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Physiological responses of the fan mussel *Pinna nobilis* to temperature: ecological and captivity implications

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Abstract

The fan mussel *Pinna nobilis* is experiencing a mass mortality event throughout the Mediterranean Sea. The survival of the remaining isolated populations and the maintenance and reproduction of individuals in captivity could determine the future of the species. This paper examines the clearance rate (CR) and oxygen consumption (VO2) of fan mussel individuals measured under 5 different temperatures (8.5, 14, 18, 23 and 28°C). The measurements of both physiological variables revealed a threshold limit of thermal tolerance at both extreme temperatures, indicating the critical situation of the remaining populations, which are located in coastal lagoons and shallow bays where these extreme temperatures usually occur. Besides, the high clearance rates observed highlight the significant ecosystem service provided by the species in terms of water filtration, especially in confined waters with low renewal rates. Routine control of the clearance rate as an early warning system is proposed for detecting ailing individuals. Such a system could also be used to improve captivity conditions.

Keywords: physiology; fan mussel; invertebrate welfare; climatic change; ecosystem services.

Introduction

The fan mussel *Pinna nobilis* is an endangered species threatened with extinction (Kersting et al., 2019) by a disease caused by *Haplosporidium pinnae* (Panarese et al., 2019; Grau et al., 2022), sometimes together with a *Mycobacterium* sp. and other pathogens (Carella et al., 2020; Lattos et al., 2021a; Lattos et al., 2021b). The die-off, which started in late 2016 (Vázquez-Luis et al., 2017), has provoked high mortalities, close to 100% of individuals throughout the Mediterranean Sea (Acarli et al., 2020; Öndes et al., 2020; Özalp & Kersting, 2020; Šarić et al., 2020; Betti et al., 2021; Katsanevakis et al., 2022). Few populations remain unaffected or partially affected in paralic environments, the only reservoirs of fan mussel populations known to date (Garcia-March et al., 2020). In the Mediterranean Sea, the effects of climatic impacts are expected to be particularly exacerbated in these environments, due to oceanographic conditions such as restricted circulation, shallow depth, and large water residence (Kennish & Paerl, 2010). Mean global warming is expected to reach 1.5°C during the following three decades (IPCC, 2018) exerting a negative effect on marine biodiversity (Jones & Cheung, 2015). The Mediterranean Sea is particularly sensitive to climate warming (Giorgi & Lionello, 2006) with heatwaves increasing in duration and intensity (Molina et al., 2020; Garrabou et al., 2022) and causing mortalities of marine benthic invertebrates (Garrabou et al., 2009; Rivetti et al., 2014; Garrabou et al., 2019; Garrabou et al., 2022).

The few fan mussel populations that can still be found in shallow bays, coastal lagoons and the Marmara Sea (Garcia-March et al., 2020; Cinar et al., 2021; Nebot-Colomer et al., 2021; Peyran et al., 2021; Acarli et al., 2022;
Katsanevakis et al., 2022) are threatened in the medium to long-term (García-March et al., 2019), but information on how resilient can they be to expected changes is lacking. Moreover, the connection between surviving populations and impacted areas (Wesselnmann et al., 2018; González-Wangi uemert et al., 2019) could be pointless because of the persistence of the disease (protozoan spores), which prevents recolonization by propagule rain after local extinction. Thus, the importance of preserving the few remaining unaffected populations is significant. However, the presence of resistant juveniles has been reported and this is promising as regards the long-term survival of the species (Ruitton & Lefebvre, 2021), coupled with the isolated recruitment events at a few locations recorded by Kersting et al. (2020).

The functional alterations of the possible disappearance of an endemic species such as P. nobilis are also uncertain, but the loss of biodiversity can alter the capacity of water ecosystems to provide food, maintain their water quality and recover from perturbations (Worm et al., 2006). In fact, one of the main roles associated with P. nobilis is its contribution to water clarity retaining large amounts of organic matter (Trigos et al., 2014), presumably by filtering large amounts of seawater, although their real capacity is still unknown. Besides, the fan mussel contributes to the increase of biodiversity by generating hard substrate that is used by other species to settle (Kuhlmann, 1998; Cosentino & Giacobbe, 2008; Rabouai et al., 2009).

