Exploring HSP90 as a Biomarker for Marine Heatwaves in *Pinctada maxima*

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Many species, particularly marine organisms, are becoming more vulnerable to marine heatwaves due to climate change. Marine species anticipate perishing during marine heatwaves, but there is a growing interest in learning why some can resist. Using *Pinctada maxima* as a model species, we were able to clone a full-length cDNA encoding *HSP90* with a calculated open reading frame of 2031 residues of amino acids and a molecular mass estimate of 78.08 kD to understand better the effects of marine heatwaves on the *HSP90* gene expression in pearl oysters. The sequence of amino acids in *P. maxima HSP90* was quite similar to the *HSP90* families of *Pinctada fucata martensii*. At 32°C and 36°C, the expression of *PmHSP90* significantly expressed and reached its highest level at 6 h, implying that in pearl oysters' response to acute marine heatwaves, *HSP90* expression rapidly increased. Pearl oysters' temperature response was relieved, as best demonstrated by the dramatically reduced expression levels of *PmHSP90* in the frequently reoccurring marine heatwaves event. Using these findings, it is possible to predict acute and repeated marine heatwaves in pearl oysters using *P. maxima HSP90* as a molecular biomarker.

Keywords: extreme weather events, marine heatwaves, biomarker, heat shock proteins, bivalve mollusks

INTRODUCTION

Since the early twentieth century, the frequency, intensity, and duration of marine heatwaves (MHWs), characterized as discrete, long-lasting, abnormally warm water episodes in the ocean, have increased significantly (Oliver et al., 2018). Amongst the most crucial elements influencing marine life and ecosystems is the combination of the atmosphere's weather patterns and the ocean's currents, waves, and current patterns (Hobday et al., 2016). "The blob" was a prime example of marine heatwaves in the Northeastern Pacific Ocean between 2014 and 2016. It is more than 3°C higher than the average sea surface temperature and exceeds annual SST records by four standard deviations (Miyama et al., 2021). There have been numerous MHWs events in the last decade, with devastating ecological and socioeconomic consequences, including off the coast of the northwest Atlantic, the Tasman Sea, and Western Australia (e.g., Chen et al., 2014; Oliver et al., 2017;

Benthuysen et al., 2018). The shelf seas off the coast of China are among the world's most decadent in marine life and fisheries (e.g., Belkin, 2009; Wu et al., 2012; Oliver et al., 2018; Li et al., 2019); The China's marginal seas have been more vulnerable to and have been significantly affected by more frequent, intense, and longer-lasting sea surface temperature extremes in the past decade, as illustrated by Belkin (2009). The South China Sea, rich in fishing grounds, has recently been identified as a "hotspot" for warming oceans (e.g., Li et al., 2019; Deng et al., 2020; Yao et al., 2020). Benthic invertebrates have been decimated, and seagrass meadows (Marbà and Duarte, 2010) have been lost due to these MHWs events (Garrabou et al., 2009).

Among the most critical marine bivalve species in the tropical regions of Asia is the pearl oyster *Pinctada maxima*. *P. maxima* aquaculture and fishing have progressed slowly over the last three decades due primarily to high juvenile mortality rates (Xu et al., 2021). Furthermore, *P. maxima's* pearl quality suffers greatly. Temperature, salinity, and pH fluctuations in seawater directly impact *P. maxima's* biomineralization and growth rates (Deng et al., 2013; He et al., 2021). The Indo-Australian archipelago, the South China Sea, and other areas where *P. maxima* are found in large numbers are essential receptacles of marine biodiversity that are now under threat from MHWs (Lind et al., 2007; Yao et al., 2020).

Understanding how pearl oysters respond and adapt to pervasive seawater temperature anomalies like MHWs is critical in this era of increased ocean warming. Marine bivalves can activate various defense mechanisms in response to environmental stressors. Some of these defense mechanisms include heat shock proteins (HSPs), which can rapidly increase expression in response to stressful conditions (Gao et al., 2008). In more detail, HSPs are a protein family that cells produce in response to stress. This grouping is based on their molecular weight and sequence homology. These families include low molecular and high molecular weight HSPs (Lindquist and Craig, 1988; Heikkila, 2010). Initially, they were referred to as "heat shocks" (Ritossa, 1962). The heat shock factor is primarily responsible for inducing the dramatic regulation of HSPs during the heat shock response (Wu, 1995). As molecular chaperones, they help to prevent the deterioration of proteins and cells in the presence of stressful conditions. According to reports (Buchner, 1999), as much as 1% of the total soluble protein in some cells is *HSP90*. Heat stress necessitates the examination of HSP90 (Tanaka and Nakamoto, 1999). According to research (Obermann et al., 1998), HSP90's ATPase activity is required to function in eukaryotes. It may be possible to understand the different responses of species to heatwaves by looking at how the molecular reactions allow them to acclimate to thermal stress. Some stress responses can increase the upregulation of heat shock proteins at the molecular level to protect biomolecules from thermal stress (Leung et al., 2019).

Therefore, to understand the physiological responses and protective mechanisms of *P. maxima* when exposed to MHWs, we explained the potential contributions of *HSP90* against MHWs varying in duration, frequency, and intensity. These findings will significantly improve our knowledge of how pearl oysters react to MHWs with variable time, frequency, and severity.

