

# RESPIRATION IN *MACROBRACHIUM ROSENBERGII* POSTLARVAE AT NORMOXIC AND HYPOXIC LEVELS<sup>1</sup>

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## ABSTRACT

Respiration patterns of *Macrobrachium rosenbergii* postlarvae (0.16–0.18 g/PL;  $n = 50$  PL) were determined at 75% oxygen saturation levels for 24 and 96 hrs, as well as at decreasing dissolved oxygen concentration levels (85, 75, 65, 45, 35, 25, and then raised to 90%) with each level lasting for 45 minutes. Measurements were conducted within 40–60 days after metamorphosis. Those measurements at 75% saturation lasting 24 hrs showed that oxygen consumption rates in darkness were higher than those during the lighted phase; such was also the case in an experiment lasting 96 hrs, which showed a cyclical regularity. A decrease in saturation levels from 85 to 65% was accompanied by a slight increase in respiration. As saturation levels further decreased below 65%, respiration correspondingly decreased. Respiration rates observed at the 90% level were lower than those in the previous saturation levels.

Growth and survival of *Macrobrachium rosenbergii* are dependent on the availability of oxygen required to sustain metabolic functions. Fluctuations in saturation levels do occur in nature as well as in intensive culture. The high mortality in pond-reared juveniles (45% within three days) reported by Green *et al.* (1977) was attributed to a drop in oxygen concentration to almost 0% during the night (0.5–0.6 ppm O<sub>2</sub> was measured early the next day). Good aquaculture practices combined with adequate knowledge in respiration rates at various oxygen saturation levels are required to prevent such massive kills. Previous determinations of postlarval and juvenile respiration rates (Clifford and Brick, 1983; Nelson and Knight, 1977; Stephenson and Knight, 1980) were conducted only within a specific saturation level, different in each investigation. In the present study, respiration rates of postlarvae at various saturation levels and durations were determined.

## MATERIALS AND METHODS

Respiration measurements on the postlarvae (PL) were conducted with the same computer-controlled closed respirometer (Fig. 1) described previously (Reyes *et al.*, 1990). Fifty PL

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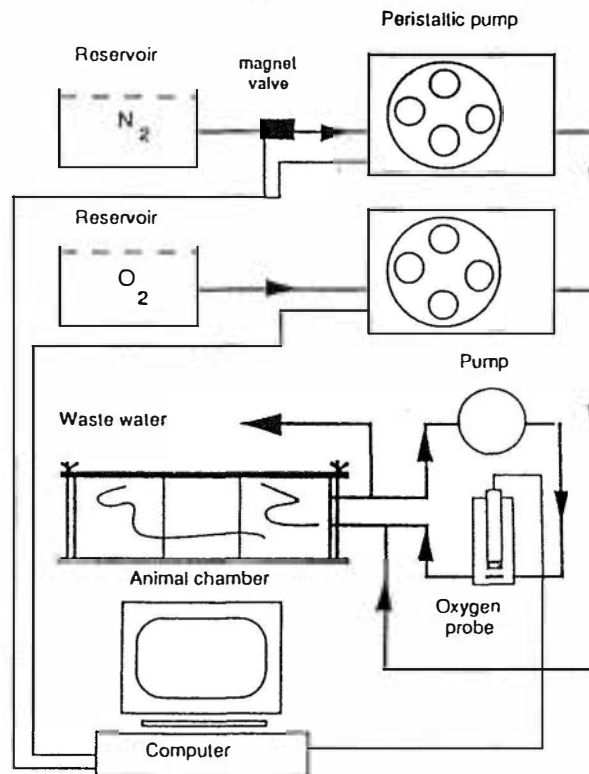


