EXPERIMENTAL STUDIES ON THE RESPIRATORY METABOLISM OF MYTILUS GALLOPROVINCIALIS (MOLLUSCA BIVALVIA) FROM THE MEDITERRANEAN SEA (GULF OF LION)

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VIE ET MILIEU, 2000, 50 (2) : 87-92

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MYTILUS GALLOPROVINCIALIS
SIZE
RESPIRATION RATES

ABSTRACT. – The bivalve *Mytilus galloprovincialis* (Lamarck, 1818) inhabiting the intertidal zone on the Mediterranean coasts may be subjected to rapid fluctuations in water oxygen concentration or temperature in the highest level of their repartition area or in brackish pools. The respiratory function plays an important role in the physiological adaptation of such species. In this paper we considered the relationships between the respiratory metabolism, some environmental factors (O_2 concentration, temperature) and internal ones (body-size). The length of the shell, fresh and dry weight of the soft tissues are measured to establish allometric relationships. *M. galloprovincialis* showed a rather constant respiration rate independently of a decreasing O_2 concentration. Conversely, the respiration rate increased with the temperature and the Q10 values > 2 reflected a good acclimatization between 6 and 19 °C. The O_2 consumption increased exponentially with the size of the mussels ($b = 0.63$) while the metabolism rate was not clearly related to the weight.

MYTILUS GALLOPROVINCIALIS
TAILLE
TAUX RESPIRATOIRE

RÉSUMÉ. – *Mytilus galloprovincialis* (Lamarck, 1818) vivant dans la zone intertidal du littoral méditerranéen, est soumise à de rapides fluctuations de concentration d'oxygène de l'eau et de température dans les niveaux supérieurs de son aire de répartition ou les mares saumâtres. La respiration joue un rôle important dans l'adaptation physiologique de cette espèce. Nous considérons ici les relations entre le métabolisme respiratoire et quelques facteurs environmentaux (concentration en O_2, température) ou internes (taille). La longueur de la coquille, les poids frais et sec du tissu mou sont mesurés afin d'établir des relations d'allométrie. *M. galloprovincialis* montre un taux respiratoire relativement constant, indépendamment de la diminution de la teneur en O_2. Au contraire le taux respiratoire croît avec la température et les valeurs de $Q10 > 2$ reflètent une bonne acclimatisation entre 6 et 19 °C. Une relation exponentielle relie la consommation d'O_2 et la taille des moules ($b = 0.63$) alors que les taux métaboliques ne sont pas clairement liés au poids.

INTRODUCTION

Mussels living in the intertidal zone are subject to rapid fluctuations in temperature due to the tidal movement (Newell & Pye 1970, Paine 1974, Widdows & Shick 1985) as well as to seasonal changes that have a strong influence on the oxygen consumption of the *Mytilus spp.* (Newell & Bayne 1973). As other bivalves, they are able to tolerate extended periods of hypoxia and anoxia, which may be induced by either shell valve closure or by the depletion of oxygen in the surrounding water (Wang & Widdows 1993). Many studies have examined the metabolic response of *Mytilus spp.* to fluctuations in oxygen tension (pO_2) (Bayne a, b 1971, Bayne 1973, Taylor & Brand 1975, Bayne *et al.* 1976, Famme 1980, Famme *et al.* 1981, Wang & Widdows 1991).

Therefore, the oxygen-independence in larger specimens of *Mytilus* is at least partly related to the water convection of the entire undifferentiated respiring surface (Vahl 1973, Famme 1980, Famme & Kofoed 1980).

Knowledge of the limits of the respiratory function is important to understand the physiological adaptation of a species, since many features of aerobic metabolism can be studied indirectly by

measuring of the rate of oxygen consumption by intact animals.

This paper is devoted to an experimental study of the metabolic rates of the Mediterranean mussel to be used in further studies on the coastal ecosystem.

Experiments were carried out on the possible changes of the respiratory metabolism due to a decrease in the oxygen concentration, to a change in the body size and to a temperature change.

MATERIAL AND METHODS

The experiments were performed in March-April 1995. The mussels of the species « *Mytilus galloprovincialis* » were collected in the Bay of Banyuls-sur-Mer (Gulf of Lion).

