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Effect of salinity and temperature on feeding physiology and scope for growth of an invasive species (*Brachidontes pharaonis* - MOLLUSCA: BIVALVIA) within the Mediterranean sea

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ABSTRACT

The Indo-Pacific mytilid *Brachidontes pharaonis* (Bivalvia, Fischer 1870) offers an excellent model for the study of “Lessepsian migration” and the successive colonization at new Mediterranean locations. This species in out competing indigenous bivalves is particularly well adapted to Mediterranean conditions and this is likely due to biological characteristics and physio-ecological plasticity. In the present paper, we report on clearance rate (CR), respiration rate (RR) and Scope for Growth (SFG) of *B. pharaonis* collected from a Western Sicilian pond (Southern Tyrrhenian, MED). Physiological variables were determined in response to a range of temperatures from 11 ° to 20 °C and a broad range of salinities from 15 to 60 psu. Salinity and temperature had a significant influence on CR of *B. pharaonis* as there was a general reduction in CR with a decline in temperature from 20 °C to 11 °C and declining salinity from 37 to 15 psu. RR showed a general temperature dependent relationship with highest RR at 20 °C. SFG showed negative values at lowest salinity of 15 psu at all tested temperatures. SFG values were generally the highest at 45 psu (at 11 °C and 20 °C), although SFG showed a maximum at 37 psu at 15 °C. SFG values were positive over the broadest range of salinities (25 to 60 psu) at 20 °C. The plasticity of the physiological rates demonstrated that *B. pharaonis* had the capacity to maintain positive SFG and tolerate a wide range of temperature/salinity conditions. Possible implications of high physiological plasticity of *B. pharaonis* in competing against indigenous bivalves are discussed.

1. Introduction

Since the opening of the Suez Canal in 1869, more than 300 tropical and subtropical species of algae, invertebrates and fish coming from the Red Sea and the Indian Ocean have dispersed through the Canal and invaded the Eastern Mediterranean (Por, 1978; Galil, 2000, 2007). These have generated evident modifications in the local communities as some invaders have locally out competed or replaced native species while some others are considered pests or cause nuisance (Baines et al., 2005). They may represent one of the major threats to native biological diversity and they can pose severe economic and ecological problems (Sarà et al., 2006). Whether a non-indigenous or invasive species persists and spreads in novel environments depends upon many factors: predator pressure may prove to be significant (Safriel et al., 1980) as also the resistance to diseases and parasites not previously encountered (Gottlieb and Schweighofer, 1996; Calvo et al., 2001), genetic variability (Mydlarz et al., 2006), breadth of trophic niche (Decottignies et al., 2007) and life history strategy (MacDonald and Bayne, 1993). However, particularly successful invaders are those, which are able to feed on a wide variety of food types (viz. “generalist feeders”), showing high fecundity and being able to tolerate broad fluctuations in salinity and temperature. These organisms being characterized by rapid growth rate and high potential of reproductive rates would be better colonizers (Kelly and Klasing, 2004). Many hypotheses have been formulated in order to identify the properties of an invasive species and for explaining what determines its success (Jansen et al., 2007). Nevertheless this is still little understood and there are few experimental studies designed to test these hypotheses. Thus, to evaluate potential risk and impacts on natural ecosystems, we need to understand the biological characteristics and ecological plasticity of invasive species (Sarà, 2006).

The Indo-Pacific mytilid *Brachidontes pharaonis* (Bivalvia, Fischer 1870) offers an excellent model for the study of this invasion called “Lessepsian migration” and the successive colonization of novel Mediterranean environments. Originally from the Indo-Pacific area, mainly south-eastern Asia (Terranova et al., 2007), it appeared to be a species tolerant to high salinity, which has colonised hard substrata as far away as the Red Sea, where it has established dense mussel mats (Safriel et al., 1980). In the last few decades, this small mytilid has been

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able to colonize many intertidal habitats from Israel to the western Mediterranean coasts. However in western Mediterranean, until recently (1994–2000; Sarà et al., 2000), *B. pharaonis* has remained confined in a few suitable habitats with similar environmental features to Red Sea Egyptian salt lakes (*sensu* Safriel et al., 1980; high salinity) such as ephemeral coastal pools and salt-ponds (Sarà et al., 2003). These extreme habitats may function as high-density hot spots (*sensu* the stepping stones hypothesised by Steinitz, 1968) for further spread into the whole western Mediterranean basin to Gibraltar. Instead, in the last five-seven years, *B. pharaonis* has been more frequently found in many marine intertidal habitats of the Tyrrhenian Sea (Sarà and Buffa, 2004; Sarà et al., 2007) where it is establishing dense beds on natural and artificial hard substrata, out competing indigenous bivalves like *Mytilaster minimus* and *Mytilus galloprovincialis* (Safriel and Sasson-frostig, 1988; Sarà et al., 2007). This may reflect that *B. pharaonis* is spreading throughout the western Mediterranean colonising not only small patches of confined hyper-salinity habitats, but also wider zones of marine intertidal environments with more oceanic features.