Fig. 1. Respirometer used in oxygen consumption measurements in postlarvae

(range of total weight = 7.95–9.16 g or 0.16–0.18 g/PL) were acclimatized prior to measurement to respirometer conditions for 3 hrs at a constant flow of 100% oxygen-saturated water maintained at  $26.1 \pm 0.1^\circ\text{C}$ . Twenty hours prior to each experiment, the postlarvae were fed with artificial dry feed (Goldfish Tetra Feed) at a ration of 10% of body wet weight; they were not fed during measurements. Oxygen consumption rates at the 75% oxygen saturation level were measured for 24 and 96 hrs (light:dark cycle at 12L:12D), with the former replicated thrice. In another set of experiments, the postlarval respiration at decreasing oxygen saturation levels from 85 to 25% (at 10% decrements) were measured for 45 minutes/saturation level. After measurements at 25%, the saturation level was raised to 90% and the respiration rates measured. All measurements at various saturation levels were conducted only under illuminated conditions. Light intensity for all experiments was  $1.25 \mu\text{mol}/\text{sq. m}/\text{sec}$ .

## RESULTS

Detailed representative examples of respiration pattern in *M. rosenbergii* postlarvae at 75% saturation level measured for 24 hrs and at different levels (90–25%) are shown in Figs. 2 and 3, respectively. Oxygen consumption levels for 24 hrs at 75% saturation were higher during the period of total darkness than during the preceding illuminated period; oxygen consumption then slightly decreased when the lights were turned on at 0700 hrs (Fig. 2). In experiments wherein the postlarvae were subjected to various saturation levels, respiration was observed to increase slightly as saturation decreased from 85 to 65%, but respiration decreased correspondingly as saturation was further reduced from 65 to 25%. Upon return

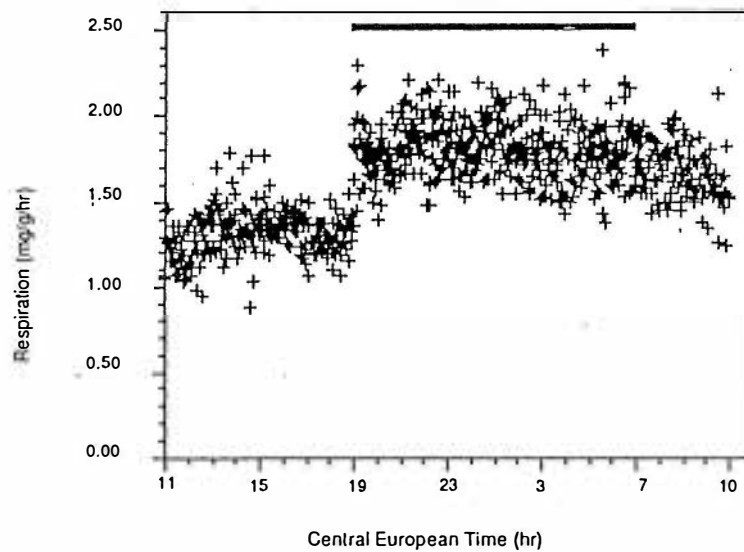


Fig. 2. A representative example of a continuous series of 24 hr oxygen consumption determinations in *M. rosenbergii* postlarvae at 75% oxygen saturation level (total wet weight = 8.36 g,  $n = 50$ )

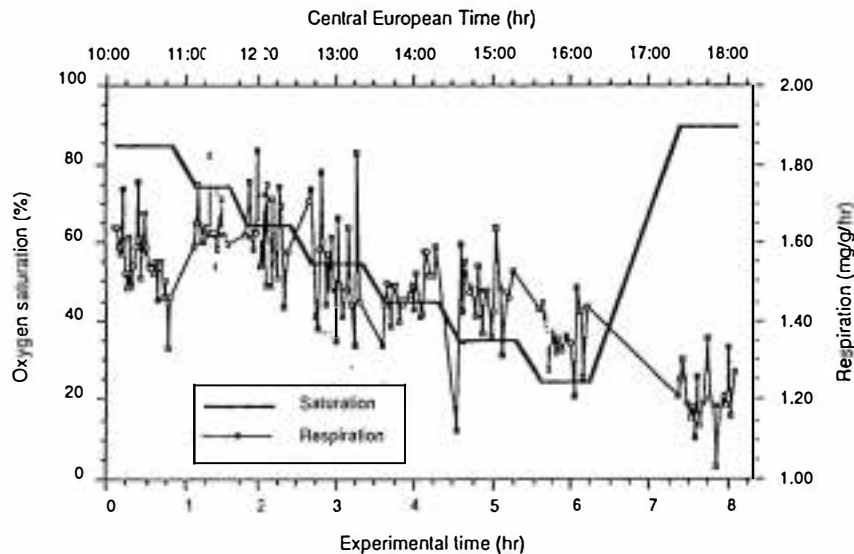


Fig. 3. A detailed example (replicate 2) showing respiration patterns in *M. rosenbergii* postlarvae (total wet weight = 8.76 g,  $n = 50$ ) at different oxygen saturation levels. Each small square represents an actual measurement within the saturation level studied.

