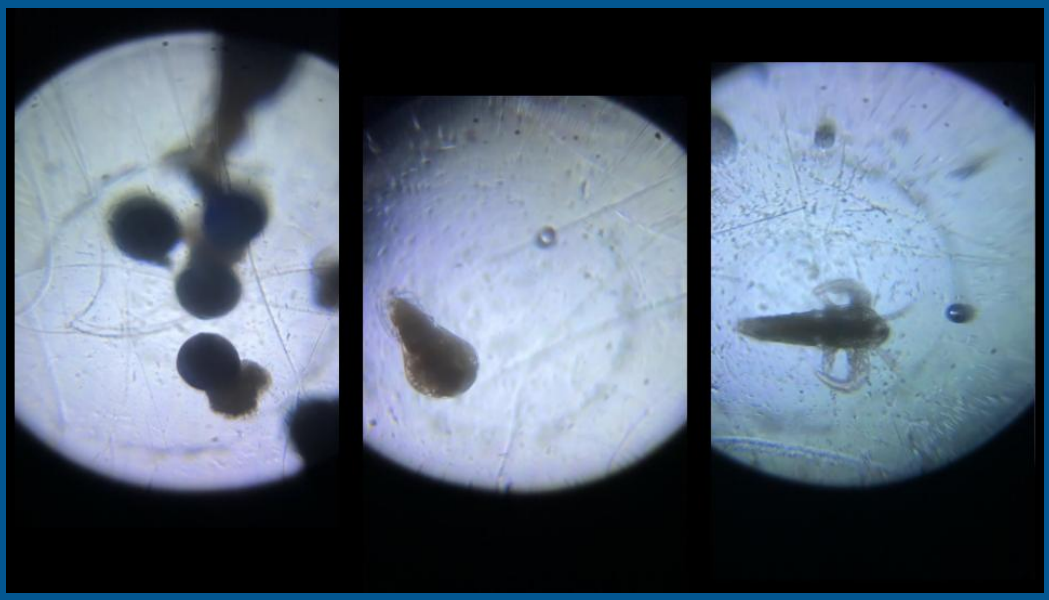
Wheaton Journal of
Developmental Biology Research

Issue 6, Winter 2021

R.L. Morris Ph.D., Editor. Wheaton College, Norton, Massachusetts.



Evidence of the effects of retinyl acetate on the development of *Artemia salina*

Nicole E. Toppses

BIO 298 / Research in Cell & Developmental Biology
Final Research Paper
29 January 2021

Evidence of the effects of retinyl acetate on the development of *Artemia salina*

Nicole E. Toppses
Research in Developmental Biology Short Report
BIO 298/ Research in Cell & Developmental Biology
Wheaton College, Norton, Massachusetts, USA
January 29, 2021

Introduction:

Retinoids act as an important morphogen in early embryonic development (Kam, Deng, Chen, Zhao 2012). Retinyl acetate is a naturally occurring fatty acid ester form of retinol with potential antineoplastic and chemopreventive activities (National Center for Biotechnology Information 2021). Retinoic acid has been involved in multiple aspects of embryo development including, eye, limb, and heart development (Goncalo C. Vihais - Neto & Oliver Pourquie 2005). Retinoic acid also determines the proximodistal axis of limb regeneration (Maden 2012). While more is known about the effects of retinoids in vertebrae development, less is known about retinoids in invertebrate development. Concentrations of retinoids have been found in *Artemia salina* and vary from 0-326 μg g^-1 dry weight (Moren, Gunderson, Hambre 2005). However, the effects of increased retinyl acetate concentration on the size of *Artemia salina* are also unknown.

The current study focused on the development of *Artemia salina*. *Artemia* can begin as metabolically inactive cysts (Stappen 1996). Once rehydrated the embryo's metabolism resumes. After about 20 hours the outer membrane of the cyst opens and the embryo appears, surrounded by the hatching membrane (Stappen 1996). It remains in the "umbrella" stage until the development of the nauplius is complete. Then it hatches and the free-swimming nauplius continues developing through its multiple larval stages. The larva grows and differentiates through 15 molts before reaching adulthood (Stappen 1996).

