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Monitoring Behavioral Responses to the Heavy Metal Cadmium in the Marine Shrimp *Hippolyte inermis* Leach (Crustacea: Decapoda) with Video Imaging

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Hubert Untersteiner, Gerwin Gretschel, Tom Puchner, Sonja Napetschnig, and Helmut Kaiser (2005) Monitoring behavioral responses to the heavy metal cadmium in the marine shrimp *Hippolyte inermis* Leach (Crustacea: Decapoda) with video imaging. *Zoological Studies* **44**(1): 71-80. In this study, the subacute toxicity of the heavy metal cadmium to *Hippolyte inermis* Leach was investigated. Subacute effects were evaluated using changes in the locomotory behavior (i.e., moving velocity and moving distance) as indicators. The locomotory activity was analyzed by means of real-time image analysis, using a video camera and a Pentium PC equipped with a standard low-cost frame grabber. For a sequence of 3000 images per treatment, where 10 shrimp were moving simultaneously, the trajectories were reconstructed as binary image sequences. The locomotory activity of the test organisms was analyzed under normal conditions (without heavy metal stress) and after application of a subacute Cd stress. Test animals were stressed by Cd of the following 3 concentrations: 1 (C_1), 2 (C_2), and 3.5 ppm (C_3). Shrimp were exposed to the heavy metal concentrations for 12 h under static conditions. At initiation (0 h) of Cd exposure, the test animals showed a significant ($p \leq 0.05$) decrease in the average swimming velocity at C_3. After 3 h of Cd exposure, the median moving velocity was for the first time highly significantly ($p \leq 0.01$) reduced with the 1 ppm Cd treatment.
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The Adriatic Sea is a complex and sensitive ecosystem in which different organisms are exposed to various anthropogenic associations of chemical compounds. The northern Adriatic Sea is strongly influenced by freshwater discharges from the River Po and adjacent rivers (Tankere and Statham 1996). Although the Adriatic Sea is not as heavily contaminated by Cd pollution as other marine ecosystems like the North Atlantic Ocean, North Pacific Ocean, Indian Ocean, and Baltic and Black Seas (Sekulić and Vertačnik 1997), investigations from Tankere and Statham (1996) indicated that the River Po-influenced region of the Adriatic (i.e., the northern part) shows higher Cd concentrations than the central and southern region. The major source for the heavy metal cadmium (Cd) is likely the River Po. It is known that Cd can be mobilized from sediments under reducing environmental conditions, and it subsequently diffuses from pore water into the water column (Zago et al. 2000). The toxic potential of Cd is enormous, since continuous exposure of marine organisms to a low concentration of Cd can result in bioaccumulation, and subsequent transfer to humans through the food web (Kljaković Gašpic et al. 2002). As a result of global concerns over the impacts of xenobiotics on aquatic ecosystems and human health, several scientific biomonitoring systems have been developed using different invertebrates and vertebrates

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as indicator organisms (e.g., the "Fish-Rheotaxis test", "Koblenz behavioral fish test", "Dreissena-Monitor", "Dynamic Daphnia test", and the "Multispecies Freshwater Biomonitor") (Knie 1978, Bocherding 1992, Schmitz et al. 1994, Gerhardt 1998, Gerhardt 1999, Untersteiner et al. 2003). In Europe and the US, numerous bioanalytical techniques for the control of water quality have been developed during the past several decades and applied at the sub- and multicellular levels of biological organization.

Table 1 gives an overview of the biological test systems (excluding plants) developed in Europe and the US during the past few decades.

In most cases of scientific biomonitoring, the acute toxicity of chemical compounds is determined using mortality rates as the parameter. To determine the sublethal effects of chemical substances, it is common to use behavioral or physiological parameters as biological endpoints, since the behavior of an organism is defined as the endpoint of a sequence of different neurophysiological processes (Lagadic et al. 1994, Gerhardt 1995, Untersteiner et al. 2003). In particular, the complex pattern of locomotory behavior can be considered as an integration of physiological, sensorial, nervous, and muscular systems (Charoy et al. 1995, Untersteiner et al. 2003).

