



**National Institute
of Oceanography and Fisheries (NIOF)**

Egyptian Journal of Aquatic Research

Volume 45, No. 2, 2019



Available online at www.sciencedirect.com

ScienceDirect

Egyptian Journal of Aquatic Research

18
H Index

Country Egypt - SJR Ranking of Egypt

Subject Area and Category
 Agricultural and Biological Sciences
 Aquatic Science
 Ecology, Evolution, Behavior and Systematics

Earth and Planetary Sciences
 Oceanography

Environmental Science
 Water Science and Technology

Publisher National Institute of Oceanography and Fisheries

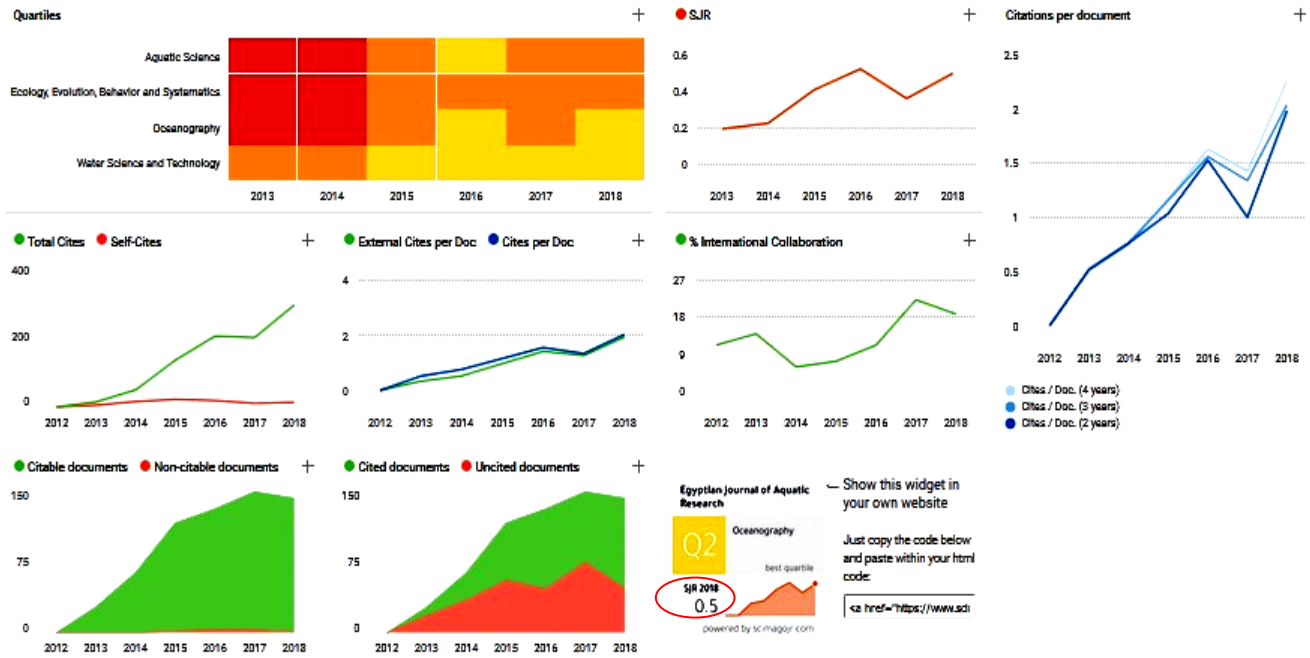
Publication type Journals

ISSN 16874285, 20903278

Coverage 2014-ongoing

Scope The Egyptian Journal of Aquatic Research is published by the National Institute of Oceanography & Fisheries. The Journal is devoted to the publication of original papers and reviews in all branches of aquatic sciences (Oceanography, Limnology, Fisheries, Aquaculture and environmental sciences)

- [Homepage](#)
- [How to publish in this journal](#)
- [Contact](#)
- [Join the conversation about this journal](#)



Editor-in-Chief

Prof. Abdalla M. Ibrahim

Faculty of Science- Ain Shams University

Email Address: drabdallai yahoo.com

Co-Editor-in-Chief

Prof. Magdy T. Khalil

Faculty of Science- Ain Shams University

Email Address: mtkhalil52 hotmail.com

Executive Assistant

Prof. Waheed M. Emam

Faculty of Science- Ain Shams University

Email Address: eased2001 yahoo.com

Prof. Abd El-Halim A. Saad

Faculty of Science- Ain Shams University

Email Address: halimsaad ymail.com

Associate Editor

Dr. Fawzia A. A. Abd El-Rahman

Faculty of Science- Ain Shams University

Email Address: fawzia_a_a yahoo.com

Advisory Board

Prof. Roger Flower

Environmental Change Research Centre (ECRC), Department of Geography Pearson Building Gower Street LONDON WC1E 6BT, UK

Email Address: rflower geog.ucl.ac.uk

Prof. Safwat H. Shakir

Chemical Engineering Department, Prairie View A&M University, Texas A&M University System, Prairie View, Texas, USA

Email Address: shshakir pvamu.edu

Prof. Richard Heckmann

Brigham Young University, Department of Biology, Provo, UT.

Email Address: biosecretary byu.edu

Prof. Irvin W. Osborne-Lee

Chemical Engineering Department, Roy G. Perry College of Engineering, Prairie View A&M University, Texas A&M University System, Prairie View, Texas, USA

Email Address: oslee pvamu.edu

Contents: Research article Open access

[Adsorption of chlorotriazine herbicide onto unmodified and modified kaolinite: Equilibrium, kinetic and thermodynamic studies](#)

Friday Onyekwere Nwosu, Oluwaseun Jacob Ajala, Fatai Olaiwola Okeola, Samad Alaya Adebayo, ... Abiodun Omodele Eletta
Pages 99-107

[Chlorophyll-*a* vertical distribution patterns during summer in the Bay of La Paz, Gulf of California, Mexico](#)

Elizabeth Durán-Campos, María Adela Monreal-Gómez, David Alberto Salas de León, Erik Coria-Monter
Pages 109-115

[Biological pollution potential in the water of Bintan-Riau Islands Province, Indonesia: First appearance of harmful algal bloom species](#)

Agung Dhamar Syakti, Fadhliyah Idris, Chandra Joei Koenawan, Rayandra Asyhar, Tri Apriadi
Pages 117-122

[Macro-benthic invertebrates as a bio-indicator for water and sediment quality in Suez Bay, Red Sea](#)

Aisha Ahmed Mohammed Belal
Pages 123-130

[Zooplankton community characteristics of the different water types in Abu Qir Bay, Alexandria, Egypt](#)

Howaida Y. Zakaria, Ahmed Radwan, Mohamed Said
Pages 131-138

[Growth attributes and biochemical composition of *Padina pavonica* \(L.\) from the Red Sea, in response to seasonal alterations of Tabuk, Saudi Arabia](#)

Abid Ali Ansari, Sulaiman Mohammad Ghanem
Pages 139-144

[Comparative study between acoustic signals and images for detecting seabed features](#)

A. Hamouda, Kh. Soliman, S. El-Gharabawy, M. Nassar
Pages 145-151

[An outbreak of *Vibrio alginolyticus* infection in aquarium-maintained dark-spotted \(*Himantura uarnak*\) and Tahitian \(*H. fai*\) stingrays](#)

Arafah M. Emam, Mahmoud Hashem, Ahmed Omar Gadallah, Mohie Haridy
Pages 153-158

[Anatomical and histological adaptations of digestive tract in relation to food and feeding habits of lizardfish, *Synodus variegatus* \(Lacepède, 1803\)](#)

Ahmed N. Alabssawy, Hassan M.M. Khalaf-Allah, Ahmed A. Gafar
Pages 159-165

[Morphometric length-weight relationships of wild penaeid shrimps in Malindi-Ungwana Bay: Implications to aquaculture development in Kenya](#)

Rashid Mohamed Kaka, Joseph Owino Jung'a, Mohamed Badamana, Renison Kahindi Ruwa, Harrison Charo Karisa
Pages 167-173

[Spatiotemporal variations in finfish assemblage and diversity indices in relation to ecological indicators of the Atrai River, Dinajpur, Bangladesh](#)

Md. Jewel Mia, Jasmin Naher, Md. Golam Azom, Md. Shamim Reza Sabuz, ... Md. Rashidul Islam
Pages 175-182

[Presence of tiger shrimp *Penaeus monodon* Fabricius, 1798 \(Penaeidae\) in the Egyptian commercial shrimp catch, Alexandria, Egypt](#)

Amal Ragaie Khafage, Somaya Mahfouz Taha, Mahmoud Ahmed Attallah
Pages 183-187

[Effects of in vitro exposure of mercury on sperm quality and fertility of tropical fish *Cyprinus carpio* L.](#)

Alfiah Hayati, Erika Wulansari, Dhea Sanggita Armando, Ari Sofiyanti, ... Manikya Pramudya
Pages 189-195

HOSTED BY



Contents lists available at ScienceDirect

Egyptian Journal of Aquatic Research

journal homepage: www.sciencedirect.com/locate/ejar

Full length article

Effects of in vitro exposure of mercury on sperm quality and fertility of tropical fish *Cyprinus carpio* L.

Alfiah Hayati [†], Erika Wulansari, Dhea Sanggita Armando, Ari Sofiyanti, Muhammad Hilman Fu'adil Amin, Manikya Pramudya

Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Jalan Mulyorejo, Surabaya 60115, Indonesia

article info

Article history:

Received 13 October 2018

Revised 11 June 2019 Accepted

11 June 2019 Available online 24

June 2019

Keywords:

DNA fragmentation

Morphology

Motility

Viability

abstract

Mercury is a widespread aquatic pollutant that adversely affects the reproductive system of male fish. Although the effects of mercuric chloride (HgCl_2) on the testicular structure, the sperm count and altered sperm morphology of fish have been investigated, the effects on sperm quality and fertilization are unknown. Therefore, the aim of this study was to investigate the effects of HgCl_2 exposure in different concentrations (0; 0.5; 1; 2.5; 5 ppm) on sperm parameters and fertility of male *Cyprinus carpio*. Sperm quality parameters, including mass and individual time of sperm motility (s), and viability (%) were measured using digital inverted microscopy. The fertilization (%) was measured by counting the number of fertilized eggs. Integrity or DNA fragmentation (%) was measured by Acridine orange test (AOT) using fluorescence microscopy. The change of sperm membrane surface was examined using scan-ning electron microscopy (SEM). The results showed that the in vitro exposure of mercury could significantly decrease some parameters of fish sperm qualities, i.e. decreased the time for mass and individual motility, viability, DNA fragmentation, and fertilization ability ($P < 0.05$) and significantly increase the malondialdehyde levels of sperm ($P < 0.05$). In addition, Hg exposure altered the morphological structure of the sperm head and interfered with the development of embryos in fish eggs.

2019 National Institute of Oceanography and Fisheries. Hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

River is an important ecosystem for human which provides water as a source for various activities such as agriculture, industry and domestic use (Moss, 2007). Water that comes from the spring has a good quality but in the process of water drainage, it will be affected by various pollutants. In recent years, river water quality in Indonesia has contaminated by many pollutants with different characteristics after passing through residential, industrial and agricultural areas. The river pollution in Indonesia has exceeded the quality standard and could negatively affect the life of aquatic biota including fish (Hayati et al., 2017a).

Mercury (HgCl_2) is one of the heavy metals found in highly polluting river water due to industrial activity. It is recognized as the most toxic heavy metal to organisms. High Hg concentrations in freshwater fish are from both aqueous and dietary containing mer-cury and decreased reproduction of adult fish (Pickhardt et al.,

2006). Furthermore, the existence of mercury at high concentrations in the water could inhibit metabolic processes leading to fish death (Vangronsveld and Clijsters, 1994). Mercury also impaired the testes function of the tropical fish *Gymnotus carapo* (L.), including the decrease in sperm count and the alternation in sperm morphology (Vergilio et al., 2014). An in vivo study conducted by Lahnsteiner et al. (2004) showed that mercury had significantly decreased the percentage of sperm motility and velocity of *Clarias gariepinus* and *Lota lota*.

The major mechanisms behind metal toxicity including mer-cury have been attributed to oxidative stress. Mercury possesses the ability to form reactive oxygen species (ROS) that cause the oxidation of DNA and lipids, DNA fragmentation, and lipid peroxidation. Moreover, malondialdehyde (MDA) will be formed after exposure to ROS which is the secondary product of lipid peroxidation (Flora et al., 2008).

Cyprinus carpio is one of the tropical fish that cultivated for human consumption. It is known as a very sensitive fish to the environmental changes. The decline in fish reproduction is associated with the decreasing of the quality and fertilization ability of sperm. Fish sperm is also used as a bioindicator of toxicity parameters (Kime et al., 2001). Previous studies showed that cadmium

Peer review under responsibility of National Institute of Oceanography and Fisheries.

[†] Corresponding author.

E-mail address: alfiah-h@fst.unair.ac.id (A. Hayati).

<https://doi.org/10.1016/j.ejar.2019.06.005>

1687-4285/ 2019 National Institute of Oceanography and Fisheries. Hosting by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

may lead to a decrease in sperm quality, fertilization ability and affect the histology in some organs of *Oreochromis niloticus* and *Barbodes* sp. (Hayati et al., 2016, 2017a,b; Nursanti et al., 2017).

In addition, Mercury in the aquatic environment affects the reproductive health of fish. Accumulation of mercury in the fish brain has resulted in hypothalamic neuron degeneration, reduced gonadotropin-secreting cells at the pituitary, and reductions in gonad size, secretion steroids, gamete production, and spawning success (Crump and Trudeau, 2009). According to Webb et al. (2006), Mercury (methylmercury) has an effect on the reproduction of white sturgeon (*Acipenser transmonatus*) in the lower Columbia River. As well, this research showed that the mean of tissue mercury concentrations was higher in muscles compared to liver and gonads.

Although the adverse effects of in vivo exposure on testicular structure, the sperm count and altered sperm morphology of fish have been investigated, the effects of in vitro exposure on sperm quality including mass and individual time of sperm motility, viability, integrity or DNA fragmentation, and fertilization are unknown. Therefore, it is essential to investigate the effects of the in vitro exposure of mercury on the sperm quality and fertility as well as to determine the concentration value of mercury that cause a decline in the fertility of *C. carpio*.