The aim of the present paper is to study the eco-physiology of *B. pharaonis* under varying physical factors (temperature and salinity) and to test the physiological tolerance of this invasive mussel to a broad range of salinities from brackish, through typical elevated Mediterranean salinities, to extreme hyper-saline conditions. *B. pharaonis* is known to be well adapted to elevated temperatures, based on its invasion via the Red Sea, but there is no information on the mussel's response to the lower temperatures found in the Mediterranean Sea. Such information will be useful when assessing the species' colonization process and predicting its future possible geographical spread. This will also be important when assessing the future potential expansion of invasive species under conditions of increasing temperature and salinity in the Mediterranean Sea, as a result of global warming, where tropical invasive species would have distinct advantage over native species.

2. Materials and methods

2.1. Sample collection and experimental set-up

Specimens of the bivalve *Brachidontes pharaonis* of similar shell length (26.4±2.0 mm) and dry weight (0.06±0.02 g) were collected from the intertidal population living in the Marsala pond (Sarà et al., 2000; Sarà, 2006, 2007) the November 4, 2000 (western Sicily [South Tyrrhenian Sea] − 37° 52' N; 12° 28' E) and transported within 24 h to the Plymouth Marine Laboratory, UK. In the laboratory, they were divided randomly into groups of 20 individuals, labelled and maintained in glass aquarium tanks under the appropriate experimental conditions. Water in the tanks was continuously aerated and changed daily, and *B. pharaonis* were fed regularly (3 times a day) with monoalgal culture of *Isochrysis galbana*.

Experiment I: Animals were held in three isothermal rooms at temperatures of 11°, 15° and 20 °C. In each room there were seawater tanks at the following salinities: 45, 37, 25 and 15 psu. Different salinities were achieved by either enhancing ambient seawater (34 psu) by the addition of sea salts (Marine Mix) or diluting by the addition of deionised water. The experimental design consisted of staggered 'acclimation' and measurements over a period of several weeks, due to a limited number of animals (n=75), equipment and total time available. The acclimation period was 7 days at each temperature and a gradual stepwise reduction in salinity with animals held for a day at each salinity from 45, 37, 25 and 15 psu. Based on earlier studies with blue mussels (*Mytilus edulis*), if there is significant physiological adaptation to temperature and salinity it should occur rapidly and largely within the first 7 days for temperature (Widdows and Bayne, 1971) and within 2 and 4 days of an abrupt increase and decrease in salinity of 15 psu (Widdows, 1985). The control group was represented by animals maintained at 45 psu and 20 °C (i.e. similar conditions to the sampling site).

Experiment II: Animals were also held at a single temperature of 20 °C and three groups were held under hyper-saline conditions of 50, 55 and 60 psu, thereby providing a range of seven salinities from brackish to hyper-saline (i.e. 15 to 60 psu) at a single temperature of 20 °C. Mortality was generally low (3.5%), and the same for most of the conditions, but it was slightly higher at 15 psu (6.6%).

For each group, clearance rate, food adsorption efficiency and respiration rate were measured according to procedures reported by Widdows and Staff (2006). Each day, fifteen animals were used to measure the physiological responses for each temperature and salinity condition. Clearance rate, or the volume of water cleared of suspended particles per hour, was measured in a closed system. Individuals were placed in separate beakers containing 2 L of filtered seawater positioned on multi-stirrer base plates to keep the water thoroughly mixed and oxygenated. After a period of 20 min, to allow the mussels to open and resume pumping, algal cells (*Isochrysis galbana*) were added to each beaker to give an initial concentration of 24,000 cells ml^{-1}. Twenty ml aliquots were sampled from each container at 20 min intervals over a period of 80 min, and the decline in cell concentration was monitored using a Coulter Counter (Model D). Control tanks, without mussels, showed no significant decline in cell concentration over the experimental period. The clearance rate was then calculated using the following equation:-

$$\text{CR}\,(1\ \text{h}^{-1}) = 2\,\text{L}\,(\log_e C_1 - \log_e C_2)\,/\text{time interval}\,(\text{h})$$

where C_1 and C_2 are the cell concentrations at the beginning and end of each time increment (i.e. every 0.33 h).

Respiration rate was determined by placing individual mussels in glass respirometers (500 ml) containing air-saturated seawater, which was stirred by a magnetic stirrer bar beneath a perforated glass plate supporting the mussel. The respirometer was sealed and the decline in oxygen concentration was measured by means of Strathkelvin oxygen electrodes (model 1302) connected to Strathkelvin oxygen meters (model 781).

