

Supplementary information

Metallic nickel nanoparticles and their effect on the embryonic development of the sea urchin *Paracentrotus lividus*

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Supplementary methods

Solubility of nickel ions from metallic nickel nanoparticles

Measurements were done with the method for dissolved metals, by acidification of the sample with nitric acid and heating for 15-20 min, followed by filtration and dilution with deionized water before analysis. In-house standard solutions were used as reference (0.002, 0.01, 0.04, 0.08, 0.8 mg/L, mixed with other elements from Inorganic Ventures Stock Standards). Each sample was run once, with quality control samples every 20 samples. Quality control uses standard deviation (x2 and x3 the SD of the mean) to define acceptance criteria.

Size determination of nickel nanoparticles in artificial seawater and deionized water by DLS

Dynamic light scattering (DLS) of metallic Ni NPs in ASW and deionized water was performed with Zetasizer Nano ZS (Malvern, UK), see Fig. S3A and B, respectively. Solutions of 0.03 mg/L, 0.3 mg/L, and 3 mg/L metallic Ni NPs were first prepared in deionized water and ASW. Sonication probe (6 min) and sonication bath (5 min) were then used to help the prepared mixture achieve a homogeneous suspension. Each sample was tested by 12 DLS runs and this has been repeated 3 times for each sample. The average of these measurements is presented in Figures 3S (A) and (B).

The peak maxima for metallic Ni NPs in ASW (Fig. S3A) were found (from Lorentzian fittings of the peaks) at 52nm, 108nm and 96 nm for 0.03 mg/L (black), 0.3 mg/L (red) and 3 mg/L (green), respectively. The peak maxima for metallic Ni NPs in deionized water (Fig. S3B) were found at 40 nm, 96 nm and 67 nm for 0.03 mg/L (black), 0.3 mg/L (red) and 3 mg/L (green), respectively. Stronger agglomeration of metallic Ni NPs was observed in ASW when compared to deionized water. The peak

maxima in deionized water for 0.03 mg/L agree well with size distribution of particles obtained with HRTEM (see Fig. 1B in the manuscript). However the peak maxima in ASW are shifted towards the higher values when compared to the same concentration of metallic Ni NPs in deionized water which is due to the presence of a passive hydronamic layer on the surface of the particles that also causes agglomeration (Lim et al. 2013). It has also been shown that the DLS estimation and error increase (in comparison to HRTEM) with the increase of the particles concentration of magnetic particles (metallic Ni NPs) like in this case (Lim et al. 2013). Furthermore, significant agglomeration of metallic Ni NPs is observed for 3 mg/L in both deionized water and ASW, because the strong shoulder is observed at 340 nm and 190 nm in ASW and deionized water (see green spectra), respectively.

Sea urchin larval culture

Adult sea urchins of the species *Paracentrotus lividus* were collected from the Adriatic Sea in the area of Rovinj (Croatia) in cooperation with the Ruđer Bošković Institute (Center for Marine Research Rovinj) and were maintained for several months in seawater tanks at 18°C at the Department of Zoology (University of Stuttgart, Germany). Artificial seawater (ASW, control seawater) was prepared with nanopore-filtered deionized water according to a published protocol (Wilt and Benson, 2004): 0.48 M NaCl, 0.01 M KCl, 0.027 M MgCl₂, 0.03 M MgSO₄, 0.01 M CaCl₂; pH 8.0, salinity 35‰. We used ASW in our experimental setup in order to rule out potential contaminations in natural seawater.

Spawning of gametes from mature specimens was achieved by injection of approximately 1 mL of 0.5 M KCl solution. Eggs were collected and washed twice with ASW, whereas sperm was collected directly from the genital plates and kept undiluted at 4°C. Sperm was diluted just before fertilization, which was carried out approximately 1 h after spawning, at 18-19°C in ASW.

ASW was supplemented with metallic Ni NPs to final concentrations of 3 mg/L, 0.3 mg/L and 0.03 mg/L, followed by treatment with ultra-sonication (Sonopuls and UW 3100, Bandelin electronic, Berlin, Germany) for 3 min in order to disperse the particles. As respective nickel salt, we tested ASW

containing $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$ with different final concentrations (0.03 mg/L, 0.3 mg/L, 1.2 mg/L, 3 mg/L, 12 mg/L and 24 mg/L). All ASW solutions were prepared freshly for each experiment, with a salinity of 35‰ and pH 8.0.

After successful fertilization and the first three cleavage divisions in ASW at 18-19°C, embryos were washed using a 40 µm gaze filter and divided in culture dishes containing ASW (control) or ASW containing either different concentrations of metallic Ni NP or nickel salt. Further embryonic development took place at 18-19°C and developmental stages were monitored using an Olympus SZH 10 binocular (Olympus, Hamburg, Germany). Separate experiments were carried out with at least duplicate samples for each condition.

