OPEN ACCESS
Vol. 6 No. 1: 65-74
May 2022
Peer-Reviewed

Akuatikisle: Jurnal Akuakultur, Pesisir dan Pulau-Pulau Kecil
EISSN 2598-8298 (online)
URL: https://ejournal.stipwunaraha.ac.id/index.php/ISLE
DOI: https://doi.org/10.29239/j.akuatikisle.6.1.65-74

Research Article

DNA damage and shell malformation in Blue Mussel, *Mytilus edulis*

Khusnul Yaqin

Departement of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar 90241, Indonesia.

Info Artikel:
Received: 12 March 2022
Accepted: 18 May 2022
Published: 18 May 2022

Keywords:
DNA damage, comet assay, blue mussel, tributyltin, antofouling agents

Correspondence:
Khusnul Yaqin
Department of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar 90245, Indonesia
khusnul@unhas.ac.id

ABSTRACT. Pollutants in waters that cause DNA damage, such as tributyltin, have been known to cause malformations in the mussel shell, which need attention to be studied. Shell malformations or deformities of blue mussels were observed in Norsminde Fjord harbour. The mean shell thickness index of blue mussels from the polluted site was greater than it from expected clean waters. DNA damages detected using the versatile comet assay which expressed as tail length and olive tail moment were evaluated in blue mussels gill cells both in situ and in vitro. The results showed that the average of DNA damages in treatments, namely the polluted site, tributyltin chloride (TBTC) (10 g/l), and hydrogen peroxide (H_2O_2) (10 M) were different from the expected unpolluted site. Although comet assay is considered as an unspecific assay, the results proposed the similarity of DNA damage character of blue mussel gill cells when exposed to tributyltin chloride and taken directly from the polluted site. The data obtained are important for assessing the environmental risks created by genotoxic agents, e.g., tributyltin, used as antifouling agents in marine paints.

1. Introduction

It is unavoidable that the development of industry around the world is proceeding successively with the extraction of nature and discharging waste into the environment, especially in aquatic habitats. Indeed, this man-made destruction of nature brings severe short and long-term effects, which endanger the sustainability of the habitat and consequently the dwellers. Some discharged products are genotoxicants or carcinogens. Numerous carcinogenic compounds have been monitored in marine sediments from polluted areas worldwide. Most of these hazardous chemicals accumulate in various trophic levels of organisms living in aquatic areas. (Bolognesi *et al*., 1996; Lee *et al*., 2020; Qin *et al*., 2020; Baines *et al*., 2021). They have significant influences in cell damage creating necrosis and neoplasia (Bebianno & Garcia da Fonseca, 2020; Medda *et al*., 2021), DNA damage (Yaqin, 2006; Asllani *et al*., 2019; Bolognesi *et al*., 2019; Pellegri *et al*., 2020; Guimarães *et al*., 2021; Camargo Filho *et al*., 2022), embryonic mortality and mutation (McLaughlin *et al*., 2020; Ganaie, 2021) and Darwinian fitness such as decrease of growth rates, reproductive output, and viability of offspring (Depledge, 2020).

Chemical-based monitoring has been used to observe and analyze pollution in the environment and thereby making a list of chemicals released in the environment. However, it only rises an idea on how many and what kind of existing pollutants are in the environment and does not depict the interaction between the existence of toxicants in an environment and the deleterious effect of organisms (Yaqin *et al*., 2011; Métais *et al*., 2019; Schuijt *et al*., 2021). Consequently, there is an urgent need to build methods that can diagnose the impact of pollutants anywhere along the hierarchy of increasing biological complexity from molecule to population. The biomarker or biological marker concept is a preferable candidate as a monitoring device to detect and assess the biological effect of pollution on organisms and the quality of the environment concomitantly (Kumari & Khare, 2018; Yaqin, 2019; Gallo & Tosti, 2020; Georgieva *et al*., 2021).

Molecular biomarkers have been regarded as early warning systems along with the recognized correlation between the genotoxic burden of aquatic ecosystems and carcinogenic effects in humans and wildlife in an epidemic study (Black & Baumann, 1991; Baines *et al*., 2021; Jeddi *et al*., 2021; Liu *et al*., 2022). Besides, the molecular biomarkers, for instance, DNA damage, serve as a good and useful early warning system of carcinogens on different organisms (Kumari & Khare, 2018; Lionetto *et al*., 2019; Ben Ameur *et al*., 2022). The basic reason for this is the capacity of carcinogens or genotoxicants when discharged in the environment to cause various types of DNA damage (Xu *et al*., 2018; Pellegri *et al*., 2020). Likewise, DNA is a predominant important substance, which is maintained by living organisms in efficient and intricate mechanisms. Significant stresses, therefore, may disturb these mechanisms and increase observable DNA damage (Lee *et al*., 2018; Fossi *et al*., 2020)

DNA damage can be reflected as DNA strand breaks are considered a sensitive and non-specific predictor of a wide range of genotoxicity exposures (Mitchelmore & Chipman,

1998; Hemalatha *et al*., 2019; Kuchařová *et al*., 2019; Poirier, 2021). The production of DNA strand breaks correlated well with mutagenic and carcinogenic properties of environmental pollutants with diverse structures (Velali *et al*., 2019; Azqueta *et al*., 2020). DNA damage may appear via profound strand breaks as a result of the alkali labile site (Hong *et al*., 2020; Møller *et al*., 2020; May *et al*., 2022) and can also occur via the action of the excision repair enzyme (Park *et al*., 1991; Speit & Hartmann, 1995; Kim *et al*., 2019; Winkelbeiner *et al*., 2020).

Comet assay is a sensitive, simple, flexible, rapid, and inexpensive technique for detecting DNA damage as DNA strand breaks in an individual cell of almost any eukaryotic organism (Azqueta & Collins, 2013; Cvetković *et al*., 2018; Tatin *et al*., 2021). Due to its ability and practical advantages, this assay has already been used as a genotoxicity test for various genotoxicants both direct and indirect-acting (Erraud *et al*., 2018; González *et al*., 2018; Gajski *et al*., 2019; Cordelli *et al*., 2021; Rajendran *et al*., 2021) for various aquatic organisms from vertebrate (žegura & Filipič, 2019; Camargo Filho *et al*., 2022; Cant *et al*., 2022; Costa, 2022; Gazo *et al*., 2022; Mahboob *et al*., 2022) to invertebrate (Erraud *et al*., 2018; Khan *et al*., 2019; Guidi *et al*., 2021; Cosentino *et al*., 2022; Ivorra *et al*., 2022)

Mussels (*Mytilus edulis* and their relatives) are a very common aquatic invertebrate species that have been used as sentinel organisms in aquatic environment monitoring since Goldberg (1975) has proposed the Mussel Watch program. Mussels were thought to fulfil what was required by environmental monitoring principles because of their behaviour as a sessile organism, wide geographical distribution, availability, and resistance to general stress. By well-known antifouling compound tributyltin (TBT) mussels showed as a par excellence sentinel organism. High larval mortality of common mussels was observed at low concentrations of TBT (Beaumont & Budd, 1984; Amara *et al*., 2018; Bryan & Gibbs, 2020). About half of the larvae induced to 0.1 µg/l were dead by 15 days and most of the surviving larvae were dying and had grown significantly slowly (Beaumont & Budd, 1984). Reduction of Darwinian fitness as reflected in deformity of the shell was first observed in Japanese oysters (*Crassostrea gigas*) from the Bay of Arcachon (Alzieu, 1991). The deformity or abnormal shell thickness mechanism occurred at TBT concentration as low as 2 ng/l (Chagot *et al*., 1990) and Phelps and Page, (1997) used it as a TBT biomonitoring device. Page et al., (1996) and Huang and Wang (1995) also noted that there was a correlation between shell deformity and TBT burden of mussel tissue, thereby it might be a biomarker of TBT exposure in *M. edulis*. However, Lundebye *et al*., (1997) carried out in situ studies in Danish waters and investigated that the TBT burden of mussel tissue was not correlated with Darwinian fitness, which was reflected as condition index and one of the biomarkers of exposure namely stress protein.

