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ACTIVITY AND PHYSIOLOGICAL SIGNIFICANCE OF THE PLEOPODS IN THE RESPIRATION OF *CALLIANASSA CALIFORNIENSIS* (DANA) (CRUSTACEA: THALASSINIDEA)

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The burrowing mud shrimp *Callianassa californiensis* lives in the severely hypoxic environment of the upper (0 to +1 foot) tidal horizon (MacGinitie, 1934). Its respiratory adaptations include a high oxygen affinity hemocyanin (Miller and Van Holde, 1974), a low respiratory rate, a low critical partial pressure, and the ability to tolerate extensive periods of anoxia (Thompson and Pritchard, 1969). Several researchers have stated that the pleopods of *Callianassa* serve a respiratory function by generating water currents within the burrow (MacGinitie, 1934, 1935; Devine, 1966; Farley and Case, 1968). Farley and Case (1968) also presented evidence for a more direct respiratory function when they found that *C. californiensis,* after enduring a period of anoxia, responded to oxygen with a sustained pleopod beat. This suggests that the pleopods are directly involved in gas exchange, perhaps serving a function analogous to that found in isopods where the pleopods account for 50%–74% of total oxygen uptake (Lockwood, 1968). Such a function for the pleopods would be unique among decapod crustaceans.

The present experiments were undertaken to clarify the role of the pleopods in gas exchange in *C. californiensis.*

Materials and Methods

Animals

Animals were collected at Morro Bay State Park, using a shovel, a clam gun, or the "Kiwi Method" which involves jumping on the mud until it liquifies, thereby causing struggling animals to float to the surface where they can be picked up by hand. Animals were placed in fresh sea water and kept in styrofoam coolers for the trip to Santa Barbara. Once in the laboratory, they were kept in mud-filled aquaria supplied with running sea water. Temperatures chosen for each experiment corresponded to the ambient sea water temperature at which the animals were maintained.

The pleopods as a respiratory surface

The contribution of the pleopods to gas exchange was measured by comparing oxygen consumption rates of animals with and without the use of pleopods.

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These rates were determined by allowing individual animals to deplete the oxygen from a Pyrex container filled with filtered sea water and closed to the atmosphere by a lucite lid. Temperature in the glass chamber was maintained at 14.2° C by means of a water jacket connected to a refrigerated water bath. Oxygen partial pressure (PO_2) within the vessel was constantly monitored with a Clark polarographic oxygen electrode (Clark, 1956) as the animal reduced the PO_2 from air saturation to unmeasurably low partial pressures (< 0.1 mm Hg). The time required for this varied from 10 to 14 hours. Streptomycin (60 mg/liter) added to the sea water inhibited bacterial growth. In order to measure the bacterial fraction of total oxygen consumption, after each run the volume of the animal was replaced with air-saturated sea water and oxygen consumption was again measured for 6–12 hours. These rates were subtracted from the total oxygen consumption to yield the consumption due to the animal.

Pleopods were immobilized by glueing them together using cyanoacrylate tissue cement thinly applied to both sides of each appendage. A small piece of polyethylene cut from a plastic bag covered all the pleopods and bound them to the abdomen in the manner of a truss. Animals were held with the thorax in sea water until the glue was dry (5 min) and then were placed in a holding tank for a minimum of 48 hours before being used. The shrimp were starved during this recuperative period. Control animals remained untouched except that they were starved for the same minimum period of time. All animals used were adult males weighing 10–15 g ($N = 9$ per group). A second set of experiments was subsequently run using each individual first as a control and then as an experimental animal, with all other techniques identical to those described above. Animals recovered for 48 hours before the second test. Since each animal was used in both the experimental and control situation, any deviation in respiratory rate could be attributed to the experimental treatment. (This served to control for any sample variance which might lead to misinterpretation of the data.)