Morphological characterization

From three separate experimental setups, triplicate samples each embracing 100 embryos per concentration were classified for normal or delayed development 24 and 48 hpf, according to Matranga and Bonaventura (2002), using a light microscope (Axioskop, Zeiss, Germany). Embryos were either fixed in 0.1% formaldehyde (final concentration) or movement was stopped with 5% MgCl_2 . Additionally, fixed embryos (48 hpf, 50 individuals per condition) were measured in length (Scheitel to the postoral rod) in triplicates with the corresponding program (NikonDsFi1, NIS-ElementsD, Nikon Instruments Europe, Amstelveen, Netherlands). Statistical analyses for significance of length measurements were performed with one-way ANOVA ($\alpha=0.05$). Light microscopic images were obtained using an Axiovert 200M microscope (software Axio Vision, Zeiss, Germany).

References

Matranga, V., Bonaventura, R., 2002. Development of *Paracentrotus lividus* embryos and larvae, in: Yokota, Y., Matranga, V., Smolenicka, Z. (Eds.), The Sea Urchin: From Basic Biology to Aquaculture, Swets and Zeitlinger, Lisse, The Netherlands, pp. 223-230.

Wilt, F.H., Benson, S.C., 2004. Isolation and culture of micromeres and primary mesenchyme cells. *Method. Cell Biol.* 74, 273-285.

Lim, J., Yeap, S., Che, H., Low, S., 2013. Characterization of magnetic nanoparticles by dynamic light scattering, *Nanoscale Res. Lett.* 8, 381.

Supplementary figures

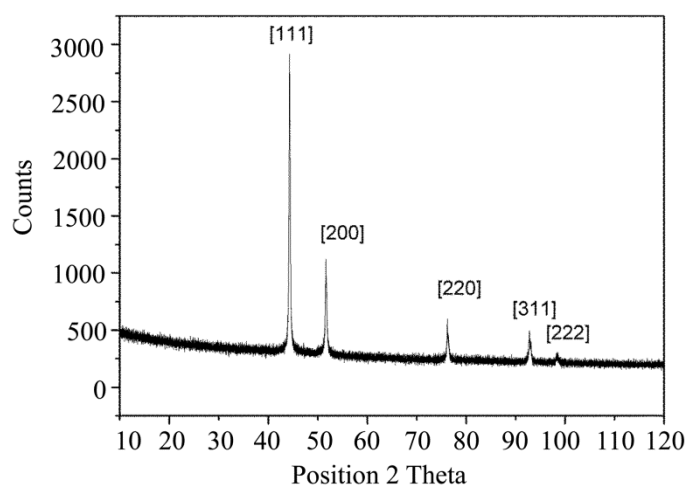


Fig. S1. X-ray diffraction pattern of metallic Ni NPs.

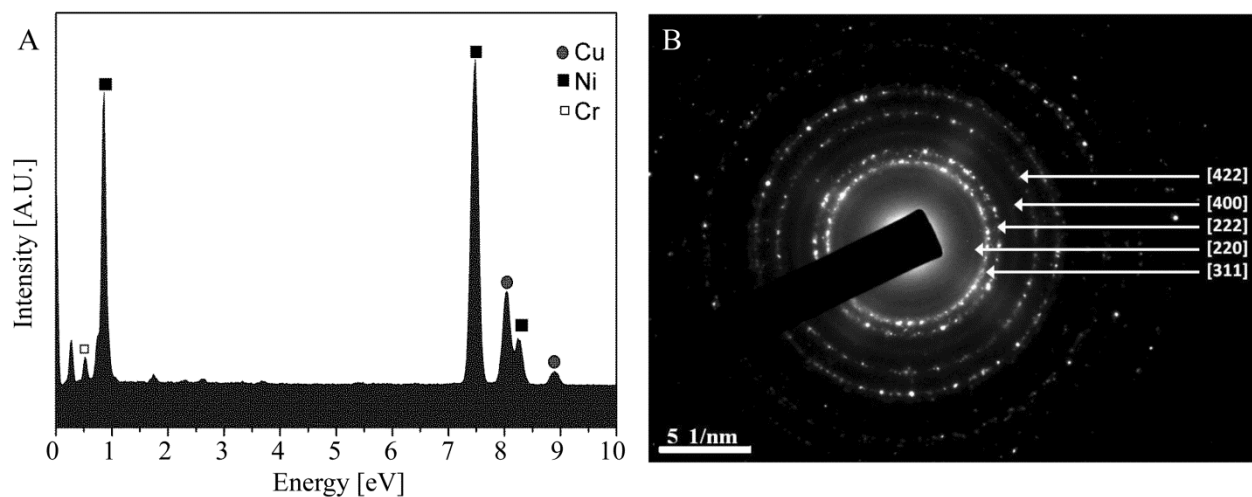


Fig. S2. Energy dispersive X-ray spectroscopy of metallic Ni NPs (A) and selected area electron diffraction of metallic Ni NPs (B).

