained at $14 \pm 0.2^\circ\text{C}$.

Statistical analyses

The six consecutive measurements of each variable (e.g., \dot{V}_W , $\dot{M}O_2$, F_P) for each worm at each treatment level (i.e., food concentration, PO_2 , temperature, and sulfide concentration) were averaged to give a single mean value for each variable at each treatment level for each worm. Prior to analysis, these values for each experiment were confirmed to have Gaussian distributions (Kolmogorov–Smirnov test) and equal standard deviations (Bartlett's test). Within each experiment, every worm was subjected to every treatment level, so the effects of these factors could be analyzed using one-way, repeated-measures analysis of variance (ANOVA). Significant differences between treatment levels ($P < 0.05$) were subsequently determined by the Tukey–Kramer multiple comparisons post test. All statistical tests were performed using commercial software (InStat 3.01, GraphPad Software).

Results

Feeding behavior

The feeding behavior of *Urechis caupo* is stereotyped and consistent (Fisher and MacGinitie 1928b). The behavior can be divided into four functional sections:

(1) attachment and secretion (Fig. 1A), (2) filtering (Fig. 1B), (3) withdrawal (Fig. 1C), and (4) grasp and consumption (Fig. 1D, E). A feeding episode starts with the worm moving up one of the vertical arms of its U-shaped burrow. *U. caupo* first expands its entire body, bringing a ring of mucous glands located posterior to the ventral setae into contact with the burrow wall. The proboscis is held upright in the center of the burrow and a series of large peristaltic waves, generated by contractions of the muscular body wall, are initiated. Simultaneously, the worm begins secretion of the mucous feeding net from the mucous glands while backing down the burrow. The waves of peristalsis are relatively uniform in size in the beginning, but subsequently the waves forward of the mucous glands inside the newly constructed net start to be reduced in size. The forward portion of the body inside the net is then contracted into a series of two or three folds that appear to position the net, keeping it taut during construction, and produce the funnel shape of the finished net. Net length varied between feeding episodes, ranging from 10 to 16 cm, and were similar in length to nets reported by Fisher and MacGinitie (1928b) and MacGinitie (1945).

When the net is completed, the worm elongates and contracts the anterior portion of its body down to approximately 25% of its normal diameter (Fig. 1A). The feeding net completely blocks the burrow, as one end of the funnel-shaped net is attached to the burrow and the other remains attached to the worm at the ring of mucous glands. Once the net is positioned, peristaltic contractions posterior to the net become large and vigorous, pulling water into the burrow and through the feeding net. As the net fills with particulate material, the peristaltic contractions increase in frequency and depth (Fig. 1B). The filtering periods observed in this study lasted 15–20 min. At the end of the feeding period, the worm breaks the connection between the net and its mucous glands with a quick backward motion. The worm then retreats a short distance down the burrow (Fig. 1C), flares and extends its proboscis, and returns up the burrow grasping the net (Fig. 1D), consuming it as it continues to move up the burrow (Fig. 1E). The consumption of the net is very rapid, taking approximately 30–60 s.

$\dot{M}O_2$, EO_2 , \dot{V}_W , F_P , and SV in normoxic, nutrient-free water

When *U. caupo* in the artificial burrows were exposed to normoxic ($21 \text{ kPa } PO_2$), nutrient-free water at 14°C , $\dot{M}O_2$ was $45 \pm 16 \text{ nmol g}^{-1} \text{ min}^{-1}$ (mean \pm SD, $n = 12$), EO_2 was 0.28 ± 0.088 , \dot{V}_W was $27 \pm 9.5 \text{ ml min}^{-1}$, F_P was $2.6 \pm 0.52 \text{ contractions min}^{-1}$, and SV was $11 \pm 2.8 \text{ ml}$. \dot{V}_W , expressed as the weight-specific rate of burrow irrigation, was $0.66 \pm 0.42 \text{ ml g}^{-1} \text{ min}^{-1}$. These data include the control data for the seven worms used in the food experiment and the control data for the five worms used in the hypoxia experiment.