MATERIALS AND METHODS

Species and Study Region

Throughout the Indo-Pacific Ocean coastal regions, the pearl oyster, *P. maxima*, is an important bivalve species. Large-scale pearl cultivation has taken place in China and Australia in order to produce the sought-after South Sea pearl (FAO, 2014). The Indo-Australian Archipelago and the Beibu Gulf are home to a vast expanse of *P. maxima*, which is an important repository for marine biodiversity that is currently threatened by MHWs (Lind et al., 2007; Liu et al., 2014; Yao et al., 2020). Fishing grounds around the Beibu Gulf in the South China Sea have recently been identified as "hot spots". Because of an increase in the severity and duration of MHWs as of late, the region has suffered greatly (Deng et al., 2020; Yao et al., 2020). There was a 26% and 14% increase in the reoccurrence and severity of MHWs impacting Beibu Gulf in 2017. As a result, the coral reefs and fisheries in the area suffered long-term damage (Zhang et al., 2020).

Collection and Acclimatization of Animals

P. maxima were raised in cages of 30 cm x 30 cm and 0.5 m water depth 34.69, 5.25 mm length; 3.33 g weight). It was collected from Liusha Bay, South China Sea, and thoroughly cleaned off any shell surface epibionts when they arrived at the lab. On the next day, flow-through systems were used to acclimate them to lab conditions, seawater temperature of 24°C, the salinity of 31, pH 8.2, and dissolved oxygen concentration (6.0mg/L) all differed significantly between Liusha Bay's seawater and that of the ocean. There was no death prior to the experiment.

Design of Experiment

Hobday et al. (2016) broad definition of MHWs was applied in this study to limit MHWs duration conditions. In our experiment, 24°C was used as the control group and 28, 32, and 36°C as the experimental groups. According to the report in the Beibu Gulf, the mean annual temperature is 24°C, and the other three temperature regimes are based on data collected by instruments during MHWs (Green et al., 2019; Xu et al., 2022). It was utilized through constructing two scenarios that mimicked MHWs occurrences' ephemeral and intermittent character in the real world by employing acute and recurrent MHWs (**Figure 1**). 150 pearl oysters were randomly assigned to one treatment in three 50-L tanks. 50 pearl oysters were acclimated in 3 tanks and then distributed at random. Each tank was replenished every 2 or 3 days with pre-equilibrated seawater to maintain good water quality. A ratio of 20,000 *Chlorella* spp. cells were supplied to pearl oysters. Each tank was equipped with an electrical thermostat to maintain water temperature. The results of temperature monitoring and mortality during the experiment can be found in the study by Xu et al. (2021).

The gills of 5 individuals from each group were chosen at random to be exposed to acute MHWs for 6 h, 12 h, 24 h, and
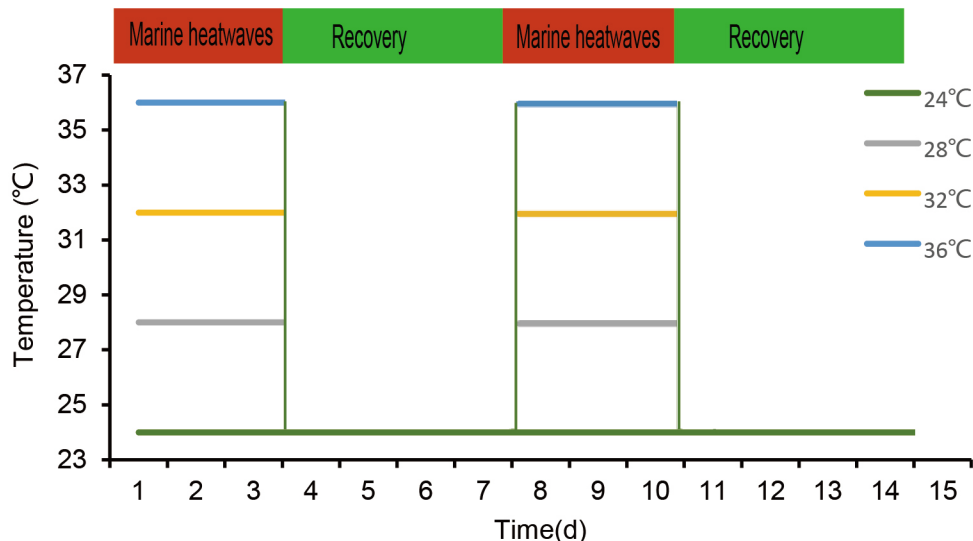
FIGURE 1 | An illustration of the study design demonstrating MHWs events with varied frequencies and intensities. The green line represents the temperature regime specified in the control, whereas the grey, yellow, and blue lines represent the 28°C, 32°C, and 36°C temperature regimes specified under MHWs conditions, respectively.

72 h. The same sample approach was applied for long-lasting MHWs at 3 days, 7 days, 10 days, and 14 days. After quick freezing in liquid nitrogen, store at -80°C before being analyzed.

