



## Data Article

# Experimental data sets on the evaluation of graphene oxide as a thyroid endocrine disruptor and a modulator of gas gland cells in Japanese medaka (*Oryzias latipes*) larvae at the onset of maturity



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## ABSTRACT

This article presents the experimental datasets obtained from the histological/histochemical studies of endocrine disrupting effects of graphene oxide (GO) on thyroid follicles and gas gland (GG) cells of Japanese medaka larvae at the onset of maturity. The experiment was conducted on one day-post hatch (dph) starved fries (orange-red variety) immersed in different concentrations of GO (2.5–20.0 mg/L) and no GO (controls) in embryo-rearing medium (ERM) for 96 h under laboratory conditions (25 ± 1 °C; light cycle 16 h light: 8 h dark). After treatment, larvae were maintained in balanced salt solution (BSS) with food and allowed depuration for 6 more weeks in a GO-free environment. On 47 dph, the larvae were anesthetized in MS 222 and their total lengths (mm) and weights (mg) were measured, and they were then cut into three small pieces (head, trunk, and tail). Head and trunk regions were fixed in 4% PFA in 20 mM PBS for 48 h at room temperature and the post-anal tail was preserved in TRI reagent and kept at –20 °C until analysis. Tissues in

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4% PFA were used for cutting 5µm thick paraffin sections in a manual rotary microtome. Sections of head regions were evaluated for thyroid follicles after hematoxylin-eosin (HE) or Periodic acid-Schiff (PAS) staining. Trunk sections were used for swim bladder (SB) inflation studies and for phenotypic sex (ovary and testis) of the larvae after HE staining. Genetic sex assessment was made from tail DNA by genotyping Y chromosome-specific male sex-determining gene *dmy*. Digital images were captured by using either an Olympus B-max 40 microscope attached to a camera with Q-capture Pro 7 software or an Olympus CKX53 microscope with DP22 camera and CellSens software. Images of thyroid follicles and GG cells were analyzed using imagej software. HE stained histological sections of thyroid follicles near the heart and branchial regions were captured and the area (µm<sup>2</sup>) of individual follicles (minimum 3) available in the entire section were measured. The heights of thyrocytes (µm) were determined directly. Manual counting of GG cells was made from the digital images captured in several regions of the SB avoiding blood cells and other cells which have indistinct nucleus and pale cytoplasm; results were expressed as the number of GG cells/mm<sup>2</sup>. Data were analyzed by GraphPad prism version 7.04. For normally distributed data, one-way ANOVA followed by post-hoc Tukey's test or unpaired parametric "t" test including Welch's correction was used. Otherwise, Kruskal-Wallis test followed by nonparametric Mann-Whitney's test as a post hoc test was used. Data were expressed as means ±SEM and the level of significance was set at  $p < 0.05$ .

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## Specifications Table

|                                |   |
|--------------------------------|---|
| Subject                        | Biological Sciences   |
| Specific subject area          | Zoology   |
| Type of data                   | Table<br>Image<br>Figure  |
| How data were acquired         | JEOL-1011 Transmission Electron Microscopy -TEM (JOEL 2010, USA) Microtome (Olympus cut 4055)<br>Haematoxylin Eosin staining (HE)<br>Periodic Acid Schiff staining (PAS)<br>Olympus B-max 40 microscope attached to a camera with Q-capture Pro-7 software (Media Cybernetics)<br>Olympus CKX53 inverted microscope with DP22 camera and CellSens software (Hunt optics & Imaging, Pittsburgh, PA, USA)<br>GraphPad Prism 7 (San Diego, CA, USA)<br><a href="http://imagej.nih.gov">imagej (imagej.nih.gov)</a> |
| Data format                    | Raw<br>Analyzed   |
| Parameters for data collection | One day post hatch Japanese medaka fries were exposed to different concentrations of GO (2.5–20 mg/L) for 96h in ERM and transferred to BSS after treatment. After 6 weeks in BSS, the larvae were sacrificed. The head region was used for histological and histochemical analysis of thyroid follicles. The trunk was used for studies on gas gland cells. The phenotypic sex was determined by gonad histology and genotypic sex by genotyping sex-specific male gene <i>dmy</i> [1–3].                      |