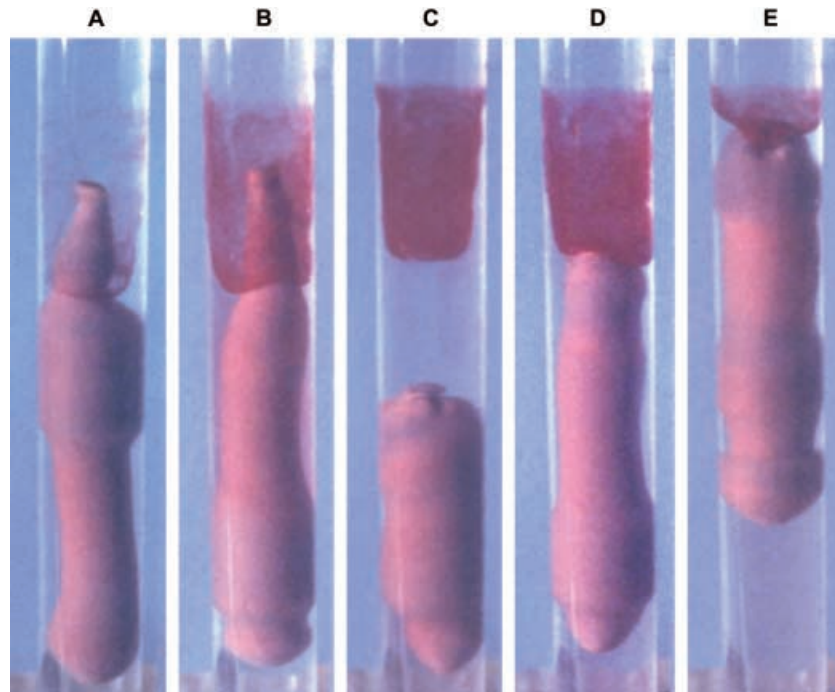


Fig. 1A–E The feeding behavior of *Urechis caupo*. **A** A commercial food suspension was added to the water to induce feeding behavior in *Urechis caupo*. When the worm spontaneously began formation of the mucous net, carmine particles were immediately introduced into the water current to make the net clearly visible. **A** The worm has attached the mucous net to the walls of the burrow and is pumping water through it. **B** Continued pumping by the worm gradually fills the net with the added carmine particles. **C** At the end of the feeding period, the worm breaks the connection between the net and its integument with a quick backward motion. **D** The worm now flares its proboscis and returns up the burrow, grasping the net. **E** The worm consumes the net and its contents as it continues to move up the burrow. The video from which this figure was derived is available at <http://www.zoology/ufl.edu/julian/urechis.html>

Influence of food concentration on $\dot{M}O_2$, EO_2 , \dot{V}_W , F_P , and SV

We found that added food at a concentration range of $0.36\text{--}36\text{ mg l}^{-1}$ had no significant effect on $\dot{M}O_2$ (Fig. 2A; $P=0.80$), EO_2 (data not shown; 0.31 ± 0.11 , $P=0.69$), \dot{V}_W (Fig. 2B; $P=0.55$), F_P (Fig. 2C; $P=0.16$), or SV (Fig. 2D; $P=0.71$), compared with the group of worms in nutrient-free water. For these data, we excluded any periods during which the worms were building a mucous net, filtering water through a net, or consuming a net, all of which we categorize as “feeding behavior.”

U. caupo did not consistently initiate feeding behavior after the addition of food, as only two of the five worms initiated feeding behavior. When this behavior was elicited, it was typically 5–10 min after the food was added and only after at least 3.6 mg l^{-1} of food had been added. We did not obtain $\dot{M}O_2$ records during feeding behavior, but we did obtain \dot{V}_W , F_P , and SV. Overall, \dot{V}_W during feeding was over twice the average rate observed during the control period (76 ml min^{-1} , range $67\text{--}99\text{ ml min}^{-1}$ during feeding compared with $33 \pm 12\text{ ml min}^{-1}$ during nonfeeding periods), and both F_P and SV were

about 1.5-fold higher than those observed during nonfeeding periods ($F_P = 5.13\text{ contractions min}^{-1}$, range $4.0\text{--}7.3\text{ min}^{-1}$ vs $3.2 \pm 0.82\text{ contractions min}^{-1}$; SV = 15 ml , range $14\text{--}17\text{ ml}$ vs $10 \pm 2.7\text{ ml}$). However, since the number of observations is small and from only two worms, no statistical comparisons were performed to determine whether these values are significantly different from nonfeeding periods. Nonetheless, the higher irrigation activity during feeding is consistent with that reported previously by Hall (1931).

Influence of hypoxia on $\dot{M}O_2$, EO_2 , \dot{V}_W , F_P , and SV

Compared with the value at normoxia ($21\text{ kPa } PO_2$), $\dot{M}O_2$ was significantly decreased at 10 kPa and $3.3\text{ kPa } PO_2$ ($P < 0.01$, $P < 0.001$, respectively, $n = 7$; Fig. 3A). Hypoxia did not significantly affect \dot{V}_W ($P = 0.23$; Fig. 3B) but did affect F_P , with F_P at $13\text{ kPa } PO_2$ being significantly higher than at 10 kPa and $3.3\text{ kPa } PO_2$ ($P < 0.01$ foreach), but not significantly different from $21\text{ kPa } PO_2$ (Fig. 3C). SV (Fig. 3D) was significantly more variable at $10\text{ kPa } PO_2$ than at the other PO_2 s ($P < 0.01$). However, the mean SV was not affected by PO_2 overall ($P = 0.16$ by Friedman’s nonparametric repeated measures test). EO_2 was 0.23 ± 0.059 at normoxia (data not shown). This value was not significantly affected by hypoxia (EO_2 was 0.31 ± 0.14 at $3.3\text{ kPa } PO_2$; $P = 0.46$).

Influence of temperature change on \dot{V}_W

Decreasing the burrow water temperature from 17°C to 10°C decreased \dot{V}_W by $43\% \pm 16\%$ ($Q_{10} = 2.2$; Fig. 4), whereas increasing the temperature from 17°C to 22°C