Physiological measurements of clearance rate (CR) in bivalves, as the volume of water filtered and cleared of particles, can be used as a biomarker of individual conditions (Toro et al., 2003) and is also the main physiological variable determining the scope for growth (SFG), representing the energy available for somatic and gonadal growth (Beiras et al., 1994; Albentosa et al., 2012b). Besides, CR exemplifies the role of bivalves in the ecosystem acting as biofilters and retaining nutrients (Dame, 1993; Odd et al., 2005; Rose et al., 2015). The measurement of oxygen consumption (VO₂) is also considered of great importance and is used to estimate metabolic expenses incurred for maintaining vital functions (Bayne & Newell, 1983), and to approximate maintenance rations required to fulfill energy requirements (Albentosa et al., 2012a). Both physiological functions, CR and VO₂, are greatly influenced by temperature (Griffiths & Griffiths, 1987), whose effect in the context of global warming could define the future of the species. In fact, Basso et al. (2015) detected higher mortality rates of individuals at 26°C compared to lower temperatures. Moreover, lower life expectancy has been observed in populations inhabiting paralic environments, indicating that more extreme environmental conditions might be one of the possible reasons (García-March et al., 2019). Moreover, temperature is a key factor for gonad maturation during the reproductive period (Utting & Millican, 1997) and related to recruitment success (Kersting & García-March, 2017), but also considered a key factor controlling the disease caused by H. pinnae, which showed a seasonal pattern and higher expression at temperatures above 13.5°C (Casbanellas-Reboredo et al., 2019). Individuals affected by H. pinnae resisted longer under lower temperatures in captivity (García-March et al., 2020), although the capacity to overcome the disease is unclear, but the use of low temperatures in captivity at the beginning of the quarantine period could help in reducing the transmission of the disease and isolate sick individuals.

In this work, CR and VO₂ were measured in P. nobilis under five different temperatures. Knowledge of basic physiological parameters such as CR and VO₂ and their variations with temperature could help to improve captivity protocols and quantify the filtration capacity and energy requirements of fan mussels. Besides, it will enhance our general understanding of ecosystem services provided by fan mussels and the vulnerability of the species, especially in the context of the populations located in paralic environments, and the consequences of climate change in the Mediterranean Sea (Diffenbaugh et al., 2007).

Material and Methods

Collection and acclimation of individuals

Eight P. nobilis individuals of similar size (=30 cm) were collected in February 2020, from Fangar Bay, in the Ebro Delta (Catalonia, NW Mediterranean). Individuals were transported fully submerged in aerated coolers to the IMEDMAR-UCV facilities in Calpe (Spain). Once in the lab, they were measured in length and width and kept for 6 months in two closed 750 L tanks, each with a closed circuit. Infection by H. pinnae was untested, given the health condition of the Fangar Bay population at the moment, although individuals were daily checked for symptoms of disease such as mantle retraction and lack of response (García-March et al., 2020). The individuals were placed in vertical position, with one third of the shell buried in coral debris substratum within individual plastic buckets. Water was maintained at 14.0 ± 0.5°C and salinity at 37.5 – 38.0 psu. During this period and during the thermal experiment, the fan mussels were continuously fed daily 16h a day with the filters deactivated. Their diet was composed by a mix of live Isochrysis galbana (T-ISO) and Chaetoceros calcitrans cultured in situ at a ratio of 50-80% and 50-20% respectively, depending on culture conditions. Each day, an initial dose of f = 2 mm³ L⁻¹ (= 45,000 cel ml⁻¹, concentration below the pseudofaeces threshold) was provided (Bayne et al., 1989; Bayne, 1993). Later, half of the initial dose was given again when the remaining concentration of microalgae reached 50% of the initial dose. During the following 8h, no food was pumped into the aquariums and the filters were activated to allow water cleaning until the next day.

Experimental design

For thermal experiments, the 8 specimens of P. nobilis were individually confined in eight equal 55 L aquariums. One extra aquarium without an individual was used
as control with the same water conditions to correct the sedimentation rate of phytoplanktonic particles in the experimental aquariums. The water was circulated from the aquaria through a sump equipped with a mechanic and biological filter, a skimmer, and a temperature regulation system and then returned to the aquaria. The fan mussels were placed in horizontal position, as no significant differences in the physiology of Pinnids were observed between laying down and standing positions (Hernandis et al., 2022).

In order to analyse the effects of temperature on P. nobilis, clearance rates (CR: L h⁻¹) and oxygen consumptions (VO₂: mg O₂ h⁻¹) at 5 temperatures were tested: 8.5, 14, 18, 23 and 28 ± 0.5°C. The lowest temperature (8.5°C) was considered the coldest temperature that can occur in shallow Mediterranean areas such as coastal lagoons (Amos et al., 2017; Marques et al., 2019; Tagliapietra et al., 2021). A water temperature of 14°C is common in winter in the Mediterranean Sea (García-March et al., 2011), and 18 and 23°C represent intermediate temperatures within the thermal comfort range of the species (Basso et al., 2015; Trigos et al., 2015). Finally, 28°C was considered as the highest temperature in the experiment for testing the upper thermal limit of the species. Similar temperatures can be observed in Fangar Bay, from where the specimens were collected, although a wider range of temperatures may occur, reaching a minimum of 6°C and a maximum above 30°C (Ramón et al., 2007). However, given the protected status of the species, these extreme temperatures were avoided in order to reduce the risk of mortality due to experimental stress or excessive bacterial growth. The experiment was performed according to the following thermal-change protocol: initially, water temperature was decreased from 14°C (acclimation temperature) to 8.5 (1 degree day⁻¹). Subsequently, the individuals were kept at this temperature for 1 week and CR and VO₂ were measured on the 7th day. Once measurements were completed, water temperature was increased to the next exposure-temperature (1 degree day⁻¹), and the physiological measurements were determined after 7 days.