Activity measurements

A pleopod beat counter was constructed from an eight inch length of PVC tubing, which had a one inch section around the area of the pleopods partially cut through and bent away, forming two flaps. A miniature photoresistor sealed in epoxy was fastened to one of the flaps. Directly across, on the other flap, a light guide connected to a dissecting lamp was inserted. Each beat of an animal's pleopods crossed the beam of light playing on the photoresistor, unbalancing a wheatstone bridge and generating an electrical pulse. A potentiometric strip chart recorder recorded the pulses.

Animals were placed in the beat counter, lightly restrained with rubber bands, and immediately submerged in a sea water-filled Pyrex vessel. Temperature was controlled at 17.2° C with a constant temperature bath or by continuously flowing sea water through the chamber at the same temperature. In all activity experiments PO_2 was constantly monitored using a Clark-type electrode. All oxygen electrodes were constructed in the laboratory using Clark's design. This gave us the ability to vary cathode diameter and electrode shape to suit a specific experiment. The electrode which was used in both activity and respiration experiments had a cathode diameter of 0.020 inches, yielding an output of one mV at

air saturation. This enabled us to monitor the output from the oxygen electrode directly on a one mV chart recorder, eliminating the need for a meter or amplifier. Electrodes were calibrated on the chart recorder using nitrogen and air saturated water. A magnetic stirring bar assured proper mixing in the chamber and sufficient stirring for the electrode to operate properly. The lowest speed possible to satisfy these two criteria was used to minimize any effect on the animal's activity. Pleopod activity was recorded as a function of PO_2 in two different ways. In the first method, the animal itself consumed the oxygen when the chamber was sealed with a lucite lid. In the second, bursts of nitrogen introduced through an airstone lowered the PO_2. No differences were noted in the data collected by the alternate methods. Animals remained in the apparatus for eight hours before experiments were begun.

In order to test the effect of anoxia on pleopod activity, as well as the previously reported oxygen receptor (Farley and Case, 1968), all variables except PO_2 were controlled in the following manner. The chamber and apparatus were the same as described above, but a flowing water system circulated oxygen-depleted water through the Pyrex vessel. Sea water passed consecutively through two columns into which nitrogen was bubbled through an airstone. After water passed through the second column, it entered a bubble trap and then flowed into the chamber. Animals were kept in this apparatus for 14–16 hours before experiments commenced. Activity was recorded for one hour after this period; then air saturated water from the sea water taps flowed through the columns and into the chamber, replacing oxygen depleted water. The animal encountered this sudden influx of air saturated water with no change whatsoever in any of the other ambient physical parameters. No pH differences in the nitrogen and air saturated water were detected.

Behavioral regulation of PO_2

In order to determine how rigorously *C. californiensis* monitored and affected the PO_2 within its immediate microhabitat, a simulated burrow was constructed of plastic tubing. An eight inch section was cut from the middle of a three foot length of 0.75 inch lucite tubing. This small middle section was fitted with a hole large enough to receive the tip of a Clark oxygen electrode. A shrimp could be coaxed into the tubing and kept within by plastic screening fitted over the ends of the small tube. This tube was replaced in the middle of the three foot length by joining the three sections with two tight fitting sleeves of surgical rubber tubing. The resulting apparatus was thus sealed to the outside except at the ends of the three foot length, with the animal confined in the middle. Since the shrimp occupied a small volume (approximately 30 ml), normal oxygen consumption rapidly depleted the PO_2 in the immediately surrounding sea water, forcing the animal to replenish the medium in order to continue normal breathing. Observation indicated that pleopod movement caused fresh sea water (higher PO_2) to flow into the confined animal's vicinity. A microcathode Clark-type oxygen electrode constantly monitored oxygen partial pressure. The diameter of the cathode (0.0006 inch) obviated the need for stirring. All experiments took place in reduced light in a seawater table filled to a depth of four inches. Ten animals were run from 12–72 hours in the apparatus.

pH and PO_2 in the burrows

Water samples were taken at low and high tide from randomly selected burrows of *C. californiensis*. Burrows were inspected for evidence of recent occupation by noting the presence or absence of fecal pellets around the opening to the surface. Stiff 0.25 inch plastic tubing was threaded into the mouth of an occupied burrow to a depth of 18–24 inches. Water was drawn up into a 50 ml syringe, whereupon the plunger was removed and the water in the syringe was tested for pH using pHydrion pH paper ($\pm$ 0.2 pH units). Oxygen concentration was "frozen" by completing the microwinkler method (Fox and Wingfield, 1938) through the addition of phosphoric acid. As high tide covered the mud flats the syringes floated, anchored by the tubing within the burrow. This allowed sampling to continue through the tidal cycle.