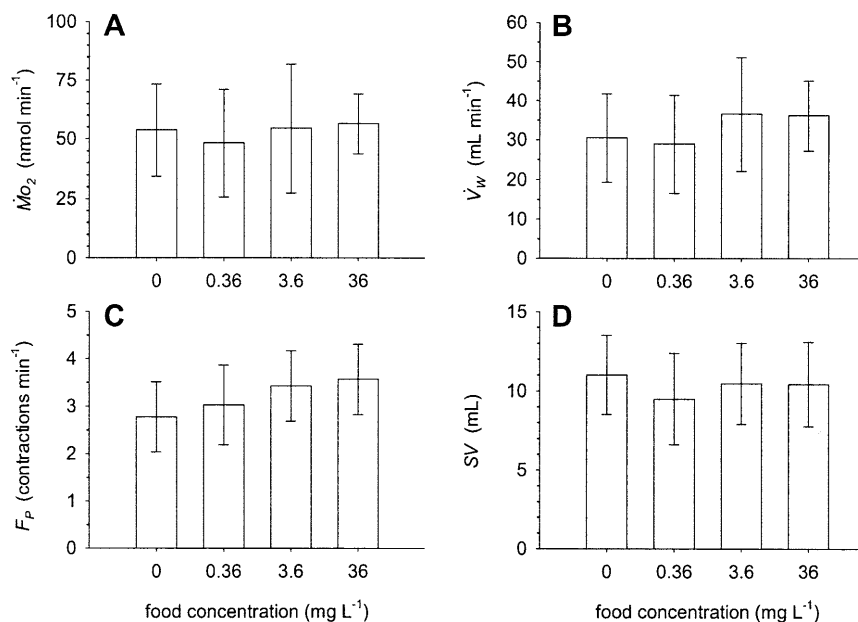


Fig. 2A–D Influence of food concentration on O_2 consumption and burrow irrigation activity. *Urechis caupo* in an artificial burrow were exposed to a commercial food suspension for 3 h, after which weight-specific O_2 consumption ($\dot{M}O_2$), burrow irrigation rate (\dot{V}_W), and body wall peristaltic frequency (F_P) were recorded for at least 1 h. Stroke volume (SV) was later calculated as \dot{V}_W/F_P . Food was added to the inflowing water to achieve a final concentration of 0.36, 3.6, or 36 mg L⁻¹. Data represent mean \pm SD, with five worms tested at every concentration. Periods during which the worms were building a mucous net, filtering water through a net, or consuming a net were excluded from these data. **A** $\dot{M}O_2$ ($P=0.80$). **B** \dot{V}_W ($P=0.55$). **C** F_P ($P=0.16$). **D** SV ($P=0.71$)

increased \dot{V}_W by $25\% \pm 8.1\%$ ($Q_{10}=1.6$). \dot{V}_{mW} at 10°C and 22°C are significantly different ($P < 0.05$), with an overall Q_{10} of 1.9.

Influence of sulfide on \dot{V}_W , F_P , and SV

Addition of $100 \mu\text{mol l}^{-1}$ sulfide caused \dot{V}_W to decrease by 58% and F_P to decrease by 50% ($P < 0.01$ for each; Fig. 5A, B). This effect was only seen with the highest sulfide concentrations, and addition of up to $50 \mu\text{mol l}^{-1}$ sulfide had no significant effect on \dot{V}_W or F_P . Stroke volume remained unchanged at all sulfide concentrations ($P = 0.61$, Fig. 5C). $\dot{M}O_2$ was not measured in these experiments because O_2 consumption from spontaneous and/or catalyzed sulfide oxidation was impossible to separate from the O_2 consumption due to the worm's metabolism.

Discussion

Feeding behavior and influence of food on irrigation behavior

Urechis caupo is one of only a few tube-dwelling animals that capture suspended food particles with a secreted

mucous net. The feeding behavior of *U. caupo* was described in detail by Fisher and MacGinitie (1928b) and later expanded by Hall (1931) and MacGinitie (1945). Four previous studies on *U. caupo* in artificial burrows reported mucous nets and feeding behavior (Fisher and MacGinitie 1928a; Hall 1931; MacGinitie 1945; Lawry 1966a), whereas one study reported that mucous nets were never observed, although “considerable mucous and fecal material were produced” (Pritchard and White 1980). None of these previous studies was successful in eliciting feeding behavior after addition of food. In the present study, feeding behavior was occasionally initiated after commercial invertebrate food suspensions were added to the water, although the presence of food had no effect on $\dot{M}O_2$, \dot{V}_W , F_P , or SV during nonfeeding periods. Similarly, Lawry (1966a) found no change in \dot{V}_W after adding a concentrated suspension of cultured diatoms (*Nitzschia* sp.) to the burrow inflow (the final concentration is unknown). On the other hand, we found that irrigation activity was higher on average when worms were pumping water through a mucous net (\dot{V}_W , F_P , and SV increased by 2.3-fold, 1.6-fold, and 1.5-fold, respectively), which agrees with the findings of Hall (1931). In contrast, the echiuran *Bonellia viridis*, which generally lives in burrows but does not use a mucous net, increases body wall peristaltic activity by only 1.2-fold during feeding (Schembri and Jaccarini 1977).

In *U. caupo*'s natural habitat, detritus is the most abundant form of carbon in the water column, and phytoplankton is the most abundant form of nitrogen (Judd 1993). The commercial food suspension we used to investigate the effect of food on burrow irrigation activity consisted primarily of casein hydrolysate and yeast extract (composition: 3.3% protein, 0.8% fat, 0.3% fiber, 95.6% moisture). We do not know whether suspensions of algae or detritus would have had a different effect. In addition, it may be important to

Fig. 3A–D Influence of hypoxia on O_2 consumption and burrow irrigation activity. *Urechis caupo* in an artificial burrow at 14°C were exposed to 13 kPa, 10 kPa, or 3.3 kPa PO_2 for 3 h, after which weight-specific O_2 consumption ($\dot{M}O_2$), burrow irrigation rate (\dot{V}_W), and body wall peristaltic frequency (F_P) were recorded for at least 1 h. Stroke volume (SV) was later calculated as \dot{V}_W/F_P . Control data (21 kPa PO_2) were obtained for each worm at the beginning of the experiment. Data represent mean \pm SD, with seven worms tested at every PO_2 . **A** $\dot{M}O_2$. The effects of 10 kPa and 3.3 kPa are significantly different from 21 kPa ($P < 0.01$, $P < 0.001$, respectively), and the effect of 3.3 kPa is significantly lower than all other values ($P < 0.001$). **B** \dot{V}_W . No effect of hypoxia ($P = 0.23$). **C** F_P . The effect of 13 kPa is significantly higher than that of 10 kPa and 3.3 kPa ($P < 0.01$ for each). **D** SV. No effect of hypoxia ($P = 0.16$ by Friedman's nonparametric repeated measures test; see text for details of analysis)