The mussels were cleaned of any epibiontic organisms and kept in aquarium tanks (30 × 20 × 15 cm). The room was cooled and the water temperature maintained at 15 ± 1 °C, to allow acclimatization of the animals prior to the experiments.

Before the experiments, the relationship between the length of the shell and the dry weight was determined on a group of 30 randomly taken mussels. The length of each animal was directly measured with a slide calibre (precision ± 0.05 mm). Thereafter the whole living tissue was taken out of the shell and weighed after removing as much as possible of the seawater. The fresh mussels were then dryed at 60 °C for 24 hours.

Oxygen uptake values were expressed as respiration rate (O_2 ml / h / weight units).

All O_2 consumption measurements were taken by the incubation method using filtered (1.2 µm) sea water aerated to saturation by air bubbling.

Isolated mussels were placed in a closed bottle (125 to 350 ml according to the size of the individual) filled with filtered seawater at 12 °C (except for the temperature experiments). A 5-hour-interval for acclimatisation was necessary in all cases, except for the decreasing O_2 concentration experiment where measurements were taken between half an hour to 24 h.

The concentration of dissolved O_2 in water was estimated by using an O_2-electrode and a Strathkelvin Oxymeter. The O_2 consumed by respiration was deduced from the differences of O_2 concentration in water collected before and after incubation. An additional experiment was done to compare polarographic and chemical measurements of the O_2 dissolved in the water.

Three sets of experiments were performed to estimate :

1) the mean respiration rate in relation to the time spent in the enclosure, i.e. in relation to the decreasing oxygen content. The mussels were selected in the range of size from 35 to 50 mm. 25 mussels were selected and placed individually in an experimental bottle. One bottle was used for each O_2 measurement. Concentration was measured every 10 minutes during the first hour, every 1h30 from 1 to 3 h, then every 2 from 3 to 24 h.

The oxygen consumption was measured with a polarographic oxygen electrode (Clark's electrode) and the chemical method (Winkler). The values obtained with both methods were compared with a parametric statistical test, the Mann-Whitney U-test (Elliott 1977);

2) the relationship between oxygen consumption and body size : mussels with different shell lengths ranging from 19.75 to 74.25 mm, were isolated and left in incubation vessels for 5 h period at 12 °C water temperature;

3) the changes in the oxygen consumption due to temperature changes : 3 series of mussels of the same size-class (40.05 mm shell length) were selected and transferred in 3 vials at 3 different water temperature baths (6, 12, 19 °C). The 3 series were acclimated for 24 h before the experiments and enclosed in the bottles at the same temperature (6, 12, 19 °C); the respiration rate was measured after 5-h period.

RESULTS

Length-Weight relationship

The relationship between dry weight and length of the shell is shown in Fig. 1. All dry weights (desiccated at 60 °C) were taken into account. For the 62 sets of observation, a good allometric relationship (R = 0.96) is observed between the length of the shell and the dry weight :

$$\text{Dry Weight} = 0.0103 * \text{Length}^{2.52} ,$$

Dry and fresh weights are significantly correlated (Fig. 1). From the average of the whole data it appears that the dry weight of individuals is equal to 19 % (± 6) of the fresh weight.

Effect of O_2 concentration on O_2 consumption

The ability of mussels to support the decreasing concentration of oxygen during incubation times up to 24 h are shown in Fig. 1. The values were pooled in five groups of O_2 levels (Table I) as suggested by the rough data that showed a significant tendency to a linear decrement (R= 0.97, n=25).

In graph 2, bottom, the mean O_2 concentrations are plotted at the medium time of each series.

The quantity of O_2 consumed, expressed as a fraction of the saturation concentration, reaches 2 % in the two first hours, 23 % after 6 h, 54 % after 14 h, 70 % after 21 h and 95 % after 24 h. The rate of O_2 consumption by mussel was then observed taking into account the time of incubation. Except for the high rate in the first half hour, 0.748 ml (± 0.04) O_2 / h / mg DW, the respiration