Materials and methods

Animals

Cyprinus carpio with adult gonads and healthy conditions were obtained from Freshwater Cultivation Management Unit, Batu City, Ministry of Fisheries, East Java Province, Indonesia. Twenty-five male fish, weighed 1–1.2 kg and aged 1–2 years were prepared for sperm collection. Five female fish, weighed 1.5 kg and aged 1.5–3 years were also prepared. Mature males and females were kept in a separate pool under temperature (23–25 LC) and photoperiod (12 h of light per day). Fish were fed with pellets twice a day. All procedures involving animal care were performed under the guidance of the cultivation unit.

Preparation of mercuric chloride (HgCl₂) solution

A stock solution of mercuric chloride (200 ppm) was prepared by diluting 0.02 g of mercuric chloride powder in 100 mL of aquadest. Then, solutions of mercuric chloride were prepared with 5 different concentrations (0; 0.5; 1; 2.5; 5 ppm).

Animal experiment

During the animal experiment, *C. carpio* were not anesthetized, but they remained quiet out of the water using cloth to cover their eyes while stripping. After rinsing with distilled water, the genital area was carefully dried. Sperm was collected in 5-ml syringes by gentle pressure. The sperm suspension was prepared by mixing fish sperm and 0.9% NaCl solution at a ratio 1:2 (v:v). Activation of fish sperm was performed by using water. Then, sperm suspension was dissolved in HgCl₂ solution with five different concentrations (0; 0.5; 1; 2.5; 5 ppm), five replications, and incubated for five seconds. HgCl₂ concentration was determined from LC50 assay conducted in a preliminary study. The result of LC50 assay was 0.214 ppm. Therefore, the concentrations used in this study were more than the LC50 result (0; 0.5; 1; 2.5; 5 ppm).

Sperm motility

Motility was objectively determined by mixing 1 drop of sperm suspension with 2 drops of tap water and observed under a digital inverted microscope (Olympus) at 100 magnification. The observed parameters were the mass duration and individual motility (seconds).

Sperm viability

Supravital staining with 1% aqueous Eosin Y and 10% aqueous Nigrosin solution (Sigma, USA) was used to examine sperm viability. A drop of fish sperm suspension was placed on a spot plate and mixed with one drop of Eosin solution. After 15 s, two drops of 10% Nigrosin solution was added and mixed thoroughly. A drop of this mixture was smeared in a glass slide and air-dried. The smears were examined under a light microscope with 100 magnification. Viable sperm cells appeared transparent and dead sperm appeared pink.

Gametes fertility

Fertilization was conducted by mixing the sperm and eggs in clean water with HgCl₂ in different concentrations and incubated for 5–10 s. After stirring using a chicken feather for 1 min, eggs were incubated for 5 min for fertilization process. The eggs were prepared in glass petri dish and placed in a 2 L aquarium. Water was constantly gently aerated at room temperature (20–23 LC). Each group consisted of 8 replications and each replication contained ± 200 eggs. Embryos were observed twice a day for three days and dead embryos (opaque eggs) were calculated.

DNA fragmentation

Dried smear was fixed in Carnoy's solution (Sigma Chemicals, St Louis, MO, USA) for at least 2 h and air-dried. Then, the smear was stained with Acridine Orange (AO) solution. After 5 min, the smear was washed with distilled water, covered with cover glass and mounted. Smears were examined using a fluorescence microscope (Olympus-FSX100, Japan) with the following filter combination: 450–490 nm excitation, 510 nm reflector and 520 nm barrier filter. The nuclei of 200–300 spermatozoa from each smear were examined and scored as green (normal) or red (DNA fragmentation).

Sperm morphology

Fish sperm was fixed in neutral buffered formalin (NBF) solution, dehydrated in ethanol series and air-dried. The dried cells were attached to stubs with carbon double-stick tape and teased apart with needles. The sample was sputter-coated with carbon and gold and was observed via FEI type Inspect S50 scanning electron microscope.

Malondialdehyde (MDA) assay

Around 150 mL of fish sperm was added into 300 mL of ice-cold phosphate buffered saline (PBS) and homogenized by brief sonication on ice (20 s). Malondialdehyde concentration was analyzed by QuantiChrom™ TBARS Assay Kit (DTBA-100) (BioAssay Systems, USA) according to the manufacturer's protocol. The absorbance was measured at 535 nm.

Data analysis

The data were analyzed using ANOVA followed by LSD test by Statistical Package for Social Studies (SPSS software version 21).

The results were presented as mean \pm SD. P-values less than 0.05 were considered statistically significant.

Results

Effects of in vitro exposure of mercury on sperm motility

Mercury exposure in high concentrations affects sperm motility of fish. The measurement results of sperm mass duration parameters showed that the control group had significantly ($P < 0.05$) the longest duration (498 ± 15.46 s) compared to the other treatments (1; 2.5; and 5 ppm). In line with the duration of mass motility, the duration of individual motility decreased when mercury exposure increased. All treatment groups showed a significant decrease compared to the control group ($P < 0.05$), except for 0.5 ppm exposure that did not differ significantly ($P > 0.05$). (Fig. 1).

Effect of mercury on the viability of sperm

The results of the collected sperm showed that the control group contained viable sperm but not all of this sperm would continue to live. The percentage of live sperm was determined by identifying the sperm with intact cell membranes. The living cell will be clear, but the non-vital cell will absorb the color.

Exposure to mercury decreases the membrane integrity so that dyes can enter sperm. The viability of sperm in the control group was $97 \pm 0.71\%$. Variations in exposure concentration could significantly reduce the sperm viability compared to the control group ($P < 0.05$). The results showed that the highest value of sperm viability was $91 \pm 0.74\%$ in the 0.5 ppm exposure and the lowest was $57 \pm 1.19\%$ after 5 ppm exposure (Fig. 2).

Effect of mercury on the fertility of sperm

The results showed that the control group had $96 \pm 2.07\%$ of eggs that successfully fertilized by sperm. However, exposure of mercury at 0.5 ppm or more had significantly decreased the ability of egg fertilization ($P < 0.05$). The number of unfertilized eggs increased due to the damage in structure and physiology of sperm and egg cells. Embryonic development was impaired because of the mercury contamination (Fig. 3).

The growth and development of embryos in eggs were normal in the control group. Successful eggs were appeared milky white, but those that fail to be fertilized would be dark. Summary of 72 h of *Cyprinus carpio* embryonic development after mercury

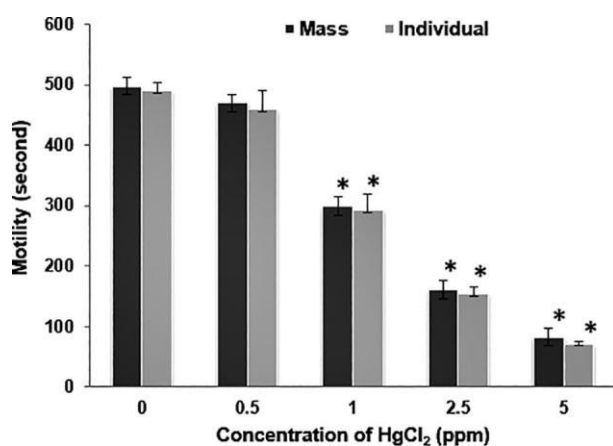


Fig. 1. The duration of mass and individual sperm after exposure to different concentrations of mercury HgCl₂. Each bar represents mean \pm SD (n = 5). $P < 0.05$ compared to the control group (0 ppm).

exposure with different concentrations is presented in Table 1. This result proved that higher concentrations of mercury inhibited the development of fish embryo.

Effect of mercury on DNA fragmentation and morphology of sperm

DNA fragmentation of sperm in the treatment groups was significantly different from the control group ($P < 0.05$). When the mercury concentration increased, DNA fragmentation of sperm was also increased (Fig. 2). Sperm that has normal DNA was appeared green and sperm with DNA fragmentation was appeared red (Fig. 4).

In addition, external investigations were conducted on each fish sperm before and after the experiment. Control sperm had no change in morphological structure (head and flagella). Meanwhile, mercury treatment group showed a change in the shape of sperm head (Fig. 5).

Effect of mercury in malondialdehyde level of fish sperm

Malondialdehyde (MDA) concentration in sperm, in relation to mercury concentration was presented in Fig. 6. The figure revealed that mercury exposure increased the level of MDA. Exposure of 0.5 ppm or more had significantly increased the MDA levels of fish sperm compared to the control group ($P < 0.05$).

Discussion

In aquatic organisms, the male reproductive system was susceptible to the side effects of heavy metals. Heavy metals that accumulated in fish tissue and cells can decrease the quality of gametes especially the sperm motility (Moss, 2007; Govind and Madhuri, 2014). Mercury is one of the abundant metals in the earth which has toxic effects. High concentrations of mercury induce free radical-mediated cytotoxicity and could be toxic for the male reproductive system (Jaishankar et al., 2014). It has been shown that exposure to mercury could decrease the sperm motility and viability due to its ability to produce ROS (Martinez et al., 2014).

Reactive oxygen species are highly reactive oxidizing oxygen derivative compound that can cause cell and tissue damage. Many of researches showed that free radicals which affect the quality of sperm could cause lipid peroxidation. Lipid peroxidation occurs in cell membranes, especially unsaturated fatty acids which are an important component of cell membrane constituents (Powers and Jackson, 2008). When free radicals are produced in vivo or in vitro inside the cells beyond the normal defense mechanism, there will be various metabolic and cellular disorders. Free radicals can damage cells by destroying the cell membrane using some mechanisms including (a) binding covalently with enzymes and/ or receptors located in the cell membrane, altering the activity of the components present in the cell membrane; (b) binding covalently to cell membrane components, altering the membrane structure and resulting in changes in membrane function and/ or altering the membrane character to be antigenic; (c) interfering with the transport system of cell membranes through covalent bonding, oxidize thiol groups, or by changing polyunsaturated fatty acids; and (d) initiating lipid peroxidation directly to polyunsaturated cell wall fatty acids. Free radicals will cause cell membrane lipid peroxidation. Lipid peroxides will form longer chains and may damage the organization of cell membranes (Sikka et al., 1995).

The success of fertilization was determined by the quality of sperm. In this study, the examination of sperm motility was divided into two parameters, the duration of the mass motility and individual motility (s). Motility of sperm occurs because of

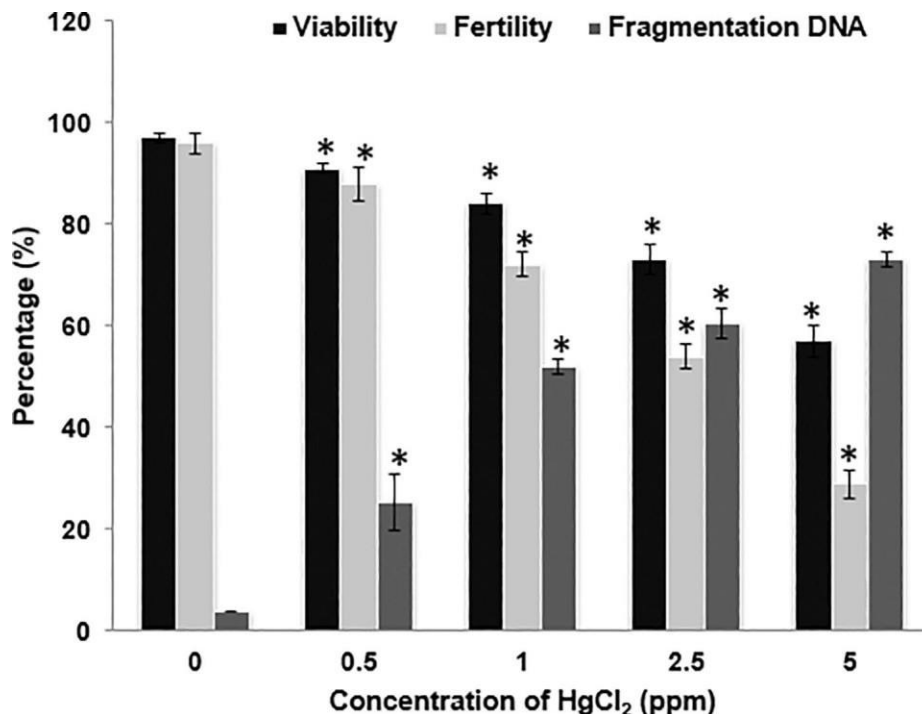


Fig. 2. Viability, fertility and DNA fragmentation of sperm after exposure to different concentrations of mercury (HgCl₂). Each bar represents mean ±SD (n = 5). * < p0.05 compared to the control group (0 ppm).

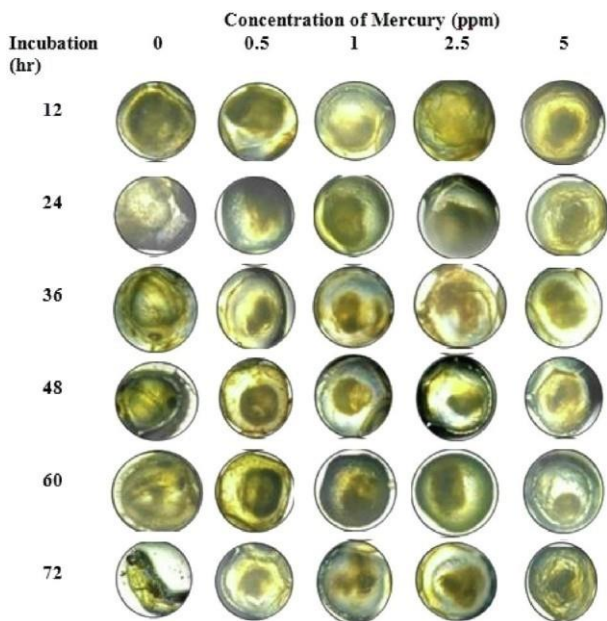


Fig. 3. Development of fish embryo after fertilization on mercury media with different concentrations.

the mitochondrial sheath in the middle of the sperm tail. Sperm tail movements depend on the ATP production by mitochondria with a source of metabolic energy derived from the fructose contained in the diluents (Ansari and Ansari, 2012).