Food absorption efficiency was measured by comparing the proportion of organic matter in the algal cells and the mussel faeces. Algal food and faecal samples were collected on washed, ashed and pre-weighed GFC filters. Salts were washed out of the filters with 3×10 ml of deionised water and the filters dried at 90 °C. They were then weighed before ashing in a furnace at 450 °C for 4 h and re-weighed. Faeces were collected from the acclimation tanks at the time of respiration measurements.

After physiological measurement the mussel tissues were removed from their shells and dried at 90 °C to obtain their dry tissue weight. The individual clearance rates (L h^{-1}) and oxygen consumption rates (µmoles O_2 h^{-1}) were converted to mass specific rates for a 'standard mussel' of 1 g dry weight using the standard weight exponent (b=0.67). Each physiological rate was then converted to energy equivalents ($\text{J h}^{-1}\ \text{g}^{-1}$) in order to calculate the energy budget and the scope for growth (SFG), which represents the difference between the energy absorbed from the food (food consumption x absorption efficiency) and the energy loss via metabolic energy expenditure.

2.2. Statistical analysis

All data were analysed by means of ANOVAs (Underwood, 1997). In the case of Experiment I, temperature (Temp; 3 levels) and Salinity (Sal, 4 levels) were treated as fixed factors. Fifteen specimens were used for each level and treated as replicates (n=15). In the Exp. 2, Salinity (Sal, 7 levels) was treated as a fixed factor and fifteen specimens were used for each level and treated as replicates (n=15). The Tukey test was used to compare the means of the different treatments. Cochran's test was used prior to the ANOVA to test the assumption of homogeneity of variances (Underwood, 1997).

3. Results

3.1. Salinity and temperature effects (Exp. 1)

Salinity and temperature had a significant influence on the clearance rate of *B. pharaonis* (ANOVA, Table 1). There was a general reduction in CR with a decline in temperature from 20 °C to 11 °C and declining salinity from 37 to 15 psu (Fig. 1). At the lowest salinity of 15 psu, CR values were very low and barely measurable at all three temperatures. At 25 psu, the CR was low at 11° and 15 °C but was significantly higher at 20 °C, although still significantly lower ($p<0.05$) than at 37 psu. At 45 psu the CR was significantly higher relative to the other lower salinities at 11 °C., whereas at 15° and 20 °C the CR was significantly lower than at 37 psu, but higher than at 15 and 25 psu. Respiration rates (RR; Fig. 2; Table 2) showed a general temperature dependent relationship (ANOVA, $p<0.001$) with highest RR at 20 °C. At each temperature there was no significant difference between the RR for animals at salinities ranging from 25 to 45 psu. Mussels at 15 psu had significantly lower RR than those at 25, 37 and 45 psu at all experimental temperatures.

Scope for growth (SFG, Fig. 3) showed negative values at lowest salinity of 15 psu at all three temperatures. SFG values were generally the highest at 45 psu (at 11 °C and 20 °C), although SFG showed a maximum at 37 psu at 15 °C. SFG values were positive over the broadest range of salinities at 20 °C.

Table 1

ANOVA carried out to test a) the interaction effects of temperature and salinity on *B. pharaonis* clearance rate (CR, l h^{-1} g^{-1}), respiration rate (RR, μmol h^{-1} g^{-1}) and scope for growth (SFG, J h^{-1} g^{-1}) [*=$P\le0.05$; **=$P\le0.01$; ***=$P\le0.001$; ns=no significant difference ($P>0.05$)] and Tukey post-hoc comparison outcome obtained for b) clearance rate, c) respiration rate and d) scope for growth

