Chapter 2
The Influence of Oil, Dispersed Oil and the Oil Dispersant SD-25, on the Heart Rate of the Mediterranean Mussel (*Mytilus galloprovincialis* L.)

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Abstract We applied an innovative technology, based on infrared light sensors, to point out the possible harmful effects of diesel oil and the oil slick dispersant Super-dispersant-25 (SD-25) on the cardiac system of the Mediterranean mussels (*Mytilus galloprovincialis* L.). Heart rate (Hr) measurements were conducted under laboratory conditions by noninvasive procedure for the registration and analysis of cardiac activity of molluscs and crustaceans. No response was observed of the Hr within a group of mussels in 2 h of exposure to 1 ml/l of diesel oil. However, a decrease of Hr in application of 10 µl/l of diesel oil dispersed by the same amount of SD-25 was recorded. Moreover, the very low concentration of SD-25, 2 µl/l and a hundred times higher one, caused a significant bradycardia which could be the evidence for high toxicity of this chemical. The resulting changes of the mussels Hr were reversible. The main advantages of the technique applied are data accuracy and real time information about changes of the environmental conditions.

2.1 Introduction

Oil spill incidents are inevitable in areas of production, transport, and the usage of oil (Etkin 2011). Oil contains polycyclic aromatic hydrocarbons (PAHs) with potent carcinogenic and mutagenic effects (Luch 2005). Blue mussels (*Mytilus edulis* L.) have a low ability to metabolize PAHs which leads to bioaccumulation in tissues (Neff 2002).

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Operational discharges from shipping and the cleaning of seashores cause an inordinate release of oil mixtures and commercial dispersants in the Adriatic Sea. Only a few dispersant formulations are widely registered and available in great quantities; one is Corexit 9527 (GESAMP 1993). Testing the influence of dispersants to a different sea species, it was found that SD-25 was less toxic than Corexit 9527 (Scarlett et al. 2005). In general, the crude oils and oil derivatives have a higher acute lethal toxicity than oil dispersants (Wells 1984).

There is a lack of available data considering the impact of SD-25 to the environment. This efficient agent has detergent properties, breaking the oil slick into fine droplets. This property makes it easier to disperse in sea conditions. SD-25 contains the following hazardous substances: 2-butoxyethanol and dioctyl sulphosuccinate (Oil Slick Dispersants ltd 2012). Accordingly, the organisms inhabiting the water column and the intertidal animals such as bivalves are particularly endangered. SD-25 was frequently used in the shipyard Bijela, Montenegro. Thus, we decided to test it on marine animals in vivo.

The Mediterranean mussel (*Mytilus galloprovincialis* L.) was suggested as a good indicator of different stress conditions in the aquatic environment (Moschino et al. 2011, Spada et al. 2013).

The human cardiovascular system quickly responded to stress, induced by oxygen depletion in arterial blood during exercises (Buchheit et al. 2004). Likewise, the occurrence of heavy metal pollution was rapidly detected by the heart of the Mediterranean mussel (Martinovic et al. 2013a) due to the activation of internal protective mechanisms that overcome this unfavourable situation. Later findings clearly determine the mussel's heart rate (Hr) as a suitable physiological parameter that provides early information about changes in environmental conditions.

One of the first methods based on infrared (IR) light sensors, used for recording the cardiac activity of molluscs and crustaceans, were designed by Depledge (1990).

We applied the innovative fiber optic method, developed by Kholodkevich et al. (2008), for the registration and analysis of the cardiac activity of invertebrates with solid skeletons. The noninvasive laser fiber-optic photopletismograph (LFOP) was used as a reliable device to provide the information about the Hr of Mediterranean mussels. Cardiac activity monitoring of eight specimens was conducted by fiber-optic IR light sensors attached to the mussel valves.

The objective of the study was to assess changes of the Hr of the Mediterranean mussel (*M. galloprovincialis* L.) from the Adriatic Sea, in short-term exposure to diesel oil, dispersed diesel oil and SD-25. Also, the aim was to create a basis for further application of IR light sensors in the biomonitoring of seawater.

2.2 Materials and Methods

Experiments were carried out at the Institute of Marine Biology Kotor on the coast of the Adriatic Sea. Sub-littoral Mediterranean mussels (*M. galloprovincialis* L.) were collected from a mussel farm in the Boka Kotorska Bay (Montenegro, 42°26′ N, 18°45′ E) at a depth of 2–3 m. After sorting, by shell length (60–70 mm) and the removal of encrusted organisms, 30 molluscs were placed in an aquarium in a temperature-controlled room for 72 h, for acclimatization to laboratory conditions (artificial salt water with constant aeration, water temperature 20 ± 1 °C and salinity 20 ± 1 g/l). Water was replaced daily.

The experimental unit for Hr registration and analysis is composed of 8-channel laser photopletismograph (LFOP), optical fibers with sensors (containing an IR light emitter and detector), 14-bit analog to digital signal converter (ADC) and a personal computer (PC; Martinovic et al. 2013b).