In this current preliminary study, I tested the hypothesis that *Artemia salina* embryos exposed to retinyl acetate from rehydration till hatching show an acceleration of growth. *Artemia salina* differentiates during embryonic development and molting stages (Stappen n.d). Retinyl acetate binds to and activates retinoid receptors, inducing cell differentiation and decreasing cell proliferation (National Center for Biotechnology Information 2021). Experiments in this current study were conducted by rehydrating *Artemia salina* in three doses of retinyl acetate. Samples were collected 58-62 hours after rehydration to measure the body length of the *Artemia*. Based on the morphogenic properties of retinoids (Kam, Deng, Chen, Zhao 2012), rehydrating *Artemia* cysts in retinyl acetate may lead to an acceleration of growth and development.

Materials and Methods:

This experiment used powdered retinyl acetate R-4632. A stock solution was prepared in DMSO at 16mg/ml. Because 16 mg/ml is 1000x the highest working solution concentration of retinyl acetate, ideally a 1/1000 dilution of DMSO would have been added to the control dish of *Artemia*.

Three doses of retinyl acetate were prepared in 1% NaCl spring water brine using serial dilutions. The 1x dosage of retinyl acetate is based on the dosage of 4µg/g of bodyweight used in a study on retinol metabolism in the Mollusk *Osilinus Lineatus* (Gesto, Castro, Reis-Henrigues, Santos 2012). The doses were 1µg/ml, 4µg/ml, and 16µg/ml. Each dose was prepared so that 20ml of liquid would be in each petri dish.

1/48th tsp (approx. 41.6mg) of *Artemia salina* cysts were added to the Petri dishes then stirred to begin rehydration. Samples of *Artemia* were observed and collected at 58-62 hours after rehydration. Several drops containing *Artemia* were placed on a foldscope slide (Foldscope Instruments 2021) using a transfer pipette. This sampling method cannot exclude the possibility of resampling animals. The addition of more *Artemia salina* to the slides decreases this possibility. *Artemia salina* were not returned to the Petri dishes after sampling to prevent resampling in future observations.

This experiment utilized a foldscope for observation and imaging. The foldscope is a paper microscope that contains a spherical lens (Foldscope Instruments 2021). The foldscope has a magnification of 140x and a resolution of 2 microns (Foldscope Instruments 2021). Images were taken by screenshotting videos of the samples using an iPhone 8 camera. The accuracy of the dimensions was limited by spherical aberration from the foldscope lens. To combat this, measurements taken in ImageJ were analyzed using screenshots of the *Artemia salina* in the most focused region. To prevent bias in measurements, control and experimental samples were imaged and analyzed identically.

The images captured at 58-62 hours after rehydration were measured in ImageJ software for each of the experimental and control dishes. The scale of the images was set in ImageJ using an image of a stage micrometer in the foldscope. The body length measured spans from the nauplius eye to the end of the tail. The average body length was calculated for each condition. In this study, only one trial for each condition was conducted. Multiple methods were beyond the scope of this preliminary study.

Data are derived from measurements of *Artemia salina* body length in 19 images from the control condition. Data are derived from measurements of *Artemia salina* body length in 14 images from the 1µg/ml retinyl acetate experimental group. Data are derived from measurements of *Artemia salina* body length in 22 images from the 4µg/ml retinyl acetate experimental group. Data are derived from measurements of *Artemia salina* body length in 24 images from the 16 µg/ml retinyl acetate experimental group.

