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# Insights into small Heat Shock Proteins: Drivers of environmental stress tolerance in selected animal species

*Review Article*

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*Abstract:*

Insects, nematodes, and aquatic animals face several biotic and abiotic stressors that can significantly affect their fitness – specifically damaging their cellular protein function. As a result, they have evolved sophisticated stress-responsive mechanisms. Certain endogenous proteins, the small Heat Shock Proteins (sHSPs), are proposed to maintain the stability and function of proteins under stress. Since the identification of the first sHSPs, an increasing number of sHSPs, mainly due to the new robust sequencing tools, continue to be identified and reported to play a critical role in the response of organisms to stress. This review explores and summarizes the contributions of the sHSPs implicated in the stress response of different animal species in unique environments. Understanding their function is crucial for advancing our knowledge of how different animal species adapt to harsh environments while maintaining cellular homeostasis.

*Key words:*
abiotic stress, biotic stress, aquatic animals, cell damage, insects, nematodes, resistance

*Apstrakt:*

**Uvidi u male proteine toplotnog šoka: pokretači tolerancije na sredinski stres kod odabranih životinjskih vrsta**

Insekti, nematode i vodeni organizmi izloženi su brojnim biotičkim i abiotičkim stresorima koji mogu značajno uticati na njihovu prilagođenost, posebno narušavajući funkciju ćelijskih proteina. Kao odgovor na to, razvilio su sofisticirane mehanizme adaptacije. Među ključnim endogenim proteinima, mali proteini toplotnog šoka (sHSPs) igraju ključnu ulogu u očuvanju stabilnosti i funkcije proteina pod stresom. Od identifikacije prvog sHSP-a, sve veći broj biva otkriven zahvaljujući naprednim sekvencirajućim alatima, a njihov značaj u stresnom odgovoru organizama postaje sve jasniji. Ovaj rad istražuje i sumira ulogu sHSP-a u prilagođavanju različitih životinjskih vrsta specifičnim ekstremnim uslovima. Razumevanje njihove funkcije ključno je za dublji uvid u mehanizme kojima se organizmi prilagođavaju nepovoljnim sredinskim faktorima, istovremeno održavajući ćelijski balans.

*Ključne reči:*
abiotički stres, biotički stres, insekti, nematode, otpornost, oštećenje ćelija, vodeni organizmi

## Introduction

Insects, nematodes, and aquatic animals are regularly exposed to biotic and abiotic stressors such as increasing temperature, salinity, and heavy metal accumulation that can significantly affect their physiology, growth and development, behavior, and survival (Häder et al., 2020; Mugwanya et al., 2022; Viitasalo & Bonsdorff, 2022). Changes in stressors above an organism's threshold can disrupt its intracellular proteins and consequently induce physiological stress, cell damage, and death (Basha et al., 2012; Mugwanya et al., 2022). For example, in the fruit fly *Drosophila virilis*, exposure of pupae to heat stress delayed their sexual maturity, whereas in adults, it caused sterility within seven days post-exposure (Walsh et al., 2021). In the face of climate change, stressors will likely become more acute or chronic and, therefore, have more impact on organisms (Guillén et al., 2022). In a simulation experiment, increased heatwaves (frequency and severity) reduced the chemosensitivity of bumblebees to sense and locate plants as their food source (Nooten et al., 2024). This can, consequently,

lead to the decline of the bumblebee population/ colony and food security (Nooten et al., 2024).

While stressors pose significant challenges to organisms, they also drive evolutionary adaptations. Organisms have evolved and can employ either behavioral adaptations to avoid or escape the stressor or physiological adaptations to endure the stressor better (Colinet et al., 2015; King & MacRae, 2015). As a physiological response, cells synthesize small heat shock proteins (sHSPs) that function as chaperons by binding to the partially folded or denatured substrate proteins, preventing them from irreversible folding/denaturation or enhancing their correct functional foldings (Gething & Sambrook, 1992; Feder & Hofmann, 1999; Sørensen et al., 2003; Basha et al., 2012; González-Tokman et al., 2020). These proteins have both constitutive and stress-inducible members (Colinet et al., 2010b), with a low molecular weight (typically 12 – 42 kDa) and a highly conserved α-crystallin domain (ACD) consisting of 80 – 100 amino acids (Morris & Aquilina, 2010). Under stress conditions, some sHSPs can accumulate and reach up to 1% of the total cellular proteins (Sun et al., 2002). Unlike other heat shock proteins (HSP) families, sHSPs do not require ATP to perform their chaperone activity (Miernyk, 1999). Thus, they can act rapidly in response to stress, and their function is less costly energy-wise.

The chaperone role of sHSPs is not only during stress exposure but also during normal development. sHSPs have been implicated in various biological processes such as cell growth, differentiation, lipid membrane polymorphism (Tsvetkova et al., 2002; Sun & MacRae, 2005; Eisenhardt, 2013), diapause (Gkouvitsas et al., 2008), and lifespan (Morrow et al., 2004). For instance, sHSP p26 in brine shrimp (*Artemia sp*.) has been reported to confer resistance to *Artemia* embryos exposed to stressors, e.g., extreme temperatures, prolonged anoxia, and desiccation during encystment and diapause (Qiu et al., 2006). It is noteworthy that in all developmental stages, p26 is expressed exclusively in the embryo stage (Willsie & Clegg, 2001) and constitutes 10 – 15 % of the total yolk protein. While the chaperone function/mechanism of p26 is reported to be by the formation of ~500KDa oligomers (Willsie & Clegg, 2002; Hibshman et al., 2023), other *in vitro* studies conducted with transfected mammalian cells cultured under stress show that it could be through apoptosis inhibition (Villeneuve et al., 2006). Transfected mammalian cells have exhibited enhanced thermotolerance (Villeneuve et al., 2006), and resistance to oxidative damage induced by hydrogen peroxide (Willsie & Clegg, 2001).

