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The respiration and hypoxic tolerance of *Nucula nitidosa* and *N. nucleus*: factors responsible for determining their distribution?

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Measurement of the respiration of *Nucula nitidosa* and *N. nucleus* determined that *N. nucleus* had a respiration rate approximately a third greater than that of *N. nitidosa*, 215.28 and 135.64 μl O_2 $g_{dfw}{}^{-1}h^{-1}$, respectively. This was calculated to be equivalent to a metabolic rate of 0.648 J individual^{-1} 24 h^{-1} for *N. nitidosa* and 1.752 J individual^{-1} 24 h^{-1} for *N. nucleus*. Estimation of the production of *N. nucleus*, from its respiration rate, revealed that for comparable populations, *N. nucleus* was approximately a third more productive than *N. nitidosa*, 30 kJ g dry flesh weight $_{(dfw)}{}^{-1}$ m^{-2} y^{-1} as opposed to 20 kJ $g_{dfw}{}^{-1}$ m^{-2} y^{-1}. Examination of the Kleiber's constant (β) obtained for each species, demonstrated that for *N. nitidosa* β fell in the range 0.75–1 and that for *N. nucleus* β fell in the range 1–1.25. This suggests, in combination with other data, that *N. nucleus* adopts an 'exploitative' functional strategy as opposed to *N. nitidosa*, which can be regarded as adopting a 'conservationist' functional strategy.

Observations on the hypoxic tolerance of both *N. nitidosa* and *N. nucleus* revealed that *N. nucleus* had a hypoxic tolerance about twice that of *N. nucleus*. The mean survival time ± standard error for *N. nitidosa* was 3.53 ±0.18 d in contrast to 7.72 ±0.21 d for *N. nitidosa*. The hypoxic tolerance of either species was not related to body size and was independent of any possible effects of starvation. These results are discussed with reference to their potential effects to determine the distribution of *N. nitidosa* and *N. nucleus*.

INTRODUCTION

The closely related protobranch bivalves, *Nucula nitidosa* (Winkworth) and *Nucula nucleus* (L.), are two of the five species of Nuculidae commonly found in soft sediments of north-eastern Atlantic seas (Tebble, 1966). The distribution of these two species has been linked to a particular sediment type, *N. nitidosa* inhabiting muddy sands and *N. nucleus* sandy/gravelly muds, and hence in the majority of localities only one of the two species are found (see Ford, 1925; Allen, 1954; Rachor, 1976; Chardy et al., 1984; Creutzberg, 1986). However, in a few sites they can be found both sympatrically and within the same locality (Hirasaka, 1927; Caspers, 1942; Holme, 1953; Walker & Rees, 1980; Wilson & Davis, 1984). At present, there are no explanations, within the literature for why these species have very differing distributions.

As nuculid protobranchs, both *N. nitidosa* and *N. nucleus* are representative of the primitive bivalve condition (Purchon, 1968) and hence they are obligate deposit feeders with the comparatively small underdeveloped gill functioning solely as a respiratory organ (Yonge, 1939, 1959; Wilson & Davis, 1984; see also Caspers, 1942; Trevallion, 1965). Wilson & Davis (1984) have measured the respiration rate of *N. nitidosa*, under various conditions, with the aim of comparing it to the respiration rate of Eulamellibranch bivalves, to determine if both the small size and comparative underdevelopment of the Nuculidae gill inferred some ecological disadvantage, such as increasing its susceptibility to hypoxia or reducing its ability to oxyregulate. They determined that the protobranch gill of *N. nitidosa* was perfectly capable of supplying oxygen to the animal at a rate equal to that of Eulamellibranchs. The measured respiration rate, ranged (at 10°C) from 111.82–215.70 μl O_2 g dry flesh weight $_{(dfw)}{}^{-1}h^{-1}$ and they concluded that the protobranch gill inferred, in terms of respiratory ability, no ecological disadvantage. To date, there are no reports within the literature pertaining to the respiration rate of *N. nucleus*.

It is possible that differences in their respiration rates and hence metabolic rates, may to some extent explain why they have differing distributions. That is, given that the organic content of the habitat occupied by *N. nucleus* is greater than that of *N. nitidosa*, *N. nucleus* may adopt an 'exploitative' strategy, in contrast to *N. nitidosa* which may have adopted a 'conservationist' strategy (see Newell, 1979). In terms of secondary production, Davis & Wilson (1985) have calculated that the production of *N. nitidosa* in Dublin Bay is 20 kJ m^{-2} y^{-1} accounting for ~23% of the total benthic productivity (see also Rachor, 1976). For *N. nucleus* there are no reports within the literature for energetic estimates of their production, although Chardy et al. (1984) have estimated production, in terms of biomass, as 9.3 g_{dfw} m^{-2} y^{-1}. For sites where *N. nitidosa* and *N. nucleus* are present, in abundance (>200 ind m^{-2}), they can be regarded as the primary organisms responsible for the

import of organic matter into the sediment (Rachor, 1976; Chardy et al., 1984; Davis & Wilson, 1985) and as an important prey species for flatfish (Blegvad, 1925; Hunt, 1925; Jones, 1952; Davis & Wilson, 1985; Ibbeken & Zander, 1999).

With regard to the susceptibility of *N. nitidosa* to hypoxia, all of the observations within the literature are anecdotal in nature. Trevallion (1965) has suggested that *N. nitidosa* can survive for up to three days and Rachor (1976) seven days (N=1) (see also Christensen, 1970). For other Nuculidae species, Moore (1931) has suggested that *N. tenuis* can survive from 5–17 d and Taylor et al. (1995) have shown that *N. sulcata* can survive for up to 21 d, whilst there are no observations for the hypoxic tolerance of *N. nucleus*. An organism's tolerance/intolerance to hypoxia is important because it can determine both its abundance and distribution (see Diaz & Rosenberg, 1995 for review). For example, Rachor (1985) has shown that *N. nitidosa* populations in the German Bight have declined in response to increasing bouts of hypoxia (see also Rachor, 1976; Kroncke & Rachor, 1992; Bris & Glemarec, 1995). For *N. nitidosa* and *N. nucleus*, variation in their hypoxic tolerances might explain the differences in their distribution, given that they are closely related species with similar modes of life and as such, Wilson & Davis (1984) have postulated that the inability of *N. nitidosa* to oxyregulate may contribute to its exclusion from very muddy sediments with their associated lowered oxygen tensions.

