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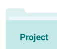

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RESEARCH ARTICLE

Ovarian growth impairment after chronic exposure to Roundup Ultramax® in the estuarine crab *Neohelice granulata*

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Abstract Adult females of the estuarine crab *Neohelice granulata* were exposed to the glyphosate formulation Roundup Ultramax® during the entire 3-month pre-reproductive period. At the end of the assay, a significant higher increment of glycemia was noted at both glyphosate concentrations assayed (0.01 and 0.2 mg/L, acid equivalent). Although no differences were observed in the gonadosomatic index, a significantly higher proportion of reabsorbed vitellogenic oocyte was observed at the highest glyphosate concentration, together with a significant decrease of vitellogenin content in the ovary. In addition, some in vitro assays were carried out by co-incubating small pieces of ovary with or without the addition of Roundup; at both concentrations tested (same as those used in vivo), a decrease in the ovarian vitellogenin content was observed, whereas the ovarian protein synthesis was significantly inhibited by glyphosate at 0.2 mg/L in the Roundup formulation used.

Keywords Roundup · Glyphosate · Crabs · Ovary · Reproduction · Vitellogenin

Introduction

Glyphosate (*N*-[phosphonomethyl] glycine) is a non-selective, systemic herbicide that inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, involved in the aromatic amino acid synthesis in plants (Lydon and Duke, 1989). In the last two decades, Argentina has exponentially increased the use of transgenic soy resistant to glyphosate (Arancibia 2013) and is currently the third world producer of soybean. In this country, environmental levels of glyphosate have been reported ranging from 0.1 to 0.7 mg/L in water, and between 0.5 and 5 mg/kg in sediments (Aparicio et al. 2013; Peruzzo et al. 2008). In other countries, levels of glyphosate as high as 3 and 7.6 mg/L have been reported (Mann and Bidwell 1999; Solomon and Thompson 2003; Giesy et al. 2000).

Neohelice (=Chasmagnathus) granulata (Decapoda, Grapsidae) is a crab species of wide distribution through most estuarine environments of Argentina and South Brazil. Particularly, the crab community settled along the 150 km of Samborombón Bay coast (Buenos Aires Province) is very conspicuous and ecologically relevant for several fish species that reproduce there (Sánchez et al. 1991). Besides, this estuarine environment receives the discharge of several rivers and channels, most of them carrying significant amounts of pesticides applied at the surrounding crops. Nevertheless, the southern edge of Samborombón Bay (Punta Rasa), which is located at the mouth of the Rio de la Plata estuary and therefore opens to the sea, has been considered a clean area in regard to pesticides and other pollutant (Comisión Administrativa del Río de la Plata 1990).

The reproductive cycle of *N. granulata* comprises three periods (López and Rodríguez 1999): pre-reproductive (winter), during which the ovary grows and mature; reproductive (spring and summer), when both spawning and hatching occur, and eventually the ovarian re-maturation takes place producing further spawns; and finally the post-reproductive period, which leads to ovarian quiescent and adult molting. During the pre-reproductive period, the ovary enters into the secondary vitellogenesis, in addition to the production of

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vitellogenin by the oocytes themselves (characteristic of the primary vitellogenesis); an active uptake of the vitellogenin synthesized in the hepatopancreas is carried out by maturing oocytes (Charmantier et al. 1997; Nagaraju, 2011). After spawning, hatching, and larval development, the megalopa returns to the coast, molting successively to several juvenile instars, and finally reaching the adult condition (López and Rodríguez 1999).

The present study was aimed at evaluating the ovarian growth, as well as some metabolic parameters related to the ovarian maturation, in *N. granulata* adult females exposed to glyphosate during the entire pre-reproductive period. For this purpose, long-term in vivo assays were carried out through a widely used commercial formulation of glyphosate. In addition, some in vitro assays were carried out in order to confirm the effects observed in vivo.

Materials and methods

Adult females of *N .granulata* were randomly collected in June 2014 (body weight = 10.69 ± 0.09, $N = 60$), at the southern edge of Samborombón Bay. All bioassays were conducted in semi-static conditions, according to the standard procedures recommended by the American Public Health Association et al. (2005). Stock solutions of Roundup Ultramax® (as soluble granules, 67.9% *w*/*w* of glyphosate a.e.; Monsanto) were prepared weekly by dissolving the appropriate amount of the chemicals in distilled water.

In vivo experiment

Small aliquots from the stock solutions were added to the test recipients, filled with the appropriate dilution water, as stated above. Two nominal, sub-lethal concentrations of 0.01 and 0.2 mg/L of glyphosate (as acid equivalent, a.e., in the Roundup Ultramax® formulation) were assayed, based on preliminary results (Frontera et al. 2011; Avigliano et al. 2014a, b). Fifteen females were assigned to each treatment, i.e., control (with no toxic added) or treated with Roundup. For all experiments, each female was isolated in a glass container filled with 400 mL of saline water prepared by diluting artificial seawater salts (Tetra Marine Salt Pro, USA) in dechlorinated tap water (hardness, 80 mg/Las equivalents of $CaCO_3$; final salinity, 12 g/L, pH = 8.0 ± 0.5) provided with constant aeration. The solution of each container was completely replaced twice a week. A temperature of 23 ± 1 °C and a photoperiod of 14:10 (L/D) were maintained throughout. All animals were fed twice a week with pellets prepared in the laboratory (Chaulet et al. 2012) in an amount equivalent to 5% of body mass, supplemented with *Elodea* sp. fresh leaves ad libitum. Females remained exposed to the glyphosate formulation or control for 90 days, starting by early July.

