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# Expression of heat shock proteins as biomarker tool in aquatic invertebrates: actual knowledge and ongoing developments for the early detection of environmental changes and ecological risks

De Jong L, Moreau X, Thiéry A.



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## Abstract

Many invertebrates are able in tolerating and compensating sublethal stress by involving metabolic pathways and reactions that allow them in surviving under adverse environmental conditions. Therefore, in the ongoing development of new tools for aquatic ecosystem biomonitoring (marine, salt marsh and freshwater), biochemical measurements that can be used as individual biomarkers of impaired biological functions in invertebrates have been proposed. Among these biomarkers, the expression of heat shock proteins (Hsps) has been widely investigated in both natural environmental stress situations (temperature seasonal variations, hypoxia...) and anthropic disturbances as chemical contaminations. Indeed, the heat shock protein family plays a key role: their induction is the signal of exposure to conditions that alter intracellular proteins. The present contribution deals with the actual knowledge in the field of heat shock proteins as biomarker tools in aquatic bioindicator invertebrates for the early detection of environmental changes and ecological risks. The growing emphasis of these biological tools is supported by their interest to complete water quality criteria based on chemical analysis and mortality of species under stress conditions. Steps for the calibration of these chaperone proteins as potential biomarker tools will be discussed. Commentary will focus on actual knowledge and further developments.

## Introduction

Heat-shock proteins (Hsps) have been evidenced by [Tissières \*et al.\* \(1974\)](#) in salivary glands of the Diptera *Drosophila melanogaster* submitted to heat shock in laboratory conditions. The name of heat-shock proteins proposed at that time has been conserved even if it has been further demonstrated that these proteins are drastically induced after different physical and chemical treatments, especially those that denature proteins ([Parsell & Lindquist, 1993](#); [Welch, 1993](#); [Freeman \*et al.\*, 1999](#)). The induction of Hsps in cells of organisms exposed to stress represents a rapid and highly conserved response to proteotoxic insult. As this ubiquitous response, observed in all organisms studied from bacteria to human ([Schlesinger \*et al.\*, 1982](#)), can be induced by pathophysiological ([Morimoto, 1991](#); [Jean \*et al.\*, 2004](#)) and environmental stresses ([Feder & Hofmann, 1999](#); [Aït-Aïssa \*et al.\*, 2003](#)), it has been proposed to consider Hsp induction as a biomarker tool for the early detection of environmental changes and ecological risks in aquatic biota ([Triebkorn \*et al.\*, 2002](#); [Yoshimi \*et al.\*, 2002](#); [Werner \*et al.\*, 2004](#); [Bodin \*et al.\*, 2004](#); [De Jong \*et al.\*, 2006](#)). The present contribution is a short review of the actual knowledge on this biomarker tool used in aquatic bioindicator invertebrates, regarded as early warning sentinels in marine salt marsh and freshwater risk assessment.

## *Biomarker concept & heat-shock proteins*

Originally defined by the committee on Biological Markers of the National Council (NCR) in 1987 biomarkers are "indicators signaling events in biological systems or samples following chemical exposure". From the NRC definition, biomarkers have been categorized in an attempt to classify responses. Three different types of markers were recognized: biomarkers of exposure to a toxicant, biomarkers of effects of exposure (Hsps belong to this category) and biomarkers of susceptibility to the effects of exposure. This first definition has been challenged by several authors (see [Schlenk, 1999](#) and [Hyne & Maher, 2003](#) for review) and it has been evidenced that some biomarkers can respond to each of these three categories. Finally, it seems that there is still a little consensus in defining biomarkers and that the definition has to be adapted to the considered biomarker. The major question is: why this biomarker is useful for environmental scientists? The answer is given by [Chapman & Guerra \(2005\)](#) stating that main focus for environmental scientists could be summarized as follow "determining pollution; assessing pollution; and providing decision-markers with the necessary information to address pollution that is adversely affecting the environment in which we live".

