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Variations in thermal performance of cardiac function of pure and hybrid *Mytilus* spp. as a factor influencing hybrid zone dynamics

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Abstract

Interspecific hybridisation within the *Mytilus* complex has been well documented. Distribution patterns of *Mytilu*s congeners in mosaic hybrid zones are hypothesised to reflect differences in their thermal tolerances. In this present study, the relative thermal performance of adult *Mytilus galloprovincialis*, *Mytilus edulis* and their hybrids, from a sympatric population within the Southwest England mosaic hybrid zone was assessed using cardiac function as a proxy for thermal performance. As indices of cardiac performance, the Arrhenius break temperature (ABT), the maximum heart rate, the slope post-ABT and the flat line temperature were used. Examination of the response of cardiac activity under emersion conditions, revealed that parental genotypes had a greater thermal limit of cardiac performance (indexed by ABT); with the ability to sustain cardiac function at temperatures, approximately 6 ^{o}C higher than for hybrids. However, the upper thermal limits of *M. galloprovincialis* and *M. edulis* did not differ between the two taxa. In contrast to previous studies which have described adult *Mytilus* hybrids as intermediate for several physiological traits, this study shows hybrid genotypes have reduced fitness compared to both parental taxa, using cardiac performance as a proxy. These findings suggest evidence of hybrid depression as a result of increased susceptibility to thermal stress, which could influence hybrid zone dynamics via selection against hybrids. Thus, the increased susceptibility to elevated temperatures in hybrids may serve as a postzygotic barrier within the *Mytilus* complex. In light of future environmental change, this could have potential implications on the distribution and structure of pure species and hybrid populations.

Keywords: *Mytilus* complex, Mosaic hybrid zone, Cardiac activity, Thermal performance, Postzygotic barrier

Introduction

Hybrid zones are regions where genetically distinct populations overlap and interbreed, producing progeny of mixed ancestry (Harrison, 1993; Gilg and Hilbish, 2003). The dynamic nature of hybrid zones provides valuable opportunities to study different stages of the speciation process and to understand the mechanisms which impede gene flow (Barton and Hewitt, 1985; Gilg and Hilbish, 2003). The maintenance and stability of hybrid zones is predominately controlled by numerous isolating mechanisms, which affects the abundance and distribution of hybrid individuals and controls gene flow between them and parental species (Jiggins and Mallet, 2000; Shields *et al.,* 2008). Incomplete reproductive isolation can occur pre-zygotically, whereby hybrid zygotes are never formed or post-zygotically, through differential viability of hybrids (Marshall *et al.,* 2002; Beaumont *et al.,* 2004). Determining the relative fitness of parental and hybrid genotypes is fundamental to assessing the maintenance and stability of hybrid zones, as well as for understanding how parental genotypes maintain their integrity (Rolán-Alvarez *et al.,*1997; Shields *et al.,* 2008).

The stability and structure of hybrid populations can be influenced by genotype-environment interactions, as environment dependent selection may lead to differences in the survival of genotypes (Moore, 1977; Springer and Heath, 2007). Consequently, the temporal and spatial variability in the abundance and distribution of hybrid genotypes, will be dependent on the fitness of hybrids relative to parental species (Shields *et al.,* 2008). However, in most systems, estimating hybrid fitness is often impractical either because the ecological characteristics (e.g. growth and reproduction) of the organism makes direct estimates of fitness logistically challenging to obtain (Harrison, 1993; Shields *et al.,* 2008), or because hybrid genotypes are only formed rarely (Wilhelm and Hilbish, 1998). As a result, measurements of fitness of hybrid genotypes, especially of marine organisms has rarely been accomplished (Wilhelm and Hilbish, 1998; Bierne *et al.,* 2002b; Shields *et al.,* 2008).

In the marine environment, hybridisation within species in the Mytilus complex has been extensively studied (Gilg et al., 2013). The two sibling species Mytilus edulis (Linnaeus 1758) and Mytilus galloprovincialis (Lamarck, 1819) occur in sympatric populations on Atlantic coasts of Europe, where they hybridise and introgress (Skibinski et al., 1983; Tolman et al., 2019). Despite extensive hybridisation between the congeners, they still prevail as two incompletely isolated genetic entities, indicating a barrier to gene flow (Skibinski et al., 1983; Bierne et al., 2002b; Rawson et al., 2003). Various pre- and postzygotic mechanisms have been proposed to contribute to reproductive isolation, such as asynchronous spawning (Gardner and Skibinski, 1990), gamete incompatibility (Miranda et al., 2010), assortative fertilisation (Bierne et al., 2006) and hybrid depression (Bierne et al., 2006). Although postzygotic isolation is considered to be one of the most likely mechanisms underpinning the stability and maintenance of hybrid zones, the nature of this process remains to be determined (Barton and Hewitt, 1985; Bierne et al., 2002b, 2006).