(continued on next page)

|                                |  |
|--------------------------------|--|
| Description of data collection | GO was obtained from Sigma (St. louis MO), and sonicated in ERM. Japanese medaka fries (1 dph) were immersed in different concentrations of GO (2.5-20 mg/L) dispersed in ERM for 96 h without food and depurated in BSS in a GO-free environment under laboratory conditions for 6 weeks with food. On 47 <sup>th</sup> dpf, the effects on thyroid follicles adjacent to gill regions and GG cells at the coronal end of SB were evaluated histologically/histochemically in 5 µm thick HE and PAS stained sections captured digitally and analyzed by imagej software. Phenotypic sex was determined histologically and the genotypic sex was identified by genotyping <i>dmy</i> gene. Statistical analysis was done using the GrapPad Prism software [1,2]. |
| Data source location           | RCMI Centre for Environmental Health, Jackson State University, Jackson, MS 39217, USA   |
| Data accessibility             | With the article   |
| Related research article       | T. E. Asala, A. K. Dasmahapatra, A. Myla, P.B. Tchounwou, Histological and Histochemical Evaluation of the effects of graphene oxide on thyroid follicles and gas gland of Japanese medaka ( <i>Oryzias latipes</i> ) larvae. Chemosphere, 286, 2022, 131719. ( <a href="https://doi.org/10.1016/j.chemosphere.2021.131719">https://doi.org/10.1016/j.chemosphere.2021.131719</a> )  |

## Value of the Data

- Fish thyroid is not a discrete encapsulated gland that imposed potential problems during TDC evaluation. Our data on histological/histochemical changes that occur in the thyroid follicles and GG cells of Japanese medaka larvae at the onset of maturity (47 dph) after GO exposure during the first fry stage appeared to be sex-specific. These innovative and cutting-edge data are valuable for evaluating GO as a potential TDC in aquatic organisms.
- Organizations such as United States Environmental Protection agency (USEPA), Organisation of Economic Cooperation and Development (OECD), European Food Safety Authority (EFSA), French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and others will benefit from these data when reviewing/developing strategies and tests to screen the TDCs disposed into the aquatic environment using sentinel animals as test models.
- Identification of TDCs using fish models is challenging as they can act at multiple levels of thyroid axis. Data on thyroid histology/histochemistry, a powerful and sensitive tool for evaluating adverse effects of chemicals on thyroid function, are lacking. The availability of such data should strengthen the importance of fish thyroid in eco-toxicological studies. As a test organism, Japanese medaka is unique and well-studied small fish model used in toxicology.
- Due to the extensive use of engineered nanomaterials in human health, it is necessary to evaluate the potential toxic effects of GO by using sentinel animal models living in different ecosystems. The cutting-edge data generated in this study are valuable in sharing the knowledge on the potential EDC effects of GO targeting thyroids of a sentinel fish species, Japanese medaka, which has unique fetatures of sex differentiation both at phenotypic and genotypic levels.
- A major goal of this research is to establish a correlation between the histopathological effects of GO on gas gland cells located at the coronal end of the swim bladder and its thyroid disruption effects which are unique and sex-specific. Such research can underscore the importance of thyroids in maintaing the volume of swim bladder and thus resonance and buoyancy of fish at different water levels during various aspects of reproductive behavior.
- The data on thyroid folicles, thyrocytes, and GG histology/histochemistry we generated through this cutting-edge research and the unique concept of sex-specific effects of TDC on thyroid architechture we developed on medaka fish larvae can be shared within the scientific communities especially among toxicologists, and reproductive/developmental biologists who may find them useful during the experimental design and analysis of their research results.

## 1. Data Description

Japanese medaka (one day post hatch) were exposed to different concentrations of GO and the histopathological evaluations of the thyroid follicles and gas gland (GG) cells were evaluated under microscopy (Supplementary Figs. 1 and 2; Supplementary Table ST1, ST2, ST3). The thyroid follicles of Japanese medaka larvae as observed on 47 dph were not encapsulated as a gland (Fig. 1). Usually, the follicles were lined by single layer of epithelial cells known as thyroid epithelial cells or thyrocytes. The centre of thyroid follicles was filled with PAS-positive colloids with the appearance of clear vesicles (Fig. 2). Moreover, the follicles were not uniform in size (please see the supplementary data table (ST1)). We have determined the size of the follicles as area ( $\mu\text{m}^2$ ) from the digital photographs of the histological sections and analysed by imagej software. Although our data indicate a non-linear effect of GO on thyroid follicles, thyrocytes height, or GG cell distribution at the coronal end of the SB [1], the average size of thyroid follicles in phenotypic male (testis) on 47 dph was  $750.40 \pm 12.88 \mu\text{m}^2$  whereas for females, the average size was  $398.70 \pm 12.36 \mu\text{m}^2$ . Similarly, for larvae having XY genotype, the follicular size of the thyroid was  $804.60 \pm 16.32 \mu\text{m}^2$  and those with XX genotype have  $492.40 \pm 16.32 \mu\text{m}^2$ . Moreover, in sex-reversed larvae, the thyroid follicles were  $636.60 \pm 20.19 \mu\text{m}^2$  in size (Table 1). Statistical analysis indicates a significant difference ( $p < 0.05$ ) between the thyroid follicular size of male (testis) and female (ovary) phenotypes as well as between XY and XX genotypes. The sex-reversed larvae (XX genotype with testis) also showed a statistical difference both with XX and XY genotypes. The heights of thyrocytes of medaka larvae on 47 dph were also determined (please see supplementary Table ST2). The average heights were  $2.650 \pm 0.016 \mu\text{m}$  for phenotypic males (testis) and  $2.530 \pm 0.026 \mu\text{m}$  for phenotypic females (Ovary). Moreover, for XY genotypes, the thyrocytes height was  $2.610 \pm 0.016 \mu\text{m}$ , and for XX, the height was  $2.640 \pm 0.019 \mu\text{m}$ . In sex-reversed larvae (testis with XX genotypes) the thyrocytes height was  $2.760 \pm 0.029 \mu\text{m}$  (Table 2). Statistical analysis established a significant difference in thyrocytes height between the larvae with phenotypic males (testis) and females (ovary) and between XY genotypes with sex-reversed (phenotypic testis with XX genotype) larvae (Table 2). The cranial end of the SB consist 3-4 layers of large uniform epithelial GG cells that have eosinophilic cytoplasm were also considered for evaluation (Please see supplementary Table ST3). However, among 40 larvae, we have tracked

