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Bioenergetics, growth and reproduction of amphipods are affected by moderately low oxygen regimes

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ABSTRACT: Low dissolved oxygen poses a major threat to coastal marine ecosystems worldwide. This study demonstrates that both growth and reproduction of the amphipod *Melita longidactyla* are impaired even by moderately low dissolved oxygen levels (3.5 to 4.5 mg O_2 l^{-1}), which are higher than levels considered to be hypoxic (2.8 mg O_2 l^{-1}). Negative growth and decreases in respiratory energy expenditure were observed after exposure to moderately low oxygen levels for 3 wk. The RNA:DNA ratio was most sensitive, and decreased significantly (by 50 to 86%) following exposure to 4.5 and 3.5 mg O_2 l^{-1} for 1 wk. Amphipods exposed to 3.5 or 4.5 mg O_2 l^{-1} for 1 wk followed by recovery in normoxia for a further week exhibited no significant change in growth, energy consumption, respiratory energy expenditure and scope for growth compared with individuals kept in normoxia for the whole period. Complete reproductive failure occurred when amphipods were exposed to 3.5 mg O_2 l^{-1} for 1 mo; however, no significant difference was found in percentage copulation, number of broods and offspring or fecundity between the normoxic control and the 4.5 mg O_2 l^{-1} treatment group, indicating that reproductive impairment occurs within a narrow range of oxygen decrease below 4.5 mg O_2 l^{-1}. *M. longidactyla* plays an important role in trophodynamics and nutrient recycling and is abundant along the Chinese coast, where dissolved oxygen is often low. Our results suggest that natural populations of this species are potentially under threat, and the present water quality objective of 4 mg O_2 l^{-1} adopted in some countries/places may not afford protection for this ecologically important species.

KEY WORDS: Amphipod · Oxygen · Growth · Reproduction · RNA:DNA ratio

INTRODUCTION

Hypoxia is generally defined as dissolved oxygen levels between 0 (anoxia) and 2.8 mg l^{-1} (Diaz & Rosenberg 1995). Hypoxia affects thousands of km^2 of coastal water worldwide, and some coastal areas (e.g. Black Sea) have become permanently hypoxic/anoxic (Rosenberg et al. 1992, Justic et al. 1993, Rabalais et al. 1994, Diaz & Rosenberg 1995, Levin 2003). Helly & Levin (2004) have estimated that the global area of permanent hypoxic shelf and bathyal sea floor is >1 million km^2. Indeed, hypoxia is now regarded as one of the most serious threats to marine populations and genetic diversity (Goldberg 1995, McIntyre 1995, Wu 1999, Gray et al. 2002). As global warming and eutrophication will further exacerbate the reduction of oxygen concentrations in marine waters, there is a pressing need to understand the functional consequences of oxygen depletion in marine ecosystems (Levin 2003).

Many animals may alter their energy utilization pattern and trade off growth and reproduction in order to cope with an increased demand for energy for maintenance during stress (Calow 1993). Indeed, individual energy budget items, such as feeding, scope for growth and metabolic rate, have often been employed as stress indicators (e.g. Senus & McLachlan 1986, Maltby et al. 1990, Das & Stickle 1993). The use of a bioenergetic approach to environmental stress studies has attracted increasing attention in the last decade (see Calow 1993). Nevertheless, very few studies have been carried out to examine how energy utilization by marine animals may be altered under hypoxia.

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Growth and reproductive success are amongst the most important Darwinian fitness traits for species survival. As such, significant impairment of growth and reproduction will lead to a decline in natural populations, thus threatening species survival and genetic diversity. Surprisingly, growth and reproductive responses of marine animals to hypoxia are poorly understood (Diaz & Rosenberg 1995, Gray et al. 2002). The paucity of data on effects of hypoxia on growth and reproduction of marine species makes it difficult or impossible to predict the ecological consequences of hypoxia for field populations (Wu 2002).

Amphipods are one of the most abundant and diverse groups of marine benthic animals in coastal waters worldwide (Hoberg et al. 1982, Geddes & Butler 1984, Faasse & Van Moorsel 2003). They serve as an important trophic link from primary producers to higher-order consumers (Chiaravalle et al. 1997) and play an important role in nutrient recycling in marine coastal systems (Camilleri 1992, Lehtonen 1995, Hoback & Barnhart 1996). Conceivably, any adverse impact on amphipods may lead to major ecological consequences for ecological functions, including alterations in energy flow and disruption of nutrient recycling processes in coastal ecosystems.

Limited information is available on how hypoxia may affect reproduction and growth of amphipods. A laboratory study by Wiklund & Sundelin (2001) showed that prolonged exposure to a low oxygen regime (3.6 ± 1.3 mg O_2 l^{-1}) caused an increase in the percentage of unfertilized/dead eggs, dead broods and a decrease in fertility in the amphipods *Monoporeia affinis* and *Potopereia femorata*, indicating that these amphipod species are sensitive to hypoxia. The results of a 7 yr field study carried out in the Baltic Sea, however, showed that the percentage of malformed embryos of the amphipod *M. affinis* was not related to oxygen concentration, while a negative correlation was demonstrated between females carrying a dead brood and oxygen concentration (Wiklund & Sundelin 2004). Chapelle & Peck (1999, 2004) hypothesized that the maximum size of amphipods is a function of oxygen availability in water. Results from the above studies provided evidence that both growth and reproduction of amphipods may be potentially affected by low dissolved oxygen. In contrast, ampeliscid amphipods are found in abundance and dominate within deep-water oxygen minimum zones (Levin 2003), indicating that some amphipod species are tolerant of hypoxia.