After completion of the experiment, the individuals were kept in captivity for further experiments.

**Physiological measurements**

**Clearance rate (CR: L g⁻¹ h⁻¹)**

Seawater recirculation in the aquariums was interrupted to measure clearance rates. Subsequently, a food dose (≈ 46816 cel ml⁻¹, ≈ 2.12 mm³ L⁻¹, POM ≈ 1.14 mg MO L⁻¹) of Isochrysis galbana (T-ISO) was added to each aquarium and the clearance rate was calculated from the depletion of particle concentration in the aquarium following the expression of Coughlan (1969):

\[
\ln(C_0) - \ln(C_t) = \left(\frac{CR}{V} + \alpha\right) \cdot t
\]

Where \(C_0\) and \(C_t\) represent particle concentration at time 0 and t, respectively, \(V\) is the water volume in the aquarium (55 L) and \(\alpha\) is the particle sedimentation rate (determined as \(\ln C_0 - \ln C_t = a \cdot t\) in a control aquarium without animal).

Particle concentration was determined using an electronic particle counter, Coulter-Multisizer III, with an aperture tube of 100 μm. The first sample (\(C_0\)) was taken immediately after the addition of the microalgae once mixed. Later, 5 more samples were taken at regular intervals, 7 to 25 min intervals, depending on the temperature (the CR at low temperatures was lower; therefore, the time between samples was higher to detect differences in microalgae concentration). This procedure was repeated four consecutive times for each individual and exposure temperature. To clean any remains of phytoplankton between replicates, water was recirculated through the system filter and through a 1 μm filter. Finally, individual clearance rate was computed as the mean CR value obtained for each individual using the 4 replicates.

**Oxygen consumption (VO₂: mg O₂ g⁻¹ h⁻¹)**

Once CR measurements were completed, the fan mussels were transferred to respirometers in order to determine consumption. Respirometers consisted of 15L plastic buckets, sealed and filled with water at the experimental temperature. Oxygen consumption was computed as the rate of reduction in dissolved oxygen concentration (DOC) in the respirometer over time. DOC was measured every 15 min (30 min at 8.5°C) using the oxygen sensor of the oceanographic probe AAQ-RINKO 177 and a circulation pump to allow water movement and guarantee correct functioning of the oxygen sensor.

Individuals were kept in the bucket until a 20% difference in oxygen concentration was detected, or a maximum of 3 hours had passed. Given the initial stress caused by handling, measurements were initiated 15 min after placing the individuals in the bucket. To maintain a constant temperature, respirometers were placed inside a 350L tank at the experimental temperature and with climate control.

**Expression of Physiological rates**

Physiological rates are usually standardized to 1g of meat dry weight (DW) using allometric coefficients to correct the inter-individual differences in size. However, since no allometric studies of the physiological variables are currently available for this species, we could not proceed to standardized physiological rates. Besides, the dissection of experimental animals was avoided given the endangered status and the critical situation of the species. Hence, the dry weight of the soft tissues of the experimental specimens was estimated using the length-dry weight regression available for P. rudis: (\(R^2 = 0.97, p < 0.001\)) the closest species for which published data is available (Hernandis et al., 2021). Therefore, physiological rates were expressed as mass-specific rates by dividing individual physiological rates by the estimated dry weight of soft-tissues.
Thermal dependence of physiological variables was analysed by computing $Q_{10}$ coefficients, using the equation where $R_1$ and $R_2$ are the physiological rates at temperature $t_1$ and $t_2$ respectively (Bayne & Newell, 1983). The $Q_{10}$ coefficient represents the dependency of an organism’s physiology on temperature changes and varies according to the thermal history of the animal, body size, activity, and reproductive conditions (Gosling, 2015). $Q_{10}$ values near 1 indicate thermal independency while a value of 2 represents doubling of the physiological rate with each 10ºC increase in temperature. In general, $Q_{10}$ values between 1 and 3 indicate non-stressful conditions whereas values > 4 indicate thermal stress (Zippay & Helmuth, 2012).

The ratio between CR and VO$_2$ was also calculated to check for variations in the physiological performance of both processes.

**Statistical analysis**

An analysis of variance (ANOVA) was used to test for differences in CR, VO$_2$ and the rate of both variables between temperatures. A one-way factor RM-ANOVA was applied with “temperature” as a fixed factor with five levels: 8.5, 14, 18, 23 and 28°C and a pair-wise t-test with paired groups was carried out to detect differences among them. Before each analysis, the Shapiro-Wilk test was used to check if data followed a normal distribution. All statistical analyses were performed using R statistical computing environment, and the results are expressed as mean ± standard error (mean ± SE).