This triggers for using another biomarker considered as a sensitive biomarker, e.g., DNA strand breaks to detect genotoxic effects of TBT. Some studies have been carried out to recognize the cytogenetic damage induced by TBT on the early stage of mussels (Jha *et al*., 2000; Mičić *et al*., 2001; Hagger *et al*., 2005). Jha *et al*., (2000) noted that TBT is capable of inducing cytogenetic damage on *M. edulis*. In hemocytes of blue mussel (*M. galloprovincialis*) TBT was inducing apoptosis at low concentrations (Mičić *et al*., 2001). However, the implication of DNA damage induced by TBT on ecologically significant factors such as Darwinian fitness of mussels is still unknown. Therefore, studying DNA damages, which are indicated in DNA strand breaks and their implication on Darwinian fitness is interesting. So, this present study aimed to determine whether a highly toxicant in water, TBT, induced DNA damage on mussel gill cells and its implication on Darwinian fitness, which was reflected as shell thickness index.

Figure 1. The site of in situ experiments (A) polluted site and (B) expected unpolluted site.

2. Materials and Methods

2.1. Samples Collection

Four hundred blue mussels 2.37 – 6.43 cm in length were collected randomly from relatively unpolluted and polluted sites, Norsminde Fjord, Denmark (Fig. 1). In the laboratory, blue mussels were kept in a well-aerated container for 2-7 days and employed for cell preparations. Blue mussels from assumed unpolluted sites were used for in vitro exposure namely H_2O_2 (hydrogen peroxide) and TBTC (tributyltin chloride) and transplantation test. The mussels from the polluted as well as from unpolluted sites were used for the determination of shell thickness index and comet assay.

2.2. Shell Thickness Index

Shell thickness index or thickness index (TI) was determined on shells for 200 blue mussels collected randomly from the unpolluted and polluted site. The thickness index is a dimensionless quantity defined as 100 times shell depth (i.e., "thickness") divided by shell length. TI was determined by measuring the greatest length and the greatest depth of the entire mussel shell with the valves closed. The depth of the shell was measured perpendicular to the plane of shell closure across both halves (Page *et al*., 1996).

2.3. Cell Preparation

Gill cells were selected for this study since they were easy to obtain, and in nature, they come into contact with relatively large volumes of seawater compared to the rest of the animal (average filtering rate for an adult mussel is approximately 2 l/h at 20 °C), thus conferring them with the potential for being a suitable target tissue for mutagen exposure (Dixon & Clarke, 1982; Venier & Canova, 1996; Pearson *et al*., 2018; Qu *et al*., 2019). The gills are initial sites of uptake TBT by *M. edulis* (Laughlin *et al*., 1986), and accumulation of TBT in gills and viscera is greater than in other parts of the bivalve mussels (Huang & Wang, 1995). Regarding cell types, the mussel gills provide an additional advantage as gill cells are relatively homogenous. Likewise, it can easily be made into a single-cell suspension without any need for chemical dissociation (Wilson *et al*., 1998).

Before the experiment gill cells were isolated as described in Wilson et al., (1998). Four pairs of gills were dissected out and placed in 2 ml Ca^{2+} -Mg^{2+} free saline (CMFS) buffer (1100 mOsm; 20 mM HEPES-NaOH pH 7.3 containing 500 mM NaCl, 12.5 mM KCl, and 5mM ethylene di-aminetetraaceticacid (EDTA)), chopped for 1 minute and washed twice. The chopped pieces were transferred into 200 ml beaker glass with 10 ml buffer saline and agitated gently under the dark condition at 1°C for 1 hour. Cell suspensions were filtered through 60 µm cell strainers to remove cell clumps. The filtrates were centrifuged at 250 x g and 2500 rpm for 5 minutes to spin down cells. The supernatant was removed and cells suspensions were harvested and ready for comet assay or in vitro test. The viability of the cell solution was tested using a 0.4 % solution of the red dye EOSIN Y.

2.4. In Vitro Exposure

The mussels collected from the expected clean site were transferred into the laboratory and treated for preparing cell suspension as outlined above. Freshly prepared cell suspensions were exposed in vitro to 10 µM of H_2O_2 and 10 µg/l of TBTC. Test compounds were added to cell suspension (1 ml final concentration) in CMFS as vehicle solvent for 1 hour at 1 °C in a dark room. Following incubation, 100 µl of cell suspension was used in the comet protocol outlined below, with the rest used for the viability determination using the red dye EOSIN-Y.

2.5. In Situ Exposure

Two models of in situ experiments were carried out. The first experiment was performed by collecting samples from the polluted site and used them for the comet assay. Transplantation was the second experiment where blue mussels from the expected unpolluted site were entered into a plastic net and fully immersed in the water column in the expected polluted site (harbour) for one month. Following incubation, animals were harvested and transferred to the laboratory for the next steps. This transplantation experiment was conducted to see whether there was a short-term effect of pollutants on the animals and to avoid confounding factors such as environmental changes in the polluted site.

2.6. Comet Assay

Three comet assay methods have been used in the ecotoxicology field namely, single, double and triple layers, the last method is called the ´sandwich method. The main difference among them is the layer of agarose used as cell suspension attachment. The double-layer method following Singh *et al*., (1988) was adopted with minor modification. Fully frosted microscope slide was coated with 100 µl of 0.85% normal-melting-point agarose (NMP, Sigma) in Kenny´s salt solution (0.4 M NaCl, 9 mM KCl, 0.7 mM K_2HPO_4, 2 mM $NaHCO_3$) at 35 °C and cover with 22 x 50 mm coverslip. The slides were placed in the refrigerator for 15 min to allow solidification. Cell suspensions in 2 ml were centrifuged at 3000 rpm and 250 X g for 5 min to create cell pellets. Cell pellets in 100 µl were resuspended in 125 µl of 0.65% low-melting-point agarose (LMP, Sigma) in Kenny´s salt solution. After the first agarose layer was solidified, cells suspended in LMP were transferred as 100 µl aliquots onto a coated fully frosted microscope slide. A coverslip was put onto the slide and agarose was allowed to solidify at the refrigerator for 15 min.

After removing the coverslip, the slides were placed in lysing solution consisting of 2.5 M NaCl, 10 mM tris,0.1 mM EDTA, 1% sarcosyl, 1% Triton X-100 and 10% DMSO, pH 10 in a Coplin jar for 1 h at the refrigerator. Following a rinse two times in electrophoresis solution (10N NaOH, 200mM EDTA, pH > 13) for removing excess salts, the slides were transferred into gel electrophoresis chamber and immersed in electrophoresis solution for 15 min to allow the unwinding of DNA. Electrophoresis was carried out in the same solution for 20 min at 25 V and 300 mA. The slides were neutralized in cold neutralization solution (0.4 M TRIS) pH 7.5, 2 x 5 min.

Before the examination, the slides were stained with 50 µl ethidium bromide (in distilled water) (10 µg/ml) for 10 min with a coverslip. The slides were examined by using a fluorescence microscope (x 1,000 magnification). 50 cells/slide were visually saved randomly and the cell images were analyzed by using CASP (Comet Assay Software Project) version 1.0. The degree of DNA damage was expressed as tail length and olive tail moment. Tail length is indicated as the length of the comet tail measured from the right border of the head area to the end of the tail (in pixel). Olive tail moment considers the tail length and fractions of the DNA in comet tail, and is calculated as a percentage of DNA in the tail x (TailMeanX – HeadMeanX) ([percent DNA in tail] x [distance between the center of gravity of DNA in the tail and the center of gravity of DNA in the head in x-direction]) (Fig. 2).

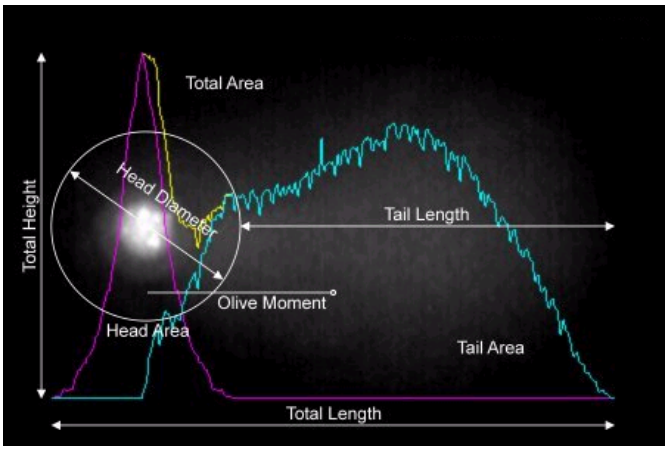

Figure 2. Comet measurements (AutoCometTM. 2002).

All the steps above were performed under artificial light to minimize DNA damage induced by UV light.