The aims of this study were as follows: the first was to measure the respiration rate of both *N. nitidosa* and *N. nucleus* so that a comparison between their respiration and metabolic rates could be made, and to estimate the total energetic production of *N. nucleus*. If the respiration (metabolism) and production of *N. nucleus* is greater than that of *N. nitidosa* then it follows that *N. nucleus* is likely to require a nutritionally richer habitat than that of *N. nitidosa* and thus this may, in part, explain the differences in their distribution. In addition, examination of the Kleiber's (1961) constant, obtained for each species, can be used, in combination with the respiration data, to assess their functional strategy, i.e. 'exploitative' vs 'conservationist'. The second aim of this study, was to examine the hypoxic tolerance of *N. nitidosa* and *N. nucleus* to determine if differences existed between the species, and if such differences could be used to explain their differing distributions. For this paper, the terms normoxia and hypoxia are in accordance with those of Diaz & Rosenberg (1995) and the phrases 'exploitative' and 'conservationist' strategy are defined as follows. For species with an abundant food supply, energy conservation may not be necessary or desirable and hence such species may adopt an 'exploitative' strategy in which growth and metabolism are high and reproduction frequent, i.e. maturation is maximal in the absence of nutritional limits. Conversely, for species with a limited food supply, metabolic adjustments to reduce energy expenditure may be critical in the maintenance of energetic gain from an environment. For species that adopt a 'conservationist' strategy, growth and metabolism will be low and reproduction infrequent (annual) in order to sustain maintenance (see Calow, 1977; Newell, 1979). Although 'exploitative' and 'conservationist' strategies can be equated to 'opportunistic' and 'equilibrium' strategies, respectively, we have deliberately avoided using these terms because they have often been equated with *r* and *k* type strategies rather than functional adaptations to a particular niche/habitat (see Grassle & Grassle, 1974; Pearson & Rosenberg, 1978; Bridges et al., 1994; Linton & Taghon, 2000 for examples).

MATERIALS AND METHODS

Collection of animals

All experiments were carried out at Kristineberg Marine Research Station, Fiskebackskil, Sweden in autumn 2000. The animals were collected (~700 of each species) within the mouth of the Gullmarsfjord off Flatholmen Island (58°24.97′E 11°15.72′N) using an Agassiz trawl, 1-mm mesh size. Five boxcores (30×30 cm) were also taken, to provide an estimate of *Nucula* densities, and individually sieved through a 1-mm square mesh sieve. Once collected the animals were stored in seawater and transported, within three hours of collection, to the marine station where the species were separated, placed in tanks filled with clean sieved (>1 mm<0.5 mm) sediment, collected sub-tidally from a beach adjacent to the station, and supplied with fresh running seawater (~10°C). Analysis of the boxcore data revealed that the mean density ± standard deviation (SD) of *Nucula nitidosa*, at the collection site, were 8.9 ±14.5 ind m^{-2} and 55.6 ±63.3 ind m^{-2} for *Nucula nucleus*. The mean ±standard error (SE) shell length, height, width, whole body (shell+flesh) dry weight and dry flesh weight of *N. nitidosa* were 8.11 ±0.11, 7.57 ±0.11, 3.80 ±0.06 mm, 0.11 ±0.01 g and 0.010 ±0.001 g, respectively. For *N. nucleus* the mean ±SE shell length, height, width, whole body dry weight and dry flesh weight of the specimens were 9.29 ±0.13, 8.76 ±0.13, 4.50 ±0.08 mm, 0.17 ±0.02 g and 0.015 ±0.001 g, respectively. The other species present in abundance at the site were *Astarte sulcata*, *Cerastoderma edule* and *Amphuria chiajei*. *Nucula sulcata* and *N. minuta* were also present but at very reduced densities (~0.2 ind m^{-2}).

Measurement of the respiration, calculation of the metabolic rate and estimation of the production of N. nitidosa *and* N. nucleus

Measurements of the respiration rate of the animals were made using a microwinkler methodology (see Barnes, 1959). All of the reagents that were used and the protocol followed were identical to that of Carpenter (1966), with the exception that phosphoric acid was used instead of concentrated sulphuric acid to dissolve the manganese precipitate (see Fox & Wingfield, 1938; Barnes, 1959). The temperature was maintained at 10°C throughout the whole procedure.

For the calculation of the oxygen consumed by each animal, the total sample volume, i.e. the water contained in each experimental bottle in the presence of the animal, was measured volumetrically for each sample. The procedure used for the control samples, taken both at the start of the experiment and at the end of the experiment, was identical, with the exception that no animals were introduced into the sample bottles. Each experiment was run in batches of five animals with five controls at the start and five controls at the end of the experiment, until the oxygen consumption of 45 animals for each species had been measured. All animals used in the experiments

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were randomly selected from the sampled population, and their shell measurements, shell width, length, height along with their whole body dry weight and dry flesh weight recorded at the end of the experiment.

Conversion of the amount of oxygen consumed by an animal ($\mu l\,h^{-1}$) into metabolic rate ($J\,h^{-1}$) can be made using the following equation derived from Schmidt-Neilsen (1997):

$$M = O \times 0.02010 \quad (1)$$

where M=metabolic rate ($J\,h^{-1}$) and O=O_2 consumed ($\mu l\,h^{-1}$).

Estimation of the theoretical production of a population from its respiration rate can be made using the equation of Engelmann (1966) as follows:

$$\log_{10} P = -0.822 + 1.163 \times \log_{10} R \quad (2)$$

where P=production ($kJ\,m^{-2}\,y^{-1}$) and R=respiration ($kJ\,m^{-2}\,y^{-1}$).