Gill surface area

Gill surface area was measured using a technique modified from Gray (1954, 1957). Filaments of various sizes were removed from several gills, representing one side of the thorax in five freshly killed animals. These were individually traced with a camera lucida. A planimeter was then used to determine the area and length of each filament. In addition, both sides of each gill were likewise traced, with length and area determined in the same manner. The number of filaments were counted for each gill used. Once the above information was obtained, the following calculation determined the gill surface area of the entire animal. A standard curve was constructed of filament length versus filament surface area using the data obtained with the planimeter [$y\ (mm^2) = 0.54 \times (mm) + 0.31$, correlation coefficient = 0.97]. A second standard curve was plotted using total gill length versus number of filaments in the gill. The area of one side of a gill was divided by its length to obtain the average width, or average length filament. The area of the average length filament then could be read off the first standard curve mentioned above. This result, when multiplied by the total number of filaments obtained from the second standard curve, yielded the surface area of one side of the gill. Bilateral symmetry was assumed in dealing with the two sides of the thorax. Thus, values for each complete gill were multiplied by two for each of the ten gills on one side of an animal's thorax to yield the total gill surface area.

Results

The pleopods as a respiratory surface

The mean respiratory rate for animals with and without the use of pleopods was calculated at seventeen partial pressures of oxygen (Fig. 1). The means at each point were compared using a *t*-test for comparison of means with unknown variance not assumed equal (Bailey, 1959). In all seventeen cases the means were not significantly different at the $P > 0.05$ level. Confidence intervals depicted in Figure 1 were calculated using a Student's *t*-distribution and were included to show the variability of the data. The metabolic rate curves were determined by the least squares method. Each group of animals had one line calculated for PO_2 values at 20 mm Hg and above and one for all PO_2 values at 20 mm Hg and