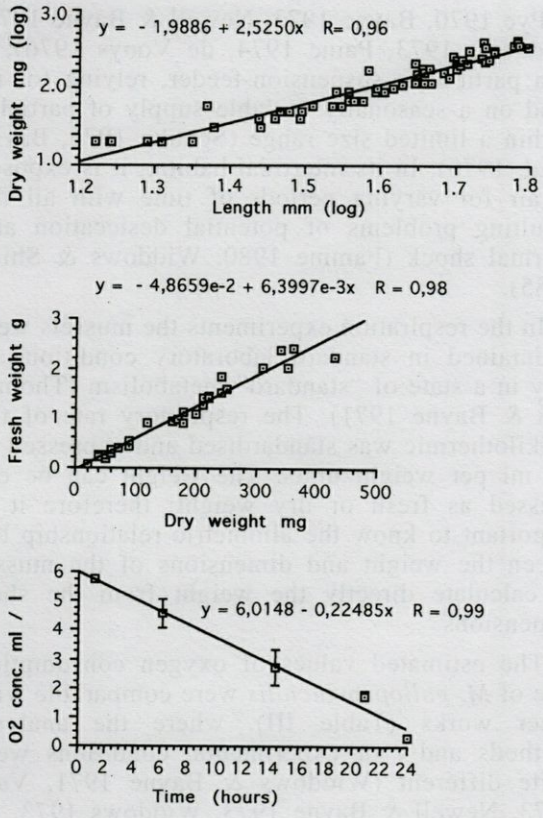

Fig. 1. – From top to bottom: *M. galloprovincialis*, allometric relatioship between the length of the shell and the dry weight of soft tissues; fresh and dry weights of the soft tissues; dissolved O_2 concentration changes in the incubation vessel within the time of experiment.

reached a constant rate of 0.211 (± 0.10) ml O_2 / h / mg DW (Fig. 2). The high rates observed at the beginning of the experiment may be hypothesised to be due to the opening of the valves after the beginning of the experiment.

Comparison of the polarographic method (Clark electrode) and chemical method (Winkler)

The possibility of bias in the measurements of the respiratory rates due to the method was investigated. Three sets of mussels (3 animals in the range of length 35-50 mm) were left in incubation for 5 h, 10 h or 15 h before analysis (Table II).

The results showed that it was possible to superimpose the respiration rates obtained with both methods. The two methods were compared with a non-parametric statistical test the Mann-Whitney U-test. The statistics showed of no-differences between the measurements using the Clark electrode or Winkler method.

Table I. – Top, decreasing O_2 concentration (± Standard error) and increasing consumption (% of the initial O_2 concentration) in relation to time of incubation (hour). Bottom, comparison of the respiration rates measured using polarographic or Winkler method (S.E : Standard error; N : number of individuals).

O2 conc. ml / l	Time	Nber of mussels	% O2 consummed
5.77 (± 0.08)	10 min. to 2 h 30	9	2 %
4.57 (± 0.5)	3 h to 10 h	6	23 %
2.71 (± 0.62)	12 h to 20 h	5	54 %
1.76 (± 0.17)	21 h to 23 h	4	70 %
0.3 -	24 h	1	95 %

	Clark electrod		Winkler method	
Time (h)	O2 ml / h / mg D.W.	S.E	O2 ml / h / mg D.W.	S.E
5	0.424	0.192	0.334	0.053
10	0.334	0.063	0.221	0.033
15	0.228	0.092	0.264	0.045
	(N=9)		(N=9)	

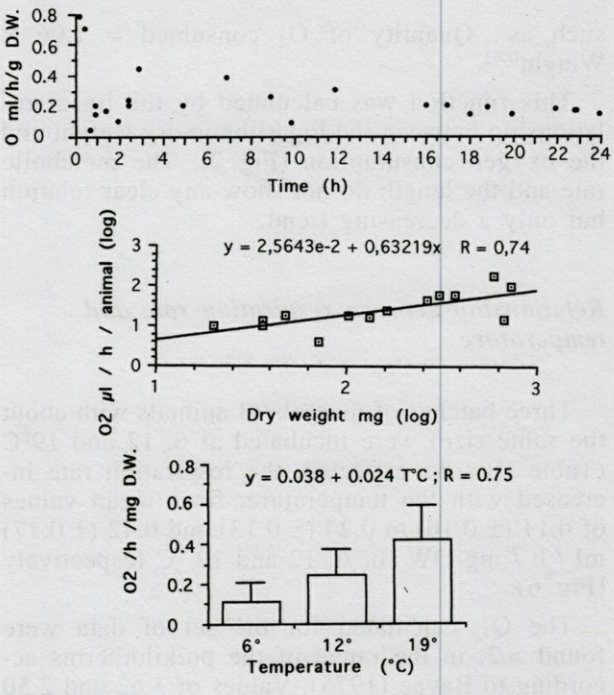

Fig. 2. – From top to bottom: metabolic rate, expressed by dry weight unit, within the curse of incubation experiment. Oxygen consumption of individuals in relation to the body weight. Temperature – Metabolic rate changes.