**Table 1**

The area of thyroid follicles distributed in the gill and adjacent regions of Japanese medaka larvae on 47 dph.

| Phenotype                      |                                 | Genotype                       |                                  | sex reversed                      |
|--------------------------------|---------------------------------|--------------------------------|----------------------------------|-----------------------------------|
| Testis                         | ovary                           | XY                             | XX                               | XX genotype with testis           |
| $750.40 \pm 12.88$<br>(n=1883) | $398.70 \pm 12.36^*$<br>(n=498) | $804.60 \pm 16.32$<br>(n=1364) | $492.40 \pm 16.32^\#$<br>(n=969) | $636.60 \pm 20.19^{\#, a}$ n=486) |

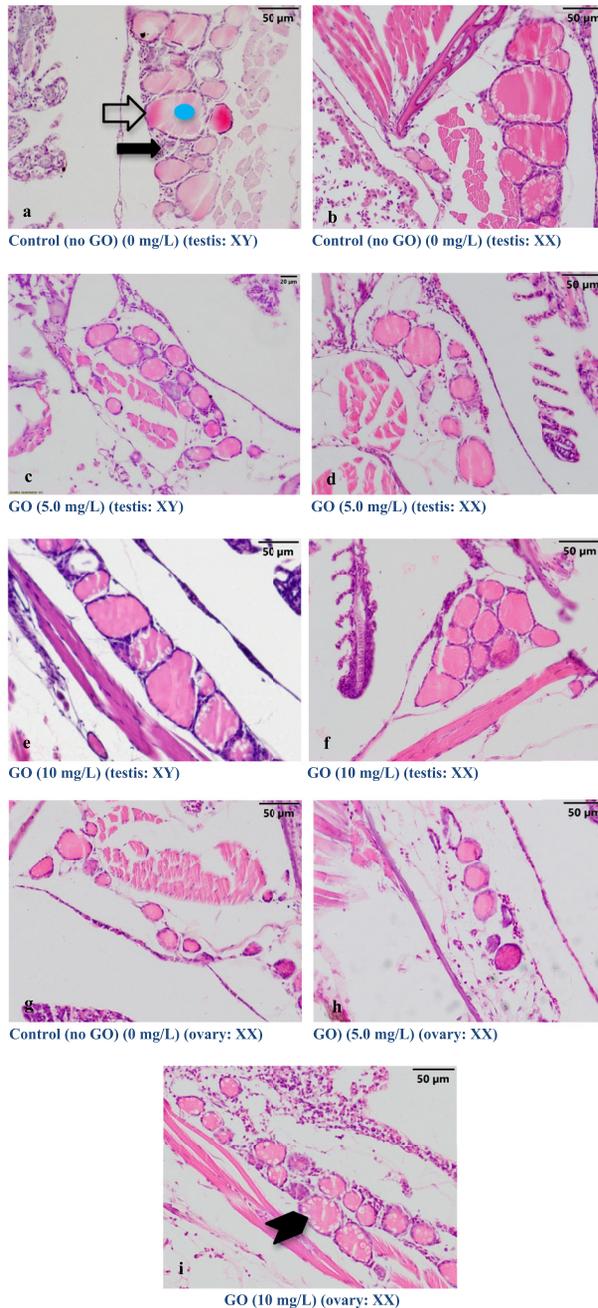
The data were expressed as means  $\pm$ SEM. n= number of thyroid follicles considered for follicular area ( $\mu\text{m}^2$ ) determination. Asterisk (\*) indicates that the data are significantly different ( $p < 0.05$ ) from phenotypic testis; Pound (#) indicates that the data are significantly different ( $p < .05$ ) from the XY genotypes and lowercase letter "a" indicates that the data are significantly different ( $p < 0.05$ ) from XX genotypes. Please see supplementary figure SF1 in Asala et al, Chemosphere 286, 2022, 131719; <https://doi.org/10.1016/j.chemosphere.2021.131719>.