Even though Mytilus congeners can hybridise successfully and give rise to viable offspring, previous work has suggested some degree of selection against hybrids in this complex (Gilg et al., 2013). For instance, Bierne et al., (2006) reported high levels of mortality in F2 laboratory raised M. galloprovincialis/M. edulis hybrid larvae. In contrast, hybrids among members of the Mytilus complex have also demonstrated increased

fitness, also known as heterosis or hybrid vigour (Beaumont et al., 1993). For instance, Bierne et al., (2002) identified positive heterosis for hybrid larval growth. Furthermore, adult hybrids of these two species have been found to have intermediate fitness; with lower fitness than M. galloprovincialis but higher fitness than M. edulis, with regard to fertility (Gardner and Skibinski, 1990), viability (Gardner and Skibinski, 1991; Gardner et al., 1993; Wilhelm and Hilbish, 1998), growth rate (Gardner et al., 1993) and parasitism (Tolman et al., 2019). Based on these studies, there is evidence to suggest that there may be extensive variation in the fitness of M. galloprovincialis/M. edulis hybrids across life stages and also across different physiological traits (Beaumont et al., 2004).

For ectothermic organisms, physiological performance is sensitive to variability in ambient temperature variation and is closely related to organismal thermal tolerance (Pörtner et al., 2006). Based on the current geographic distribution and speciation pattern of the Mytilus congeners, their tolerances are expected to differ (Hilbish et al., 2002; Braby and Somero, 2006). M. galloprovincialis, which originates from the Mediterranean (Gosling, 1992), is considered to be the most tolerant of warm environments. Whereas M. edulis is a cold-temperate species, that occupies regions of North America and Europe (Edwards and Skibinski, 1987; Hilbish et al., 1994). Since the taxa typically occupy contrasting thermal regimes, their physiological response to temperature is likely to be a crucial factor governing the capacity of these taxa to exploit their respective habitats (Hilbish et al., 1994). The physiological implications of these contrasting evolutionary histories consist of different thermal performance characteristics, such as differences in heat tolerance of cardiac function (Braby and Somero, 2006; Dowd and Somero, 2013). In consequence, based upon their evolutionary histories with regard to environmental temperatures, it has been suggested that temperature may play a fundamental role in controlling distribution patterns in Mytilus hybrid zones (Braby and Somero, 2006), including those in Britain (Hilbish et al., 2002) and Europe (Bierne et al., 2002b).

M. edulis and M. galloprovincialis form a well-known mosaic hybrid zone in Southwest England, characterised by alternating patches of pure species and hybrid populations (Skibinski et al., 1983; Bierne et al., 2002; Gilg and Hilbish, 2003). This pattern is hypothesised to be evidence of physiological adaptation to localised abiotic environmental conditions (Gardner and Skibinski, 1988; Gardner, 1994; Braby and Somero, 2006). In support of this, allozyme studies within this hybrid zone have observed spatial distributions of alleles at diagnostic loci, suggesting the influence of environmental factors (Gardner and Skibinski, 1988; Gardner, 1994). Temperature, wave exposure (Gardner et al., 1993; Gardner and Skibinski, 1988) and salinity (Skibinski et al., 1983) have been suggested, to be the main abiotic factors determining differences in fitness within this Mytilus hybrid zone (Riginos and Cunningham, 2005; Shields et al., 2008).

The aim of this study was to elucidate the role of temperature in determining blue mussel distribution patterns in hybrid zones, by assessing the physiological performance of adult mussels identified as M. galloprovincialis, M. edulis or hybrids, using heart rate as a proxy for fitness. To address this aim, the secondary contact mosaic Mytilus hybrid zone in Croyde Bay, North Devon was used as a model. This site is representative of the Southwest England hybrid zone (Tolman et al., 2019). Croyde Bay is depicted by size-dependent variation in genotype frequencies, whereby alleles specific to the M. galloprovincialis phenotype increase in frequency with age, due to differences in mortality between the two species (Gardner and Skibinski, 1988; Gardner, 1994). To assess

thermal limits of physiological performance, cardiac responses to elevated temperatures were measured under emersion conditions, which is when mussels typically experience thermal stress in the field (Sorte et al., 2011; Bjelde and Todgham, 2013). Cardiac activity has been demonstrated to be an effective index for whole organism physiological performance (Hochachka and Somero, 2002), for a variety of intertidal molluscs (Stenseng et al., 2005; Braby and Somero, 2006; Bjelde and Todgham, 2013). Given that hybrids have been shown to be intermediate between the two parental taxa across various physiological traits (Hilbish et al., 1994; Tolman et al., 2019), it was predicted that hybrids would exhibit intermediate cardiac thermal tolerance. Moreover, since the M. galloprovincialis phenotype is in favour of selective mortality within this hybrid zone and the warm-adapted evolutionary history of this species, it was predicted that M. galloprovincialis would be able to maintain cardiac function at higher temperatures compared to M. edulis.

Materials and Methods

Animal collection and maintenance

Mussels from a population including *M. galloprovincialis*, *M. edulis* and their hybrids, were collected from the low shore of Croyde Bay in North Devon, United Kingdom (51.1346° N, 4.2342° W) during low tide. Low rates of introgression have been found in the population at this site (Gardner and Skibinski, 1988; Gardner *et al.,* 1993). Based on initial morphological identification, *M. galloprovincialis* (Lamarck, 1819), *M. edulis* (Linnaeus, 1758) and putative hybrids were sorted into roughly equal numbers, within a size range of 28-34 mm external shell length. Genotype frequencies for marker allozyme loci are approximately equal between the parental taxa, within this size range (Gardner and Skibinski, 1988; Tolman *et al.,* 2019).