Relationship between the respiration rate and size or weight

The relation between the mussel dry weight and the oxygen consumption were computed and expressed as a function of the dry weight of soft parts (W) by an allometric equation $Y = a W^b$

Table II. – Changes of respiration rate in relation to temperature.

water T °C	shell length mm	dry weight g	oxygen ml / h / anim.	oxygen ml / h / mg dry weight
6	43.25	0.232	0.0100	0. 043
	39.05	0.104	0.0240	0.231
	37.55	0.135	0.0081	0.060
12	43.25	0.232	0.0210	0.091
	39.05	0.104	0.0340	0.327
	37.55	0.135	0.0400	0.296
19	43.25	0.232	0.0560	0.241
	39.05	0.104	0.0590	0.567
	37.55	0.135	0.0620	0.459

such as : Quantity of O_2 consumed = 1.06 × Weight$^{0.63}$.

This function was calculated by the linear relationship between the logarithmic dry weight and the oxygen consumption (Fig. 2). The metabolic rate and the length do not show any clear relation but only a decreasing trend.

Relationship between respiration rate and temperature

Three batches of mussels (3 animals with about the same size) were incubated at 6, 12 and 19°C (Table III). As expected, the respiration rate increased with the temperature, from mean values of 0.11 (± 0.10) to 0.24 (± 0.13) and 0.42 (± 0.17) ml / h / mg DW for 6, 12 and 19 °C respectively (Fig. 6).

The Q_{10} calculated for the set of data were found > 2, in the range of the poikilotherms according to Bayne (1975). Values of 3.62 and 2.50 were obtained between 6-12 °C and 12-19 °C respectively.

DISCUSSION

Physiological ecology is the study of how an animal adapts itself to a particular function in a specific environment. *Mytilus galloprovincialis* is a benthic (littoral and near sublittoral) semi-sessil species (Bayne *et al.* 1976), exposed to wide fluctuations in salinity and temperature (Newell & Pye 1970, Bayne 1973, Newell & Bayne 1973, Widdows 1973, Paine 1974, de Vooys 1976); it is a particulate suspension-feeder, relying for its food on a seasonally variable supply of particles within a limited size range (Schulte 1975, Bayne *et al.* 1976). In its intertidal habitat, it is exposed to air for varying periods of time with all the resulting problems of potential desiccation and thermal shock (Famme 1980, Widdows & Shich 1985).

In the respiration experiments the mussels were maintained in standard laboratory conditions to stay in a state of "standard" metabolism (Thompson & Bayne 1971). The respiratory rate of the poikilothermic was standardised and expressed as O_2 ml per weight units. The weight can be expressed as fresh or dry weight; therefore it is important to know the allometric relationship between the weight and dimensions of the mussel to calculate directly the weight from the shell dimensions.

The estimated values of oxygen consumption rate of *M. galloprovincialis* were comparable with other works (Table III), where the analysis methods and / or experimental conditions were quite different (Widdows & Bayne 1971, Vahl 1973, Newell & Bayne 1973, Widdows 1973, de Vooys 1976).

The respiration rate was constant when the oxygen concentration in the closed bottles decreased to values as low as approximately 2 ml /l. The O_2 concentration in the range of 100 % to about 5 % of saturation appears to be without any effect on the metabolism rates of mussels. This confirms the absence of relationship between the respiratory rates and the oxygen concentration in the water described by Taylor & Brand (1975). *M. galloprovincialis* tolerates extended periods of hypoxia and anoxia (Bayne 1971a), which may be induced by either shell valve closure (Famme *et al.* 1981) or by the depletion of oxygen in the surrounding water (Wang & Widdows 1993). *M. galloprovincialis* has the ability to maintain constant oxygen consumption down to very low pO_2 value, and to lower the critical pO_2 *via* fermentative pathways of ATP production necessary for the metabolic activity (Zwaan *et al.* 1991).