By now, it is clearly established that biomarkers are biological parameters measuring alterations in behaviors, physiology, biochemistry, cell integrity, genomic structure and expression ([Lagadic \*et al.\*, 1997](#)). During the

past 25 years, many researchers have focused their efforts to develop and apply biomarkers in ecotoxicology and, now, there is a consensus to consider that they provide useful informations in the ecological risk assessment. The main idea is that they will provide early warning detection signal before that irreversible damages occur at the population and/or community levels. Even "opponents" to these biological tools state that "*Biomarkers may contribute to ecological risk assessment in two main areas. The first is in exposure assessment of long-lived and/or rare species. The second is in testing hypotheses about mechanism of chemical impacts at different levels of biological organization*" (Forbes *et al.*, 2006). Despite the pertinent informations collected until now, there is still no standardized protocol developed for of these promising tools in monitoring programs. In fact, with the growing knowledge on biomarker potentialities, researchers have been set against the necessity to define a range of calibration criteria of which the application of a biomarker tool in field studies depends. These criteria could be summarized as follow: (1) the detection method must be simple and sensitive; (2) the biomarker response must allow discriminating polluted from unpolluted situations; variations in the biomarker response according to xenobiotic dose and time exposures must be considered; (3) the variability due to non-toxic factors (specific variability, abiotic factors as seasonal variations, fluctuating temperature or oxygen levels...) must be understood (Mayer *et al.* 1992; Engel and Vaughan, 1996; Moreau *et al.*, 2008).

As many organisms are able to synthesize proteins that offer some protection from cellular damages, the quantification of their induction, when calibrated, will be a valuable biomarker tool. Among these proteins Hsps, which are involved in cellular protein homeostasis and repair, have been particularly investigated for several reasons: (1) Hsp genes and proteins are present in all organism studied to date; (2) Hsps are induced by numerous stressors. Table 1 sum up major works dealing with Hsps in marine, salt marsh and freshwater invertebrates. Heat-shock proteins are classified and named according to their molecular weight. Extensive studies on model species have revealed four major families of Hsps: Hsp90 (85-90 kDa), Hsp70 (68-73 kDa), Hsp60 and low molecular weight Hsp (16-47 kDa). Unlike other Hsps, low molecular weight Hsps have no known constitutive function and are only induced during stress (Ciocca *et al.*, 1993; Sharp *et al.*, 1994). For example, in *Anemonia viridis* (marine Cnidarian) differential expression of Hsp28 and Hsp29 is correlated with thermotolerance. These cnidarians induce low molecular Hsps following heat shock but do not express them constitutively (Sharp *et al.*, 1994).

Many studies on Hsps have dealt with thermal stress and especially response to heat shock has been widely investigated in numerous animal species (Table 1). In most cases, induction of Hsp70 and Hsp90 were detected. Expression of Hsps proteins is now considered to confer resistance to organism against global changes and their pattern of expression which is supposed to offer markers to detect general warming is now proposed to be included in international environmental programs (Lejeune *et al.*, 2006).

Beside to thermal stress as inducers, researchers have also studied the induction of Hsps after xenobiotic expositions under laboratory conditions. The complexity of the biological answers (i.e. induction of Hsps) results of the following points:

- (1) the expression differs according the species considered even within the same genus,
- (2) for the same species, the expression differs according the tissue considered (ex: mantle and gills for bivalves),
- (3) for the same specie and for the same tissue, the expression may differs according age and sex,
- (4) different methods of detection have been employed and so results between different studies are often not comparable.

Therefore, the despite fundamental results and findings no real effort of calibration of such ecotoxicological biomarkers have been assay even in marine species such *Mytilus edulis* or *Mytilus galloprovincialis* that have been widely investigated.

### ***Hsp expressions in assessment of the global warming effects & species biogeographical distribution***

Chapple *et al.* (1998) have shown that thermal seasonal changes are closely related with levels of Hsp70 in *Mytilus edulis*. In the same species and in polluted area, Bodin *et al.* (2004) have demonstrated seasonal variations of Hsp70 expression. They have also shown a relation between Hsp70 expression and heavy metal concentrations.