RNA Extraction and cDNA Synthesis

Total RNA was extracted following the instructions of Trizol Reagent (Invitrogen, USA). A NanoDrop ND1000 UV light source spectrophotometer and a 1% agarose gel electrophoresis was used to measure the RNA's concentration, purity, and integrity. Creating templates with (5', 3') RACE accompanying Amplification kit was the SMARTerTM RACE cDNA; RNaseH generates RT-qPCR cDNA templates. *PmHSP90* partial sequence of was retrieved from database analyzing the transcriptome, and Primer Premier 5 software was used to designed customized

primers (**Table 1**). Polymerase chain reaction amplification was performed to verify the intermediate fragment, and RACE technology was used to obtain 3'- and 5'-end sequences. Electrophoresis in 1% agarose gel was used to detect the amplified products. The purification and recovery kit isolated and purified target gene fragments then linked them to the, PMD19-T vector thereafter converted them to DH5 cells. LA (containing ampicillin Apm+) was used to select the positive cloned bacteria, and Sangon Biotech was used to sequence them. The PCR fragment (3' 5')-RACE sequences were aligned to construct the nucleotide with the complete sequence of the presumed *HSP90* cDNA from pearl oysters. Before doing cluster analysis, the collected sequences were amplified to ensure accuracy.

TABLE 1 | *PmHSP90* primers used in the study.

Primer	Primer sequence (5'-3')	A
HSP90-F	AATACTTGCGATACTACTCCTCAC	Middle clone
HSP90-R	GACTGATGTCTAATGATTGGC	Middle clone
5'-HSP90-outer	GCAATCTCGTCAAAGAGTTCTACACAC	5'RACE
5'-HSP90-inner	GGCAAGTCCTCAGAGTCTACAACACC	5'RACE
3'-HSP90-outer	AAGTAAACCCTGACCATTCCATC	3'RACE
3'-HSP90-inner	GAGACACAAACAACCGAGACAAC	3'RACE
UPM (long)	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	RACE
UPM (short)	CTAATACGACTCACTATAGGGC	RACE
NUP	AAGCAGTGGTATCAACGCAGAGT	RACE
M13-47	CGCCAGGGTTTTCCCAGTCACGAC	PCR detection of colony PRC
M13-48	AGCGGATAACAATTTCACACAGGA	PCR detection of colony PRC
HSP90-qF	AAAAGGTGGTGGTATCCAACAGG	RT-PCR
HSP90-qR	TGATGGAATGGTCAGGGTTTACTT	RT-PCR
βactin-F	CGGTACCACCATGTTCTCAG	Reference gene
βactin-R	GACCGGATTCATCGTATTCC	Reference gene

Bioinformatics Analysis

DNAMAN spliced *PmHSP90's* full-length sequence. Utilization of the NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/orffinder/), amino acid sequence and ORF of *PmHSP90* were predicted. Physicochemical parameters were decided using the Expasy-protparam (http://web.expasy.org/protparam/). It is used to analyze the hydrophilicity of amino acids using an online tool Expasy-ProtScale (http://web.expasy.org/protscale/). (PSITE-Search) Prosite patterns with statistical analysis can be used to predict amino acid sub-basic functional sites. SignalP4.1 predicted amino acid sequence of signal peptides, and SWISS-MODEL (https://swissmodel.expasy.org/interactive) generated the three-dimensional structure from which SMART (http://smart.embl-heidelberg.de/) was used to discover domains. *PmHSP90* sequences were compared using Clustal W and NCBI BLAST was used determine the nucleotide and protein sequence similarity. The phylogenetic tree was constructed using the neighbor- joining method MEGA X software.

Analysis of Relative Quantitative Real-Time PCR

Total RNA was extracted using Trizol reagent according to the manufacturer's instructions. PrimeScript RT Reagent Kit was used to generate cDNA. The RT-qPCR was performed using the Roche LightCycler 480 equipment and the SYBR(R) Ex TaqTM Premix. The comparative Ct method ($2^{-\Delta\Delta Ct}$) was used to analyze the relative expression of these genes by taking *β-actin* as an endogenous control.

Statistical Analysis

This study used SPSS 22.0 to validate the research data. Shapiro and Wilk employed these two F-tests to assess the data's normality and homogeneity. We also utilized two-way ANOVA to investigate how different MHWs scenarios affect expression levels (*P*<0.05).

RESULTS

Sequence Analysis of *PmHSP90*

RACE technology cloned the cDNA of *PmHSP90* gene with a 2383 bp length, a 2031 bp ORF, with 676 amino acids encoded, a (5'- 3') UTR, 25 bp, and 352 bp respectively (**Figure 2**). PmHSP90 was predicted to have a 78.08 kDa molecular weight and a 4.94 theoretical isoelectric point. 76.26 as the PmHSP90 protein aliphatic index, and 34.69 as the instability index, suggesting that it is a stable protein, according to ExPASy-ProtParam analysis. PmHSP90 has an ATP/GTP-binding site (IGQFGVGFYSAYLVAD). According to predicted amino acid sub-basic functional sites, three *HSP90* protein family signatures (LGTIAKSGT, IKLYVRRVFI, GVVDSEDLPL) and the characteristic cytoplasmic motif MEEVD were identified. A hydrophilic protein's average coefficient of hydrophilicity (GRAVY) is -0.811. PmHSP90 has an *HSP90* domain at positions 142- 667, according to SMART analysis (**Figure 2**).

