Phuket mar. biol. Cent. Res. Bull. **60**: 95-98 (1995)

SCOPE FOR GROWTH OF *PERNA VIRIDIS* AS A MEASUREMENT OF POLLUTION EFFECTS IN PHUKET HARBOUR, THAILAND

By John Widdows
Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth, PL1 3DH, England

ABSTRACT

Mussels (*Perna viridis*) were transplanted to two sites (Phuket Harbour - polluted; Jetty of the Phuket Marine Biological Center - reference) for a period of 6 days. The mean scope for growth of mussels from Phuket Harbour was positive but significantly reduced (c. 50%) compared to the reference site. This was due to the inhibition of clearance (= feeding) rate (c. 30% reduction). In addition, the workshop demonstrated that the scope for growth technique could be readily applied to indigenous tropical bivalve species, adapted to prevailing laboratory conditions and acquired and applied by regional research scientists during the time scale of a training workshop.

INTRODUCTION

A UNEP Workshop on 'Biological effects of pollutants' was held at the Phuket Marine Biological Center (PMBC) in Thailand on 16-25 November 1993. The overall objectives of this workshop were:

To assess the application of methods, used in temperate regions, for assessing the harmful biological effects of pollutants in a tropical environment.

- To develop the scientific skills of the personnel that are engaged in toxicological studies and the assessment of pollution effects in the East Asian Seas region.
- To improve the comparability of results of scientific studies and assessments of the biological effects of pollutants in the participating countries of the East Asian Seas region.
- To build-up a network of scientists and institutions in the countries of the region.

The aims of this particular study were:

- To train scientists in the measurement and application of 'scope for growth' of bivalves.
- To compare the scope for growth of the mussel *Perna viridis* transplanted to two sites,

Phuket Harbour (polluted) and PMBC jetty (clean reference).

Growth provides one of the most sensitive measures of stress in an animal, since growth represents an integration of major physiological responses and specifically the balance between processes of energy acquisition (feeding and digestion) and energy expenditure (metabolism and excretion). Each of these physiological responses can be converted into measures of energy flow (J h^{-1}) and alterations in the amount of energy available for growth and reproduction (termed scope for growth) can be quantified by means of the balanced energy equation. Therefore scope for growth provides an instantaneous measure of the energy status of an animal, which can range from maximum positive values under optimum conditions, declining to negative values when the animal is severely stressed and utilising body reserves.

Previous laboratory and field studies have shown bivalves, and particularly mussels, to be ideal animals for pollution assessment using reductions in scope for growth as a measure of increasing stress which is directly related to the accumulation of chemical contaminants in their body tissues (for review see Widdows and Donkin, 1992).

MATERIALS AND METHODS

Mussels (*Perna viridis*) were collected from Phuket fish market and recovered in the laboratory for 2 days before being placed in cages (70 individuals per cage) and suspended (2m deep) at Phuket Harbour and PMBC jetty. After 6 days the mussels were collected for measurement of scope

for growth under 'standardised conditions' at PMBC [*i.e.* temperature 28°C; salinity 32 p.s.u.; seston 1.34 mg L^{-1}; particulate organic matter (POM) 0.83 mg L^{-1}].

Feeding rate measurement.

Clearance rate, defined as the volume of water cleared of particles per mussel per hour, was determined in a "closed system" by measuring the exponential decline in the concentration of suspended algal cells over a period of one hour. The concentration of algal cells in sea water was determined spectrofluorometrically because an electronic particle counter (*e.g.* Coulter Counter) was not available. In preliminary studies, natural sea water (at PMBC) was scanned to establish the optimal excitation and emission wavelengths for algal cell fluorescence (Ex. 340 nm; Em. 682 nm) and the range over which the fluorescence was linear. A calibration curve was established on each occasion by dilution of natural sea water with filtered sea water and this demonstrated that fluorescence was linear and measurable down to a 10% dilution. In addition, it was established that fluorescence was stable following temperature equilibration of samples to the operating temperature of the spectrofluorometer (*i.e.* at 24°C in an air-conditioned room). Due to the more limited range of detection for the fluorometer compared to the electronic particle counter, the duration of the clearance rate measurement was reduced from 2h to 1h.

Food absorption efficiency.

The efficiency with which food is absorbed from the ingested food material (seston) was measured by the ratio method of Conover (1966).

$$\text{Absorption efficiency} = (F - E) / [(1 - E) F]$$

where F = ash-free dry weight : dry weight ratio of the food (seston) and E = ash - free dry weight : dry weight ratio of the faeces. The amount of suspended particulate matter or seston concentration (mg L^{-1}) at PMBC was determined by filtering known volumes of sea water through washed, ashed and pre-weighed glass fibre filters (Whatman GFC) (a minimum of five samples were collected). Mussel faeces accumulated overnight in tanks receiving flowing sea water were sampled by pipetting onto washed ashed and weighed GFC filters. Salts were washed out of the filters with distilled water (3 x 5 ml). The filters were oven dried at 90°C for > 24 h and weighed. They were then ashed in a furnace at 450°C for 6 h and weighed again in order to calculate the weight of organic material combusted. Filters were stored in desiccators prior to weighing and blank GFC filters were weighed at each stage in order to correct for any weight changes.