to a normoxic level of 90%, respiration was lower than at all preceding saturation levels (Fig. 3).

Figure 4 shows hourly averages of oxygen consumption determinations at 75% saturation levels in three replicates, each lasting 24 hrs. In all replicates, respiration levels for the first nine hours of measurement (1100–1900 hrs) did not differ significantly ( $t$  test,  $P > 0.05$ ). In replicates 1 and 2, which were measurements on the same postlarvae, oxygen consumptions throughout the entire period of darkness were significantly higher than in the lighted phase, but in replicate 3 (consisting of a different batch of postlarvae comparatively in the same weight range, 7.95–8.91 g/50 PL, as the previous replicates) respiration progressively decreased from 2200 hrs onwards, and increased after the lights were turned on at 0700 hrs. In a separate experiment, a comparison of the results obtained in three replicates wherein

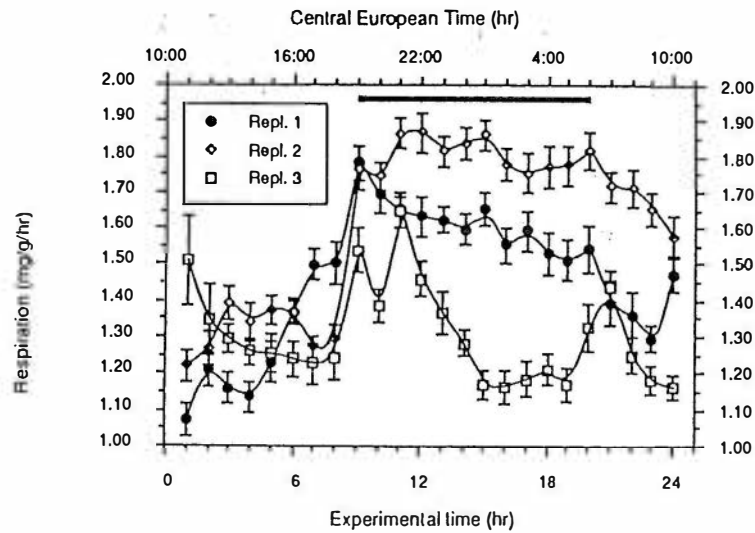


Fig. 4. Respiration in *M. rosenbergii* postlarvae (range of total wet weight = 7.95–8.91 g,  $n = 50$ ) at 75% oxygen saturation level within a 24 hr period. The thick horizontal line at the upper margin indicates the period of darkness, whereas the vertical lines with horizontal bars are the 95% confidence limits for each hourly computed average.

the postlarvae were subjected to different oxygen saturation levels showed no significant differences in the values between 85 and 25% (as indicated by overlapping 95% confidence intervals). Only the respiration rates in replicate 1 at 90% deviated significantly from the other two replicates (Fig. 5).

Postlarval respiration determined in three replicates at different saturation levels is summarized in Fig. 5 and Table 1. Replicates were not significantly different from each other using ANOVA ( $P > 0.05$ ), also demonstrated by the overlapping 95% confidence limits. Mean respiration rate at 90% is significantly different from the rates measured at 45–85% level. Average oxygen consumption at 25% was significantly different from those measured between 55 and 85%, but not from the rates at 35 and 45% (Table 1).