Results:

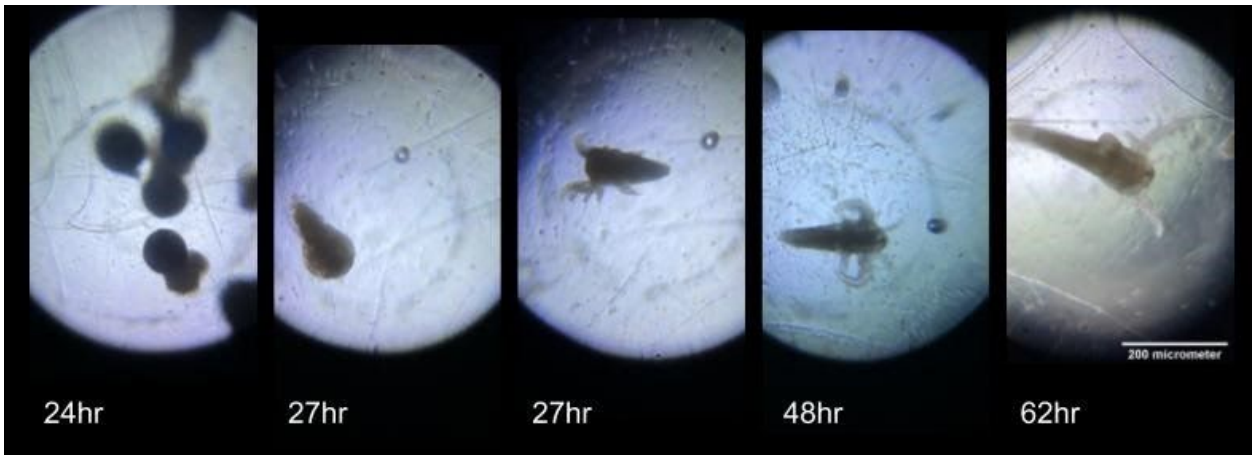


Figure 1. Developmental stages of *Artemia salina*. Images are of the control group under a foldscope at 140x. The 27 hr images are of the same nauplius, the left is in the "umbrella" stage and the right is 1-2 minutes after hatching.

Both the experimental and control conditions were successful in hatching *Artemia*. There was a large number of *Artemia* in both the control and experimental conditions at 27 hours and even more at 38 hours after hydration.

