GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

REVIEW

Endocrine disruption in echinoderms

Ecotox- 04/2019

ARPA-ACUA Deliverable T2. E4

Estación de Ciencias Mariñas de Toralla (ECIMAT) - *Universidade de Vigo*

24 Octubre 2019

Universidade Vigo

Ficha Técnica

Título

Review: Endocrine disruption in echinoderms.

Proyecto

ARPA-ACUA: **A**lternativas ambientalmente **R**espetuosas para **P**olímeros y sus **A**ditivos químicos en el medio **A**cuático ***(CTM2016-77945-C3)***

Autores

Ricardo Beiras, *Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo*.

Pedro Campoy, *Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo.*

Contacto

Ricardo Beiras, *Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo*.

Email: rbeiras@uvigo.es

Web: http://ecotox.es/

Fecha	**Versión**	**Nº de págs.**
24 de octubre de 2019	1	19

Código: Entregable T2.E4

Este documento debe ser citado como:

Beiras R., Campoy P., 2019. Review: Endocrine disruption in echinoderms. ARPA-ACUA Project. Deliverable T2-E4, 19 pp.

1. Endocrine disruption in echinoderms.

In this work selected aspects of endocrine disruption on which limited information is available will be reviewed, in particular those concerning echinoderms. The importance of developing echinoderms studies is largely due to the limited knowledge on the endocrine physiology of echinoderm species that represent important components of marine ecosystems. Moreover, little is known on the molecular targets for the action of endocrine disrupters in these organisms, and only in few marine invertebrate species the genomics and proteomics approaches are being developed.

1.1.Sex steroid hormones.

Sexual differentiation, gametogenesis, and reproduction of vertebrates are controlled by **sex steroid hormones**: androgens, estrogens and progestogens (see Fig. 1) synthetized from cholesterol in the gonads of adults. Foreign compounds, called endocrine disrupting compounds (EDCs) can either interfere with the metabolism of these and other hormones or act as their mimics and bind to their receptors, thus impairing these fundamental processes for life. According to the World Health Organization, "an **endocrine disrupter** is an exogenous substance or mixture that alters function of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." Chemicals acting as estrogens are called **estrogenic**. Many natural compounds are known to have abortive properties, and farmers have long known for instance that grazing sheep on new growth clover reduce pregnancies. We know now this is due to the action of phytoestrogens that can disrupt the normal metabolism of sexual hormones. However, only recent concern has been raised about the potential endocrine disrupting effects of synthetic chemicals such as **detergents**, **cosmetics components**, or **plastic additives** increasingly present in our environment (from Beiras, 2018). Endocrine disruptors typically enter the marine environment via effluents from sewage treatments plants, and from industry and agricultural runoff.

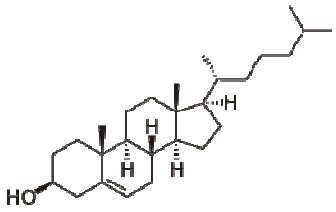
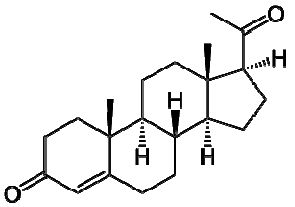
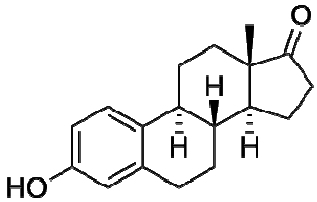
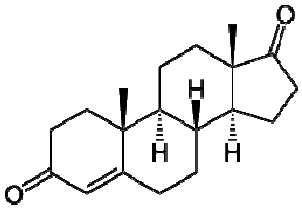
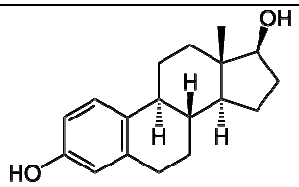
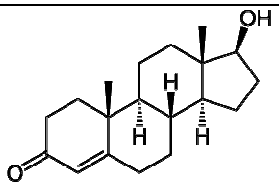
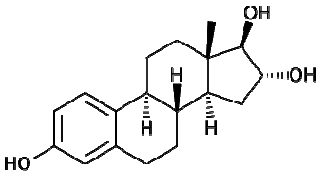
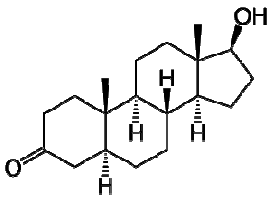
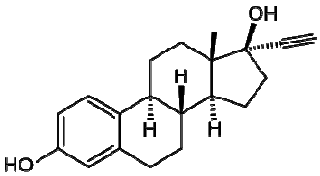
 Cholesterol		
Sex steroids		
Progestogens	**Estrogens**	**Androgens**
 Progesterone (P4)	 Estrone (E1)	 Androstenedione
	 17β-estradiol (E2)	 Testosterone
	 Estriol (E3)	 5α-dihydrotestosterone
	 Ethinyl-estradiol (EE2) (synthetic)	