In order to validate nominal glyphosate concentrations, water samples (15 mL) were taken at 0 and 72 h, i.e., the period for water replacement in all test containers. After derivatization at pH = 9 with 9-fluorenylmethylchloroformate (FMOC-CL), glyphosate concentrations were measured by high-pressure liquid chromatography, coupled to an Agilent mass spectrophotometry detector, model VL. An X-SELECT C_{18} chromatographic column was used. A mixture of $MeOH/NH_4$ (5 mM) 9 mM was chosen as mobile phase, with a flow rate of 0.5 mL/min.

At the end of the 3-month assay, females were weighed to determine body weight, and for every surviving animal, a small sample of hemolymph from the base of their appendages was extracted using a 29G needle and a tuberculin syringe. Animals were then sacrificed by means of ice water anesthetizing in order to quickly dissect muscle and ovary tissue for further analysis. Glycogen, total lipids, and protein content were determined in chelae muscle. Glycogen was extracted by the method of Van Handel (1965) and quantified as glucose as indicated below for hemolymph, after performing acid hydrolysis with HCl and subsequent neutralization with Na_2CO_3. Total lipids were extracted using the method of Folch et al. (1957) and quantified by the method of Fring and Dunn (1970) by measuring absorbance of the sulfo-phospho-vanillin complex at a wavelength of 530 nm using olive oil as standard. Protein extraction was performed by the addition of 30% KOH to each sample, followed by 2-h incubation at 100 °C. Protein concentration was assessed according to Lowry et al. (1951) using bovine albumin as a standard and measuring absorbance at 650 nm. Hemolymphatic glucose level was quantified by means of the glucose oxidase method (kit from Wiener Laboratories, Buenos Aires, Argentina).

Immediately after being dissected, ovaries were weighed in order to determine the gonadosomatic (GSI) index as GSI = (gonad wet weight/body weight) × 100. Samples of ovaries were then fixed in Bouin solution for 4 h at room temperature, dehydrated in alcohol series, and finally embedded in paraplast. Then, 5-μm sections were prepared and stained with hematoxylin and eosin. For each animal, a representative section of the ovary was analyzed to determine the relative proportions of normal and abnormal oocytes. Previtellogenic, intermediate, and vitellogenic oocytes were characterized according to their size and degree of basophilia. Each type of oocyte, including those under reabsorption, was systematically counted in at least three ovarian sections. For oocyte area, both major and minor diameters of the oocytes showing their nuclei were estimated by means of a micrometric ocular lens, calibrated against a Leitz-Wetzlar plate with 1/100 mm spacing, to calculate the oocyte area as $(\pi/4) \times$ major diameter × minor diameter, as in Rodríguez

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et al. (1994). In order to restrain biological variability, only the non-molted and non-ovigerous females were considered for all the estimated variables.

An ELISA assay was employed to determine the vitellogenin (Vg) levels in both ovary and hemolymph. A primary antibody against native Vg was obtained by inoculating rabbits with purified Vg, according to previous studies (Dreon et al. 2003; García et al. 2008). Anti-IgG from rabbit was conjugated with BIOARS Lab. Peroxidase was used as the secondary antibody. Purified Vg in a 1/500 dilution was used to prepare the standard (0–210 ng). Fifty microliters of either the standard or sample was placed, in triplicate, in a 96-well plate (Nunc-Immunoplate Polisorp). Samples were previously diluted in coating buffer (15 mM sodium carbonate, 35 mM sodium bicarbonate, pH = 9.6). Both primary and secondary antibodies were diluted (1/500) in PBS + 0.05% Tween + 6% powder milk. Absorbance was measured in all wells at 415 nm by using an ELISA plate reader (BIO-Rad Lab., Model 680); 2-20-azino-di-3-ethylbenzthiazoline sulfonic acid was used as chromogen.

In vitro experiment

Stock female crabs (maintained under the same conditions used for controls of the in vivo assay) were used for the in vitro experiments. Ovarian explants (approximately 1 × 0.5 cm each) were incubated for 24 h in CO_2 chambers at 27 °C and in constant darkness. Each female provided a similar piece of ovary for one vial of every group of the same experiment (blocked design). Medium 199 was prepared using powdered medium with L-glutamine and Earle's salts (Sigma Chemical Co.), dissolved in crustacean saline (Cooke et al., 1977), and modified to compensate for the salts already present in the culture medium. In addition, as in previous studies (Sarojini et al. 1997; Rodríguez et al. 2000), 6 mg of penicillin-G per 100 mL of medium was added to prevent bacterial growth, and the pH was adjusted to 7.4 with 0.5 N NaOH.