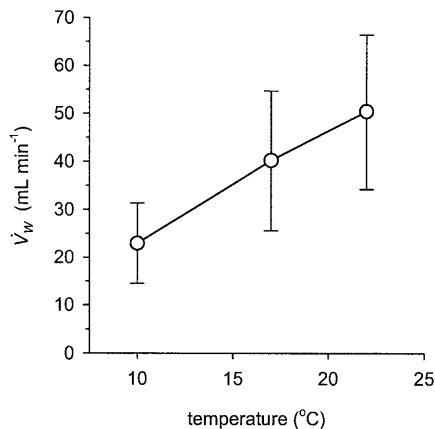
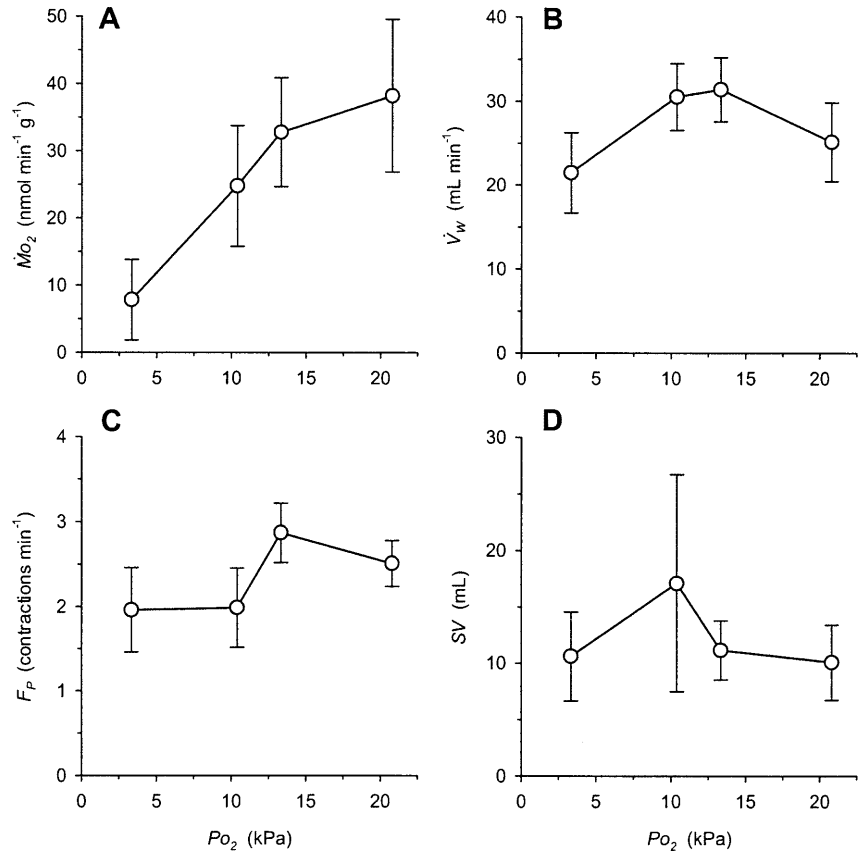


Fig. 4 Influence of temperature change on burrow irrigation rate; *Urechis caupo* in an artificial burrow were held for 12 h at 17°C after which burrow irrigation rate (\dot{V}_W) was measured. The temperature was then adjusted to either 10°C or 22°C for 3 h after which \dot{V}_W was again measured for at least 1 h. The temperature was then adjusted back to 17°C for 12 h and the experiment was repeated at the remaining temperature. Data represent mean \pm SD, with four worms tested at every temperature. Q_{10} values are as follows: 2.2 from 10°C to 17°C, 1.6 from 17°C to 22°C, and 1.9 from 10°C to 22°C. \dot{V}_W at 10°C and 22°C are significantly different ($P < 0.05$)