***Figure 1**. Molecular structure of steroid sex hormones and their precursor, cholesterol.*

2. Estrogenic endocrine disruption.

Most information on the biological effects and mechanisms of action of EDCs has been focused on vertebrates. (From Yamaguchi et al, 2005): In vertebrates, natural estrogen, such as **estradiol** (E2), regulates the estrogen-responsive genes by binding to a specific **estrogen receptor** (**ER**). Then the estrogen - ER complex interacts with the estrogen - responsive elements (EREs) of the target promoter genes to modulate their transcriptional activity. Among the estrogen-responsive genes, **vitellogenin** (**Vtg**), an estrogen-inducible

phosphoprotein and complex precursor protein of egg yolk, is synthesized in the liver after stimulation of ovarian estrogens, transported to the ovary through the blood stream and incorporated into the oocytes in teleost fish. A high level of plasma and/or hepatic Vtg is observed in sexually mature females, whereas Vtg levels in males and sexually immature fish are normally very low. However, a number of environmental estrogens, such as alkylphenolic compounds [Bisphenol A, nonylphenol…], phytoestrogens, synthetic estrogens, and pesticides [methoxychlor] also induce Vtg synthesis in both males and females. Therefore, **Vtg production in male or juvenile fish has become a useful biomarker for detecting estrogenic contamination of the aquatic environment** (Sumpter and Jobling, 1995). Previous studies demonstrated significant increased plasma Vtg in male goldfish (*Carassius auratus*) fed the phytoestrogen-enriched fish diet (Ishibashi et al., 2004). Moreover, they reported that bisphenol A (BPA) and its metabolite 4-methyl-2,4-bis(4-hydroxyphenyl) pent-1-ene (MBP) have ER binding potency, and MBP showed significantly higher hepatic Vtg induction in male medaka than BPA (Watanabe et al., 2003). Measurement of estrogen-responsive protein such as Vtg has included Western blotting analysis, radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), and all have been commonly used in various teleost fish, such as common carp (*Cyprinus carpio*), fathead minnow, zebrafish and medaka (Nilsen et al., 2004), and also in *Cyprinodon variegatus* (see Hemmel et al. 2002). On the other hand, the **induction of Vtg gene expression occurs almost immediately after estrogenic compound exposure, in comparison with the induction of Vtg protein production** (Arukwe et al., 2001). However, little is known about the expression patterns of estrogen-responsive genes such as those encoding for **Vtg** and **ER** in male fish exposed to xenoestrogens, although the **measurement of specific mRNA coding for these biomarkers provides a particularly sensitive monitor** of endocrine status in fish.

3. Role of sex steroids in echinoderms.

It is general accepted that endocrine-disrupting chemicals are partially responsible for reproductive and development alterations in wildlife. Donahue and Jennings (1937, cited by Wasson & Watts 2013) found that an oil-soluble substance extracted from the ovaries of *Lytechinus variegatus* induced vaginal growth in ovariectomized rats. Since then, considerable evidence has been published supporting that vertebrate-type **sex steroids**, progesterone, estrogens (17β-estradiol and estrone) and androgens (testosterone) **are synthesized by echinoderms** (Botticelli et al. 1961; Dieleman & Schoenmakers 1979; Wasson et al. 2000a, b; reviewed by Wasson & Watts 2013). Lavado et al. (2006a) and Wasson & Watts (2013) reviewed the metabolic pathways involved in the synthesis of estrogens in echinoderms (see

Fig. 2). However, estradiol and testosterone **levels are maturity-stage and sex independent**, and no clear patterns of seasonal differences in neither testosterone or estradiol content were found (Wasson et al. 2000a, Lavado et al., 2006ab in *Paracentrotus lividus* and *Antedon mediterranea*; Barbaglio et al. 2007 in *P. lividus*).

In addition, 80 to 99.8% of testosterone and estradiol in *A. mediterranea* were in **esterified** form (Lavado et al. 2006b), as a consequence of phase II activity (acyl-transferases) (Lavado et al. 2006a). In vertebrates esterified steroids are assumed to be inactive (do not bind to the estrogen receptor), but in echinoderms no ER were identified up to date and ER-independent mechanisms of activity for the esterified steroids cannot be discarded.