Vitellogenin content of ovarian explants was measured in two independent experiments run with glyphosate added to the culture medium at a concentration of 0.01 or 0.2 mg/L, together with a paired control with no glyphosate added. At the end of the 24-h incubation period, the total content of vitellogenic proteins (vitellogenin and vitellins) was determined using the same methodology described for the in vivo assay.

In a third in vitro experiment, made at the same glyphosate concentrations and control above mentioned, tritiated leucine was added to the culture medium at the start of the incubation in order to estimate the synthesis of ovarian protein by its incorporation to the acid-precipitable protein fraction, following the methodology previously used for *N. granulata* and other crustacean species (Eastman-Reks and Fingerman 1985; Zapata et al. 2001; Rodríguez et al. 2002). Briefly, an aliquot of 30 μL from the 3H–leucine stock solution (1:10 dilution from 1 mCi/mL; NEN Life Science, Inc.) was added to each vial, to reach a total activity of 3 μCi. At the end of the incubation period, the ovarian explants were weighed and homogenized in 2 mL cold 10% trichloroacetic acid (TCA) followed by centrifugation at 5000×*g* for 10 min at 4 °C, then washed twice with cold TCA, resuspended, and decanted into a Millipore suction filtration funnel using 0.22-μm nitrocellulose filter disks. After filtration, the disks were air-dried for 1 h and submerged in scintillation fluor solution (Optiphase Hi Safe 2) overnight at 4 °C. Radioactivity was measured in a Beckman scintillation counter. Uptake of labeled leucine by the ovary was expressed on an ovarian wet-weight basis (CPM/mg ovary).

Proportion of survival, molted, or ovigerous females were compared between experimental groups by the Fisher exact test (Sokal and Rohlf 1981). For all the continuous variables analyzed, a one-way ANOVA followed by LSD multiple comparisons (Sokal and Rohlf 1981) was used for testing differences between experimental groups and control. When no homogeneity of variance was confirmed, the variance heterogeneity was incorporated to the statistical model by using the R studio 3.3.1 program (Zuur et al., 2009).

Results

Table 1 shows the measured glyphosate concentrations, which were close to the nominal ones, hereafter referred. A low mortality (up to 6.7%) was observed during the in vivo experiments, while the percentage of females that became ovigerous was as high as 20% (Table 2). In any case, no significant differences with control values were observed ($p > 0.05$).

Concerning energy reserves, no differences ($p > 0.05$) were observed in the muscle in either protein (overall mean = 38.99 ± 2.56 mg/g) or lipid content (overall mean = 1.17 ± 0.12 mg/g); although not statistically significant ($p > 0.05$), a clear decrease in muscle glycogen content

Table 1 Nominal versus measured concentrations of glyphosate (as acid equivalent) in the Roundup Ultramax® formulation

Glyphosate concentration (mg/L)	Measured concentration (mg/L)		
	0 h	72 h	Overall mean
0 (control)	0.0000 0.0000	0.0000 0.0000	0.0000
0.01	0.0136 0.0111	0.0090 0.0083	0.0105
0.2	0.1886 0.2075	0.1564 0.1523	0.1762

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Table 2 Survival, molting, and ovigerous percentages at the end of both experiments

Glyphosate concentration (mg/L)	Ni	% mortality	% molting	% ovigerous	Nf
0 (control)	15	0.00	0	20	12
0.01	15	6.67	0	13.33	12
0.2	15	13.33	6.67	20	9

No significant differences ($p < 0.05$) with respect to control were observed, in any case

Ni initial number, *Nf* final number, considering only the non-molted, non-ovigerous surviving females

was observed at the highest concentration of glyphosate, compared to control, concomitantly with a significantly higher ($p < 0.05$) increase of glycemia (Fig. 1). Vitellogenin levels are shown in Fig. 2 for the in vivo assay. A significantly ($p < 0.05$) lower vitellogenin content was noted at the highest glyphosate concentration. No differences among treatments ($p > 0.05$) were detected in the level of circulating vitellogenin, in any case.

No significant ($p < 0.05$) differences among treatment were noted in the gonadosomatic index (overall mean = 2.34 ± 0.47). At 0.2 mg/L, a significantly ($p < 0.05$) higher proportion of reabsorbed vitellogenic oocyte was observed (Fig. 3). Although not statistically significant ($p > 0.05$), a lower area was observed in the vitellogenic normal oocytes from females exposed to 0.2 mg/L of glyphosate (Fig. 4). Examples of the histological sections analyzed are shown by Fig. 5; a reactional atresia could be observed in ovaries from glyphosate-exposed females, characterized by the proliferation of follicular cells inside the vitellogenic oocytes under reabsorption, in order to participate in the phagocytosis of vitellum.

Figure 6 shows the results obtained from the in vitro assay. The ovarian vitellogenin content was significantly ($p < 0.05$) lower than control at both glyphosate concentrations tested (Fig. 6a). On the other hand, the incorporation of leucine to ovarian proteins was significantly ($p < 0.05$) lower at 0.2 mg/L as compared to control (Fig. 6b).