The high evolutionary conservation observed in sHSPs across nematodes, insects, fish, and other organisms underscores their importance in fundamental cellular processes. Their amino acid sequence alignment of sHSPs reveals how these proteins are conserved across these taxa. In nematodes, particularly in *Caenorhabditis elegans*, the conservation of sHSPs is evident. For instance, multiple amino acid sequence alignment of CeHsp12 using MAFFT v7.*490* algorithm (Katoh et al., n.d.; Katoh & Standley, 2013) in Geneious Prime software 2024.0.7 showed over 86% mean pairwise similarity across various species within the *Caenorhabditis* genus (**Fig. 1),** similarly, in fish, such as *Carassius sp., Cyprinus sp., Sinocyclochellus sp., Ctenopharyngodon sp.,* and *Culter sp.,* the sHSP family is well-conserved, with Hsp20 showing over 95% pairwise similarity in sequence alignment (**Fig. 2**). Moreover, in insects, *Amyelois transitella,*

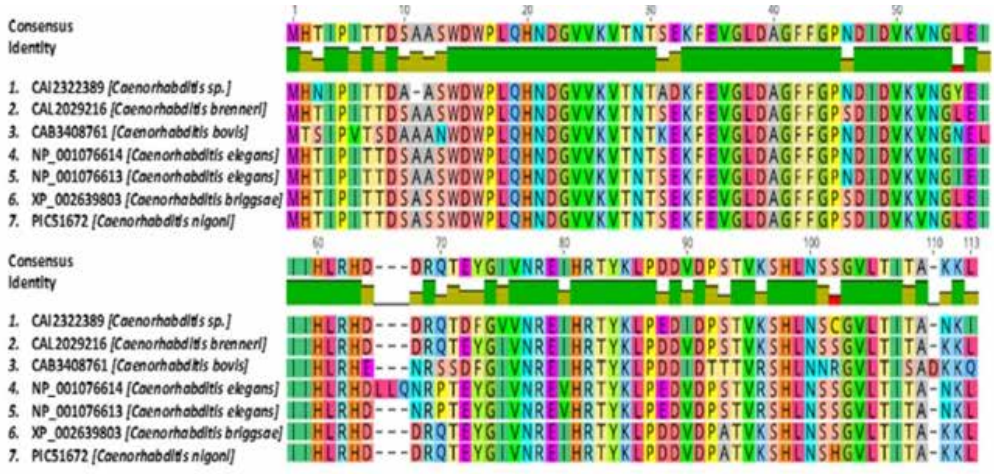


**Fig. 1.** Amino acid sequence alignment of CeHsp12 among different nematode species. The alignment was performed using the MAFFT algorithm in Geneious Prime software 2024.0.7. The mean pairwise identity over all pairs in the column is depicted-green: 30% to 100% identity; red: <30% identity

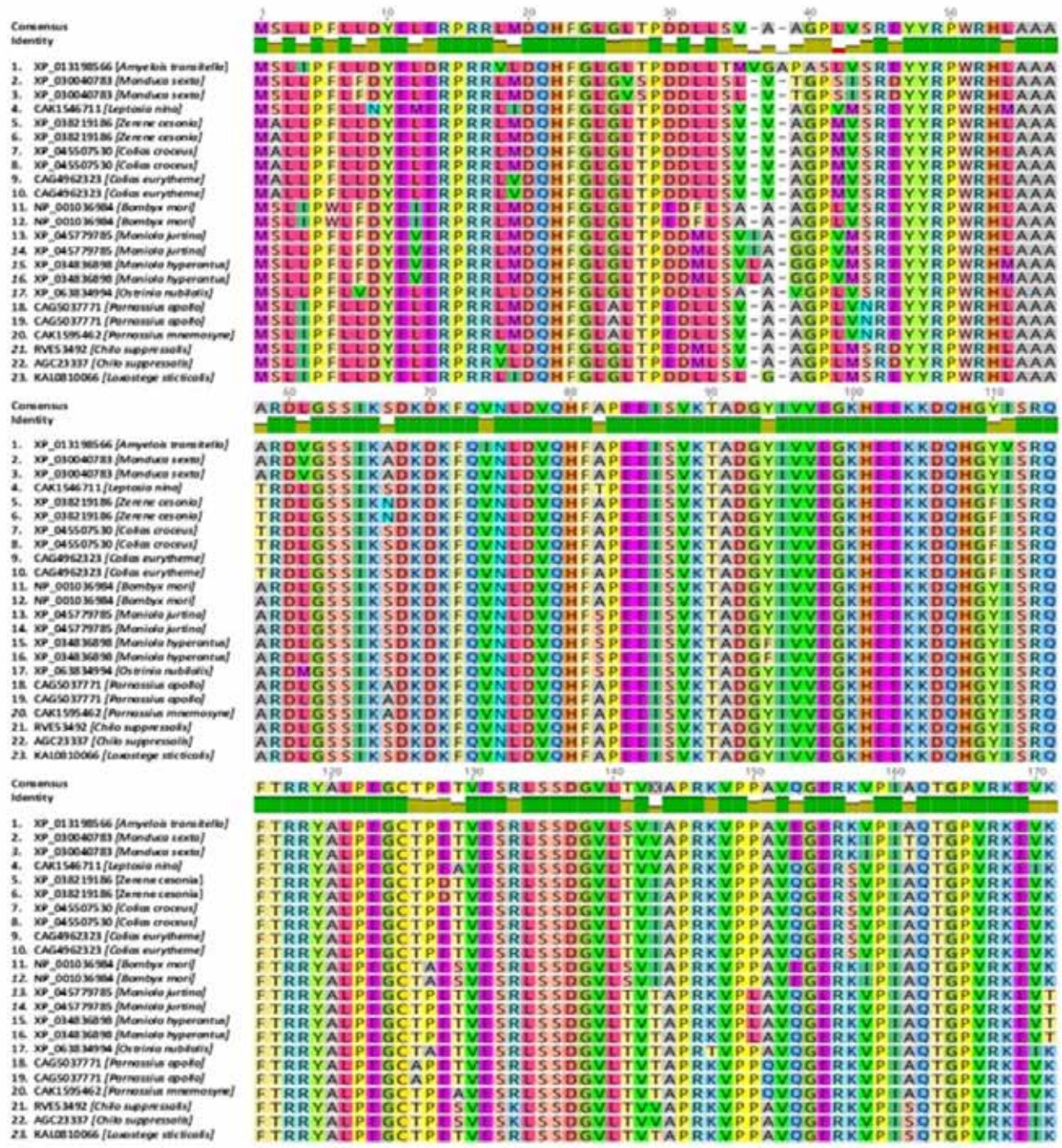

**Fig. 2.** Amino acid sequence alignment of sHsp19 among different insect species. The alignment was performed using the MAFFT algorithm in Geneious Prime software 2024.0.7. The mean pairwise identity over all pairs in the column is depicted-green: 30% to 100% identity; red: <30% identity

*Manduca sexta, Leptosia nina, Zerene cesonia, Colias sp., Bombyx mori, Maniola sp., Ostrinia nubialist, Parnassius sp., Chilo sp.* and *Loxostege sticticalis*, all exhibit more than 90% pairwise similarity of sHsp19 (**Fig. 3**).