2.7. Statistical Analysis

For statistical analysis, data were arcsinh transformed and a level of 0.05 was used as a significance level. One-way analysis of variance (ANOVA) was used to determine to mean differences of all treatments in comet assay and shell thickness index. The Bonferroni procedure was employed for the post hoc test.

3. Result and Discussion

3.1. Shell Thickness Index

Shell thickness index or thickness index (TI) is an index that depicts deformity or mal growth of mussel shell. The larger the value of the index is the more rounded the shell

shape. Table 1 shows the result of TI means of blue mussels from expected clean and polluted sites. Based on One-Way ANOVA there is a difference in means of TI between samples that were taken from the expected clean and polluted site at level $p < 0.01$ (Table 1). It was indicated that mussels from the polluted site have a more rounded shell shape than those from expected clean sites. This reveals that pollutants, which occur in the harbour, may create a shell calcification anomaly or deformity of mussel shells (Fig. 3).

Table 1. Shell thickness index (mean ± SEM) of blue mussels, *M. edulis* from clean and polluted sites.

Site	Shell thickness index
Clean	31.311 ± 0.523
Polluted	36.205 ± 0.777**

** $p < 0.01$

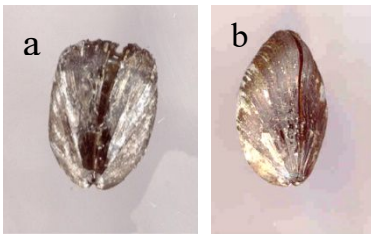

Figure 3. Blue mussel shells occurred in polluted (a) and clead (b) sites.

Norsminde Fjord is a shallow inlet, which is used as a harbor for small boats, situated just south of Aarhus. Therefore, this fjord is expected as a place where TBT was widespread. From a preliminary study, it was known that many malformed blue mussels have rounded shell shapes. This study revealed that TI as TBT biomarkers is greater in blue mussels from harbour than TI in blue mussels from expected clean water. It may indicate that even TBT has been banned to use for ships with a length, of not more than 25 m since 1993 in Danish waters, the severe effects of TBT on sedentary animals like blue mussels remain. Indeed, it is very hard to make a judgment that TBT occurred in this place created shell deformity of blue mussels solely since this study did not analyze TBT and other xenobiotics such as PAH (polyaromatic hydrocarbons) discharged mainly in the harbour. However, Alzieu as early as 1975 (Alzieu, 1998) observed deformity incidence occurring in the *Crassostrea gigas* farm of the Arcachon Bay along the French Atlantic Coastline. TBT contamination of the local breeding waters was found to be responsible for failure to reproduce and for anomalies occurring in the shell calcification of adult oysters leading to stunted growth. The existence of the bad effects of TBT in some waters is still visible even though its use has been banned for a long time (Gallo & Tosti, 2020).

Many studies have been conducted to show the relationship between tributyltin contamination in waters with shell deformity in various bivalves, such as Japanese oyster (*C. gigas*) (Waldock & Thain, 1983), Portuguese oyster (*C. anguluta*) (Phelps, 1990), and *M. edulis* from a marina area (Stephenson *et al.*, 1986). Page *et al.*, (1996) also detected that there was a correlation between TI of blue mussels with tributyltin concentration in mussels tissue. However, the mechanism by which, tributyltin creates anomalies occurring in the shell calcification is still unknown. The altered mechanism of calcification resulted in shell anomalies at TBT concentrations as low as 2 ng/l *(*Chagot *et al.,* 1990). Alzieu, (1991) found that the organotin compound created oyster shell abnormalities consisting of water-like chambering with the formation of interlamellar jelly. The observation by Krampitz *et al*., (1983) revealed that gelatinous contents of the chambers differed from normal organic matrix protein in having lower levels of aspartic acid, glycine, and serine, and a higher level of threonine. This lead to the hypothesis, that the target of tributyltin could be the organic matrix. This hypothesis agrees with the results of Allemand *et al.* (1998) who demonstrated that the primary target of tributyltin in calcifying animals is protein synthesis, whose inhibition leads to the inhibition of organic matrix and consequently creates malicious calcification. Likewise, TBT used to expose the Hooded oyster *Saccostrea cucullata*, disrupts the calcium homeostatic process through the VDAC-3 protein present in the mantle and this is considered to be a key mechanism of TBT in disrupting shell calcification with the result of bivalve shell deformity (Khondee *et al*., 2016).

3.2. DNA Damage

The red dye EOSIN-Y was used to test the viability of the cells. Using this dye the viability of the cells was consistently above 90 % for all treatments.

After electrophoresis, the presence of DNA strand breaks causes a fraction of DNA to migrate from the nucleoid core toward the anode by forming a comet (Singh *et al*., 1988). DNA damage expressed as DNA fragments migrating to the tail region were measured by tail length and olive tail moment as comet parameters.

In this study, the expected unpolluted site was used as a control and compared by other in situ experiments, polluted area, and transplantation experiments, an in vitro experiment, TBTC (10 µg/l), and H_2O_2 (10 µM) exposure. The comet did not follow a normal distribution (Anderson *et al.* 1996), and the data were transformed using arcsinh transformation procedure to stabilize these. The transformed data were analyzed by one-way ANOVA to show if there were different means among the experiments and proceed to post hoc test using the Bonferroni procedure. The level of DNA damage in gill cells of blue mussels by various exposures was shown in Fig. 4, 5, and Table 2. The results of One-way ANOVA showed that there was a significantly different mean among the exposures ($p < 0.01$).

Table 2. Comet parameters, tail/length, and tail olive moment (mean ± SEM) in blue mussel gill cells from different experiments. Comparison between clean areas as controls and the treatments (transplantation, polluted area, TBTC, and H_2O_2).

Experiments	DNA damage	
	Tail length	Tail olive moment
Clean	28.0093 ± 0.0768	2.2788 ± 0.0727
Transplantation	42.8006 ± 0.0948**	4.3695 ± 0.1070**
Polluted	108.2138 ± 0.0669**	12.9196 ± 0.0792**
TBTC (10 µg/l)	95.8958 ± 0.0703**	11.4798 ± 0.0854**
H_2O_2	109.1630 ± 0.0752**	20.1105 ± 0.0928**

** $p < 0.01$

Figure 4. The level of DNA damage of blue mussel gill cells from different exposure.

Gill cells of blue mussels from clean water showed undamaged cells to mainly low with a small number of cells with considerable DNA damage. Relatively small increases in the number of cells damaged and the extent of DNA damages were detected in blue mussels that were transplanted to the polluted site for one month. The level of DNA damage and the number of cells damages extended exceedingly for blue mussels from the polluted site and blue mussels exposed to TBTC (10 µg/l). The effect of TBTC leading to DNA damage in this study agrees with Gabbianelli *et al.*, (2002) who demonstrated that TBTC led to a significant increase in the extent of DNA damage as measured by three tail parameters (tail length, tail intensity, tail moment) and the number of cell damages. The coordination number and the nature of the organic group attached to the central tin atom determine the binding ability of organotin(IV) compounds to DNA (Devi & Pachwania, 2018).

The highest level of DNA damages and the number of cell damages were revealed by exposing gill cells of blue mussels to H_2O_2 (10 µM) as a positive control (Fig. 4, 5 & Table 2). Increasing the percentage of cell damages indicated as the percentage of DNA fragments in the tail has been pointed out in this treatment. Hydrogen peroxide (H_2O_2) has been frequently used as a positive control compound in many comet assay studies investigating its application to various cell types (Singh *et al.*, 1988; Gajski *et al.*, 2019; Odongo *et al.*, 2019; žegura & Filipič, 2019; Erikel *et al.*, 2020; Muruzabal *et al.*, 2021). This compound is considered to cause base modifications directly, particularly following the Fenton reaction to form the highly reactive hydroxyl radical (OH) (Halliwell, 2001; Madkour, 2019; Lelieveld *et al.*, 2021). Mitchelmore *et al.*, (1998) showed that the addition of H_2O_2 produced a significant concentration-dependent increase in the percentage of DNA in the comet tail.