Mussels were then returned to the laboratory, where their shells were cleaned of epifauna and then they were assigned randomly to six 10 L aquaria containing aerated seawater at preexposure conditions (temperature: 15 ^{o}C, salinity: 36, 12 h:12 h L:D cycle) for three weeks. Mussels were fed with a commercial bivalve diet (Shellfish Diet 1800 Reed Mariculture, United Kingdom) daily and half the tank volume was exchanged with seawater three times a week, cleaning out accumulated pseudofeces. Prior to assessing cardiac performance, mussels were putatively identified as *M. galloprovincialis*, *M. edulis* or hybrids based on morphological characteristics of their shells. Genetic identification was carried out subsequent to the assays, consequently final sample sizes were reduced.

Assessment of cardiac activity and thermal performance assays

Cardiac activity was monitored non-invasively via plethysmography, which has been previously used with mollusks and other intertidal organisms (Burnett *et al.,* 2013; Bjelde and Togham, 2013). A CNY70 sensor (Vishay Intertechnologies), comprising of an infrared emitter and phototransistor was attached to the dorsal side of each mussel's shell, directly over the pericardial sac (Moyen *et al.,* 2019), using Blue-Tack (Bostik, United Kingdom). The IR sensor was connected to an amplifier (AMP-O3, Newshift, Leira, Portugal) and a USB-6009 (National Instruments) was used to convert the signals into a digital format. Throughout the ramps, heart rate data was recorded every 5 min at a sampling rate of 40 Hz, using the software DAQExpress (National Instruments).

After sensor attachment, each mussel was placed in a separate glass beaker (volume = 50 mL), covered with parafilm and placed in a programmable water bath (GRANT GP200, Grant Instruments, United Kingdom). Five mussels from the same species were tested simultaneously in each ramp. Temperature ramps (*n* = 20 per genotype) were conducted in air and began with a 20 min hold at 15 ^{o}C to minimise post-handling stress. Afterwards, temperature was increased gradually at a constant rate of 10 ^{o}C h^{-1} until all mussels' hearts had stopped beating (38-41 ^{o}C). A wide range of heating rates have been used in previous studies to determine thermal limits; with similar studies using heating rates between 6 ^{o}C h^{-1} and 13 ^{o}C h^{-1} (Dong and Williams, 2011; Logan *et al.,* 2012; Tagliarolo and McQuaid, 2015; Moyen *et al.,* 2019). Air temperature was recorded continuously via a thermocouple (Omega HH806AV) which had been inserted into an empty mussel shell and secured with cyanoacrylate glue (Loctite, United Kingdom). After the completion of assays, mantle tissue (<2 mg) was dissected from each mussel and stored at -20 ^{o}C for genetic identification.

Genetic identification

Congeners of *Mytilus* are difficult to distinguish due to similarity in morphological traits, so all mussels were genotyped post-experimentation (Braby and Somero, 2006). The diagnostic marker *Glu-5'* has been used extensively for identifying species within the *Mytilus* complex (Wood *et al.,* 2003; Tolman *et al.,* 2019). The *Glu-5'* locus amplifies a different sized fragment for *M. edulis* (180 bp) and *M. galloprovincialis* (126 bp). A *M. edulis/M. galloprovincialis* hybrid amplifies both sized fragments (120 bp and 180 bp). DNA was extracted from <2 mg of mantle tissue using the HotSHOT protocol. Tissue was digested in 100 µL of alkaline lysis solution (25 mM NAOH and 0.2 mM disodium EDTA) at 95 ^{o}C for 30 min and cooled on ice for 5 min, after which 100 µL of neutralising solution (40 mM Tris-HCL) was added. PCR reactions were carried out in a 12.5 µL volume containing: 0.75 µL DNA, 6.25 µL 2x MyTaq Mix (Bioline) and 0.25 µL of each primer: *Me15* (5′-CCAGTATACAAACCTGTGAAGA-3′) and *Me16* (5′-TGTTGTCTTAATAGGTTTGTAAGA-3' (Inoue *et al.,* 1995). The cycling conditions included an initial denaturation step at 94 ^{o}C for 5 min, followed by 30 cycles of 94 ^{o}C for 30 s, 56 ^{o}C for 30 s, 70 ^{o}C for 1 min 30 s and 72 ^{o}C for 5 min. PCR products were visualised on a 2.5 % agarose gel, stained with SYBR Safe.

At the Glu-5' locus it is possible for backcrosses to appear homozygous (Tolman *et al.,* 2019). However, low rates of introgression have been found in the population used in this study (Gardner *et al.,* 1993), therefore giving adequate assurance in the markers ability to detect pure and hybrid individuals.

Cardiac performance analysis

Automating counting of heartbeats has been found to introduce errors in previous studies, due to the variability in the heartbeat signals within individuals (Burnett *et al.,* 2013). However, manual counting can also be questionable, with regard to practicality and reliability (Kaufmann *et al.,* 2011). On account of this, heart rates were approximated using the software ARTiiFACT (Kaufmann *et al.,* 2011), which combines automated peak detection with manual detection of artefacts. Manual correction was applied after automatic detection, so that miscounting could be avoided, whereby small sections of the signal were evidently abnormal, reflecting movement of the mussels instead of heartbeats. One heartbeat was characterised by the time between two large peaks. Sometimes there will be one to two peaks after the largest peak, which together

represent one heartbeat (Burnett *et al.,* 2013; Moyen *et al.,* 2019). The heartbeats were counted for 60 s at every 1 ^{o}C and expressed as beats min^{-1}. Data acquired using this method can vary in quality (Burnett *et al.,* 2013), thus, any recordings of individuals with undetectable or fragmented heartbeats were removed from analysis.