Other HSP families, mainly classified based on molecular weight, are excellently reviewed (Tissières et al., 1974; Sørensen et al., 2003). In insects, nematodes, and aquatic animals, the first sHSPs were recognized and extracted from *D. melanogaster* in 1974 (Tissières et al., 1974), *C. elegans* in 1983 (Russnak et al., 1983), and brine shrimp (*Artemia* sp.) in 1999 (Liang & MacRae, 1999), respectively. Since then, because of genomics and transcriptomics, an increasing number of sHSPs has continued to be reported in several of these organisms such as the Diamondback moth (*Plutella xylostella)*, *C. elegans*, *Meloidogyne hapla, Penaeus*

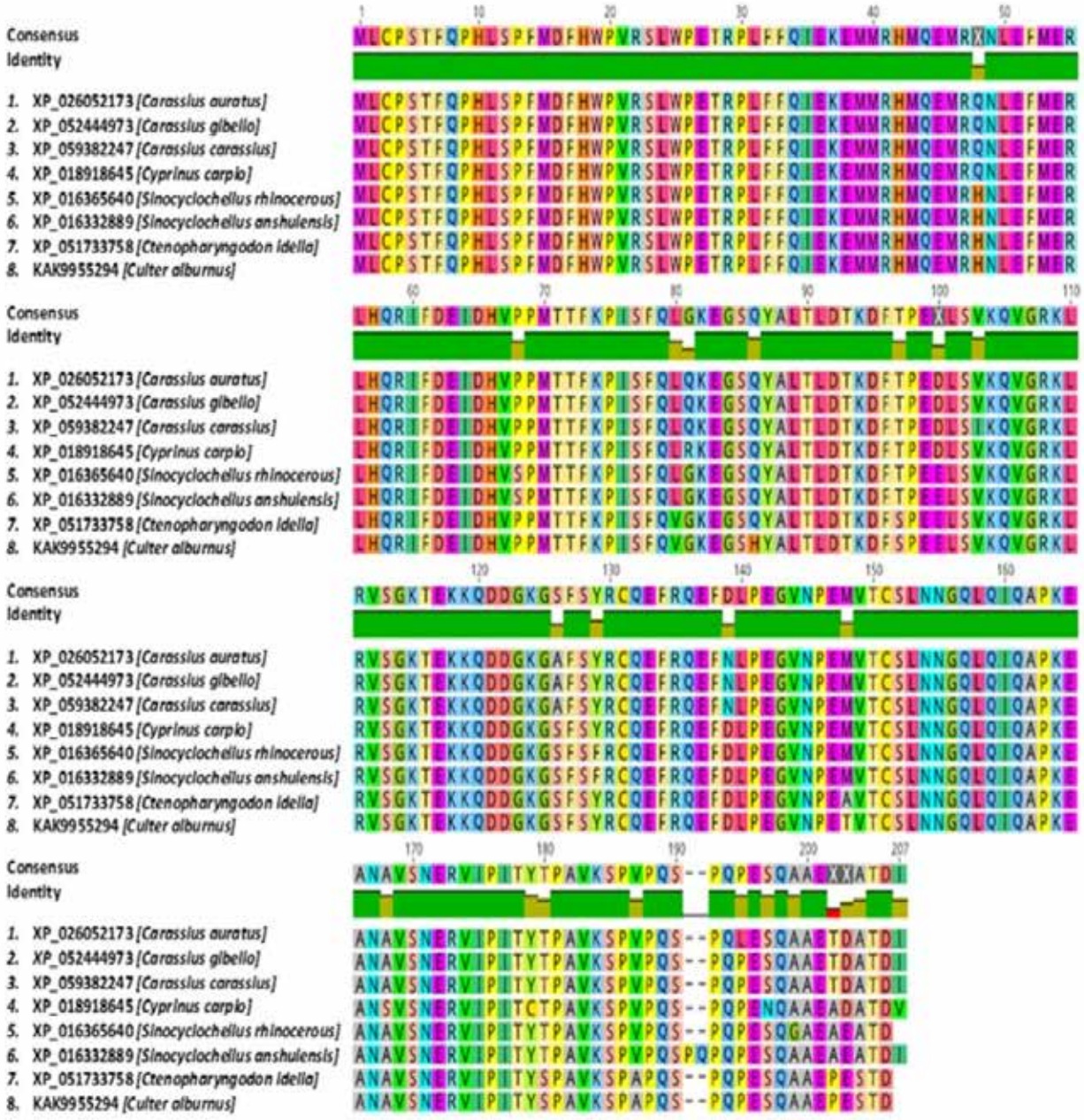


**Fig. 3.** Amino acid sequence alignment of sHsp20 among different fish species. The alignment was performed using the MAFFT algorithm in Geneious Prime software 2024.0.7. The mean pairwise identity over all pairs in the column is depicted—green: 30% to 100% identity; red: <30% identity

*monodon, V. philippinarum* and the goldfish (*Carassius auratus*) (Chen & Zhang, 2015; Chen et al., 2022; Flis et al., 2024). Excellent reviews of sHSP's role and association in physiological responses to stress have been made particularly; in insect responses to temperature extremes, crowding, starvation, and hypoxia/ anoxia (Denlinger et al., 2001; King & MacRae, 2015; Wang et al., 2016; González-Tokman et al., 2020), however, no comprehensive reviews on the role of sHSPs in nematodes and aquatic animals have been made in response to abiotic and biotic stress. As such, this review summarizes what is known, to date, about several sHSPs involved in the response of insects, nematodes, and aquatic animals to abiotic and biotic stressors. Understanding their function is crucial for advancing our knowledge in agricultural pest management, climate change adaptation, and biotechnology. We explore how sHSPs contribute to the resilience of these organisms under various environmental stressors, highlighting their relevance in mitigating environmental stress and maintaining cellular homeostasis.

## Small Heat Shock Proteins

### *Temperature extremes*

Temperature is one of the most important determining factors of insect abundance and distribution (Bale et al., 2002; King & MacRae, 2015). Exposure to high temperatures, even for a short time, can negatively affect insect growth and development (Abdullah, 1961; Gilbert & Raworth, 1996). For instance, exposure of the fruit fly *Zeugodacus tau* to high temperatures (34°C and 38°C) for 12 hours exhibited changes in their mating behavior, antioxidant defense, and detoxifying enzymes in a sex-specific manner (Li et al., 2023). Li et al. (2023) further noted that high-temperature exposure promoted mating, a behavioral adaptation strategy for survival under environmental stress. Insects produce sHSPs as one of the various physiological responses to exposure to temperature extremes (Feder & Hofmann, 1999; González-Tokman et al., 2020). Several sHSPs have been associated with affording heat tolerance, heat acclimation, and hardening (Dahlgaard et al., 1998; Huang et al., 2007). For example, in a study tracking the level of sHSPs in outdoor larvae of the gall fly, *Eurosta solidaginis*, the sHSP αB-crystallin increased in expression levels prior to and during the cold winter weather (approx. at -10°C to 10°C from December to February) suggesting that it contributes to protection from cold (Zhang et al., 2011). Within certain limits of temperature stress, insects can recover from stress-induced coma when the stress is removed. In *Drosophila melanogaster*, two genes (Hsp22 and Hsp23) were upregulated during recovery (at 25°C) but not during the cold exposure itself (at 0°C for 12 h) (Colinet et al., 2010a). Besides, observations of upregulation and involvement of sHSPs in thermal tolerance have also been studied through gene knockdown by RNAi. For instance, the thermal tolerance of the soil-dwelling beetle *Gastrophysa atrocyanea* to daily exposure to high temperatures reaching 50°C for 12 minutes, as indicated by its viability, lowered to 9% when two sHSPs genes (i.e., sHSPs 21 and 23) were silenced compared to the > 67% viability in the control (Atungulu et al., 2006).