Homologous and Structural Analysis

Table 1s displays the species information from the various alignments and the phylogenetic tree. Based on amino acid sequence alignment, the multiple sequence alignment of PmHSP90 as well as other bivalves HSP90 reveals that HSP90 has substantial homology among bivalves. *Pinctada fucata martensii* HSP90 has 95.41 percent homology with PmHSP90 (**Figure 3**). According to the evolutionary tree, PmHSP90 patterns with bivalves mostly resembling *P. fucata martensii* and *P. penguin*, whereas mammals, and fish are further away (**Figure 4**).

mRNA Expression in Different Tissues

PmHSP90 distribution and expression levels were assessed in the tissues using RT-qPCR in normal conditions. Hepatopancreas, and gills had the higher levels of *PmHSP90* expression, respectively, in *P. maxima*, while the adductor muscle had the lowest levels of *PmHSP90* expression (**Figure 5**).

mRNA Expression After Marine Heatwaves Stimulation

When comparing the experimental groups to the control groups during the acute exposure, the expression level in gills was significantly higher in the groups, notably those at 36°C.In the 28°C groups, the expression level of *PmHSP90* reached its maximum at 24 h after being stimulated by the MHWs; in the 32°C groups, it reached its maximum at 6 h after being stimulated by the MHWs (**Figure 6**). The analysis of variance results demonstrated how respectively the temperature of the MHWs and the duration of stress had a significant impact on gene expression (*P*<0.05), and that their combination had a significant impact (*P*<0.05) as elaborated in (**Table 2**). Upon reoccurring MHWs exposure, revealed that the level of *HSP90* expression was lower than that observed in the first MHWs, as well as the fold alterations between experimental and control groups were less than in the initial MHWs (**Figure 7**). The analysis of variance demonstrated, the MHWs' temperature as well as the period of stress had a significant impact on gene expression (*P*<0.05), as did their interaction (*P*<0.05) (**Table 2**).

DISCUSSION

Recently, the intense, frequent, and longer-lasting MHWs have been emerging as a significant threat to marine bivalves and the ecosystems they support (Deng et al., 2020). Previous studies have demonstrated the impacts of MHWs on pearl oysters and indicated the rapid acclimation of this species to various MHWs events (He et al., 2021; Xu et al., 2021; Xu et al., 2022). The rarity of heatwaves in nature has limited research into how they affect marine ecosystems, but what is known is that they have significant effects on marine life in a relatively short time. Nonetheless, organisms' adaptive and recovery capacities, which allow them to cope with thermal stress such as oxygen consumption, and filtration rate (Viergutz et al., 2007; Zhao et al., 2019; Marroni et al, 2021), have been largely overlooked
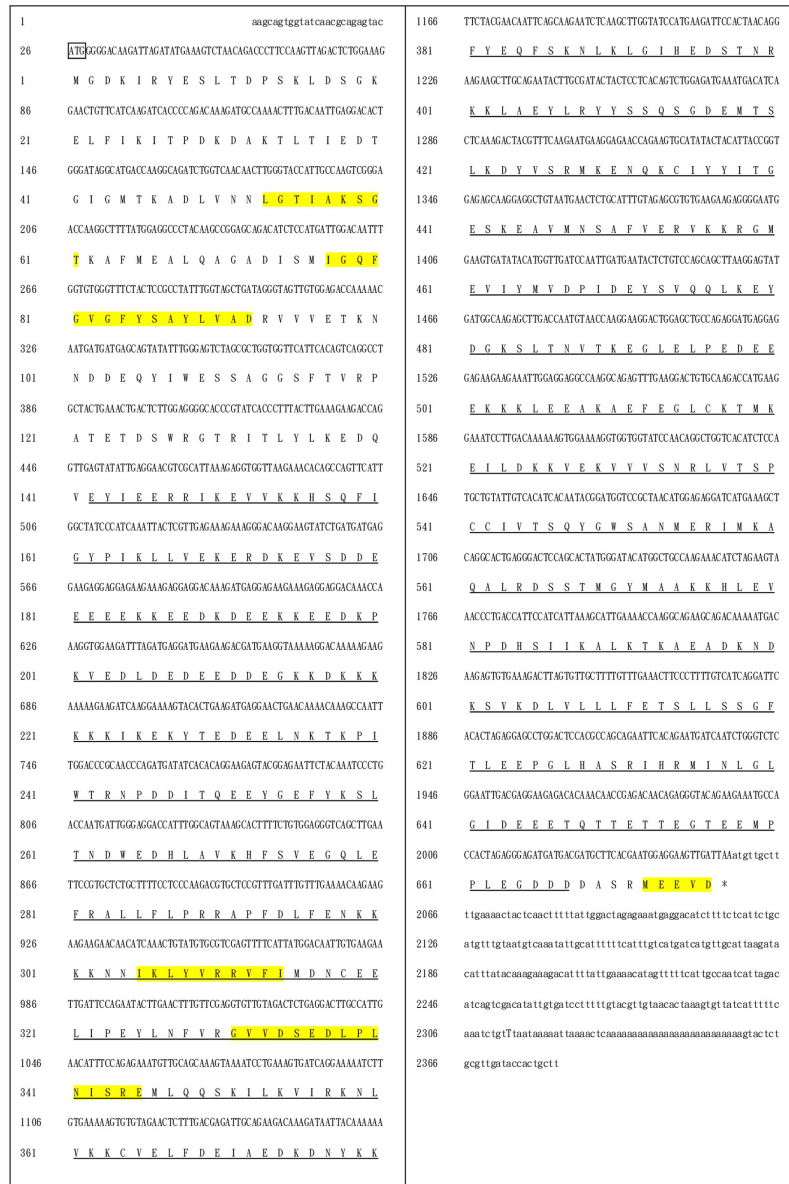
FIGURE 2 | *Pm-HSP90* nucleotide sequence analysis. The 5' and 3' UTRs are denoted by small letters, while the deduced amino acid sequences are indicated by capital letters. The 5'-UTR start codon was in a box, and the stop codons (TAA) were labeled with an asterisk (*). The HSP90 characteristic motif was highlighted on a yellow background, and the underlined indicates the HSP90 domain.