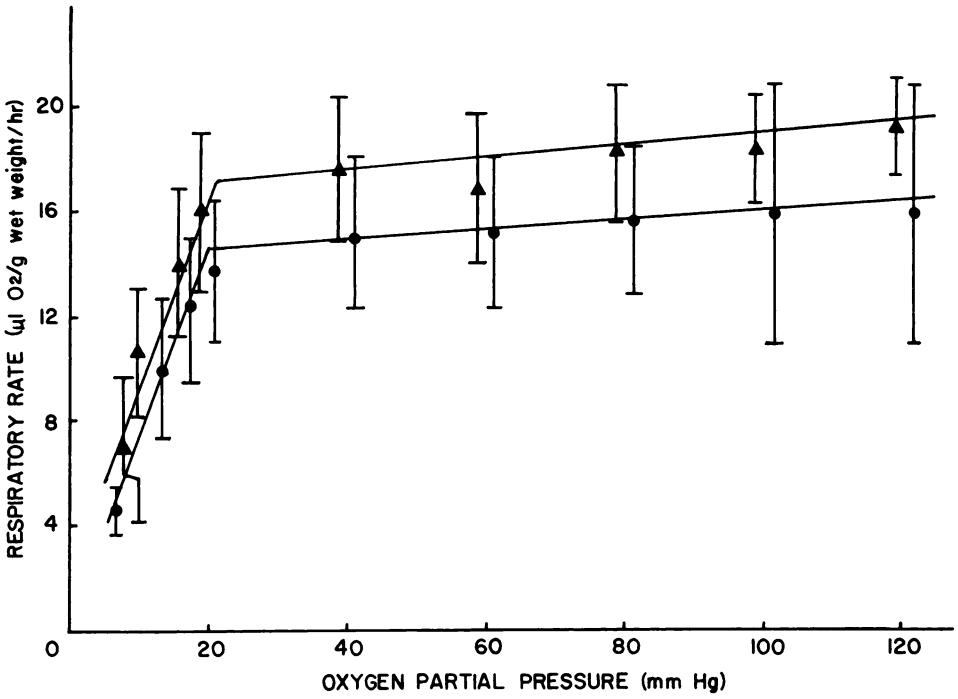

FIGURE 1. Relationship of respiratory rate to oxygen partial pressure in animals with (circle) and without (triangle) the use of pleopods. Error bars indicate 95% confidence limits around each point. Each value is the mean rate of nine animals. Regression lines through the points were calculated using the least squares method. One line was calculated for points 20 mm Hg and above, one line was calculated for points 20 mm Hg and below. The intersection of the lines was called the critical oxygen partial pressure (P_c). Lines were not significantly different. For animals with the use of pleopods, r is 0.86 for the line above 20 mm Hg, and r is 0.94 for values below; mean weight is 12.9 ± 1.3 ($\bar{x} \pm$ s.d.). For animals without the use of pleopods, r is 0.86 for above 20 mm Hg, and r is 0.97 below; mean weight is 12.1 ± 1.7. See text.

below. The intersection of the two lines was called the critical oxygen partial pressure (P_c), the partial pressure of oxygen below which the metabolic rate ceases to be independent of external oxygen concentration (Prosser, 1973). The P_c for control animals was approximately 17 mm Hg, that for animals without the use of pleopods was approximately 20 mm Hg. The variation about the regression lines makes this difference meaningless.

Data collected using individual animals yielded similar results. These respiratory rates were tested using the Wilcoxon test for paired comparisons, at nine PO_2 values above the P_c. Significance levels of the data for the three animals tested were all $>> P = 0.05$, strongly supporting the null hypothesis: treatment and control are the same. It should be mentioned that the metabolic rates obtained in this set of experiments were substantially lower than those reported by Thompson and Pritchard (1969), despite the fact that the temperature at which they were run was 4.2° C higher. This seemingly irreconcilable conflict can perhaps be explained by the fact that the animals used in this study averaged

12.9 ± 1.3 g as compared with 5.3 ± 1.5 g used by Thompson and Pritchard (1969), and were starved for a longer period of time. Since the current study was internally controlled, this conflict does not affect the conclusions drawn from the results.

Activity measurements

The figures in Table I represent pleopod activity levels at three different partial pressures of oxygen, expressed as the percentage of total time active and rate of beat. Since the pleopods of *C. californiensis* beat intermittently, there is variability in both the total time active and the rate of pleopod beat. Intermittent irrigation is a common phenomenon in tubicolous intertidal forms and has been demonstrated in polychaetes (Mangum, 1964), amphipods (Gamble, 1970), stomatopods (Johnson, 1936), and in another species of *Callianassa, C. filholi* (Devine, 1966).

It is interesting to note here that *C. californiensis* varies its percentage of time active, while keeping its rate of beat relatively constant over the PO_2 ranges shown (Table I). Although the amount of time spent in activity is lessened at zero PO_2 when compared with activity at the P_c and above, it is not absent. When in anoxia, animals will struggle when touched. Apparently these shrimp do not enter a true quiescent or lethargic state as was reported by Waterman and Travis (1953) for *Limulus,* and Gray (1957) for *Ocypode,* but do reduce total activity.

A second factor which these data suggest is that these animals do alter their pleopod activity in response to oxygen partial pressure as was reported by Farley and Case (1968). Maximum activity occurs at PO_2 values approaching the P_c. During high tide water of high oxygen concentration is available. Maximal pleopod activity at PO_2 values around the P_c could replace the oxygen depleted water surrounding an animal before aerobic respiration becomes ineffective.

Low tide conditions were simulated in the lab by maintaining the animal at very low oxygen concentrations for 14–16 hours. This produced a different

TABLE I

Relationship of activity to oxygen partial pressure (PO_2) at three PO_2 intervals, expressed as the percentage of total time active and beats per minute (bpm). Values are for individual animals determined over the PO_2 increments shown. Means are not given, due to individual differences.