Although many publications exist which deal with Cd toxicity to different aquatic invertebrate and vertebrate species (Viarengo et al. 1997, Fichet et al. 1998, Rasmussen and Andersen 2000, Rainbow et al. 2000, Adami et al. 2002, Kljaković Gašpic et al. 2002, Filipović and Raspor 2003), no one has studied the Cd sensitivity of *Hippolyte inermis* Leach (Crustacea: Decapoda). This organism meets various requirements and therefore appears to potentially be suitable for use in environmental biomonitoring. It is very abundant in an easily accessible and ecologically extremely important habitat, i.e., seagrass meadows composed of *Posidonia oceanica* and *Cymodocea nodosa* (Gambi et al. 1992). It can be sampled easily and in high numbers by divers, and it can be maintained in tanks in a healthy condition until use (Zupo 2000).

The aim of this work was therefore to study acute and subacute toxicity of the trace metal Cd to the marine crustacean species *H. inermis* and to test its suitability as a test organism in the context of classical biomonitoring.

MATERIALS AND METHODS

Study sites and test organisms

The test organisms were collected from a seagrass habitat (*Cymodocea nodosa* (Ucria)) in the bay of Valsaline, Pula, Croatia (44°51'044"N, 13°50'080"E) (Fig. 1) at water temperatures of between 25 and 27°C.

For every toxicological trial, specimens were sampled by 2 divers from a depth of 4~6 m by pulling a simple hand dredge (with a frame of 15 x 20 cm, a net length of 105 cm, and a mesh size of

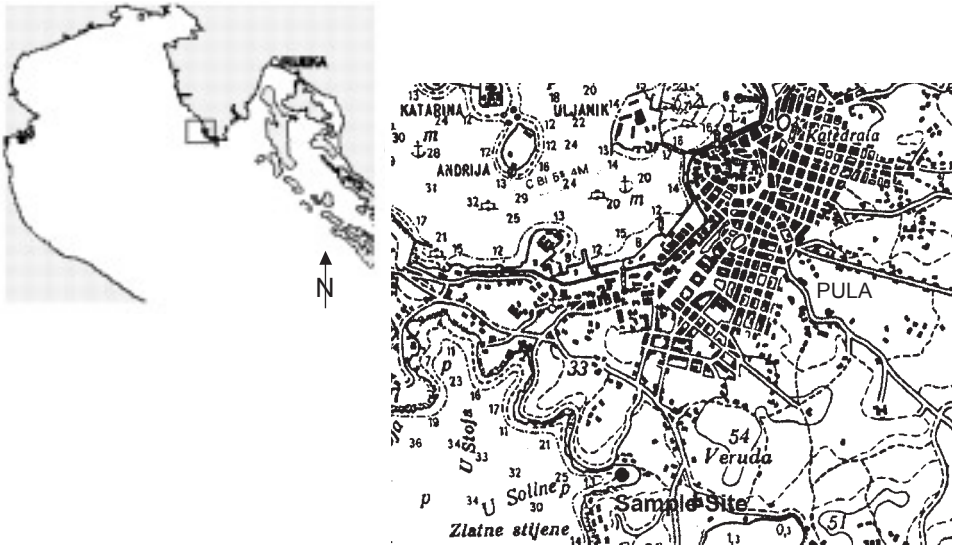

Fig. 1. Location of the sampling sites in the Bay of Valsaline, Pula, Croatia (44°51'044"N, 13°50'080"E).