					CR			RR			SFG		
Source				df	MS	F	P	MS	F	P	MS	F	P
a)													
Temperature=Temp				2	98.5	69.4	***	1067.4	73.9	***	177.4	17.5	***
Salinity = Sal				3	137.6	96.9	***	188.3	7.6	***	867.8	85.5	***
Temp x Sal				6	27.06	19.1	***	40.3	8.9	***	132.4	13.1	***
Residuals				168	1.3			11.3			10.2		
Cochran's Test							ns			ns			ns
b)													
CR	T	11	11	11	11	15	15	15	15	20	20	20	20
T x	S	15	25	35	45	15	25	35	45	15	25	35	45
11	15	-											
11	25	ns	-										
11	35	**	ns	-									
11	45	ns	ns	ns	-								
15	15	ns	ns	ns	ns	-							
15	25	ns	ns	***	ns	***	-						
15	35	ns	*	***	ns	***	ns	-					
15	45	ns	ns	***	ns	**	ns	ns	-				
20	15	ns	ns	***	ns	***	ns	ns	ns	-			
20	25	***	***	***	***	***	***	***	***	***	-		
20	35	***	***	***	***	***	***	**	***	***	ns	-	
20	45	*	***	***	***	***	ns	ns	ns	ns	**	ns	-
c)													
RR	T	11	11	11	11	15	15	15	15	20	20	20	20
T x	S	15	25	35	45	15	25	35	45	15	25	35	45
11	15	-											
11	25	ns	-										
11	35	ns	ns	-									
11	45	ns	ns	ns	-								
15	15	ns	ns	ns	ns	-							
15	25	***	ns	***	ns	***	-						
15	35	***	*	***	ns	***	ns	-					
15	45	***	ns	***	ns	***	ns	ns	-				
20	15	***	ns	***	ns	***	ns	ns	ns	-			
20	25	***	***	***	***	***	***	***	***	***	-		
20	35	***	***	***	***	***	***	***	***	***	ns	-	
20	45	***	***	***	***	***	ns	ns	ns	ns	**	ns	-
d)													
SFG	T	11	11	11	11	15	15	15	15	20	20	20	20
T x	S	15	25	35	45	15	25	35	45	15	25	35	45
11	15	-											
11	25	ns	-										
11	35	ns	ns	-									
11	45	***	***	***	-								
15	15	ns	ns	ns	***	-							
15	25	ns	ns	ns	***	ns	-						
15	35	***	***	***	***	***	***	-					
15	45	***	***	***	ns	***	***	***	-				
20	15	ns	ns	***	***	ns	ns	***	***	-			
20	25	***	***	ns	ns	***	***	***	ns	***	-		
20	35	***	***	***	ns	***	***	***	ns	***	ns	-	
20	45	***	***	***	ns	***	***	ns	ns	***	***	ns	-

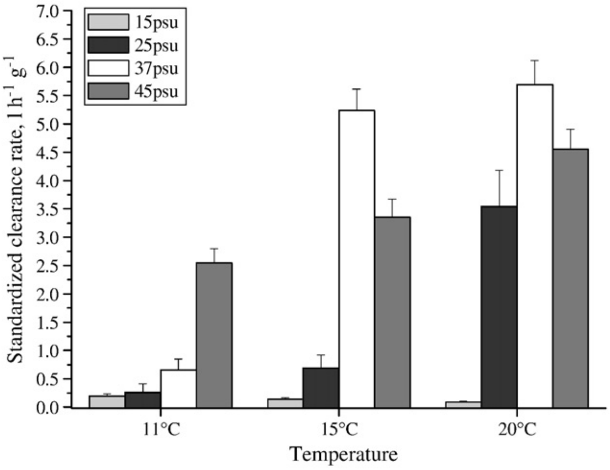

Fig. 1. Combined effects of salinity (15, 25, 37 and 45 psu) and temperature (11, 15 and 20 °C) on the clearance rate (l h^{-1} g^{-1}) of *Brachidontes pharaonis*.

3.2. The effect of salinity at a temperature of 20 °C (Exp. 2)

Results from Exp. 2 showed a marked and significant salinity dependent relationship in the response of this Lessepsian bivalve to salinities between 15 and 50 psu (CR: Fig. 4). There is a significant linear relationship for CR, which peaks at 50 psu ($p<0.001$) and then declines significantly (ANOVA $p<0.05$) by 30–35% at the extreme hyper-salinity of 60 psu. Respiration rates showed a less well-defined relationship but with minimum RR values at 15 psu (ANOVA, $p<0.05$), relatively constant values between 25 and 50 psu, and maximum values, ~2-fold higher, at 55 psu (Fig. 5).

SFG (Fig. 6) showed a clear linear increase between 15 and 50 psu ($p<0.001$) reaching maximum values at 50 psu, but with marked reductions in SFG at the highest salinities of 55 and 60 psu ($p<0.05$).

Table 2

ANOVA carried out to test a) the salinity effect at the constant temperature of 20 °C on *B. pharaonis* clearance rate (CR, l h^{-1} g^{-1}), respiration rate (RR, μmol h^{-1} g^{-1}) and scope for growth (SFG, J h^{-1} g^{-1}) [*=P≤0.05; **=P≤0.01; ***=P≤0.001; ns=no significant difference (P>0.05)] and Tukey post-hoc comparison outcome obtained for b) clearance rate, c) respiration rate and d) scope for growth