Observations on the hypoxic tolerance of Nucula nitidosa *and* N. nucleus

Measurement of the hypoxic tolerance of *N. nitidosa* and *N. nucleus* was made in separate experiments as follows. Ten litres of autoclaved filtered (0.2 μm) seawater (10°C) was vigorously bubbled with filtered (0.2 μm) N_2, in a semi-enclosed vessel, for 6 h prior to the start of the experiment, i.e. to ensure that the seawater contained as little oxygen as possible. Two hundred and forty, 9 ml (nominal) bottles were then filled with the seawater and a randomly selected animal placed into each. One hundred and eighty of the bottles were then capped, ensuring that the bottle remained under the surface of the water at all times (treatment animals) and the remaining 60 bottles left uncapped (control animals). In addition, 35 bottles were filled with seawater and the bottles capped (water control). Five of these bottles were immediately analysed for their oxygen content using the microwinkler procedure as previously described. All of the bottles were placed together under a 12:12 h L:D cycle and maintained at 10°C for the duration of the experiment.

Every day, both the treatment and control animals were inspected, individually, to ascertain whether or not they were alive. If an animal was found to be dead, it was removed from the experiment and its death recorded along with its shell height, width, length, whole dry weight, flesh dry weight and the oxygen concentration of the water. Every three days, from the start of the experiment, five randomly selected water controls were removed and the oxygen concentration determined. The end of the experiment was determined by either the death of all of the treatment animals or by time limitations, as for *N. nucleus*. It should be noted that where an experiment ends before all of the animals have died and/or where animals are removed during an experiment such observations are regarded as censored. In the analysis that is used, such cases are included until they become censored whereupon they are excluded from all future calculations. Where cases exist at the end of an experiment, the lifetime of the organism is calculated up to that point in time, including the remaining cases (for a full discussion see Klein & Moeschberger, 1998).

RESULTS

Measurement of the respiration, calculation of the metabolic rate and estimation of the production of Nucula nitidosa *and* N. nucleus

Examination of the control data, using a Student's *t*-test, for each block of measurements, revealed that there were no discernible differences between the oxygen concentration of the controls taken at the start of an experiment and those measured at the end of an experiment. Linear

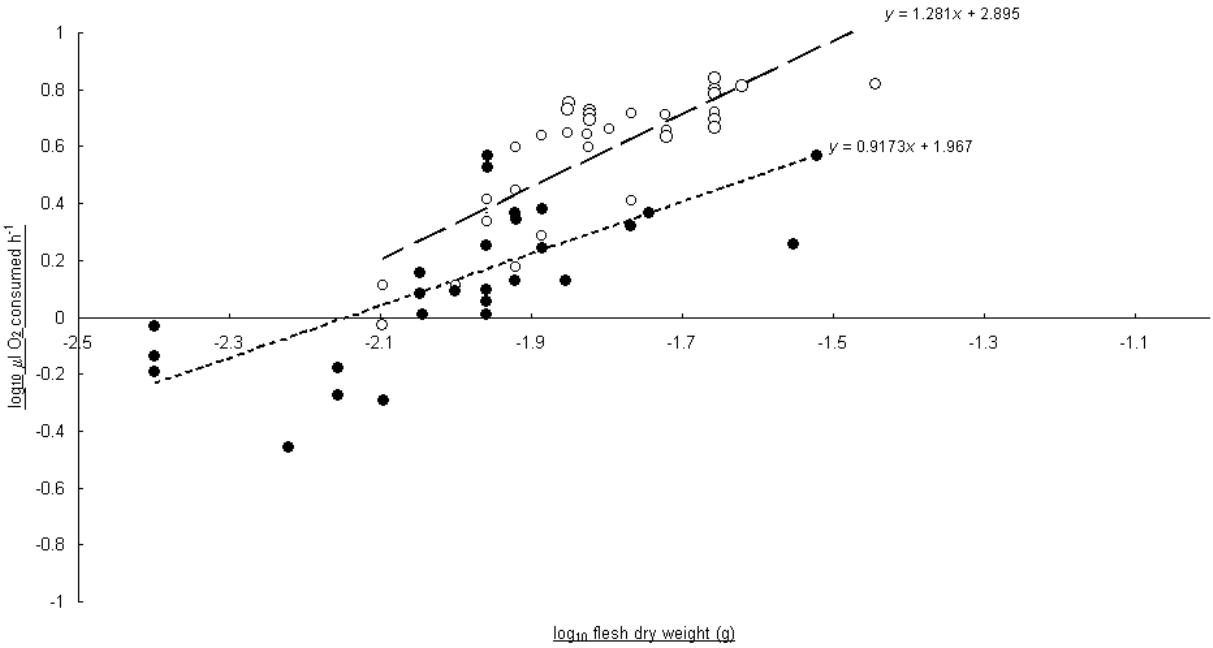

Figure 1. Linear regression of the respiration data for *Nucula nitidosa* and *N. nucleus*. ●, *N. nitidosa*; ○, *N. nucleus*; – – – –, *N. nitidosa*; — —, *N. nucleus*.

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Table 1. *Calculated regression equations for the respiration and morphology of* Nucula nitidosa *and* Nucula nucleus.