Table 1. Overview of developed and applied biological assays (completed and modified after Gerhardt 1999)

Biological organization	Endpoint of biological response	References
Biomarker		
stress proteins (e.g., Hsp27 and Hsp60), metabolic enzymes (e.g., hexokinase and dehydrogenase), biotransformation enzymes (cytochrome P450 enzyme system or mixed-function oxygenase system)	induction, catalytic activity	Schramm et al. 1999
Fish cell lines		
cells of connective tissue (e.g., R1 cells and RTG-2 cells), hepatocytes (PLHC-1 and RTL-W 1 cells)	acute cell death, cell vitality, morphological alterations, biochemical alterations	Braunbeck et al. 1992 Braunbeck 1995
Bacteria		
Vibrio fisheri (NRRL-B-11177)	bioluminescence	Krebs 1992, Link 1992, Steinhäuser 1992, Klein 1992, Bulich et al. 1996
Synechococcus sp. (PCC6301)	electron transport	Stein 1992
Escherichia coli	respiration	Stein 1992
Salmonella typhimurium	genotoxicity	Nakamura et al. 1987
Mussels		
Dreissena polymorpha	bioaccumulation shell movements	Mersh et al. 1993 Bocherding, Volpers 1994, Hoffmann et al. 1994
Corbicula fluminea	shell movements	Ham and Peterson 1994
Anodonta cygnea	shell movements	Englund et al. 1994
Mytilus galloprovincialis *Adamussium colbecki* *Mytilus trossulus* *Crassostrea gigas*	metallothionein concentration in tissue, metal concentration in tissue, morphological alterations	Viarengo et al. 1997, Rainbow et al. 2000, Fichet, Miramand 1998
Praunus flexuosus	copper metabolism	Garnacho et al. 2001
Crustacea		
Daphnia spp. *Artemia franciscana* *Gammarus* spp. *Balanus improvisus* *Hippolyte inermis*	motility, swimming velocity, swimming direction, phototaxis, trace metal concentrations in tissue	Knie 1978, Baillieul Scheunders 1998, Blübaum-Gronau and Hoffmann 1998, Fichet et al. 1998, Rainbow et al. 2000, this work
Echinoderms		
Paracentrotus lividus	bioaccumulation, morphological alterations during development	Fichet et al. 1998
Polychaetes		
Arenicola marina	mortality and volume regulation	Rasmussen and Andersen 2000
Other invertebrates		
different aquatic organisms (multiple species)	motility, ventilation (based on quadropole impedance conversion technology)	Gerhardt 1998 1999 2001
Craniota		
different species of fish	rheotaxis, motility ventilation, heart rate, locomotion	Spieser et al. 1994
Fundulus heteroclitus	mortality	Hurk et al. 1998
Gnathonemus petersii *Apteronotus albifrons*	electric organ discharge (EOD)	Thomas et al. 1996

1 mm) through the seagrass meadow (10~15 cm above ground). Sampling by divers proved to be highly efficient (~15 to 30 individuals/min). Impact on the seagrass habitat was minimal compared to conventional dredging by boat. The test animals were immediately transferred to tanks and sorted by body size. For the toxicological trial test, animals of the same body size class (~1.4 cm total length from the tip of the rostrum to the posterior medial notch) were used. The acute toxicity tests (the range-finding and definitive tests) were conducted with 100 shrimp (20 per group), while the ethotoxicological trials were conducted with 40 animals (10 per group).

To assess the ambient pollution levels of cadmium, a water sample was taken for chemical analysis in the laboratory (Table 2).

Cadmium dilutions

A 36‰ standard seawater solution (Aqua marina) was prepared for use in the toxicological tests. For a time period of 1 h before beginning the toxicological experiment, the standard seawater solution was well aerated using an aquarium air pump. In the ethotoxicological experiment, the test organisms were stressed by sublethal cadmium concentrations of 0 (C), 1 (C_1), 2 (C_2), and 3.5 mg/L (C_3). Dilutions were produced from a 1000 ppm cadmium standard solution (Merck). The highest Cd concentration (C_3) was based on the range of 12 h LC_{50} values determined in preliminary acute toxicity tests.

Measurement of locomotory activity

The design of the ethotoxicological experiment is presented in figure 2. The equipment was placed in a climate-controlled room to maintain a constant temperature of 27 ± 0.5°C during the trials, in order to ensure similar temperature conditions as found in the seagrass habitat of the shrimp.