(Leung et al., 2019). In the current study, we used RACE technology to clone the full-length *PmHSP90*. The expression pattern of *HSP90* molecular mechanisms responses of pearl oysters to MHWs was analyzed.

Levels of HSP90 in Different Tissues

The quantification of the expression pattern analysis of *HSP90* was revealed in the adductor muscle, gills, hepatopancreas, and mantle tissues of *P. maxima* under no MHWs condition (**Figure 5**). *HSP90* was commonly expressed in all examined tissues, implying that *HSP90* was necessary for cellular balance.

However, the extent to which they were told differed by tissue (Rungrassamee et al., 2010). There was primarily an expression of *HSP90* in the hepatopancreas and gills. The level of expression significantly increased, and this matched the study's findings of Cheng et al. (2019), explaining that the gills are both respiratory organs and feeding organs because bivalves are filter feeders; as a result, they are better able to detect changes in their surroundings than other tissues. In addition to creating reactive oxygen species and xenobiotic detoxification, the hepatopancreas plays a vital role in immunological defense, digestion, absorption of nutrients,
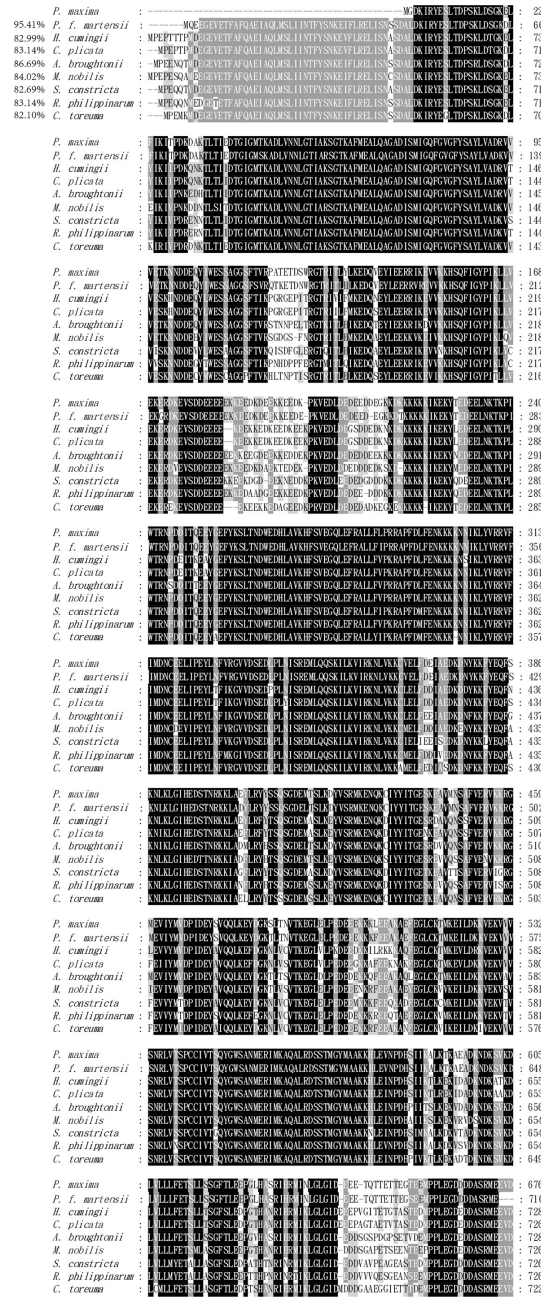
FIGURE 3 | The deduced amino acid sequences of HSP90 from *P. maxima* and other organisms were aligned multiple times. Black indicates complete homology, grey indicates homology more significant than 80%, and white indicates homology greater than 60%.

and the release of hormones (Chaurasia et al., 2016; Park et al., 2020). Because of its metabolic activity, the hepatopancreas is susceptible to environmental alterations (Rungrassamee et al., 2010; Qian et al., 2012). However, gill tissue was further explored because the gills are involved with both respiration and ciliary suspension feeding hence exploring the gills tissue under acute and recurrent MHWs could fully investigate HSP90 as a biomarker for MHWs.