N. nitidosa									
Y	x	Gradient (β)	Constant (α)	r^2	$\alpha=0$*	$\beta=0$**	$\beta=0.75$**	$\beta=1.00$**	$\beta=1.25$**
Log_{10} oxygen consumption (μl O_2 h^{-1})	Log_{10} dry flesh weight (g)	0.917 ±0.17	1.967 ±0.34	0.51	No ($P \leqslant 0.001$)	No ($P \leqslant 0.001$)	Yes ($P \geqslant 0.40$)	Yes ($P \geqslant 0.52$)	No ($P \leqslant 0.001$)

Y	x	Gradient (β)	Constant (α)	r^2	$\alpha=0$*
Oxygen consumption (μl O_2 h^{-1})	Whole dry weight (g)	44.641	−3.164	0.42	No ($P<0.001$)

N. nucleus									
Y	x	Gradient (β)	Constant (α)	r^2	$\alpha=0$*	$\beta=0$**	$\beta=0.75$**	$\beta=1$**	$\beta=1.25$**
Log_{10} oxygen consumption (μl O_2 h^{-1})	Log_{10} dry flesh weight (g)	1.281 ±0.17	2.895 ±0.32	0.64	No ($P \leqslant 0.001$)	No ($P \leqslant 0.001$)	No ($P \leqslant 0.001$)	Yes ($P > 0.30$)	Yes ($P > 0.93$)

Y	x	Gradient (β)	Constant (α)	r^2	$\alpha=0$*
Oxygen consumption (μl O_2 h^{-1})	Whole dry weight (g)	25.783	−1.214	0.46	No ($P \leqslant 0.001$)

*, denotes calculated from regression analysis of variance; **, denotes calculated from *t*-tests (Zar, 1999).

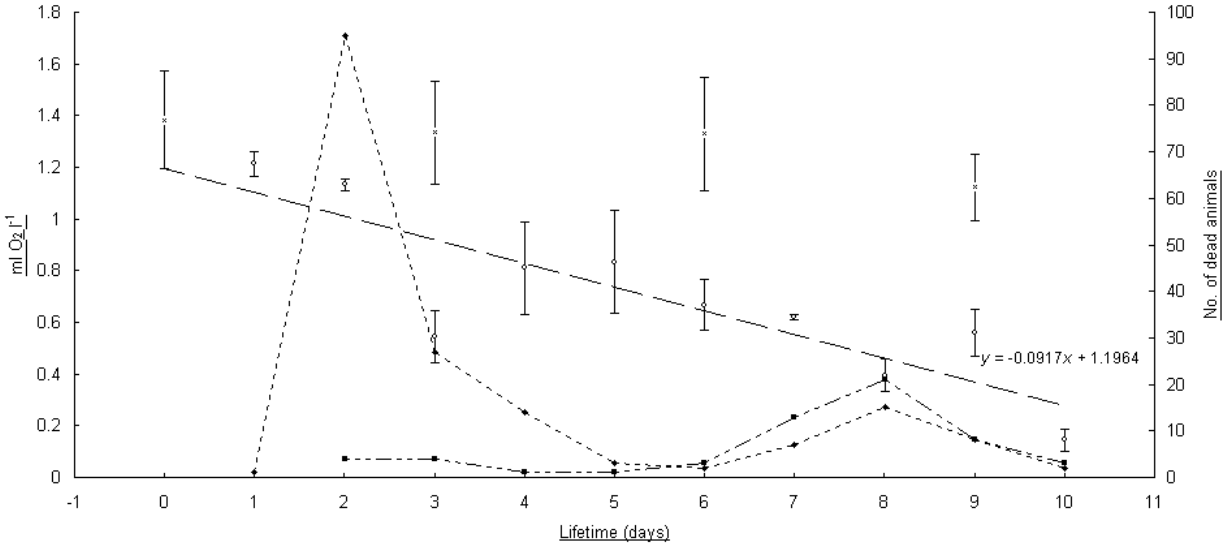

Figure 2. The mean and standard error of the oxygen concentration of the control and treatment samples compared with the number of *Nucula nitidose* that have died on each day. ○, mean oxygen concentration of the treatment samples; *, mean oxygen concentration of the control water samples; – –●– –, number of treatment animals that have died; – –■– –, number of control animals that have died; — —, calculated regression line (mean oxygen concentration).

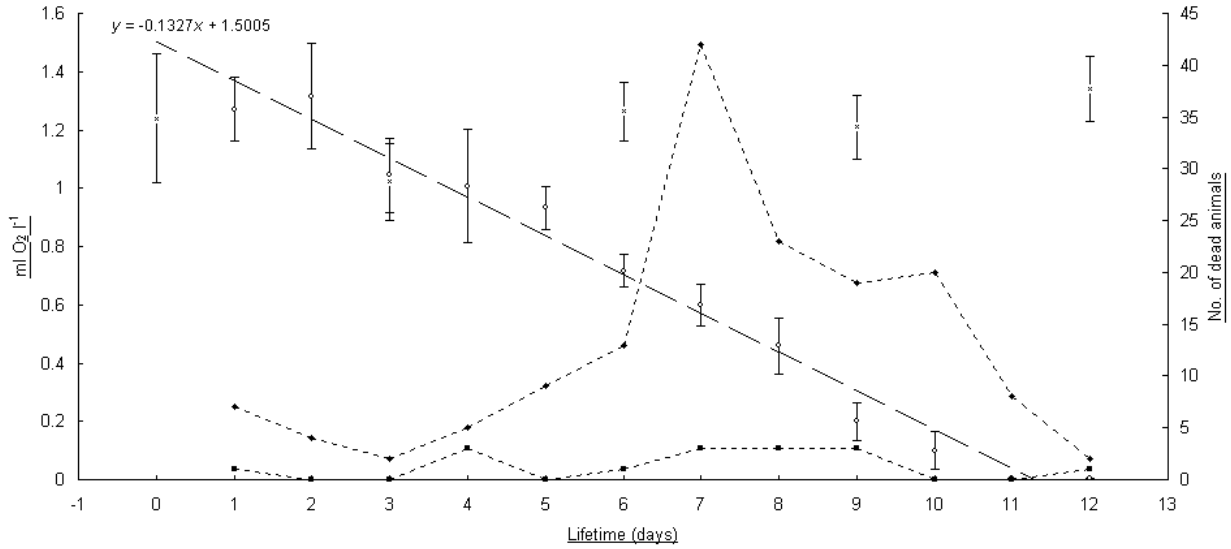

Figure 3. The mean and standard error of the oxygen concentration of the control and treatment samples compared with the number of *Nucula nucleus* that have died on each day. ○, mean oxygen concentration of the treatment samples; *, mean oxygen concentration of the control water samples; – –●– –, number of treatment animals that have died; – –■– –, number of control animals that have died; — —, calculated regression line (mean oxygen concentration).