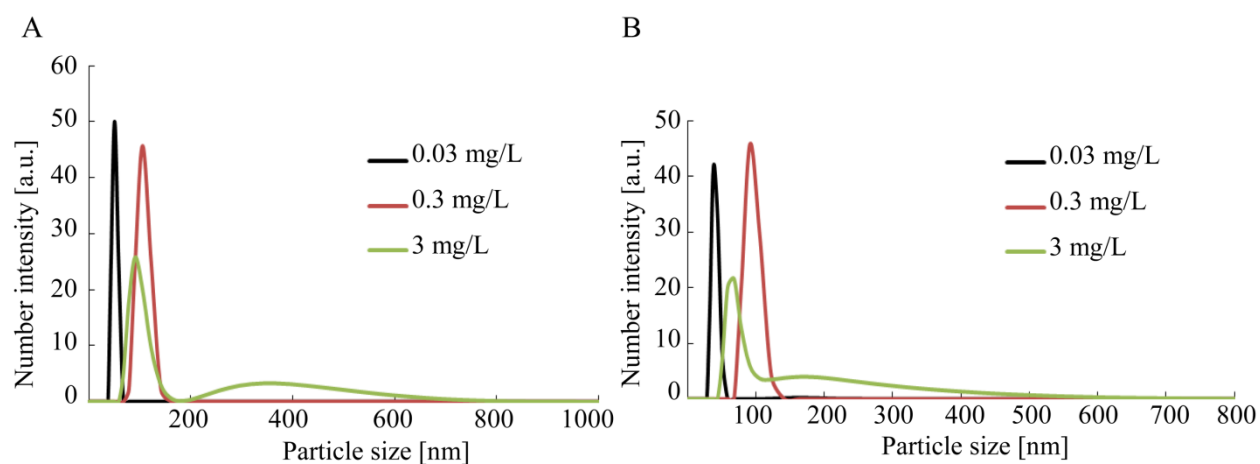


Fig. S3. Dynamic light scattering shows the number average of particle size of different concentrations of metallic Ni NPs in artificial seawater (ASW, A) and deionized water (B).

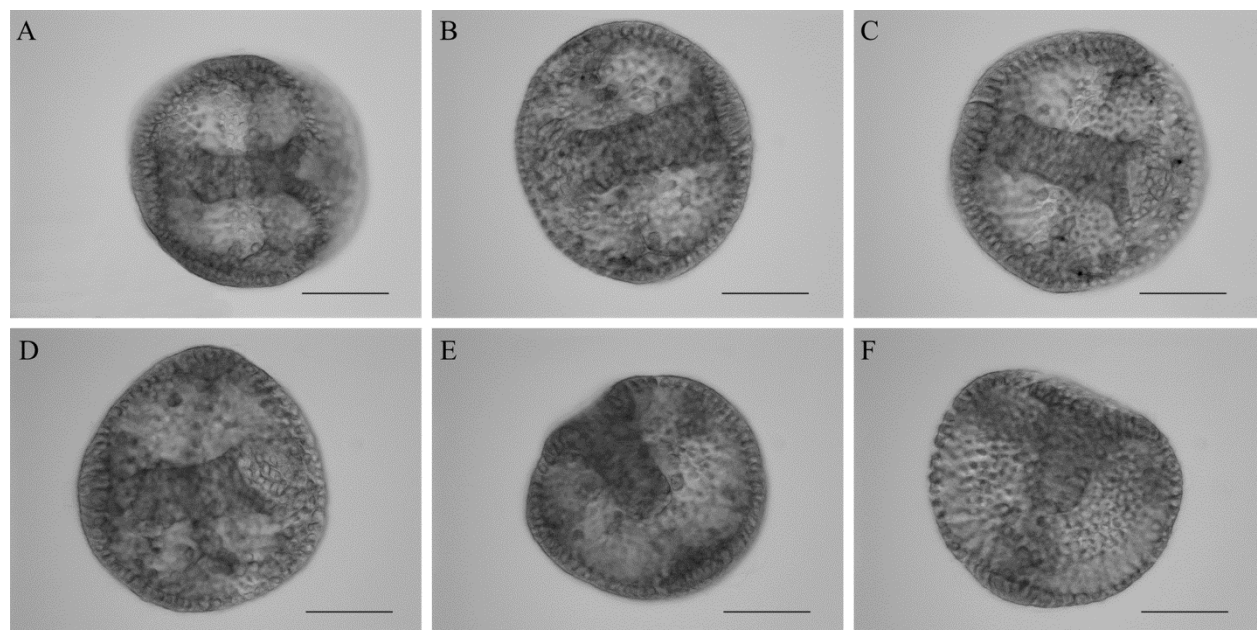


Fig. S4. *P. lividus* embryos 24 hpf (scale bar 50 μm), representative images. A: ASW; B: 3 mg/L metallic Ni NPs; C: 3 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$; D: 1.2 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$; E: 12 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$; F: 24 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$.