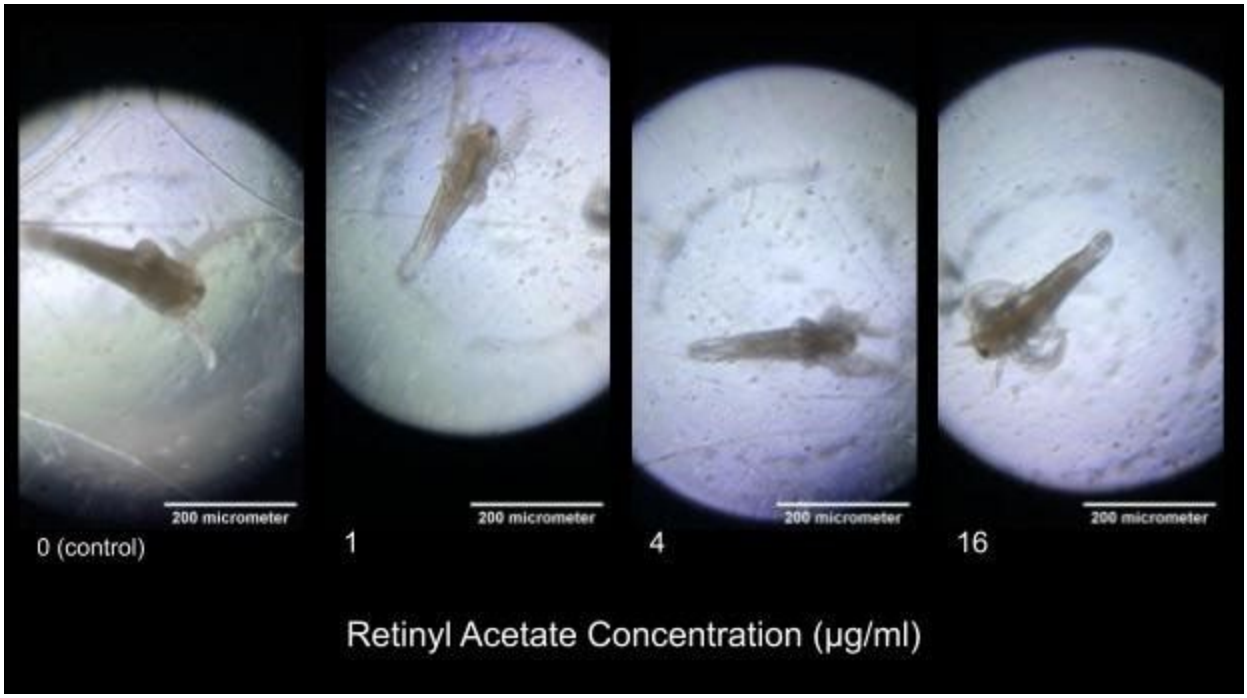


Figure 2. The body length of *Artemia salina* images measured from 58-62 hrs after rehydration. The body length measured spans from the top of the head at the nauplius eye to the end of the tail.

The measurements were collected between 58-62 hours after rehydration. Most of the *Artemia* sampled resembled the *Artemia* at 62 hrs in Figure 1. Some *Artemia* were in earlier stages similar to the *Artemia* at 48hrs in Figure 1.

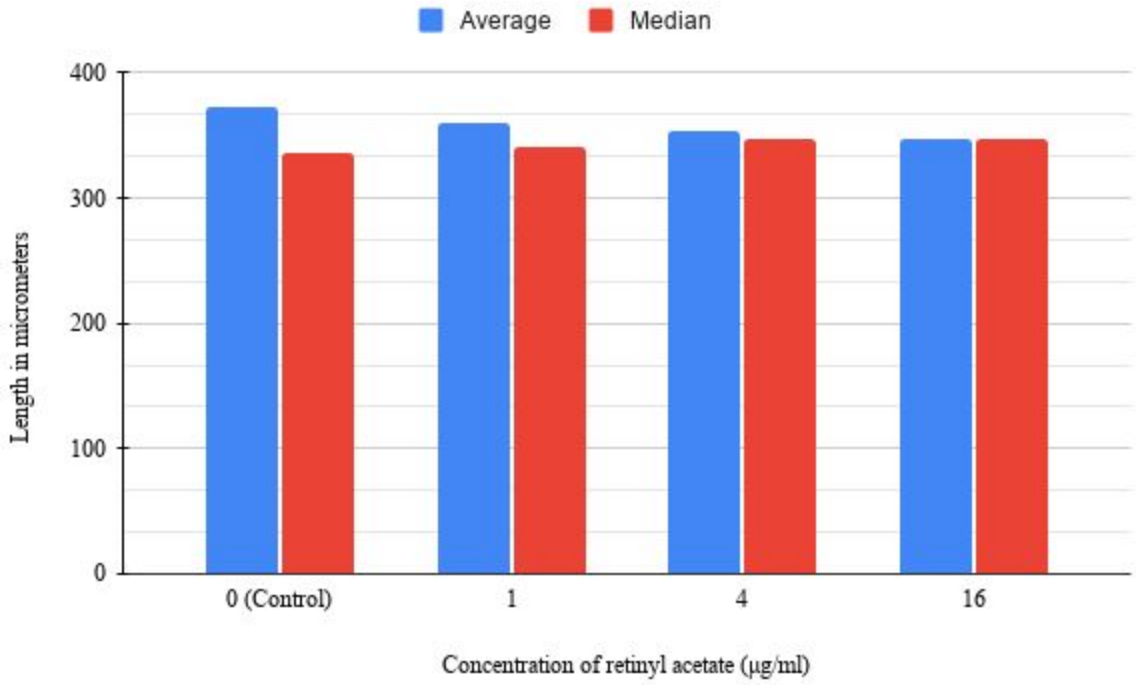


Figure 3. The measurement data from the average body length of *Artemia* measured from 58-62 hours after rehydration. The median values are next to the average for comparison of variance within each sample. Notice how the median and average length values are closer together in 4µg/ml and 16µg/ml.