The cardiac performance of parental and hybrid genotypes under ramping increases in temperature was assessed by four indices (1) the highest temperature which caused a rapid decline in heart rate (Arrhenius break temperature, ABT) (2) the temperature at which heart rate had completely ceased (Flatline temperature, FLT) (3) the maximum heart rate recorded (MaxHR) at final ABT and (4) the slope of the decline in heart rate following the ABT (PostABT). Upper critical temperatures of heart rate were determined via Arrhenius plots, as described previously (Stillman and Somero, 1996). Arrhenius plots were generated (ln bpm versus reciprocal temperature (K); Fig. 1) and the inflection point was identified as a peak in heart rate, followed by a rapid decrease in slope (Bjelde and Todgham, 2013). Two regression lines of best fit were drawn over the data and the intersection of the increasing and decreasing slopes was used to determine the ABT in degrees Celsius (Stenseng *et al.,* 2005; Bjelde and Todgham, 2013). Calculations of these indices were informed by segmented regression analysis, using the package "Segmented" (Muggeo, 2008).

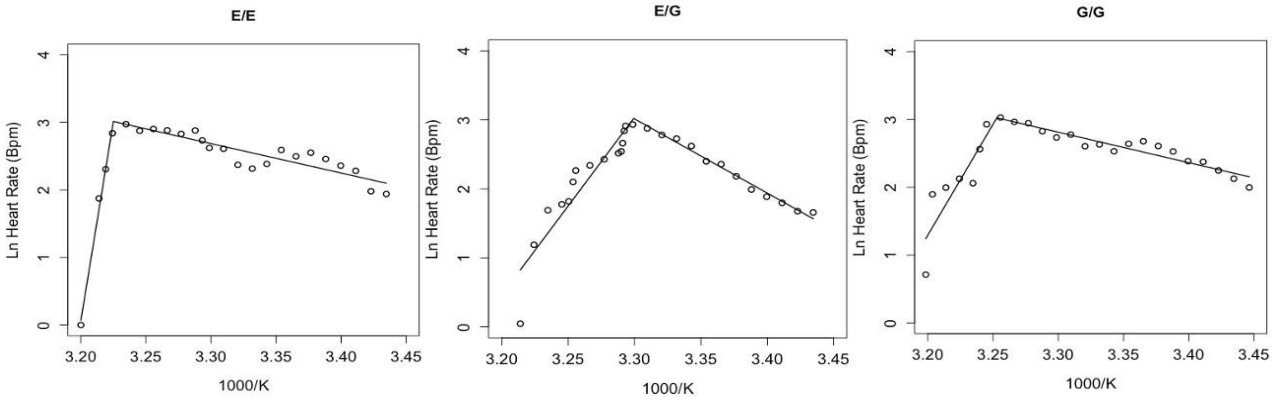

Fig. 1: Representative examples of Arrhenius plots of individuals with a single break in cardiac performance, identified as *Mytilus edulis* (E/E), hybrid (E/G) or *Mytilus galloprovincialis* (G/G). Regression lines are plotted using segmented regression analysis and the intercept of the two lines represents the Arrhenius break temperature (ABT).

In some individuals, multiple breaks in heart rate were detected during elevated temperatures (Fig. 2), which has also been documented in mussels (Braby and Somero, 2006; Moyen *et al.,* 2019) and other intertidal organisms such as *Lottia digitalis* (Bjelde and Todgham, 2013). Breaks in heart rate were defined as any inflection of the Arrhenius plots, whereby heart rate steadily decreased and then increased again until the final break in heart rate, expressed as the upper critical limit of cardiac function (ABT) (Pasparakis *et al.,* 2016). The total number of breaks in heart rate (1-2) per individual mussel were calculated using the same regression analysis as for ABT. For individuals with two break points, the final break point in cardiac performance, before a rapid decline in heart rate was used in subsequent statistical analysis (Fig. 2).