Evidence supporting a role of steroids in gametogenesis comes chiefly from starfish. Both estrone and estradiol injected daily for 16 days caused an increase in egg diameter and protein content of the ovaries caused an increase in egg diameter and protein content of the ovaries in starfish (Takahashi and coworkers[1] , Barker & Xu 1993[2]). Concerning sea-urchins, injection of estradiol to adult *P. lividus* for 10 weeks did not influence the maturation stage of the gonads and the development of the gametes (Sugni et al. 2012). In *L. variegatus* fed with estradiol the ovarian index was 54% greater due to increased volume occupied by nutritive phagocytes but egg diameter was smaller, while progesterone increased the testis index by 56% (Wasson et al. 2000c). Varaksina & Varaksin[3] found that injection of estradiol dipropionate increased ovarian index, number of mature oocytes, and protein synthesis in ovaries during gametogenesis but not in mature individuals (stage III “before spawning”). However, the same treatment also increased protein synthesis in male sea-urchin gonads (Varaksina & Varaksin 2001).

Levels of steroids detected in *L. variegatus* sea-urchin were low compared to starfish, and this might reflect paracrine-like mechanisms in cell signaling (i.e. interaction between a messenger and a cell in close proximity to the place of synthesis of the messenger), as compared to endocrine-like mechanisms proposed to regulate gonad function in asteroids. Wasson et al. (2000a) speculate that the different anatomy of starfish, where nutrients are distantly stored in the pyloric ceca of the digestive system, in contrast with echinoids where nutrients are stored directly in the gonads, may explain the lower levels of steroids in the latter.

---

[1] Takahashi (1982a): daily injection of estrone for 16 days significantly increased ovarian protein in *Asterina pectinifera*; but: Takahashi (1982b): same treatment in testes also increased gonad index

[2] Injecting estradiol-17β (1.2 µl of 1 × 10−7 M per 10 g body weight) or estrone (1.2 µl of 2 × 10−7 M) daily into the starfish *Sclerasterias mollis* for 16 days at early stage one of the gametogenic cycle caused an increase in the estrone and progesterone levels in the ovaries. There was a significant increase in the sizes of oocytes and protein levels in the ovaries of treated animals, but no effect on gonad index.

[3] Increase in ovarian index and number of mature oocytes is reported by Varaksina & Varaksin 1987 cited by Wasson& Watts (2013); increase in protein synthesis reported by Varaksina & Varaksin (2002).

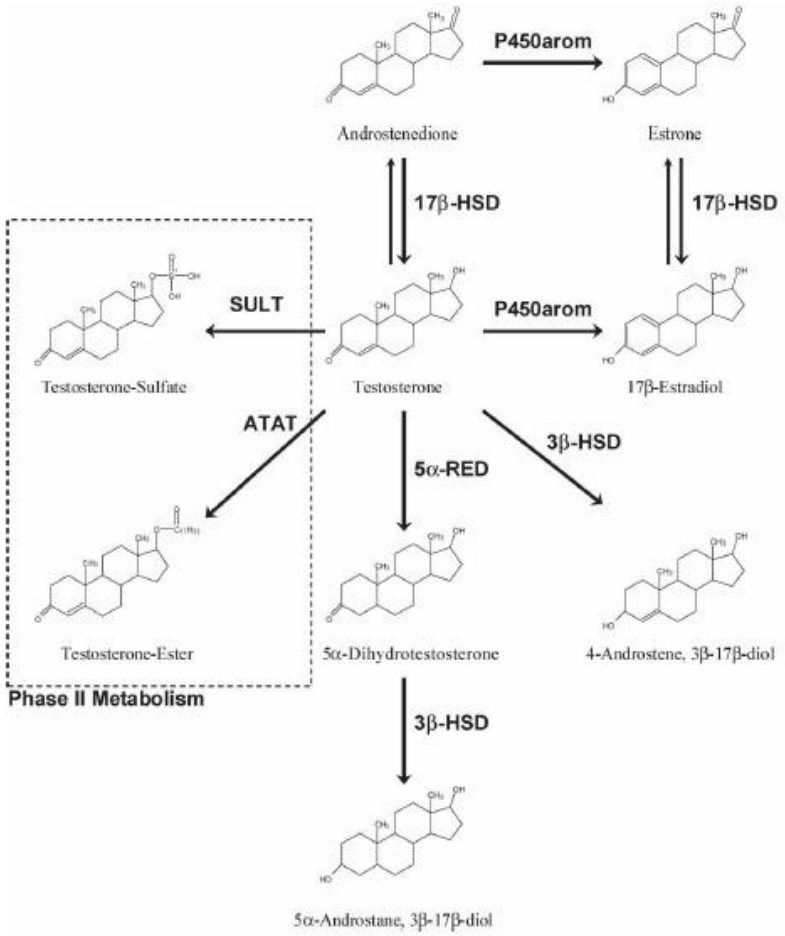
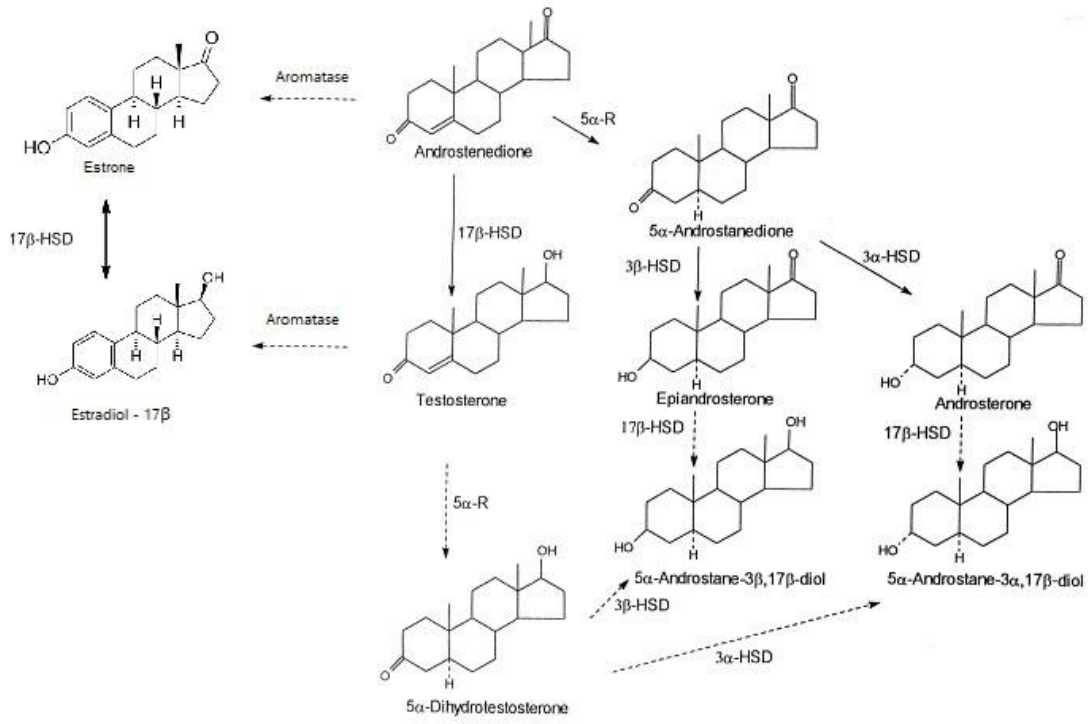