Levels of HSP90 Following Acute MHWs

In the current study, we examined the analysis of the expression pattern of *HSP90* in *P. maxima* under acute (**Figure 6**) and recurrent (**Figure 7**) MHWs scenarios. We found a significant upregulation in the expression levels of *HSP90* over the entire course of MHWs compared to the control period, revealing that *HSP90* plays a vital role in indicating organisms that are going
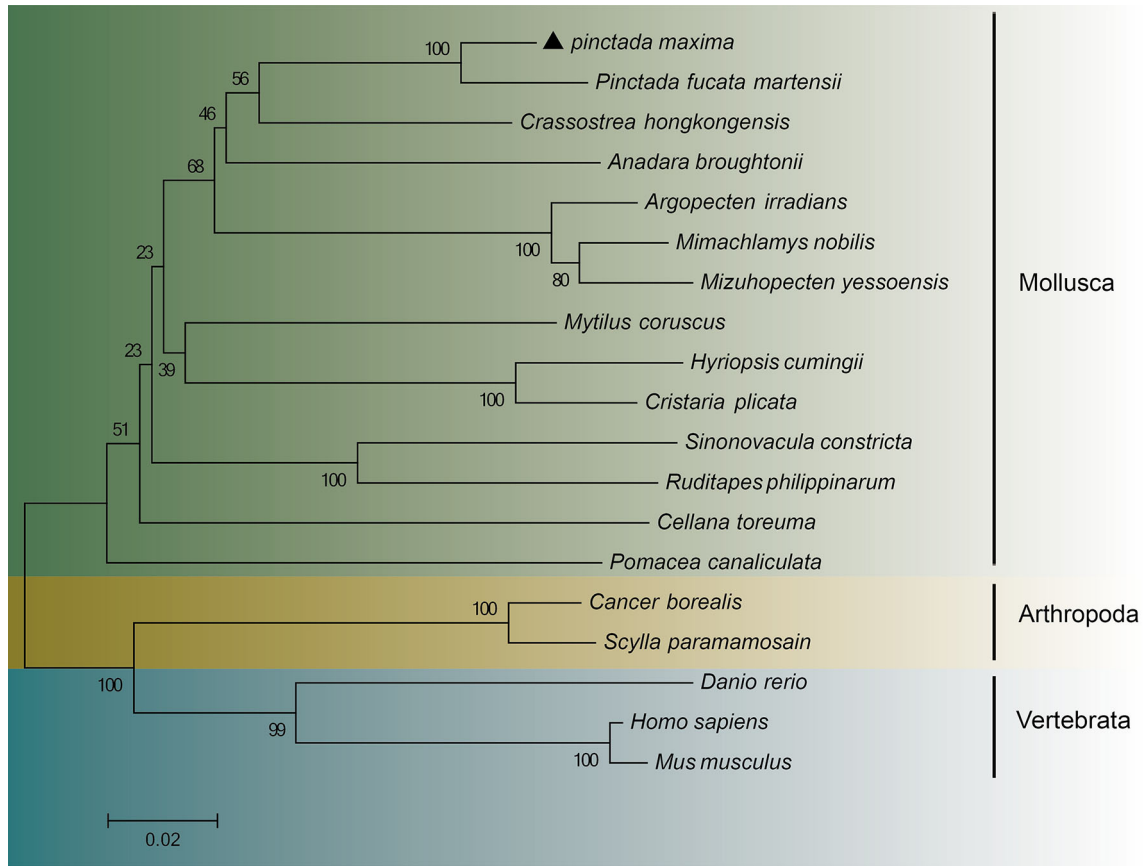
FIGURE 4 | A phylogenetic tree based on amino acid sequences deduced from the HSP90 gene of *Pinctada maxima* and other species, constructed using the neighbor-joining method. *"Pinctada maxima"* was labeled "▲". **Table 2** displays the GenBank accession numbers for all HSP90.

through stress. *HSP90* is a chaperone that plays a role in several processes. The up-regulation of *HSP90* is a defense mechanism because HSP90 could be attached to misfolded or damaged proteins to return them to their shape in their originality (Ramaglia, 2004; Gao et al., 2008).