In 2001, Choresch *et al.* have reported that the sea anemone *Anemonia viridis* exhibits a seasonal pattern of Hsp60 expression according to changes in seawater temperature, with significantly higher level of Hsp60 in summer. Considering the worldwide degradation of coral reefs, due to global climate change and human perturbations, they have suggested "*the development of an early warning system for ambient assessment of the*

health of marine benthic communities" and proposed Hsp60 as "a possible biomonitor in marine invertebrates to predict their ability to survive future possible short-term and long-term changes in seawater temperature".

Sorte and Hofmann (2005) have investigated physiological traits responsible for determining the tide-height and latitudinal distributions of Northeastern Pacific *Nucella* congeners (Gasteropoda). Their results have shown that the level of total, not stress inducible, Hsp70 was a good predictor of thermotolerance and that there were species-specific differences in the relationship between Hsp70 expression and thermotolerance. They have suggested that "*Hsp70 expression may be important in conferring thermotolerance in Nucella species in nature and that higher levels of molecular chaperones may underlie increased thermotolerance between conspecifics.*"

Recently, a field study, conducted over 4 years, has clearly demonstrated that Hsp responses are extremely fine, when considering specific expressed isoforms, and allow detecting any minor temperature change between seasons or between years in the "thermophilic" marine cave mysid *Hemimysis margalefi* (Crustacea, Mysidacea) (Lejeusne *et al.*, 2006). For example, the authors have reported that expression of Hsp60 is closely related to seasonal changes in temperature and that the higher expressions during summer suggest more protein damage at that time. This study, conducted in the Northwestern Mediterranean sea, evidences that Hsps are good biomarkers to study the effects of warming episodes or heat stress in the wild. The influence of secondary factors has been also considered: (i) in *H. margalefi*, Hsp expression is not sex related; (ii) percentage of immature individuals and percentage of gravid females better explain variability in Hsp45 and Hsp60 amounts, respectively; (iii) abiotic factors (pH, salinity, dissolved O<sub>2</sub> and light intensity variations) usually reported to be rather stable in marine caves may only explain part of the unresolved observed variations; (iv) good and stable water quality of the study site avoid influence of pollutants in the expression of these stress proteins. The results provide strong background for the use of Hsps as biomarkers of biological and ecological effects of global warming on marine communities. This study also demonstrated that, when well conducted, field research lead to a better understanding of Hsp expression patterns in wild populations and are more informative than laboratory experiments for development and the calibration of biomarkers. Such studies must be especially encouraged and supported as they offer new early warning biological tools for one of the most preoccupant present problem in ecology, the global climate warming. For example, in the Mediterranean sea, a warming trend with a higher frequency of exceptional meteorological-hydrological events has already led to northward migrations of thermophilic species, to diseases and to mortalities inducing local extinctions or species shifts (Pérez *et al.*, 2000; Chevaldonné and Lejeusne, 2003). This kind of research could be enlarged to others key species to monitor ecological risk assessment as global warming. It seems now well established that biomarkers of short-term effects reflecting the mechanisms by which the health of organism is altered are relevant to predict the effects of long-term perturbations (Lagadic, 2002).

Another study, carried out under experimental conditions in two Polychaeta species, *Marenzelleria neglecta* and *M. viridis*, shows that acclimation to variations in salinity had no explicit effect on Hsp synthesis, and that *M. viridis* was thermally more sensitive than its sibling species (Blank *et al.*, 2006). The authors suggest that temperature, alone or in combination with other abiotic factors, plays a far greater role in the biogeographical distribution in *Marenzelleria* spp. than has been estimated so far. Thus, according to them, the induction and synthesis of stress proteins in the Polychaeta species will be helpful to understand biogeographical distribution.

### ***Hsp expressions in the assessment of xenobiotic exposure effects***

During the last decades, aquatic ecosystems have been subject to increased contamination from both inorganic, e.g. heavy metals, and organic compounds, e.g. polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), pesticides, medicinal compounds from urine wastes... Pollution caused by these contaminants represents one of the major factors of environmental stress in aquatic environments, where 'environmental stress' define all the external factors that alter the physiological processes of a living organism. Nowadays, most aquatic biota is exposed to moderate and chronic multifactor pollution and adverse effects on these ecosystems are difficult to assess. That is why, during the last 25 years, substantial efforts have focus on development of sensitive biomarkers for ecological risk evaluation. However, Forbes *et al.* (2006) have claimed that the validation process for application of biomarkers in ecotoxicology and ecological risk assessment is extremely difficult, if not impossible. These have also stated that biomarkers "*have failed to demonstrate their usefulness, as indicated by their minimal incorporation into national and international risk assessment protocols*". Handy *et al.* (2003) have already report several important steps before transferring biomarkers