Data quantification showed that the average size of the *Artemia* decreased at higher concentrations of retinyl acetate. As seen in Figure 3, the control group had the highest average body length. The average size of the *Artemia* hatched in retinyl acetate decreased about 10 µm between each concentration.

The variance in *Artemia* size within the sample appeared to decrease as the concentration of retinyl acetate increased. The control samples observed had *Artemia* in various sizes while the sample observed from the 16µg/ml had *Artemia* closer in size to one another. This observation was quantified after measuring images and calculating the standard deviation. The standard deviation for the control group was 73.80. The standard deviation for the experimental group rehydrated in 1µg/ml is 70.12. The standard deviation for the experimental group rehydrated in 4µg/ml is 57.86. The standard deviation for the experimental group rehydrated in 16µg/ml is 46.37.

Discussion:

The results of this preliminary study do not support the hypothesis that *Artemia salina* embryos exposed to retinyl acetate from rehydration till hatching show an acceleration of growth. This evidence suggests that perhaps, *Artemia salina* embryos exposed to retinyl acetate from rehydration to hatching show an inhibition of growth. The average body length in the *Artemia* at 58-62 hours after rehydration appeared to decrease as the dosage of retinyl acetate increased.

The possibility of growth inhibition may be related to the mechanism of retinyl acetate. Retinyl acetate binds to and activates retinoid receptors, inducing cell differentiation and decreasing cell proliferation (National Center for Biotechnology Information 2021). The ability to decrease cell proliferation suggests that increased retinyl acetate could lead to an inhibition of body length growth.

There also appeared to be less variance in measurements of *Artemia salina* rehydrated in retinyl acetate. This evidence suggests that perhaps *Artemia* rehydrated in retinyl acetate may differentiate at a consistent rate. Retinyl acetate's ability to induce cell differentiation may be a potential cause for body length consistency among samples rehydrated at higher retinyl acetate concentrations. This possibility poses more questions for future studies. While retinyl acetate may potentially inhibit growth due to the possible decrease in cell proliferation, could the anatomy and sizes of the *Artemia salina* sample exposed to retinyl acetate be more uniform?

In future studies, I would make improvements to this experimental setup. Some sources of error include distortion due to spherical aberration of foldscope lens and resampling *Artemia*. Usage of a microscope with a combination of positive and negative lens elements with various thicknesses would help combat spherical aberration (Abramowitz, Spring, Davidson 2021). I would also include a DMSO carrier control in the setup. More trials and more sampling at consistent time intervals would help to refine the experiment.

Retinoids have many potential functions and effects in development in both vertebrates and invertebrates. The evidence from this preliminary study on *Artemia salina* raises more questions for future studies on retinyl acetate in embryonic development and regeneration.

References:

Abramowitz, Spring, Davidson (n.d) Lenses and Geometrical Optics - Common Optical Defects in Lens Systems (Aberrations) | Olympus LS. (n.d.). Retrieved January 29, 2021, from https://www.olympus-lifescience.com/en/microscope-resource/primer/lightandcolor/opticalaberrations/

Albalat, R. (2009, December 10). The retinoic acid machinery in invertebrates: Ancestral elements and vertebrate innovations. *Molecular and Cellular Endocrinology*, Vol. 313, pp. 23–35. https://doi.org/10.1016/j.mce.2009.08.029

Dionisio, K. L., Phillips, K., Price, P. S., Grulke, C. M., Williams, A., Biryol, D., … Isaacs, K. K. (2018). Data Descriptor: The Chemical and Products Database, a resource for exposure-relevant data on chemicals in consumer products. *Scientific Data*, *5*. https://doi.org/10.1038/sdata.2018.125

Foldscope Instruments. (2021) User Guide . Retrieved January 28, 2021, from https://www.foldscope.com/user-guide