		CR			RR			SFG		
Source	df	MS	F	P	MS	F	P	MS	F	P
a)										
Salinity	6	72.1	33.3	***	610.4	28.0	***	329.93	21.5	***
Residuals	98	2.2			21.8			15.4		
Cochran's Test				ns			ns			ns
b)										
CR	15	25	35	45	50	55	60			
15	-									
25	***	-								
35	***	***	-							
45	***	ns	ns	-						
50	***	***	ns	ns	-					
55	***	***	ns	ns	ns	-				
60	***	ns	ns	ns	***	ns	-			
c)										
RR	15	25	35	45	50	55	60			
15	-									
25	***	-								
35	ns	ns	-							
45	ns	ns	ns	-						
50	***	ns	ns	***	-					
55	***	***	***	***	***	-				
60	***	ns	ns	***	ns	***	-			
d)										
SFG	15	25	35	45	50	55	60			
15	-									
25	***	-								
35	***	ns	-							
45	***	***	ns	-						
50	***	***	***	ns	-					
55	***	ns	ns	***	***	-				
60	***	ns	ns	ns	***	ns	-			

4. Discussion

Previous studies on the physiological responses of the Mediterranean *Brachidontes pharaonis* (Sarà et al., 2000) have highlighted a “complex response” resulting from a synergy of trophic, chemical and physical factors. Measurement of such responses can provide the key to understanding the high plasticity of this organism, which is able to colonize a wide variety of habitats at different latitudes around the world (Terranova et al., 2007). For about 10–15 years, after its first discovery in western Sicily in 1994 (Gianguzza et al., 1996), *B. pharaonis* was confined to the western Sicilian salt ponds (Sarà et al., 2006), where it occurred at high densities (~11,000 ind. per m^2). During this period, several thorough field surveys did not highlight new colonization nuclei along the coasts of the southern Tyrrhenian Sea (Sarà and Buffa, 2004). However, since approximately 2000 – 2002, *Brachidontes pharaonis* has been discovered in numerous sites along Sicilian coasts at lower densities than in salt ponds and always associated with native

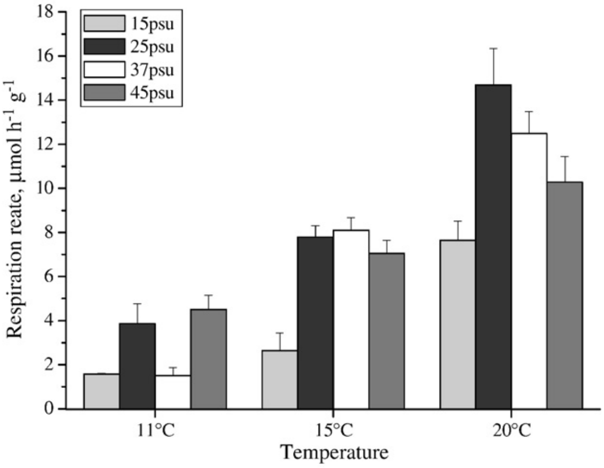

Fig. 2. Combined effects of salinity (15, 25, 37 and 45 psu) and temperature (11, 15 and 20 °C) on the respiration rate (μmol O_2 h^{-1} g^{-1}) of *Brachidontes pharaonis*.

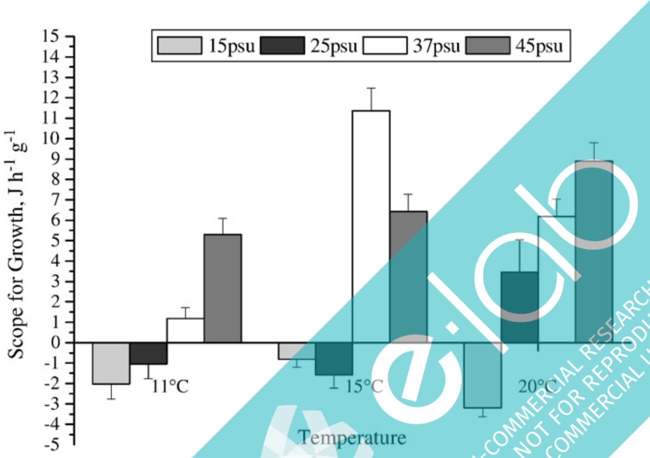

Fig. 3. Combined effects of salinity (15, 25, 37 and 45 psu) and temperature (11, 15 and 20 °C) on the scope for growth (J h^{-1} g^{-1}) of *Brachidontes pharaonis*.

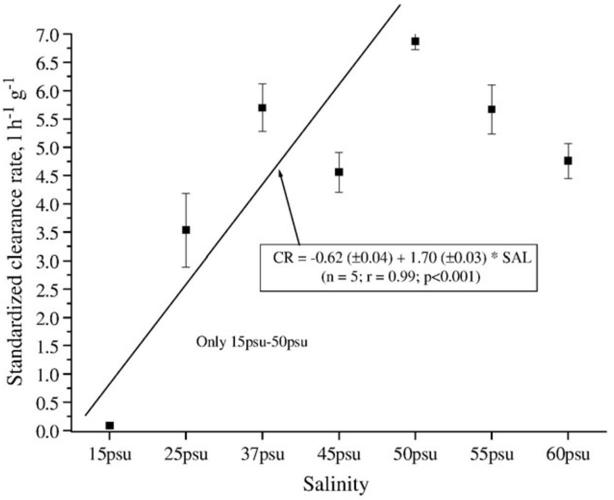

Fig. 4. Effect of salinity (15, 25, 37, 45, 50, 55 and 60 psu) on the clearance rate (l h^{-1} g^{-1}) of *Brachidontes pharaonis*.