**Table 2**

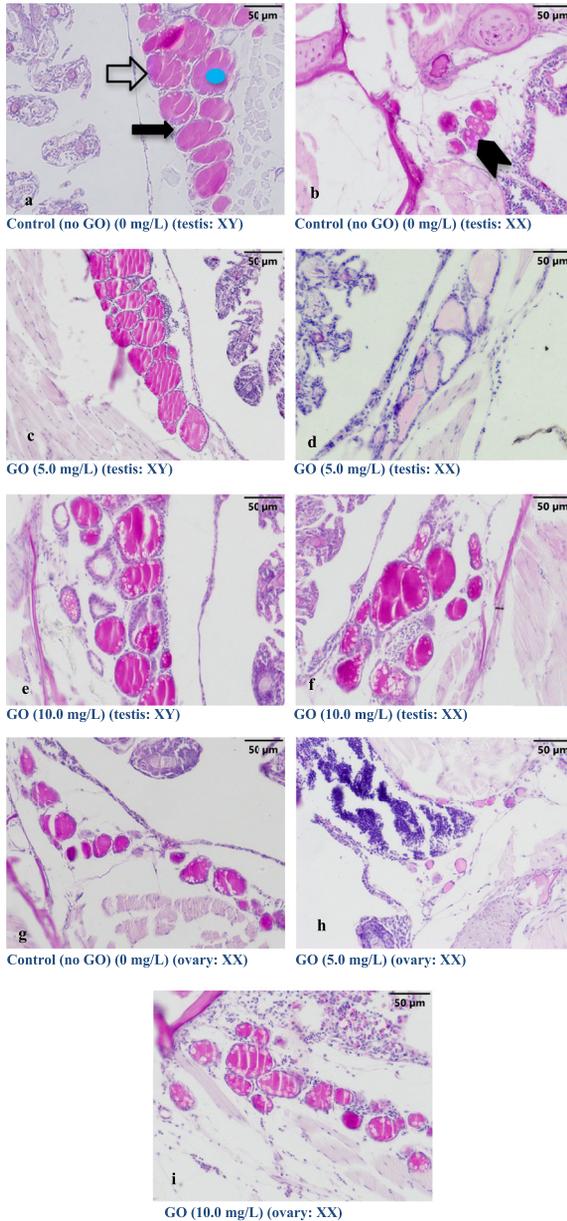
The Height of thyrocytes of Japanese medaka larvae on 47 dph.

| Phenotype                    |                               | Genotype                     |                              | sex reversed                   |
|------------------------------|-------------------------------|------------------------------|------------------------------|--------------------------------|
| Testis                       | Ovary                         | XY                           | XX                           | XX genotype with testis        |
| $2.65 \pm 0.016$<br>(n=2850) | $2.53 \pm 0.026^*$<br>(n=795) | $2.61 \pm 0.016$<br>(n=2067) | $2.64 \pm 0.019$<br>(n=1580) | $2.76 \pm 0.029^\#$<br>(n=785) |

The data were expressed as means  $\pm$ SEM. n= number of thyrocytes considered for follicular area ( $\mu\text{m}^2$ ) determination. Asterisk (\*) indicates that the data are significantly different ( $p < 0.05$ ) from phenotypic testis; Pound (#) indicates that the data are significantly different from the XY genotypes. Please see supplementary figure SF2 in Asala et al, Chemosphere 286, 2022, 131719; <https://www.doi.org/10.1016/j.chemosphere.2021.131719>.



**Fig. 1.** Representative photomicrographs of the thyroid follicles of medaka larvae (47 dph) located within the pharyngeal region. Follicles (HE stain) are lined by flattened to cuboidal epithelial cells and are filled with colloid. Fig. 1a–i are representative of HE-stained photomicrographs from the medaka larvae with different phenotypes (testis and ovary) and genotypes (XY and XX) exposed either to medium only (control; no GO; Fig. 1a,b, and g) or to different GO concentrations (5 and 10 mg/L). The representative figures of other two concentrations of GO (2.5 and 20 mg/L) used in this study were presented previously<sup>1</sup>. The concentrations of GO used (mg/L), as well as the phenotypes (testis/ovary) and the genotypes (XY/XX) are indicated below the figures. Empty arrows indicate thyrocytes; filled arrows indicate interstitial connective tissue; arrow heads indicate vacuoles and the blue circles indicate colloids.