**Results**

**Biometric data**

The mean size of individuals was 29.4 ± 2.2 cm of shell length and 12.0 ± 1.0 cm of shell width. The estimated mean DW was 5.7 ± 1.0 g (using the length-dry weight regression available for *Pinna rudis* (Hernandis et al., 2021)). The mean size of individuals was 29.4 ± 2.2 cm of shell length and 12.0 ± 1.0 cm of shell width. The estimated mean DW was 5.7 ± 1.0 g (using the length-dry weight regression available for *Pinna rudis* (Hernandis et al., 2021)).

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<table>
<thead>
<tr>
<th>Individual</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>DW* (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.30</td>
<td>11.45</td>
<td>5.62</td>
</tr>
<tr>
<td>2</td>
<td>32.20</td>
<td>13.20</td>
<td>7.09</td>
</tr>
<tr>
<td>3</td>
<td>31.40</td>
<td>12.00</td>
<td>6.67</td>
</tr>
<tr>
<td>4</td>
<td>31.10</td>
<td>12.40</td>
<td>6.51</td>
</tr>
<tr>
<td>5</td>
<td>29.40</td>
<td>11.30</td>
<td>5.66</td>
</tr>
<tr>
<td>6</td>
<td>26.50</td>
<td>12.00</td>
<td>4.38</td>
</tr>
<tr>
<td>7</td>
<td>26.20</td>
<td>10.40</td>
<td>4.26</td>
</tr>
<tr>
<td>8</td>
<td>29.10</td>
<td>13.20</td>
<td>5.52</td>
</tr>
</tbody>
</table>

*Estimated dry weight obtained using the length-dry weight regression available for *Pinna rudis* (Hernandis et al., 2021).

**Physiological measurements**

Mean clearance rate (CR; L g$^{-1}$ h$^{-1}$) of fan mussels increased sharply with water temperature (one-way ANOVA, $F = 38.17$, $p < 0.01$, Fig. 1A). In the comparison between temperatures, the pair-wise t-test showed that in the cold temperature range (8.5 to 14°C) mean CR varied from $0.55 ± 0.15$ to $4.27 ± 0.54$ L g$^{-1}$ h$^{-1}$, $p < 0.01$ and $Q_{10} = 56.59 ± 16.75$ (Table 2). In the mild temperature range (14 to 23 ºC), the clearance rate increased to $5.68 ± 0.86$ at 18°C and to $7.84 ± 1.20$ L g$^{-1}$ h$^{-1}$ at 23°C ($Q_{10}$ approximately 2, Table 2), with only significant differences ($p < 0.05$) between 14 and 23°C. Finally, the thermal sensitivity of the clearance rate increased significantly in the warmest range (23 to 28°C): CR rose significantly from $7.86 ± 1.20$ to $14.55 ± 1.34$ L g$^{-1}$ h$^{-1}$ for 6 individuals, $p < 0.05$ and $Q_{10} = 6.03 ± 2.64$ (Table 2). The other two maintained their valves almost closed during the day of CR measurements and showed a reduction of their filtration below $4$ L g$^{-1}$ h$^{-1}$.

For 28°C, a spawning event was observed before the experiment, where all individuals released gametes (7 males and 1 female). The experiment was postponed for 48h to allow the individuals to recover.

Mean values of mass-specific oxygen consumption (VO$_2$: mg O$_2$ g$^{-1}$ h$^{-1}$) also increased significantly with temperature (one-way ANOVA, $F = 111.00$, $p < 0.01$, Fig. 1B). In the comparison between temperatures, the pair-wise t-test showed that in the cold temperature range (8.5 to 14°C), VO$_2$ evidenced high thermal dependency ($Q_{10} = 6.60 ± 1.00$, Table 2), significantly increasing from values of $0.26 ± 0.02$ to $0.70 ± 0.04$ mg O$_2$ g$^{-1}$ h$^{-1}$ ($p < 0.01$). At mild temperatures (14 to 23°C), VO$_2$ showed relatively reduced thermal dependence ($Q_{10}$ of approximately 1.7, Table 2) significantly increasing to $0.85 ± 0.06$ mg O$_2$ g$^{-1}$ h$^{-1}$ at 18°C and to $1.10 ± 0.09$ mg O$_2$ g$^{-1}$ h$^{-1}$ at 23°C ($p < 0.05$). Contrary to the trend displayed by CR, the VO$_2$ of fan mussels did not significantly increase in the upper thermal range (23 to 28°C), which varied from $1.10 ± 0.09$ mg O$_2$ g$^{-1}$ h$^{-1}$ at 23°C to $1.25 ± 0.03$ mg O$_2$ g$^{-1}$ h$^{-1}$ at 28°C ($p > 0.05$).