technology from research and development framework to a regulatory one. Among them, the need of standard operating procedures, coordinated by central body such as the International Standards Organization (ISO) or Organization for Economic Cooperation and Development (OECD), is a key impediment to the utilization of biomarkers. If it is true that biomarkers are still not used as ecotoxicological tools in the day to day management of aquatic environments, a fundamental question arises: why is it so difficult to use biomarkers in such management programs? An answer could be obtained when considered all previous works (Table 1). It is evident that most studies have been conducted under laboratory controlled experimental conditions and not *in situ* conditions. Hsp responses to xenobiotics have mostly been studied by laboratory works dealing with acute short-term exposures to single contaminants while long-term responses are often neglected in these experiments. This concern was already pointed out by Depledge *et al.* (1992) and Handy *et al.* (2003) for the global biomarker approach. According to Handy *et al.* (2003) "*long-term laboratory studies may serve as a reference point for developing biomarkers of chronic exposure or effect in the field*". Long-term studies will be more informative on the adverse biological effects of contaminants. They will better reflect the field exposures that are characterized by low levels of diverse array of contaminants via both water and dietary route. As invertebrates play a key role in food webs, biomarker responses in the aquatic invertebrates are pertinent for risk assessment. Laboratory experiments are necessary to understand and to make sure that the expression of Hsps results from the exposition to a specific xenobiotic. However, despite offering clues about their induction following chemical stressors, results obtained in controlled conditions failed to be translated to field studies. As pointed out by Lejeune *et al.* (2006) "*most published works study the influence of an acute or repetitive stress on the physiology of organisms raised or stressed in the laboratory. Although such approaches are helpful in providing specific biological responses to a give (controlled) stress, they are less representative and less sensitive than fieldworks. Therefore, they are not sufficient to adequately assess the combination of biotic and abiotic factors found in natural conditions.*"

It is known that pollutants interfere with organism integrity at the biochemical level with consequent adverse effects at the individual level such as growth, reproduction and survival. However, these biochemical parameters have a reduced long-term ecological relevance at the population and community levels. Indeed, such early warning biomarkers would be useful for detecting sublethal pollution before changes in community structure or species composition occur. This concept of an early detection of adverse effects is illustrated by Sanders *et al.* (1991). They have shown a significant induction of Hsp60 in *Mytilus edulis* than in controls in response to copper exposure. Their data reveal that Hsp60 induction is detected for substantially lower copper concentrations compared with those leading to physiological injuries, i.e. scope-for-growth. Another example is given by the study of De Jong *et al.* (2006) in which expression of Hsp70 is induced in chloride cells of mayfly larvae collected in highway retention pond during an osmotic shock, resulting from a large increase in Na<sup>+</sup> and Cl<sup>-</sup> concentrations after the de-icing road surface. As salinity is obviously a major stress for animals living in freshwater habitats, it was suggested a protective role of Hsp70. The osmotic shock disrupts biochemical processes, modifies cellular homeostasis and can cause stunted growth or death. In this study, NaCl pond concentrations were 30 times lower than those leading to chloride cells ultrastructural damages that result in impaired osmoregulation processes. Therefore, in this case, induction of Hsp70 in these target cells can also be considered as an early biological signal. In addition, the induction of stress proteins is often maximal in target organs and/or cells (De Jong *et al.*, 2006), and thus, expression pattern of biomarkers would be relevant to better understand cellular mechanisms involved during xenobiotic exposure. So, studies on biomarkers will have given informations about toxicological pathways of pollutants in living organisms.