Gesto, M., Castro, L. F. C., Reis-Henriques, M. A., & Santos, M. M. (2012). Retinol Metabolism in the Mollusk Osilinus lineatus Indicates an Ancient Origin for Retinyl Ester Storage Capacity. PLoS ONE, 7(4), e35138. https://doi.org/10.1371/journal.pone.003513

Gundersen, T. E. (2006). Methods for detecting and identifying retinoids in tissue. *Journal of Neurobiology*, *66*(7), 631–644. https://doi.org/10.1002/neu.20243

Kam, R. K. T., Deng, Y., Chen, Y., & Zhao, H. (2012, March 22). Retinoic acid synthesis and functions in early embryonic development. *Cell and Bioscience*, Vol. 2, p. 11. https://doi.org/10.1186/2045-3701-2-11

Kamm, J. J. (1982). Toxicology, carcinogenicity, and teratogenicity of some orally administered retinoids. *Journal of the American Academy of Dermatology*, *6*(4), 652–659. https://doi.org/10.1016/S0190-9622(82)70054-

Kanungo, J. (2016). Retinoic Acid Signaling in P19 Stem Cell Differentiation. *Anti-Cancer Agents in Medicinal Chemistry*, *17*(9). https://doi.org/10.2174/1871520616666160615065000

Li, X., Long, X. yang, Xie, Y. jie, Zeng, X., Chen, X., & Mo, Z. cheng. (2019, October 1). The roles of retinoic acid in the differentiation of spermatogonia and spermatogenic

disorders. *Clinica Chimica Acta*, Vol. 497, pp. 54–60. https://doi.org/10.1016/j.cca.2019.07.013

Monaghan, J. R., Athippozhy, A., Seifert, A. W., Putta, S., Stromberg, A. J., Maden, M., … Voss, S. R. (2012). Gene expression patterns specific to the regenerating limb of the Mexican axolotl. *Biology Open*, *1*(10), 937–948. https://doi.org/10.1242/bio.20121594

Monaghan, J. R., & Maden, M. (2012). Visualization of retinoic acid signaling in transgenic axolotls during limb development and regeneration. *Developmental Biology*, *368*(1), 63–75. https://doi.org/10.1016/j.ydbio.2012.05.015

Moren, M., Gundersen, T. E., & Hamre, K. (2005). Quantitative and qualitative analysis of retinoids in Artemia and copepods by HPLC and diode array detection. *Aquaculture*, *246*(1–4), 359–365. https://doi.org/10.1016/j.aquaculture.2005.01.017

National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 638034, Retinyl acetate. Retrieved January 28, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Retinyl-acetate.

Page, K. C., Heitzman, D. A., & Chernin, M. I. (1996). Stimulation of c-jun and c-myb in rat Sertoli cells following exposure to retinoids. *Biochemical and Biophysical Research Communications*, *222*(2), 595–600. https://doi.org/10.1006/bbrc.1996.0789

Piatchanina, T. V., & Sidorik, E. P. (1990). Rol' belkovykh retseptorov vitamina A v mekhanizmakh protivoopukholevogo de ĭ stviia retinoidov. *Eksperimentalnaa Onkologia*, Vol. 12, pp. 8–13.

Reichrath, J., Lehmann, B., Carlberg, C., Varani, J., & Zouboulis, C. C. (2007, February). Vitamins as hormones. *Hormone and Metabolic Research*, Vol. 39, pp. 71–84. https://doi.org/10.1055/s-2007-958715

Ross, S. A., McCaffery, P. J., Drager, U. C., & De Luca, L. M. (2000). Retinoids in embryonal development. *Physiological Reviews*, Vol. 80, pp. 1021–1054. https://doi.org/10.1152/physrev.2000.80.3.1021

Stappen (1996) 4.1. Introduction, biology and ecology of Artemia. Retrieved January 24, 2021, from http://www.fao.org/3/w3732e0m.htm