Significant differences were observed in the CR/VO$_2$ ratio (Fig. 1C) (one-way ANOVA, $F = 25.13$, $p < 0.01$). In the comparison between temperatures, the pair-wise
Fig. 1: Mean values ± standard error of: A) Mass-specific clearance rates (CR; L g⁻¹ h⁻¹) of *Pinna nobilis* individuals in relation to water temperature (n = 8, at 28 ºC n = 6). B) Mass-specific oxygen consumption (VO₂; mg O₂ g⁻¹ h⁻¹) of *P. nobilis* individuals in relation to water temperature (n = 8). C) CR/VO₂ ratios at the different experimental temperatures for *P. nobilis* individuals (n = 8). Letters A - D represent statistical different groups (n = 6). A black point and solid vertical lines indicate mean and standard error respectively.

Table 2. Q₁₀ coefficient ± standard error (SE) results for physiological measurements of clearance rate (CR) and oxygen consumption (VO₂) in *Pinna nobilis* individuals.

<table>
<thead>
<tr>
<th>Temperature (ºC)</th>
<th>CR Q₁₀ ± SE</th>
<th>VO₂ Q₁₀ ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.5 — 14</td>
<td>56.59 ± 16.75</td>
<td>6.60 ± 1.00</td>
</tr>
<tr>
<td>14 — 18</td>
<td>2.68 ± 0.59</td>
<td>1.67 ± 0.18</td>
</tr>
<tr>
<td>18 — 23</td>
<td>2.20 ± 0.36</td>
<td>1.72 ± 0.23</td>
</tr>
<tr>
<td>23 — 28</td>
<td>6.03 ± 2.64</td>
<td>1.45 ± 0.24*</td>
</tr>
</tbody>
</table>

*No significant differences were found in oxygen consumption (VO₂) within the range 23–28 ºC, therefore the Q₁₀ between these temperatures could be considered as 1.
t-test did not reveal significant differences (p > 0.05) in the comparison at mid temperatures (14, 18 and 23°C), where a relatively constant value of ≈ 6.5 was observed. However, significant differences were observed (p < 0.01) compared to both 8.5 and 28°C, where the ratio decreased to 2.1 and increased to 11.9, respectively.

Discussion

Thermal tolerance and physiological measurements

Temperature is one of the main factors affecting metabolism in bivalves (Newell & Branch, 1980) and thus influencing their filtration capacity (Fuchs & Specht, 2018; Specht & Fuchs, 2018). Clearance rate (CR) typically increases with temperature up to the limit of tolerance for the species when a rapid decline is observed (Griffiths & Griffiths, 1987). Accordingly, in this study, the CR of P. nobilis increased with temperature, reaching a maximum at 28°C. The mass-specific CRs in P. nobilis recorded during this study (from a mean of 0.55 to 14.55 L g⁻¹ h⁻¹) were higher than in other bivalve groups such as mussels, scallops, oysters, and cockles (from 2.47 to 3.57 L g⁻¹ h⁻¹ according to the meta-analysis performed by Cranford et al. (2011)). Yet, higher CRs of 25.88 L g⁻¹ h⁻¹ at 28°C have been reported for Pinctada margaritifera (Pouvreau et al., 1999). In the context of other parameters of the Pinnidae, the CR recorded at 18°C in this study (5.68 L g⁻¹ h⁻¹) was similar to that indicated by Hernandez et al. (2022) for P. rudis (5.29 L g⁻¹ h⁻¹) at that temperature. However, Nieves-Soto et al. (2013) observed much lower CRs at all temperatures in Atrina tuberculosa (0.37, 1.39, and 3.96 L g⁻¹ h⁻¹ respectively at 17, 22.5 and 28°C) compared to P. nobilis in the present study (5.68, 7.86 and 14.55 L g⁻¹ h⁻¹ respectively at 18, 23 and 28°C), which could be related to methodological differences in the flow-through system used to measure CR.