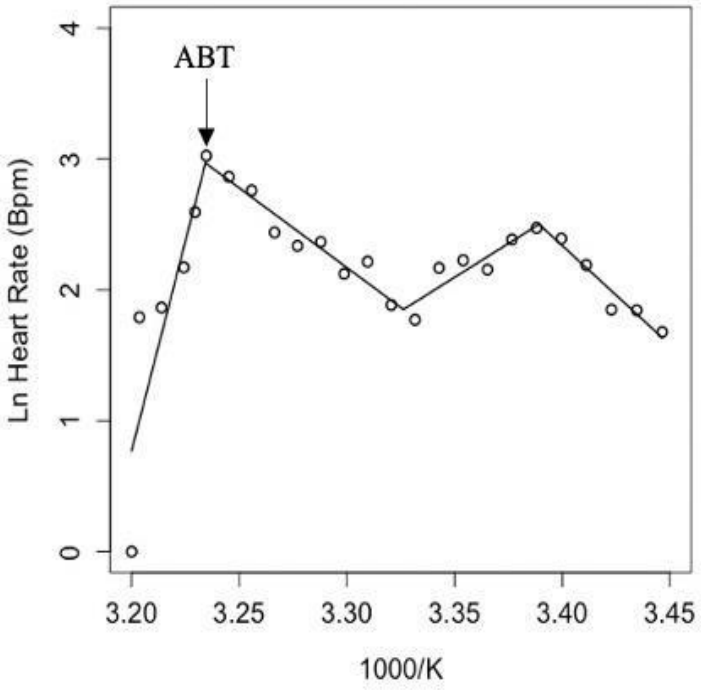

Fig. 2: Example of an Arrhenius plot, of an individual identified as *Mytilus edulis* with two breaks in cardiac performance. Regression lines are plotted using segmented regression analysis. Arrhenius break temperature (ABT) is shown as the final break in cardiac performance, before a rapid decline in heart rate.

Statistical analysis

Analysis of co-variance (ANCOVA) was used to test for a batch effect, this had no significant effect on the cardiac performance indices measured and was therefore not included as a covariant for further analysis. To determine where cardiac performance indices (ABT, Post-ABT Slope and MaxHR) differ between parental and hybrid genotypes, one-way ANOVAs were performed. This was followed by Tukey's HSD multiple comparisons test at ($P < 0.05$), to discern the differences among genotypes. In order to test whether multiple breaks in cardiac performance affected final ABT, a two-way ANOVA was conducted with genotype and number of breaks as fixed factors and final ABT as the response variable. A Kruskal-Wallis rank test was used to analyse FLT. Assumptions of homogeneity of variance and normality were met following Levene's test and Shapiro-Wilks ($P > 0.05$), unless stated otherwise. Statistical analyses were conducted using RStudio, v. 1.2.5042 (R Core Team, 2020).

Results

The upper critical thermal limit of cardiac function as indexed by ABT, differed among parental and hybrid genotypes (ANOVA, $F(2,13)= 9.029$, $P =0.003$; Fig.1a). According to the Tukey HSD post hoc test, G/G ($P=0.005$) and E/E ($P =0.009$) were able to maintain cardiac function at significantly higher temperatures, compared to their hybrids (E/G), which had a mean ABT approximately 6 °C lower than for parental genotypes. However, there were no differences in ABTs observed between G/G and E/E ($P > 0.05$). Furthermore, there was no independent effect of the number of breaks on ABT (Two-way

ANOVA, $F(1,11)= 0.020$, $P > 0.05$; Fig. 4) and no interactive effects of genotype and number of breaks in cardiac performance on ABT (Two-way ANOVA, $F(1,11)= 0.226$, $P > 0.05$; Fig. 4).

By contrast, FLT was not significantly different across genotypes (Kruskal-Wallis, $c^2 = 0.187$, df = 2, $P > 0.05$; Fig.1b). However, a similar trend to ABT (Fig. 3a) can be observed, whereby some hybrids experienced cessation of cardiac function (FLT) at lower temperatures than parental genotypes. There was a significant difference in the gradient of decline in heart rate after ABT among genotypes (ANOVA, $F(2,13) = 4.716$, $P = 0.029$; Fig. 3c), with E/G having a greater mean slope in declining heart rate in comparison with a more gradual decline of heart rate for G/G ($P = 0.023$). In addition, there were differences among genotypes in the MaxHR the mussels exhibited during the ramping protocol (ANOVA, $F(2,13)= 4.716$, $P = 0.044$; Fig. 3d). Whilst final ABTs between E/E and G/G were similar (35.7±1.59 °C vs 35.9±0.65 °C, respectively), G/G had a significantly lower MaxHR than E/E ($P = 0.046$) when cardiac function collapsed (16.53±1.24 bpm vs 20.73±0.66 bpm).