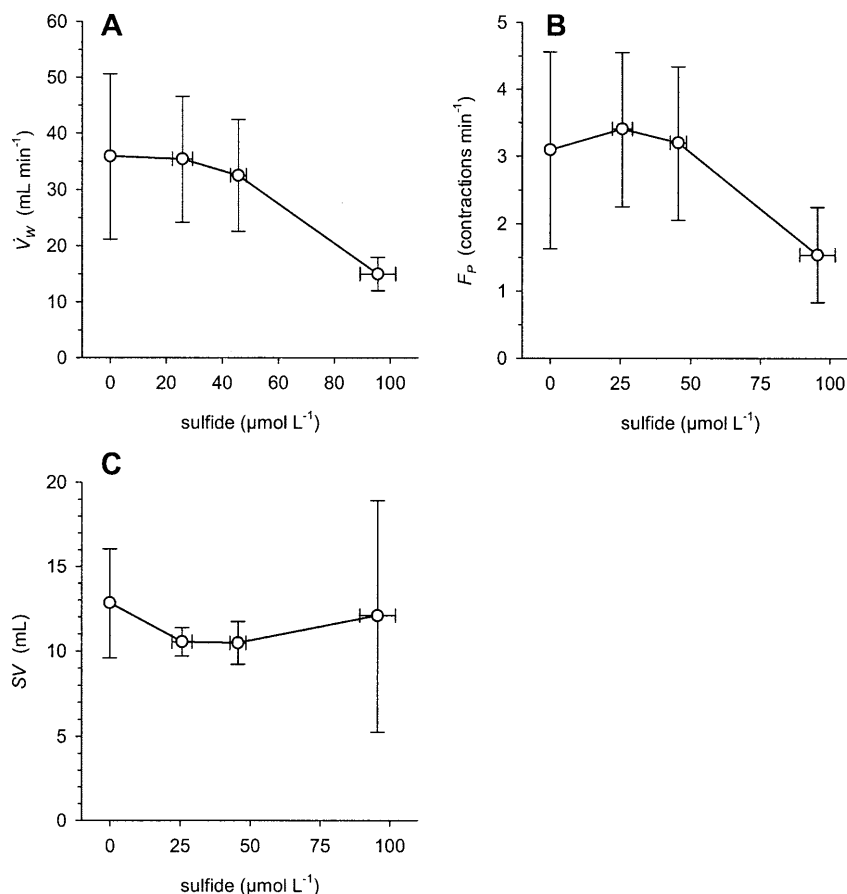
distinguish between a response to a pulse of food and a response to a more natural condition for *U. caupo*, in which food particles are virtually always present, although in varying concentrations (Judd 1993).

Influence of sulfide and temperature on irrigation behavior

We observed a relatively large effect of temperature on \dot{V}_W between 10°C and 22°C, suggesting that short-term changes in temperature that are within the range expected to occur with tides, and perhaps even with daily fluctuations, cause a clear change in \dot{V}_W in *U. caupo*. We do not know whether the same trends would occur after physiological acclimation to seasonal temperature changes in the natural environment. Temperature effects on metabolism and long-term temperature acclimation are likely to be complex. For example, although *Arenicola marina* shows pronounced temperature sensitivity (Seymour 1972), the related polychaete *Abarenicola affinis* shows temperature independence of \dot{V}_W , with Q_{10} values of approximately 1 (Barrow and Wells 1982).

Addition of up to 50 $\mu\text{mol l}^{-1}$ sulfide had no significant effect on burrow irrigation activity in our study. Since the average sulfide concentration in the burrows of intertidal worms during low tide is 40 $\mu\text{mol l}^{-1}$, with a maximum recorded value of 66 $\mu\text{mol l}^{-1}$ (Arp et al. 1992), burrow irrigation activity appears to be insensitive to the concentrations of sulfide encountered by intertidal *U. caupo* in the natural environment. Despite the large number of publications examining the physiological adaptations of animals in sulfidic habitats, to our knowledge only two other publications have reported

Fig. 5A–C Influence of sulfide on burrow irrigation activity. *Urechis caupo* in an artificial burrow at 14°C were exposed to 25 $\mu\text{mol l}^{-1}$, 50 $\mu\text{mol l}^{-1}$, or 100 $\mu\text{mol l}^{-1}$ sulfide (approximate values) in nominally normoxic water for 3 h, after which burrow irrigation rate (\dot{V}_W) and body wall peristaltic frequency (F_P) were recorded for at least 1 h. Stroke volume (SV) was later calculated as \dot{V}_W/F_P . Control data (no sulfide) were obtained for each worm at the beginning of the experiment. Data represent mean \pm SD, with four worms tested at every sulfide concentration. **A** \dot{V}_W . Value at 100 $\mu\text{mol l}^{-1}$ sulfide is significantly lower than control ($P < 0.01$). Values at 25 $\mu\text{mol l}^{-1}$ and 50 $\mu\text{mol l}^{-1}$ sulfide are not significantly different from control. **B** F_P . Value at 100 $\mu\text{mol l}^{-1}$ sulfide is significantly lower than control ($P < 0.01$). Values at 25 $\mu\text{mol l}^{-1}$ and 50 $\mu\text{mol l}^{-1}$ sulfide are not significantly different from control. **C** SV. No effect of sulfide ($P = 0.61$)



the effect of sulfide on burrow irrigation behavior. In the first study, Miron and Kristensen (1993) found that sulfide injected into the burrows of three closely related nereid polychaetes (*Nereis virens*, *N. diversicolor*, and *N. succinea*) did not have a consistent effect on \dot{V}_W (rate during irrigation bursts) but did affect the duration of active and rest periods, although the response was quite variable. More recently, Wohlgemuth et al. (2000) examined the effect of 25–500 $\mu\text{mol l}^{-1}$ sulfide on \dot{V}_W in *Arenicola marina* during normoxia, hypoxia, (16 kPa, 10 kPa, and 6 kPa PO_2), and anoxia. As in our study, Wohlgemuth et al. recorded the irrigation behavior of animals exposed to sulfide for several hours, whereas Miron and Kristensen looked at acute effects immediately following injection of sulfide into the burrow. Incubation with a low sulfide concentration (27 $\mu\text{mol l}^{-1}$) had no effect on \dot{V}_W in *A. marina*, whereas higher sulfide concentrations reduced \dot{V}_W by up to 50%, which is similar to our observation that *U. caupo* reduces \dot{V}_W by 58% during exposure to 100 $\mu\text{mol l}^{-1}$ sulfide.