Table 2. *Comparison of respiratory rates of protobranch and lamellibranch molluscs.*

Species	Mean/range respiration (μl O_2 g_{dfw}^{-1} h^{-1})	Temperature (°C)	Source
Nuculidae			
Nucula nitidosa	135.64	10	This study
Nucula nitidosa	118.81–15.70	10	Wilson & Davis (1984)
Nucula nucleus	215.28	10	This study
Nucula tenuis	547.00	10	Bayne & Thurberg (1988)
Nucula sulcata	35.17	10	Taylor et al. (1995)
Lamellibranchs			
Pecten maximus	200.13	10	Brand & Roberts (1973)
Cerastoderma edule	~240.00	10	McMahon & Wilson (1981)
Tellina tenuis	~200.00	10	McMahon & Wilson (1981)

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regression of the $\log_{10}$ transformed data, following the removal of obvious outliers (i.e. O_2 consumption $\geqslant$ initial O_2) for the oxygen consumed by the specimens revealed that for both species there was a statistically significant relationship between oxygen consumption and dry flesh weight, N=31 for *N. nitidosa* and N=33 for *N. nucleus* (Figure 1 and Table 1). Comparison between the slopes of the regressions calculated, for each species, using a *t*-test (Zar, 1999), revealed that for the size range measured *N. nucleus* had a greater respiration rate than that of *N. nitidosa* (t=16.20, $P \leqslant 0.001$) (Figure 1). Calculation of the point where the two regression lines crossed, determined that it was equal to 0.0028 g_{dfw} for the x axis and 0.43 μl O_2 h^{-1} for the y axis. The mean ±SE respiration rate of *N. nitidosa*, for an average sized animal (mean ±SE dry flesh weight=0.010 ±0.001 g) was 1.36 ±0.16 and 3.62 ±0.11 μl O_2 h^{-1} for *N. nucleus* (mean ±SE dry flesh weight=0.015 ±0.001 g). This is equivalent to a respiration rate of 135.64 μl O_2 $g_{dfw}{}^{-1}h^{-1}$ for *N. nitidosa* and 215.28 μl O_2 $g_{dfw}{}^{-1}h^{-1}$ for *N. nucleus*. In terms of the population sampled this equates to a respiratory demand of 12.40 μl O_2 $m^{-2}h^{-1}$ for *N. nitidosa* and 201.27 μl O_2 $m^{-2}h^{-1}$ for *N. nucleus*. Similarly, linear regression of the oxygen consumed against whole body dry weight produced a statistically significant regression, for both species, although the scatter of the data was quite large (Table 1). Comparative analysis of the Kleiber's (1961) constant (β), obtained for each species, for β=0.75, β=1 and β=1.25, using *t*-tests (Zar, 1999), determined that for *N. nitidosa* β fell in the range 0.75–1 and for *N. nucleus* 1–1.25 (Table 1). This reveals that for *N. nucleus*, respiration is effectively, directly proportional to body mass (Schmidt-Nielsen, 1997) and that the growth rate of *N. nucleus* is greater than that of *N. nitidosa* (Riisgard, 1998), which in part is indicative of an 'exploitative' strategy. For *N. nitidosa*, respiration is proportional to body mass but not directly.

Using the calculated regression equation for dry flesh weight (Table 1), the equation of Schmidt-Neilsen (1997) and assuming that the average dry flesh weight of *N. nitidosa* and *N. nucleus* is 0.010 and 0.015 g, respectively, then the metabolic rate is equal to 0.027 J $ind^{-1}h^{-1}$ for *N. nitidosa* and 0.073 J $ind^{-1}h^{-1}$ for *N. nucleus*. This is equivalent to a metabolic rate of 0.648 and 1.752 J ind^{-1} 24 h^{-1} or 236.52 and 639.48 J $ind^{-1}y^{-1}$ for *N. nitidosa* and *N. nucleus*, respectively. For the populations sampled this is equal to an energetic demand of 2.1 kJ $m^{-2}y^{-1}$ by *N. nitidosa* and 35.6 kJ $m^{-2}y^{-1}$ by *N. nucleus*.

To estimate the production if we take the equation of Engelmann (1966) and assume that the respiratory demand for the sampled populations are 2.1 kJ $m^{-2}y^{-1}$ and 35.6 kJ $m^{-2}y^{-1}$ for *N. nitidosa* and *N. nucleus*, respectively, i.e. from above, then the theoretical production of *N. nitidosa* is 0.4 kJ $m^{-2}y^{-1}$ and 9.6 kJ $m^{-2}y^{-1}$ for *N. nucleus*. For a dense population of *N. nucleus*, as described by Chardy et al. (1984), (the mean respiratory biomass= 16.6 g_{dfw} $m^{-2}y^{-1}$) it is equal to ~271 kJ $m^{-2}y^{-1}$.

Observations on the hypoxic tolerance of Nucula nitidosa *and* N. nucleus

The initial oxygen concentration ±SE of the seawater at the start of the experiment was 1.38 ±0.19 and 1.24 ±0.22 ml O_2 l^{-1} for the *N. nitidosa* and *N. nucleus* experiments, respectively. That is, the seawater can be regarded as hypoxic at the start of the experiment (Diaz & Rosenberg, 1995). Comparison of the oxygen concentrations of the water controls, during the experiments, using a one-way analysis of variance (ANOVA), determined that for both species the oxygen concentration of the control water did not change over the duration of the experiment (F=0.387, P=0.764 and F=0.835, P=0.519 for *N. nitidosa* and *N. nucleus*, respectively) (Figures 2 & 3). For *N. nitidosa* all of the treatment