In this study, both tail length and olive tail moment were considered as appropriate parameters for quantifying DNA strand breaks. The mean of DNA strand breaks expressed by two parameters showed a similar sensitivity at level $p < 0.01$ (Tabel 2). However, the Bonferroni procedure showed that the tail length endpoint did not reveal the difference in means among polluted sites, 10 µg/l of TBTC and 10 µM of H_2O_2 exposure. On the other hand, in terms of olive tail moment polluted site and 10 µg/l of TBTC exposure revealed a similar degree of DNA damages, whilst the effect of H_2O_2 (10 µM) on gill cells of blue mussels shown increasing significantly in DNA damages level (Table 2). In many cases, tail moment and olive tail moment are more precise as endpoints than tail length, because these parameters consider not only the length of comet tail but also the intensity of DNA in the tail and the mean distance of DNA migration in the tail (Olive *et al.*, 1990; Dutta & Bahadur, 2019; Cui *et al.*, 2021; Gajski *et al.*, 2021; Rajendran *et al.*, 2021).

The extent of DNA damage taken place along with an increasing number of gill cell damages of transplanted blue mussels described that transplanting method could be used for assessing the impact of such geno-toxicants like tributyltin to marine animals. It was also explained that transplanting mussels facilitated measuring biomarkers like DNA damage and made this tool more flexible when used for manipulative field testing and hypothesis testing (Bolognesi *et al.*, 2019; Gecheva *et al.*, 2020; Perovic *et al.*, 2020; Dos

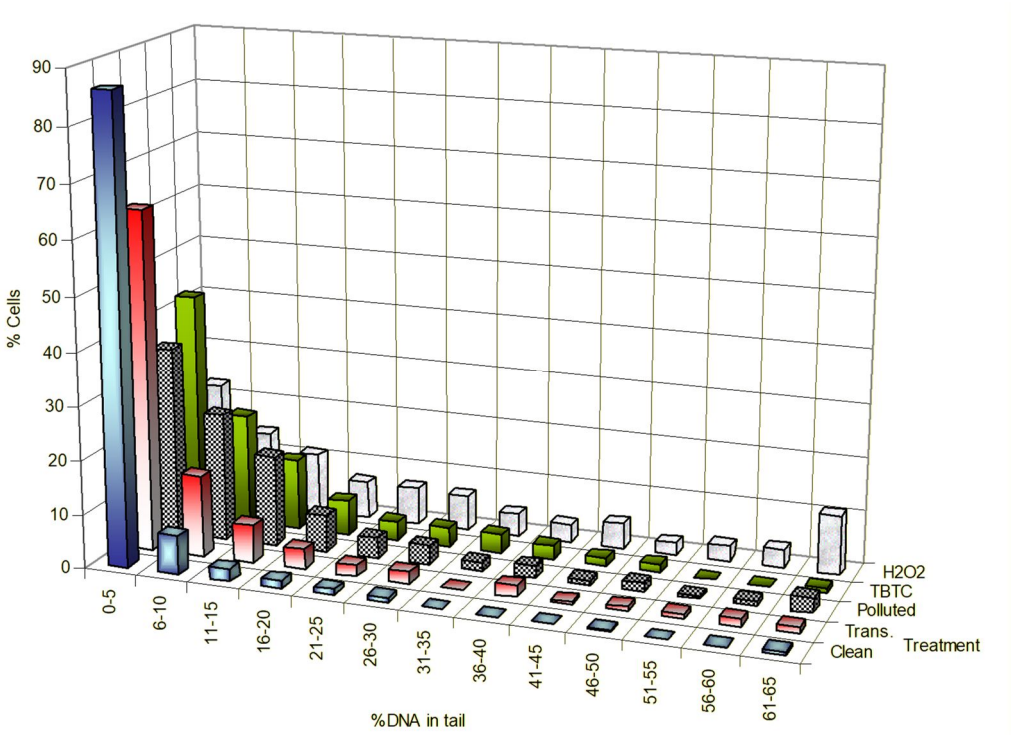

Figure 5. Distribution of DNA damage based on percentage DNA in the comet tail of blue mussels gill cells in different treatments.

Santos *et al*., 2022; Sussarellu *et al*., 2022). Despite that the level of DNA damage in blue mussel gill cells for this experiment was not more than those in gill cells of blue mussels taken from the polluted site directly, the result also described the time of transplanting exposure was appropriate for exposing mussels in the in-situ experiment. volume

A field study by taking mussels from the polluted site directly described that DNA damage of gill cells was more severe than those from transplanting experiments and expected unpolluted sites. D′costa *et al*., (2018) showed a relationship between DNA single-strand breaks and environmental contaminants in the field. This result suggested that Norsminde fjord harbor contains potential genotoxicants. In addition, the results depiucted that there was no difference in means between the levels of DNA damage in term olive tail moment in gill cells when taken from polluted site as when exposed to TBTC (10 µg/l). On the other hand, the extent of DNA damages in gill cells when exposed to H_2O_2 (10 µM) differed from those exposed by TBTC (10 µg/l) and taken from the polluted site. However, it is uncertain from this study to suggest that the main genotoxicant in Norsminde Fjord harbour which induced DNA damage on gill cells of mussels was tributyltin. This suggestion was needed to evaluate tributyltin contain and other potential genotoxicants in the water column, sediment, and mussel tissues, which was not performed in this study. Moreover, the results indicated that DNA damages on gill cells exposed by TBTC (10 µg/l) have a similar character, in the light of the olive tail moment, with those of blue mussels taken from the polluted site. It might indicate that the effect of TBTC on these gill cells was similar to the effect of genotoxicants contained in the harbour.

Evaluation of the genotoxic potential of tributyltin on marine invertebrates like blue mussels using comet assay could give important information on the DNA alteration induced by this compound. Tributyltin has been observed to cause programmed cell death or apoptosis in human cell lines and tissue of marine sponge, *Geodia hydronium* (Batel *et al.,* 1993). Apoptosis was also detected in *M. galloprovincialis* exposed to tributyltin chloride (Mičić *et al*., 2001). The study showed that in TBT-treated mussels, DNA fragmentation and degradation to higher-order DNA structures appeared to be the critical lesions responsible for the induction of apoptosis. Cytogenetic damages (sister chromatid exchanges and chromosomal aberration) in larvae of blue mussel induced by TBT have also been evaluated (Jha *et al*., 2000). In addition, a study on rainbow trout erythrocytes using comet assay showed that TBTC has a profound genotoxic effect, whilst DBTC (dibutyltin chloride) creates lesser DNA damage and DNA damage is completely absent for MBTC (monobutyltin chloride) (Tiano *et al*., 2001). Likewise, a study on the adverse effects of different organotin compounds on DNA of *Sparus aurata* (freshwater fish) erythrocytes using comet assay showed different genotoxicities among TBTC, DBTC, and MBTC (Gabbianelli *et al*., 2002). TBTC and DBTC have pronounced effects on tail length, tail intensity, and tail moment, though TBTC is more efficient in producing DNA damage. MTBC caused a fast genotoxic effect that did not change with incubation time.

The current study has evaluated the genotoxic effect of TBT, and other pollutants both in situ and in vitro that caused DNA damage on blue mussel gill cells. However, the cause of the DNA damage is not yet established, especially for TBT. DNA damage induced by TBT may act through an indirect mechanism by perturbation of Ca^{2+} homeostasis. It has been reported that intracellular Ca^{2+} was increased by 10 –5 M TBT exposure in *Ciona intestinalis* (Urochordata) ovaries (Puccia *et al*., 2001), which played an important role in inducing DNA cleavage in apoptotic cells. Another possibility is a perturbation of mitochondrial activity. It is possible that TBT disturbance of the mitochondrial proton gradient could divert electrons from the respiratory chain, leading to the formation of reactive oxygen species (Puccia *et al*., 2001). In gill cells of *M. edulis* (Wilson *et al.,* 1998), *Lithophaga lithophaga* (Essawy *et al.,* 2021), *Donax incarnatus* (Dias *et al.,* 2021), and *M. coruscus* (Xu *et al*., 2018), oxidative DNA damage generated by reactive oxygen species has been attributed to the high and variable DNA damage.

4. Conclusion

The results of this study demonstrated that deformity has been detected on blue mussels living in Norsminde fjord harbor that might be caused by a mixture of genotoxicants, probably dominated by tributyltin. The evaluation of DNA damage using comet assay is a simple and effective method to assess the existence of genotoxicants in both in situ and in vitro experiments. Therefore, the results obtained are important for assessing genotoxicants that produced a severe impact on marine animals. Further studies on tributyltin contained in the environment and tissue of animals are requested to specify which genotoxicant causes an exact severe impact on the animals since this assay is an unspecific but versatile tool for detecting the presence of genotoxicants.