In marine mussels, the extraction of the oxygen from water occurs primarily at the gill surface. The gill area, therefore, serves a dual function, in feeding and respiration (Bayne *et al.* 1976).

The relationship between body size and oxygen consumption highlights a value of "b" in agreement with Krüger (1960), Vahl (1973) & Famme (1980), although their "b" values are slightly higher than our data.

The positive correlation between metabolism and dry weight shows that the increase of the body size does not induce an increase of the

Table III. – Comparison of Oxygen consumption of *Mytilus edulis* according to experimental conditions.

Animal	Ref.	Méthod	Experiments	Oxygen consumption
M.edulis	*Widdows et al. 1971*	(Radiometer)	running water 2.4 l / h	0.24-0.35 ml/h/g DW
		Clark electrod	15 °C	
	Vahl. 1973	(Radiometer)	incubation	0.350 ml/h/g DW
			10 °C	
	Newell et al. 1973	Gaz Analyseur	running water 2.7 l / h	0.30-0.40 ml/h/g DW
		(Beckman)	15 °C	
	Widdows 1973	«	running water 3.0 l / h	0.50 ml /h/g DW
			15 °C	
	De Vooys 1976		running water ≥ 1000 l /h	
			10 °C	0.07 ml /h/g FW
			20 °C	0. 15 ml /h/g FW
M. gallo-provincialis	Present data	Clark electrod (Strathkelvin)	incubation 12° C	0.202 ± 0.074 ml/h/ gDW

metabolism of similar amplitude. Nevertheless, it is well known that a larger size of the bivalve is correlated with a wide gill area resulting from an increase in length and in density of filaments. However, the gill area increase does not always correspond to the higher metabolism rate (Vahl 1973). This could, therefore, explain the lack of a strong direct linear correlation between oxygen consumption and dry weight as to the function.

Beyond the influence of the internal convection, the oxygen gradients through the tissues are determined mainly by the oxygen tension at the surface and the oxygen consumption of the tissues. These findings show that the relationship between body weight and the degree of independence of the metabolic rate is not only conditioned by the higher weight-specific oxygen consumption of smaller species, but also by the oxygen translation distance (Famme 1980).

Temperature is one of the major environmental variables that influence the rates of metabolic activity in poikilotherms. However, mussels like many other littoral invertebrates, though apparently unable to regulate their rate of heat loss or gain from the environment, are able to vary their respiratory and feeding rate in such a way as to maintain them relatively independent of the environmental temperature (Bayne *et al.* 1976, Widdows 1973, Schulte 1975).

The calculated Q_{10} values, between 2.4 and 3.9, providing an index of the dependence of the physiological rate on temperature, reflect a change of the metabolic activity due to variations of temperature with a short-time adaptation (Newell & Bayne 1973). The best acclimatisation activity (Q_{10} = 2.4) is observed in the range of the seasonal *in situ* temperature where mussels were living (12 – 19 8), the highest stress occurring at the lowest temperatures (Newell *et al.* 1970; Bayne *et al.* 1976).

The present data confirm that *M. galloprovincialis* is able to compensate its respiration metabolism against environmental changes. This capacity of some degree of compensation is due to its intertidal habit, where it is exposed to air for varying periods of time with the resulting problems of potential desiccation, thermal shock and oxygen lack (Bayne *et al.* 1976).

M. galloprovincialis is, in fact, amply distributed in all the Mediterranean Sea where it occurs at highest density in the intertidal zone (Paine 1974, Gosling 1984). Littoral and near sublittoral habit represents a refuge for the benthic semi-sessil species, with the lower limits set by biotic factors of competition and predation and the upper limits determined by environmental stressors (Paine 1974).

ACKNOWLEDGEMENTS. – This research was supported by the ERASMUS project. We thank the Laboratoire Arago for the use of their laboratory and other facilities. The authors wish to thank Prof. S Fonda Umani for her usefull suggestions in the draft of the manuscript. Thanks are also due to two anonymous referees for their comments.

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Reçu le 7 juillet 1999; received July 7, 1999
Accepté le 15 décembre 1999; accepted December 15, 1999