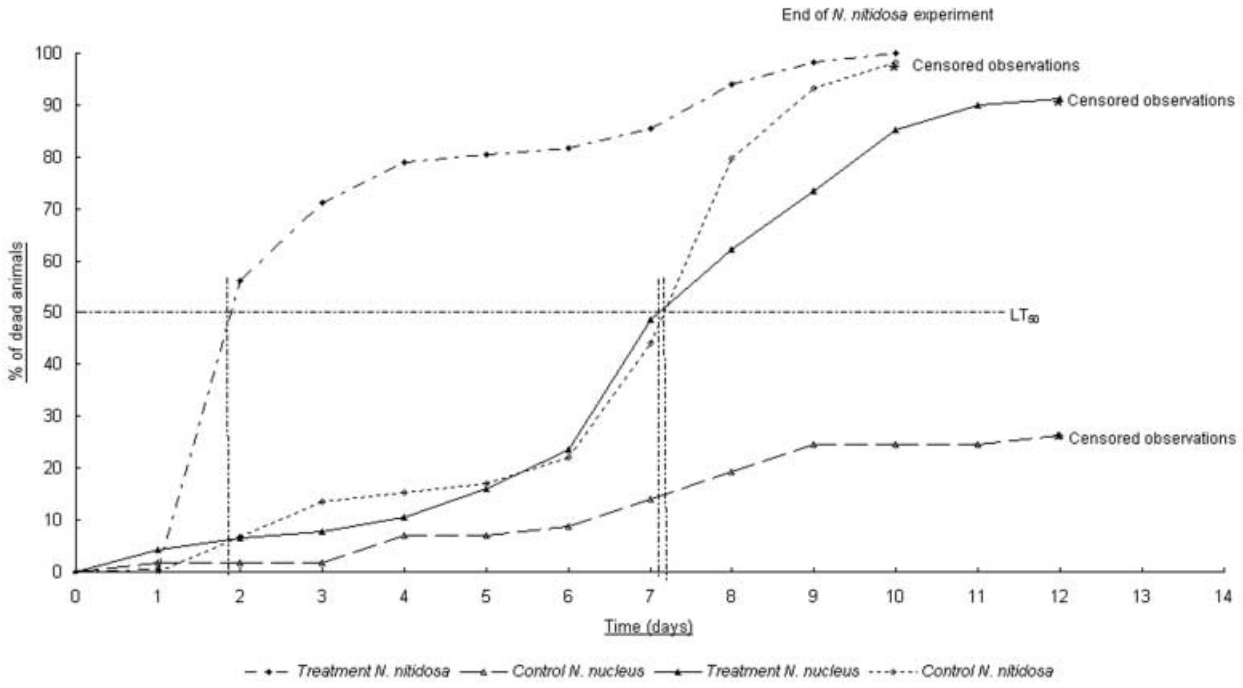

Figure 4. Cumulative percentage of dead animals for the *Nucula nucleus* and *N. nitidosa* hypoxia experiments.

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Table 3. *Comparison of the hypoxic tolerance of a variety of bivalves.*

Species	LT_{50}	Limit (d)	Source	Habitat	Relative oxygen concentration
Nuculidae					
Nucula hanleyi	Unknown	Unknown	N/A	Sandy gravel[1]	High
Nucula nitidosa	2	10	This study	Muddy sand (high sand content)[1]	High–medium
Nucula nitidosa	Not stated	7	Rachor (1976)	Muddy sand (high sand content)[1]	High–medium
Nucula nitidosa	Not stated	3	Trevallion (1965)	Muddy sand (high sand content)[1]	High–medium
Nucula nucleus	7	12+	This study	Sandy/gravelly mud (medium–low sand content)[1]	Medium
Nucula tenuis	Not stated	17	Moore (1931)	Sandy mud (low sand content)[1]	Medium–low
Nucula sulcata	14	21	Taylor et al. (1995)	Mud[1]	Low
Lamellibranchs					
Cerastoderma edule	~ 4	Not stated	Theede (1973)	Muddy sand (high sand content)[2]	High–medium
Abra alba	8	Not stated	Dries & Theede (1974)	Sandy mud (low sand content)[2]	Medium
Abra elliptica	~ 62	Not stated	Dries & Theede (1974)	Muddy/gravelly sand–mud (variable sand content)[2]	High–low, populations at depth may be prone to frequent hypoxia
Macoma balthica	~ 21	Not stated	Dries & Theede (1974)	Sandy/gravelly mud (low sand content)[2]	Medium–low, especially low for some intertidal populations
Arctica islandica	~ 83	Not stated	Dries & Theede (1974)	Sand–mud (variable sand content)[2]	High–low, populations at depth may be prone to frequent hypoxia

[1], Allen (1954); [2], Tebble (1966).

animals had died by day ten of the experiment, whereas for *N. nucleus* only ~90% of the treatment animals had died by day 12 of the experiment, which had to be curtailed because of time limitations, i.e. all of the remaining animals are regarded as censored observations.

Analysis of the survival data for the two species over time, using the Kaplan–Meier procedure (see Klein & Moeschberger, 1998) with log rank comparison, and accounting for the censored observations for *N. nucleus*, determined that for both species the survival rate of control animals was significantly different from that of the treatment animals ($P \leqslant 0.001$ for *N. nitidosa* and *N. nucleus*) (Figure 4). For *N. nitidosa* the mean ±SE survival time for the treatment animals was 3.53 ±0.18 d (median=2 d, LT_{50}2 d) whereas the mean survival time for the control animals was 7.08 ±0.28 d (median=8 d, LT_{50}7 d) (Figure 4). In contrast for *N. nucleus* the mean ±SE survival time for the treatment animals was 7.72 ±0.21 d (median=8 d, LT_{50}7 d) whilst the survival time for the control animals was 10.65 ±0.36 d (median >12 d, LT_{50} > 12 d) (Figure 4).