Ten shrimp of each Cd treatment were placed into inert petri dishes (with an inside diameter of 85 mm) under static conditions, i.e., with no water exchange during the trial. Test organisms were carefully transferred out by means of a micropipette while minimizing stress to the organisms. Above the petri dishes, a video camera (JVC model no. GR-SX9E) was installed, which enabled the observation and recording of the behavioral parameters of the test animals. The animals were visible as dark silhouettes on a uniformly illuminated background (Fig. 3). The obtained images were digitized, processed, and analyzed by means of image-processing techniques. At first, images were recorded using a conventional video recorder. In the 2nd step, the videotapes were digitized using a low-cost frame grabber placed in a Pentium PC. The frame grabber consists of an A/D converter, which enables the digitization of images in real time (25 frames/s, i.e., an interval of 4 x 10^{-2} s between 2 frames). For further processing of the digitized images, Image J (vers. 1.30 v) software was used. It is a public domain Java image-processing program inspired by the National Institute of Health, USA for Macintosh computers. As a downloadable application, it runs on a PC with Java 1.1 or later virtual machines. Images were manipulated as series of single images, or so-called stacks. After thresholding the organisms, pixels (pixels covered by the organisms) were set to black (with a gray value of 0) and all other pixels (the background) were set to white (with a gray value of 255); by

Table 2. Physicochemical parameters of the seawater sample taken in the Bay of Valsaline, Pula, Croatia

Parameter	Values
Cd (µg/l)	0.46 ± 0.01
Ca (mg/l)	469 ± 4
Mg (mg/l)	1340 ± 20
° (dH)	375
pH	7.4
Salinity (‰)	36

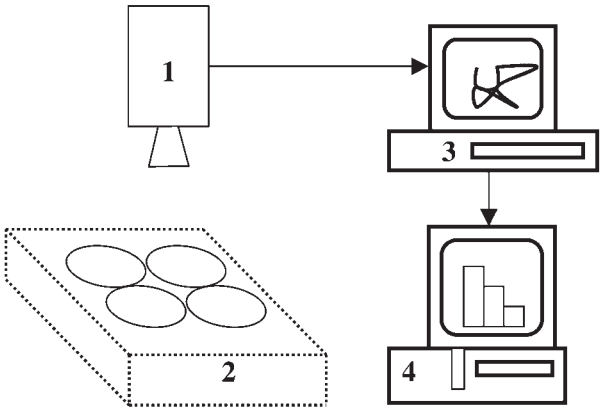

Fig. 2. Experimental setup: 1, video camera; 2, pad with petri dishes; 3, video recorder with monitor showing locomotory behavior; 4, Pentium PC with an integrated frame grabber and analytical software.

means of a special Image J plug-in (Multi-object tracker), an automated, simultaneous tracking of the test organisms during the entire stack was possible. The temporal resolution between 2 frames of the stack was set to 40 ms (for the real-time analysis). The program recorded the coordinates and automatically reconstructed the trajectories of the test animals. Although the Image J plug-in "multi-object tracker" is a very efficient tool for automatically recording the coordinates of the shrimp, some manual corrections and overwork were necessary, when 2 or more of the test organisms were superimposed upon each other, because the program plug-in is incapable of distinguishing between the pixels of 1 animal and the fused pixels of 2 or more animals. However, optimization of the Image J plug-in code was used to resolve the problem of the fused pixels. The obtained data were stored and exported to a statistical software package for further processing.

Statistical analysis

The 12 h LC_{50} values were calculated by means of probit analysis. Since the collected data showed no normal distribution, differences between the observed locomotory activities (moving velocity and distance moved) were tested using the Friedman test. In case of significant ($p < 0.05$) results from this test, differences between the 2 groups were tested by means of the Wilcoxon-Wilcox test in each case. The validity of an ethotoxicological trial was tested by means of survival analysis (Fig. 4). The acceptability criterion was defined as the survival of 90% of test animals in the controls during the trial. The statistical package SPSS for Windows (vers. 9.0) was used for calculating these tests.