Currently, there is *HSP90* relative expression at different time points in gill tissue during acute MHWs. After exposure to thermal stress at 36°C, up-regulation of *HSP90* was identified in the gills within the first 6 h, maximum expression level peaked at 6 h after the heat exposure, and in contrast, after the heat exposure at 12h–72h (**Figure 6**), *HSP90* expression declined continuously in the gills, A similar study explains that it could be as a result of modifying biochemical reactions and energy resource allocations involved in protein synthesis, such as catalase and other HSP (Lin et al., 2018). This expression indicates that HSP90 may be vital in responding to acute temperature stress. Protein folding relies on HSP90, one of the systems that protect eukaryotic and prokaryotic cells from heatwave stress. (Wandinger et al., 2008). Previous research has also revealed similar trends in the data in crustacean heat
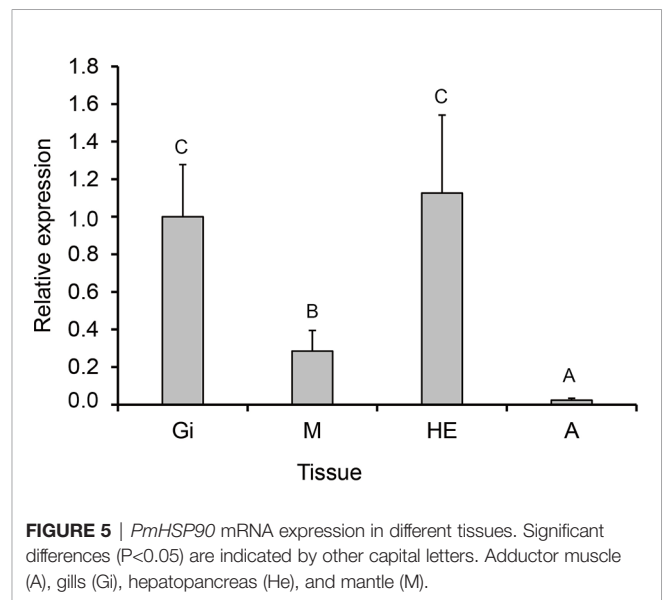

FIGURE 5 | *PmHSP90* mRNA expression in different tissues. Significant differences (P<0.05) are indicated by other capital letters. Adductor muscle (A), gills (Gi), hepatopancreas (He), and mantle (M).
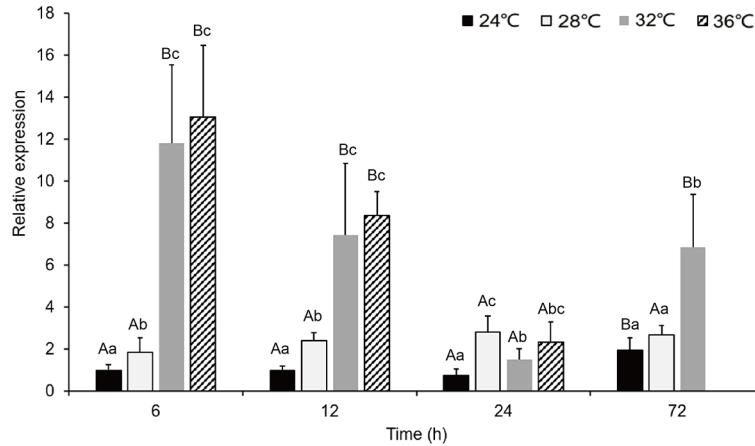
FIGURE 6 | The expression of *PmHSP90* mRNA in *P. maxima* gills after acute MHWs event. capital letters indicate significant differences between four temperature treatments (P<0.05), and small letters indicate significant differences between four temperature levels each time. The experiment was halted because pearl oysters exposed to a temperature anomaly of 36°C suffered mortality after 24 h.

TABLE 2 | The relative expression of HSP90 in pearl oysters exposed to acute and recurrent MHWs scenarios was studied using two-way ANOVA.

Scenarios	Factors/interactions	df	F	P
Acute MHWs	Temperature	3	94.418	0.000
	Time	3	30.465	0.000
	Temperature*time	8	11.402	0.000
Recurrent MHWs	Time	3	23.477	0.000
	Temperature	2	14.952	0.000
	Time*temperature	6	7.485	0.000
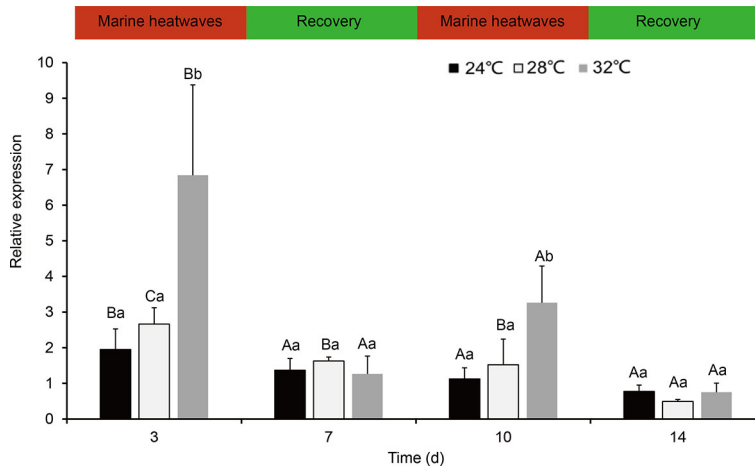
FIGURE 7 | The expression levels of *PmHSP90* mRNA in P. maxima gills following repeated exposure to MHWs event. Capital letters represent significant variations between distinct periods within each temperature treatment, whereas small letters dnote significant differences within each interval of time, between four distinct temperatures (P<0.05). It was discontinued after 24 hours because pearl oysters exposed to a 36°C temperature anomaly suffered mortality.

shock proteins (HSPs) such as *HSP90* of *Fenneropenaeus chinensis, HSP90* of *Penaeus monodon HSP40*, and *HSP90* of *Marsupenaeus japonicus* shown to have thermal tolerance (Ju-Ngam et al., 2021). Farcy et al. (2007) reported that environmental temperature fluctuations could activate stress responses in muscle cells. Kim et al. (2009) also explained that there were up-regulation patterns of *HSP90* when subjected to acute temperature elevation. To the extent that heat stress reaction to MHWs was alleviated, implying that *P. maxima* can adapt quickly to heat stress after acute MHWs exposure (He et al., 2021; Xu et al., 2021; Xu et al., 2022).