The amphipod *Melita longidactyla* (family Melitidae) is abundant along the entire Chinese coast, spanning from tropical to temperate waters (Huang 1994). With rapid coastal and urban development, hypoxia has been frequently reported over large areas along Chinese coastal waters (Lin & Li 2002, Peng & Zhou 2002), and potentially may affect this amphipod species. In this study, we hypothesize that low dissolved oxygen will adversely affect the growth, bioenergetics, reproductive behavior, reproductive success and reproductive output of *M. longidactyla*. These effects may pose a significant threat to natural populations of this ecologically important species over large areas.

MATERIALS AND METHODS

Source, sampling and acclimation of experimental animals. Amphipods *Melita longidactyla* were collected from fish farms at Sam Mun Tsai, Hong Kong, during their reproductive season (May to September). In addition, the green alga *Ulva lactuca*, which is used by the amphipods as a refuge and food source in their natural habitat, was also collected. In the laboratory, *M. longidactyla* were acclimated in aerated, running seawater (20°C, 30‰, 12:12 h light:dark cycle) for at least 1 wk before the start of the experiment. During acclimation, amphipods were fed to satiation with *U. lactuca*. Active growing juveniles (wet wt of 3.5 to 4.5 mg) were sorted for growth experiments, while adults (wet wt of 8.0 to 10.0 mg) were selected for reproductive experiments.

Set-up of oxygen-control systems. Control systems were set up in the laboratory to provide constant, controlled levels of ambient dissolved oxygen throughout the entire experimental period. A detailed design of the system is described in Zhou et al. (2001). Oxygen levels were checked 3 times daily using a blood gas analyzer (Ciba Corning 278 Blood Gas System). Temperature and salinity were maintained at 20 ± 2°C and 30 ± 2‰, and dissolved oxygen at ±0.2 mg O_2 l^{-1} of the desired level throughout the experiment. A preliminary experiment was first carried out to determine the hypoxic tolerance of *Melita longidactyla*. Ten amphipods each were exposed to 0.2, 0.5, 1.2, 1.6, 1.8, 2.0, 2.5, 3.0 and 3.5 mg O_2 l^{-1}. Mortality of amphipods in each treatment was checked twice daily and the 48 h LC_{50} (lethal concentration killing 50% of exposed individuals) determined (Fig. 1). Observation was further extended to 1 wk, and the results showed that significant mortality occurred when *M. longidactyla* was exposed to <3 mg O_2 l^{-1} for 1 wk, indicating that this species is sensitive to low oxygen levels. As a result, amphipods were exposed to 7.5, 4.5 and 3.5 mg O_2 l^{-1} in our experiments (equivalent to 100, 60.8 and 47.3% saturation, or 128, 79 and 61 torr, respectively). Twenty replicates were set up for each oxygen level, and each replicate consisted of 10 amphipods held inside a transparent tube (length: 8.5 cm, diameter: 6.5 cm) submerged in the reservoir. Both ends of the tube were covered with a mesh (pore size: 0.5 mm diameter) to prevent faecal loss and escape of animals.

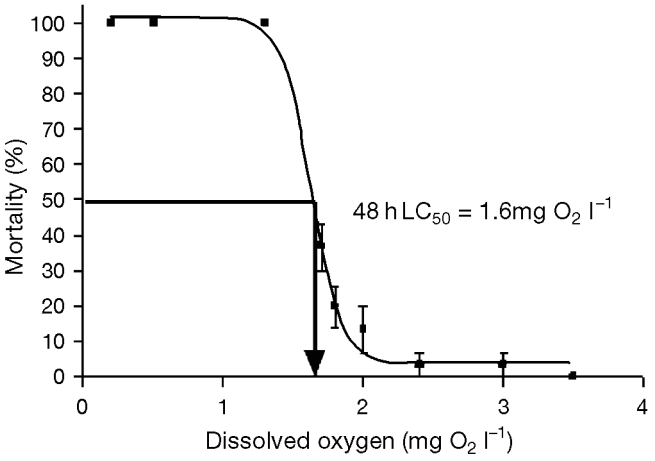

Fig. 1. *Melita longidactyla*. LC_{50} (48 h) under different dissolved oxygen regimes (mean ± 1 SD)