**Fig. 2.** Representative photomicrographs of the thyroid follicles of medaka larvae (47 dph) used for histochemical evaluation. Histochemical studies were conducted by PAS reactions. Follicles are filled by PAS-positive colloids and the vacuoles are lined near the periphery of the follicles (close to thyrocytes). Fig. 2a–i are representative PAS-stained pictures from the medaka larvae with different phenotypes (testis and ovary) and genotypes (XY and XX) exposed either to medium only (control; no GO; Fig. 2a, b, and g) or to different GO concentrations (5 and 10 mg/L). The representative figures of other two concentrations of GO (2.5 and 20 mg/L) used in this study were presented previously<sup>1</sup>. Fig. 2a shows the colloid (filled in blue circle), thyrocytes (empty arrow), and a vacuole (Fig. 2b) indicated by arrowhead. The concentration of GO used (mg/L), as well as the phenotypes (testis/ovary) and the genotypes (XY/XX) are indicated below the figures. Empty arrows indicate thyrocytes; filled arrows indicate interstitial connective tissue; arrow heads indicate vacuoles and the blue circles indicate colloids.

**Table 3**

The distribution of GG cells at the coronal end of SB in Japanese medaka larvae on 47 dph.

| Phenotype                 |                            | Genotype                 |                            | sex reversed              |
|---------------------------|----------------------------|--------------------------|----------------------------|---------------------------|
| Testis                    | ovary                      | XY                       | XX                         | XX genotype with testis   |
| 8171 ± 223.9<br>(n = 134) | 9415 ± 322.8*<br>(n = 498) | 7410 ± 199.4<br>(n = 77) | 9573 ± 287.1#<br>(n = 117) | 9645 ± 470.8#<br>(n = 60) |

The data (number/mm<sup>2</sup>) were expressed as mean ± SEM. n= number of GG cells. Asterisk (\*) indicates that the data are significantly different (p<0.05) from phenotypic testis; Pound (#) indicates that the data are significantly different (p < 0.05) from the XY genotypes. Please see supplementary figure SF3 in Asala et al, Chemosphere 286, 2022, 131719; <https://www.doi.org/10.1016/j.chemosphere.2021.131719>.

**Table 4**

The area of Thyroid follicles, height of thyrocytes, and the distribution of GG cells in Japanese medaka larvae females in two stages of maturity.

|                                      | Stage 0                  | Stage 1                     |
|--------------------------------------|--------------------------|-----------------------------|
| Thyroid follicles (μm <sup>2</sup> ) | 403.50 ± 23.07 (n = 182) | 405.10 ± 15.80 (n = 316)    |
| thyrocytes (μm)                      | 2.59 ± 0.04 (n = 344)    | 2.48 ± 0.03 (n = 45)        |
| GG cells (number/mm <sup>2</sup> )   | 7081.0 ± 425.90 (n=9)    | 9852.00 ± 340.4 0 (n = 48)* |

The data were expressed as mean ± SEM. n= numbers used for analysis. Asterisk (\*) indicates that the data are significantly different (p<0.05) from larvae with stage 0 ovary. Please see supplementary figures SF1, SF2, and SF3 in Asala et al, Chemosphere 286, 2022, 131719; <https://doi.org/10.1016/j.chemosphere.2021.131719>.