Eight mussels with attached sensors were maintained for 24 h, before the Hr measurements. Installing the sensors on the mussels valves above the heart area by use of harmless epoxy adhesive, allows IR light beam to pass through the shell illuminating the heart and circulatory vessels. The reflected light, modified by heart contractions, was received by an IR detector within the sensor. The obtained heartbeat signal was amplified, filtered, and digitalized by ADC and then sent to the PC.

Simultaneous recording of cardiac activity of eight individual sea shells was used for the analysis of Hr variations, caused by the applications of diesel oil, dispersed oil and the oil dispersant SD-25 as functional loadings. The oil slick dispersant Superdispersant-25 (SD-25) has all the approvals required for use at sea, on beaches and rocky shores (Oil Slick Dispersants ltd 2014).

Three different concentrations of functional loadings (10 µl/l of dispersed diesel oil; 2 and 100 µl/l of SD-25) were applied by pipette to an aquarium with 10 l of artificial seawater. For the preparation of dispersed diesel oil, we used 10 µl/l of diesel oil combined with a 1:10 water solution of 10 µl/l of SD-25. The oil mixture was placed in an automatic shaker for 12 h to enable better dispersion. After 2 h of exposure to the functional loadings, it was washed out and replaced with clean seawater.

VarPulse original software with variation pulsometry method was used to study the statistical analyzes of cardiac intervals (Kholodevich et al. 2008).

To determine the initial values of the Hr as a baseline, recording of mussels cardiac activity was started 2–3 h before the functional loading application. The Hr baseline was important as a reference point for the analysis of later-occurred Hr changes induced by the impact of tested substances.

The recovery time of an organism after stress caused by deterioration of the environmental conditions is calculated as the time needed for the restoration of Hr values before the experimental changes (Martinovic et al. 2013b). The calculation of recovery time was based on result graphs.

2.3 Results

There was no change in cardiac activity of the Mediterranean mussels in the action of 1 ml/l of diesel oil. Hr was consistently retained on the baseline level, all the time of exposure.

The influence of 10 µl/l of diesel oil, dispersed by the same concentration of the oil slick dispersant SD-25, caused a decrease in cardiac activity of mussels. The mean Hr baseline value within a group of eight mussels was stable at 18.7 beats/min with low standard deviation (SD; Fig. 2.1a). During the exposure period (*between*