Acknowledgement

This study was supported by The Danish Agency for Development Assistance (DANIDA). I would like to thank Vibeke Simonsen and Janeck Scott-Fordsmand for the useful guidance and discussion on this study. Thank Hans Løkke the director of the National Environmental Research Institute (DMU), Denmark for providing a laboratory to conduct comet assay analysis. I also thank Anni Christiansen for kindly helping during laboratory work.

References

Allemand, D.; Tambutté, É.; Girard, J.-P. & Jaubert, J., 1998. Organic matrix synthesis in the scleractinian coral Stylophora pistillata: role in biomineralization and potential target of the organotin tributyltin. *The Journal of experimental biology*, 201(13): 2001–2009, ISSN: 1477-9145.

Alzieu, C., 1991. Environmental problems caused by TBT in France: assessment, regulations, prospects. *Marine Environmental Research*, 32(1–4): 7–17, ISSN: 0141-1136.

Alzieu, C., 1998. Tributyltin: case study of a chronic contaminant in the coastal environment. *Ocean & Coastal Management,* 40(1): 23–36, ISSN: 0964-5691.

Amara, I.; Miled, W.; Slama, R. Ben & Ladhari, N., 2018. Antifouling processes and toxicity effects of antifouling paints on marine environment. A review. *Environmental toxicology and pharmacology,* 57: 115–130, ISSN: 1382-6689.

Asllani, F. H.; Schürz, M.; Bresgen, N.; Eckl, P. M. & Alija, A. J., 2019. Genotoxicity risk assessment in fish (*Rutilus rutilus*) from two contaminated rivers in the Kosovo. *Science of the total environment,* 676: 429–435, ISSN: 0048-9697.

Azqueta, A. & Collins, A. R., 2013. The essential comet assay: a comprehensive guide to measuring DNA damage and repair.

Archives of toxicology, 87(6): 949–968, ISSN: 1432-0738.

Azqueta, A.; Ladeira, C.; Giovannelli, L.; Boutet-Robinet, E.; Bonassi, S.; Neri, M.; Gajski, G.; Duthie, S.; Del Bo, C. & Riso, P., 2020. Application of the comet assay in human biomonitoring: An hCOMET perspective. *Mutation Research/Reviews in Mutation Research,* 783: 108288, ISSN: 1383-5742.

Baines, C.; Lerebours, A.; Thomas, F.; Fort, J.; Kreitsberg, R.; Gentes, S.; Meitern, R.; Saks, L.; Ujvari, B. & Giraudeau, M., 2021. Linking pollution and cancer in aquatic environments: A review. *Environment International,* 149: 106391, ISSN: 0160-4120.

Batel, R.; Bihari, N.; Rinkevich, B.; Dapper, J.; Schäcke, H.; Schröder, H. C. & Müller, W. E. G., 1993. Modulation of organotin-induced apoptosis by the water pollutant methyl mercury in a human lymphoblastoid tumor cell line and a marine sponge. *Marine Ecology Progress Series,* : 245–251, ISSN: 0171-8630.

Beaumont, A. R. & Budd, M. D., 1984. High mortality of the larvae of the common mussel at low concentrations of tributyltin. *Marine pollution bulletin,* 15(11): 402–405, ISSN: 0025-326X.

Bebianno, M. J. & Garcia da Fonseca, T., 2020. Fate and effects of cytostatic pharmaceuticals in the marine environment. In: *Fate and Effects of Anticancer Drugs in the Environment*, Springer, pp. 295–330.

Ben Ameur, W.; El Megdiche, Y.; Ennaceur, S.; Mhadhbi, T.; Ben Hassine, S.; Annabi, A.; de Lapuente, J.; Driss, M. R.; Borràs, M. & Eljarrat, E., 2022. Biomarkers responses and polybrominated diphenyl ethers and their methoxylated analogs measured in Sparus aurata from the Lagoon of Bizerte, Tunisia. *Environmental Science and Pollution Research,* : 1–15, ISSN: 1614-7499.

Black, J. J. & Baumann, P. C., 1991. Carcinogens and cancers in freshwater fishes. *Environmental Health Perspectives,* 90: 27–33, ISSN: 0091-6765.

Bolognesi, C.; Cirillo, S. & Chipman, J. K., 2019. Comet assay in ecogenotoxicology: applications in *Mytilus* sp. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 842: 50–59, ISSN: 1383-5718.

Bolognesi, C.; Rabboni, R. & Roggieri, P., 1996. Genotoxicity biomarkers in M. galloprovincialis as indicators of marine pollutants. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology,* 113(2): 319–323, ISSN: 0742-8413.

Bryan, G. W. & Gibbs, P. E., 2020. Impact of low concentrations of tributyltin (TBT) on marine organisms: a review. *Metal ecotoxicology,* : 323–361, ISSN: 1003069975.

Camargo Filho, C. B.; dos Santos, A. R.; Ferrari, J. L.; Preto, B. de L.; Kunz, S. H.; Senhorelo, A. P.; Burak, D. L. & Rossi Junior, J. L., 2022. Evaluating the Trace Element Concentration in Sediments and Assessing Their Genotoxicity in Ichthyofauna of a Coastal Lagoon in Southeastern Brazil. *Diversity*, 14(2): 151, ISSN: 1424-2818.

Cant, A.; Bonnard, M.; Porcher, J.-M.; Prygiel, J.; Catteau, A.; Delahaut, L.; Palluel, O.; Turiès, C.; Geffard, A. & Bado-Nilles, A., 2022. Integration of Genotoxic Biomarkers in Environmental Biomonitoring Analysis Using a Multi-Biomarker Approach in Three-Spined Stickleback (*Gasterosteus aculeatus* Linnaeus, 1758). *Toxics*, 10(3): 101, ISSN: 2305-6304.

Chagot, D.; Alzieu, C.; Sanjuan, J. & Grizel, H., 1990. Sublethal and histopathological effects of trace levels of tributyltin fluoride on adult oysters Crassostrea gigas. *Aquatic Living Resources,* 3(2): 121–130, ISSN: 0990-7440.

Cordelli, E.; Bignami, M. & Pacchierotti, F., 2021. Comet assay: a versatile but complex tool in genotoxicity testing. *Toxicology Research,* 10(1): 68–78, ISSN: 2045-4538.

Cosentino, S.; Aureli, F. & Iannilli, V., 2022. Bisphenols A and Its Analogues Induce Genotoxic Damage in Marine and Freshwater Amphipods. *Environmental Advances*, : 100183, ISSN: 2666-7657.

Costa, P. M., 2022. Current aspects of DNA damage and repair in ecotoxicology: a mini-review. *Ecotoxicology*, 31(1): 1–11, ISSN: 1573-3017, DOI: 10.1007/s10646-021-02487-2, Available: <https://doi.org/10.1007/s10646-021-02487-2>.

Cui, Z.; Luan, X.; Li, S.; Zhao, X.; Lin, Z.; Li, J.; Gao, W.; Zheng, L.; Ma, Z. & Xie, J., 2021. Genotoxicity detection of oil-containing drill cuttings by Comet assay based on a demersal marine fish Mugilogobius chulae. *Ecotoxicology and Environmental Safety,* 208: 111655, ISSN: 0147-6513.

Cvetković, V. J.; Takić Miladinov, D. & Stojanović, S., 2018. Genotoxicity and mutagenicity testing of biomaterials. In: *Biomaterials in clinical practice*, Springer, pp. 501–527.

D′costa, A. H.; Shyama, S. K.; MK, P. K. & Furtado, S., 2018. The Backwater Clam (*Meretrix casta*) as a bioindicator species for monitoring the pollution of an estuarine environment by genotoxic agents. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 825: 8–14, ISSN: 1383-5718.

Depledge, M. H., 2020. The rational basis for the use of biomarkers as ecotoxicological tools. In: *Nondestructive biomarkers in vertebrates*, CRC Press, pp. 271–295, ISBN: 036781370X.

Devi, J. & Pachwania, S., 2018. Recent advancements in DNA interaction studies of organotin (IV) complexes. *Inorganic Chemistry Communications,* 91: 44–62, ISSN: 1387-7003.

Dias, R.; D′Costa, A.; Praveen Kumar, M. K. & Shyama, S. K., 2021. DNA damage and biochemical responses in estuarine bivalve *Donax incarnatus* (Gmelin, 1791) exposed to sub-lethal concentrations of an organophosphate pesticide monocrotophos. *Environmental Monitoring and Assessment*, 193(6): 1–12, ISSN: 1573-2959.