Discussion

Both concentrations used in the current study were within the environmental range of glyphosate reported for several water bodies of Argentina (Peruzzo et al. 2008; Aparicio et al. 2013). In this country, glyphosate application has been steadily increasing over time (Arancibia 2013); currently, approximately 200 million liters over more than 20 million ha (mainly soybean crops) are treated with glyphosate (Aparicio et al. 2013). Since glyphosate has not shown to be acutely toxic for aquatic fauna, the study of its chronic accumulative effects bears a special meaning. This has been precisely the focus of this paper, therefore spreading along the entire period of ovarian growth in the estuarine crab *N. granulata.*

As an overall indicator of metabolic condition, glycerin and glycogen content in several tissues were measured. The increased in glycemia levels, observed at all assayed concentrations of glyphosate, is indicative of unspecific stress, as reported for several other crustacean species (Sokolova et al. 2012). Nevertheless, since the decrease observed in the muscle glycogen content was not statistically significant, the

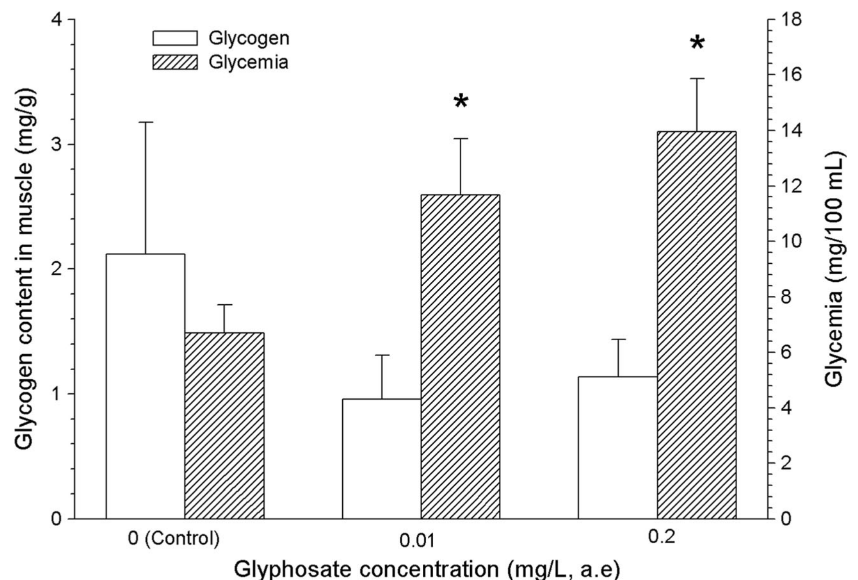

Fig. 1 Mean value (± SE) of muscle glycogen and glycemia, measured at the end of the in vivo experiment. Asterisk indicates significant differences ($p < 0.05$) with respect to control. Number of females (Nf) is indicated in Table 2

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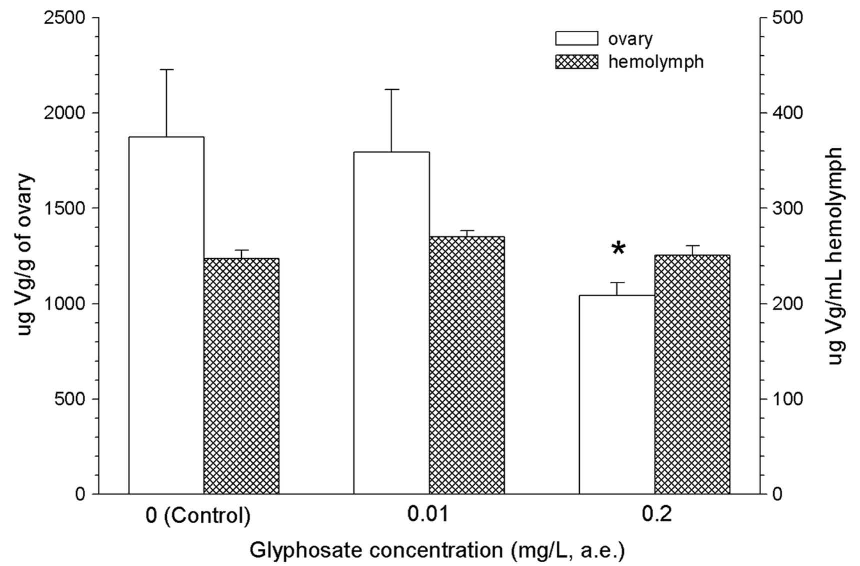

Fig. 2 Mean vitellogenin content (± SE) in the ovary (**a**) and hemolymph (**b**). Number of females (Nf) is indicated in Table 2. Asterisk indicates significant differences ($p < 0.05$) with respect to the respective control

animals seemed to have suffered a moderate stress, which would imply a discrete utilization of energy reserves to cover the extra maintenance costs related to damage repair, slightly reducing the energy available for growth and reproduction (Sokolova et al. 2012). However, since there was no significant decrease in either muscle protein or lipid reserves, no solid evidence was found for a reduction in energy reserves allocated in somatic tissues, which could eventually reduce the availability of energy for reproduction.