Animal	PO_2 (mm Hg)					
	100–70		40–15		0	
	Time active (%)	Rate (bpm)	Time active (%)	Rate (bpm)	Time active (%)	Rate (bpm)
1	27.3	26	42.7	22	9.2	18
2	31.9	40	47.9	40	13.7	44
3	77.2	29	77.2	15	28.6	28
4	29.5	14	45.7	15	10.0	15
5	35.2	37	56.7	47	1.0	40

TABLE II

Initiation of ventilation. Values are for individual animals. Activity is given for animals before and after admission of oxygen (see text).

	Before admission of O_2			After admission of O_2			
Animal	PO_2 of system (mm Hg)	Time active (%)	Rate (bpm)	Latency of response (sec)	Time active (%)	Rate (bpm)	Threshold (PO_2 mm Hg)
1	18.6	100	30	20	100	41	27.0
2	22.7	100	26	26	100	50	41.4
3	7.8	0	0	34	100	35	27.3
4	0	40	22	20	100	49	12.0

kind of behavior (Table II). These animals responded to an influx of oxygenated water, analogous to an incoming tide, by sustained rapid beating of the pleopods. Increases in both the percentage of time active (in 50% of the animals) and rate of beat (100% of animals) accompany readmitted oxygen. The difference in quality and quantity of activity between the results of Table I and Table II suggest that long term oxygen deprivation constitutes a stress situation, despite the animal's extensive anaerobic capabilities. In contrast, preliminary experiments with shrimp kept at very low PO_2 for 10–30 minutes gave variable results, often with no response at all to readmitted oxygen.

Behavioral regulation of PO_2

C. californiensis used its pleopods to influence the oxygen partial pressure in the area immediately surrounding it. However, all ten animals observed in this experiment showed only a very coarse regulatory capability (Fig. 2). The data was treated by dividing the total time for each experiment into one half hour increments, and noting the minimum PO_2 within each increment. These incremental minimum PO_2 values were used to obtain a bar graph showing their relative frequency of occurrence during an experiment. Individual runs all showed the same trends; namely, PO_2 dropped to zero on several occasions, sometimes for up to thirty minutes, with the great majority of the total time spent above the P_c. For this reason Figure 2 incorporates all the data obtained, with equal weight given to each run. We feel that this best represents the trends suggested by the data.

This experiment was originally designed to obtain a definite threshold for an oxygen receptor, on the assumption that a shrimp would not permit the oxygen concentration to drop below this point. Since the PO_2 dropped to zero in all cases, it seems that short term exposure to hypoxic conditions causes little or no stress.

pH and PO_2 in the burrows

Table III shows the values obtained for burrow oxygen concentrations and pH at low and high tides. Mean PO_2 varied from 0.59 ± 0.15 ml/liter at low tide to 1.31 ± 0.54 ml/liter at high tide (mean $\pm$ s.d.). pH varied from 6.3