Previous authors did not investigate the effect of sulfide on F_P or SV, so our finding that 100 $\mu\text{mol l}^{-1}$ sulfide reduces \dot{V}_W by affecting F_P but not SV is novel, and it demonstrates that the effect of sulfide on \dot{V}_W at this concentration may be due primarily to an effect on the activity of the putative pacemaker (Lawry 1966b). The insensitivity of SV confirms the findings of an earlier study in which contractions of isolated muscle strips

from *U. caupo* were inhibited only by very high sulfide concentrations in vitro (K_d greater than 1 mmol l^{-1} , Julian et al. 1998). However, this previous study also demonstrated that spontaneous rhythmic activity in nerve-muscle preparations in vitro was relatively insensitive to sulfide below 1 mmol l^{-1} , so the effect of sulfide on F_P in the present study is likely due to interactions more complex than those occurring in isolated tissues in vitro.

Influence of hypoxia on irrigation behavior and $\dot{M}O_2$

To our knowledge, this is the first report of burrow irrigation behavior in response to hypoxia in echiuran worms, although a few studies have been performed on burrow-dwelling polychaetes (van Dam 1937; Dales et al. 1970; Barrow and Wells 1982; Kristensen 1983b; Toulmond and Tchernigovtzeff 1984; Wohlgemuth et al. 2000). In an early study on *A. marina*, van Dam (1937) reported an eightfold increase in \dot{V}_W during hypoxia. In more recent studies on *A. marina*, Toulmond and Tchernigovtzeff (1984) and Wohlgemuth et al. (2000) found a much more modest increase of less than twofold when PO_2 was reduced from 21 kPa to between 5 kPa and 11 kPa. In contrast, Barrow and Wells (1982) observed that for *Abarenicola affinis* in a closed system, \dot{V}_W decreased almost proportionally with PO_2 .

It is not clear from our study whether *U. caupo* can be termed an oxyconformer or a partial oxyregulator. If the latter, the P_{CR} (critical respiratory PO_2 below which $\dot{M}O_2$ rapidly falls) appears to be above 13 kPa. By the use of a closed system, where O_2 consumption by the worm produced the progressive hypoxia, Pritchard and White (1980) suggested that *U. caupo* is a partial oxyregulator, but they did not provide a P_{CR} . Using flow-through systems, Eaton and Arp (1993) suggested that *U. caupo* is an oxyregulator with a P_{CR} of about 6 kPa PO_2 , whereas Julian et al. (1996) provided data indicating a clear oxyconforming pattern. Neither of these recent studies was made with worms in burrows in which irrigation of water through the burrow would have been completely dependent on the worm's activity. Therefore, our data demonstrate that *U. caupo* in a flow-through burrow that approximates the natural condition exhibits only weak regulation of O_2 uptake.

If the burrow irrigation rate is lower than the hindgut ventilation rate, then the hindgut will rebreathe some water during each ventilation cycle. During hindgut ventilation, the hindgut is gradually filled with 50 ml of water by a series of many small cloacal "sips," with a complete inhalation-exhalation cycle occurring approximately every 2 min (i.e., 25 ml min^{-1} ; Julian et al. 1996). This is almost identical to the burrow irrigation rate (27 ml min^{-1}). Therefore, the burrow irrigation rate appears to be matched with the hindgut ventilation rate, and so virtually all of the water passing the worm also enters the hindgut.

The metabolic cost of pumping and O_2 extraction

We noted a strong linear correlation between $\dot{M}O_2$ and \dot{V}_W at normoxia, with an apparent relationship of 44 nmol O_2 consumed per milliliter water pumped (Fig. 6, $r^2=0.55$). A similar correlation has been noted previously for *U. caupo* (Hall 1931; Pritchard and White 1980), as well as for other burrow-dwelling worms. It has been suggested that this relationship represents the "metabolic cost" of pumping water, and that this value can be as high as 50% of the total metabolic rate in *U. caupo* (Pritchard and White 1980). However, measurements of hydraulic pressures during burrow irrigation and theoretical estimations of pumping cost in *U. caupo* (Chapman 1968; Jørgensen et al. 1986), as well as other burrowing worms (Riisgård and Banta 1998; see Riisgård and Larsen 1995 for review), suggest that the actual cost of pumping is much lower, perhaps even less than 5% of metabolic rate. Jørgensen et al. (1986) have proposed that the correlation between $\dot{M}O_2$ and \dot{V}_W is due primarily to a diffusive boundary layer across the body wall and therefore does not represent metabolic cost. Regardless of the actual cost, the fact remains that *U. caupo* consumes much more oxygen when it is pumping water at a faster rate (in fact, the relationship is almost proportional), and this trend persists even at the highest \dot{V}_W .

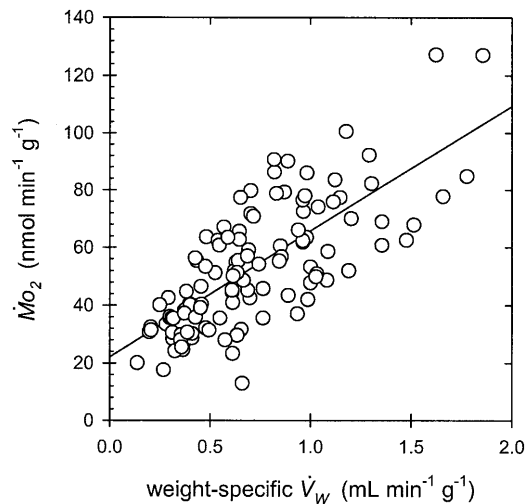


Fig. 6 Effect of burrow irrigation rate on O_2 consumption. Weight-specific O_2 consumption ($\dot{M}O_2$) from Fig. 3 are plotted against weight-specific burrow irrigation rate (\dot{V}_W). Equation for linear correlation: $y = 43.6x + 22.0$, $r^2 = 0.55$. Plotting as whole-animal \dot{V}_W instead of weight-specific \dot{V}_W does not change the correlation coefficient ($r^2 = 0.56$ vs 0.55)