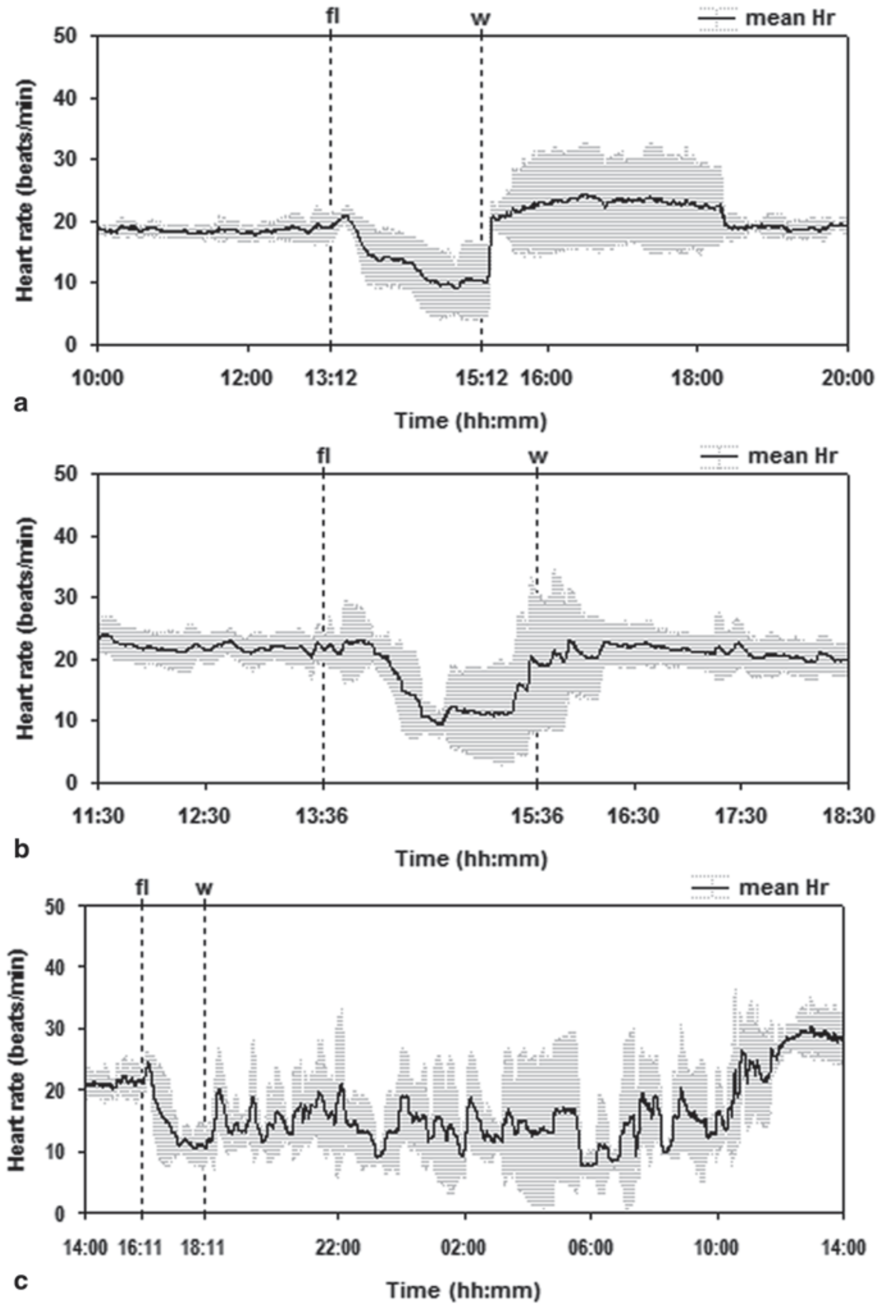

Fig. 2.1 Mean heart rate with standard deviation within a group of Mediterranean mussels *M. galloprovincialis* L. in 2-h exposure to **a** 10 µl/l of diesel oil, dispersed by 10 µl/l of SD-25, **b** 2 µl/l of SD-25, and **c** 200 µl/l of SD-25. *Hr* heart rate, *fl* functional loading, *w* washout

dashed lines), a slight increase of the Hr was observed, six minutes after functional loading application, followed by a descent to a minimal value of 9.1 beats/min and with a higher SD. The minimal Hr value was more than 50 % lower compared to the initial point. After the washout, the Hr was increased sharply with a significantly higher SD. The recovery time was calculated as the period from the moment of washout to achieving stable Hr values with a low SD. This was 3 h and 12 min.

The lowest concentration of SD-25 that impacted cardiac activity of the mussels, was only 2 μl/l. Also, we observed a large decrease in Hr activity from 23.1–9.6 beats/min followed by higher SD (Fig. 2.1b). Before the moment of washout, the mean Hr value was increased to 20.2 beats/min due to the spontaneous onset of Hr recovery. After the washout, the time of Hr recovery was only 46 min.

Five minutes after the application of 200 μl/l of SD-25, the initial Hr value rose for a short time, then sharply dropped from 23.2 to 10.4 beats/min (Fig. 2.1c). During the exposure, high concentration of SD-25 caused a great amount of foam in the aquarium due to strong dispersant activity. After the washout, Hr had ascending trend with high fluctuations retained for a long period. Also, SD was high and unstable. Accordingly, Hr recovery time was very long, 18 h and 40 min.

2.4 Discussion

The absence of Hr response in the action of 1 ml/l of diesel oil could be prescribed to the relatively short exposure period, associated with the insolubility of oil in seawater. In a recent study, published by Bakhmet et al. (2009), long-term exposure of oil pollution to blue mussels (*M. edulis* L.) caused changes of Hr.

Possible reasons were offered for the long-lasting decrease of the Hr of mussels during the exposure to different toxic polluters. Valves closure accompanied with blocking neuromuscular transduction was suggested by Kholodkevich et al. (2009) and stimulation of inhibitory cholinergic nerves of the heart (Curtis et al. 2001).

In our opinion, decrease of the Hr in action of dispersed diesel oil and SD-25 could be the low oxygen consumption when the mussel valves are closed and also the impact of hazardous compounds of SD-25. However, spontaneous Hr recovery observed in the addition of the lowest dose of SD-25 indicates that, in spite of the presence the toxicants, mussels overcome the stress that leads to conclusion that SD-25 poses mechanism of action that does not affect neurophysiologic pathways.

Associated with oil, SD-25 caused a longer recovery time to stress than separately. After dispersion, oil was able to reach mussel tissues, and despite low concentration, it was possible to contribute in resulting reaction of the mussel's heart.

Long-term fluctuations of the Hr in the action of 200 μl/l of SD-25 can be prescribed to insufficient washout of chemical due to great the quantity of foam. Thus, the baseline Hr value was achieved after the period required for water filtration by mussels.

2.5 Conclusions

We cannot argue that the direct influence of diesel oil is not dangerous for seashells, based on the absence of cardiac activity response within 2 h of exposure. However, oil slicks present a serious problem due to their mechanical impact on the entire ecosystem.

Dispersed diesel oil is more hazardous to organisms inhabiting the water column due to its solubility in seawater.

Due to a significant response in cardiac activity of the Mediterranean mussels (*M. galloprovincialis* L.) in the delivery of the 2 μl/l of SD-25, this could be the evidence for high toxicity of oil slick dispersant. Furthermore, the usage of SD-25 and similar substances needs to be reduced.

The fast response of the mussels Hr to the deterioration of environmental conditions, results obtained by IR light-based technology, may become an important tool for testing the impact of hazardous substances that are present in the port areas.

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