The effect of temperature on the metabolism and CR of bivalves is directly associated with VO₂ (Buxton et al., 1981) because of the higher energetic expenditure with increased CR (Navarro et al., 1992; Velasco & Navarro, 2002). Accordingly, an increase of VO₂ with temperature was observed in P. nobilis over the entire investigated range (8.5-23°C), although no significant differences were detected from 23 to 28°C. However, Trigos et al. (2015) observed a higher VO₂ at 20°C than at 25°C. A possible explanation could be that different individuals were used for experimental conditions at 20 and 25°C, and that those results were given per individual, not in standardized values. In fact, they observed lower values of oxygen consumption per individual at 16°C (3.1 mg O₂ ind⁻¹ h⁻¹) compared to those found in this study at 14°C (4.2 mg O₂ ind⁻¹ h⁻¹, equivalent to 0.70 mg O₂ g⁻¹ h⁻¹) even though the individuals of this study were smaller and the temperature was lower. On the contrary, Basso et al. (2015) observed a much higher VO₂ compared to that of this study, with values above 1.5 mg O₂ g⁻¹ h⁻¹ at 20 and 23°C, and over 3.0 mg O₂ g⁻¹ h⁻¹ at 26°C compared to values of 1.25 mg O₂ g⁻¹ h⁻¹ in this study at 28°C. The variations between Basso et al. (2015) and our study could be explained by differences in size. The individuals used by Basso et al. (2015) were smaller (maximum length of 9.48 cm) than those of our study, and a higher mass-specific oxygen consumption can be expected in smaller individuals (Bayne & Newell, 1983). Alternatively, differences in VO₂ could be due to variations in the thermal optimum induced by differences in acclimation temperature (Newell et al., 1977; Buxton et al., 1981), which was 20°C in Basso et al. (2015) vs. 14°C in our work. Each of these arguments agrees with the results observed by Hernandis et al. (2022) in P. rudis; the authors found that medium-sized individuals (18.4 cm), acclimated at 18°C, showed an intermediate VO₂ (1.61 mg O₂ g⁻¹ h⁻¹ at 18°C in P. rudis vs. 0.92 mg O₂ g⁻¹ h⁻¹ at 18°C in this study and 1.55 – 3.51 mg O₂ g⁻¹ h⁻¹ at 20°C in Basso et al. 2015a).

The Q₁₀ values of fan mussels were around 2 for both, CR and VO₂, over intermediate temperature ranges (14-18°C and 18-23°C), thus indicating thermal dependency but non-stressful conditions for both variables. Similar Q₁₀ values for CR and VO₂ have been obtained for other bivalves at comparable temperatures. In Mytilus spp. Q₁₀ values below 3 have been obtained with acclimatization temperatures ranging from 10 to 22°C (Widdows, 1973; Van Erkom Schurink & Griffiths, 1992; Barbariol & Razoul, 2000), indicating, occasionally, thermal independence (Q₁₀ ≈ 1) that has been associated with individuals living in intertidal conditions (Eckert et al., 1988). Furthermore, for Ostrea spp. Q₁₀ values near 2 have been observed at temperatures from 10 to 23°C (Rodhouse & O’Kelly, 1981; Sytnik & Zolotniiski, 2014). Over more extreme temperature ranges, fan mussels showed higher Q₁₀ values, indicating stressful conditions at 8.5 and 28°C, as observed for other bivalve species such as Pinctada mazatlantica and Venerupis pullastra at similar temperatures (Albentosa et al., 1994; Saucedo et al., 2004) with Q₁₀ values > 4 that indicate thermal dependence (Zippay & Helmuth, 2012), and suppression of CR at 8.5°C was observed. This suppression of CR at 8.5°C could explain the extremely high Q₁₀ of 56.59 observed over the 8.5-14°C range. It should be noted that individuals were acclimated at 14°C during a 6-month period; this could have affected optimum thermal performance and, therefore show a better fit at 14°C than at other temperatures (Bayne, 2017). From 14 to 23°C, the CR/VO₂ ratio showed lower variability with the relationship between the two variables remaining close to a flat line. However, bigger differences were observed for this ratio at 8.5 and 28°C. CR almost doubled from 23 to 28°C while no significant increase in VO₂ was observed, thus suggesting the implication of anaerobic metabolism for maintaining the energy expenses of this higher filtration activity. Thermal tolerance limits are defined by the upper and lower “pejus temperature” – the limit of optimum hemolymph oxygenation, where processes such as growth and reproduction are negatively affected (Pörtner, 2002). Beyond those limits, the aerobic scope is reduced toward the upper and lower “critical temperatures”, where the occurrence of anaerobic metabolism is necessary to meet the energy metabolism demand (Bayne, 2017). In this ex-
periment, the high increase of CR but not VO$_2$ at 28°C suggests the implication of anaerobic metabolism in supplying the extra energy required; thus, the critical temperature would have been exceeded. In marine invertebrates, anaerobic metabolism in oxygenated waters could imply approach to the threshold thermal tolerance limit (Anestis et al., 2007) with the resistance of individuals becoming a matter of time depending on their starvation tolerance (Pörtner, 2002). Response to extreme temperatures can vary significantly between individuals (Bayne, 2017) and it was observed that two fan mussels drastically reduced their filtration activity, which is considered detrimental (Williams et al., 2016; Bayne, 2017). Similarly, Basso et al. (2015) observed thermal stress at 26°C with higher mortality in stabled $P$. nobilis juveniles compared to lower temperatures of 20 and 23°C during long-term exposure (36 days). Prado et al. (2019) also observed high mortality of fan mussel individuals when temperatures were above 25°C, which triggered infection by *Vibrio mediterranei*. At low temperatures (8.5°C), a clear decrease in CR and VO$_2$ was observed. The lower CR/VO$_2$ ratio at this temperature suggests low gill activity, which could cause starvation and low oxygen uptake (Pörtner, 2002), indicating stressful conditions for the species. In both scenarios, 8.5 and 28°C, a catabolic process could be playing a major role as a response to insufficient energy obtained through respiration, as previously observed for $P$. nobilis individuals under inadequate diets (Prado et al., 2021a). It is important to consider that the inherent stress of captivity could make $P$. nobilis even more vulnerable to high temperatures and associated diseases compared to individuals in the open sea (Dickens et al., 2010). However, no mortality was observed in our experiment. The shorter exposure to each extreme temperature (one week) was probably too short to cause excessive pathogen growth or stress, leading to mortality.