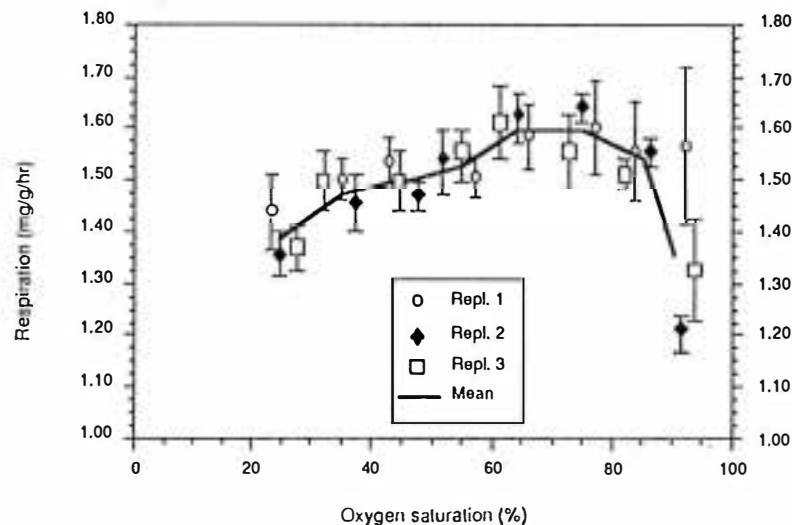


Fig. 5. Mean oxygen consumption rates of *M. rosenbergii* postlarvae (range of total wet weights = 8.65–9.16 g,  $n = 50$ ) at different oxygen saturation levels. Vertical lines with horizontal bars are the 95% confidence limits of the computed average for each saturation level. The thick curve represents the average of three replicates.

Table 1. Respiration rates of *M. rosenbergii* postlarvae at various saturation levels

Saturation (%)	Respiration (mg/g/hr)			
	repl. 1	repl. 2	repl. 3	Mean
90	1.566	1.206	1.329	1.367 <sup>c</sup>
85	1.588	1.544	1.514	1.549 <sup>a</sup>
75	1.603	1.643	1.557	1.601 <sup>a</sup>
65	1.586	1.619	1.616	1.607 <sup>a</sup>
55	1.507	1.539	1.554	1.533 <sup>a</sup>
45	1.539	1.472	1.502	1.504 <sup>ab</sup>
35	1.501	1.453	1.494	1.483 <sup>abc</sup>
25	1.438	1.363	1.374	1.392 <sup>bc</sup>

Means with the same superscript are not significantly different from each other at 5% level (two-way ANOVA, DMRT;  $p < 0.05$ ;  $cv = 4.51\%$ ). Differences between replicates are not significant (ANOVA,  $P > 0.05$ ).

A continuous series of oxygen consumption determinations lasting for 96 hrs showed a repetition of the rate increase tendency during darkness, with maximal peak values (between 1.50 and 1.70 mg/g/hr) when the lights were turned off at 1900 hrs (Fig. 6). Thereafter, respiration rates decreased to a comparatively lower range (1.05–1.30 mg/g/hr) during the illuminated period, with the exception of four smaller peaks (rates between 1.30 and 1.50 mg/g/hr), the first at 0800 hrs, the second at 1600 hrs, the third and fourth at 1400 hrs (at 21, 30, 52, and 76 hrs from the start of the experiment, respectively). In comparison, the respiration curves on the second, third and fourth days (in this context, the term 'day' refers to a 24 hr interval) have more similarity to each other in terms of relative increase or decrease with reference to time (slope of the curve within a particular time interval). The first 24 hr period differed from the successive days, as observed in the sudden respiration decrease