The results showed that the higher concentration of mercury decreased the duration of mass motility and individual motility. Treatment group of 0.5 ppm mercury exposure showed a significant difference although the mercury concentration was small. Mercury was very toxic so that even in small concentrations, it

could affect the quality of sperm. Sperm motility was decreased because sperm membranes that were rich in unsaturated fatty acids were particularly vulnerable to ROS attacks. Increase of mercury concentration caused a rapid formation of ROS. High levels of ROS would affect the integrity of sperm DNA and sperm membrane flexibility by oxidizing the membrane and decreasing sperm motility. The integrity of cell membranes was crucial to the metabolic processes that depend on ATP energy supply. Damaged membranes inhibited the ATP production by disruption of outflow and electron transport required in cell metabolism, so cells could not metabolize properly (Jaishankar et al., 2014; Martinez et al., 2014).

The ability of ROS to decrease the sperm motility through membrane lipid peroxidation and production of MDA, resulted in a decreasing of sperm tails flexibility and movement. Furthermore, mercury could bind to sperm tail proteins that affect sperm movement and beat-cross frequency or could bind to enzymes affecting sperm cell metabolism that could lead to a decrease in the duration of motility of mass and individual sperm (Dietrich et al., 2010).

Viability was one of the sperm quality parameters that is required to examine the fertilization function of sperm. It is the ability to survive and to allow sufficient time for sperm to reach the place of fertilization. Based on observations and data analysis, the higher the concentration of mercury, the more sperm will die. Higher mercury concentration could increase the level of toxicity that caused lack of nutrients for sperm to survive. Mercury was also able to produce ROS that directly damages the DNA of sperm by attacking purine and pyrimidine bases. Reactive oxygen species could initiate the occurrence of apoptosis in sperm, causing the activation of caspase enzymes to degrade sperm DNA that can decrease the survival ability of sperm (Tremallen, 2008).

Fertility was the ability of a fish sperm to fertilize an egg. In the process of fertilization, merging the sperm core with the egg core occurs in the cytoplasm to form a zygote. Not all of the fertilized eggs had developed and hatched into larvae, this was included in the type of eggs with poor quality and the pollution of heavy metals (Nica et al., 2012; Mojer, 2015). The present results showed that

Table 1
Summary of 72 h of *Cyprinus carpio* embryonic development after mercury exposure with different concentrations.

Incubation (h)	Concentration of mercury (ppm)				
	0	0.5	1	2.5	5
12	Gastrula period. Early differentiation indicates development of the head and tail	Showed the mass of clustered eggs	The eggs were damaged	The eggs were damaged	The eggs were damaged
24	The segmentation period. Eggs turned whitish, the tail and head were slightly seen	The eggs were damaged	The eggs were damaged	Dead embryo	Dead embryo
36	The end of segmentation period. The tail and head were seen, eyes were clearly seen	Dead embryo	Dead embryo	Dead embryo	Dead embryo
48	The beginning of the pharyngula period. Head, eye and tail region were clearly seen	Dead embryo	Dead embryo	Dead embryo	Dead embryo
60	The end of pharyngula stage. Head, eye and tail were more elongated than before	Dead embryo	Dead embryo	Dead embryo	Dead embryo
72	The larvae, pectoral fins were slightly seen	Dead embryo	Dead embryo	Dead embryo	Dead embryo

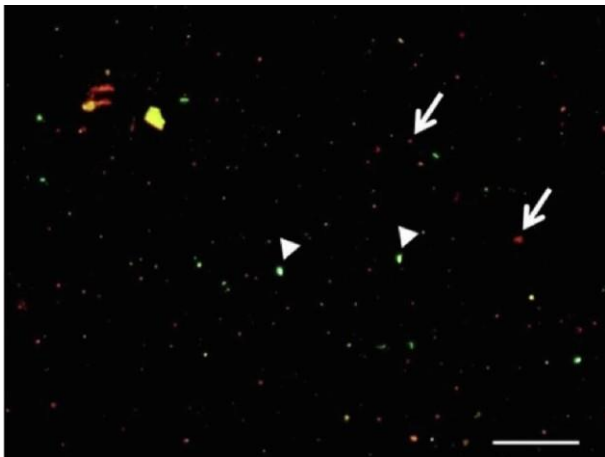


Fig. 4. Fragmentation of sperm DNA after $HgCl_2$ exposure (200). Sperm that has DNA fragmentation (\blacktriangle), normal sperm (\blacktriangleright). Scale bar = 100 nm.

0.5 ppm mercury had significantly decreased the success of sperm fertilization. This result happened because of the very toxic nature of mercury and the increase of ROS, which may affect the fertility and the development of fish embryos. In the treatment group with the highest concentration (5 ppm mercury), it showed a very low fertility percentage. A study conducted by [El-Greisy and El-Gamal \(2015\)](#) also reported that heavy metal pollution specifically cadmium could affect the development of the embryos of common carp. The decrease in fertility percentage occurred because of the mercury contamination of the egg that affected the quality of sperm, so it could inhibit the occurrence of fertilization in fish eggs

([Fürböck et al., 2009](#); [Martinez et al., 2014](#); [Ansari and Ansari, 2015](#)).

In the control group, fertilized egg appeared to have a complete embryonic development. Embryo development was influenced by the stability of the DNA of sperm which was organized to keep chromosomes stable. The organization of this DNA not only allows a very tight packed genetic information to be transferred to the eggs but also ensures that the DNA is delivered in a physical and chemical form that enables the embryo to develop for easy access to genetic information. Moreover, abnormalities in sperm chromatin and DNA fragmentation of fish were influenced by environmental factors. The fertile sperm has a stable DNA, capable of decondensation at just the right time in the fertilization process and transmits the DNA completely. The genetic material in the sperm was damaged when the chromosome was in condensation or during maturation of the sperm nucleus, or aneuploid chromosome. The main factors causing sperm DNA damage include several mechanisms: abnormal chromatin packing, ROS, and apoptosis. Detection of damage or fragmentation of oxidative DNA in sperm was related to sperm morphology structure, function, and infertility.

Oxidative stress which changes the DNA integrity of sperm is one of the failure causes of sperm fertilization. This change caused abnormality in sperm function, especially affecting motility, morphology, and sperm viability ([Zini et al., 2008](#)). Changes in DNA integrity have a risk of increasing DNA fragmentation, that was associated with both in vivo and in vitro fertility potential. There was an increase in DNA fragmentation in sperm (40%). On the other hand, from the results of this study, there was a positive correlation between motility and sperm viability, which indicated that the sperm motility was depended on the sperm viability. Meanwhile, there was a negative correlation found between sperm

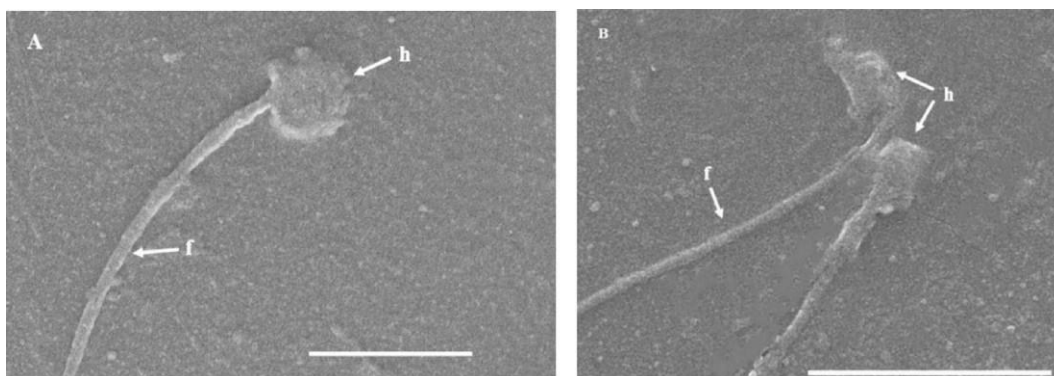
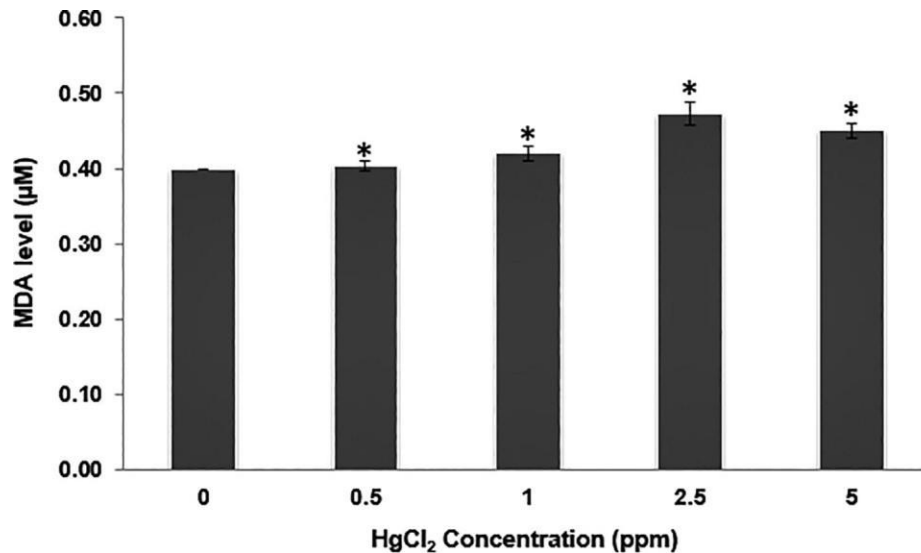


Fig. 5. Sperm morphology was observed with scanning electron microscopes (SEM). A, normal sperm; B, abnormal sperm (abnormal head morphology); h, head; f, flagellum; (15,000). Scale bar = 3 μ m (normal sperm) and 5 μ m (abnormal sperm).

Fig. 6.



Malondialdehyde levels of fish sperm after exposure to different concentrations of HgCl₂. Each bar represents mean ± SD (n = 5). P < 0.05 compared to the control group (0 ppm).

motility with DNA fragmentation and MDA level. Oxidative stress could increase lipid peroxidation, resulting in an increase of MDA level and also indicate the infertility (Hosen et al., 2015). In particular, semen with a high percentage of damaged spermatozoa (e.g. denatured DNA) has a very low potential for natural fertility. In external aquatic fertilization animals, DNA fragmentation of sperm was probably occurred because of internal factors, such as apoptosis in seminiferous tubular epithelium, defects in chromatin remodeling during spermatogenesis and free radical production during sperm migration from seminiferous tubules to other (uro-genital) male reproductive tracts. The external factors that affect DNA fragmentation of sperm are changes in the aquatic environment such as pollution and changes in water temperature, acidity, and dissolved oxygen level (Hayati et al., 2017c). The success of sperm fertilization in egg cells is also influenced by the integrity of DNA in sperm including the process of fertilization and the development of embryos in eggs. The result of this study showed a high success rate of fertilization (96%) with low DNA fragmentation (4%) in the control group.

To conclude, It was found that mercury (HgCl₂) exposure at 0.5 ppm and above could lower the sperm motility (mass and individual duration), viability, gamete fertilization, and could increase the DNA fragmentation as well as the MDA levels of *C. carpio* sperm. Exposure to mercury was also found to change the shape of sperm head and it could impair the embryonic development of *C. carpio*.

Acknowledgement

Author would like to thank for Faculty of Science and Technology, University of Airlangga, Indonesia which provides funding for Superior Moss, B., 2007. Water pollution by agriculture. *Philos. Trans. R. Soc. London B* 363 (1491), 659–666. <https://doi.org/10.1098/rstb.2007.2176>.