Growth and bioenergetics. Each treatment consisted of 3 replicates and each replicate contained 10 amphipods. Excess food (*Ulva lactuca*) was provided throughout the experimental period. Growth (dry wt, mg), the RNA:DNA ratio and scope for growth were measured for 3 different oxygen concentrations (i.e. 7.5, 4.5 and 3.5 mg O_2 l^{-1}) for each replicate (n = 10 in each replicate) over the periods of 1 and 3 wk. The RNA:DNA ratio of tissue samples (10 amphipods pooled) was determined for each replicate following the method of Wright & Hetzel (1985). Scope for growth of amphipods was measured following the method developed by Maltby et al. (1990). Respiration rate was measured by placing 5 amphipods in aerated seawater (20°C) in a 50 ml syringe. Oxygen levels in seawater were measured initially and again after 150 min using a Blood Gas Analyzer (Ciba Corning 278 Blood Gas System). This time interval was selected to obtain a difference of about 0.4 mg O_2 l^{-1} between 0 and 150 min since such a small difference in dissolved oxygen would not be expected to affect the oxygen consumption rate of the experimental animals. Oxygen consumption was then expressed in mg O_2 mg dry wt^{-1}. Food consumption was estimated by determining mg dry wt of *U. lactuca* consumed per mg dry wt of amphipod, and then converting this to energy equivalents by multiplying by the calorific value of *U. lactuca*. Faecal production was, however, not estimated since results of a preliminary experiment showed that the energy value of faecal material is low at 2.66 J mg^{-1} and the faecal production was only about 0.32 mg faeces mg dry wt^{-1} d^{-1} (which is equivalent to 0.85 J mg dry wt^{-1} d^{-1}).

Ulva lactuca contains ~9.6% protein and 1.5% lipid (ash free wt). Thus, the main bulk of *U. lactuca* consists of carbohydrate (~89%). Respiratory energy expenditure was, therefore, estimated using a conversion factor of 1 mg O_2 consumed being equivalent to 14.1 J (Elliott & Davison 1975), assuming that carbohydrate is the main energy source.

Reproduction. Replicate plastic containers (1.5 l) were set up, each containing 10 males and 10 females and covered with a 0.5 mm mesh. Three replicate containers were exposed to each level of dissolved oxygen, i.e. 3.5, 4.5 and 7.5 mg O_2 l^{-1}, for 4 wk (20°C, 30‰, 12:12 h light:dark cycle). During the 4 wk period, excess food (*Ulva lactuca*) was provided, and experimental animals in each replicate were observed 3 times daily. During copulation, the male remains mounted on the female for more than 24 h. Each copulating pair was isolated and put into a plastic tube (length: 8.5 cm, diameter: 6.5 cm) covered with a 0.5 mm mesh at both ends, and immersed in the experimental tanks of the same oxygen level. Copulating females carry eggs in their abdomen. Juveniles produced by each pair of amphipods were counted daily and isolated, after which the parent pair was returned to the oxygen control system.

The following reproductive parameters were also determined as reproductive biomarkers for each replicate: (1) percentage of copulating pairs, (2) percentage of reproducing females, (3) number of viable progeny per brood, (4) number of broods per reproducing female and (5) total fecundity, defined as: (no. of reproducing female) × (no. of viable progeny per brood) × (no. of brood per reproducing female).

Recovery from low dissolved oxygen. Amphipods (4.0 ± 0.3 mg) were exposed to 3.5, 4.5 and 7.5 mg O_2 l^{-1} for 1 wk, then returned to normoxia (7.5 mg O_2 l^{-1}) for a further week. Six replicates were set up per treatment, and each replicate contained 10 individuals. Excess *Ulva lactuca* was provided as food and refuge throughout the entire experimental period and the algae was renewed weekly. Three replicates were sampled from each treatment after exposure to various levels of oxygen for 1 wk. Another 3 replicates were sampled at the end of the additional 1 wk recovery period. Growth, the RNA:DNA ratio and scope for growth in the control and various treatment groups were determined for each replicate using the methods described above and then compared.

Statistical analysis. Data were tested for homogeneity using Bartlett's test. One-way analysis of variance (ANOVA) was used to test the null hypothesis that there was no significant difference in the mean values of each parameter between the 3 levels of dissolved oxygen. Percentage data were arcsine-square-root-transformed before statistical analysis. Whenever any significant difference was found, pairwise comparisons were made using Tukey's test to identify the difference between mean values (Zar 1999). Statistics were performed using the statistical software Statistica (Statsoft).

RESULTS

Low dissolved oxygen tolerance

The dose-response relationship between amphipod mortality and dissolved oxygen is shown in Fig. 1. Mortality occurred rapidly when dissolved oxygen dropped below 2.5 mg O_2 l^{-1}. The 48 h LC_{50} of *Melita Longidactyla*, as determined by probit analysis, was 1.6 mg O_2 l^{-1}.

Growth

After 1 wk, negative growth was measured for all 3 oxygen levels, and no significant differences among groups were detected (ANOVA: $p < 0.05$; Fig. 2). After 3 wk, positive growth was found in the 7.5 mg O_2 l^{-1} control, while negative growth continued in the 3.5 and 4.5 mg O_2 l^{-1} treatment groups. Amphipods in the 3.5 mg O_2 l^{-1} treatment suffered a significant reduction in growth (254%) compared with that of the 7.5 mg O_2 l^{-1} control (Tukey's test: $p < 0.05$). Compared with the 7.5 mg O_2 l^{-1} control, the RNA:DNA ratios significantly decreased, by 85.7 and 50.2% respectively, upon exposure to 3.5 and 4.5 mg O_2 l^{-1} for 1 wk (Tukey's test: $p < 0.05$ and $p < 0.01$, respectively) After 3 wk, the RNA:DNA ratio in the 3.5 mg O_2 l^{-1} treatment was 82.6% lower than that in the 7.5 mg O_2 l^{-1} control, whereas the ratio was 74.1% lower in the 4.5 mg O_2 l^{-1} treatment (Tukey's test: $p < 0.001$) (Fig. 2).