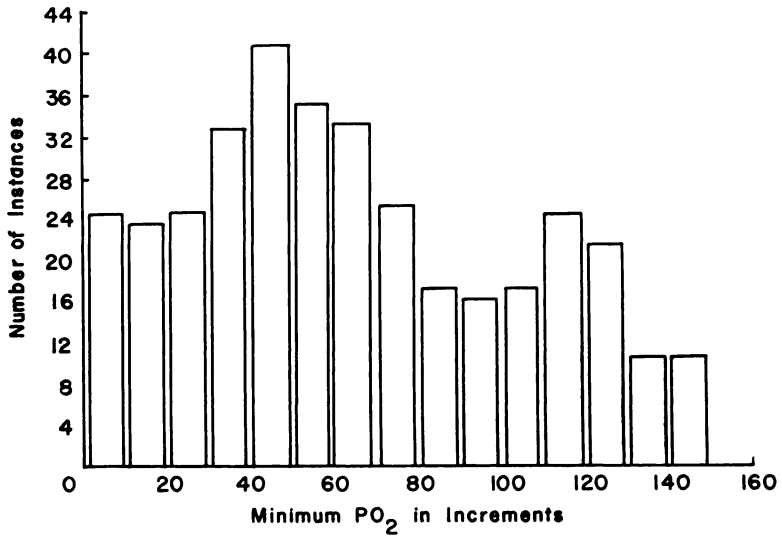

FIGURE 2. Behavioral regulation of PO_2. Abscissa shows the minimum PO_2 found (in a given half hour treatment). Ordinate shows the number of instances a given PO_2 was recorded. Data is from ten animals. See text.

to 7.0. These should be regarded only as approximate values due to the variation in exposure which is caused by tides of different heights. This particular low tide was +0.4 which is relatively high. It caused the burrows to be exposed for approximately 5.5 hours. MacGinitie (1935) reports that *Callianassa* can be exposed for as much as 18 hours in Elkhorn Slough, California, in which case

TABLE III

Oxygen concentration and pH in C. californiensis *burrows at low and high tide. Values are for individual burrow samples. Surface water pH is 7.0; oxygen concentration of surface water is 4.25 ml/liter, temperature, 12.0° C. Means are given with standard deviation.*

Low Tide		High Tide	
O_2 conc (ml/liter)	pH	O_2 conc (ml/liter)	pH
0.60	6.2	0.83	7.0
0.60	6.3	1.55	7.0
0.66	6.4	0.46	7.0
0.23	6.1	1.55	7.0
0.77	6.2	1.20	6.8
0.60	6.4	1.55	7.0
0.66	6.3	1.20	7.0
0.70	7.4	1.13	7.0
0.66	6.2	2.61	7.0
0.60	6.2	1.45	7.0
0.59 ± 0.15	6.30 ± 0.2	1.31 ± 0.54	7.0 ± 0.2

the PO_2 at low tide would certainly drop to zero. The pH would probably be substantially lower as well. Interestingly, immediately after the receding tide exposed the sampled burrows, oxygen concentrations dropped to low tide values. This indicates that even though the burrows of *C. californiensis* at Morro Bay are quite permanent, due to the high clay content of the mud there (Torres, personal observation), there is no barrier to equilibration with interstitial water. Burrows showed no evidence of lining upon close examination. However, in the upper 18 inches they did show a ring of lighter-colored sand about 1 mm in thickness surrounding the burrow walls, indicating a higher PO_2 than the surrounding anaerobic mud.

Gill surface area

Gill surface areas obtained from five shrimp yielded a mean value of 4.13 ± 0.72 cm²/g wet body weight ($\bar{x} \pm$ s.d.). This figure includes measurements from three adult males and two adult females, ranging from 14.6 mm to 17.0 mm in carapace length, and 4.8 to 6.6 g wet body weight. It is not known whether there is a change in weight specific gill surface area between very small individuals and adults, as has been reported by Belman and Childress (1976) for *Gnathophausia ingens* (Crustacea, Mysidacea), and Gray (1957) for several brachyuran crabs.

Discussion

C. californiensis differs from its congener *C. affinis* and its close relative *Upogebia pugettensis* in that it is not a suspension feeder (MacGinitie, 1930, 1934, 1937, 1939). Rather, *C. californiensis* sifts through the mud in its burrow for detritus. In its constant foraging for food, which includes extending and reworking tunnels, *C. californiensis* often encounters situations of reduced or zero oxygen, even during high tide. Further, due to its high position in the intertidal zone (0 to +1 foot), it is exposed to hypoxia for longer periods of time than most other mud flat dwellers. MacGinitie (1935) reports exposure of these animals for up to 18 hours in Elkhorn Slough, California, and we have observed exposure periods of up to 12 hours at Morro Bay on several occasions.