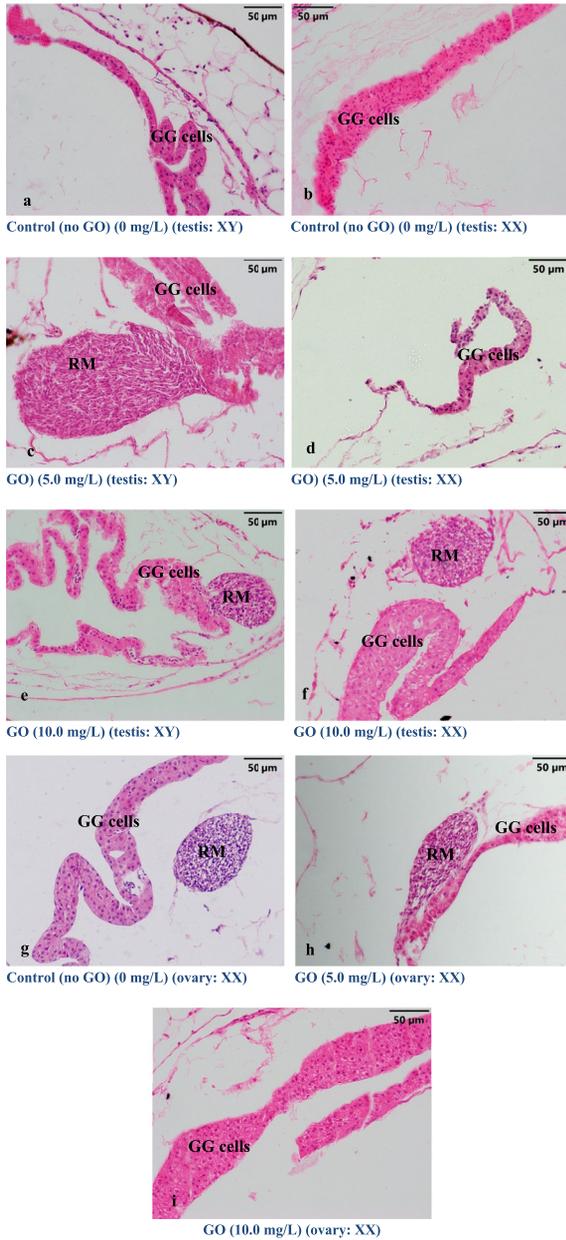
only 20 experimental fish for counting of GG cells. The GG was connected to RM by blood capillaries (Fig. 3). The number of GG cells/ mm<sup>2</sup> in male phenotypes (testis) were 8171.0 ± 223.9/ mm<sup>2</sup> and in phenotypic females the GG cell number was 9415.0±322.8/ mm<sup>2</sup>. In XY genotypes, the number of GG cells was 7410.0 ± 199.4 and in XX, the number was 9573±287.1/ mm<sup>2</sup>; in sex-reversed larvae (XX genotypes with testis) the number of GG cells was 9645.0 ± 470.8/mm<sup>2</sup> (Table 3). Statistical analysis indicates a significant difference in GG cell number of phenotypic males (testis) with phenotypic females and between XY and XX genotypes (Table 3). In sex-reversed larvae (phenotypic testis in XX genotype) the number of GG cells showed significant difference with XY genotypes and not with the XX genotypes (Table 3). We have also compared the areas of thyroid follicles (size), height of thyrocytes, and the distribution of GG cells at the coronal end of swim bladder between two stages (stage 0 and stage 1) of ovarian maturity [4]. The area of thyroid follicles in females with stage 0 ovary were 403.50 ± 23.07 and the larvae with stage 1 ovary were 405.1±15.8 μm<sup>2</sup> (Table 4). The heights of thyrocytes in larvae (female) with stage 0 ovary were 2.59 ± 0.04 and for stage 1 ovary were 2.48±0.03 μm. The GG cell distribution in female phenotypes with stage 0 ovary was 7081.0 ± 425.9 and for stage 1 ovary was 9852.0±360.4/ mm<sup>2</sup>. Statistical analysis showed a significant difference p < 0.05) between stage 0 and stage 1 ovaries of female phenotypes only in GG cell distribution at the coronal end of SB (Table 4).

## 2. Experimental Design, Materials and Methods

The experiments conducted in this study were approved by the Institutional Animal Care and Use Committee (IACUC Protocol number IBC 08-01-17 and IBC 09-01-17) of the Jackson State University, Jackson, MS.

### 2.1. Animal maintenance and exposure to GO

The animal maintenance and details of the medaka colony maintained at the Medaka fish culture facility at the Jackson State University were described previously [1–3,5,6]. Briefly, the



**Fig. 3.** Representative photomicrographs of the GG cells found at the coronal end of SB of medaka larvae (47 dph). Fig. 3a–i are representative HE-stained photomicrographs of the GG cells of the medaka larvae with different phenotypes (testis and ovary) and genotypes (XY and XX) exposed either to medium only (control; no GO; Fig. 3a, b, and g) or to different concentrations (2.5–20 mg/L) of GO. Photomicrographs of SB from two concentrations 5 mg/L and 10 mg/L are presented. The data indicate the columnar shaped GG cells arranged either single or multiple layers with bright basophilic nuclei and eosinophilic cytoplasm. The concentration of GO used (mg/L), as well as the phenotypes (testis/ovary) and the genotypes (XY/XX) are indicated below the figures. GG= gas gland cells found in the coronal end of the SB. RM= rete mirabile (supply blood to GG cells/SB). The empty holes observed in the GG cellular area are the sites where the blood vessels from RM can enter into the SB.