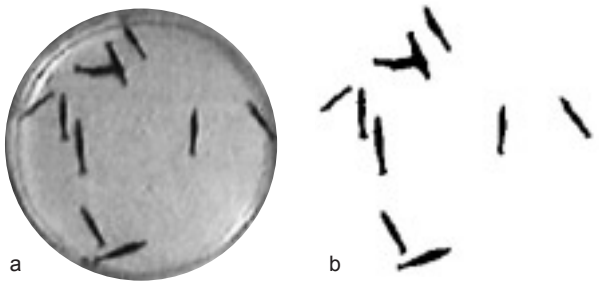

Fig. 3. (a) Silhouette of 10 shrimp, (b) pixels of the organisms after thresholding.

RESULTS

Chemical analysis of the water sample showed that the research site (the Bay of Valsaline) is less polluted with cadmium, and so it could be assumed that our research organisms were not adapted to Cd. As a result of the acute toxicity tests (the range-finding and definitive tests) a 12 h LC_{50} value of 3.45 mg/l Cd was calculated. Table 3 gives an overview of the toxicological data calculated for *H. inermis*.

The ethotoxicological trial with *H. inermis* was defined as valid when the animals in the control group showed a ≥ 90% survivability. To determine this survivability, a survival analysis was done. The results are shown in figure 4.

At initiation (0 h) of the ethotoxicological trial, the swimming activity highly significantly differed between groups (** $p \leq 0.01$). The median moving velocity was significantly reduced from 0.63 mm/s (controls) to 0.00 mm/s (3.5 ppm Cd treatment) (* $p \leq 0.05$). The median distance moved was significantly reduced from 75.74 mm (controls) to 0.00 mm in the 3.5 ppm Cd treatment (* $p \leq 0.05$). After 1 h of Cd exposure, median swimming activity highly significantly differed between groups (** $p \leq 0.01$). The median distance moved was significantly reduced from 40.03 mm (controls) to 0.00 mm in the C_3 group (* $p \leq 0.05$). After 3 h of Cd exposure, median moving velocity was for the first time highly significantly reduced in the 1 ppm Cd treatment (0.25 mm/s in the control group vs. 0.06 mm/s, ** $p \leq 0.01$). The median distance moved

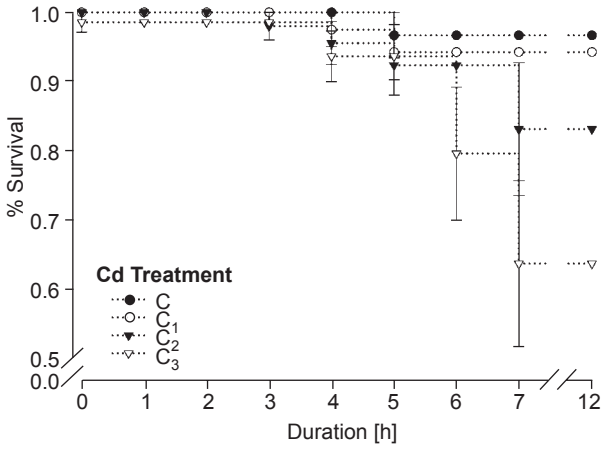

Fig. 4. Survival analysis for assessing the validity for the ethotoxicological trial. A trial was considered valid when the survivability of the controls was at least 90%.C, 0 ppm Cd; C_1, 1.0 ppm Cd; C_2, 2.0 ppm Cd; C_3, 3.5 ppm Cd. Ordinate (y-axis), Survival (%); Abscissa (x-axis) = Exposure time (h).

was highly significantly reduced from 29.58 mm (controls) to 5.44 mm (C_1 group). Tables 4 and 5 provide an overview of the calculated descriptive data of the ethotoxicological trial.

DISCUSSION

In general, *H. inermis* reacts very sensitively to artificial environmental conditions. After a time period of 12 h, it is not possible to make reliable ethotoxicological trials under static conditions, because the mortality of the controls increases very rapidly. The presumed cause is the relatively high water temperature of 27°C compared to 18°C used by Zupo (2000). However, for a time period ≤ 12 h the ethotoxicological results were reliable (Fig. 4). It can be observed that our test organisms reacted very sensitively to cadmium stress. During a time period of 3 h, we were able to measure the effects of Cd. The survivability decreased with increasing Cd concentration (Fig. 4). From the observed data and based on the stress response mechanisms of *Daphnia magna* described by Wolf et al. (1998) we derived the following processes of the stress response of *H. inermis* (Fig. 5): an adaptation reaction, a protection reaction, and 1st- and 2nd-order escape reactions.