By integrating multiple endpoints at different ecologically relevant levels of organization within one test organism, it should be possible to gain understanding in how each level within this organism responds to toxic exposure and how responses at these levels are interrelated. Agell *et al.* (2004), in a study combining field and laboratory investigations have examined the effect of copper on several endpoints: the production of heat-shock proteins, defense metabolites, growth rates and presence of resistance forms in the colonial ascidian *Pseudodistoma crucigaster*. They performed a transplant experiment to a copper polluted harbor and laboratory experimental copper exposures. They failed to find any Hsp responses, despite a negative growth, in transplanted animals and in animals exposed to copper levels comparable to those measured in the harbor. They suggest an inhibition of Hsp induction possibly due to a too high stress level related to very high copper concentrations. When exposed to lower copper levels, however, an induction of Hsp78 was observed in *P. crucigaster*. These results demonstrate that preliminary sensitive analyses are needed to determine the range of

pollutant concentrations allowing valuable responses of this biomarker in a given species. They conclude that Hsp expression can be a good biomarker for pollution in these organisms when stress levels are below a threshold.

In risk assessment of chronic multifactor pollution, it is necessary to previously conduct integrated *in situ* studies that combine biological and physico-chemical markers. This kind of approach has been successful for the calibration step of other stress proteins belonging to the multixenobiotic resistance system (MXR) (Saez *et al.*, 2008). In insect aquatic larvae, MXR protein membrane density was positively correlated with PAH but not with PCB concentrations measured in stream sediments. Such field approach could also be developed for Hsps and positive experimental data already exist to encourage investigations on the relationships between Hsp expression and PCB levels in the field. For example, [Wiens \*et al.\* \(2000\)](#) report that PCB118 induces Hsp90 in the octocoral marine species *Dendronephthya klunzingeri*. PCB congeners, PCB77, PCB118 and PCB153, are also able to induce constitutive Hsp73 in the marine sponge *Suberites domuncula* ([Schröder \*et al.\*, 1999](#)). Others laboratory experiments have shown that cadmium exposure induces Hsp70 in *Mytilus californianus* ([Eufemia & Epel, 2000](#)) and in *Mytilus edulis* ([Tedengren \*et al.\*, 2000](#); [Radlowska & Pempkowiak, 2002](#)). However, in field research, as these proteins seems to be induced by many chemical stressors (Table 1), the Hsp70 response is probably difficult to interpret in the context of chronic multifactor pollution. Further studies that will consider specific isoforms and especially low molecular weight Hsps will probably offer suitable interpretation that could be related to specific class of pollutants ([Dhainaut \*et al.\*, 1997](#)). As the amino-acid sequence of these Hsps is less conserved between species than that of the ubiquitous Hsp70 family, such investigations must be carried out only in pertinent species. A pertinent species, in the present context, is either a species reared in unpolluted conditions and then transplanted in the field (caging) or a species that lives in the considered study area. In marine ecosystems, oysters seem to be good candidates for the Hsp biomarker tool calibration to assess both the global warming and xenobiotic exposure. Indeed, in *Ostrea edulis*, heat shock (1h exposure at 32°C) induces the appearance of a new Hsp isoform of approximately 69 kDa, which was not present in control animals ([Piano \*et al.\*, 2002, 2004](#)). Moreover, the expression of two constitutive Hsp isoforms, Hsp77 and Hsp72 was not or poorly affected by heat. Field studies are needed to characterize the seasonal variations of Hsp expressions: is induced isoform expression affected by the seasonal variations of physico-chemical parameters? Are constitutive Hsps induced during exposure to specific family of xenobiotics? For all these questions and because oysters could be maintained in laboratory and also kept in cages in the field, further studies on the expression pattern of Hsps must be encouraged in this species. Specific Hsp responses could also be obtained taking in consideration the tissue specificity. This latter and temporal patterns of Hsp induction have therefore been observed in *O. edulis* ([Piano \*et al.\*, 2004](#)), in agreement with similar observations reported in other organisms in response to stress ([Sanders \*et al.\*, 1994](#), [De Jong \*et al.\*, 2006](#)).

## Conclusion

In marine ecosystems, many studies, including pertinent field research, have pointed out the possibility to use Hsp induction in the monitoring of global warming changes. Considering these investigations, a better knowledge of the mechanisms of Hsp induction in sentinel organisms will certainly lead to the development of valuable tools for ecological risk assessment.