### *Desiccation stress*

Maintaining water balance is a critical requirement for insects (Hadley, 1994), as dehydration can lead to significant physiological injuries such as protein denaturation, reactive oxygen species (ROS) production, nucleic acid damage, and membrane damage through lipid peroxidation (Hansen et al., 2006) and, consequently, death (Lopez-Martinez et al., 2008). Due to the severity of the cellular damage resulting from dehydration, it is better prevented than repaired. Thus, it is advantageous for the insect to initiate an early response to dehydration. Several sHSPs have been identified to offset or respond to dehydration/rehydration changes – suggesting their importance in preventing possible protein denaturation. For example, the sHSP, smHsp, in the larvae of *Belgica antarctica*, an insect that lives in Antarctica, reacted to varying levels of dehydration and rehydration (Lopez-Martinez et al., 2008). This Antarctic midge larva spends most of its time frozen in ice, but during summer, the ice melts, exposing it to potential dehydrating conditions such as intense sunlight and wind. Under fast dehydration (75% RH for 36 h) – quick loss of water, sHSP mRNA was increased, and levels continued to be high during rapid rehydration (done by submerging larvae in water for two hours), indicating involvement in preparation for stress exposure. Several other cases of sHSPs involvement in desiccation are presented in **Tab. 1.**

### *Pesticides and heavy metals stress*

Exposure to pesticides is a significant contributor to terrestrial insect population decline (Sánchez-Bayo & Wyckhuys, 2019; van Klink et al., 2020). Pesticide exposure is linked to increased ROS production in cells, which, if not neutralized or regulated to low levels, targets proteins whose amino acid residues are oxidation-sensitive and consequently can change protein functional structure and/or cause aggregation (Kannan & Jain, 2000; Reichmann et al., 2018; Martelli et al., 2020; Li et al., 2022). Besides exposure to targeted insecticides, insects can also be impacted by exposure to non-insect targeted pesticides, such as herbicides, due to sharing a common habitat with the target pests (Cheng et al., 2018; D'Ávila et al., 2018). For instance, glyphosate, the most used herbicide worldwide, negatively affects insect physiology, delays the growth and development of larvae, and disrupts the foraging behavior of worker honeybees *Apis mellifera* (Farina et al., 2019). Also, glyphosate impaired the habituation of mosquito larvae (*Aedes aegypti*) under field conditions (Baglan et al., 2018).

Insects respond to pesticide exposure by overproducing sHSPs. ROS activates heat shock factor (HSF1) to stimulate the expression of these sHSPs (Rajak & Roy, 2018). sHSPs contribute to repairing damage from pesticide-induced oxidative stress (Otaka et al., 2006), stimulate the activity of antioxidant-related genes and antioxidant enzymes, or hinder the activation of ROS transcription factors (Somensi et al., 2017; Yu et al., 2019; Shan et al., 2020). In *Apis cerana*, a knockdown of the sHSP gene, *AccsHSP21.7*, decreased its resistance to oxidative stress resulting from exposure to glyphosate, resulting in a significantly increased

**Table 1.** Expression of sHSPs in insects in response to several stressors

| Insect species | Small Heat Shock Protein/gene | Expression pattern | References |
|---|---|---|---|
| | ***Temperature stress*** | | |
| Corn stalk borer (*Sesamia nonagrioides*) | SnoHsp19.5 and SnoHsp20.8 | mRNA levels for both genes were upregulated (within 15 min) by heat shock at 40 °C and when larvae recovered (at 25°C) after cold shock treatment (- 5 °C to 17 °C). | Gkouvitsas et al. (2008) |
| Leaf beetle (*Gastrophysa atrocyanea*) | sHsps 21 and sHsps 23 | RNAi knockdown of mRNA of both genes decreased viability from > 67% to 9% when exposed to daily heat shock of 50 °C for 12 minutes. | Atungulu et al. (2006) |
| *Liriomyza sativae* | ls-hsp19.5, ls-hsp20.8 and ls-hsp21.7 | mRNA levels of all were significantly induced by cold treatment (0 °C to - 15 °C for 1 hr) with *ls-hsp20.8* mRNA displaying the greatest sensitivity. This suggests that different sHSPs may respond to various stress intensities. | Huang et al. (2009) |
| *Eurosta solidaginis* | α- crystallins and β-crystallins | The sHSPs αB-crystallin increased in protein levels just prior to and during a 3-month winter season at approx. -10 °C to 10 °C. Protein levels of both αA and αB crystallin were highly induced in response to thaw conditions (3 °C). | Zhang et al. (2011) |
| Silkworm (*Bombyx mori*) | shsp19.9, shsp20.1, shsp20.4, shsp20.8, shsp21.4, shsp23.7 and shsp21.4. | mRNA levels of all genes were upregulated by heat stress (45°C for 35 minutes) except shsp21.4, which was downregulated. | Sakano et al. (2006), Li et al. (2012) |
| Red flour beetle (*Tribolium castaneum*) | Tchsp18.3 | mRNA levels of the gene were upregulated in response to heat stress (45°C for 1 – 12 h) but not to cold stress (4°C for 1 – 12 h). | Xie et al. (2019) |
| Fruitfly *(Drosophila melanogaster)* | Hsp22 and Hsp23 | The removal of the gene's mRNA by RNAi interrupted recovery (time to recover and mobility following recovery) from chill injury thus showing that upregulation of the genes is required for recovery, but not during the cold stress itself. | Colinet et al. (2010a) |

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|---|---|---|---|
| Flesh fly *(Sarcophaga cras-sipalpis)* | Hsp23 | Deletion of *genes'* mRNA reduced cold hardiness. | Rinehart et al. (2007) |
| Western flower thrip *(Frankliniella occidentalis)* | FoHSP11.6 and FoHSP28.0 | mRNA levels of both genes were induced by both low (-10°C to 0°C for 1 h) and high (39°C and 41°C for 1 h) temperatures with maximum expression levels attained after 0.5 – 1 h of temperature stress exposure. Also, thermotolerance was reduced when both genes were silenced by RNAi. | Yuan et al. (2022) |
| *Chilo suppressalis* | Cshsp19.0 | mRNA level of the gene was upregulated as a response to heat and cold stress extremes in a range of –11°C to 43 °C (with 27 °C as a control) for exposure duration of 2 h. | Dong et al. (2021) |
| *Spodoptera frugiperda* | SfsHsp21.3, SfsHsp20, SfsHsp20.1, SfsHsp19.3, and SfsHsp29. | All mRNAs of the genes were significantly upregulated at both temperature extremes (42°C and 4°C for a duration between 0 – 150 minutes) except two genes (*SfsHsp20.1* and *SfsHsp19.3)* in the adult males that did not respond to the 4°C treatment. | Yang et al. (2021) |
| ***Pesticide and heavy metal stress*** | | | |
| *Apis cerana cerana* | *AccsHSP21.7* | A knockdown of the sHSP gene's mRNA decreased the resistance of the insect to a commercial herbicide glyphosate, resulting in significant mortality. | Huang et al. (2023) |
| Fall armyworm (*Spodoptera frugiperda*) | sHsp19.07, sHsp20.7 and sHsp19.74. | mRNA levels of all genes were upregulated following exposure to the Chlorantraniliprole pesticide. Though sHsp19.74 reached maximum mRNA expression levels faster (8 h after exposure) than the rest (12h), its levels plummeted at 12 h after exposure, suggesting a momentary responsiveness of sHSPS to pesticide treatment. | Samanta et al. (2021) |