During the adaptation reaction, a part of the metabolic energy is used to restore this imbalance. The protection reaction is characterized by decreased motility. During this phase, spontaneous muscular activity becomes depressed due to higher maintenance costs resulting in higher metabolic rates in certain non-muscular tissues (Heath 1995, Knops et al. 2001). The 1st-order escape reaction is characterized by increased ventilation and motility, whereas the 2nd-order escape reaction is characterized by very greatly increased ventilation activity and motility due to powerful beats of the pleon. This energy mobilization during the 2nd-order escape reaction is the last chance for the animal to escape unfavorable environmental conditions, or otherwise it will die (irreversible toxicological effects). A protection reaction was shown by the test animals exposed to lower Cd concentrations (C_1). Test animals of the C_2 group also showed a protective reaction at the initial Cd exposure (at 0 and 1 h). After 2 h of Cd exposure, the test animals of the C_2 group showed a typical 1st-order escape reaction. In this phase, they utilized their metabolic energy for increased muscle activity. Similar stress response mechanisms have been described for other crustaceans, like daphnids (Wolf et al. 1998, Untersteiner et al. 2003).

In the amphipod *Gammarus pulex* (L.), Gerhardt (1999) observed a general behavioral response pattern to toxicants, the so-called "stepwise stress model". The model describes how in reaction to a toxic compound, an organism shows a sequence of behavioral stress responses above their respective threshold of resistance. Within a species-specific tolerance range, organisms can regulate their behavior (e.g., ventilation behavior) in order to keep their body functions unaffected. The model further states that beyond an organism's resilience range, toxic effects appear, such as hyperactivity, resulting in exhaustion, decreased activity, and ultimately lethality. To avoid such toxic effects, organisms are able to switch to another stress-response behavior.

This behavioral plasticity of organisms as a

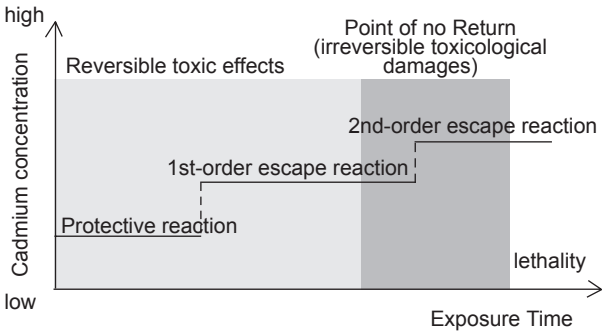

Fig. 5. Stress response model for *Hippolyte inermis* to Cd stress. With increasing toxicant concentration, an organism switches from 1 behavior to the next behavior according to its respective range of regulation.

Table 3. Calculated 12 h LC values for the biological endpoint of mortality

LC value (ppm)		95% Confidence limits (ppm)		Pearson goodness-of-fit	
		lower	upper	Chi-squared	*p* value
12-h LC_{10}	1.26	0.62352	2.09784		
12-h LC_{50}	3.45	2.67498	4.61758	0.458	0.977
12-h LC_{90}	5.64	4.51534	8.36748		