References

- Ansari, S., Ansari, B.A., 2012. Alphamethrin toxicity: Effect on the reproductive ability and the activities of phosphatases in the tissues of zebrafish, *Danio rerio*. *Int. J. Life Sci. Pharma Res.* 2 (1), 89–100.
- Ansari, S., Ansari, B.A., 2015. Effects of heavy metals on the embryo and larvae of Zebrafish, *Danio rerio* (Cyprinidae). *Scholars Acad. J. Biosci.* 3 (1B), 52–56.
- Crump, K.L., Trudeau, V.L., 2009. Mercury-induced reproductive impairment in fish. *Environ. Toxicol. Chem.* 28 (5), 895–907. <https://doi.org/10.1897/08-151.1>.
- Dietrich, G.J., Dietrich, M., Kowalski, R.K., Dobosz, S., Karol, H., Demianowicz, W., Glogowski, J., 2010. Exposure of rainbow trout milt to mercury and cadmium alters sperm motility parameters and reproductive success. *Aquat. Toxicol.* 97 (4), 277–284. <https://doi.org/10.1016/j.aquatox.2009.12.010>.
- El-Greisy, Z.A., El-Gamal, A.H.H., 2015. Experimental studies on the effect of cadmium chloride, zinc acetate, their mixture and the mitigation with vitamin C supplementation on hatchability, size and quality of newly hatched larvae of common carp, *Cyprinus carpio*. *Egypt. J. Aquat. Res.* 41, 219–226. <https://doi.org/10.1016/j.ejar.2015.03.007>.
- Flora, J.S., Mittal, M., Mehta, A., 2008. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J. Med. Res.* 128 (4), 501–523.
- Fürbäck, S., Lahnsteiner, F., Patzner, R.A., 2009. A fine structural review on the spermatozoa of Cyprinidae with attention to their phylogenetic implications. *Histol. Histopathol.* 24 (10), 1233–1244. <https://doi.org/10.14670/HH-24.1233>.
- Govind, P., Madhuri, S., 2014. Heavy metals causing toxicity in animals and fishes. *Res. J. Anim. Vet. Fish. Sci.* 2 (2), 17–23.
- Hayati, A., Pratiwi, H., Khoiriyah, I., Winarni, D., Sugiharto, 2016. Histopathological assessment of cadmium effect on testicles and kidney of *Oreochromis niloticus* in different salinity International Biology Conference (IBOC). AIP Conf. Proc. 1854, 1–8. <https://doi.org/10.1063/1.4985405>.
- Hayati, A., Abdizen, M.M., Antien, R.S., Solikha, B.M., Maulidiah, N., Tiantono, N., Widyana, H., Sugiharto, Winarni, D., 2017a. Bioaccumulation of heavy metals in fish (*Barbodes sp.*) tissues in the Brantas River, Indonesia. *J. Appl. Environ. Biol. Sci.* 7 (3), 139–143.
- Hayati, A., Giarti, K., Winarsih, Y., Amin, M.F.F., 2017b. The effect of cadmium on sperm quality and fertilization of *Cyprinus carpio* L. *J. Trop. Biodivers. Biotechnol.* 2, 45–50.
- Hayati, A., Tiantono, N., Mirza, M.F., Putra, I.D.S., Abdizen, M.M., Seta, A.R., Solikha, B.M., Fu'adil, M.H., Putranto, T.W.C., Affandi, M., Rosmanida, 2017c. Water quality and fish diversity in the Brantas River, East Java, Indonesia. *J. Biol. Res.* 22 (2), 43–49. <https://doi.org/10.23869/bphjbr.22.2.20172>.
- Hosen, B., Islam, R., Begum, F., Kabir, Y., Howlader, M.H.Z., 2015. Oxidative stress induced sperm DNA damage, a possible reason for male infertility. *Iran. J. Reprod. Med.* 13 (9), 525–532.
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Breegowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.* 7 (2), 60–72. <https://doi.org/10.2478/intox-2014-0009>.
- Kime, D.E., Van Look, K.J.M., McAllister, B.G., Huykens, G., Rurangwa, E., Ollevier, F., 2001. Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish. *Comp. Biochem. Physiol.* 130 (4), 425–433. [https://doi.org/10.1016/S1532-0456\(01\)00270-8](https://doi.org/10.1016/S1532-0456(01)00270-8).
- Lahnsteiner, F., Mansour, N., Berger, B., 2004. The effect of inorganic and organic pollutants on sperm motility of some freshwater teleost. *J. Fish Biol.* 65, 1283–1297. [10.1111/j.1095-8649.2004.00528.x](https://doi.org/10.1111/j.1095-8649.2004.00528.x).
- Martinez, C.S., Escobar, A.G., Torres, J.G., Brum, D.S., Santos, F.W., Alonso, M.J., Wiggers, G.A., 2014. Chronic exposure to low doses of mercury impairs sperm quality and induces oxidative stress in rats. *J. Toxicol. Environ. Health* 77 (1–3), 143–154. <https://doi.org/10.1080/15287394.2014.867202>.
- Mojer, A.M., 2015. Phenotypic study for embryonic and larval development of common carp (*Cyprinus carpio* L., 1758). *Mesopotamian J. Mar. Sci.* 30 (2), 98–111.
- Nica, A., Cristea, V., Gheorghel, D., Hoha, G.V., Enache, I.B., 2012. Embryonic and larval development of Japanese ornamental carp *Cyprinus Carpio* (Linnaeus, 1758). *Lucra stiintifice – seria zootehnie* 58, 116–120.
- Nursanti, L., Nofitasari, E., Hayati, A., Hariyanto, S., Irawan, B., Soegianto, A., 2017. Effects of cadmium on metallothionein and histology in gills of tilapia (*Oreochromis niloticus*) at different salinities. *Toxicol. Environ. Chem.* 99 (5–6), 926–937. <https://doi.org/10.1080/02772248.2017.1315120>.
- Pickhardt, P.C., Stepanova, M., Fisher, N.S., 2006. Contrasting uptake routes and tissue distributions of inorganic and methyl-mercury in mosquito fish (*Gambusia affinis*) and redear sunfish (*Lepomis microlophus*). *Environ. Toxicol. Chem.* 25, 2132–2142. <https://doi.org/10.1897/05-595R.1>.
- Powers, S.K., Jackson, M.J., 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol. Rev.* 88 (4), 1243–1276. <https://doi.org/10.1152/physrev.00031.2007>.
- Sikka, S., Rajasekaran, M., Hellstrom, W.J.G., 1995. Role of oxidative stress and antioxidants in male infertility. *J. Androl.* 16 (6), 464–468. <https://doi.org/10.1002/j.1939-4640.1995.tb00566.x>.
- Tremellen, K., 2008. Oxidative stress and male infertility – a clinical perspective. *Hum. Reprod. Update* 14 (3), 243–258. <https://doi.org/10.1093/humupd/dmn004>.
- Vangronsveld, J., Clijsters, H., 1994. Toxic effects of metals. In: Farago, M.E. (Ed.), *Plants and the Chemical Elements: Biochemistry, Uptake, Tolerance and Toxicity*. VCH Publishers, Weinheim, German, pp. 150–177. 302 pp.
- Vergilio, C.S., Moreira, R.V., Carvalho, C.E.V., Melo, E.J.T., 2014. Effect of in vitro exposure to mercury on male gonads and sperm structure of the tropical fish *tuvira Gymnotus carapo* (L.). *J. Fish Dis.* 37, 543–551.

- Webb, M.A., Feist, G.W., Fitzpatrick, M.S., Foster, E.P., Schreck, C.B., Plumlee, M., Wong, C., Gundersen, D.T., 2006. Mercury concentration in gonad, liver, and muscle of white sturgeon, *Acipenser transmontanus* in the lower Columbia River. *Arch. Environ. Contam. Toxicol.* 50 (3), 443–451. <https://doi.org/10.1007/s00244-004-0159-0>.
- Zini, A., Boman, J.M., Belzile, E., Ciampi, A., 2008. Sperm DNA damage is associated with an increased risk of pregnancy loss after UVF and ICSI: systematic review and meta-analysis. *Hum. Reprod.* 23 (12), 2663–2668. <https://doi.org/10.1093/humrep/den321>.

ALUR PUBLIKASI DARI SUBMIT HINGGA TERBIT

EES Registration for ejar

Egyptian Journal of Aquatic Research <eesserver@eesmail.elsevier.com> Fri, Oct 5, 2018, 1:07 PM

to me, alfiahayati64

*** Automated email sent by the system ***

Dear Mrs. Alfiah Hayati,

You have received this email to confirm that you can now access the Elsevier Editorial System (EES) - the online submission and peer review tracking system for Egyptian Journal of Aquatic Research – with your [Elsevier profile](#). Your Elsevier profile may also be used to access other Elsevier products.

Please note: Your **username is the **email address** to which this message was sent.**

When you register for another Elsevier journal, you can link the new journal to your Elsevier profile. Doing this will automatically copy the contact and log-in information to your new account. This means that you will be able to log into all of your Elsevier journals using the same username and password.

You can update your password and other personal information by selecting the 'change details' option on the menu banner. On this Change Details screen you can also provide journal-specific information, such as personal keywords and classifications, or opt out of receiving marketing mail.

When you log into EES, you may click the 'My EES Hub' link on the menu banner at the top of the page to go to a landing page that offers several benefits:

- A helpful overview lists any activities pending in your account
- Any unlinked journal accounts registered to an email address matching that of your consolidated account are listed, so that you can easily add these to your consolidated account
- Once you link additional journals to your consolidated account, you can switch between them without logging out
- A search option helps you find other EES journals

Kind regards,

Elsevier Editorial System
Egyptian Journal of Aquatic Research

If you need further assistance, please visit our customer support site at <http://help.elsevier.com/app/answers/list/p/7923> Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Your PDF has been built and requires approval

Egyptian Journal of Aquatic Research <eesserver@eesmail.elsevier.com> Sat, Oct 6, 2018, 9:24 PM

to me, alfiahayati64

*** Automated mail sent by the system ***

Egyptian Journal of Aquatic Research

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia

Authors: Alfiah Hayati, Dr.; Erika Wulansari, S.Si.; Dhea Sanggita Armando, S.Si.; Ari Sofiyanti, S.Si.; Muhammad Hilman Fu'adil Amin, M.Si.; Manikya Pramudya, M.Si.

Dear Alfiah,

The PDF for your submission, "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia" has now been built and is ready for your approval. Please view the submission before approving it, to be certain that it is free of any errors. If you have already approved the PDF of your submission, this e-mail can be ignored.

To approve the PDF please login to the Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

Your username is: alfiah-h@fst.unair.ac.id

Then click on the folder 'Submissions Waiting for Author's Approval' to view and approve the PDF of your submission. You may need to click on 'Action Links' to expand your Action Links menu.

You will also need to confirm that you have read and agree with the Elsevier Ethics in Publishing statement before the submission process can be completed. Once all of the above steps are done, you will receive an e-mail confirming receipt of your submission from the Editorial Office. For further information or if you have trouble completing these steps please go to:

http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Please note that you are required to ensure everything appears appropriately in PDF and no change can be made after approving a submission. If you have any trouble with the generated PDF or completing these steps please go to: http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Your submission will be given a reference number once an Editor has been assigned to handle it.

Thank you for your time and patience.

Kind regards,

Editorial Office

Egyptian Journal of Aquatic Research

For further assistance, please visit our customer support site at <http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Submission Confirmation

Egyptian Journal of Aquatic Research <eesserver@eesmail.elsevier.com> Sat, Oct 6, 2018, 9:28 PM

to me, alfiahayati64

*** Automated email sent by the system ***

Dear Alfiah,

We have received your article "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia" for consideration for publication in Egyptian Journal of Aquatic Research.

Your manuscript will be given a reference number once an editor has been assigned.

To track the status of your paper, please do the following:

1. Go to this URL: <https://ees.elsevier.com/ejar/>
2. Enter these login details:

Your username is: alfiah-h@fst.unair.ac.id

If you need to retrieve password details, please go to: http://ees.elsevier.com/EJAR/automail_query.asp

3. Click [Author Login]

This takes you to the Author Main Menu.

4. Click [Submissions Being Processed]

Thank you for submitting your work to this journal.

Kind regards,

Elsevier Editorial System
Egyptian Journal of Aquatic Research

Please note that the editorial process varies considerably from journal to journal. For more information about the submission-to-publication lifecycle, click here:

http://help.elsevier.com/app/answers/detail/p/7923/a_id/160

For further assistance, please visit our customer support site at

<http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Editor query EJAR

Elzahrae Elmasry <eesserver@eesmail.elsevier.com>

Oct 8, 2018, 6:27 PM

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, lala24.512z, marwa_ismaiel, mahmoud_ejar, salah_niof

Journal title: Egyptian Journal of Aquatic Research

Corresponding author: Mrs. Alfiah Hayati

Article title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia

Manuscript number:

Dear Dr. Alfiah Hayati,

Good day to you.

We thank you for being interested to submit your manuscript to our journal the "Egyptian Journal of Aquatic Research" EJAR.

We would like to inform you of the new process of peer-review and production according to EJAR policy starting from 2016.

After your initial submission, the manuscript is forwarded to 5 reviewers for correction and comments.

The reviewing process will be terminated once 2 reviewers among the 5 reply with their evaluation. If the manuscript is accepted you will receive an email from our part with the acceptance and a due payment of 300 US \$ that should be transferred via western union service or Moneygram service to the name of an editorial member. The details of the receiver will be forwarded to you later.

If your MS requires English editing/proofreading by a professional English editing service, we provide such service for an extra charge of 50 USD. This service is optional and you can use any other service if you have access to one.

Please note that the bank account transfer is not a valid option we only use western union service or Moneygram, so please before approving payment, check with your institution's administration, if they accept such method of payment in order to avoid any delays after the acceptance of your manuscript. After money transfer, the comments will be forwarded to you for revision. The revision is then sent to a second stage review for checking the detailed response of the authors. When all revisions are done, the manuscript will be sent for final production.

Please note that due to a large number of submissions, your manuscript, if accepted, might be published online by the second half of 2018. However, until that time if the reviewing process is fast and your article is accepted, it will appear as an article in press and you can download it and use it according to your needs.

If you approve our journal policy and you wish to proceed with the reviewing process, please note that:

- 1- A technical check is done to your manuscript so that the layout, sections and the cover letter are all correct. The cover letter should state that your work is authentic and is not currently submitted or reviewed or published in any other journal.
- 2- Kindly remove all authors' names and affiliations from the manuscript's file.
- 3- Your article should not exceed 15 pages including tables, figures, and diagrams. Font used Times new roman, size 12, line spacing 1.5. Please add line number to your manuscript.

- 4- your plagiarism percentage should not exceed 15%.
- 5- You are required to send us not less than 5 international reviewers (not from your home country) within the same field of your manuscript. (if not added in your first submission, kindly send them in an email to the below email addresses at the end of this email)
- 6- If applicable, you are requested to use citations published in EJAR related to your work.

If any of the above criteria are not complete or there is an unjustified delay in the response of the corresponding author, we regret that we will be forced to reject your paper.
I hope these criteria and timeline would suit your agenda.

Looking forward that you would confirm your acceptance of the above to proceed with the reviewing and publication process.

We thank you once again for your interest to submit your work to EJAR.

* Please cc in your reply the following emails: _

fatma_abdelrazek@hotmail.com; cassiopea23@yahoo.com; lala24.512z@gmail.com; marwa_ismaiel@ymail.com; mahmoud_ejar@yahoo.com; salah_niof@yahoo.com

Best Regards.

Sincerely,

Mahmoud Ahmed Attallah

Editorial Office of EJAR

alfiah hayati <alfiah-h@fst.unair.ac.id>

Oct 9, 2018, 8:11 AM

to eesserver, cassiopea23, fatma_abdelrazek, lala24.512z, marwa_ismaiel, mahmoud_ejar, salah_niof

Dear Editor,

I accept new journal policy of EJAR. Could you give me some times to revise and fullfil your enquirement? After I revise, where should I send my revision?

Looking forward for your answer.

Thank you

Best Regards. Dr.

Alfiah Hayati

z.m. elmasry <cassiopea23@yahoo.com>

Oct 9, 2018, 1:37 PM

to me, fatma_abdelrazek, lala24.512z, marwa_ismaiel, mahmoud_ejar, salah_niof

Dear dr. Alfiah,

Thank you for your reply,

I will send you back your manuscript to remove the old files and to upload the new edited version.

Please add as well line numbering to the manuscript.

With kind regards

Sincerely

Elzahrae Elmasry
Editorial Office of EJAR

Please edit your submission

Egyptian Journal of Aquatic Research <eeserver@eesmail.elsevier.com> Tue, Oct 9, 2018, 5:03 PM

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, radwaabdelkader, marwa_ismaiel, mahmoud_ejar, salah_niof

Re: Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia

Dear Mrs. Hayati,

Your submission entitled "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia" has been received by Egyptian Journal of Aquatic Research.