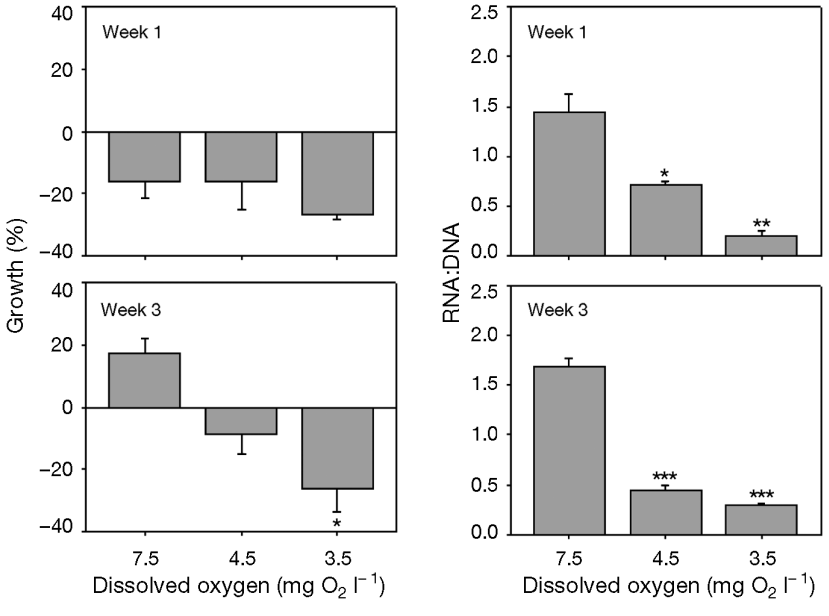

Fig. 2. *Melita longidactyla*. Percentage growth (measured by weight gain) and the RNA:DNA ratio of *M. longidactyla* upon exposure to different dissolved oxygen concentrations for 1 and 3 wk (mean ± 1 SD; values significantly different from the normoxic control (Tukey's test) are indicated by asterisks: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

Bioenergetics

Energy consumed, respiratory energy expenditure and scope for growth over the study period are expressed as J mg^{-1} tissue dry wt and are shown in Figs. 3 to 5. There was no significant difference in energy consumed, respiratory energy expenditure and scope for growth after 1 wk of exposure to 3.5 and 4.5 mg O_2 l^{-1} compared to the 7.5 mg O_2 l^{-1} control. Significant decreases in respiratory energy expenditure were found after 3 wk relative to the 7.5 mg O_2 l^{-1} control (3.5 mg O_2 l^{-1}: 70.5%; 4.5 mg O_2 $^{-1}$: 51%) (Tukey's test: $p < 0.05$), but there were no changes in energy consumption and scope for growth at either oxygen level (ANOVA: $p < 0.05$).

Reproduction

Copulation ceased after exposure to 3.5 mg O_2 l^{-1} for 4 wk, and all measures of reproductive success and output were reduced to 0. No significant difference in any of these reproductive parameters was found at 4.5 mg O_2 l^{-1} relative to the 7.5 mg O_2 l^{-1} control (Turkey test: $p < 0.001$) (Fig. 6).

Recovery from low dissolved oxygen

Exposure to 3.5 and 4.5 mg O_2 l^{-1} for 1 wk followed by recovery in normoxia (7.5 mg O_2 l^{-1}) for a further week resulted in no significant change in growth relative to amphipods kept in the normoxic control throughout the same period (Fig. 7). A significant increase in the RNA:DNA ratio was, however, clearly evident when *Melita longidactyla* was returned to normoxic levels after exposure to 4.5 mg O_2 l^{-1} (ANOVA: $p < 0.05$). No significant differences from the 7.5 mg O_2 l^{-1} control in energy consumption, respiratory energy expenditure and scope for growth were observed when the amphipods were returned to 7.5 mg O_2 l^{-1} (normoxia) after exposure to 3.5 and 4.5 mg O_2 l^{-1} (ANOVA: $p < 0.05$) (Fig. 7).

DISCUSSION

The LC_{50} of *Melita longidactyla* to hypoxia was much lower than that of other amphipod species, showing that *M. longidactyla* is relatively sensitive to low dissolved oxygen levels (Table 1).