Table 4. Descriptive data of the effects of Cd on locomotory activity (distance moved, mm) of *Hippolyte inermis* for different durations of Cd Exposure. AM, arithmetic mean; SD, standard deviation; Sig, statistical significance: n.s., not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Animal	Start time (0 h)				Exposure time (1 h)				Exposure time (2 h)				Exposure time (3 h)			
no.	C	C_1	C_2	C_3	C	C_1	C_2	C_3	C	C_1	C_2	C_3	C	C_1	C_2	C_3
1	1613.71	267.58	0.00	0.00	236.81	70.38	0.00	1.44	0.00	25.84	31.43	10.88	21.25	6.29	0.00	0.03
2	68.17	59.16	60.69	0.00	42.67	38.59	0.00	0.00	115.43	200.13	21.76	504.05	20.74	4.59	0.00	0.00
3	0.00	4.59	205.53	205.53	37.40	25.33	27.73	0.00	82.43	31.62	121.40	0.00	239.78	16.66	217.26	0.00
4	4.59	4.59	226.27	0.00	28.05	64.63	0.00	0.00	0.00	21.76	0.00	0.00	507.12	0.00	0.00	0.02
5	26.86	14.96	0.00	0.00	244.46	0.00	0.00	0.00	0.00	7.48	652.63	0.00	0.00	0.00	26.86	0.00
6	427.21	8.09	0.00	2.89	6.29	15.98	0.00	4.59	25.33	7.48	0.00	0.00	78.88	4.59	96.05	0.00
7	83.30	34.76	91.12	0.00	0.00	32.81	339.32	27.71	32.83	0.00	121.06	0.00	534.14	10.37	0.00	0.01
8	167.28	4.59	30.60	0.00	3.27	10.88	0.00	0.00	3.40	0.00	118.83	0.00	6.29	16.15	39.78	0.00
9	64.94	103.87	66.64	13.26	97.48	10.88	0.00	0.00	151.98	0.00	0.00	0.00	10.88	6.29	31.11	0.04
10	116.11	79.57	21.59	0.00	194.31	5.10	0.00	0.00	0.00	0.00	0.00	0.00	37.91	2.89	96.90	0.00
AM	257.22	58.18	70.24	22.17	89.07	27.46	36.71	3.37	41.14	29.43	106.71	51.49	145.70	6.78	50.80	0.01
SD	492.50	81.61	82.95	64.56	98.78	24.30	106.68	8.67	55.80	61.14	199.17	159.05	209.86	5.92	69.35	0.01
Median	75.74	24.86	45.64	0.00	40.03	20.66	0.00	0.00	14.37	7.48	26.60	0.00	29.58	5.44	28.99	0.00
Sig.		n.s.	n.s.	**		n.s.	*	***		n.s.	n.s.	n.s.		**	n.s.	***

Table 5. Descriptive data of the effects of Cd on the locomotory activity (velocity, mm/s) of *Hippolyte inermis* for different durations of Cd exposure. AM, arithmetic mean; SD, standard deviation; Sig., statistical significance: n.s., not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Animal	Start time (0 h)				Exposure time (1 h)				Exposure time (2 h)				Exposure time (3 h)			
no.	C	C_1	C_2	C_3	C	C_1	C_2	C_3	C	C_1	C_2	C_3	C	C_1	C_2	C_3
1	13.45	2.23	0.00	0.00	1.97	0.59	0.00	0.01	0.00	0.22	0.26	0.09	0.18	0.05	0.00	0.00
2	0.57	0.49	0.51	0.00	0.36	0.32	0.00	0.00	0.96	1.67	0.18	4.20	0.17	0.04	0.00	0.00
3	0.00	0.04	1.71	1.71	0.31	0.21	0.23	0.00	0.69	0.26	1.01	0.00	2.00	0.14	217.26	0.00
4	0.04	0.04	1.89	0.00	0.23	0.54	0.00	0.00	0.00	0.18	0.00	0.00	4.23	0.00	0.00	0.00
5	0.22	0.12	0.00	0.00	2.04	0.00	0.00	0.00	0.00	0.06	5.44	0.00	0.00	0.00	26.86	0.00
6	3.56	0.07	0.00	0.02	0.05	0.27	0.00	0.04	0.21	0.06	0.00	0.00	0.66	0.04	96.05	0.00
7	0.69	0.29	0.76	0.00	0.00	0.27	2.83	0.23	0.27	0.00	1.01	0.00	4.45	0.09	0.00	0.00
8	1.39	0.04	0.26	0.00	0.03	0.09	0.00	0.00	0.03	0.00	0.99	0.00	0.05	0.13	39.78	0.00
9	0.54	0.87	0.56	0.11	0.81	0.09	0.00	0.00	1.27	0.00	0.00	0.00	0.09	0.05	31.11	0.00
10	0.97	0.66	0.18	0.00	1.62	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.02	96.90	0.00
AM	2.14	0.48	0.59	0.18	0.74	0.24	0.31	0.03	0.34	0.25	0.89	0.43	1.21	0.06	50.80	0.00
SD	4.10	0.68	0.69	0.54	0.82	0.20	0.89	0.07	0.46	0.51	1.66	1.33	1.75	0.05	69.35	0.00
Median	0.63	0.21	0.38	0.00	0.33	0.24	0.00	0.00	0.12	0.06	0.22	0.00	0.25	0.05	28.99	0.00
Sig.		n.s.	n.s.	**		n.s.	*	**		n.s.	n.s.	n.s.		**	n.s.	***