During low tide exposure, low oxygen and low pH conditions prevail. In this study, burrow water samples contained oxygen concentrations as low as 0.23 ml/liter, with pH values of 6.1 to 6.4. The mean oxygen concentration of 0.59 ± 0.15 ml/liter is equivalent to a PO_2 of 21 mm Hg, closely approaching the P_c of *C. californiensis* (Fig. 1; and Thompson and Pritchard, 1969) and illustrating the fact that even a "high" low tide of +0.4 is sufficient to reduce burrow PO_2 values to a point where aerobic respiration is insufficient.

Callianassa affinis, which inhabits burrows in the rocky intertidal communities of Southern California has to deal with similar low oxygen conditions at low tide. Congleton (1974) reports PO_2 values of 0.5 and 3 mm Hg in samples of burrow water, with interstitial water ranging from 0.8 to 6.1 mm Hg. Neither *C. affinis* nor *C. californiensis* secrete a lining on their burrows and therefore have no barrier to rapid equilibration between burrow and interstitial water at low tide. *Upogebia pugettensis* does possess a tunnel lining, which results in a

higher oxygen concentration in its burrow (0.58 ± 0.26 ml/liter) than surrounding interstitial water (0.15 ml/liter) (Thompson and Pritchard, 1969).

Values for burrow oxygen at high tide were surprisingly low, with a mean concentration of 1.31 ± 0.54 ml/liter. These data suggest little interchange between burrow and surface waters when the tide comes in, without active animal intervention. The 0.25 inch tubing used to sample burrows did not prevent water from flowing downward, but it certainly excluded ghost shrimp from the area where it was inserted. It is unlikely that the pleopods of *C. californiensis* can maintain a current strong enough to irrigate its entire extensive burrow habitat, but it is possible that animal movement plays a large part in surface water-burrow interchange, and further local irrigation results in a sufficiently oxygenated immediate microhabitat.

Sustained rapid pleopod beating after long term anoxia (Table II; and Farley and Case, 1968) supports the notion that *C. californiensis* must encourage the flow of surface water into its oxygen-deprived burrow. Several animals were observed in the field forcing water in or out of the burrows directly after being covered by the incoming tide. This is easily discerned by a stream of muddy water flowing from a burrow, or a shifting movement at the mouth of the burrow. These movements were observed in the lab several times and are easily seen when the tide has covered the burrows one to two inchs in depth. Such activity at the surface is closely related to feeding (MacGinitie, 1934), as well as being very possibly involved in respiration. The previously hypothesized function of the oxygen receptor (Farley and Case, 1968) as being a means by which animals might minimize their period of anoxia, thus has added value in signaling the possibility of renewed feeding at the surface.

It is difficult to say what, if any, effects changing pH has on the animal. A preliminary experiment with the effects of CO_2 concentration on activity in one animal showed that high CO_2 concentration, corresponding to a pH of 5.0 induced quiescence, but anything closer to 7.0 had no noticeable effect. It may be that external pH at the levels found in the burrow has little effect on *C. californiensis.*

C. californiensis feeding behavior must be included in any consideration of the animal's relationship to its environment. Since it is a deposit feeder, it often constructs new tunnels (Peterson, 1972), making numerous encounters with hypoxia unavoidable. Some of these encounters may be minimized by irrigation with the pleopods, since the animal does respond to lowered oxygen with increased pleopod activity (Table I; and Farley and Case, 1968). However, when building a new tunnel, there will often be no oxygenated water available for circulation. In these situations *C. californiensis* may continue to function anaerobically to satisfy its energy needs. Short periods of anaerobiosis do not seem to tax this animal, as was observed both in preliminary experiments on oxygen perception and in the behavioral PO_2 regulation experiments (Fig. 2). It is not surprising that ghost shrimp may sometimes rely on anaerobiosis for energy, for hypoxic conditions would otherwise severely restrict the animal's movements, even during high tide.

Long periods of anoxia, such as that produced by minus tides, warrant an eventual reduction in total activity (Table I). A similar response to declining oxygen (preliminary hyperactivity, followed by quiescent behavior), has also

been noted in *Typhlogobius californiensis* (Teleostei: Gobiidae) (Congleton, 1974). *Typhlogobius* is a commensal in the burrows of *Callianassa affinis,* and is subject to the same types of respiratory stress as *C. californiensis.* In both cases, minimal activity during prolonged anoxia may serve to reduce oxygen debt.