bivalves (Sarà et al. submitted). Thus, this invasive species with its highly flexible requirements has been finding suitable conditions, which it can exploit within a diverse and variable Mediterranean intertidal zone (*sensu* Helmuth et al., 2002). Present findings support this hypothesis, as *B. pharaonis* seems to have a large capacity to survive in a wide range of temperature / salinity conditions. The physiological rates measured throughout the controlled experiments reflect this plasticity and indicate that this small mytilid can cope with and is resistant to a broad range of temperatures and salinities. The CR of *B. pharaonis* demonstrated that this species can maintain suspension feeding over a broad range of salinities between 25 and 45 psu, and temperatures between 11 °C to 20 °C at 45 psu. Only under conditions of low temperature (11 °C) and reduced salinity (i.e. <37 psu) were the clearance rates close to zero. This suggests that *B. pharaonis* can physiologically regulate and maintain feeding and food acquisition in a broad range of environments, from estuaries to salt ponds, found along the Mediterranean coastline. The only published data on CR responses of *Brachidontes* derive from field measurements in a salt pond and were obtained using the biodeposition method (Sarà et al., 2000). However, physiological responses measured under ambient field conditions were not consistent with the temperature and salinity effects measured in this study under laboratory controlled conditions.

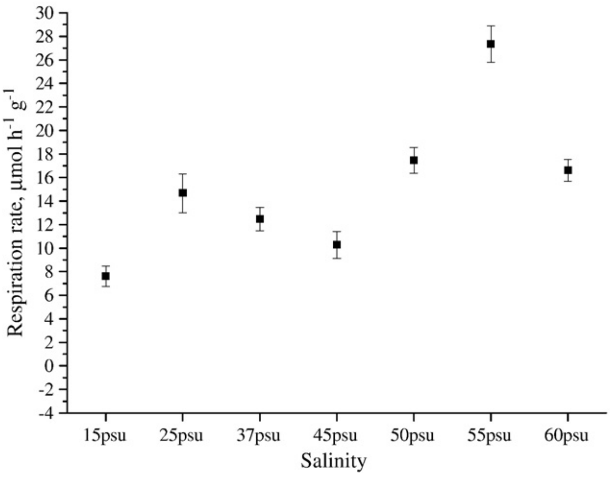

Fig. 5. Effect of salinity (15, 25, 37, 45, 50, 55 and 60 psu) on the respiration rate (μmol O_2 h^{-1} g^{-1}) of *Brachidontes pharaonis*.

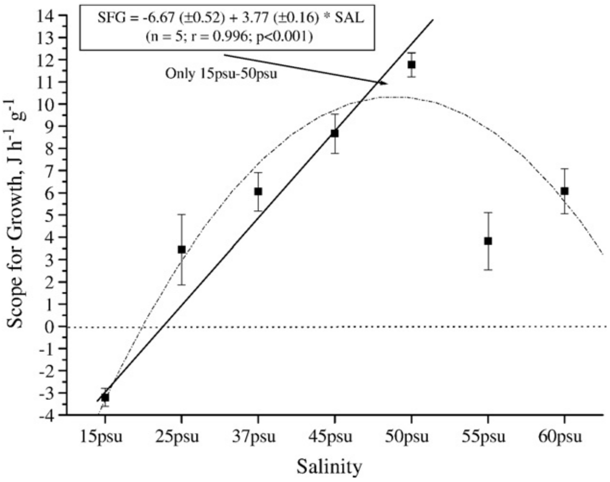

Fig. 6. Effect of salinity (15, 25, 37, 45, 50, 55 and 60 psu) on the scope for growth (J h^{-1} g^{-1}) of *Brachidontes pharaonis*.