Bivalves are able to acclimate to temperature variations; they usually display short-term response – acute response – in the following hours-days (few hours - 2 days) (Cuison et al., 2005; Peteiro et al., 2018). Subsequently, the compensation process allows the individuals to acclimate to the new temperature, which starts after $\approx$ 3 days (Cuison et al., 2005) and requires several days to achieve ($\approx$ 7 to 21 days) (Bayne, 2017; Peteiro et al., 2018). This acclimation allows to extend the temperature range over which a positive energy balance is achieved. In this sense, Beiras et al. (1995) observed that oyster spat acclimated at 20°C showed a decrease in CR after 1 day at 14°C. However, after 8 and 16 days at this temperature, the individuals were able to compensate the thermal change partially by increasing CR and slightly decreasing the metabolic cost ($Q_{10}$ of 3.55, 2.50, 1.68 respectively for day 1, 8 and 16). Nevertheless, there are limits to acclimation (Bayne, 2017). In our work, one week was considered sufficient to compensate each temperature, almost entirely. An extension of the exposure time would have involved a higher and unnecessary risk for the individuals given the species status. Besides, after one week at each temperature, high $Q_{10}$ ($> 6$) values were still observed at 8.5°C (for CR and VO$_2$) and 28°C (for CR), presumably indicating that fan mussels were hardly able to further physiologically compensate at these temperatures. Furthermore, the results obtained are conservative considering that more extreme temperatures can be found in the paralytic environments where the remaining populations survive, over a temperature range that could reach limits as low as $\approx$ 6°C during winter, and above 30°C in summer (Pérez-Ruzafa et al., 2005; Ramón et al., 2007; Amos et al., 2017; Marques et al., 2019; Tagliapietra et al., 2021); therefore, harder conditions for the fan mussel can be expected at those sites. It is also important to note that individuals could have experienced higher stress compared to that under natural conditions, as regards the speed of increase in temperatures during the experiment. However, 8.5°C was the first temperature tested after a long acclimation period to 14°C; therefore, the accumulated stress at this temperature was lower. As for 28°C, despite the faster increase in temperatures compared to natural conditions, normal physiological results ($Q_{10}$) were recorded at intermediate temperatures (14, 18 and 23°C), with physiological results comparable to those other bivalve species such as $P$. rudis (Hernandis et al., 2022). Although, additional stress due to the experimental design was expected, the main effect could be associated with exposure to extreme temperatures.

Regarding the spawning event observed in all individuals, it could be associated with stress conditions and triggered by the high temperatures (Barber & Blake, 2006). It is relevant to mention that, given the current situation of the species, captivity reproduction should be a priority for the fan mussel, although this is beyond the scope of the current work.

The high mass-specific CR observed in $P$. nobilis coupled with its large size confirms the assumption that fan mussels are outstanding water filters. Besides, if we consider that the species can reach up to 120cm (Zavodnik et al., 1991) in length and that lengths of over 50cm are commonly observed (Garcia-March et al., 2019), higher CR per individual can be expected for much larger adults than those used in this study (≈ 30cm of shell length). The environmental impact in terms of water filtration of fan mussels would be substantial, especially for large populations in confined waters which, in addition, are the only areas with individuals enduring the disease, like the Mar Menor lagoon (Nebot-Colomer et al., 2021; Cortés-Melendreras et al., 2022), the Ebro Delta (Prado et al., 2021b), several coastal lagoons in the Gulf of Lion (Peyran et al., 2021), the Marano lagoon (Curiel et al., 2020) and the Sea of Marmara (Acarli et al., 2022). These paralytic environments are particularly susceptible to eutrophication because of high anthropogenic impacts and lower water renewal compared to the open sea (Kennish & Paerl, 2010); therefore, the relevance of fan mussel water filtration is greater. In Alfacs Bay, where the fan mussels were collected for this study, the estimated population in 2011-2012 was 90,303 individuals (Prado et al., 2014). The bay receives nutrients from agriculture irrigation (Mañosa et al., 2001; Falco et al., 2010), has an approximate volume of 150 hm$^3$ (Jordana et al., 2015) and the water renewal time is 45 days in the inner parts of the
bay, decreasing considerably near the mouth (Cerralbo et al., 2019). Considering the observed CR, we roughly estimate that 2.06% of the Alfas de Bay volume would have been filtered in a month at 23°C.