Cross-comparison between the survival data obtained for the species, using the Kaplan–Meier procedure with log rank comparisons, revealed that the survival rates of both the treatment and control animals for each species, were significantly different ($P \leqslant 0.001$) (Figure 4). In effect, the survival rate of *N. nucleus* under hypoxic/anoxic conditions is ~2 times greater than that of *N. nitidosa*. In addition, the survival rate due to starvation (i.e. as is thought to occur in the control animals) of *N. nucleus* is at least ~1.5 times greater than that of *N. nitidosa*.

Correlation, using Spearman's rho (r_{sc}), of the shell length, height, width, whole body dry weight and dry flesh weight data to the survival data failed to produce any statistically significant correlations for either species, i.e. death due to hypoxia does not appear, for these experiments, to be related to body size. Linear regression of the lifetime of *N. nitidosa* and *N. nucleus* vs the mean oxygen concentration of the water samples, at the time of death, produced a statistically significant regression for both species (r^2=0.97, F=23.03, I=0.01 for *N. nitidosa* and r^2= 0.74, F=430.86, P=0.001 for *N. nucleus*), i.e. the O_2 concentration of the water for the treatment animals dropped over time as they respired, leading to their eventual death (Figures 2 & 3). Analysis of the number of animals dying per day, against the mean O_2 concentration for the animals that died that day, again using Spearman's rho, to determine if there was a threshold O_2 concentration lower than that at the start of the experiment that caused death, produced no statistically significant correlation for either species (r_{sc}= 0.351, P=0.290 and r_{sc}= −0.439, P= 0.133 for *N. nitidosa* and *N. nucleus*, respectively), i.e. the initial O_2 concentration of the water was sufficient, without any further reduction in its oxygen concentration, to cause death due to hypoxia (Figures 2 & 3).

DISCUSSION

Measurement of the respiration rate of the two nuculid species determined that *Nucula nucleus* had a greater respiratory demand, within the size range measured, than that of *N. nitidosa* (Table 2). Comparison between our results recorded for *N. nitidosa*, and those measured by Wilson & Davis (1984), using oxygen electrodes, reveal no real difference (Table 2). With regard to measurements of the respiration of other nuculid species, Bayne & Thurberg (1988) and Taylor et al. (1995) have estimated the respiration of *N. tenuis* and *N. sulcata* as 547.00 and 35.17 μl O_2 $g_{dfw}^{-1} h^{-1}$, respectively (Table 2). Taylor et al. (1995) suggested that the extremely low value for the respiration of *N. sulcata* was probably due to its mode of life, i.e. it was a fairly inactive deposit feeder, and/or due to its adaptation to its normal habitat, muds with a low oxygen concentration (see also Allen, 1978). However, it is our opinion that the respiratory values recorded by Taylor et al. (1995) are uncommonly low. This conclusion is supported by the respiratory values recorded for certain lamellibranch bivalves (Table 2), which are of the same order as those recorded here, by Wilson & Davis (1984) and by Bayne & Thurberg (1995). In terms of the respiratory ability of the primitive nuculid gill it appears to be as effective, as suggested by Wilson & Davis (1984), as a lamellibranch gill (Table 2) and it can be concluded that it offers no physiological disadvantage in terms of respiratory ability.

Calculation of the metabolic demand of *N. nitidosa* and *N. nucleus* determined, as for the respiration rates, that *N. nucleus* had a higher metabolic demand, 1.752 in comparison to 0.648 J ind^{-1} 24 h^{-1}, than *N. nitidosa*, which is to be expected. In terms of the amount of organic input into the benthos, dense populations of *N. nucleus* place a greater load on the available resources than comparable populations of *N. nitidosa*. Examination of the Kleiber's (1961) constant (β) determined that it was different for each species, β falling in the range 0.75–1 for *N. nitidosa* and 1–1.25 for *N. nucleus*. Riisgard (1998) has shown that for young or fast growing species, β will be similar to 1, i.e. respiration is directly proportional to body mass, whereas for older or slow growing species it is more likely to be similar to 0.75. This suggests that *N. nucleus* is a faster growing species than *N. nitidosa*, which in turn is, reflected by its higher respiration rate, as above, and therefore its metabolism.

Correspondingly, estimation of the secondary production of *N. nucleus* revealed that for the population sampled, the production of *N. nucleus* was ~25 times greater than that of *N. nitidosa*, 0.4 kJ $m^{-2} y^{-1}$ for *N. nitidosa* as opposed to 9.6 kJ $m^{-2} y^{-1}$ for *N. nucleus*. For a dense population of *N. nucleus*, as recorded by Chardy et al. (1984), the production of such a population was calculated as ~271 kJ $m^{-2} y^{-1}$. Corresponding estimates for the secondary production of *N. nitidosa*, by Rachor (1976) and Davis & Wilson (1985) are 26 kJ $m^{-2} y^{-1}$ and 20 kJ $m^{-2} y^{-1}$, respectively. If production between the aforementioned populations are standardized, i.e. production g_{dfw}^{-1} $m^{-2} y^{-1}$, then the production of *N. nucleus* is approximately a third greater than that of *N. nitidosa*, i.e. production kJ $g_{dfw}^{-1} m^{-2} y^{-1}$ for *N. nitidosa* is ~ equal to 20 for both Davis & Wilson (1985) and Rachor (1976) and ~equal to 30 for Chardy et al. (1984). This result is not surprising given that the respiration of *N. nucleus* is approximately a third greater than that of *N. nitidosa*. With regard to fisheries and the import of matter into the sediment, populations of *N. nucleus* are more likely to be, at appropriate densities, a more important source of food for flatfish and import more material

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into the benthos, than corresponding populations of *N. nitidosa*.