strategy against toxic stress as it is explained by the stepwise stress model has also been observed in other crustaceans, like the freshwater prawn *Macrobrachium nipponense* (Crustacea: Palaemonidae) (Gerhardt et al. 2002). As the 1st stress reaction, both *G. pulex* and *M. nipponense* show an increase in velocity followed by increased gill ventilation. Although the stepwise stress model describes the avoidance reaction (attempts to escape from the unfavorable site) as the 1st behavior of the stress response, it seems that a link of our stress model (based on stress patterns described by Wolf et al. 1998) to the well-reasoned "stepwise stress model" is possible. However, to verify this link a thorough quantification of additional behavioral parameters, like ventilation time and-ventilation frequency, which was not done in our experiments with *H. inermis*, is necessary. Furthermore it seems that the 1st stress reaction of our model, the "adaptation reaction" can be linked to the "regulation phase" of the stepwise stress model. Nevertheless more efforts have to be made to describe physiological processes of these phases of the stress response in order to make a satisfactorily correlation between the "adaptation reaction" of our stress model for *H. inermis* and the "regulation phase" of the stepwise stress model and to generalize the stress model for various aquatic organisms.

Locomotory behavior plays an important role in the evaluation of toxic compounds released into an ecosystem, since the complex pattern of locomotory behavior can be considered an integration of physiological, sensorial, nervous, and muscular systems (Charoy et al. 1995).

It is known that heavy metals influence a broad spectrum of physiological processes in organisms. In particular, effects of heavy metals on the nervous system are very important since the nervous system regulates and coordinates locomotory behavior. Findings of Baillieul and Blust (1999) showed that the beat frequency of the 2nd antennae, which are responsible for swimming activity in *D. magna* decreased with increasing cadmium concentration due to neurological failure. A correlation between heavy metal concentrations and toxic neurological effects in mussels was shown by Salanki (1992). Other physiological effects of the non-essential metal Cd have been discussed by several authors. Effects of Cd on changes in genetic variability and allele frequency at the population level of the gastropod *Littorina brevicula* were investigated by Kim et al. (2003). Viarengo et al. (1997) used the enzyme metallothionein of Mediterranean and Antarctic molluscs, *Mytilus galloprovincialis* and *Adamussium colbecki*, *respectively*, as an indicator of heavy metal stress. Metallothioneins are metal-binding proteins whose neosynthesis represents a specific response of different organisms to heavy metal stress. An increasing heavy metal concentration in cells stimulates the synthesis of apothioneins which bind metal cations in a non-toxic form, thus reducing their deleterious effects (Viarengo 1997). It can be assumed that metallothioneins play an important role during the adaptive and protective reactions of organisms.

Our results showed that *H. inermis* reacts very sensitively to Cd pollution. If sampled in seasons with lower water temperatures and reared at temperatures around 18°C to increase longevity under artificial conditions, *H. inermis* may prove useful as a standardized test organism for biomonitoring. However, further studies are required in order to verify the hypothesis that the marine seagrass shrimp (*Hippolyte inermis*) meets all quality criteria demanded of standardized test organisms in aquatic ecotoxicology.

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