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| Diamondback moth (*Plutella xylostella*) | Fourteen sHSPs (sHSP27.5, sHSP28.9, sHSP21.6, sHSP18.8, sHSP19.22, sHSP21.8, sHSP21.9, sHSP22.1, sHSP23.4, sHSP19.5, sHSP20.06, sHSP20.09, sHSP19.23, sHSP20.1) | Fourth instar larvae were exposed to various pesticides and heavy metals for 24 hr. sHSP mRNA level responses were as below: Beta-cypermethrin pesticide significantly upregulated all except sHSP20.09, whereas chlorfenapyr pesticide downregulated all except sHSP28.9. Expression responses to Indoxacarb and Cantharidin were irregular. Exposure to H2O2 for 24 h downregulated five sHSPs (sHSP19.22, sHSP19.23, sHSP21.6, sHSP22.1, and sHSP23.4)Copper (Cu2+) downregulated three sHSPs (sHSP20.1 sHSP22.1, sHSP28.9) and upregulated seven sHSPs (sHSP19.22, sHSP19.23, sHSP20.06, sHSP20.09, sHSP21.8, sHSP21.9, sHSP27.5). Manganese (Mn2+) upregulated four sHSPs (sHSP20.1, sHSP21.6, sHSP22.1, sHSP28.9) and upregulated all the rest. Nickel (Ni2+) upregulated (sHSP19.22, sHSP19.5, sHSP20.06, sHSP20.09), not induced (sHSP20.1, sHSP21.8, sHSP21.9), and the rest were down-regulated. Gene expression response to Lead (Pb2+) was irregular. | Chen & Zhang (2015) |
| *Daphnia magna* | eleven sHSP genes (termed DmsHSP1 - DmsHSP11) | Insect exposure to heavy metals ($Cd^{2+}$, $Cu^{2+}$, and $Zn^{2+}$) upregulated mRNA levels of DmsHSP1 and DmsHSP5. RNAi knockdown of genes DmsHSP1–21, except DmsHSP11–12.8, increased susceptibility to heavy metal stress exposure. | Li et al. (2022) |
| *Chironomus riparius* | hsp17, hsp21, hsp22, hsp23, hsp24, hsp27, and hsp34 | Following acute exposure to Cadmium (Cd), mRNA levels of hsp23, hsp24, hsp27, and *hsp34* were upregulated, whereas levels of *hsp17* and *hsp21* remained unaltered. This indicates that sHSPs have diverse roles during response to Cd. | Martín-Folgar & Martínez-Guitarte (2017) |

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|---|---|---|---|
| Chinese rice grasshopper (*Oxya chinensis*) | OcGrp78, OcHsp70, OcHsp90, and OcHsp40 | Following exposure to Cadmium (Cd), mRNA expression levels of all genes increased, reaching a maximum within a short period (6 h), albeit decreasing significantly after 12 h. | Zhang et al. (2015) |
| | ***Crowding stress*** | | |
| Migratory locusts (*Locusta migratoria L*) | Hsp20.5, Hsp20.6, and Hsp20.7 | mRNAs of all sHPS were more expressed in gregarious phases (representing high population density) compared to solitary phases (representing low population density) | Wang et al. (2007) |
| Australian plague locust (*Chortoicetes terminifera)* | Hsp20.5 and Hsp20.7 | Crowding (during the gregarious phase) resulted in a 2 – 3 fold significant upregulation of mRNA levels of both genes. | Chapuis et al. (2011) |
| | ***Starvation stress*** | | |
| Mulberry pyralid caterpillar (*Glyphodes pyloalis*) | GpHSP19.5, 20, 20.2, and 21.6, GpHSP21.8 and GpHSP21.4 | Genes were upregulated time-dependently, reaching maximum levels on the sixth day of food deprivation. On the contrary, mRNA expression levels of two GpsHSPs (GpHSP21.8 and GpHSP21.4) demonstrated intermittent downregulation in comparison to the control at 2 or 4 days following the starvation period. | Chu et al. (2020) |
| Fruitfly (*Drosophila melanogaster*) | Hsp27 | sHSP was knocked out, and flies showed a significant decrease in resistance to starvation. | Hao et al. (2007) |
| Housefly (*Musca domestica)* | MdomHSP27, MdomHSP10, MdomHSP27.1, and MdomHSP27.2 | The mRNA expression of MdomHSP27 was significantly downregulated after a 6h starvation period, whereas mRNA expression of other 3 MdomHSPs (*Mdom*HSP10, *Mdom*HSP27.1 and *Mdom*HSP27.2) were not significantly affected | Tian et al. (2018) |
| Red flour beetle (*Tribolium castaneum*) | Tchsp18.3 | When mRNA of sHSP gene was knocked down, the lifespan of adult beetles was reduced by 15.8% (they died within 18 days after starvation) compared to the control group. | Xie et al. (2019) |

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|---|---|---|---|---|
| *Pteromalus puparum* | PpHSP20 | mRNA levels of the sHSP increased significantly after 6 h of starvation but declined after 24 h | Wang et al. (2012) |
| Diamondback moth (*Plutella xylostella*) | sHSP20.1, sHSP21.6, sHSP22.1, and sHSP28.9 | mRNA expression levels of all sHSPs were significantly downregulated following food starvation for 21 h. | Chen & Zhang (2015) |

mortality rate (Huang et al., 2023). The promoter region of the AccsHSP21.7 gene was rich in binding sites for oxidative stress transcription factors CREB (cAMP Response Element-Binding Protein) and HSF (Heat Shock Factor) (Huang et al., 2023). CREB can help the insect save energy by transitioning to the diapause stage (King & MacRae, 2015) e.g., under hypoxia conditions (Wang et al., 2020), whereas HSF turns on heat shock genes that subsequently drive more sHSP synthesis (Brunquell et al., 2016).