Levels of HSP90 Following Recurrent MHWs

A high-temperature environment stimulates a change in the relative expression of HSP90 in *P. maxima*. *HSP90* in the gills of *P. maxima* showed to increase significantly after three days of repeated heat stress, but the *HSP90* in the gills decreased rapidly when the duration of heat stress was prolonged after three days (**Figure 7**). The findings are consistent with those of Rungrassamee et al. (2010), which reported that *HSP90* expression steadily decreased to the same level as in the untreated condition during the recovery phase following heatwave stress. The relative expression of *LvHSP90* increased at 6 h, peaked at 24 h, and then fell progressively to the control at 96 h. (Qian et al., 2012). These results indicate that the heat tolerance of the gills of *P. maxima* is time-limited and that it induces *HSP90* expression to alleviate heat stress. In a previous study, high temperatures could cause the synthesis of *HSP90* mRNA and *HSP90* protein to protect cells (Lei et al., 2009). However, this long-term stress may result in permanent cell damage. *P. maxima* response during constant exposure and recovery from MHWs suggest that metabolic exchange is essential in stress responses and acclimatization (He et al., 2021). However, it is pretty costly to maintain protein structure under pressure; increased HSP expression in long-term stress has repercussions (Sørensen et al., 2003). Its chronic heat stress has been shown to cause downregulation patterns detected in aposymbiotic larvae (Meyer et al., 2011).

Furthermore, heat stress accelerates metabolism. Thus, more ATP might have led to a continuation of HSP90 consumption. HSP90 protein expression is reduced when the consumption of HSP90 is greater than the synthesis of HSP90 (Yu et al., 2021). Interestingly, further research on the molecular basis of how HSP90 was activated under heat stress revealed that trans activations of heat shock proteins were mediated *via* the heat shock factor 1 (HSF1) and heat shock elements (HSE) interactions (Wu, 1995). Inactive HSF1 is localized in the cytosol, but upon heat stress (Kim et al., 2005), translocates to the nucleus and is directly coupled to large structural rearrangements in *HSP90* regulated through inhibition, HSF1 activation increases *HSP90* expression (Lackie et al., 2017). For some reason, HSF1 appears to be released from the activator by *HSP90* when the temperature rises slightly (Fabbri et al., 2008). This demonstrates that HSP90 could be further explored under combined stressors such as MHWs and hypoxia.

Future Exploration

The current study also opens up the possibility of a future investigation of transgenerational effects as a result of *HSP90* expression in different tissues under MHWs being inherited as a result of persistent exposure, which is based on a recent review by Zhao et al. (2020), according to which acidic exposure causes a considerable increase in metabolism in clams with no parental exposure to low pH, possibly shifting the energy requirement for calcification and slowing shell growth (Leung et al., 2020). The current study paves the way for further research into the combined consequences of global warming, MHWs, and ocean acidification on bivalves (Wang et al., 2015; Zhao et al., 2018). More research is needed to understand better how MHWs and ocean acidification interacts to harm individual organisms and the entire ecosystem. This knowledge could also lead to further research on bivalve plasticity, particularly *P. maxima*, in the heat shock response to MHWs. In one of the few cases, *Mytilus edulis* transplanted from the Baltic to the North Sea showed stress protein levels similar to North Sea mussels (Tedengren et al., 1999). Experimenting with *Nucella ostrina* whelks in experimental cages exposed and protected from waves and a location with reduced quality of prey revealed HSP70 expression that changed over time and between different ecosystems and locations (Dahlhoff et al., 2001). The above mentioned future explorations could lead to a better understanding and new scientific discovery of how MHWs impacts bivalve species, *P.maxima* in particular.

CONCLUSION

The study concludes that *HSP90* was expressed in different tissues of the pearl oyster *P. maxima HSP90* was directly implicated in the sensitivity to temperature fluctuations. The analysis of the *HSP90* expression pattern in *P. maxima* under acute and recurrent MHWs studies revealed the production of *HSP90* in *P. maxima* gill tissues, which aids in acclimation and survival against MHWs. The distribution of the *PmHSP90* expression provides essential information for analyzing its biological function in *P. maxima*. *HSP90* expression in *P. maxima* is a possible biomarker because it can be utilized as a physical assessment in stress risk prediction. Early stress identification allows for better therapy selection and evaluation of therapeutic approaches. After all, MHWs will continue to influence ecosystems regardless of whether steps are taken to address some of their causes. It is critical to understand how pearl oysters will respond to future environmental stressors such as MHWs.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

FM: Methodology, Investigation, Writing- Original draft preparation YX: Methodology, Investigation. GH: Methodology. FL: Methodology. XL: Methodology, Formal analysis. KY: Resources, Formal analysis. RM: Methodology. JL: Methodology. YD: Conceptualization. LZ: Conceptualization, Writing-original draft preparation, Project administration. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.913920/full#supplementary-material

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