For assessment of xenobiotic exposure effects considerable efforts must be devoted to consider Hsps as a reliable biomarker tool in aquatic biota. Integrated *in situ* studies that combine biological and physico-chemical markers for evaluation of environmental risks must be developed for the monitoring of aquatic ecosystems. Indeed, as it has been stated by [Bodin \*et al.\* \(2004\)](#), studies involving integrated responses of biomarkers as a function of natural variables and chemical mixtures in specific natural habitats would appear to be the most suitable approach for future monitoring of environmental stress.

To relate an observed biological effect to a specific pollutant or even a class of pollutants remains a very difficult task, due to the usually unknown, complex and often highly variable concentrations of pollutants in aquatic ecosystems. In this context, multimarker tools have to be developed and new biochemical methods will be helpful. Recently, [Dondero \*et al.\* \(2006\)](#) have described the design and implementation of a novel low-density oligonucleotide microarray: the “Mytox-chip”. It consists of 24 mussel genes involving both normalizing elements and stress response related genes, each represented on the array with one or two different 50-mer oligonucleotide-probe reporters spotted in replicated samples on glass-activated slides. As target genes were selected on the basis of their potential involvement in mechanisms of pollutant and xenobiotic response,

this method can represent a valid tool to improve the knowledge in the field of molecular toxicology for mussels. The authors explained that *"the integration of biological effects of pollutants with both gene and protein expression profiles will provide a more correct interpretation of how molecular adaptations take place and how a stress syndrome develops in challenged organisms. Furthermore, since transcription patterns were significantly modulated by exposure to pollutants, and since mussels are established sentinel organisms often employed in marine biomonitoring programs, the 'Mytox-chip' appears to be a technology with a high potential."* Microarray technology has to be developed for many model species. In this ongoing topic research [Hansen et al. \(2007\)](#) have proposed an expressed sequence tags library from the marine copepod *Calanus finmarchicus* exposed to different oil-industry related environmental stressors, as a tool for future toxicogenomic studies.

To assess water quality and pollution in field conditions, transplanted organisms or field collected organisms must be used. *In situ* exposures will provide some insights into understanding and predicting effects of chemicals on natural communities under realistic exposure conditions and would, thereby, contribute towards improving the interpretation of laboratory to *in situ* extrapolation. To recognize biomarkers as monitoring tools, it is important to find a complementary suite of and to use multivariate analysis for response interpretations that could combine biological responses and physico-chemical field parameters, including priority contaminant concentrations. [Handy et al. \(2003\)](#) propose to *"take advantage of techniques developed for human epidemiology in using a suite of biomarker responses to describe "syndromes" that are diagnostic of a particular pollution scenario"*.

It is now evident that a calibration of biomarkers is needed before to consider them as interpretable warning signals for ecotoxicological risk assessment. Calibration must focus on the choice of representative species, field studies, and simple reproducible methods offering the ability to investigate a battery of specific biomarkers. In regards to actual knowledge, it seems that Hsp expression could be included among such biomarkers.