For example, in the field-based study clearance rates showed a negative relationship with temperature in the range from 8.5 °C to 30 °C: the lower the temperature, the higher the clearance rate. These studies also showed that CR was influenced by seasonal changes in the quality and quantity of food available and this probably explained the apparent differences in the relationships between CR and temperature. To date, there is no comparable information on the influence of temperature on the physiological responses of Mediterranean mussels such as the Mediterranean black mussel *Mytilus galloprovincialis* and the small mytilid *Mytilaster minimus*, which are among the potential competitors in the Mediterranean intertidal zone. *M. galloprovincialis* is largely distributed at higher Mediterranean latitudes where food availability (expressed as chlorophyll-*a* concentrations) is higher than southern latitudes (Margalef, 1985) and where the climatic drivers, such as local irradiance during low-tide exposure, is lower (*sensu* thermal theory, Helmuth, 1999; Jansen et al., 2007; Zenone and Sarà, 2007). In contrast, *Mytilaster* is a small mytilid colonising rocky intertidal coasts and represents the structuring species of southern Mediterranean coasts (Safriel and Sasson-frostig, 1988), but no data is currently available about its physiological performances. Clearance rates of *M. galloprovincialis* adults (from south coast of France), estimated under oligotrophic conditions in the laboratory, appear to be higher than *B. pharaonis*, reaching about 17.5 l g^{-1} h^{-1} (or 6.3 l h^{-1} for 0.36 g dry wt mussel; Maire et al., 2007). In addition, juveniles of *M. galloprovincialis* of similar length to our experimental specimens had lower CR (~3 l h^{-1}; Perez Camacho et al., 2000) compared to *B. pharaonis*. Similar values were found in New Zealand black mussels (CR ~2.5 l h^{-1}; Helson and Gardner, 2007). The relationship between CR and temperature in Mediterranean black mussels has been estimated only on a few occasions (e.g. Schulte, 1975). *M. galloprovincialis* has an apparent optimum temperature between 15 °C and 25 °C, whilst there is almost complete cessation of CR above 25 °C (Schulte, 1975). Below 10–15 °C, *M. galloprovincialis* showed a marked decline in clearance rate (Schulte, 1975) until complete cessation at 5 °C. This type of relationship between temperature and filtration rate by mussels has also been shown for other species (Schulte, 1975; Widdows, 1976). Unfortunately, in the present study CR was not examined over such a wide temperature range as salinity, but *B. pharaonis* is likely to follow a similar normal-shaped curve as other bivalves with a decline in clearance rates at higher and lower temperature extremes. Furthermore, Sarà et al. (2006) showed that *B. pharaonis* not only survives in the salt pond conditions in western Sicily, but also *B. pharaonis* continues to suspension feed and does not stop growing during the summer (30–35 °C) and during the winter (<9 °C) periods. This would suggest that the temperature tolerance curve and physiological extreme points of this species are higher than other Mediterranean mussels (e.g. *M. galloprovincialis*; Schulte, 1975).

The effect of salinity on the CR of marine bivalves follows a similar curve to that of temperatures. Coastal and estuarine mussels (*Mytilus edulis*) from the north-east Atlantic region, showed physiological responses including CR were independent of salinities from 30 down to 20 psu, and declining sharply below 20 psu (Widdows, 1985). However, there is little information on the upper salinity tolerance of *Mytilus edulis* and other mussels. In contrast, *B. pharaonis* seems to be well adapted to higher salinity conditions with the maximum CR between 37 and 55 psu, while still actively filtering at 60 psu. At lower salinities (<25 psu) there was a negative effect on CR with near cessation at 15 psu (Figs. 1 and 4). This suggests that salinity thresholds of this invasive organism are likely to be higher than those of other bivalves, thus allowing *B. pharaonis* to survive at elevated salinities that are usually lethal for the most of other intertidal bivalves (Safriel and Sasson-frostig, 1988). The tolerance to hypersaline conditions is further supported by observations that *B. pharaonis* is common in Salt Lakes of Sinai Peninsula (Safriel et al., 1980) and has survived for over two decades in salt ponds of Western Sicily (>45–50 psu; Sarà et al., 2003, 2006). However, the curve plotted in Fig. 4, suggests that the CR of *B. pharaonis* will still be active at salinities >60 psu, but there are no data confirming the upper tolerance limit when there is a near cessation of CR. However, a salinity of 60 psu corresponds to extreme conditions where there is almost a total absence of life, apart from a few crustaceans such as *Artemia salina* and salt bacteria (Croghan, 1958).