breeding colony of Japanese medaka fish (orange red varieties, Hd-rR strain) was maintained in the Aquatic Toxicology Laboratory at the Plant Science building at Jackson State University, Jackson, MS, USA. The colony was originally obtained from the Medaka culture facility of the University of Mississippi (UM), University, MS, USA by a protocol transfer agreement. We have used 35 L tanks containing 25 L balanced salt solution (BSS; 17 mM NaCl, 0.4 mM KCl, 0.3 mM MgSO<sub>4</sub>, 0.3 mM CaCl<sub>2</sub>, pH 7.4) at 25 ± 1 °C with 16h L: 8h D light cycle for the maintenance of the breeders and others (embryos and larvae) under development. The medium (BBS) was filtered through disposable bio and carbon filters, recirculated through pumps, and replaced every two- three weeks if the pH (7-8.5) and ammonia concentration (1-3 ppm) exceeded the limits. The fish were able to breed successfully in this culture condition and the egg collection was made within 1-3 h after the light was turned on. The fertilized eggs (embryos) were gently separated from the clutch and reared in embryo-rearing medium (ERM; 17 mM NaCl, 0.4 mM KCl, 0.6 mM MgSO<sub>4</sub>, 0.36 mM CaCl<sub>2</sub>, pH 7.4) in the laboratory (25 ± 1 °C with 16h L: 8h D light cycle). The embryos generally hatched within 10-14 days after post fertilization (dph). The hatched embryos after 14 dpf were excluded from GO exposure experiment. The development and staging of the experimental embryos and larvae were previously described [7].

Medaka fries (one day post-hatch Stage 40, 1<sup>st</sup> fry stage) were exposed by immersion to different concentrations (2.5, 5, 10, 20 mg/L in ERM) of GO (Sigma-Aldrich, St. Louis, MO) continuously for 96 h in the laboratory (25 ± 1 °C; 16h L: 8h D light cycle. GO obtained from commercial source (2 mg/mL or 2,000 mg/L, dispersed in water) was diluted to desired a concentration (20 mg/L which is equivalent to 20 µg/mL; 100-fold) by ERM and sonicated for 5 min (2s on-1s off pulse, 225 w) using a probe sonicator (ultrasonicator LPX 750, Cole Palmer, Chicago, IL, USA) in ice temperature and further diluted to 10.0, 5.0, and 2.5 mg/L by ERM. After sonication and required dilution 1 ml of the GO in ERM was added directly to the fries maintained in a 13 × 75 mm glass tubes on a water bath at 25±1 °C under 16h light and 8 h dark light cycle. The highest concentration of GO used in this study (20 mg/L) was selected from a published article by Mullick Choudhury et al. [8] on graphene nanoribbons (GNRs) exposure experiment on medaka embryos. Previously, we have checked the particle size of sonicated nanomaterial (GO in ERM) by TEM [2]. Briefly, after sonication and transfer to a copper grid, GO solution was dried at room temperature for at least for 24 h and then analyzed by TEM using the JEOL-1011 Transmission Electron Microscopy system available in the Electron Microscopy Core Laboratory at the Jackson State University. This instrument is equipped with 0.2 nm lattice resolution with magnification of 50 to 1,000,000 under the accelerating voltage of 40 to 100 kV, a camera system, and a computer with LC monitor for image analysis. The fries during GO treatment (2.5-20 mg/L in ERM) and the controls (no GO, only ERM) remained fasting (no exogenous food). The media of the larvae (both GO exposed and control) was refreshed every day. On 5 dph, survived fries were transferred to BSS and maintained in 500 mL glass jars (4 fries/ 200 mL BSS) and maintained in a GO-free environment in the laboratory. The mortality of the cultured fries/larvae was checked every day and the media changed once a week or even earlier, if necessary. The larvae (stages 42-43) were sacrificed after 42 days (47 dph) post-treatment when the external secondary sexual characters of medaka larvae began to develop. Before sacrifice, the larvae were anesthetized in MS 222 (Sigma-Aldrich; St. Louis, MO; 100 mg/L) and the length (cm) and weight (mg) were recorded [2]. The head and trunk regions of the body were preserved in 4% paraformaldehyde (PFA) in 20 mM PBS for histological and histochemical evaluation of thyroid follicles; trunk part used for the study of GG cells located on the coronal end of swim bladder and also for the histological observation of Phenotypic sex [2]. The post anal tails were preserved in 500 µL TRI reagent (Sigma-Aldrich, St. Louis, MO) and kept at -20 °C until used for genotyping the Y chromosome-specific male sex determining gene, *dmy*.