However, before we can proceed with the review process we ask you to address the following:

Dear dr. Alfiah,

Thank you for your reply,

I am sending back your manuscript to remove the old files and to upload the new edited version according to the requested criteria.

Please add as well line numbering to the manuscript.

With kind regards

Sincerely

Elzahrae Elmasry
Editorial Office of EJAR

Please log onto Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

1. Go to the menu item "Submissions/Revisions Sent Back to Author".
2. Click "Edit Submission/Revision".
3. Click on the relevant submission step on the left-hand menu;
4. Provide or modify the item/information as requested.
5. Go to "Attach Files" and "Build PDF for my Approval".
6. View and Approve your new PDF file including the changed item(s), or if needed, Edit again.

Thank you for submitting your work to the journal, and if you have any questions, please don't hesitate to contact me.

Yours sincerely,

Egyptian Journal of Aquatic Research

alfiah hayati <alfiah-h@fst.unair.ac.id>

Wed, Oct 10, 2018, 7:16 AM

to **ejar**, cassiopea23, fatma_abdelrazek, radwaabdelkader, marwa_ismaiel, mahmoud_ejar, Mohamedsalah6

Dear Editor,

We will revised the manuscript as soon as possible.
Thank you
Best Regards, Dr. Alfiah Hayati

Your PDF has been built and requires approval

Egyptian Journal of Aquatic Research
<eesserver@eesmail.elsevier.com>

Sat, Oct 13, 2018, 8:13
AM

to me, alfiahayati64

*** Automated mail sent by the system ***

Egyptian Journal of Aquatic Research
Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L. In Indonesia
Authors: Alfiah Hayati, Dr.; Erika Wulansari, S.Si.; Dhea Sanggita Armando, S.Si.; Ari Sofiyanti, S.Si.; Muhammad Hilman Fu'adil Amin, M.Si.; Manikya Pramudya, M.Si.

Dear Alfiah,

The PDF for your submission, "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L. In Indonesia" has now been built and is ready for your approval. Please view the submission before approving it, to be certain that it is free of any errors. If you have already approved the PDF of your submission, this e-mail can be ignored.

To approve the PDF please login to the Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

Your username is: alfiah-h@fst.unair.ac.id

Then click on the folder 'Submissions Waiting for Author's Approval' to view and approve the PDF of your submission. You may need to click on 'Action Links' to expand your Action Links menu.

You will also need to confirm that you have read and agree with the Elsevier Ethics in Publishing statement before the submission process can be completed. Once all of the above steps are done, you will receive an e-mail confirming receipt of your submission from the Editorial Office. For further information or if you have trouble completing these steps please go to:

http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Please note that you are required to ensure everything appears appropriately in PDF and no change can be made after approving a submission. If you have any trouble with the generated PDF or completing these steps please go to: http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Your submission will be given a reference number once an Editor has been assigned to handle it.

Thank you for your time and patience.

Kind regards,

Editorial Office

Egyptian Journal of Aquatic Research

For further assistance, please visit our customer support site at

<http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Editor handles EJAR-D-18-00198

Egyptian Journal of Aquatic Research
<eesserver@eesmail.elsevier.com>

Sat, Oct 13, 2018, 6:35 PM

to me, alfiahayati64

Ms. Ref. No.: EJAR-D-18-00198

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L. In Indonesia
Egyptian Journal of Aquatic Research

Dear Alfiah,

Your submission "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L. In Indonesia" will be handled by Editor in Chief Fatma Aly Abd El Razek.

You may check the progress of your paper by logging into the Elsevier Editorial System as an author at <https://ees.elsevier.com/ejar/>.

Enter these login details:

Your username is: alfiah-h@fst.unair.ac.id

If you need to retrieve password details, please go to:

http://ees.elsevier.com/EJAR/automail_query.asp

Thank you for submitting your work to this journal.

Kind regards,

Elsevier Editorial System
Egyptian Journal of Aquatic Research

For further assistance, please visit our customer support site at <http://help.elsevier.com/app/answers/list/p/7923>
Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

A manuscript number has been assigned: EJAR-D-18-00198

Egyptian Journal of Aquatic Research
<eesserver@eesmail.elsevier.com>

Sat, Oct 13, 2018, 6:35 PM

to me, alfiahayati64

Ms. Ref. No.: EJAR-D-18-00198

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia

Egyptian Journal of Aquatic Research

Dear Alfiah,

Your submission "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia" has been assigned manuscript number EJAR-D-18-00198.

To track the status of your paper, please do the following:

1. Go to this URL: <https://ees.elsevier.com/ejar/>

2. Enter your login details

3. Click [Author Login]

This takes you to the Author Main Menu.

4. Click [Submissions Being Processed]

Thank you for submitting your work to Egyptian Journal of Aquatic Research.

Kind regards,
Elzahrae Elmasry, MSc
Editorial Office
Egyptian Journal of Aquatic Research

Please note that the editorial process varies considerably from journal to journal. To view a sample editorial process, please click here:

http://ees.elsevier.com/eeshelp/sample_editorial_process.pdf

For further assistance, please visit our customer support site at

<http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Editor query EJAR EJAR-D-18-00198

Elzahrae Elmasry <eesserver@eesmail.elsevier.com>

Sun, Apr 7, 5:19 PM

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismaiel, mahmoud_ejar, salah_niof

Journal title: Egyptian Journal of Aquatic Research

Corresponding author: Mrs. Alfiah Hayati

Article title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish
Cyprinus carpio L. In Indonesia

Manuscript number: EJAR-D-18-00198

Dear dr. Hayati,

Please note that the reviewers completed the review of your manuscript and advised its acceptance after revision.

The current status of your manuscript is: pending for payment (300 USD).

You are requested to make the fee transfer via the western union or MoneyGram services (The bank transfer option is not valid) to the following receiver:

The receiver's information:

Name: Elzahrae Mahmoud Abdelkader Elmasry

Address: 16 Ameer Elbehar st., Bolkly, Alexandria, Egypt

Id #: 27605160200783

Mobile: +201229526899

And please send a scanned copy of the transaction having the MTCN number, the sender's full name and the country from where the money was sent.

I shall wait for your confirmation about payment so that I would be able to send you the reviewers' comments

to proceed further with the reviewing process.

If you have any questions, please don't hesitate to contact me.

With kind regards,

Sincerely,

Elzahrae Elmasry

Editorial Office of EJAR

alfiah hayati <alfiah-h@fst.unair.ac.id>

Sun, Apr 7, 7:17 PM

to Elzahrae

Dear Editor,

Thank you for your information. I will transfer the publication fee tomorrow via western union and send the scanned copy of document that you want.

Sincerely,

Alfiah Hayati

Biology Department

Faculty of Science and Technology

Universitas Airlangga
Kampus C Mulyorejo, Surabaya, East Java, Indonesia

alfiah hayati <alfiah-h@fst.unair.ac.id>

Mon, Apr 8, 2:13 PM

to Elzahrae

Dear Editor of Egyptian Journal of Aquatic Research, Elzahrae Elmasry

Here, I attach the scan copy of transaction with MTCN number

Sender's full name: Alfiah Hayati

Sender's country: Indonesia

We look forward to hearing from you soon. Thank you

Attachments area

Your Submission

Egyptian Journal of Aquatic Research <eesserver@eesmail.elsevier.com> Mon, Apr 8, 2:51 PM

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismail, mahmoud_ejar, salah_niof

Ms. Ref. No.: EJAR-D-18-00198

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia

Dear Alfiah,

The reviewers have commented on your above paper. They indicated that it is not acceptable for publication in its present form.

However, if you feel that you can suitably address the reviewers' comments (included below), I invite you to revise and resubmit your manuscript.

Please carefully address the issues raised in the comments.

If you are submitting a revised manuscript, please also:

- a) outline each change made (point by point) as raised in the reviewer comments AND/OR
- b) provide a suitable rebuttal to each reviewer comment not addressed

To submit your revision, please do the following:

1. Go to: <https://ees.elsevier.com/ejar/>
2. Enter your login details
3. Click [Author Login]

This takes you to the Author Main Menu.

4. Click [Submissions Needing Revision]

I look forward to receiving your revised manuscript.

Yours sincerely,

Fatma Aly Abd El Razek

Editor in Chief

Egyptian Journal of Aquatic Research

Reviewers' comments:

Reviewer #2: The present paper evaluate the Hg toxicity in sperm quality and fertility of a fish species *Cyprinus carpio* exposed to progressive concentrations of mercury chloride (0; 0.5; 1; 2.5; 5 ppm) after in vitro treatment. The thematic is relevant, however, in this form; this paper is not indicated for publication. There authors do not cited the literature that already demonstrated the Hg adverse effects in sperm or fecundation. There is not evidenced in the text about the innovation potential of the results for the scientific area. The experimental design with the fish species *Cyprinus carpio* is not applied only for Indonesia as indicated in title and in some parts of the text. There is no explanation about the concentrations used in the present study (0; 0.5; 1; 2.5; 5 ppm) - These levels have some relation with the environmental Hg levels found in Indonesia fishes?. There is no indication of the statistical analysis in the graphical results. There is a need to improve the quality of the scanning electron microscopy and light images. There is no scale bars in microscopy images to indicate the magnification used.

Some punctual comments:

Linha 5/ Page 1 and Line 1 / Page 2: The author cited in the text the contamination of heavy metals, and used mercury as example, describing HgCl₂, that is a specific compound, not the metal itself.

Lines 22 to 24/ Page 2: The processes of how the superficial runoff contributes as source of metals to river waters were not very well explained.

Lines 19 to 21 / Page 3: The authors described studies that with cadmium effects in decrease in sperm quality and fertilization ability. However, no further studies were mentioned about the Hg effects in these parameters for different fish species.

- Is not described how the mercuric chloride solution was prepared for the present study.

- The duration of the Hg exposure is not clear.

Line 26 / Page 3: There is no mention about the project registration in some Ethics Committee of Animals Use.

Reviewer #3: This work present interesting information about the effects of *Cyprinus carpio* sperm exposed to mercury. The test was divided on in vitro sperm exposure and fertilized eggs exposure. Results are showing the affectations are reduction of sperm motility, viability and fertilization. However, as in the manuscript is mentioned, several investigations have been done on the mercury effect on quantity and quality of the sperm. For the authors, the difference is that their study was performed in Indonesia. I ask myself what is the difference if experiments were performed at laboratory under controlled conditions? Considering tis observation title and objectives should change.

The comprehension of the document is not easy because the syntax needs to be improved.

The revision of previous investigations is limited and not all citations appears on the reference section and some references are not cited. Similar investigations have been done on Poland, Canada and EU, and they could increase the background of the work.

The methods are not clear enough to give a comprehensive view of the work done. Many basic details are missing and this decrease the reliability of the manuscript. The description of the statistical analysis is excessively resumed and the presentation of the result do not explain clearly from where the data are coming. All the material and methods section need to be meticulous explain it, as well the results (only $p < 0.5$ was included). It is important to remember: the reliability of science depends on the possibility of the experiments repetition, which for this manuscript would be complicated.

Graphs are adequately, but it will improve if the frame and grid are removed. Although, the pictures of the embryo development are well arranged and differences between treatment can be observed, an explanation of them would emphasize the results.

Reviewer #4: The major doubts concern:

Line 62-: More information about trash fish employed could be provided.

Line 91-: in the experimental design, total fish employed seems not to correspond in a correct way.

Line 93-: were the fish fed until apparent satiation?

Tables: If the ANOVA process is performed, why any kind of standard deviation or coefficient of variation is reported? without them it's difficult to evaluate the significance of differences among the means and discuss the results.

All the points reported should be addressed before final acceptance.

Editor's comments:

Please note that your manuscript needs English revision. We provide such service for an extra charge of 50 USD. This service is optional and you can use any other service of your choice, just please provide us with a certificate that your article was revised with a professional English proofreading service or by a Native speaker.

Please note that the editorial process varies considerably from journal to journal. To view the submission-to-publication lifecycle, click here:

http://help.elsevier.com/app/answers/detail/p/7923/a_id/160

For further assistance, please visit our customer support site at <http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Editor query EJAR EJAR-D-18-00198R1

Elzahrae Elmasry <eesserver@eesmail.elsevier.com> Thu, Apr 18, 3:42 PM

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismaiel, mahmoud_ejar, salah_niof

Journal title: Egyptian Journal of Aquatic Research

Corresponding author: Mrs. Alfiah Hayati

Article title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. In Indonesia

Manuscript number: EJAR-D-18-00198R1

Dear Dr. Alfiah Hayati

Thanks for your email,

Regarding your query, the English editing will be done after uploading the revised version of your manuscript.

Let me know if you need any further assistance.

Yours sincerely,

Marwa Ismaiel

Egyptian Journal of Aquatic Research

alfiah hayati <alfiah-h@fst.unair.ac.id>

Thu, Apr 18, 4:38 PM

to Elzahrae

Dear Editor,

Thank you for your information. We will upload the revised manuscript

Sincerely,
Dr. Alfiah Hayati

Your PDF has been built and requires approval

Egyptian Journal of Aquatic Research <eesserver@eesmail.elsevier.com> Mon, Apr 22, 8:56 PM

to me, alfiahayati64

*** Automated mail sent by the system ***

Egyptian Journal of Aquatic Research

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Authors: Alfiah Hayati, Dr.; Erika Wulansari, S.Si.; Dhea Sanggita Armando, S.Si.; Ari Sofiyanti, S.Si.;

Muhammad Hilman Fu'adil Amin, M.Si.; Manikya Pramudya, M.Si.

Dear Alfiah,

The PDF for your submission, "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has now been built and is ready for your approval. Please view the submission before approving it, to be certain that it is free of any errors. If you have already approved the PDF of your submission, this e-mail can be ignored.

To approve the PDF please login to the Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

Your username is: alfiah-h@fst.unair.ac.id

Then click on the folder 'Submissions Waiting for Author's Approval' to view and approve the PDF of your submission. You may need to click on 'Action Links' to expand your Action Links menu.