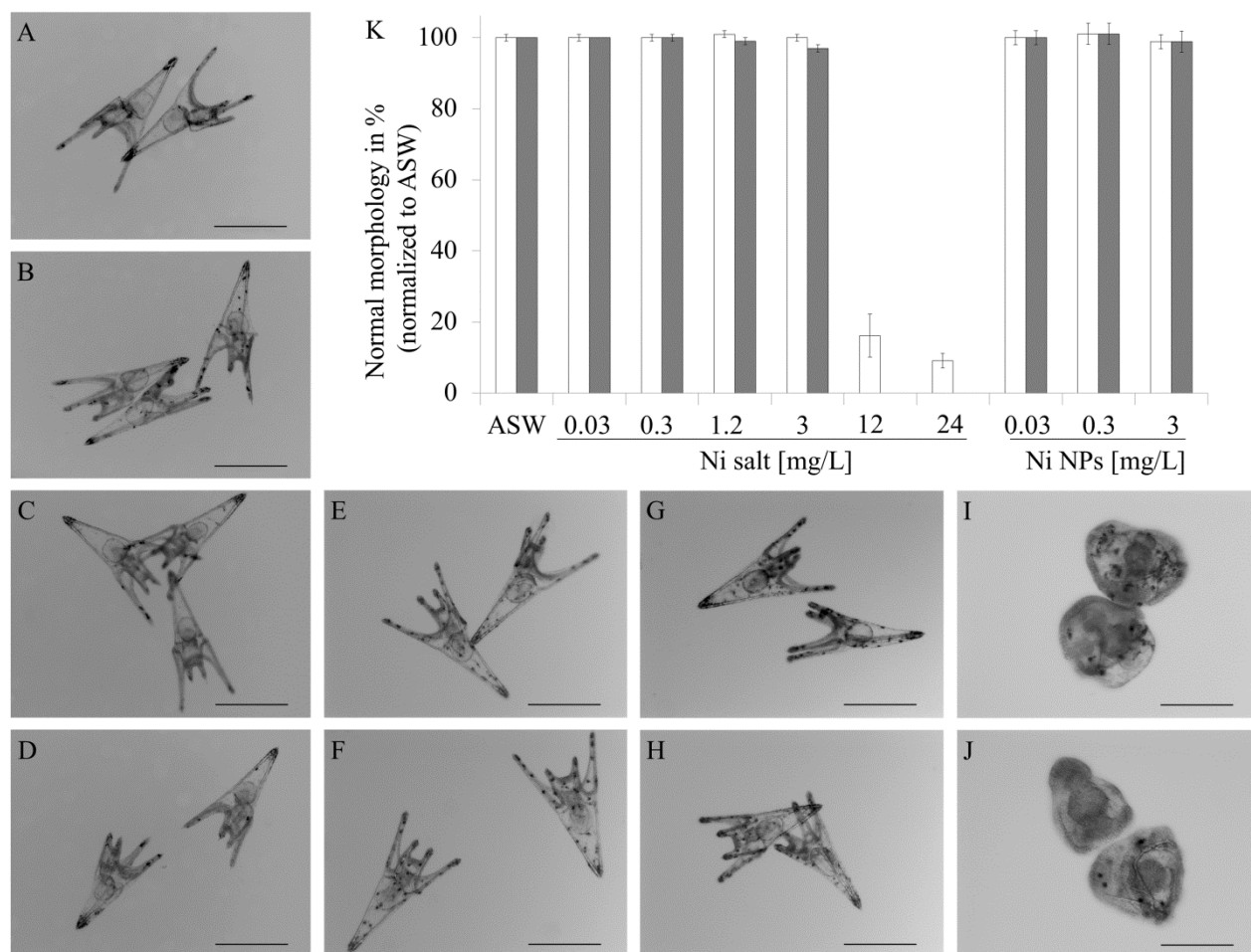


Fig. S5. *P. lividus* embryos 48 hpf (A-H scale bar 200 μm ; I and J scale bar: 100 μm). A: ASW; B: 0.03 mg/L metallic Ni NPs; C: 0.3 mg/L metallic Ni NPs; D: 3 mg/L metallic Ni NPs; E: 0.03 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$; F: 0.3 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$; G: 1.2 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$; H: 3 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$; I: 12 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$; J: 24 mg/L $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$. Percentage of embryos 24 hpf (white bars) and 48 hpf (grey bars) with normal morphology normalized to ASW as 100% (K).