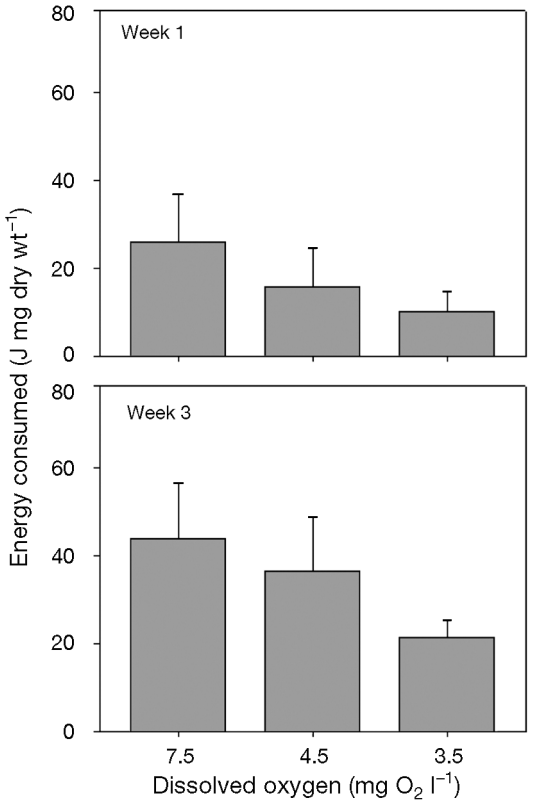

Fig. 3. *Melita longidactyla*. Energy consumed upon exposure to different dissolved oxygen concentrations for 1 and 3 wk mean + 1 SD)

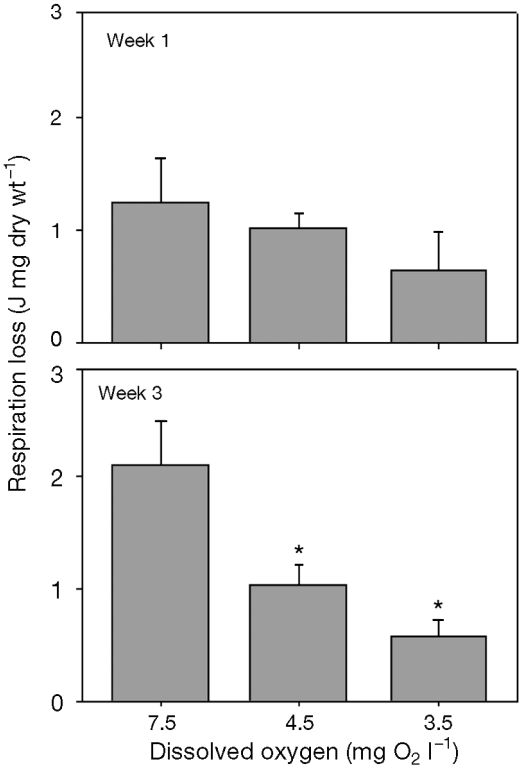

Fig. 4. *Melita longidactyla*. Respiratory energy expenditure upon exposure to different dissolved oxygen concentrations for 1 and 3 wk (mean + 1 SD); *: values significantly different from the normoxic control (Tukey's test, $p < 0.05$)

The relative tolerances and sensitivities of different organisms to alterations in environmental factors appear to be related to the characteristics of their natural habitats (Theede et al. 1969, Stickle et al. 1989). Compared with marine ecosystems, freshwater ecosystems are often subject to larger fluctuations of dissolved oxygen and the occurrence of hypoxia may be more frequent; thus freshwater species should be more tolerant and have better-developed hypoxia adaptations than their marine counterparts. In Hong Kong, *M. longidactyla* is generally found in algal tufts where the water current is strong and, in agreement with our results, they should be sensitive to low oxygen levels.

Chapelle & Peck (2004) hypothesized that oxygen availability limits the maximum potential size attainable in benthic gammaridean amphipods on an evolutionary time scale. Using quantile regression, McClain & Rex (2001) found that the maximum size in deep-sea turrid gastropods increases with increasing oxygen levels. Our results show that both growth and the RNA:DNA ratio of *Melita longidactyla* were reduced at lower oxygen levels, thus lending further support to the hypothesis and findings postulated above. The RNA:DNA ratio appeared to be a sensitive biomarker as it readily responded following exposure to 4.5 mg O_2 l^{-1} for 1 wk (Fig. 2). No response was discernible in the other parameters (e.g. percentage weight gain, respiratory energy expenditure, food consumption and scope for growth) under the same conditions. Likewise, a fast recovery response in the RNA:DNA ratio was also detected when *M. longidactyla* was returned to normoxia after exposure to low oxygen concentrations (4.5 mg O_2 l^{-1}) for 1 wk (Fig. 7). Peakall (1992) reported that RNA:DNA ratios are useful but non-specific indicators of recent growth and general nutritional conditions in a variety of animals including mollusks, crustaceans and fish. Furthermore, RNA:DNA ratios were positively correlated with the somatic growth (Sambhu & Jayaprakas 1997) and ovarian growth (Mohamed & Diwan 1992) of the prawn *Penaeus indicus*, as well as the egg productivity of the copepod *Paracalanus* sp. (Nakata et al. 1994). Notably, Zhou et al. (2001) studied the growth and scope for growth of the common carp *Cyprinus carpio* under hypoxia and found that the RNA:DNA ratios were correlated with reduced growth. In contrast to the RNA:DNA ratio, reduction in growth of *M. longidactyla* (in terms of weight gain) was much less sensitive to changes in dissolved oxygen.