Dixon, D. R. & Clarke, K. R., 1982. Sister chromatid exchange: a sensitive method for detecting damage caused by exposure to environmental mutagens in the chromosomes of adult *Mytilus edulis*. *Marine Biology Letters,* (3).

Dos Santos, F. S.; Neves, R. A. F.; Crapez, M. A. C.; Teixeira, V. L. & Krepsky, N., 2022. How does the brown mussel *Perna perna* respond to environmental pollution? A review on pollution biomarkers. *Journal of Environmental Sciences,* 111: 412–428, ISSN: 1001-0742.

Dutta, S. & Bahadur, M., 2019. Comet assay genotoxicity evaluation of occupationally exposed tea-garden workers in northern West Bengal, India. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 844: 1–9, ISSN: 1383-5718.

Erikel, E.; Yuzbasioglu, D. & Unal, F., 2020. Genotoxic and antigenotoxic potential of amygdalin on isolated human lymphocytes by the comet assay. *Journal of Food Biochemistry,* 44(10): e13436, ISSN: 0145-8884.

Erraud, A.; Bonnard, M.; Duflot, A.; Geffard, A.; Danger, J.-M.; Forget-Leray, J. & Xuereb, B., 2018. Assessment of sperm quality in palaemonid prawns using Comet assay: methodological optimization. *Environmental Science and Pollution Research*, 25(12): 11226–11237, ISSN: 1614-7499.

Essawy, A. E.; Osman, G. Y.; Morshedy, R. M. El; Al-Nasser, A. S. & Sheir, S. K., 2021. Immune responses, DNA damage and ultrastructural alterations of gills in the marine mussel Lithophaga lithophaga exposed to CuO nanoparticles. *Environmental Science and Pollution Research*, : 1–16, ISSN: 1614-7499.

Fossi, M. C.; Leonzio, C. & Peakall, D. B., 2020. The use of nondestructive biomarkers in the hazard assessments of vertebrate populations. In: *Nondestructive biomarkers in vertebrates*, CRC Press, pp. 3–34, ISBN: 036781370X.

Gabbianelli, R.; Villarini, M.; Falcioni, G. & Lupidi, G., 2002. Effect of different organotin compounds on DNA of gilthead sea bream (*Sparus aurata*) erythrocytes assessed by the comet assay. *Applied organometallic chemistry,* 16(4): 163–168, ISSN: 0268-2605.

Gajski, G.; Ravlić, S.; Godschalk, R.; Collins, A.; Dusinska, M. &

Brunborg, G., 2021. Application of the comet assay for the evaluation of DNA damage in mature sperm. *Mutation Research/Reviews in Mutation Research,* 788: 108398, ISSN: 1383-5742.

Gajski, G.; žegura, B.; Ladeira, C.; Pourrut, B.; Del Bo, C.; Novak, M.; Sramkova, M.; Milić, M.; Gutzkow, K. B. & Costa, S., 2019. The comet assay in animal models: From bugs to whales–(Part 1 Invertebrates). *Mutation Research/Reviews in Mutation Research*, 779: 82–113, ISSN: 1383-5742.

Gallo, A. & Tosti, E., 2020. Reproductive Processes of Marine Animals as Biomarker for Environmental Stress Impact. *Encyclopedia of Marine Biotechnology,* : 3283–3298.

Ganaie, H. A., 2021. Mechanism of Toxicity in Aquatic Life. *Freshwater Pollution and Aquatic Ecosystems: Environmental Impact and Sustainable Management*, : 109, ISSN: 1000291138.

Gazo, I.; Naraine, R.; Lebeda, I.; Tomčala, A.; Dietrich, M.; Fraňek, R.; Pšenička, M. & Šindelka, R., 2022. Transcriptome and Proteome Analyses Reveal Stage-Specific DNA Damage Response in Embryos of Endangered Sturgeon. .

Gecheva, G.; Yancheva, V.; Velcheva, I.; Georgieva, E.; Stoyanova, S.; Arnaudova, D.; Stefanova, V.; Georgieva, D.; Genina, V. & Todorova, B., 2020. Integrated monitoring with moss-bag and mussel transplants in reservoirs. *Water*, 12(6): 1800.

Georgieva, E.; Velcheva, I.; Yancheva, V.; Stoyanova, S.; Vasileva, T.; Bivolarski, V.; Todorova, B. & Iliev, I., 2021. A Review on Multi-Biomarkers in Fish for the Assessment of Aquatic Ecosystem Contamination with Organic Pollutants. *Ecologia Balkanica*, 13(2), ISSN: 1313-9940.

González, E. A. P.; Miana, V. V & Palermo, A. M., 2018. Optimization of Alkaline Single Cell Gel Electropohoresis (Comet Assay) Protocol for Cells from Oral Cavity. *Optimization*, 26(4).

Guidi, P.; Bernardeschi, M.; Palumbo, M.; Scarcelli, V.; Genovese, M.; Protano, G.; Vitiello, V.; Pontorno, L.; Bonciani, L.; Buttino, I.; Chiaretti, G.; Pellegrini, D.; Fiorati, A.; Riva, L.; Punta, C.; Corsi, I. & Frenzilli, G., . 2021. *Cellular Responses Induced by Zinc in Zebra Mussel Haemocytes. Loss of DNA Integrity as a Cellular Mechanism to Evaluate the Suitability of Nanocellulose-Based Materials in Nanoremediation.* vol. 11, Nanomaterials , ISBN: 2079-4991 .

Guimarães, A. T. B.; Estrela, F. N.; Pereira, P. S.; de Andrade Vieira, J. E.; de Lima Rodrigues, A. S.; Silva, F. G. & Malafaia, G., 2021. Toxicity of polystyrene nanoplastics in Ctenopharyngodon idella juveniles: a genotoxic, mutagenic and cytotoxic perspective. *Science of The Total Environment,* 752: 141937, ISSN: 0048-9697.

Hagger, J. A.; Depledge, M. H. & Galloway, T. S., 2005. Toxicity of tributyltin in the marine mollusc *Mytilus edulis*. *Marine pollution bulletin,* 51(8–12): 811–816, ISSN: 0025-326X.

Halliwell, B., 2001. Food-derived antioxidants: how to evaluate their importance in food and in vivo. *Handbook of antioxidants,* 690.

Harayashiki, C. A. Y.; Márquez, F.; Cariou, E. & Castro, Í. B., 2020. Mollusk shell alterations resulting from coastal contamination and other environmental factors. *Environmental Pollution,* 265: 114881, ISSN: 0269-7491.

Hemalatha, D.; Nataraj, B.; Rangasamy, B.; Shobana, C. & Ramesh, M., 2019. DNA damage and physiological responses in an Indian major carp Labeo rohita exposed to an antimicrobial agent triclosan. *Fish physiology and biochemistry,* 45(4): 1463–1484, ISSN: 1573-5168.

Hong, Y.; Han, H.-J.; Lee, H.; Lee, D.; Ko, J.; Hong, Z.; Lee, J.-Y.; Seok, J.-H.; Lim, H. S. & Son, W.-C., 2020. Deep learning method for comet segmentation and comet assay image analysis. *Scientific Reports*, 10(1): 1–12, ISSN: 2045-2322.

Huang, G. & Wang, Y., 1995. Effects of tributyltin chloride on marine bivalve mussels. *Water Research*, 29(8): 1877–1884, ISSN: 0043-1354.

Ivorra, L.; Cruzeiro, C.; Ramos, A.; Tagulao, K. & Cardoso, P. G., 2022. How can environmental conditions influence dicofol genotoxicity on the edible Asiatic clam, Meretrix meretrix? *Environmental Pollution*, 293: 118467, ISSN: 0269-7491.

Jeddi, M. Z.; Hopf, N. B.; Viegas, S.; Price, A. B.; Paini, A.; van Thriel, C.; Benfenati, E.; Ndaw, S.; Bessems, J. & Behnisch, P. A., 2021. Towards a systematic use of effect biomarkers in population and occupational biomonitoring. *Environment International,* 146: 106257, ISSN: 0160-4120.

Jha, A. N.; Hagger, J. A. & Hill, S. J., 2000. Tributyltin induces cytogenetic damage in the early life stages of the marine mussel, *Mytilus edulis*. *Environmental and molecular mutagenesis,* 35(4): 343–350, ISSN: 0893-6692.

Khan, M. I.; Zahoor, M.; Khan, A.; Gulfam, N. & Khisroon, M., 2019. Bioaccumulation of Heavy Metals and their Genotoxic Effect on Freshwater Mussel. *Bulletin of Environmental Contamination and Toxicology,* 102(1): 52–58, ISSN: 1432-0800, DOI: 10.1007/s00128-018-2492-4, Available: <https://doi.org/10.1007/s00128-018-2492-4>.