In terms of the physiological differences between *N. nitidosa* and *N. nucleus* respiration (metabolism), growth and production in *N. nucleus* is greater than that of *N. nitidosa*. This suggests that in terms of the functional strategies adopted by the two nuculid species *N. nucleus* adopts an 'exploitative' strategy and *N. nitidosa* a 'conservationist' strategy, in comparison to each other. Additional evidence for such a supposition is provided from data on their respective reproductive cycles where spawning is an annual event in *N. nitidosa*, and a bi-annual event in *N. nucleus* (Lebour, 1938; Allen, 1954; Rachor, 1976; Davis & Wilson, 1983; Chardy et al., 1984). It is possible that the distribution of the two species can be related to resource partioning and hence functional adaptation to a particular environment. *Nucula nucleus* inhabits comparatively organically rich sediments, i.e. muddy, whereas *N. nitidosa* inhabits much poorer sediments, i.e. sandy. For both species, occupation of a particular habitat, where food is limited for *N. nitidosa* and unlimited for *N. nucleus*, is reflected in their functional strategy. The excess of nutritional resources in the habitat occupied by *N. nucleus* allows it to adopt an 'exploitative' strategy, i.e. maturation is maximal in the absence of nutritional limits, whereas the nutritional limits of the habitat occupied by *N. nitidosa* results in it adopting a 'conservationist' strategy, i.e. the ability to make metabolic adjustments to ensure that there is sufficient energetic gain for maintenance, is critical in a nutritional poor habitat. Instances of other closely related species, that differ in their distributions, adopting different functional strategies according to the nutritional properties of their habitat are fairly common within the literature (Calow, 1977; Branch & Newell, 1978; Newell, 1979), especially so, concerning opportunistic and equilibrium species (see Grassle & Grassle, 1974; Bridges et al., 1994; Lington & Taghon, 2000). In addition, Buhl-Mortensen & Hoisaeter (1993) have suggested that there is a correlation between the organic content of sediment and the functional strategy adopted by a species (see also Pearson & Rosenberg, 1978). Whether or not there is interspecific competition between the two species for resources, where they co-exist, is outside the scope of this paper however, as will be discussed, there is a second factor, hypoxic tolerance, that can be further used to explain their differing distributions.

Examination of the hypoxic tolerance of *N. nitidosa* and *N. nucleus* determined that *N. nucleus* had ~two times the hypoxia tolerance of *N. nitidosa* (Table 3). For *N. nitidosa* the maximum survival time was 10 d whereas for *N. nucleus* it was >12 d. In terms of the animals' ability to withstand starvation, i.e. the control treatment, the mean survival time for *N. nitidosa* was 7.08 d and greater than 12 d for *N. nucleus*. For both species there was no correlation of lifetime with body mass, shell length etc. and/or sample water oxygen concentration with the number of animals dying. That is, death due to hypoxia, in both species, was independent of body size and/or the relative level of hypoxia.

With regard to the existing observations within the literature, for the hypoxic tolerance of *N. nitidosa*, our results are within those suggested by other authors (Table 3). In comparison to other nuculid species *N. nitidosa* appears to be the least able to tolerate hypoxia followed by *N. nucleus*, *N. tenuis* and then by *N. sulcata* (Table 3). Although there is a general lack of information within the literature, with regard to the hypoxic tolerance of molluscan fauna, in terms of the data that is available, *N. nitidosa* has a hypoxic tolerance approximately equal to that exhibited by the common cockle, *Cerastoderma edule*, whilst *N. nucleus* has a hypoxic tolerance equal to that of *Abra alba* (Table 3). For both species, their hypoxic tolerance is low when compared to the observations for other molluscs (Table 3) (see also Theede, 1973; Dries & Theede, 1974; Diaz & Rosenberg, 1995).

The observed differences in the hypoxic tolerances of *N. nitidosa* and *N. nucleus* may in part explain differences in their distribution. The particular presence or absence of a nuculid species can be related to the mud/organic content of the sediment present at a site and hence its oxygen concentration, both within the sediment and at the sediment water interface, as illustrated in Table 3. Observation of the hypoxic tolerance of north-eastern Atlantic nuculids, where recorded, reveals that there appears to be a negative relationship between the potential for a low oxygen concentration in sediment and the hypoxic tolerance of the bivalves. In effect, the relatively low hypoxic tolerance of *N. nitidosa* probably prevents it from colonizing areas inhabited by *N. nucleus*, which are probably subject to periodic bouts of hypoxia. This supposition is further substantiated by the observation of Wilson & Davis (1984) who have noted that in Dublin Bay *N. nitidosa* is replaced in softer sediments by *N. nucleus* and *A. alba*, *Abra* having a hypoxic tolerance equal to that of *N. nucleus* (Table 3). In addition, Rachor (1985) and Kroncke & Rachor (1992) have shown that populations of *N. nitidosa* in the German Bight have declined in response to an increase in the frequency of localized hypoxic events (see also Rachor, 1976; Bris & Glemarec, 1995).

The structuring effects of hypoxic events on community composition have been well documented (see Diaz & Rosenberg, 1995 for review). In general, aperiodic hypoxic events will serve to remove species sensitive to hypoxia, thereby increasing the abundance and opportunities for more tolerant species. Such events are likely to have no long term effects on the structure of a community, and may be indistinguishable from the more normal natural variation in community structure, but are likely to generate sites with a higher diversity and uncommon faunal compositions to that found in consistently aerobic sediments. In contrast, periodic hypoxic events will structure a community such that only hypoxia tolerant species are found. For the sites where *N. nitidosa* and *N. nucleus* occur sympatrically, e.g. for the population we sampled, such sites are likely to experience aperiodic events of hypoxia as is the case for the Gullmarsfjord (see Diaz & Rosenberg, 1995). The difference between the two species in terms of their hypoxic tolerance may arise from differences in their respiratory pigments, haemocyanin vs haemoglobin (see Morse et al., 1986; Taylor et al., 1995) and/or from the relative concentrations of the pigment within their haemolymph. However, until further work is carried out both the former and latter arguments are little more than speculation.

In summary, both the hypoxic tolerance and functional strategy adopted by *N. nitidosa* and *N. nucleus* are different. It is likely that the functional strategy that is adopted,

hypoxic tolerance and/or interspecific competition act to determine the distribution of the two species relative to each other. As such, differences in the functional strategies and hypoxic tolerances of nuculid species may to a large extent explain their differing distributions.

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