Respiration rate measurement.

Rates of oxygen consumption by individual mussels were measured in closed glass respirometers (500 ml) over a period of 40 min. The rate of decline in oxygen concentration was measured using a calibrated oxygen electrode (Strathkelvin 1302) connected to an oxygen meter (Strathkelvin Model 781b) and output onto a chart recorder.

After physiological measurement the mussels tissues were dried at 90°C and weighed.

Calculation of Scope for Growth.

Mass-specific physiological rates responses were converted into energy equivalents and used in the balanced energy equation :

$$P = (C \times Ab) - R$$

where P = Scope for growth or the energy available for growth and reproduction;
C = Food energy consumed; [clearance rate ($l\ g^{-1}\ h^{-1}$) x POM (mgL^{-1}) x 23 J mg^{-1} ash free dry weight]
Ab = Food absorption efficiency;
R = Respiratory energy expenditure; [µmol $O_2\ g^{-1}\ h^{-1}$ x 0.456 J µmol O_2^{-1}]

Further details of scope for growth methodology are presented in Widdows and Salkeld (1993) and Widdows (1993).

All participants on the workshop successfully completed measurement of the physiological responses of mussels collected from the two sites, the computation of the results and the calculation of scope for growth.

RESULTS AND DISCUSSION

The physiological responses of mussels after 6 days at the two sites are summarised in Table 1. There was a statistically significant ($P < 0.05$) reduction in the scope for growth of mussels from Phuket Harbour compared to those from PMBC jetty (*i.e.* c. 50% lower). This was primarily due to the decline in feeding or clearance rate (*i.e.* Phuket Harbour 30% lower than PMBC jetty). There were no significant differences recorded in the rates of respiration or the absorption efficiencies of the two groups. Mussels at both sites had positive scope for growth values which indicates that although the clearance rates of mussels from Phuket Harbour were reduced there was sufficient seston and food available in the water column to support growth. However, the ability to discriminate between the two sites would probably have been enhanced by a longer period of exposure (*i.e.* > 6 days). The uptake kinetics of most chemical contaminants would indicate that the majority of toxicants require several weeks to reach a steady state between toxicant concentration in the tissues and that in the water (Widdows and Donkin, 1992).

Table 1. Physiological responses and scope for growth of mussels (*Perna viridis*) transplanted to two sites for 6 days (Mean ± 95% C.I.; n = 15)

Physiological Responses	*PMBC Jetty (Reference)*	*Phuket Harbour*
Clearance rate (L g^{-1} h^{-1})	4.58 ± 0.9	3.19 ± 0.46
Absorption efficiency	0.86 ± 0.02	0.84 ± 0.03
Respiration rate (µmol O_2 g^{-1} h^{-1})	57.9 ± 9.6	58.7 ± 6.8
Scope for growth (J g^{-1} h^{-1})	48.3 ± 15.2	25.5 ± 7.5

There were no chemical analyses of contaminants accumulated in the mussel tissues in this study and therefore a quantitative toxicological interpretation of the physiological responses is not possible. However, in all previous field studies (see Widdows and Donkin, 1992) petroleum hydrocarbons from both urban and shipping sources have formed a major environmental contaminant and a significant component of the total toxicant load within mussels. Hydrocarbons are known to both accumulate rapidly (steady state reached within hours or days depending on the molecular weight and hydrophobicity) and exert inhibitory effects on the feeding rate of mussels through the mechanism of narcosis (Donkin and Widdows, 1990). Consequently, hydrocarbons are not only likely to be a major contaminant in Phuket Harbour, but they will be rapidly accumulated within the 6 days of exposure and are therefore likely to be the major cause of the observed reduction in scope for growth of mussels from Phuket Harbour.

In conclusion, this study has shown that it is possible to detect and quantify pollution effects using the scope for growth of mussels transplanted to a site in Phuket Harbour for a relatively short period of time (*i.e.* within the time constraints imposed by the workshop). Furthermore, the results not only indicate the degree of scope for growth but how close the mussels are to the lethal limit. This workshop has also demonstrated that the scope for growth technique is easily applied to indigenous bivalves in tropical regions, is readily adapted to prevailing laboratory conditions and regional research scientists can successfully acquire and apply the procedures during the time scale of a training workshop.

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