For the Mar Menor, the largest saltwater lagoon in Europe (598.8 hm²), the water filtration service provided by fan mussels would have been higher (up to ca. 10% of the lagoon volume in a month at 23°C) due to the lower water renewal of 384 days (Umgiesser et al., 2014) and the much larger population reported, with up to 1.6 million of estimated individuals in 2013-2014 (Giménez-Casaldueño et al., 2020). However, the threat of eutrophication is constant (Velasco et al., 2006; Robledano Aymerich et al., 2008) and caused the collapse of the lagoon in late 2015 (García-Ayllón, 2018) resulting in the loss of over 99% of the population (Nebot-Colomer et al., 2021; Cortés-Melendreras et al., 2022).

Unfortunately, these enclosed areas are more prone to extreme temperatures than the open sea because of the prevailing oceanographical conditions (Quintana et al., 2018). In summer, the water temperature in these areas can exceed the 28°C, while temperatures as low as 8°C are also commonly reported during the winter (Yalçın et al., 2017; Marques et al., 2019; Romero et al., 2020; Llorca et al., 2021; Prado et al., 2021b; Tagliafierro et al., 2021). In fact, the harsh conditions of these areas have been pointed out as one of the reasons of the lower life expectancy of fan mussel individuals inhabiting them (García-March et al., 2019). Overreaching the thermal comfort at 8.5 and 28°C could increase the impact of additional environmental pressures, and highlight the critical situation of the species in paralic environments, the remaining reservoirs. All this taking place in a context of climate change, increased heatwaves, and weather extreme conditions in duration and intensity (Cramer et al., 2018; IPCC, 2018; Abbasnia & Toros, 2019), where these environments will be especially affected. These sites are of high importance for the natural recovery of the species through larval export (Kersting, 2020) which could be as well negatively affected by these stressful conditions.

### Implications for Pinna nobilis in captivity

The die-off affecting *P. nobilis* has been associated with *H. pinnae* at temperatures above 13.5°C (Cabanelas-Reboredo et al., 2019; García-March et al., 2020), similar to the mortality of captive individuals by Vibrio infection at temperatures >17 °C (peaked at 25°C) (Prado et al., 2019; Andree et al., 2020; Prado et al., 2020). Given the observed results, a temperature of ≈13°C could be considered as suitable for fan mussel maintenance during disease control. The use of lower temperatures such as 8.5°C is not suitable for the species, although it has been shown to be an effective way of stopping or at least delaying mortality caused by *H. pinnae* (García-March et al., 2020) for a limited period of time.

One of the major complications in captivity maintenance is that it relies on the early detection of ailing individuals. In fact, although concern about the welfare of invertebrates is on the rise, it rarely includes (Horvath et al., 2013; Carere & Mather, 2019) species other than cephalopods (Mather, 2020). But the lack of knowledge on the management and cultivation of Pinnids (Chávez-Villalba et al., 2022) together with the critical situation of the species increases the need to implement welfare conditions for individuals in captivity. Given the actual situation of *P. nobilis*, the development of adequate welfare indicators for the maintenance of individuals in captivity is an urgent issue for conservation biology. Mantle retraction is currently the most commonly used indicator of fan mussel distress, although it might be a late indication of disease and reveal an already severe condition usually close to death of the individual (Prado et al., 2019). Also, a slow gaping response can be understood as a deterioration of health but is complicated by the subjectivity of the observer (García-March et al., 2008), differences of response related to water temperature and/or the duration of the captivity period, as individuals seem to get used to manipulation (authors pers. observation). On the contrary, CR provides an easy quantitative measure for individual monitoring in captivity as an early response and non-invasive method compared to other physiological variables, thus minimizing the stress associated with manipulation before the animal suffers further damage. CR has also been used as a biomarker of organic and inorganic contaminants, salinity, and toxicity caused by microalgae in other bivalves (Toro et al., 2003; Moezzi et al., 2013; Domínguez et al., 2020; Gao et al., 2021). Periodical control measurements could provide valuable data on individual condition and reveal early symptoms of discomfort or disease that allow the implementation of management measures, and the isolation of ailing individuals to prevent the spread of pathogens in the tank. Animals could be separated and placed in individual aquaria, which would enable the measurement of CR without further manipulation. The measurement of V̇O2 could also provide additional information about the underlying processes such as physiological compensation (Kim et al., 2001; Bayne, 2017), although this would require further handling.

**Compliance with Ethical Standards:** The authors declare that they have no conflicts of interest. The individuals were collected with permission from the competent authorities (permission reference: SGPM/BDM/AUTSPP/05/2020).

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