Nevertheless, the current study showed a significant lower content of vitellogenin in the ovary, in relation with both a lower area of normal vitellogenic oocytes and a significantly ($p < 0.05$) higher percentage of reabsorbed vitellogenic oocytes. Moreover, these effects were observed at 0.2 mg/L of glyphosate, a concentration within the environmental range reported for argentine impacted areas (Peruzzo et al. 2008). Reabsorption in the ovary could be characterized as a reactional atresia in a significant fraction of vitellogenic oocytes by effect of the Roundup formulation used; this kind of atresia, contrary to the autolytic, involves the active participation of follicular cells and has been previously described in females of the studied species exposed to the herbicide 2,4-D (Rodríguez et al. 1994). Reabsorption of mature oocytes has been previously observed in the studied species by effect of several pollutants, including pesticides (Rodríguez et al. 1994) and heavy metals (Kogan et al. 2000). Atretic oocytes have also been reported in several frog species chronically exposed to Roundup® (Howe et al. 2004). A reduction in sperm quality was also observed in fish exposed to the same formulation (Benck Soso et al. 2007).

The so-called stress hormones are mediating the use of several energy stores during the resistance stage to environmental

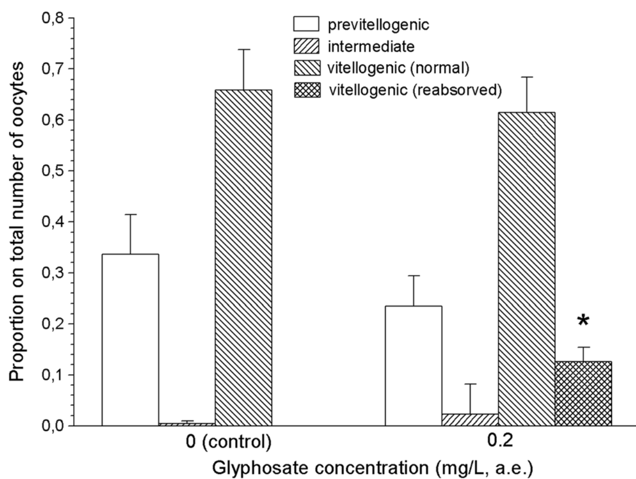

Fig. 3 Proportion (mean ± SE) of each oocyte type in the ovary, at 0.2 mg/L of glyphosate. Asterisk indicates significant differences ($p < 0.05$) with respect to control. Number of females (Nf) is indicated in Table 2

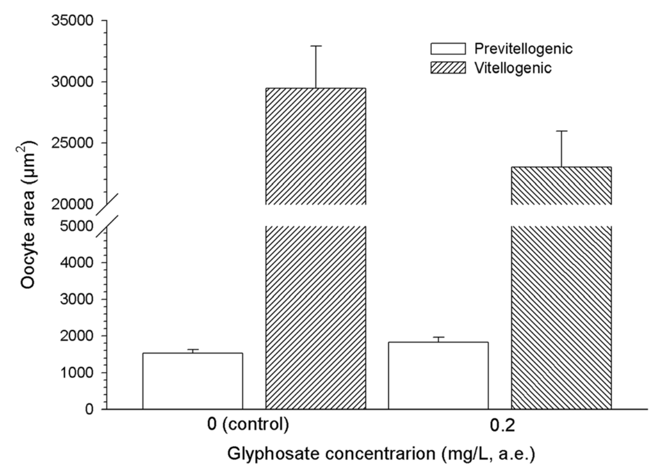

Fig. 4 Mean oocyte area (± SE) in the ovary for both normal previtellogenic and vitellogenic oocytes, at 0.2 mg/L of glyphosate. Asterisk indicates significant differences ($p < 0.05$) with respect to control. Number of females (Nf) is indicated in Table 2

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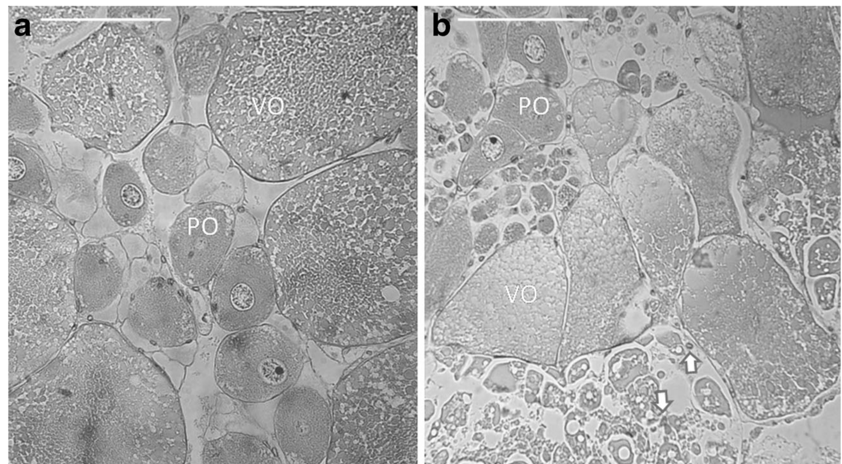