We found that the O_2 extraction coefficient (EO_2 , or the fraction of available O_2 removed from the burrow irrigation flow) averaged 0.27 in *U. caupo*, which is much higher than the previously reported "rough estimate" of 0.05–0.08 (Pritchard and White 1980). Furthermore, we found that EO_2 was not strongly dependent on PO_2 (from 3.3 kPa to 21 kPa), indicating that *U. caupo* tends to remove a constant fraction of the available oxygen from the water irrigated through its burrow, even during ambient hypoxia. Animals with a higher O_2 extraction coefficient are able to obtain O_2 more efficiently, at least in terms of water processing, than animals with lower coefficients. A similar relationship, $\dot{V}_W/\dot{M}O_2$, has been termed the "water convection requirement" (Dejours 1981; Toulmond and Tchernigovtzeff 1984), where animals with a lower convection requirement may be considered as more efficient in O_2 extraction. The water convection requirement for *U. caupo* is 23 l water pumped per millimole O_2 consumed (data from the inverse of Fig. 6). This value was essentially independent of absolute irrigation rate.

Because the concentration of suspended particles in the water column is generally quite low, it is known that the amount of water that burrow dwelling, suspension-feeding invertebrates process for feeding is generally much larger than required for their O_2 demand. Consequently, suspension-feeding invertebrates typically have low O_2 extraction coefficients and very high water convection requirements; for example, Jørgensen et al. (1986) suggest 340 l per mmol O_2 as representative for marine filter feeders in temperate coastal waters. By comparison, *U. caupo*'s O_2 extraction coefficient is very much higher than would be expected, and its water convection requirement (23 l per mmol O_2) is much lower than expected. Although O_2 extraction by

U. caupo appears to be very efficient for a suspension feeder, it is still less efficient than that found for many deposit-feeding, burrow dwellers. For example, the polychaete *Arenicola marina* extracts between 50% and 85% of the available O_2 down to as low as 5.3 kPa PO_2 and has a water convection requirement at normoxia of 5 l per mmol O_2 (Toulmond and Tchernigovtzeff 1984).

Estimates of natural daily irrigation rates

The 12 *U. caupo* exposed to normoxic, nutrient-free water in our experiments irrigated the burrow at a daily rate of 39 l day^{-1} . If we accept Hall's (1931) assumption that one-fifth of burrow irrigation time is spent during feeding periods, and if we assume that \dot{V}_W is twofold higher during such activity compared with nonfeeding periods, then our estimate of the total "daily" burrow irrigation rate would be 47 l day^{-1} (31 l day^{-1} nonfeeding, 16 l day^{-1} feeding).

To date, no measurements have been reported of burrow irrigation rates by *U. caupo* in its natural habitat, and until \dot{V}_W is measured under natural conditions, we cannot be certain of the normal value. For example, Kristensen (1983a, 1989) found that the \dot{V}_W in two Nereids was dependent on whether the animals were in sediment tubes or artificial burrows, with \dot{V}_W being greater in artificial burrows. In contrast, Krüger (1964) found that \dot{V}_W of *A. marina* kept in artificial burrows differed only slightly from \dot{V}_W measured in the field under natural conditions. Therefore, it is worth considering whether *U. caupo* in our experiments were exhibiting normal irrigation behavior.

Although we attempted to make the experimental burrow similar to a natural burrow, and although we tried to keep the worm from being disturbed, it is possible that some aspects of the burrow construction (e.g., burrow dimensions, flow resistance) or the experimental conditions (e.g., artificial sea water, absence of commensals, presence of ambient light) were not ideal, and that this difference might have caused a change in irrigation behavior. One specific concern is that our values of \dot{V}_W , SV, and F_P are much lower than reported for *U. caupo* by Jørgensen (1955, 1966) and Jørgensen et al. (1986), raising the possibility that burrow irrigation was depressed in our experiments.

However, two findings suggest that the worms in our study did not exhibit depressed burrow irrigation activity. First, \dot{V}_W under normoxic conditions in our study is in general agreement with that reported by all other investigators (Hall 1931; Lawry 1966a; Pritchard and White 1980). Although SV and F_P were smaller for the one worm studied by Lawry (1966a) at 13°C , Lawry stated that his method of measuring peristaltic contractions could not resolve frequencies greater than 1.2 min^{-1} , suggesting that his reported frequencies are underestimates and his stroke volumes are overestimates. One previous study (Julian and Arp 1992) reported a value of 12.5 min^{-1} for F_P , but this was for

U. caupo kept in a short, fenestrated burrow that provided no resistance to water flow. Second, the weight-specific $\dot{M}O_2$ under normoxic, nutrient-free conditions in our study was no lower (and, in fact, was somewhat higher) than that reported previously for *U. caupo* (Hall 1931; Pritchard and White 1980; Eaton and Arp 1993; Julian et al. 1996). In large part, our higher value for $\dot{M}O_2$ is probably due to the smaller average size of our worms ($42 \pm 9.8 \text{ g}$) compared with other studies, which generally used worms of approximately 60 g. However, other factors, such as duration of acclimation, may also have contributed (see Julian et al. 1996 for further discussion). In any case, this indicates that the conditions under which we maintained our animals prior to the experiments and the procedures of the experiments themselves did not depress the metabolic rate of our animals.