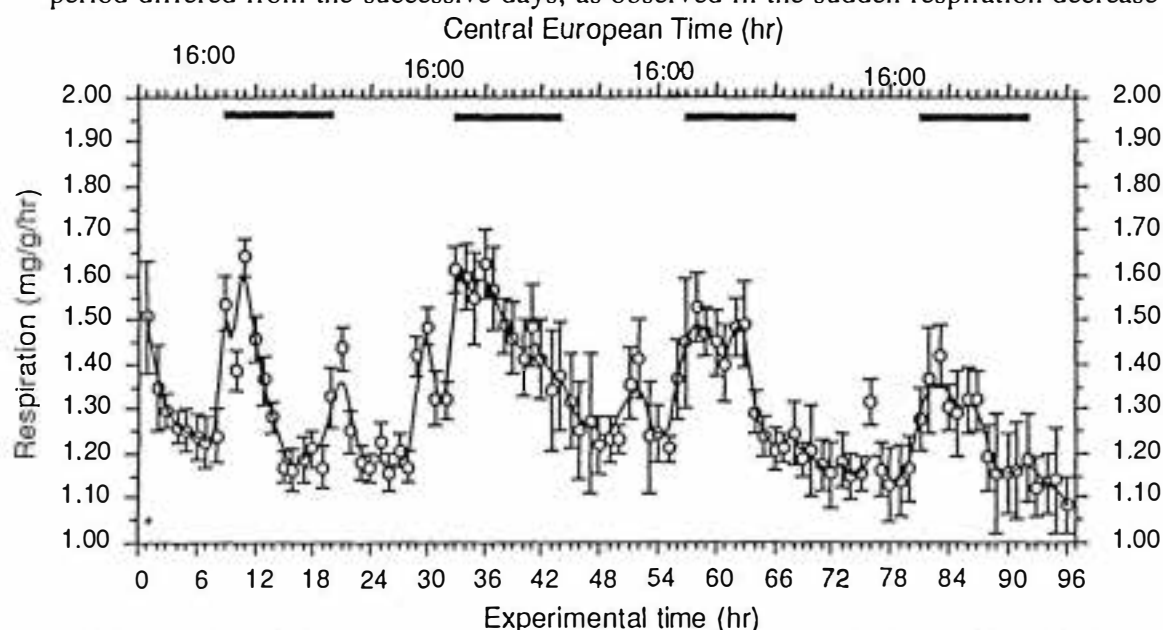


Fig. 6. Respiration in *M. rosenbergii* postlarvae (total wet weight = 8.91 g,  $n = 50$ ) measured continuously for 96 hrs. Vertical lines with horizontal bars are the 95% confidence limits for each hourly average of measurements, whereas the four thick horizontal lines represent the dark periods within the time frame.

from 1.65 to 1.15 mg/g/hr between 2100 and 100 hrs. When the lights were turned on the rates increased, then decreased after 2 hrs, exhibiting a minor peak which was not repeated in successive days. With reference to maximal and minimum values, the first and second days are comparatively the same; such values were noted to decrease over the third and fourth days.

## DISCUSSION

After metamorphosis in brackish water, naturally occurring *M. rosenbergii* postlarvae migrate to fresh water, where they grow to maturity. In their natural habitats, upstream movement may be stimulated by less optimal temperature and salinity conditions associated with changing conditions in seasonal weather (John, 1957). In ponds, movement of postlarvae is rather restricted and stressful conditions may be detrimental. This has been demonstrated in reported cases of juvenile mortalities associated with oxygen depletion in ponds (Green *et al.*, 1977; Scott *et al.*, 1988). Data from the present study indicate that postlarval respiration is oxygen-dependent, in contrast to oxygen-independent adult respiration (Reyes *et al.*, 1990). Knight (1976) suggested that a 60% oxygen saturation level is well in excess of the critical level at which the respiration rate becomes oxygen-dependent. Our findings showed that at saturation levels below 65% respiration rates and saturation rates decreased parallel to each other; such declines became significant at 25% (Table 1). During the experiments hypoxia-related mortalities were not observed even at 25%, indicating that the postlarvae can tolerate hypoxic levels within a brief period. Based on the significantly lower mean respiration rate upon return to normoxia (90%), it appears that the oxygen debt ensuing from hypoxia was not paid back.

Ranges of observed values at 75% determined for 24 and 96 hrs were within 1.0–1.9 mg/g/hr, well above the adult respiration rates (0.2–0.7 mg/g/hr) (Reyes *et al.*, 1990). Observed respiration trends (Figs. 6 and 7) not only confirm findings by Nelson *et al.* (1977) that oxygen consumption significantly increases during the dark period, but also suggest that photoperiod-related cyclical fluctuations are characteristic of the postlarval metabolism. Elevated respiration rates in darkness coincide with the nocturnal activity of the postlarvae.