Fig. 5 Histological sections of the ovary, from the in vivo experiment. **a** control, **b** exposed to 0.2 mg/L of glyphosate. Arrows indicate follicular cells participating in the reabsorption of vitellogenic oocytes (VO). PO, previtellogenic oocytes. Scale bars = 50 μm

stress (Meyer et al. 1992). The crustacean hyperglycemia hormone (CHH) has been proposed as the stress hormone of crustaceans (Chang et al. 1999; Elwood et al. 2009); its multifunctional role, including some effects on the ovary of some crustaceans, has been reported (De Kleijn and Van Herp, 1998; Fanjul-Moles 2006). In the current study, the significant chronic increase of glycemia is expected to be caused by augmented and sustained levels of circulating CHH. Although several isoforms of CHH have been reported to stimulate the ovarian growth at specific stages of ovarian maturation (De Kleijn and Van Herp, 1998; Gu et al. 2000), other isoforms have shown inhibitory effects on the ovary (Sook Chung et al. 2010). Therefore, it should not be discarded the possibility that a chronic secretion of CHH from the eyestalks (or eventually from another sites) had impacted on the ovary of the studied species, therefore leading to oocyte reabsorption.

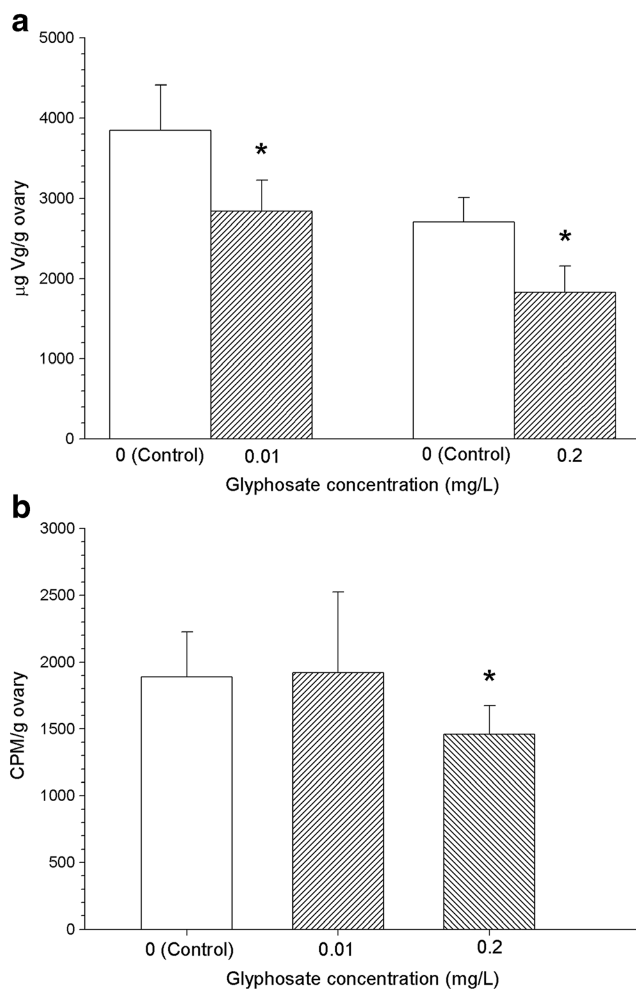

Fig. 6 Results from the in vitro experiments. **a** Mean vitellogenin content in ovarian explants incubated for 24 h with glyphosate at 0.01 and 0.2 mg/L (independent experiments). Number of replicates = 6. **b** Incorporation of tritiated leucine to ovarian proteins, at the same glyphosate concentrations. Number of replicates = 12. In all cases, asterisks indicate significant differences ($p < 0.05$) with respect to the respective control

Concomitantly with the results observed in vivo, in vitro experimental results showed a clear effect of glyphosate on ovarian growth, in terms of the total vitellogenin content as well as in protein synthesis, mainly related to vitellogenin synthesis in growing oocytes. Although the assayed glyphosate concentrations in vials were likely much higher than the effective glyphosate concentrations in hemolymph (i.e., the internal medium surrounding the ovary during the in vivo assay), it should be considered that the in vitro exposure time (24 h) was substantially shorter than the 3-month in vivo exposure. On the other hand, during the short duration of the in vitro assay, de novo synthesis of vitellogenin certainly takes place. In fact, an early expression of vitellogenin ARNm (i.e., after 3 h of incubation) has been reported in the crustacean hepatopancreas (Reddy Buchi et al. 2016). Moreover, as reported previously, either an increment or decrement of vitellin synthesis was reported in crustacean ovarian explants after 24 h of in vitro incubation under several experimental conditions, including exposure to pollutants (Eastman-Reks and Fingerman 1985; Rodríguez et al. 2000, 2002; Zapata et al. 2001; Cahansky et al. 2011).