Values for \dot{V}_W and SV reported by Jørgensen (1955) were not from a direct measurement of water flow but rather were calculations based on F_P (recorded as $8\text{--}9 \text{ min}^{-1}$) and visual measurements of the dimensions of one 21-g *U. caupo* during a feeding period. These data were then the basis for similar calculations in Jørgensen (1966) and Jørgensen et al. (1986). In the original publication, the estimates were 300 ml min^{-1} for \dot{V}_W and 36 ml for SV (Jørgensen 1955; also see Jørgensen 1966). However, the calculations were later modified to account for "piston width," yielding (from the same data set) 213 ml min^{-1} for \dot{V}_W and 25 ml for SV (Jørgensen et al. 1986). Jørgensen (1966) suggested that the main difference between his values and those in Hall (1931), which at the time was the only other study of irrigation rate in *U. caupo*, was likely to be the lower resistance of his artificial burrow in comparison with Hall's. However, since then Pritchard and White (1980) have used a burrow that was similar to that used by Jørgensen (although they directly measured \dot{V}_W) and they obtained irrigation values similar to those of other investigators. Another possible explanation for the higher irrigation rate reported by Jørgensen (1955, 1966) is that his data represent measurements from one worm during feeding at 20°C , and this measurement period may not have been representative. In our experiments, we found that the irrigation rate was quite variable both between worms and over time for the same worm. For one worm in our study, \dot{V}_W during feeding at 14°C was about 100 ml min^{-1} . Therefore, given the large temperature effect we observed, we might reasonably expect \dot{V}_W to exceed 150 ml min^{-1} during feeding at 20°C (the temperature used by Jørgensen), although perhaps only occasionally. Finally, Jørgensen's estimate of SV assumed the absence of backflow (leak) between the "piston" portion of the body and the inner wall of the burrow tube. To what extent such a leak exists in *U. caupo* has yet to be tested, and although it would likely be small, backflow leak would cause an overestimate of SV (and therefore \dot{V}_W).

Burrow irrigation by *U. caupo* is likely to have a substantial effect on the ecology of the mudflat

environment, especially in areas of high population density, so an accurate estimate of irrigation rate is especially valuable. If we assume that \dot{V}_W for a single *U. caupo* is 47 l day⁻¹, and at an average burrow density of 61 m⁻² (Arp et al. 1992, for Elkhorn Slough, Monterey Bay, California), daily burrow irrigation by a population of *U. caupo* would be about 2,750 l m⁻² day⁻¹ (2.75 m³ m⁻² day⁻¹). If we assume each *U. caupo* filters 16 l water per day through a mucous net, then a population of *U. caupo* in one square meter could clear 976 l seawater per day of all suspended particles and even many macromolecules. Additionally, actively ventilated burrows extending deep into the sediment act to increase the surface area and thickness of a mudflat's oxidized zone, thereby substantially altering the physical and chemical nature of the mudflat. Finally, *U. caupo* burrows are typically home to a variety of commensal animals, including the crab *Scleroplax granulata*, the clam *Cryptomya californica*, the scale worm *Hesperonoe adventor*, and the goby fish *Clevelandia ios*, all of which, with the exception of the goby, appear to be permanent residents of the burrow (Fisher and MacGinitie 1928b). It is not known whether oxygen consumption by burrow commensals and the microbial biota surrounding the burrow has an effect on burrow irrigation activity, but the effect may be small, since \dot{V}_W in *U. caupo* was relatively insensitive to hypoxia.

Conclusion

We found that burrow irrigation by *U. caupo* persists relatively unchanged, despite changes in food concentration, ambient PO₂, or sulfide concentration, with temperature being the only factor we examined that strongly affects burrow irrigation rate. Therefore, with the exception of temperature, it is likely that burrow irrigation by *U. caupo* is relatively insensitive to the major environmental variables it is likely to encounter in its burrow.

U. caupo pumps 23 l water for each millimole O₂ consumed, which is much lower than expected for a suspension-feeding invertebrate. Accordingly, the O₂ extraction coefficient is much higher than expected. Mudflats are productive, energy-rich environments, but below the first few millimeters, the sediment is anoxic and strongly reducing, and high sulfide concentrations typically exist in the interstitial water. Because *U. caupo* is adapted to both hypoxia and sulfide, it is able to survive in this environment and thereby gain access to higher concentrations of suspended food than are available to suspension feeders in less productive environments. Additionally, since *U. caupo*'s mucous net captures particles as small as 4 nm in diameter with close to 100% efficiency (MacGinitie 1945), *U. caupo* should be able to clear virtually all suspended organic material from water irrigated through the burrow while it is filter-feeding. Therefore, *U. caupo*'s efficient feeding mechanism, combined with its access to a highly productive environment, may allow it to process much less water for

feeding than would otherwise be expected for such a large suspension-feeding invertebrate.

To take full advantage of this lower water-processing requirement, *U. caupo* needs to extract O₂ with greater efficiency than is achieved by other suspension feeders. This is achieved both by simple diffusion across the body wall and the unique water lung function of the hindgut, which can extract more than 70% of the available O₂ even during extreme hypoxia (Julian et al. 1996). Since hindgut ventilation rate is matched with burrow irrigation rate, it may be the hindgut's ventilation requirement that determines the burrow irrigation rate, and not the concentration of suspended nutrients in the water.

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