You will also need to confirm that you have read and agree with the Elsevier Ethics in Publishing statement before the submission process can be completed. Once all of the above steps are done, you will receive an e-mail confirming receipt of your submission from the Editorial Office. For further information or if you have trouble completing these steps please go to:

http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Please note that you are required to ensure everything appears appropriately in PDF and no change can be made after approving a submission. If you have any trouble with the generated PDF or completing these steps please go to: http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Your submission will be given a reference number once an Editor has been assigned to handle it.

Thank you for your time and patience.

Kind regards,

Editorial Office

Egyptian Journal of Aquatic Research

For further assistance, please visit our customer support site at

<http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Editor handles EJAR-D-18-00198R1

Inbox x



Egyptian Journal of Aquatic Research
<eeserver@eesmail.elsevier.com>

Tue, Apr 23, 4:00 AM

to me, alfiahayati64

Ms. Ref. No.: EJAR-D-18-00198R1

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish
Cyprinus carpio L.

Egyptian Journal of Aquatic Research

Dear Alfiah,

Your submission "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of
Tropical Fish Cyprinus carpio L." will be handled by Editor in Chief Fatma Aly Abd El Razek.

You may check the progress of your paper by logging into the Elsevier Editorial System as an
author at <https://ees.elsevier.com/ejar/>.

Enter these login details:

Your username is: alfiah-h@fst.unair.ac.id

If you need to retrieve password details, please go to:

http://ees.elsevier.com/EJAR/automail_query.asp

Thank you for submitting your work to this journal.

Kind regards,

Elsevier Editorial System
Egyptian Journal of Aquatic Research

For further assistance, please visit our customer support site at <http://help.elsevier.com/app/answers/list/p/7923>
Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Your Submission

Egyptian Journal of Aquatic Research

Wed, May 29, 3:40 PM

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismaiel, mahmoud_ejar, salah_niof

Ms. Ref. No.: EJAR-D-18-00198R1

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L. Egyptian Journal of Aquatic Research

Dear Alfiah,

The reviewers have commented on your above paper. They indicated that it is acceptable for publication after minor revision.

I invite you to revise and resubmit your manuscript.

Please carefully address the issues raised in the comments.

If you are submitting a revised manuscript, please also:

- a) outline each change made (point by point) as raised in the reviewer comments AND/OR
- b) provide a suitable rebuttal to each reviewer comment not addressed

To submit your revision, please do the following:

1. Go to: <https://ees.elsevier.com/ejar/>
2. Enter your login details
3. Click [Author Login]

This takes you to the Author Main Menu.

4. Click [Submissions Needing Revision]
I look forward to receiving your revised manuscript.

Yours sincerely,
Fatma Aly Abd El Razek
Editor in Chief
Egyptian Journal of Aquatic Research

Reviewers' comments:

Reviewer #3: The quality of the manuscript improved substantially. The author or authors followed the observations made in their vast majority. The current document has greater consistency and it is possible to read it more easily. However, there are still small observations to be made that may improve the quality of the publication, which I enclose in the file with comments and suggestions. Figure four includes a series of photographs of the development of fertilized eggs and shows the Hg effects at this early stage of development. Although, the figure is not accompanied by the descriptive table of events and alterations during these 72 hours. Maybe this was not the goal of the authors but if they have already the photographs a little extra work could be of great value to increase the quality and extension of the document.

Reviewer #4:

Accept.

Editor's comments:

Please login to your EES account to be able to download the attachment of the reviewer.

Please note that the editorial process varies considerably from journal to journal. To view the submission-to-publication lifecycle, click here: http://help.elsevier.com/app/answers/detail/p/7923/a_id/160 . For further assistance, please visit our customer support site at <http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Submission Confirmation for EJAR-D-18-00198R2

Inbox x



Egyptian Journal of Aquatic Research Fri, May 31, 7:49 AM

to me, alfiahayati64

*** Automated email sent by the system ***

Ms. Ref. No.: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Egyptian Journal of Aquatic Research

Dear Alfiah,

This message is to acknowledge that we have received your revised manuscript for reconsideration for publication in Egyptian Journal of Aquatic Research.

You may check the status of your manuscript by logging into the Elsevier Editorial System as an author at <https://ees.elsevier.com/ejar/>.

Thank you for submitting your work to Egyptian Journal of Aquatic Research.

Kind regards,

Elsevier Editorial System
Egyptian Journal of Aquatic Research

For further assistance, please visit our customer support site at <http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer

support representatives.

Please edit your submission EJAR-D-18-00198R2

Egyptian Journal of Aquatic Research

Mon, Jun 3, 4:13 PM

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismaiel, mahmoud_ejar, salah_niof

Re: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Dear Mrs. Hayati,

Your submission entitled "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has been received by Egyptian Journal of Aquatic Research.

However, before we can proceed with the review process we ask you to address the following:

Please note that the current pages number of your article is 18, and the journal limit is only 15 pages.

Please decrease the pages no. today or your article will be delayed in publication to the next issue of September 2019.

Please log onto Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

1. Go to the menu item "Submissions/Revisions Sent Back to Author".
2. Click "Edit Submission/Revision".
3. Click on the relevant submission step on the left-hand menu;
4. Provide or modify the item/information as requested.

5. Go to "Attach Files" and "Build PDF for my Approval".

6. View and Approve your new PDF file including the changed item(s), or if needed, Edit again.

Thank you for submitting your work to the journal, and if you have any questions, please don't hesitate to contact me.

Yours sincerely,
Marwa Ismaiel

alfiah hayati <alfiah-h@fst.unair.ac.id>

Mon, Jun 3, 4:20 PM

to **Egyptian**

Dear Editor,
Thank you for your information. I will revise it today

alfiah hayati <alfiah-h@fst.unair.ac.id>

Mon, Jun 3, 7:03 PM

to Egyptian

Dear Editor,
We have revised the manuscript but the system have not sent notification email yet to us. Do you get our revision? I want to make sure that our revised manuscript is already in your hand.
We look forward to hearing from you
Thank you

Your PDF has been built and requires approval

Egyptian Journal of Aquatic Research Mon, Jun 3, 6:46 PM

to me, alfiahayati64

*** Automated mail sent by the system ***

Egyptian Journal of Aquatic Research

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Authors: Alfiah Hayati, Dr.; Erika Wulansari, S.Si.; Dhea Sanggita Armando, S.Si.; Ari Sofiyanti, S.Si.; Muhammad Hilman Fu'adil Amin, M.Si.; Manikya Pramudya, M.Si.

Dear Alfiah,

The PDF for your submission, "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has now been built and is ready for your approval. Please view the submission before approving it, to be certain that it is free of any errors. If you have already approved the PDF of your submission, this e-mail can be ignored.

To approve the PDF please login to the Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

Your username is: alfiah-h@fst.unair.ac.id

Then click on the folder 'Submissions Waiting for Author's Approval' to view and approve the PDF of your submission. You may need to click on 'Action Links' to expand your Action Links menu.

You will also need to confirm that you have read and agree with the Elsevier Ethics in Publishing statement before the submission process can be completed. Once all of the above steps are done, you will receive an e-mail confirming receipt of your submission from the Editorial Office. For further information or if you have trouble completing these steps please go to:

http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Please note that you are required to ensure everything appears appropriately in PDF and no change can be made after approving a submission. If you have any trouble with the generated PDF or completing these steps please go to: http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Your submission will be given a reference number once an Editor has been assigned to handle it.

Thank you for your time and patience.

Kind regards,

Editorial Office

Egyptian Journal of Aquatic Research

Revised Manuscript Notification-Alfiah Hayati

alfiah hayati <alfiah-h@fst.unair.ac.id>

Mon, Jun 3, 7:15 PM

to eesserver, Fatma, Elzahrae, Marwa, Mahmoud, Mohamedsalah6

Dear Editor,

We have revised the manuscript but the system have not sent notification email yet to us. Have you recieved our revision? We want to make sure that our revised manuscript is already in your hand.

We look forward to hearing from you

Thank you

z.m. elmasry

Mon, Jun 3, 8:27 PM

to me, Fatma, Marwa, Mahmoud, Mohamedsalah6

Dear Dr. Alfiah,

We confirm receiving your revision.

With kind regards

Sincerely

Elzahrae Elmasry

Editorial Office of EJAR

alfiah hayati <alfiah-h@fst.unair.ac.id>

Mon, Jun 3, 8:59 PM

to Elzahrae

Thank you for your information

Re: Please edit your submission EJAR-D-18-00198R2 [190603-007120]
The Egyptian Journal of Aquatic Research Tue, Jun 4, 12:06 PM

□

Dear Mrs. Hayati,

Thank you for contacting us.

I confirm that we have received your revised submission.
Please feel free to contact us if you have any queries.

Thanks,

Azghar Zaman

Journal Manager

Global Journals Production

Tel: +91 44 4299 4815

ELSEVIER

Ascendas International Tech Park (Crest, 12th Floor), Taramani road, Taramani, Chennai 600 113

Find out some simple ways to [share your research data](#), including features that are directly available when you submit your research article to an Elsevier journal.

For assistance, please visit our [Customer Support site](#) where you can search for solutions on a range of topics and find answers to frequently asked questions.

From: alfiah hayati

Date: 03/06/2019 12.03 PM

Dear Editor,

We have revised the manuscript but the system have not sent notification email yet to us. Do you get our revision? I want to make sure that our revised manuscript is already in your hand.

We look forward to hearing from you

Thank you

On Mon, Jun 3, 2019, 4:20 PM alfiah hayati <alfiah-h@fst.unair.ac.id> wrote:

Dear Editor,

Thank you for your information. I will revise it today

On Mon, Jun 3, 2019, 4:13 PM

Egyptian Journal of Aquatic Research <eesserver@eesmail.elsevier.com> wrote:

Re: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Dear Mrs. Hayati,

Your submission entitled "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has been received by Egyptian Journal of Aquatic Research.

However, before we can proceed with the review process we ask you to address the following:

Please note that the current pages number of your article is 18, and the journal limit is only 15 pages.

Please decrease the pages no. today or your article will be delayed in publication to the next issue of September 2019.

Please log onto Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

1. Go to the menu item "Submissions/Revisions Sent Back to Author".
2. Click "Edit Submission/Revision".
3. Click on the relevant submission step on the left-hand menu;
4. Provide or modify the item/information as requested.
5. Go to "Attach Files" and "Build PDF for my Approval".
6. View and Approve your new PDF file including the changed item(s), or if needed, Edit again.

Thank you for submitting your work to the journal, and if you have any questions, please don't hesitate to contact me.

Yours sincerely,

Marwa Ismaiel

Egyptian Journal of Aquatic Research

This communication is confidential and may be privileged. Any unauthorized use or dissemination of this message in whole or in part is strictly prohibited and may be unlawful. If you receive this message by mistake, please notify the sender by return email and delete this message from your system. Elsevier B.V. (including its group companies) shall not be liable for any improper or incomplete transmission of the information contained in this communication or delay in its receipt. Any price quotes contained in this communication are merely indicative and may not be relied upon by the individual or entity receiving it. Any proposed transactions or quotes contained in this communication will not result in any legally binding or enforceable obligation or give rise to any obligation for reimbursement of any fees, expenses, costs or damages, unless an express agreement to that effect has been agreed upon, delivered and executed by the parties.

©2019, Elsevier BV. All rights reserved.

Please edit your submission EJAR-D-18-00198R2

Egyptian Journal of Aquatic Research

Mon, Jun 10, 6:11 PM (11 days ago)

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismaiel, mahmoud_ejar, salah_niof

Re: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Dear Mrs. Hayati,

Your submission entitled "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has been received by Egyptian Journal of Aquatic Research.

However, before we can proceed with the review process we ask you to address the following:

I will send you the English-revised version of your article. Please track the changes carefully and upload it ASAP (Today if possible).

Please log onto Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

1. Go to the menu item "Submissions/Revisions Sent Back to Author".
2. Click "Edit Submission/Revision".
3. Click on the relevant submission step on the left-hand menu;
4. Provide or modify the item/information as requested.
5. Go to "Attach Files" and "Build PDF for my Approval".
6. View and Approve your new PDF file including the changed item(s), or if needed, Edit again.

Thank you for submitting your work to the journal, and if you have any questions, please don't hesitate to contact me.

Yours sincerely,

Marwa Ismaiel

Re: Please edit your submission EJAR-D-18-00198R2

Marwa Ismaiel

Mon, Jun 10, 6:13 PM (11 days ago)

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, mahmoud_ejar, salah_niof

Dear Dr. Hayati,

Kindly find attached the English-revised version of your article. Please track the changes carefully and upload the corrected file as fast as you can (Today if possible).

Best Regards,

Sincerely,

Marwa Ismaiel

Editorial officer of the Egyptian Journal of Aquatic Research.

On Monday, June 10, 2019, 01:11:10 PM GMT+2, Egyptian Journal of Aquatic Research
<eesserver@eesmail.elsevier.com> wrote:

Re: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Dear Mrs. Hayati,

Your submission entitled "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has been received by Egyptian Journal of Aquatic Research.

However, before we can proceed with the review process we ask you to address the following:

I will send you the English-revised version of your article. Please track the changes carefully and upload it ASAP (Today if possible).

Please log onto Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

1. Go to the menu item "Submissions/Revisions Sent Back to Author".
2. Click "Edit Submission/Revision".
3. Click on the relevant submission step on the left-hand menu;
4. Provide or modify the item/information as requested.
5. Go to "Attach Files" and "Build PDF for my Approval".
6. View and Approve your new PDF file including the changed item(s), or if needed, Edit again.

Thank you for submitting your work to the journal, and if you have any questions, please don't hesitate to contact me.

Yours sincerely,

Marwa Ismaiel
Egyptian Journal of Aquatic Research

Attachments area

alfiah hayati <alfiah-h@fst.unair.ac.id>

Tue, Jun 11, 9:38 AM (10 days ago)

to Marwa

Dear Editor,

We have sent the revised manuscript without track changes in the system. Here we attach the revised manuscript with track changes.

Thank you, we are looking forward hearing from you.

Sincerely,
Dr. Alfiah Hayati
Biology Department
Faculty of Science and Technology
Universitas Airlangga
Kampus C Mulyorejo, Surabaya, East Java, Indonesia

Attachments area



Re: Please edit your submission EJAR-D-18-00198R2

Marwa Ismaiel

Tue, Jun 11, 3:08 PM (10 days ago)

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, mahmoud_ejar, Mohamed

Dear Dr. Hayati,

We need your swift response regarding the sent modifications, in order to include your article in the current issue.