The buildup of heavy metals (HMs) beyond their required range is toxic and has been linked to oxidative stress, as the buildup enables the formation of free radicals (Jomova & Valko, 2011; Jaishankar et al., 2014). Also, metals such as cadmium (Cd) can disrupt the proteins involved in DNA repair and DNA damage signaling (Hartwig et al., 2002). In addition, Cd has a high affinity for and interacts with the thiol groups in cysteine residues, disrupting/denaturing protein structure and consequently stimulating the expression of chaperone proteins such as sHSPs (Martín-Folgar & Martínez-Guitarte, 2017). In *Daphnia magna*, a model insect for investigating HM toxicity, sHSPs like DmsHSP1 and DmsHSP5 were upregulated when the insect was exposed to HMs (Cadmium ions ($Cd^{2+}$), copper ions ($Cu^{2+}$), and zinc ions ($Zn^{2+}$), perhaps preventing potential protein agglutination and denaturation (Li et al., 2022a). RNAi knockdown of these genes (*i.e.* DmsHSP1 and DmsHSP5) increased susceptibility to HM stress exposure (Li et al., 2022). It is noteworthy that besides metallothionein (MT), sHSPs are being explored as possible bioindicators of metal contamination because of their sensitivity to small changes in cell homeostasis and their conserved nature in many species (Denlinger et al., 2001; Morales et al., 2011; Martín-Folgar & Martínez-Guitarte, 2017).

### *Hypoxia/anoxia stress*

Several adult insects or during certain stages of their life cycle live in habitats with consistent or bursts of oxygen levels below the normal 20.94%, such as ground burrows, decaying organic matter, aquatic ecosystems, flood-prone soils, and high altitudes (Hoback & Stanley, 2001). Under hypoxic conditions, organisms employ short- and long-term mechanisms to increase their access to oxygen or decrease their oxygen demand, and failure can result in tissue damage or death (Liu et al., 2006; Michaud et al., 2011). Therefore, the ability to survive in hypoxic or anoxic conditions is necessary for the survival of these insects. Michaud et al. (2011) and Zhang et al. (2011) reported an association of sHSP upregulation with insect exposure to hypoxia/anoxia conditions. In *S. crassipalpis*, which typically burrows underground (Basson & Terblanche, 2010), mRNA levels of three sHSPs (*hsp*25, *hsp*23, and *hsp*18) were upregulated during hypoxia (3% oxygen) treatment application, and declining 2 h post-treatment application (Michaud et al., 2011). In larvae of gall fly, *Eurosta solidaginis,* the protein levels of both αA and αB crystallin increased in response to anoxia (exposure period of 24 h under nitrogen gas at 15°C) (Zhang et al., 2011). These studies suggest that the respective sHSPs are involved in anoxic/hypoxic stress response with a potential role in maintaining protein homeostasis during and shortly after stress exposure.

### *Crowding and starvation stresses*

Generally, when organisms are crowded, they are at risk of being stressed (Wang et al., 2007), and consequently, their growth and development processes are affected. For instance, in the migratory locust species *Locusta migratoria L,* the fecundity of solitary locusts was higher compared to that of gregarious locusts (representative of crowding) (Albrecht et al., 1959). As a stress factor, population density has been shown to affect the physiology of organisms, such as inducing the production of sHSPs, as shown in Tab. 1. In *L. migratoria L*, mRNAs of three sHSPs (Hsp20.5, Hsp20.6, and Hsp20.7) were more expressed in gregarious phases compared to solitary phases (Wang et al., 2007). It is worth noting that insects can experience multiple stressors in crowded conditions, including starvation, pathogen infection and desiccation. Therefore, given

that sHSP induction is in response to any stressor that causes protein unfolding (Parsell & Lindquist, 1993), it is difficult to decipher the contributions of a single stressor to sHSP induction (Wang et al., 2007). Within the context of starvation, when an insect is deprived of food, its energy homeostasis can be damaged (Chu et al., 2020). sHSPs have been implicated in responding to this stress sensitively (**Tab. 1**). For instance, In the 4-day-old larvae of the housefly *Musca domestica,* the expression of MdomHSP27 was significantly down-regulated after a six-hour starvation period (Tian et al., 2018). Under conditions of starvation, silencing of TcHSP*18.3 in Tribolium castaneum* (Xie et al., 2019), and *hsp27* gene in *Drosophila melanogaster* (Hao et al., 2007) reduced the lifespan of the insect by 15.8% and 39% compared to the control. These findings suggested that sHSPs can be sensitively responsive to starvation in insects.

### *Parasitism stress*

Parasitism can induce sHSPs in host organisms (Rinehart et al., 2002), for instance eliciting sHSPs in response to the venom injection. Envenomation of *Pieris rapae* by the endoparasitic wasp *Pteromalus puparum* stimulates the expression of hsp20 in the host (Zhu et al., 2013). Also, envenomation of the larvae of *Plodia interpunctella* by the ectoparasitoid *Bracon hebetor* stimulated upregulation of sHSP (*shsp*) (Shim et al., 2008). These results suggest that sHSPs could be involved in immune responses to parasite attack.

### *UV radiation stress*

Some studies have indicated a correlation between UV-induced stress and the expression of sHSPs in insects. UV radiation, such as UV-A, photo-oxidizes photosensitizers inside insect cells resulting in the production of large quantities of ROS that ultimately impair the function of proteins (Cadet et al., 2005; Meng et al., 2009; Meyer-Rochow et al., 2002). For instance, UV-A irradiation on *Spodoptera frugiperda* adults resulted in increased expression levels of five sHSPs genes (SfsHsp21.3, SfsHsp20, SfsHsp20.1, SfsHsp19.3, and SfsHsp29) indicating that they play a role in molecular response mechanisms (Yang et al., 2021). Similarly, expression levels of Hsp22.6 and Hsp27.6 in the honeybee *Apis cerana cerana* (Liu et al., 2012; Zhang et al., 2014), as well as those of Hsp27 and Hsp21.8b in the beetle *Tribolium castaneum*, were upregulated upon exposure to UV radiation (Sang et al., 2012; Xie et al., 2018).

## Small Heat Shock Proteins in nematodes

### *Heat stress*

Heat stress is a major environmental challenge for nematodes, particularly with the increasing impact of global climate change. Increasing temperatures can cause protein denaturation and malfunction, which in turn activates the heat shock response (Kayastha et al., 2024). During this process, sHSPs are upregulated to aid in the refolding of damaged proteins, thus boosting the thermotolerance of nematodes (Kyriakou et al., 2022). Research has demonstrated that *C. elegans* relies on sHSPs for survival under high-temperature conditions, highlighting their critical role in protecting against heat-induced stress (Zevian & Yanowitz, 2014). For instance, CeHsp16.1 and CeHsp16.2 are among the first proteins to be upregulated in response to heat shock at 35°C. Their deletion significantly reduces survival under prolonged heat stress, specifically at 35°C over 2 hours (Strayer et al., 2003). Other nematodes also exhibit a robust heat shock response. For example, in *Meloidogyne hapla*, sHSPs MhHsp12.2, MhHsp6, MhHsp1, and MhHsp43, are induced by heat stress with higher expression levels at 35-40°C (Flis et al., 2024). In *Bursaphelenchus xylophilus*, Bx-sHSP16A, Bx-sHSP16B, Bx-sHSP21, and BxsHSP25 are upregulated in response to both heat and cold stress, playing a dual role in temperature adaptation (Wang et al., 2016). In *Strongyloides ratti*, the expression of SrHsp17 is significantly elevated during heat stress and when exposed to pathogens, particularly in the infective larval stage whereas sHSP18 and sHSP22 are up-regulated at 37°C in third-stage larvae of the filarial nematode *Brugia pahangi* (Jecock & Devaney, 1992; Younis et al., 2011). Although these findings strongly suggest a protective role, they primarily indicate correlation rather than definitive causation.