Khondee, P.; Srisomsap, C.; Chokchaichamnankit, D.; Svasti, J.; Simpson, R. J. & Kingtong, S., 2016. Histopathological effect and stress response of mantle proteome following TBT exposure in the Hooded oyster *Saccostrea cucullata*. *Environmental pollution,* 218: 855–862, ISSN: 0269-7491.

Kim, D. V; Makarova, A. V; Miftakhova, R. R. & Zharkov, D. O., 2019. Base excision DNA repair deficient cells: From disease models to genotoxicity sensors. *Current Pharmaceutical Design*, 25(3): 298–312, ISSN: 1381-6128.

Krampitz, G.; Drolshagen, H. & Deltreil, J. P., 1983. Soluble matrix components in malformed oyster shells. *Experientia*, 39(10): 1105–1106, ISSN: 1420-9071.

Kuchařová, M.; Hronek, M.; Rybáková, K.; Zadák, Z.; Štětina, R.; Joskova, V. & Patková, A., 2019. Comet assay and its use for evaluating oxidative DNA damage in some pathological states. *Physiological research,* 68(1): 1–15, ISSN: 0862-8408.

Kumari, K. & Khare, A., 2018. Integration of Biomarker approach in pollution monitoring programme of aquatic ecosystem. In: *Biosynthetic Technology and Environmental Challenges,* Springer, pp. 331–354.

Laughlin, R. B.; French, W. & Guard, H. E., 1986. Accumulation of bis (tributyltin) oxide by the marine mussel Mytilus edulis. *Environmental science & technology*, 20(9): 884–890, ISSN: 0013-936X.

Lee, C.-C.; Hsieh, C.-Y.; Chen, C. S. & Tien, C.-J., 2020. Emergent contaminants in sediments and fishes from the Tamsui River (Taiwan): Their spatial-temporal distribution and risk to aquatic ecosystems and human health. *Environmental Pollution,* 258: 113733, ISSN: 0269-7491.

Lee, S.; John, B.; Gene, J.; Gerald, M.; William, R.; John, S. & Scott, S., 2018. DNA Alterations. In: *Biomarkers,* CRC Press, pp. 125–154, ISBN: 1351070274.

Lelieveld, S.; Wilson, J.; Dovrou, E.; Mishra, A.; Lakey, P. S. J.; Shiraiwa, M.; Po¨schl, U. & Berkemeier, T., 2021. Hydroxyl Radical Production by Air Pollutants in Epithelial Lining Fluid Governed by Interconversion and Scavenging of Reactive Oxygen Species. *Environmental science & technology,* 55(20): 14069–14079, ISSN: 0013-936X.

Lionetto, M. G.; Caricato, R. & Giordano, M. E., 2019. Pollution Biomarkers in Environmental and Human Biomonitoring. *The Open Biomarkers Journal,* 9(1).

Liu, S.; Yan, L.; Zhang, Y.; Junaid, M. & Wang, J., 2022. Polystyrene nanoplastics exacerbated the ecotoxicological and potential carcinogenic effects of tetracycline in juvenile grass carp (*Ctenopharyngodon idella*). *Science of The Total Environment,* 803: 150027, ISSN: 0048-9697.

Lundebye, A.-K.; Langston, W. J. & Depledge, M. H., 1997. Stress proteins and condition index as biomarkers of tributyltin exposure and effect in mussels. *Ecotoxicology*, 6: 127–136.

Madkour, L. H., 2019. Function of reactive oxygen species (ROS) inside the living organisms and sources of oxidants. *Pharm.*

Sci. Anal. Res. J, 2: 180023.

Mahboob, S.; Ahmed, Z.; Khan, M. F.; Saho, C.; Virik, P.; Al-Mulhm, N. & Baabbad, A. A. A., 2022. Ecogenotoxicological studies for an early toxicity screening and monitoring in Epinephalus chlorostigma and Scamberomorus commerson. *Saudi Journal of Biological Sciences,* ISSN: 1319-562X.

May, S.; Hirsch, C.; Rippl, A.; Bürkle, A. & Wick, P., 2022. Assessing Genotoxicity of Ten Different Engineered Nanomaterials by the Novel Semi-Automated FADU Assay and the Alkaline Comet Assay. *Nanomaterials*, 12(2): 220.

McLaughlin, M. C.; Blotevogel, J.; Watson, R. A.; Schell, B.; Blewett, T. A.; Folkerts, E. J.; Goss, G. G.; Truong, L.; Tanguay, R. L. & Argueso, J. L., 2020. Mutagenicity assessment downstream of oil and gas produced water discharges intended for agricultural beneficial reuse. *Science of The Total Environment,* 715: 136944, ISSN: 0048-9697.

Medda, N.; De, S. K. & Maiti, S., 2021. Different mechanisms of arsenic related signaling in cellular proliferation, apoptosis and neo-plastic transformation. *Ecotoxicology and Environmental Safety,* 208: 111752, ISSN: 0147-6513.

Métais, I.; Châtel, A.; Mouloud, M.; Perrein-Ettajani, H.; Bruneau, M.; Gillet, P.; Jrad, N. & Mouneyrac, C., 2019. Is there a link between acetylcholinesterase, behaviour and density populations of the ragworm Hediste diversicolor? *Marine pollution bulletin,* 142: 178–182, ISSN: 0025-326X.

Mičić, M.; Bihari, N.; Labura, Ž.; Müller, W. E. G. & Batel, R., 2001. Induction of apoptosis in the blue mussel *Mytilus galloprovincialis* by tri-n-butyltin chloride. *Aquatic toxicology,* 55(1–2): 61–73, ISSN: 0166-445X.

Mitchelmore, C. L.; Birmelin, C.; Livingstone, D. R. & Chipman, J. K., 1998. Detection of DNA strand breaks in isolated mussel (*Mytilus edulis* L.) digestive gland cells using the "comet" assay. *Ecotoxicology and Environmental Safety,* 41(1): 51–58, ISSN: 0147-6513.

Mitchelmore, C. L. & Chipman, J. K., 1998. DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis,* 399(2): 135–147, ISSN: 00275107, DOI: 10.1016/S0027-5107(97)00252-2.

Møller, P.; Stopper, H. & Collins, A. R., 2020. Measurement of DNA damage with the comet assay in high-prevalence diseases: current status and future directions. *Mutagenesis*, 35(1): 5–18, ISSN: 0267-8357.

Muruzabal, D.; Sanz-Serrano, J.; Sauvaigo, S.; Treillard, B.; Olsen, A.-K.; López de Cerain, A.; Vettorazzi, A. & Azqueta, A., 2021. Validation of the in vitro comet assay for DNA cross-links and altered bases detection. *Archives of toxicology,* 95(8): 2825–2838, ISSN: 1432-0738.

Odongo, G. A.; Skatchkov, I.; Herz, C. & Lamy, E., 2019. Optimization of the alkaline comet assay for easy repair capacity quantification of oxidative DNA damage in PBMC from human volunteers using aphidicolin block. *DNA repair,* 77: 58–64, ISSN: 1568-7864.

Olive, P. L.; Banáth, J. P. & Durand, R. E., 1990. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the" comet" assay. *Radiation research,* 122(1): 86–94, ISSN: 0033-7587.

Page, D. S.; Dassanayake, T. M. & Gilfillan, E. S., 1996. Relationship Between Tissue Concentrations of Tibutyltin and Shell Morphology in Field Populations of Mytilus edulis. *Bulletin of environmental contamination and toxicology,* 56(3): 500–504, ISSN: 0007-4861.

Park, J. K.; Lee, J. S.; Lee, H. H.; Choi, I. S. & Dai Park, S., 1991. Accumulation of polycyclic aromatic hydrocarbon-induced single strand breaks is attributed to slower rejoining processes by DNA polymerase inhibitor, cytosine arabinoside in CHO-K1 cells. *Life sciences,* 48(13): 1255–1261, ISSN: 0024-3205.

Pearson, H. B. C.; Dallas, L. J.; Comber, S. D. W.; Braungardt, C. B.; Worsfold, P. J. & Jha, A. N., 2018. Mixtures of tritiated water, zinc and dissolved organic carbon: Assessing interactive bioaccumulation and genotoxic effects in marine mussels, Mytilus galloprovincialis. *Journal of Environmental Radioactivity,* 187: 133–143, ISSN: 0265-931X.