Best Regards,

Sincerely,

Marwa Ismaiel

Editorial officer of the Egyptian Journal of Aquatic Research.

On Tuesday, June 11, 2019, 10:05:22 AM GMT+2, Egyptian Journal of Aquatic Research

<eesserver@eesmail.elsevier.com> wrote:

Re: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Dear Mrs. Hayati,

Your submission entitled "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has been received by Egyptian Journal of Aquatic Research.

However, before we can proceed with the review process we ask you to address the following:

- In line 19: Please change the citation "Hayati et al., 2017d" to be "Hayati et al., 2017b".
- Subsequently, Please update the 3 references of Hayati et al., 2017 a,b,c in the references list, as their arrangement are surely changed after you changed them in the text. Please carefully revise this part.
- After you make the update, In line 19: you add "*Barbodes* sp." to the text, that found in Hayati et al 2017 "Bioaccumulation of heavy metals in fish (*Barbodes* sp.) tissues in the Brantas" please refer to the citation in the correct order of Hayati et al 2017 a or b or c.
- Please remove the old attachments of figures and tables " that attached separately" in order not to confuse the production system.

Let me know if you have any queries.

Please log onto Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

1. Go to the menu item "Submissions/Revisions Sent Back to Author".
2. Click "Edit Submission/Revision".
3. Click on the relevant submission step on the left-hand menu;
4. Provide or modify the item/information as requested.
5. Go to "Attach Files" and "Build PDF for my Approval".
6. View and Approve your new PDF file including the changed item(s), or if needed, Edit again.

Thank you for submitting your work to the journal, and if you have any questions, please don't hesitate to contact me.

Yours sincerely,

Marwa Ismaiel

Egyptian Journal of Aquatic Research

alfiah hayati <alfiah-h@fst.unair.ac.id>

to Marwa

Tue, Jun 11, 8:28 PM (10 days ago)

Dear Editor,
I will do that

alfiah hayati <alfiah-h@fst.unair.ac.id>

to Marwa

Tue, Jun 11, 8:51 PM (10 days ago)

Dear Editor,
I have edited the manuscript. I sent the "without track changes" version to system. Here I attach edited manuscript with track changes.
Thank you. I am looking forward hearing from you.

Attachments area

Please edit your submission EJAR-D-18-00198R2

Egyptian Journal of Aquatic Research Tue, Jun 11, 3:05 PM (10 days ago)

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismaiel, mahmoud_ejar, salah_niof

Re: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Dear Mrs. Hayati,

Your submission entitled "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has been received by Egyptian Journal of Aquatic Research.

However, before we can proceed with the review process we ask you to address the following:

- In line 19: Please change the citation "Hayati et al., 2017d" to be "Hayati et al., 2017b".
- Subsequently, Please update the 3 references of Hayati et al., 2017 a,b,c in the references list, as their arrangement are surely changed after you changed them in the text. Please carefully revise this part.
- After you make the update, In line 19: you add "Barbodes sp." to the text, that found in Hayati et al 2017 "Bioaccumulation of heavy metals in fish (*Barbodes* sp.) tissues in the Brantas" please refer to the citation in the correct order of Hayati et al 2017 a or b or c.
- Please remove the old attachments of figures and tables " that attached separately" in order not to confuse the production system.

Let me know if you have any queries.

Please log onto Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

1. Go to the menu item "Submissions/Revisions Sent Back to Author".
2. Click "Edit Submission/Revision".
3. Click on the relevant submission step on the left-hand menu;
4. Provide or modify the item/information as requested.
5. Go to "Attach Files" and "Build PDF for my Approval".
6. View and Approve your new PDF file including the changed item(s), or if needed, Edit again.

Thank you for submitting your work to the journal, and if you have any questions, please don't hesitate to contact me.

Yours sincerely,
Marwa Ismaiel

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismaiel, mahmoud_ejar, salah_niof

Re: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Dear Mrs. Hayati,

Your submission entitled "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has been received by Egyptian Journal of Aquatic Research.

However, before we can proceed with the review process we ask you to address the following:

Dear Dr. Alfiah

Please change the citations in line 19 page 2 to be (Hayati et al., 2016; Hayati et al., 2017a; Hayati et al., 2017b; Nursanti et al., 2017).

You didn't update the rearrangement Of Hayati 2017 correctly in the reference list, please replace them with the following order:

Hayati, A., Abdizen, M.M., Antien, R.S., Solikha, B.M., Maulidyah, N., Tiantono, N., Widyana, H., Sugiharto, Winarni, D. 2017a. Bioaccumulation of heavy metals in fish (*Barbodes* sp.) tissues in the Brantas River, Indonesia. *Journal of Applied Environmental and Biological Sciences*. 7(3), 139-143.

Hayati A., Giarti, K., Winarsih, Y. Amin, M.F.F. 2017b. The effect of cadmium on sperm quality and fertilization of *Cyprinus carpio* L. *Journal of Tropical Biodiversity and Biotechnology*. 2, 45-50.

Hayati, A., Tiantono, N., Mirza, M.F., Putra, I.D.S., Abdizen, M.M., Seta, A.R., Solikha, B.M., Fu'adil M.H., Putranto, T. W. C., Affandi, M., Rosmanida. 2017c. Water quality and fish diversity in the Brantas River, East Java, Indonesia. *Journal of Biological Researches*. 22(2), 43-49.
<http://dx.doi.org/10.23869/bphjbr.22.2.20172>

Awaiting your swift response.

Best Regards,
Sincerely,

Marwa Ismaiel
Editorial officer of the Egyptian Journal of Aquatic Research.

Your PDF has been built and requires approval

Egyptian Journal of Aquatic Research Tue, Jun 11, 8:35 AM (10 days ago)

to me, alfiahayati64

*** Automated mail sent by the system ***

Egyptian Journal of Aquatic Research

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Authors: Alfiah Hayati, Dr.; Erika Wulansari, S.Si.; Dhea Sanggita Armando, S.Si.; Ari Sofiyanti, S.Si.; Muhammad Hilman Fu'adil Amin, M.Si.; Manikya Pramudya, M.Si.

Dear Alfiah,

The PDF for your submission, "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has now been built and is ready for your approval. Please view the submission before approving it, to be certain that it is free of any errors. If you have already approved the PDF of your submission, this e-mail can be ignored.

To approve the PDF please login to the Elsevier Editorial System as an Author:

<https://ees.elsevier.com/ejar/>

Your username is: alfiah-h@fst.unair.ac.id

Then click on the folder 'Submissions Waiting for Author's Approval' to view and approve the PDF of your submission. You may need to click on 'Action Links' to expand your Action Links menu. You will also need to confirm that you have read and agree with the Elsevier Ethics in Publishing statement before the submission process can be completed. Once all of the above steps are done, you will receive an e-mail confirming receipt of your submission from the Editorial Office. For further information

or if you have trouble completing these steps please go to:
http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Please note that you are required to ensure everything appears appropriately in PDF and no change can be made after approving a submission. If you have any trouble with the generated PDF or completing these steps please go to: http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

Your submission will be given a reference number once an Editor has been assigned to handle it.

Thank you for your time and patience.

Kind regards,

Editorial Office

Egyptian Journal of Aquatic Research

3

Egyptian Journal of Aquatic Research

Tue, Jun 11, 7:46 PM (10 days ago)

to me, alfiahayati64

Egyptian Journal of Aquatic Research

Tue, Jun 11, 8:45 PM (10 days ago)

to me, alfiahayati64

Editor handles EJAR-D-18-00198R2

Egyptian Journal of Aquatic Research

Tue, Jun 11, 9:18 PM (10 days ago)

to me, alfiahayati64

Ms. Ref. No.: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish
Cyprinus carpio L.

Egyptian Journal of Aquatic Research

Dear Alfiah,

Your submission "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L." will be handled by Editor in Chief Fatma Aly Abd El Razek.

You may check the progress of your paper by logging into the Elsevier Editorial System as an author at <https://ees.elsevier.com/ejar/>.

Enter these login details:

Your username is: alfiah-h@fst.unair.ac.id

If you need to retrieve password details, please go to:

http://ees.elsevier.com/EJAR/automail_query.asp

Thank you for submitting your work to this journal.

Kind regards,

Elsevier Editorial System

Egyptian Journal of Aquatic Research

Your Submission

Egyptian Journal of Aquatic Research

Tue, Jun 11, 9:19 PM (10 days ago)

to me, alfiahayati64, fatma_abdelrazek, cassiopea23, marwa_ismaiel, mahmoud_ejar, salah_niof

Ms. Ref. No.: EJAR-D-18-00198R2

Title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L., Egyptian Journal of Aquatic Research

Dear Alfiah,

I am pleased to inform you that your paper "Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L." has been accepted for publication in Egyptian Journal of Aquatic Research.

Below are comments from the editor and reviewers.

Thank you for submitting your work to Egyptian Journal of Aquatic Research.

Yours sincerely,

Fatma Aly Abd El Razek
Editor in Chief
Egyptian Journal of Aquatic Research

For further assistance, please visit our customer support site at <http://help.elsevier.com/app/answers/list/p/7923>. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

alfiah hayati <alfiah-h@fst.unair.ac.id>

Tue, Jun 11, 9:31 PM (10 days ago)

to **Egyptian**

Dear Editor in Chief,

Thank you for your information. We are pleased to hear that. We are looking forward hearing from you about the publication of this paper.

Sincerely,

Dr. Alfiah Hayati
Biology Department
Faculty of Science and Technology
Universitas Airlangga
Kampus C Mulyorejo, Surabaya, East Java, Indonesia

Track your article [EJAR_348] accepted in The Egyptian Journal of Aquatic Research

Elsevier - Article Status <Article_Status@elsevier.com>

Wed, Jun 12, 2:05 AM (9 days ago)

to me

Please note this is a system generated email from an unmanned mailbox.
If you have any queries we really want to hear from you via our 24/7 support at <http://help.elsevier.com>

Article title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L.
Reference: EJAR348
Journal title: The Egyptian Journal of Aquatic Research
Corresponding author: Mrs. Alfiah Hayati
First author: Dr. Alfiah Hayati

Dear Mrs. Hayati,

Your article Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L. will be published in The Egyptian Journal of Aquatic Research.

To track the status of your article throughout the publication process, please use our article tracking service:

<https://authors.elsevier.com/tracking/article/details.do?aid=348&jid=EJAR&surname=Hayati>

For help with article tracking: http://help.elsevier.com/app/answers/detail/a_id/90

Yours sincerely,
Elsevier Author Support

HAVE A QUERY?

We have 24/7 support to answer all of your queries quickly.
<http://help.elsevier.com>

UNRIVALLED dissemination for your work

When your article is published, it is made accessible to more than 15 million monthly unique users of ScienceDirect, ranging from scientists, researchers, healthcare professionals and students. This ensures that your paper reaches the right audience, wherever they may be on the globe, and that your research makes the greatest impact possible.

> Find new research yourself at: www.sciencedirect.com

SENDER INFORMATION

This e-mail has been sent to you from Elsevier Limited, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, United Kingdom. To ensure delivery to your inbox (not bulk or junk folders), please add Article_Status@elsevier.com to your address book or safe senders list.

PRIVACY POLICY

Please read our privacy policy <http://www.elsevier.com/privacypolicy> [T-12b-20150414]

Publication of your article [EJAR_348] in The Egyptian Journal of Aquatic Research is on hold due to file problems

Jayasrs@elsevier.com

Jun 15, 2019, 12:27 PM (6 days ago)

to me

Our reference: EJAR 348
Article reference: EJAR_EJAR-D-18-00198
Article title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L.
To be published in: The Egyptian Journal of Aquatic Research

Dear Mrs. Hayati,

Congratulations on having your article accepted.

We have now received your manuscript in production and would like to begin the typesetting process.

Unfortunately we have encountered a problem with the electronic files you provided and cannot process your article further until the following issues are resolved:

* In the supplied manuscript, the Title Page for your paper is not included. Please email a Title Page for your manuscript that contains the following information: full title, all authors and their affiliations and complete contact information for the corresponding author.

We would be grateful if you could kindly address the problem as quickly as possible, ideally within 48 hours, by replying to this message.

Further information on acceptable file formats can be found at <http://www.elsevier.com/guidepublication>. Please quote the reference for your article, EJAR 348, in all of your messages to us.

Thank you for your help with this issue; I look forward to hearing from you soon.

Kind regards,

Jayasree S

Data Administrator

Elsevier

E-Mail: Jayasrs@elsevier.com

HAVE QUESTIONS OR NEED ASSISTANCE?

For further assistance, please visit our Customer Support site, where you can search for solutions on a range of topics and find answers to frequently asked questions. You can also talk to our customer support team by phone 24 hours a day from Monday-Friday and 24/7 by live chat and email.

Get started here: <http://service.elsevier.com/app/home/supporthub/publishing>

Copyright © 2015 Elsevier B.V. | Privacy Policy <http://www.elsevier.com/privacypolicy>

Elsevier Limited, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, United Kingdom,
Registration No. 1982084

alfiah hayati <alfiah-h@fst.unair.ac.id> Jun 15, 2019, 1:47 PM (6 days ago)

to Jayasrs

Dear Editor, here we attach the title page for our paper. Thank you for your information. We are looking forward hearing from you.

Attachments area

alfiah hayati <alfiah-h@fst.unair.ac.id>

Jun 15, 2019, 10:03 PM (6 days ago)

to Jayasrs

Dear Editor,

Here we attach the title page of our paper. Thank you for your information. We are looking forward hearing from you.

Sincerely,

Dr. Alfiah Hayati
Biology Department
Faculty of Science and Technology
Universitas Airlangga
Kampus C Mulyorejo, Surabaya, East Java, Indonesia

S, Jayasree (ELS-CON)

Jun 17, 2019, 6:06 PM (4 days ago)

Dear Dr.

Thank you for your Email,

This is to confirm that we have received title page of your article. We will get back to you in case of anything might need.

If I could be further assistance, feel free to contact me. I'm glad to assist you.