### *Cold stress*

Although often less discussed, cold stress can also impact nematodes, particularly in environments with seasonal temperature fluctuations. Cold exposure can affect the fluidity of cellular membranes and the stability of proteins (Fonseca et al., 2019). sHSPs are involved in the cold shock response of organisms, helping to preserve protein stability under low temperatures (Sadura & Janeczko, 2024). Evidence suggests that sHSPs are upregulated in nematodes exposed to cold environments, aiding in their ability to maintain cellular functions and protect proteins from cold-induced damage (Peter & Candido, 2002). For example, in *C. elegans*, heat shock factor 1 (hsf-1) triggered inducing the nematode into an arrested state of development (diapause) at 4°C, and this is essential for maintaining cellular stability under cold temperatures (Horikawa et al., 2024). Similarly, in *M. hapla*, MhHsp12.2 plays a dual role, being upregulated both during heat and cold shock.

The expression of this gene at low temperatures (-20°C) enhances cold tolerance, allowing the nematode to survive periods of cold exposure during the winter season (Flis et al., 2024). Additionally, in *Panagrolaimus davidi*, a cold-adapted nematode from Antarctic environments, sHSPs are crucial in ensuring survival at subzero temperatures, by maintaining protein stability and membrane fluidity under freezing conditions (Thorne et al., 2014).

#### *Desiccation stress*

Desiccation, or dehydration, also poses a serious threat to nematode survival. During dehydration, cellular structures, including proteins, are at risk of damage. sHSPs play a vital role in enhancing desiccation tolerance by stabilizing proteins and cellular structures and preventing irreversible damage during water loss (Hibshman et al., 2023). Nematodes can survive extreme desiccation, which helps them maintain cellular integrity during periods of water scarcity (Adhikari et al., 2010). In *C. elegans*, the dauer larvae stage is particularly adapted to survive harsh desiccation conditions and exhibits high levels of sHSP p16 (Erkut et al., 2013). Thus, sHSPs enable nematodes to thrive in environments where water availability is unpredictable, making them essential for desiccation tolerance. Similarly, in *Panagrolaimus superbus*, a model species for desiccation research, both PsHsp17.1, and PsHsp20 are upregulated during desiccation and dehydration (Tyson et al., 2012).

### *Anhydrobiosis and hypoxia stress*

In extreme cases, such as anhydrobiosis (a state of suspended animation in response to complete desiccation) and hypoxia (low oxygen conditions), sHSPs have been found to play a protective role. During anhydrobiosis, where nematodes experience extreme dehydration, sHSPs help stabilize cellular components, preventing damage during the transition to and from the dried and hypoxic state (Lee & Lee, 2013). In *Heterorhabditis indica, Dnj-13,* which is a class B J-protein belonging to *hsp40* family, is upregulated when subjected to anhydrobiotic conditions (Balakumaran et al., 2022) allowing the nematode to survive complete dehydration for extended periods. Similarly, under hypoxic conditions, sHSPs contribute to cellular protection by maintaining protein homeostasis. For example, in *C. elegans*, the sHSP hsp-16.1 and hsp-16.2 were upregulated following a one-hour exposure to hypoxic conditions (Hong et al., 2004). While sHSPs likely play a significant role in stabilizing cellular components during these stresses, it is important to note that anhydrobiosis is a complex process involving the coordinated action of multiple genes in nematodes (Evangelista et al., 2017).

#### *Chemical stress*

Nematodes, like many organisms, are frequently exposed to a variety of chemical stressors in their environments, including HMs, pesticides, and other toxic compounds (Kim et al., 2014). In *C. elegans*, sHSPs are often co-expressed with other antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), providing a coordinated defense against oxidative damage caused by HM stress (Sampayo et al., 2003). Ezemaduka et al. (2017b), showed that the expression of CeHSP17 increases when *C. elegans* are exposed to toxic HMs like Cd and Zn. As earlier mentioned in the previous section, these metals disrupt protein folding and lead to the formation of misfolded protein aggregates. sHSPs such as CeHSP17 play a critical role in preventing irreversible aggregation and facilitating the disaggregation of misfolded proteins. Pesticides and other xenobiotic compounds are widely present in agricultural soils, posing a significant chemical stress challenge to nematodes. sHSPs have been implicated in the defense against pesticide-induced stress. For example, exposure to the synthetic pyrethroid insecticide cypermethrin leads to oxidative damage and stress responses in *C. elegans*. Shashikumar & Rajini, 2010, demonstrated that cypermethrin exposure at non-lethal concentrations induces the expression of HSP-16 in a concentration- and time-dependent manner. Marked upregulation of HSP-16 was observed throughout the nematode's body after 24 hours of exposure, suggesting its involvement in mitigating cypermethrin-induced stress. While these findings highlight the potential role of HSP-16 in responding to pesticide stress, the study did not provide direct evidence of reduced protein aggregation or enhanced cellular recovery in the presence versus the absence of HSP-16. Further research is required to clarify the mechanistic role of sHSPs in protecting against pesticide-induced damage.

## Small Heat Shock Proteins in aquatic animals

#### *Heavy metal, temperature, and salinity stress*

Poor disposal of industrial waste often leads to contamination and accumulation of heavy metals (HMs) in aquatic ecosystems (Gaur et al., 2020; Sonone et al., 2021). Cadmium, considered the most dangerous HM to aquatic animals, is the most common contaminant from industrial waste (Khan et al., 2022). Even at low concentrations, this HM can accumulate in the vital organs of aquatic animals, leading to their death (Moiseenko &