## 2.2. Histopathology and histochemistry

The head [ $n = 8$  per test concentration (0-control, 2.5, 5, 10, and 20 mg/L) for a total 40 medaka larvae], and trunk ( $n = 8$  each for control, 2.5, 5, and 20 mg/L, and  $n = 7$  for 10 mg/L,

for a total 39 medaka larvae) regions were fixed in 4% PFA in 20 mM PBS for 48 h (changed once after 24 h), and were washed thoroughly in water. The washed tissues were dehydrated in graded alcohol (30–100%), cleared in xylene, and embedded in paraffin (58–60 °C). Serial paraffin sections at 5 µm thickness were cut in a manual rotary microtome (Olympus cut 4055). Microscopic evaluation of thyroid follicles was made on the sections of the head regions of all 40 experimental fish after hematoxylin and eosin (HE) staining (Fig. 1a–i). Some of the thyroid sections from all the experimental fish groups were used for Periodic acid-Schiff (PAS) staining (Fig. 2a–i). Histopathology of GG and thus SB inflation was studied in the trunk tissue (Fig. 3a–i). Unfortunately, we lost one of the trunk tissue of one larvae exposed to 10 mg/L GO during sectioning. Therefore, the phenotypic analysis of thyroid follicles or thyrocytes was made only on 39 larvae (all groups have 8 larvae including control, however, in 10 mg/L GO, the number of larvae was reduced to 7). However, during genotypic analysis all 40 larvae were considered for thyroid follicles or thyrocytes analysis. For GG, among 40 experimental fish, we were successfully able to track GG cells distributed at the anterior end of the SB in only 20 experimental fish [Please see the supplementary Table ST3]. The digital photomicrographs of the sections were taken using an Olympus B-max 40 microscope attached to a camera with Q-capture Pro 7 software (Media Cybernetics, Inc, Rockville, MD). Some of the photomicrographs were taken using Olympus CKX53 inverted microscope with DP22 camera and CellSens software (Huntoptics & Imaging, Pittsburg, PA, USA). Histopathological evaluation of the thyroid follicles was done following a protocol previously described by Fournie et al. [9]. Imagej software (<http://www.imagej.nih.gov/ij>) was used for the analysis of digital images of thyroid follicles near gills/ heart and the GG cells restricted at the coronal end of SB. Due to uneven distribution and size variations, the thyroid follicles adjacent to branchial region and near to heart were considered and the area (µm<sup>2</sup>) of the individual follicles (minimum 3) available in the entire section were determined. The fields selected for follicular measurement in both controls and GO-exposed larvae were random, though restricted near the gills and heart. The follicles are lined by single layered squamous/or cuboidal epithelium (thyrocytes) facing lumen of the follicle. The heights of the thyrocytes (µm) were directly measured from the base of the thyrocytes (attached to the periphery of the follicle) towards the center (generally three to ten thyrocytes/ follicle were measured). The columnar shaped GG cells consist large basophilic nuclei and eosinophilic cytoplasm. During manual counting of GG cells the digital images of in both control and GO-exposed larvae were randomly captured from several regions of the SB. We carefully excluded blood cells and other cells which have indistinct nucleus and pale cytoplasm during manual counting. The GG cell data were expressed as the number of GG cells/mm<sup>2</sup> of the SB, though the GG cells are mostly restricted at the coronal end of SB.

### 2.3. Statistical analysis

Data analysis was done using GraphPad prism version 7.04 software (GraphPad Prism, San Diego, CA). Descriptive analysis was done to evaluate thyroid follicles (area), thyrocytes (height), and GG cells (number/mm<sup>2</sup>) in each concentration group. D'Agostino-Pearson (DP) or Shapiro-Wilks (SK) test was used to determine the normality of the data. The statistical significance was considered at  $p < 0.05$ . The data when normally distributed and met the precepts of using a parametric test, we used one-way ANOVA followed by post-hoc Tukey's test or unpaired parametric "t" test including Welch's correction. If the normality test indicated that the data did not meet the criteria of using a parametric test, we performed the Kruskal-Wallis test followed by Mann-Whitney's test (non-parametric test) as a post hoc test and the level of significance adopted as  $p < 0.05$ . The data were expressed means  $\pm$  SEM.

### Ethics Statement

No human subjects were involved in this research.

The Institutional Animal Care and Use Committee (IACUC) of the Jackson State University, Jackson, MS, USA, approved all experimental protocols (Protocol number IBC 08-01-17 and IBC 09-01-17).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

## CRedit Author Statement

**Tolulope E. Asala:** Formal analysis, Visualization, Investigation, Software, Writing – review & editing; **Asok K. Dasmahapatra:** Conceptualization, Formal analysis, Visualization, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing; **Anitha Myla:** Formal analysis, Visualization, Investigation, Software, Writing – original draft, Writing – review & editing; **Paul B. Tchounwou:** Conceptualization, Visualization, Investigation, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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## Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.dib.2021.107625](https://doi.org/10.1016/j.dib.2021.107625).

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