The respiratory adaptations of *Typhlogobius* and *C. californiensis* are very similar despite their widely different phyletic origins. A 1.4 g *Typhlogobius* has a respiratory rate of 21 μl O_2/hr at 15° C (Congleton, 1970), as compared with a rate of $\sim$ 15 μl O_2/g wet weight/hr in *C. californiensis* at 14.2° C. Oxygen consumption in *Typhlogobius* is much lower than that reported for *C. californiensis* by Thompson and Pritchard (1969); however both this study and that of Congleton used animals in a postabsorptive state of starvation, with experiments conducted at a similar temperature, allowing for better comparison. Further similarities include a similar P_c (9–16 mm Hg for *Typhlogobius;* 10–20 mm Hg for *C. californiensis*), and a large anaerobic capacity ($\sim$ 4 days for *T. californiensis;* 5.7 days for *C. californiensis*).

The characteristics of aerobic respiration in both *C. californiensis* and *Typhlogobius* are probably indicative of adaptations to high rather than low tide oxygen conditions. A low respiratory rate is useful in conserving what limited oxygen is available in a burrow; a low P_c allows aerobic respiration to continue in low oxygen areas within the burrow at high tide. After exposure, extremely low oxygen concentrations preclude the possibility of aerobic respiration. It is for this reason that a highly efficient system of oxygen extraction is not needed. Oxygen is either available, or it is not, depending on the tidal position.

C. californiensis shows no extrabranchial oxygen exchange across the pleopods, which on inspection would be ideally suited for this purpose (Fig. 2). These appendages have a large hemocoel, as well as a large surface area, and would add considerably to the gas exchange surface of the animal. The fact that they serve mainly in propelling water may indicate that additional respiratory surface is unnecessary for survival. It is corroborated to some extent by the low gill surface areas of *Callianassa* (4.13 cm^2/g). This value is lower than any yet reported in the literature for a totally aquatic crustacean (Gray, 1957; Hughes, Knights, and Scammel, 1969).

A large gill surface area and a highly efficient system of oxygen extraction are more typical of either very active Crustacea such as the portunids (Gray, 1957), or animals from a stable low oxygen environment such as *Gnathophausia ingens,* the oxygen minimum layer mysid (Childress, 1971; Belman and Childress, 1976), or *Pleuronocodes planipes,* the galatheid red crab (Quetin and Childress, 1976). Oxygen concentrations as low as 0.20 ml/liter are typical of oceanic oxygen minimum layers (Schmidt, 1925; Sewell and Fage, 1948; Banse, 1964). However, many crustaceans which inhabit zones of oxygen minimum are believed to live entirely aerobically (Childress, 1971; 1975) and often have little or no capability for anaerobiosis (Childress, 1975; Torres, unpublished data).

Total dependence on the limited oxygen available for respiration is allowed for by the stability of an oxygen minimum layer. In contrast, the relative instability of the high intertidal environment of *Callianassa* would preclude anything but minimum dependence on environmental oxygen, that is, facultative anaerobiosis.

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SUMMARY

1. The pleopods of *C. californiensis,* a potential site for extrabranchial oxygen exchange, do not contribute significantly to oxygen consumption.

2. *C. californiensis* has a gill surface area of 4.13 ± 0.72 cm^2/g wet body weight, the lowest value yet reported for a totally aquatic crustacean.

3. *C. californiensis,* when placed in simulated burrow conditions, regulates the PO_2 very loosely in its immediate microhabitat, using its pleopods.

4. Field studies of pH and PO_2 values in burrows of *C. californiensis* indicate that animal movement may play a large part in water exchange between the surface and burrow.

5. Activity studies suggest that oxygen is not critical to *C. californiensis* on a short term basis. Perception of oxygen after long deprivation may signal the possibility of renewed feeding and activity at the surface of its burrow.

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