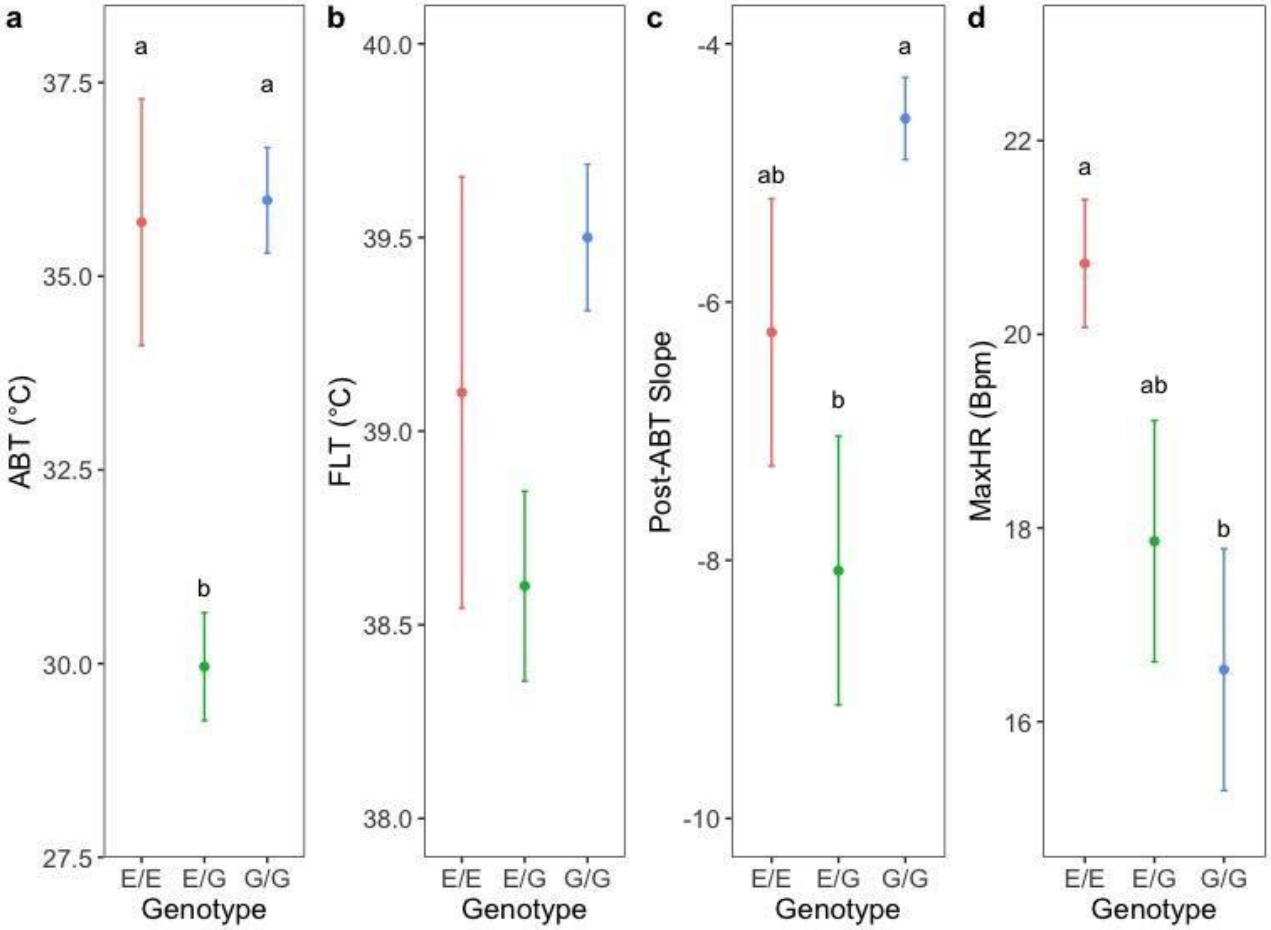

Figure 3: Cardiac performance indices of members of the *Mytilus* complex under a constant temperature ramp (10 oC h-1) a) Arrhenius break temperature (ABT (oC)) b) Flatline temperature (FLT (oC)) c) Gradient of decline in heart rate after final ABT (Post-ABT) d) Maximum heart rate (Max HR (Bpm)) of mussels identified as *M. edulis* (E/E, n = 5), *M. galloprovincialis* (G/G, n =7) and their hybrids (E/G, n = 5). Error bars represent mean ± SE and letters above error bars represent a significant difference between genotypes (P < 0.05).