One possibility to explain the in vitro results is a direct inhibitory effect of glyphosate on the synthesis pathway of vitellins, inside the oocytes. However, no precedent of this

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direct effect has been previously reported for glyphosate. An alternative hypothesis might be that glyphosate acts as an endocrine disruptor on the ovary. In mammals, some evidences have been reported about the possible role of glyphosate as en endocrine disruptor, interfering with the synthesis and/or signaling of steroids (Richard et al. 2005; Gasnier et al. 2009). A reduction in the 17β-estradiol levels has been also reported in fish after 40 days of exposure to Roundup® (Benck Soso et al. 2007). A previous study carried out on *N. granulata* during ovarian re-maturation (i.e., in ovigerous females) yielded some results indicating the possibility of glyphosate acting as endocrine disrupter on one or more hormones controlling ovarian growth (Avigliano et al. 2014a).

We conclude that Roundup, at concentrations within the environmental range, was able to reduce the vitellogenin content of the ovary during the pre-reproductive maturation period. Such reduction showed to be related to oocyte reabsorption, observed in vivo, as well as to the inhibition of protein synthesis in the ovary, observed in vitro. Additionally, the studied herbicide caused a marked increment in glycemia at the end of the in vivo exposure.

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References

American Public Health Association, American Water Works Association, Water Pollution Control Federation (2005) Standard methods for the examination of water and wastewaters, 21st edn. American Public Health Association, Washington DC

Aparicio VC, De Gerónimo E, Marino D, Primost J, Carriquiriborde P, Costa JL (2013) Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. Chemosphere 93:1866–1873

Arancibia F (2013) Challenging the bioeconomy: the dynamics of collective action in Argentina. Technol Soc 35:79–22

Avigliano L, Álvarez NB, Mac Loughlin C, Rodríguez EM (2014a) Effects of glyphosate on egg incubation, larvae hatching and ovarian re-maturation, in the estuarine crab *Neohelice granulata*. Environ Toxicol Chem 33:1879–1884

Avigliano L, Fassiano AV, Medesani DA, Ríos de Molina MC, Rodríguez EM (2014b) Effects of glyphosate on growth rate, metabolic rate and energy reserves of early juvenile crayfish, *Cherax quadricarinatus* M. B Environ ContamToxicol 92:631–635

BenckSoso A, Gil Barcello LJ, Ranzani-Paiva MJ, Kreutz LC, Mezzalira Quevedo R, Anziliero D, Lima M, Bolognesi da Silva L, Ritter F, Calliari Bedin A, Finco JA (2007) Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundiá (*Rhamdiaquelen*). Environ Toxicol Phar 23:308–313

Cahansky AV, Medesani DA, Chaulet A, Rodríguez EM (2011) *In vitro* effects of both dopaminergic and enkephalinergic antagonists on the ovarian growth of *Cherax quadricarinatus* (Decapoda, Parastacidae), at different periods of the reproductive cycle. Comp Biochem Physiol 158A:126–131

Chang ES, Chang SA, Keller R, Reddy PS, Snyder MJ, Spees JL (1999) Quantification of stress in lobsters: crustacean hyperglycemic hormone, stress protein and gene expression. Am Zool 39:487–495

Charmantier G, Charmantier-Daures M, Van Herp F (1997) Hormonal regulation of growth and reproduction in crustaceans. In: Fingerman M, Nagabhushanam R, Thompson MF (eds) Recent advances in marine biotechnology. Oxford & IBH Publishing Co, New Delhi, pp 109–161

Chaulet A, Medesani DA, Freitas J, Cervino A, Cervino N, Rodríguez EM (2012) Induction of somatic growth in juvenile crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae), by ecdysone and insulin growth factor. Aquaculture 370–371:1–6

Comisión Administradora del Río de la Plata (1990) Estudio para la evaluación de la contaminación en el Río de la Plata. Argentine Hydrographic Service Navy, Buenos Aires

Cooke IM, Haylett BA, Weatherby TM (1977) Electrically elicited neurosecretory and electrical responses of the isolated crab sinus gland in normal and reduced calcium salines. J Exp Biol 101:125–149

De Kleijn DPV, Van Herp F (1998) Involvement of the hyperglycaemic neurohormone family in the control of reproduction in decapod crustaceans. Invertebr Rep Dev 33:263–272

Dreon MS, Heras H, Pollero RJ (2003) Metabolism of ovorubin, the major egg lipoprotein from the apple snail. Mol Cell Biochem 243:9–14

Eastman-Reks SB, Fingerman M (1985) *In vitro* synthesis of vitellin by the ovary of the fiddler crab *Uca pugilator*. J Exp Zool 233:111–116

Elwood RW, Barr S, Patterson L (2009) Pain and stress in crustaceans? App Anim Behav Sci 118:128–136

Fanjul-Moles ML (2006) Biochemical and functional aspects of crustacean hyperglycemic hormone in decapod crustaceans: review and update. Comp Biochem Physiol 142C:390–400

Folch J, Lees M, Stanley GHS (1957) A simple method for isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509

Fring CS, Dunn RT (1970) A colorimetric method for determination of total serum lipids based on the sulfo-phospho-vanillin reaction. Am J Clin Pathol 53:89–91