<i>Organisms</i>	<i>Stressors</i>	<i>Hsp 90 family</i>	<i>Hsp 70 family</i>	<i>Hsp 60 family</i>	<i>Hsp 20-40</i>	<i>Laboratory conditions (LC) or Field studies (FS)</i>	<i>References</i>
<b>Marine organisms</b>							
<b>Rotifers</b>							
<i>Brachionus plicatus</i>	Copper, tributyltin (TBT)				Hsp58	LC	Cochrane <i>et al.</i> , 1991
<b>Chaetognaths</b>							
<i>Spadella cephaloptera</i>	Wound healing		+			LC	Jean <i>et al.</i> , 2004
<b>Sponges</b>							
<i>Geodia cydonium</i>	Thermal stress (heat & cold shocks)					LC	Krasko <i>et al.</i> , 1997
<i>Suberites domuncula</i>	PCB77, PCB118, PCB153				+ (mRNA) Hsp73 (C), Hsp75 (I)	LC	Schröder <i>et al.</i> , 1999
<b>Cnidarians</b>							
<i>Anemonia viridis (Hexacoral)</i>	Heat shock					LC	Sharp <i>et al.</i> , 1994
<i>Anemonia viridis</i>	Seasonal temperature variations					LC & FS	Choresch <i>et al.</i> , 2001
<i>Dendronephthya klunzingeri (Octocoral)</i>	Thermal stress	Hsp90				LC & FS	Wiens <i>et al.</i> , 2000
	PCB118	Hsp90				LC	
	Cadmium	NS				LC	
<b>Polychaeta</b>							
<i>Marenzelleria viridis &amp; M. neglecta</i>	Thermal stress				Hsp75, Hsp86	LC	Blank <i>et al.</i> , 2006
<b>Molluscs</b>							
<b>Bivalvia</b>							
<i>Mytilus edulis</i>	Copper					LC	Sanders <i>et al.</i> , 1991, 1994
<i>Mytilus edulis</i>	Tributyltin (TBT)					LC	Steinert & Pickwell, 1993
<i>Mytilus edulis</i>	Heat shock					LC	Chapple <i>et al.</i> , 1997
<i>Mytilus edulis</i>	Seasonal temperature variations					FS	Chapple <i>et al.</i> , 1998
<i>Mytilus edulis</i>	Chlorine exposure					LC	Lawrence & Nicholson, 1998
<i>Mytilus edulis</i>	Heat shock, cadmium					LC	Tedengren <i>et al.</i> , 2000
<i>Mytilus edulis</i>	Cadmium, lead, copper					LC	Radlowska & Pempkowiak, 2002
<i>Mytilus edulis</i>	Salinity and cadmium	Hsp90				FS	Lyons <i>et al.</i> , 2003
<i>Mytilus edulis</i>	Heat shock, cadmium					LC	Pruski & Dixon, 2007
<i>Mytilus californianus</i>	Cadmium, arsenite, DDE, PCP, heat shock					LC	Eufemia & Epel, 2000
<i>Mytilus galloprovincialis</i>	Seasonal temperature variations					FS	Minier <i>et al.</i> , 2000
<i>Mytilus galloprovincialis</i>	Seasonal variations & chronic pollution					FS	Bodin <i>et al.</i> , 2004
<i>Mytilus galloprovincialis</i>	50 Hz magnetic field					LC	Malagoli <i>et al.</i> , 2004
<i>Mytilus galloprovincialis</i>	Hg <sup>2+</sup>	+				LC	Franzellitti & Fabbri, 2006
<i>Mytilus galloprovincialis</i>	CH <sub>3</sub> Hg <sup>+</sup>					LC	Franzellitti & Fabbri, 2006
<i>Modiolus modiolus</i>	Seasonal temperature variations					FS	Lesser & Kruse, 2004