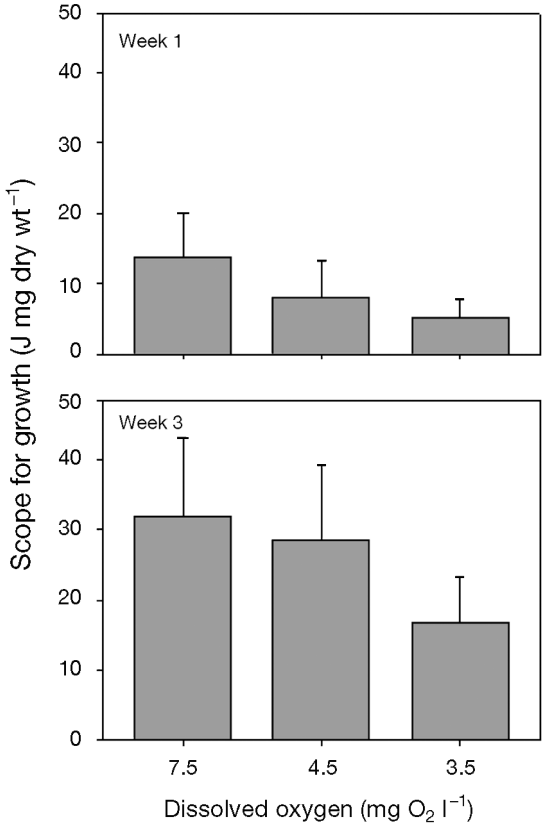

Fig. 5. *Melita longidactyla*. Scope for growth upon exposure to different dissolved oxygen concentrations for 1 and 3 wk (mean + 1 SD)

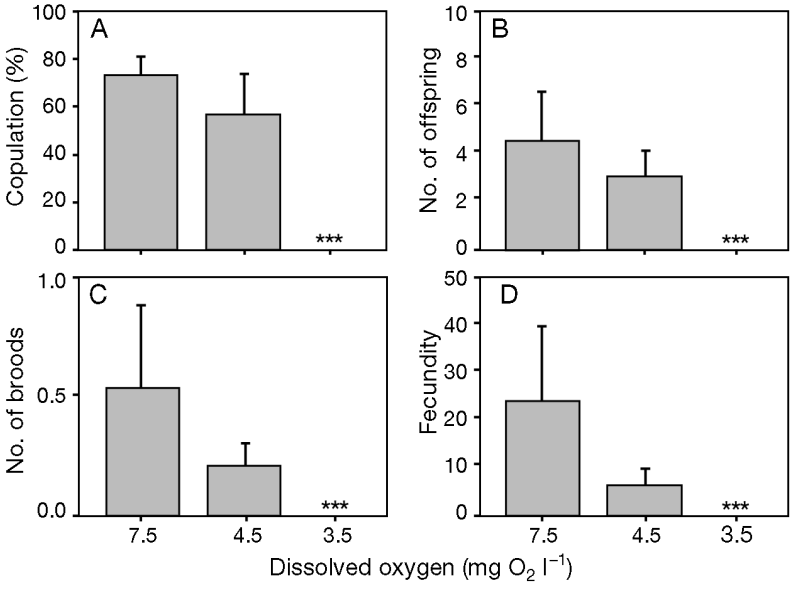

Fig. 6. *Melita longidactyla*. (A) Percentage of copulating pairs, (B) number of viable offspring/brood, (C) number of broods/reproducing female and (D) total fecundity upon exposure to different dissolved oxygen concentrations for 4 wk (mean + 1 SD; ***: values significantly different from the normoxic control (Tukey's test, $p < 0.001$)

Significant reductions in energy consumption and scope for growth per individual (data not shown) were found in *Melita longidactyla* when exposed to 3.5 mg O_2 l^{-1} for 3 wk, but no difference was observed when these 2 parameters were expressed in energy per unit body weight. This suggests that the reduced energy consumption was caused by a decrease in body weight in the low dissolved oxygen treatment groups. No significant reduction in energy intake/food consumption under low dissolved oxygen was reported for the brittle star *Amphiura filiformis* (exposed to 1.8 mg O_2 l^{-1} for 12 d) (Nilsson & Skold 1996) or for the isopod *Saduria entomon* (exposed to 3.45 mg O_2 l^{-1} for 60 h) (Sandberg 1997). In contrast, other studies have shown food consumption to be the most sensitive energy budget item in response to stress. For example, food energy intake by the amphipod *Gammarus pulex* was reduced by 70% when exposed to 5 mg O_2 l^{-1} for 6 d (Maltby et al. 1990).

Respiratory energy expenditure decreased when *Melita longidactyla* was exposed to 4.5 mg O_2 l^{-1} for 3 wk (Fig. 4). Compared with energy consumption and scope for growth, respiratory energy expenditure is more sensitive to low dissolved oxygen. In agreement, Hoback & Barnhart (1996) also found that respiration of the amphipod *Gammarus pseudolimnaeus* was sensitive to low oxygen and began to decrease within 4 h of exposure to 5.7 mg O_2 l^{-1}. Reduction in respiratory energy expenditure is also common following exposure to other stressors. Maltby et al. (1990) found that energy absorbed, respiratory energy expenditure and scope for growth of the amphipod *Gammarus pulex* were all reduced when exposed to ammonia. In contrast, Das & Stickle (1993) showed higher respiration rates when the lesser blue crab *Callinectes similes* was exposed to hypoxia.