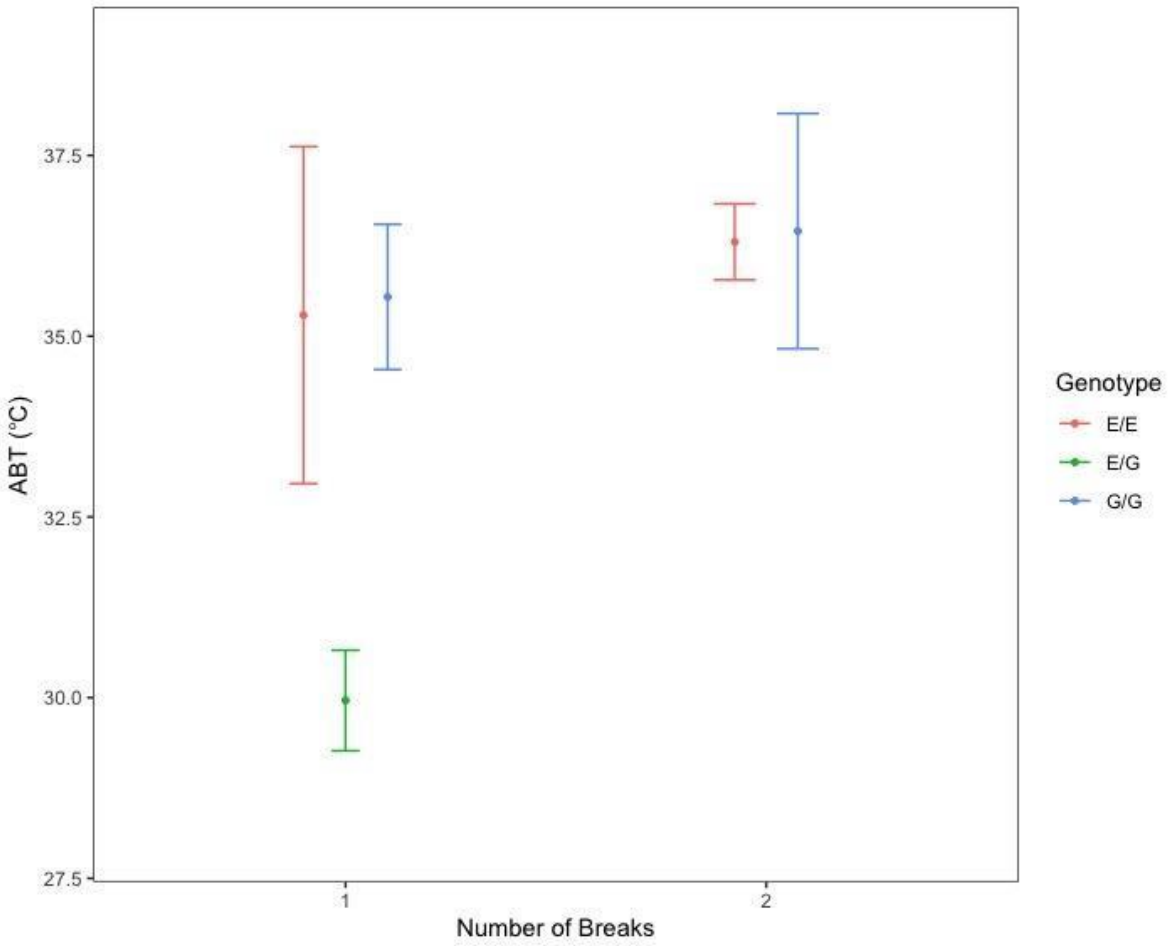

Figure 4: Arrhenius break temperatures (ABT) in heart rate separated by the total number of breaks in heart rate that mussels identified as *M. edulis* (E/E, one: *n* = 3, two: *n* = 2), *M. galloprovincialis* (G/G, one: *n* = 5, two: *n* = 2) and their hybrids (E/G, one: *n* = 5) exhibited throughout a constant temperature ramp (10 ^{o}C h^{-1}). Error bars represent mean ± SE.

Discussion

In this study, the relative thermal performance of *Mytilus galloprovincialis, Mytilus edulis* and their hybrids was assessed using cardiac performance indices. The results showed that cardiac responses varied among genotypes. Mussels identified as hybrids exhibited a lower thermal limit of cardiac function relative to both parental species, suggesting that hybrids are more susceptible to thermal stress. In contrast to predictions, the upper thermal limits of cardiac function did not differ between *M. galloprovincialis* and *M. edulis*, which indicates that they both have a similar capacity to cope with elevated temperatures.

To cope with the pervasive effects of thermal stress during emersion periods, intertidal organisms have evolved physiological adaptations that enable them to maintain performance and survival (Hochachka and Somero, 2002; Olabarria *et al.,* 2016). Given the divergent evolutionary histories of the congeners to contrasting thermal regimes, their adaptive strategies are expected to differ (Hilbish *et al.,* 1994, 2002). In comparison with previous studies (Hilbish *et al.,* 1994; Braby and Somero, 2006), the results from this study did not indicate a distinct physiological advantage of *M. galloprovincialis* genotypes over *M. edulis* at elevated temperatures. However, there are notable differences in their

response to thermal stress which suggests that they are employing physiological mechanisms that differ. For instance, individuals identified as *M. edulis* demonstrated 1.25-fold higher heart rates in order to withstand temperatures as high as *M. galloprovincialis*. Cold-adapted species typically demonstrate intrinsically higher rates of cardiac function than warm-adapted species, in order to cope with the effects of temperature (Lockwood and Somero, 2011). This concurs with findings of Braby and Somero (2006), conducted within the Californian hybrid zone, whereby, under elevated temperatures, *M. galloprovincialis* was found to exhibit a lower intrinsic rate of cardiac activity relative to *M. edulis* and *M. trossulus.* Thus, differences in maximum heart rate between the two parental taxa is likely reflective of different adaptive thermal strategies, that are in line with the evolutionary history of *M. galloprovincailis* to warmer conditions (Hilbish *et al.,* 2002; Lockwood and Somero, 2011).

In the Southwest England hybrid zone, adult *M. edulis/M. galloprovincialis* hybrids often exhibit an intermediate fitness level, usually with higher fitness than *M. edulis* (Gardner, 1994; Hilbish *et al.,* 1994; Gardner, 1996; Wilhelm and Hilbish, 1998; Tolman *et al.,* 2019). In contrast, the findings from this study indicate that hybrid genotypes have reduced fitness relative to both parental species, using cardiac performance as a proxy. The increased susceptibility of hybrids to thermal stress suggests evidence of hybrid depression, which has previously been observed in larval viability and performance studies. For example, the larval success of hybrid crosses has been shown to be lower than for parental species (Beaumont *et al.,* 1993; Bierne *et al.,* 2002a). Furthermore, in *M. edulis* and *M. galloprovincialis* hybridisation trials, Beaumont *et al.,* (2004) reported slower growth in hybrid veliger larvae compared with pure species, when exposed to acute elevated temperatures. Hybrid depression may occur as a result of hybrid offspring being not as well physiologically adapted to varying environmental temperatures in comparison to parental species, who have acquired adaptive differences that increase their fitness in their current environment (Charlesworth and Willis, 2009; Lamare *et al.,* 2018). Hybrids also demonstrated a more rapid rate of decline in heart rate after ABT, which was considered as a collapse in cardiac function (Bjelde and Todgham, 2013). This indicates that hybrids were not able to control cardiac activity after their ABT. In addition, some hybrid individuals experienced cessation of heart rate at lower temperatures, which further implies that the cardiac activity of hybrids is comparatively more compromised than parental genotypes at elevated temperatures.