Respiration rate is a temperature-dependent process in mussels with evidence of some degree of acclimation to temperature change (Widdows, 1973; Bayne et al., 1976). At high temperatures, beyond the temperature-specific maximum respiration rate, there is a marked decline in oxygen consumption. This is caused, in part, by oxygen limitation, due to reduction of oxygen partial pressures, and a reduction in ventilation rate (i.e. oxygen supply). This will result in a rapid decline in respiration rates and an increase utilisation of anaerobic metabolic pathways (Portner, 2001, 2002; Tang et al., 2005; Moschino and Marin, 2006; Jansen et al., 2007). The amount of energy respired by *B. pharaonis* during our controlled experiments, although measured over a relatively narrow range between 10 and 20 °C, followed the temperature dependent relationship. This pattern is common in many bivalves from *Donax vittattus* (Ansell, 1973) to *Mytilus* spp. (Sukhotin et al., 2003). In comparison to *Mytilus edulis* (Widdows, 1973) the measured respiration rates of *B. pharaonis* are relatively low at 20 °C, which suggests there is plenty capacity to increase their metabolic rate and continue growing at higher temperatures. However, the reduction in respiration rate at lower winter temperatures may allow *B. pharaonis* to conserve energy and survive at low temperatures, but due to the high metabolic cost of synthesis will limit its capacity to grow (Widdows and Hawkins, 1989). Unfortunately in the present study we were unable to investigate the response of *B. pharaonis* to a wider range of temperatures, beyond a relatively low maximum of 20 °C. This prevents us from assessing the upper temperature limit on the physiological responses of this species and we therefore have to infer from field observations and other species. Since *B. pharaonis* normally survive at water temperature over 30–35 °C in Sinai Salt Lakes and western Sicilian salt ponds, maintaining positive growth rates (Sarà et al., 2000, 2006), then respiration of this species is unlikely to decline markedly over the range 25 to 35 °C, which is in contrast to other bivalves (Jansen et al., 2007). The rates of oxygen uptake by a co-generic species (e.g. *Brachidontes demissus*), increase linearly with temperature up to 35.2 °C (Head, 2003). Therefore, the measured respiration vs. temperature relationship did not reach a critical or lethal level, suggesting that the tolerance limits of *B. pharaonis* are likely to lie above 35 °C which would be the lethal limit for native bivalves.

The relationship between salinity and respiration in bivalves has been studied in the past (Pierce, 1971; Bayne et al., 1976; Shumway and Koehn, 1982; Widdows, 1985; Hutchinson and Hawkins, 1992; Tomaru et al., 2002; Järnegren and Altin, 2006). There is a general trend for RR to increase with salinity with evidence of a peak at 55 psu. This will reflect an increase in maintenance costs, some of which will be involved with changes in the osmoconcentration of extra- and intra-cellular fluids (e.g. free amino acids [FAA] and ions). However, the slight increases in RR will also reflect increased metabolic costs associated with growth (Fig. 6). Bivalves are osmoconformers and the effect of salinity on the amino acid concentration in euryhaline bivalves has been demonstrated by Allen (1961) and Livingstone et al. (1979) in *Mytilus edulis.* Recent transplant experiments (Sarà, 2006) have shown that *B. pharaonis* is able to survive under very extreme physical conditions and this tolerance is achieved in part by the high food availability in the salt ponds. Consequently, when the food supply was very low then mortality was high (Sarà, 2006). This indicates that in the wild, growth and survival of *B. pharaonis* are influenced by food availability. High food levels enable it to cope with stress induced by extreme salinity and temperature regimes. The present results would support this hypothesis with positive values of SFG in most cases (Figs. 3 and 6). Negative SFG values were only recorded under conditions of combined low salinity and low temperature. *B. pharaonis* SFG was positive at 20 °C from 25 psu up to 60 psu, and only around 15 psu showed negative values (see Fig. 6). These observations for *B. pharaonis* are consistent with environmental conditions in most coastal environments in southern Mediterranean, from estuaries to marine intertidal and subtidal habitats, where there is a mean temperature of 20 °C and salinity ranges from 20 to 40 psu. Consequently, *B. pharaonis* will experience suitable conditions for survival, growth and presumably reproduction almost everywhere in Mediterranean, particularly after forming stable colonization nuclei (*sensu* Sarà et al., 2007). The only limiting factors to its mass spreading may be represented by food and space availability. Food quality and quantity should not represent limiting factors for *B. pharaonis* dispersal. It is able to assimilate a wide range of foods (Sarà, 2006) from detritus, which would highly refractory for many bivalves (e.g. seagrass; Sarà, 2007) to labile fractions (e.g. resuspension of benthic microflora and phytoplankton). In addition, local human activities (e.g. sewage, drainage etc.) provide another huge source of potential food (Evgenidou and Valiela, 2002). Spatial patches for recruitment could also be increasingly available, particularly as a result of altered physical conditions within the Mediterranean area, due to changes in meteorological and oceanographic conditions (Tsimplis and Rixen, 2002). For example, salinity, temperature, storms and wave activity during high tides, and local irradiance, elevated air temperatures and precipitation at low tides, are detrimental for native species and intertidal biodiversity (sensu Helmuth, 1999; Helmuth et al., 2002; Worm et al., 2006). These factors are contributing to the clearance of habitat patches in the intertidal (*sensu* Jansen et al., 2007). Consequently, less thermally tolerant indigenous species (e.g. *M. galloprovincialis* and *M. minimus*) would abandon habitat patches in favour of more tolerant organisms (e.g. *B. pharaonis*) like *B. pharaonis* or other invasive species.

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