Scope for growth was not affected when *Melita longidactyla* was exposed to 3.5 mg O_2 l^{-1} for 3 wk (Fig. 5). Likewise, Das & Stickle (1993) found no change in scope for growth in the blue crab *Callinectes sapidus* upon exposure to 2 higher levels of hypoxia in their experiment, while a linear decline in scope for growth occurred with the severity of hypoxia in the oyster drill *Stramonita haemastoma*. Our results show that the RNA:DNA ratio is more sensitive than scope for growth to hypoxic stress in amphipods. In contrast, scope for growth was found to be more sensitive than the RNA:DNA ratio in responding to hypoxia in fish (Zhou et al. 2001).

Reproductive impairment of *Melita longidactyla* was clearly evident under low dissolved oxygen conditions. Both reproductive

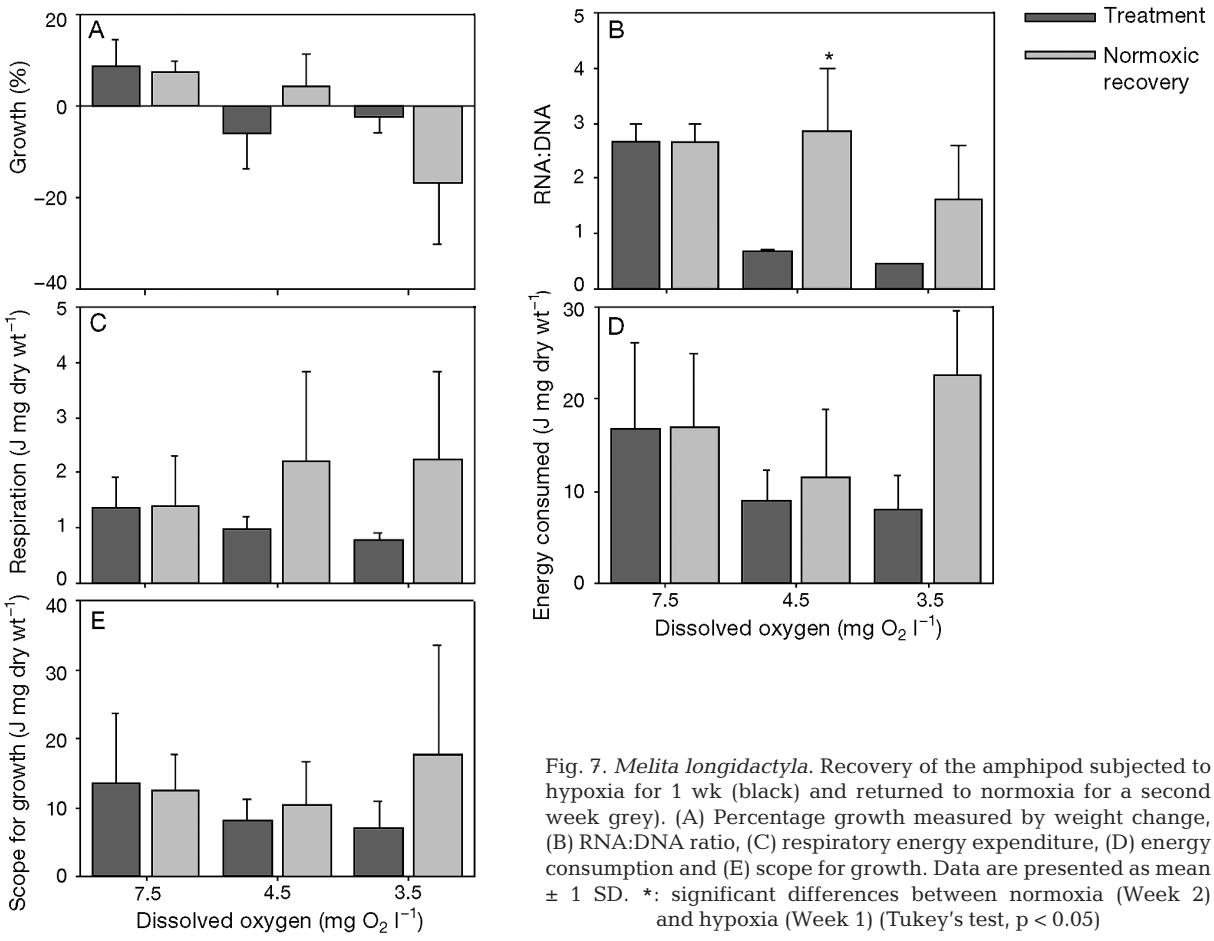

Fig. 7. *Melita longidactyla*. Recovery of the amphipod subjected to hypoxia for 1 wk (black) and returned to normoxia for a second week grey). (A) Percentage growth measured by weight change, (B) RNA:DNA ratio, (C) respiratory energy expenditure, (D) energy consumption and (E) scope for growth. Data are presented as mean ± 1 SD. *: significant differences between normoxia (Week 2) and hypoxia (Week 1) (Tukey's test, $p < 0.05$)

behavior (copulation attempts) and reproductive output (number of reproducing females and number of juveniles per female) were affected (Fig. 6). McCahon et al. (1991) showed that 55% of copulating pairs of the amphipod *Gammarus pulex* separated upon exposure to 1.0 mg O_2 l^{-1} for 24 h. Furthermore, Hoback & Barnhart (1996) showed that more than 50% of copulating pairs of the amphipod *Gammarus pseudoliminaeus* separated when exposed to 2 mg O_2 l^{-1} for 24 h. Only viable eggs and broods were measured in the present study, whereas Wiklund & Sundelin (2001) showed that exposure to a lower level of oxygen (3.6 ± 1.3 mg O_2 l^{-1}) resulted in an increase in the number of dead eggs and also in a higher percentage of females carrying dead broods. An analysis of 7 yr of field data in the Baltic Sea (Wiklund & Sundelin 2004) further confirmed a negative correlation between female amphipods carrying a dead brood and oxygen concentration of bottom water. Taken together, it appears that even moderately low levels of oxygen (above hypoxic levels) can cause reproductive impairment in amphipods. The resulting reduction in growth and the number of viable broods and offspring will decrease the fitness of *M.*