Frontera JL, Vatnick I, Chaulet A, Rodríguez EM (2011) Effects of glyphosate and polyoxyethylenamine on growth and energetic reserves in the freshwater crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae). Arch Environ Con Toxicology 61:590–598

García F, Cunningham ML, Garda H, Heras H (2008) Embryo lipoproteins and yolk lipovitellin consumption during embryogenesis in *Macrobrachium borellii* (Crustacea: Palaemonidae). J Comp Physiol 151B:317–322

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE (2009) Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262:184–191

Giesy JP, Dobson S, Solomon KR (2000) Ecotoxicological risk assessment for roundup herbicide. Rev Environ Contam 167:35–120

Gu PL, KL Y, Chan SM (2000) Molecular characterization of an additional shrimp hyperglycemic hormone: cDNA cloning, gene organization, expression and biological assay of recombinant proteins. FEBS Lett 472:122–128

Howe CM, Berrill M, Pauli BD, Helbing CC, Werry K, Veldhoen N (2004) Toxicity of glyphosate-based pesticides to four North American frog species. Environ Toxicol Chem 23:1928–1938

Kogan M, López Greco LS, Romano LA, Rodríguez EM (2000) Effects of cadmium on somatic and gonadal growth of juvenile females of the estuarine crab *Chasmagnathus granulata* (Brachyura, Grapsidae). Zool Stud 39:344–350

López Greco LS, Rodríguez EM (1999) Annual reproduction and growth of adult crabs, *Chasmagnathus granulata* (Crustacea, Brachyura, Grapsidae). Cah Biol Mar 40:155–164

Lowry OH, Rosenbrough NJ, Randal RJ (1951) Protein measurements with the Folin phenol reagent. J Biol Chem 183:265–275

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Lydon J, Duke SO (1989) Pesticide effects on secondary metabolism of greater plants. Pestic Sci 25:361–373

Mann RM, Bidwell JR (1999) The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. Arch Environ Con Toxicology 36:193–199

Mayer FL, Versteeg DJ, McKee MJ, Folmar LC, Graney RL, McCume DC, Rattner BA (1992) Physiological and nonspecific biomarkers. In: Huggett RJ, Kimerle RA, Mehrle Jr PM, Bergman HL (eds) Biomarkers: biochemical, physiological and histological markers of anthropogenic stress. Society of Environmental Toxicology and Chemistry special publications series, Lewis publishers, Boca Raton, pp 5–85

Nagaraju GPC (2011) Reproductive regulators in decapod crustaceans: an overview. J Exp Biol 214:3–16

Peruzzo PJ, Porta AA, Ronco AE (2008) Levels of glyphosate in surface waters, sediments and soil associated with direct sowing soybean cultivation in north pampasic region of Argentina. Environ Pollut 156:61–66

Reddy Buchi S, Vaadala S, Hosamani N, Reddy Pamuru R, Reddy Pamanji S (2016) Regulation of vitellogenesis by selected endocrine modulators in crab *Oziothelphusa senex senex*, with special reference to methyl farnesoate. Aquacult Rep 3:24–30

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005) Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ Health Persp 113:716–720

Rodríguez EM, Schuldt M, Romano L (1994) Chronic histopathological effects of parathion and 2,4-D on female gonads of *Chasmagnathus granulata* (Decapoda, Brachyura). Food Chem Toxicol 32:811–818

Rodríguez EM, López Greco LS, Fingerman M (2000) Inhibition of ovarian growth by cadmium, in the fiddler crab *Uca pugilator* (Decapoda, Ocypodidae). Ecotox Environ Saf 46:202–206

Rodríguez EM, López Greco LS, Medesani DA, Laufer H, Fingerman M (2002) Effect of methyl farnesoate, alone and in combination with other hormones, on ovarian growth of the red swamp crayfish, *Procambarus clarkii*, during vitellogenesis. Gen Comp Endocrinol 125:34–40

Sánchez F, Mari N, Lasta C, Giangioble A (1991) Alimentación de la corvina rubia (*Micropogonias furnieri*) en la Bahía de Samborombón. Frente Marítimo 8:43–50

Sarojini R, Nagabhushanam R, Fingerman M (1997) An *in vitro* study of the inhibitory action of methionine enkephaline on ovarian maturation in the red swamp crayfish, *Procambarus clarkii*. Comp Biochem Physiol 117C:207–210

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, New York

Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA (2012) Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance n aquatic invertebrates. Mar Environ Res 79:1–15

Solomon KR, Thompson DG (2003) Ecological risks assessment for aquatic organisms from overwater uses of glyphosate. J Toxicol Environ Health 6B:289–324

Sook Chung J, Zmora N, Katayama H, Tsutsui N (2010) Crustacean hyperglycemic hormone (CHH) neuropeptides family: functions, titer, and binding to target tissues. Gen Comp Endocrinol 166:447–454

Van Handel E (1965) Estimation of glycogen in small amount soft tissue. Anal Biochem 1:256–265

Zapata V, López Greco LS, Rodríguez EM (2001) Effect of copper on hatching and development of larvae of the estuarine crab *Chasmagnathus granulata* (Decapoda, Brachyura). Environ Toxicol Chem 20:1579–1583

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York

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