Thank you,

Kind regards,

[Jayasree.S](#)

Data Administrator

Editorial-Production

Department Elsevier

B.V Radarweg 29

1043 NX Amsterdam The Netherlands

Fax: +31 2048 52799/+91 4442197793

E-mail: Jayasrs@elsevier.com

From: alfiah hayati <alfiah-h@fst.unair.ac.id>

Sent: 15 June 2019 20:33

To: S, Jayasree (ELS-CON) <jayasrs@elsevier.com>

Subject: Re: Publication of your article [EJAR_348] in The Egyptian Journal of Aquatic Research is on hold due to file problems

S, Jayasree (ELS-CON)

Jun 17, 2019, 6:06 PM (4 days ago)

Dear Dr.

Thank you for your Email,

This is to confirm that we have received title page of your article. We will get back to you in case of anything might need.

If I could be further assistance, feel free to contact me. I'm glad to assist you.

Thank you,

Kind regards,

[Jayasree.S](#)

Data Administrator

Editorial-Production Department

Elsevier B.V

Radarweg 29

1043 NX Amsterdam

The Netherlands

Fax: +31 2048 52799/+91 4442197793

E-mail: Jayasrs@elsevier.com

From: alfiah hayati <alfiah-h@fst.unair.ac.id>

Sent: 15 June 2019 20:33

To: S, Jayasree (ELS-CON) <jayasrs@elsevier.com>

Subject: Re: Publication of your article [EJAR_348] in The Egyptian Journal of Aquatic Research is on hold due to file problems

Production has begun on your article [EJAR_348] in The Egyptian Journal of Aquatic Research

Jayasrs@elsevier.com

Jun 18, 2019, 1:55 PM (3 days ago)

Our reference: EJAR 348

Article reference: EJAR_EJAR-D-18-00198

Article title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L

To be published in: The Egyptian Journal of Aquatic Research

Dear Mrs. Hayati,

Thank you for choosing to publish in The Egyptian Journal of Aquatic Research. Please read this e-mail carefully as it contains important information.

FINALIZE PUBLISHING YOUR ARTICLE:

We work hard to publish our authors' articles online as quickly and efficiently as possible, therefore processing of your accepted manuscript for publication has already begun. To ensure that we publish your article in accordance with your wishes, please now complete the forms found here:

<http://authors.elsevier.com/authorforms/EJAR348/76bb4276408b3c4cc1a57ca13e0505f9>

If this link does not work, please copy the entire URL (noting that it may run on to a second line in this message) into your browser. You should log in with your Elsevier Profile credentials, which you may have already created when submitting your article.

CHECK YOUR CONTACT DETAILS:

Please check that your details listed below are correct so we can contact you if needed:

Mrs. Alfiah Hayati

Universitas Airlangga
Department of Biology
Faculty of Science and Technology
Campus C, Jalan Mulyorejo
Surabaya 60115
Indonesia

Phone: +62 81330950399

Fax: not available

E-mail: alfiah-h@fst.unair.ac.id

YOUR REFERENCE NUMBER:

Lastly, to help us provide you with the best service, please make a note of your article's reference number EJAR 348 and quote it in all of your messages to us.

Thank you for your cooperation. Please contact us if you have any questions.

Kind regards,

Jayasree S

Data Administrator

Elsevier

E-Mail: Jayasrs@elsevier.com

HAVE QUESTIONS OR NEED ASSISTANCE?

For further assistance, please visit our Customer Support site, where you can search for solutions on a range of topics and find answers to frequently asked questions. You can also talk to our customer support team by phone 24 hours a day from Monday-Friday and 24/7 by live chat and email.

Get started here: <http://service.elsevier.com/app/home/supporthub/publishing>

Copyright © 2015 Elsevier B.V. | Privacy Policy <http://www.elsevier.com/privacypolicy>

Elsevier Limited, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, United Kingdom, Registration No. 1982084

Rights and Access form completed for your article [EJAR_348]

Inbox x



Elsevier - Author Forms <Article_Status@elsevier.com>

Jun 18, 2019, 3:02 PM (3 days ago)

to
me

Dear Mrs. Hayati,

Thank you for completing the Rights and Access Form for your article *Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish Cyprinus carpio L* on June 18, 2019.

The Order Summary is attached to this email.

Your article will be free for everyone to read online as soon as it is published.

If you have any questions, please do not hesitate to contact us. To help us assist you, please quote our article reference EJAR348 in all correspondence.

Now that your article has been accepted, you will want to maximize the impact of your work. Elsevier facilitates and encourages authors to share their article responsibly. To learn about the many ways in which you can share your article whilst respecting copyright, visit: www.elsevier.com/sharing-articles.

Kind regards,

Elsevier Researcher Support



Have questions or need assistance?

Please do not reply to this automated message.

For further assistance, please visit our [Elsevier Support Center](#) where you search for solutions on a range of topics and find answers to frequently asked questions.

You can also talk to our researcher support team by phone 24 hours a day from Monday-Friday and 24/7 by live chat and email.

© 2018 Elsevier Ltd | **Privacy Policy** <http://www.elsevier.com/privacypolicy>

Elsevier Limited, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, United Kingdom, Registration No. 1982084. This e-mail has been sent to you from Elsevier Ltd. To ensure delivery to your inbox (not bulk or junk folders), please add article_status@elsevier.com to your address book or safe senders list.

Attachments area

Marwa Ismaiel Jun 21 2019

3:09 PM (31 minutes ago)

to **z.m., Fatma, Mahmoud, Mohamed**, me

Dear Dr. Alfiah

Good day to you,

Kindly send me the original files of Fig. 1,2 & 6 " that can be edited".

I will highly appreciate your swift response.

Marwa Ismaiel

4:01 PM (10 minutes ago)

to me, z.m., Fatma, Mahmoud

Dear Dr. Alfiah

I really appreciated your swift response.

Thank you

alfiah hayati <alfiah-h@fst.unair.ac.id>

4:04 PM (7 minutes ago)

to Marwa

Dear Editor,

Here we attach the new file for Figure 6.

Attachments area

Your article [EJAR_348] - is now available online

Elsevier - Article Status <Article_Status@elsevier.com> 8:51 AM (3 hours ago)

Please note this is a system generated email from an unmanned mailbox.
If you have any queries we really want to hear from
you via our 24/7 support at <http://service.elsevier.com>

Article title: Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish
Cyprinus carpio L.

Article reference: EJAR348

Journal title: The Egyptian Journal of Aquatic Research

Corresponding author: Mrs. Alfiah Hayati

First author: Mrs. Alfiah Hayati

Online publication complete: 24-JUN-2019

DOI information: 10.1016/j.ejar.2019.06.005

Dear Mrs. Hayati,

We are pleased to inform you that the final corrections to your proofs have been made. Further corrections are no longer possible. Your article is now published online at:

<https://doi.org/10.1016/j.ejar.2019.06.005>

Since your article is being published Open Access, access to your full article is not restricted in any way. This article can already be cited using the year of online availability and the DOI as follows: Author(s), Article Title, Journal (Year), DOI.

WHAT HAPPENS NEXT

You will be automatically notified by e-mail once the full bibliographic details are available.

To track the status of your article throughout the publication process, please use our article tracking service:

<https://authors.elsevier.com/tracking/article/details.do?aid=348&jid=EJAR&surname=Hayati>

Kind regards,
Elsevier Author Support

ADVANCING WOMEN

Advancing women in science and libraries in the developing world:

Every year, the Elsevier Foundation provides grants to institutions around the world, with a focus on support for the world's libraries and for scholars in the early stages of their careers. Since 2002, The Elsevier Foundation has awarded more than 60 grants worth millions dollars to non-profit organizations focusing on helping the world's libraries, nurse faculties, and women scholars during their early and mid-careers. Maybe we can help you.

See the latest call for funding applications at: www.elsevierfoundation.org

HAVE QUESTIONS OR NEED ASSISTANCE?

For further assistance, please visit our Customer Support site where you search for solutions on a range of topics and find answers for frequently asked questions. You can also talk to our customer support team by phone 24 hours a day from Monday-Friday and 24/7 by live chat and email. Get started at > <http://service.elsevier.com>

© 2016 Elsevier Ltd | Privacy Policy <http://www.elsevier.com/privacypolicy>

Elsevier Limited, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, United Kingdom, Registration No. 1982084. This e-mail has been sent to you from Elsevier Ltd. To ensure delivery to your inbox (not bulk or junk folders), please add article_status@elsevier.com to your address book or safe senders list. [T-6b-20151509]

Track Your Accepted Article

The easiest way to check the publication status of your accepted article

Effects of In-vitro Exposure of Mercury on Sperm Quality and Fertility of Tropical Fish *Cyprinus carpio* L.

Article reference EJAR348
Journal The Egyptian Journal of Aquatic Research
Corresponding author Alfah Hayati
First author Alfah Hayati
Received at Editorial Office 13 Oct 2018
Article revised 11 Jun 2019
Article accepted for publication 11 Jun 2019
DOI [10.1016/j.ejar.2019.06.005](https://doi.org/10.1016/j.ejar.2019.06.005)

Last update: 24 Jun 2019

[Share via email](#)

Status comment

- Your corrected proof is now available online.



ISSN 1687-4285

Bibliographic information

Volume/Issue
Will appear soon

Fwd: Your paper is online: Effects of in vitro exposure of mercury on sperm quality and fertility of tropical fish *Cyprinus carpio* L.

alfiah hayati <alfiah-h@fst.unair.ac.id>

06.43 (2 menit yang lalu)

----- Forwarded message -----

Dari: Dan from Peerus <dan@peer.us>

Date: Rab, 26 Jun 2019 11:14 PM

Subject: Your paper is online: Effects of in vitro exposure of mercury on sperm quality and fertility of tropical fish *Cyprinus carpio* L.

To: Alfiah Hayati <alfiah-h@fst.unair.ac.id>

Dear Alfiah Hayati,

Your paper **Effects of in vitro exposure of mercury on sperm quality and fertility of tropical fish *Cyprinus carpio* L.** has just been published in **Egyptian Journal of Aquatic Research**, **congratulations!** You can now view the **reading performance of your paper** through ReaderTracker.

[Find your paper on Peerus](#)

What is Peerus ?

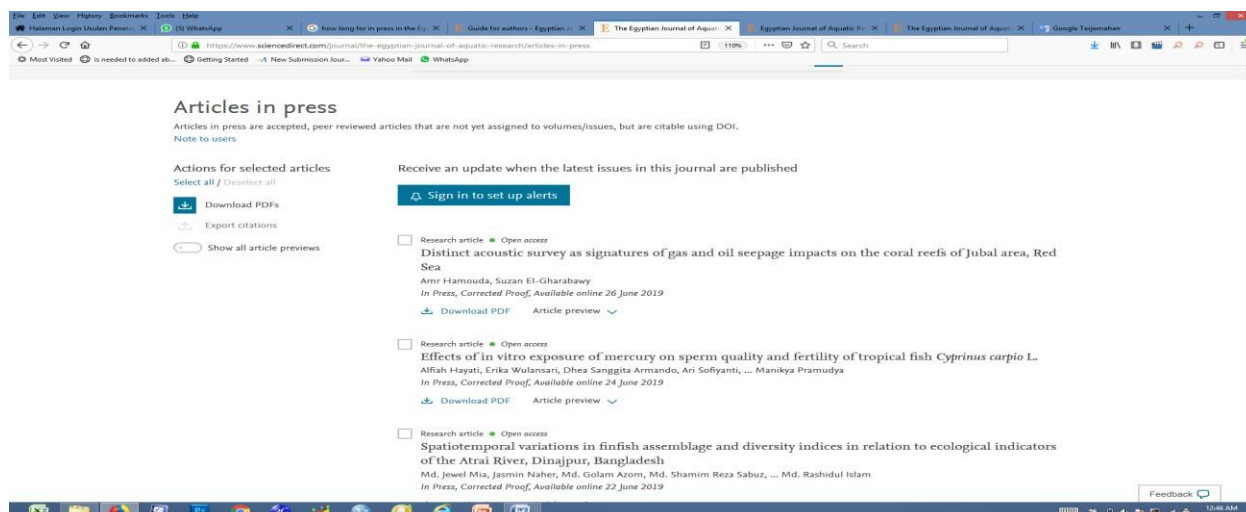
Peerus is a paper monitoring app that will keep you up-to-date with the latest publications in your research field. It brings to your attention anything that's published with the terms you want to track, in any of the journals covered by Peerus. 25 000 journals covered!

Save time in your research with Peerus !

By this email, DSRT informs you that:

- your article has been indexed by Peerus: your name is published on our website to inform other scientists about your work
- by joining you will be granted access to the latest publications in your research field either through the webapp or by emails.

To contact you, we have collected on Google your name and your email address. You have the right to access, object and erase your personal data by [contacting us](#).
by clicking on emails'link, you hereby accept our [TCU](#)



<https://www.sciencedirect.com/journal/the-egyptian-journal-of-aquatic-research/articles-in-press>

Note to users:

Articles in press are peer reviewed, accepted articles to be published in this publication. When the final article is assigned to volumes/issues of the publication, the article in press version will be removed and the final version will appear in the associated published volumes/issues of the publication. The date an article was first made available online will be carried over. Please be aware that, although articles in press do not have all bibliographic details available yet, they can already be cited using the year of online publication and the DOI, as follows: author(s), article title, Publication (year), DOI.

Please consult the journal's reference style for the exact appearance of these elements, abbreviation of journal names and use of punctuation.

There are three types of Articles in Press:

- **Accepted manuscripts:** articles that have been peer reviewed and accepted for publication by the editorial board. They have not yet been copy edited and/or formatted in the publication house style, and may not yet have full ScienceDirect functionality, e.g., supplementary files may still need to be added, links to references may not resolve yet, etc.
- **Uncorrected proofs:** articles that have been copy edited and formatted, but have not been finalized yet. They still need to be proof-read and corrected by the author(s) and the text could still change before final publication.
- **Corrected proofs:** articles that contain the authors' corrections. Final citation details, e.g. volume and/or issue number, publication year and page numbers, still need to be added and the text might change before final publication.

ISSN: 1687-4285

Copyright © 2019 National Institute of Oceanography and Fisheries. Production and hosting by Elsevier B.V. All rights reserved