Table 1. LC_{50} values of some crustaceans to hypoxia

Organism	LC_{50} (mg O_2 l^{-1})	Duration (h)	Condition	Source
Amphipods				
Hyalella azteca	0.03	24	20°C, freshwater	Sprague (1963)
	<0.30	96	16.8°C, freshwater	Nebeker et al. (1992)
Gammarus faciatus	4.30	24	20°C, freshwater	Sprague (1963)
G. lacustris	<0.20	168	12.9°C, freshwater	Nebeker et al. (1992)
G. limnaeus	<3.00	96	Freshwater	Gaufin (1973)
	2.80	480		
G. pseudolimnaeus	2.20	24	20°C, freshwater	Sprague (1963)
Females	2.00	48	15°C, freshwater	Hoback (1996)
Males	1.28	48		
Juveniles	1.05	48		
Melita longidactyla	1.60	48	20°C, 30‰	Present study
Crabs				
Callinectes sapidus	4.70	672	24°C, 30‰	Das & Stickle (1993)
C. similis	1.91	672	24°C, 30‰	Das & Stickle (1993)

longidactyla in a low-oxygen environment and should have subsequent effects on total fecundity and recruitment of the natural population.

Based on the RNA:DNA ratio, *Melita longidactyla* recovered from exposure to 4.5 mg O_2 l^{-1} when returned to normoxia, but failed to recover within a week when exposed to a lower level of dissolved oxygen, i.e. 3.5 mg O_2 l^{-1} (Fig. 7). It is not known whether amphipods exposed to a lower level of dissolved oxygen could recover when returned to normoxia for a longer period. It is also unclear whether there would be recovery of reproductive factors following low dissolved oxygen levels. These questions are important in assessing the impact of low dissolved oxygen levels on this species, and merit further investigation. It is generally assumed that animals first restore their energy reserve, then growth and finally reproduction during recovery from stress (McCauley et al. 1990). Short-term exposure to low dissolved oxygen may not, therefore, have permanent effects on development, growth and mortality (Baker & Mann 1994). Indeed, Das & Stickle (1993) showed that blue crabs recovering from various hypoxic levels to normoxia quickly increased their feeding and molting rates. It is also noteworthy that effects of hypoxia (or low oxygen) on animals may be compounded by other environmental factors such as temperature and contaminants (Wiklund & Sundelin 2001, 2004). Thus, effects of low oxygen regimes on *M. longidactyla* may be even more pronounced in the natural environment where these confounding factors are significant.

Low oxygen levels commonly occur not only along the coast of China, but also worldwide where *Melita longidactyla* is abundant. For example, water quality monitoring in Tolo Harbour, Hong Kong, where *M. longidactyla* is abundant, frequently recorded dissolved oxygen levels lower than 4 mg O_2 l^{-1} at 5 out of 7 monitoring stations (EPD 2002). Obviously, growth and reproduction of resident *M. longidactyla* populations may be adversely impacted and their survival threatened. It is noteworthy that 4 mg O_2 l^{-1} is being adopted as a marine water quality objective by some states/countries (e.g. Hong Kong, Alaska, the former USSR and part of China) (Alaska Department of Environment Conservation 1979, NEPA 1998, EPD 2002). Based on the results of this study, the water quality objective of 4 mg O_2 l^{-1} does not afford adequate protection to *M. longidactyla*. It is therefore important that these water quality objectives are reviewed critically to protect the marine environment. The use of biological indicators or key species, such as *M. longidactyla*, may provide a simple and scientifically sound basis for setting and revising water quality objectives, supplementing chemical data. Furthermore, the biomarkers utilized in this study may be employed to provide ecologically relevant guidelines for ecosystem protection.

This study demonstrates that both growth and reproduction of amphipods are impaired by moderate dissolved oxygen levels (3.5 to 4.5 mg O_2 l^{-1}), which may occur in their natural habitat. Since amphipods play an important role in ecosystem structure and function, any adverse effects of low dissolved oxygen on this important species may further disrupt trophodynamics (Chiaravalle et al. 1997) and nutrient recycling, thereby leading to major alterations in ecological functions (Austen & Wibdom 1991).

A recent study found that hypoxia can be an endocrine disruptor and teratogen in fish (Wu et al. 2003, Shang & Wu 2004). It would be instructive to investigate whether low oxygen levels could also cause disruption of reproductive and growth hormones (e.g. ecdysteroids and methyl farnesoate), and malformation of embryos in amphipods and other invertebrates.

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