Gashkina, 2020; Das et al., 2023;). As mentioned in previous sections, HM stress leads to oxidative stress in exposed tissues due to the accumulation of ROS (Tamás et al., 2017; Georgiadou et al., 2018). Moreover, HMs and metalloids negatively impact protein homeostasis and the viability of cells by interfering with the protein folding processes in cells (Tamás et al., 2014; Balali-Mood et al., 2021). sHSPs have been reported to play a vital role in alleviating the stress effects of HMs in certain aquatic animals. MnHSP28.6 was found to predominately accumulate in the muscles of the oriental river prawn (*Macrobrachium nipponense*) upon exposure to Cd and Cu ions and hence thought to be involved in animal response to HM (Yuan et al., 2019). Moreover, *in vitro* expression of this sHSP in *Escherichia coli* conferred host cells impressive protection against hydrogen peroxide ($H_2O_2$) compared to control *E. coli* suggesting its responsibility in alleviating oxidative stress induced by $H_2O_2$ (Yuan et al., 2019). In another study, Zhang et al. (2013) demonstrated that exposing razor clam (*Sinonovacula constricta*) to HM stress (Cd and lead (Pb)) led to an increased accumulation of ScsHSP in haemocytes, gonads, and mantle hence confirming sHSP's linkage to tolerance responses to HM toxicity stress in *S. constricta*. Likewise, Yang et al. (2012) observed increased expression of sHSP *Lc*HSP27 in the liver and brain of yellow croaker (*Larimichthys crocea*) exposed to Cd ions. Moreover, LcHSP27 expression was enhanced in the liver under high and low temperatures thus indicating its role in protecting the liver from multiple stress factors. In another study, Li et al. (2010) reported improvement in the level of expression of VpsHSP-2 in Manila clam (*Venerupis philippinarum*) exposed to Cd ion stress. A previous study on hard clam (*Meretrix meretrix*) showed that exposing the animal to Cd stress led to increased expression of mRNA levels of Mm-HSP 20 in the digestive gland and hemocytes hence indicating its role in HM detoxification and protection against oxidative stress (Li et al., 2013). Li et al. (2022) exposed *Daphnia magna* to different levels of heavy metals (Cd, Cu, and Zn), and the authors observed the expression of eleven sHSPs (DmsHSP1 – DmsHSP11) under HM stress. Exposing the animals to $Cu^{2+}$ strongly induced the expression of DmsHSP1 while it was moderately induced under $Cd^{2+}$ and $Zn^{2+}$ exposure. The authors also observed that DmsHSP5 was induced in all the HM stress.

Temperature stress is one of the major abiotic stress factors affecting the survival of aquatic animals. Changes in the environmental temperatures below or above the organism's thermal threshold cause oxidative stress that could hinder the survival and general well-being of the organism (Mugwanya et al., 2022). The expression of sHSPs in tissues of stressed animals could aid in the alleviation of temperature stress effects in the organisms. For instance, Bildik et al. (2019) investigated changes in the expression of HSPs (sHSP 30) in gilt-head bream (*Sparus aurata*) exposed to high and low temperatures and observed a two-fold increase in the expression of HSP30 under higher temperatures (27°C), which they correlated to improved thermal tolerance in fish. In another study, Chen et al. (2022) observed an increase in the relative mRNA expression of sHSP20 in the spleen of goldfish (*Carassius auratus*) exposed to high temperatures (32°C), which correlated with fish thermal tolerance. Currie (2000) demonstrated that acclimatization of rainbow trout (*Oncorhynchus mykiss*) to higher temperatures (25°C) led to increased expression of sHSP30 in all tissues except blood. Likewise, Liu et al. (2019) reported increased expression of sHSP30 mRNA levels in the gills of *O. mykiss* exposed to temperature stress (25°C), indicating its association with heat stress tolerance in fish. A previous study on pool barb (*Puntius sophore*) showed increased expression of HSP47 when exposed to temperature stress (41°C) (Mahanty et al., 2017).

Salinity stress in aquatic animals leads to the accumulation of ROS in tissues, an over-accumulation of which leads to cell damage and apoptosis. Expression of certain sHSPs in aquatic animals exposed to high or low salinity stress has been shown to enhance the stress tolerance of the organism. Shekhar et al. (2013) investigated stress responses of *P. monodon* exposed to low salinity stress and observed increased expression of HSP21 in tissues thus suggesting its role in salinity stress adaptive mechanism in shrimp. A recent study by Zarei et al. (2024) has shown that an increase in mRNA expression levels of HSP27 was associated with immune responses and cell survival in Sterlet Sturgeon (*Acipenser ruthenus*) under salinity stress.

### *Pathogenic infection*

sHSPs induced by biotic stress play a significant role in protein folding, enhancement of immune responses, and protection against diseases caused by various pathogenic organisms and viruses (Muthusamy et al., 2017). Although several studies have previously reported on the induction of sHSPs by abiotic stress factors, little information is known about the induction of sHSPs by abiotic stress factors. Certain studies have suggested that members of the HSP20, and HSP37, among others, occur in crustaceans and several fish species. Chen et al. (2022) conducted a study on goldfish (*C. auratus*) to understand how pathogen infection can

induce the expression of sHSPs in animal tissues. The authors observed that intraperitoneal injection of lipopolysaccharide (LPS) or polyinosinic-polycytidylic acid (Poly I:C) induced the expression of HSP20 in the spleen, thus improving the tolerance of the fish to stress. Arockiaraj et al. (2012) reported that the expression of MrHSP37 in freshwater prawns (*Macrobrachium rosenbergii*) triggered an immune response upon exposure to pathogen infection (hypodermal and hematopoietic necrosis virus). Li et al. (2010) demonstrated that exposing Manila clam (*V. philippinarum*) to a pathogen challenge (*Vibrio anguillarum*) induced the upregulation of VpsHP-1in haemocytes and increased 1.5-fold and 9.9-fold 6 h and 96 h post-infection, respectively. Likewise, the mRNA expression level of VpsHP-2 increased by 8.7-fold compared to the control group 24 h post-infection.

Certain studies have, however, shown a down-regulation of sHSPs in tissues of aquatic animals exposed to biotic stress factors. For instance, Huang et al. (2008) demonstrated that when challenged with the white spot syndrome virus, *Penaeus monodon* has a significantly decreased expression of HSP21 thus indicating that the gene regulation of sHSP 21 was negatively affected by the virus.

## Conclusions

Environmental stressors pose significant challenges to the survival, fitness, and distribution of insects, nematodes, and aquatic organisms, particularly in the context of climate change and increased anthropogenic pressures. Small Heat Shock Proteins (sHSPs) play an essential role in the resilience of these organisms. This review highlights the broad and highly conserved role of sHSPs across species in responding to various biotic and abiotic stressors discussed above. Future research should aim to unravel further the molecular mechanisms and pathways regulated by sHSPs, particularly in the context of multiple simultaneous stressors. Integrating genomics, proteomics, and transcriptomics approaches will be key to uncovering the full spectrum of sHSP functions. Moreover, exploring the evolutionary conservation of sHSPs across taxa will provide deeper insights into their potential as universal stress-resilience mechanisms. Ultimately, enhancing our understanding of sHSPs will not only advance fundamental biological knowledge, but also inform practical applications in fields such as agriculture, conservation biology, and environmental monitoring, helping to safeguard biodiversity and ecosystem stability in an era of unprecedented environmental change.

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