<i>Perna perna</i>	Zinc		NS	+		LC	Franco <i>et al.</i> , 2006
<i>Bathymodiolus azoricus</i>	Hydrothermal vent extreme conditions		+			LC & FS	Pruski & Dixon, 2007
<i>Macoma nasuta</i>	Polluted sediments (DDT & metabolites)		Hsp70			LC	Werner <i>et al.</i> , 2004
<i>Ostrea edulis</i>	Cd & Zn		Hsp70			LC	Piano <i>et al.</i> , 2004
<i>Ostrea edulis</i>	Heat shock		Hsp69(I)			LC	Piano <i>et al.</i> , 2005
<i>Argopecten irradians</i>	infection by <i>Vibrio anguillarum</i> : immune response		+			LC	Song <i>et al.</i> , 2006
<i>Chlamys farreri</i>	Cd <sup>2+</sup> , Pb <sup>2+</sup> & Cu <sup>2+</sup>	+				LC	Gao <i>et al.</i> , 2007
<i>Gasteropoda</i>							
<i>Nucella ostrina</i> & <i>N. canaliculata</i>	Thermotolerance: geographic distribution		Hsp70			LC	Sorte & Hofmann, 2005
<b>Crustaceans</b>							
<i>Eurytemora affinis</i> (estuarine Copepoda)	Heat shock		+			LC	Bradley <i>et al.</i> , 1992
<i>Calanus finmarchicus</i> (Copepoda)	Heat shock		+			LC	Voznesensky <i>et al.</i> , 2004
<i>Asellus aquaticus</i>	Heat shock			+		LC	Anneli-Korhonen & Lagerspetz, 1996.
<i>Hemimysis margalefi</i> (Peracarida, Mysidacea)	Seasonal temperature variations			+	Hsp45	FS	Lejeune <i>et al.</i> , 2006
<i>Procambarus clarkii</i>	Heat shock	+	+			LC	Rochelle <i>et al.</i> , 1991; Sheller <i>et al.</i> , 1998
<i>Penaeus monodon</i>	Heat shock		Hsp86			LC	Cimino <i>et al.</i> , 2002
	Hypoosmotic stress		Hsp86, Hsp100			LC	Cimino <i>et al.</i> , 2002
<i>Homarus americanus</i> (larvae)	Heptachlor exposure			+			Snyder & Mulder, 2001
<i>Cherax quadricarinatus</i>	Heat shock		Hsp86, Hsp97		Hsp25	LC	Cimino <i>et al.</i> , 2002
<b>Echinoderms</b>							
<i>Paracentrotus lividus</i> (embryo)	Cadmium exposure	+	Hsp70, Hsp72	Hsp56	Hsp25, Hsp28	LC	Roccheri <i>et al.</i> , 2004
<b>Ascidian</b>							
<i>Pseudodistoma crucigaster</i>	Copper		Hsp78				Agell <i>et al.</i> , 2004
<b>Salt marsh organisms</b>							
<b>Crustaceans</b>							
<i>Artemia sp</i> (larvae)	Heat shock		Hsp68		Hsp31	LC	Miller & McLennan, 1988
<i>Artemia franciscana</i>	Heat shock		HSC70, Hsp67			LC	Frankenberg <i>et al.</i> , 2000
<b>Freshwater organisms</b>							
<b>Sponges</b>							
<i>Ephydatia fluviatilis</i>	Organic micropollutants extracted from polluted water & high temperature		+			LC	Müller <i>et al.</i> , 1995
Endemic Baikalian sponges	Heat stress		+			LC	Efremova <i>et al.</i> , 2002
<i>Baikalospongia intermedia</i>	Wastewater from Paper Plant		+			LC	Efremova <i>et al.</i> , 2002

<b>Planarians</b>					
<i>Dugesia (Girardia) schubarti</i>	Copper			-	Guecheva <i>et al.</i> , 2003
<i>Dugesia (Girardia) schubarti</i>	Heat shock			+	Guecheva <i>et al.</i> , 2003
<b>Molluscs</b>					
<i>Dreissena polymorpha</i>	Copper, tributyltin (TBT).		+	+	Clayton <i>et al.</i> , 2000
<b>Crustaceans</b>					
<i>Daphnia magna</i> (Cladoceran)	Heat shock			+?	LC Bond <i>et al.</i> , 1993
	AgCl & SDS	+		+	LC Bradley, 1993.
<b>Insects</b>					
<i>Chironomus tentans</i> (larvae)	nonylphenol, bisphenol-A, 17 $\alpha$ -ethynyl estradiol, bis(2-ethylhexyl) phthalate, endosulfan, paraquat dichloride, chloropyrifos, fenitrothion, cadmium chloride, lead nitrate, potassium dichromate, benzo[ <i>a</i> ]pyrene, carbon tetrachloride.			HSC70, HSP70	LC Lee <i>et al.</i> , 2006
<i>Cloeon dipterum</i> (larvae)	NaCl (osmotic shock)			+	FS De Jong <i>et al.</i> , 2006

Table 1. List of major works dealing with Hsp induction in response to stressors in marine, salt marsh and freshwater invertebrates.

(C) : constitutive form; (I): inducible form; + : Induction of Hsp but molecular weight not given; -: Non induced

Note: Given references are not exhaustive but are representative of current Hsp researches in aquatic invertebrates

DDE: 1-1 dichloro-2,2-bis(p-chlorophenyl)ethylene, PCP: pentachlorophenol; NS: Non significant.

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