Some individuals identified as *M. galloprovincialis* and *M. edulis* exhibited variable patterns in cardiac activity during elevated temperatures, characterised by multiple breaks in cardiac performance; whereby heart rate would rapidly decrease, but shortly recover and increase again before ABT (Pasparakis *et al.,* 2016). Breaks in cardiac activity, aside from the break point at the upper critical temperature threshold has not been routinely documented within the literature (Pasparakis *et al.,* 2016). However, this pattern has been observed in some intertidal molluscs (Braby and Somero 2006; Bjelde and Todgham 2013; Pasparakis *et al.,* 2016). For example, *Echinolittorina malacanna* demonstrated periodic heart suppression in response to warming between 30 ^{o}C and 45 ^{o}C (Marshall *et al.,* 2011). As suggested by Bjelde and Todgham (2013), multiple breaks in cardiac performance could be a strategy to conserve energy at elevated temperatures, through which heart rate can vary between periods of thermal sensitivity and insensitivity (Marshall *et al.,* 2011).
In support of this, numerous studies have suggested that ectothermic organisms are able to regulate physiological activity to a considerable extent (Bjelde and Todgham, 2013;

Tagliarolo and McQuaid, 2015). It is notable that the number of breaks in cardiac performance had no effect on the ABT of either species, thus, in this case it does not seem to be a physiological mechanism for increasing upper temperature tolerance. Contrastingly, hybrids of the two parental taxa did not exhibit this pattern. This may be due to the fact that they have lost the capacity to regulate cardiac activity. Given that oxygen consumption was not measured at the same time as heart rate, it is not possible to determine if metabolic rate also decreased when heart rate decreased. Therefore, in order to elucidate the adaptive significance and mechanisms underlying this potential energy conservation strategy, future studies should aim to measure cardiac responses alongside other physiological variables, such as anaerobic and aerobic metabolic products.

The *Mytilus* population at the low shore of Croyde Bay exhibits size-dependent variation in genotype frequency, with directional selection in favour of *M. galloprovincialis* (Gardner and Skibinski, 1988; Gardner *et al.,* 1993). Based on the findings from this study, differences in cardiac performance between *M. galloprovincialis* and *M. edulis* under thermal stress, within the chosen size range, does not explain the size-selection mortality that occurs within this hybrid zone. This suggests that there must be other factors which are contributing to viability differences. Previous work has indicated that this could be based on factors such as the stronger immune response of *M. galloprovincialis* to bacterial infection, at lower metabolic cost than *M. edulis* (Tolman *et al.,* 2019) and the energetic advantage of *M. galloprovincilais* over *M. edulis* at elevated temperatures (Hilbish *et al.,* 1994).

The maintenance of pure genotypes within the Southwest England hybrid zone, suggests that there must be some degree of lower fitness of hybrid genotypes at the postzygotic stage, as gene flow is not occurring freely (Barton and Hewitt,1985; Gardner, 1997; Bierne *et al.,* 2002b). It has been suggested that both parental species maintain their genetic integrity, despite extensive hybridisation, as a result of adaptation to contrasting environments (Gardner, 1996). The reduced fitness observed among hybrid genotypes under thermal stress could serve as a postzygotic barrier, which may contribute to maintaining reproductive isolation between the *Mytilus* congeners (Hilbish *et al.,* 1994). Variations in cardiac function capacities among genotypes, may play a fundamental role in determining environmental optima, tolerance limits and thus, distribution patterns within hybrid zones (Braby and Somero, 2006; Somero, 2011).

In light of the findings from this study, this raises questions about the potential impacts of climate change on *Mytilus* hybrid zones. Changes in environmental conditions, such as projected increases in global sea surface temperatures (IPCC 2014) and prevalence and severity of heatwaves (Olabarria *et al.,* 2016) may alter the outcome of hybridisation, by affecting the relative fitness of pure species and hybrids (Chunco 2014). The fitness of hybrids is usually environmentally dependent, with hybrids outperforming parental species in some environments, but not others (Barton and Hewitt 1985). Thus, the dynamics of hybrid fitness is likely to be particularly sensitive to changes in environmental conditions (Chunco, 2014). Given the susceptibility of hybrids to thermal stress, global warming may impact *Mytilus* hybrid zone dynamics via extrinsic selection against hybrid individuals.

Conclusions

In summary, this study provides evidence for physiological differentiation between pure and hybrid genotypes with respect to temperature, with hybrids having a lower cardiac tolerance to elevated temperatures relative to both parental species. This finding further adds to previous studies, which have suggested some degree of selection against hybrids within the *Mytilus* complex. In order to understand the underlying mechanisms of hybrid zone maintenance and dynamics, assessing the performance of hybrids is essential. Given the potential implications of climate change on hybrid zone dynamics, a greater understanding of the thermal physiology of hybrid progeny is required to predict the future of hybridisation within the *Mytilus* complex.

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