Computed mean respiration rates for every 24 hrs were 1.304, 1.387, 1.312 and 1.210 mg/g/hr for the first, second, third and fourth days, respectively, indicating a slight increase

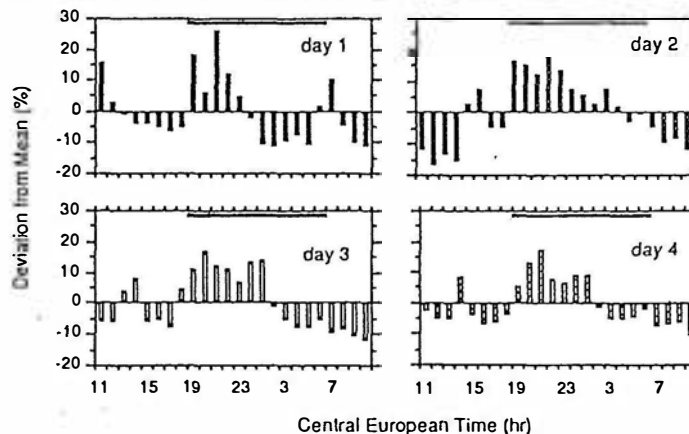


Fig. 7. Percentage deviation from the 24 hr mean respiration rate at 75% oxygen saturation for four consecutive days. The 24 hr averages are 1.304, 1.387, 1.312 and 1.210 mg/g/hr for days 1, 2, 3 and 4, respectively. The thick dark horizontal line at the upper margin of each graph represents the dark period of the light regime.

in rates during the second day, after which respiration rates decreased. These averages are lower than the first ( $1.461 \pm 0.193$  mg/g/hr) and second ( $1.619 \pm 0.230$  mg/g/hr) replicates of measurements lasting for 24 hrs only. The third replicate (from an entirely different set of individuals, but of the same number and weight range) was also considered as the measurement for the first day of the 96 hr experiment since the same conditioning as for the foregoing two replicates was met. The mean 24 hrs respiration rate for the third replicate ( $1.304 \pm 0.132$  mg/g/hr) overlapped the lower deviation of the other two, but the respiration decreased more rapidly during the night (Fig. 4). Statistical analysis of the three replicates showed that 24 hr averages were significantly different from each other (*t* test and Kolmogorov-Smirnov test,  $P < 0.05$ ), but as could be seen in Fig. 4, respiration rates were within the same levels prior to 2000 hrs in all the replicates conducted. This suggests that activity during the light phase falls within a narrow range, but differs from one group of individuals to another during darkness, even when at the same saturation level.

Since the postlarvae were not fed during measurements, oxygen consumption rates in the 96 hr measurement also reflected metabolism in fasting individuals. This fasting effect was evident in the comparatively lower peak rates after 48 hrs; by the 96th hr, the respiration range was approximately 1.05–1.15 mg/g/hr. This range is nevertheless not significantly different from those observed at the 24th, 48th and 72nd hrs. Clifford and Brick (1983) studied substrate metabolism in fasting juveniles (mean weight = 0.68 g) and obtained weight-normalized oxygen consumption rates of 1.450 and 1.348 mg/g/hr after starvation periods of four and eight days, respectively. The 7% depression produced by doubling the fasting time was, however, found to be statistically insignificant (covariance analysis,  $P > 0.05$ ). On the basis of high RQ and O:N values, the authors suggested that the juveniles relied more heavily upon carbohydrate reserves for immediate energy requirements during the first four days of fasting, but noted a subsequent two-fold increase in lipid and protein oxidation as the fasting time doubled. They estimated that 0.1 g juveniles (weight range of postlarvae in the present study) fasting for four days catabolized 0.9% body protein per day and pointed out that the higher weight-specific metabolism in small individuals would precipitate a more rapid depletion of carbohydrate reserves, hastening the transition to increased protein and lipid catabolism.

Weight-specific oxygen consumption rates within the specified time frames and conditions presented herein provide further basic biological information, useful not only in pond culture per se, but also in planning postlarval transport to grow-out ponds. On the basis of the mean respiration rates provided, one can make a good estimate of stocking densities which would minimize hypoxia-related mortalities.

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