Pellegri, V.; Gorbi, G. & Buschini, A., 2020. DNA damage detection by Comet Assay on *Daphnia magna:* Application in freshwater biomonitoring. *Science of The Total Environment,* 705: 135780, ISSN: 0048-9697.

Perovic, S.; Sljukic, B.; Šrut, M.; Perovic, A. & Klobučar, G. I. V, 2020. Evaluation of DNA damage in haemolymph of freshwater mussels Unio pictorum from Lake Skadar. *Biologia*, 75(3): 431–436, ISSN: 1336-9563.

Phelps, H. L., 1990. Tributyltin in Portugal: Biomonitoring using the shell thickness index. In: *Proceedings of the Third International Organotin Symposium, Washington, DC, Marine Technology Society*, pp. 205–206.

Phelps, H. L. & Page, D. S., 1997. Tributyltin biomonitoring in Portuguese estuaries with the Portuguese oyster (Crassostrea angulata). *Environmental technology,* 18(12): 1269–1276, ISSN: 0959-3330.

Poirier, M. C., 2021. DNA damage in cetaceans: A mini review. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 870: 503392, ISSN: 1383-5718.

Puccia, E.; Mansueto, C.; Cangialosi, M. V; Fiore, T.; Di Stefano, R.; Pellerito, C.; Triolo, F. & Pellerito, L., 2001. Organometallic complexes with biological molecules. XV. Effects of tributyltin (IV) chloride on enzyme activity, Ca2+, and biomolecule and synthesis in Ciona intestinalis (Urochordata) ovary. *Applied organometallic chemistry,* 15(3): 213–220, ISSN: 0268-2605.

Qin, N.; He, W.; Liu, W.; Kong, X.; Xu, F. & Giesy, J. P., 2020. Tissue distribution, bioaccumulation, and carcinogenic risk of polycyclic aromatic hydrocarbons in aquatic organisms from Lake Chaohu, China. *Science of the Total Environment,* 749: 141577, ISSN: 0048-9697.

Qu, M.; Ding, J.; Wang, Y.; Chen, S.; Zhang, Y. & Di, Y., 2019. Genetic impacts induced by BaP and Pb in *Mytilus coruscus*: Can RAPD be a validated tool in genotoxicity evaluation both in vivo and in vitro? *Ecotoxicology and Environmental Safety,* 169: 529–538, ISSN: 0147-6513.

Rajendran, M. S.; Prabhakaran, R.; Vignesh, S. & Nagarathinam, B., 2021. Comet Assay: Is it a Sensitive Tool in Ecogenotoxicology? In: *Environmental Biotechnology 4,* Springer, pp. 181–199.

Schuijt, L. M.; Peng, F.-J.; van den Berg, S. J. P.; Dingemans, M. M. L. & Van den Brink, P. J., 2021. (Eco) toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. *Science of the Total Environment,* 795: 148776, ISSN: 0048-9697.

Singh, N. P.; McCoy, M. T.; Tice, R. R. & Schneider, E. L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental cell research*, 175(1): 184–191, ISSN: 0014-4827.

Speit, G. & Hartmann, A., 1995. The contribution of excision repair to the DNA effects seen in the alkaline single cell gel test (comet assay). *Mutagenesis*, 10(6): 555–560, ISSN: 1464-3804.

Stephenson, M.; Smith, D.; Goetel, J.; Icnikawa, G. & Martin, M., 1986. Growth abnormalities in mussels and oysters from areas with high levels of tributyltin in San Diego Bay. In: *OCEANS'86,* IEEE, pp. 1246–1251.

Sussarellu, R.; Chouvelon, T.; Aminot, Y.; Couteau, J.; Loppion, G.; Dégremont, L.; Lamy, J.-B.; Akcha, F.; Rouxel, J. & Berthelin, C., 2022. Differences in chemical contaminants bioaccumulation and ecotoxicology biomarkers in *Mytilus edulis* and *Mytilus galloprovincialis* and their hybrids. *Environmental Pollution*,

292: 118328, ISSN: 0269-7491.

Tatin, X.; Muggiolu, G.; Sauvaigo, S. & Breton, J., 2021. Evaluation of DNA double-strand break repair capacity in human cells: Critical overview of current functional methods. *Mutation Research/Reviews in Mutation Research,* 788: 108388, ISSN: 1383-5742.

Tiano, L.; Fedeli, D.; Moretti, M. & Falcioni, G., 2001. DNA damage induced by organotins on trout-nucleated erythrocytes. *Applied organometallic chemistry,* 15(7): 575–580, ISSN: 0268-2605.

Velali, E.; Pantazaki, A.; Besis, A.; Choli-Papadopoulou, T. & Samara, C., 2019. Oxidative stress, DNA damage, and mutagenicity induced by the extractable organic matter of airborne particulates on bacterial models. *Regulatory Toxicology and Pharmacology,* 104: 59–73, ISSN: 0273-2300.

Venier, P. & Canova, S., 1996. Formation of DNA adducts in the gill tissue of *Mytilus galloprovincialis* treated with benzo [a] pyrene. *Aquatic toxicology,* 34(2): 119–133, ISSN: 0166-445X.

Waldock, M. J. & Thain, J. E., 1983. Shell thickening in Crassostrea gigas: organotin antifouling or sediment induced? *Marine pollution bulletin,* 14(11): 411–415, ISSN: 0025-326X.

Wilson, J. T.; Pascoe, P. L.; Parry, J. M. & Dixon, D. R., 1998. Evaluation of the comet assay as a method for the detection of DNA damage in the cells of a marine invertebrate, *Mytilus edulis* L. (Mollusca: Pelecypoda). *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 399(1): 87–95, ISSN: 0027-5107.

Winkelbeiner, N.; Wandt, V. K.; Ebert, F.; Lossow, K.; Bankoglu, E. E.; Martin, M.; Mangerich, A.; Stopper, H.; Bornhorst, J. & Kipp, A. P., 2020. A multi-endpoint approach to base excision repair incision activity augmented by parylation and DNA damage levels in mice: Impact of sex and age. *International journal of molecular sciences*, 21(18): 6600.

Xu, K.; Tang, Z.; Liu, S.; Xia, H.; Liu, L.; Wang, Z. & Qi, P., 2018. Effects of low concentrations copper on antioxidant responses, DNA damage and genotoxicity in thick shell mussel *Mytilus coruscus*. *Fish & shellfish immunology,* 82: 77–83, ISSN: 1050-4648.

Yaqin, K., 2006. Ecotoxicological Assessment of Aquatic Genotoxicity Using the Comet Assay. *HAYATI Journal of Biosciences,* 13(3), ISSN: 20864094, DOI: 10.1016/S1978-3019(16)30305-9.

Yaqin, K., 2019. *Petunjuk praktis aplikasi biomarker sederhana.* 1st ed., Makassar: UPT, Unhas Press, 54 p., ISBN: 9789795302209.

Yaqin, K.; Lay, B.; Riani, E. & Masud, Z., 2011. Hot spot biomonitoring of marine pollution effects using cholinergic and immunity biomarkers of tropical green mussel (*Perna viridis*) of the Indonesian waters. *Journal of Toxicology,* 3(14): 356–366.

žegura, B. & Filipič, M., 2019. The application of the Comet assay in fish cell lines. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 842: 72–84, ISSN: 1383-5718, DOI: https://doi.org/10.1016/j.mrgentox.2019.01.011, Available: <https://www.sciencedirect.com/science/article/pii/S1383571818303103>.

Khusnul Yaqin, Program Studi Manajemen Sumberdaya Perairan, Jurusan Perikanan, Fakultas Ilmu Kelautan dan Perikanan Universitas Hasanuddin, Sulawesi Selatan 90245, Indonesia, Email: khusnul@unhas.ac.id
URL ID-orcid: http://orcid.org/0000-0002-6050-8725
URL Google Scholar: https://scholar.google.co.id/citations?user=21lUj3wAAAAJ&hl=en
URL Sinta Dikti: https://sinta.kemdikbud.go.id/authors/detail?id=6002758&view=overview

How to cite this article:
Yaqin, K. 2022. DNA damage and shell malformation in Blue Mussel, *Mytilus edulis*. *Akuatikisle: Jurnal Akuakultur, Pesisir dan Pulau-Pulau Kecil* 6(1): 65-74. https://dx.doi.org/10.29239/j.akuatikisle.6.1.65-74