***Figure 2**. Schematic representation of the steroid synthesis and metabolism pathways in Paracentrotus lividus, Lavado et al. (2006a) (above), and in Lytechinus variegatus, Wasson & Watts (2013) (below)*



4. Mayor Yolk Protein, MYP.

In echinoids[4] the main yolk precursor protein is **MYP** (major yolk protein or major yolk precursor[5]) while in asteroids it seems to be **vitellogenin** (Prowse & Byrne 2012, Alqaisi et al. 2016, but see also Zazueta-Novoa et al. 2016). However, in both cases these proteins are expressed in both sexes (for sea-urchin see Unuma et al. 1998). Exposure of echinoid coelomocytes to 10^{-6}M estradiol failed to induce MYP synthesis (Harrington & Ozaki 1986). Exposure of sea-urchin larvae to β-estradiol failed to interfere with MYP gene expression, although at the six-armed stage (but not at later stages) estrone did suppress MYP expression (Kiyomoto et al. 2008).

In sea-urchins, MYP, a glycoprotein of 170–180 kDa, is the most abundant protein in the yolk granules of **eggs**, and it is also present in in the **nutritive phagocytes**[6] (NP) of the gonad in both sexes (Walker et al. 2013)[7]. During egg embryogenesis the MYP is partially replaced by cleavage products (Scott & Lennarz 1989; see also Kari & Rottmann 1985). Therefore, MYP was related to the vitellogenin of vertebrates and insects. However, MYP aminoacid sequence is not homologous to vertebrate Vtg but rather to iron-binding **transferrin**[8] (Brooks & Wessel 2002, Yokota et al. 2003). According to Unuma et al. (2010), MYP has **two isoforms** with slightly different molecular masses: the coelomic fluid MYP (180 kDa) would be a precursor of the egg MYP (170 kDa)[9]. The precursor form initially called vitellogenin[10], constitutes 70% of the protein within the coelom. This fits with previous findings by Harrington & Easton (1982) who had described the MYP in the coelomic fluid as a precursor of the egg MYP. Antibodies for the egg MYP and this coelomic protein cross-react.

The MYP may be synthesized in the digestive tract, exported to the coelomic fluid through the coelomic spherule cells, and from there a portion further exported to the gonads, or synthetized de novo in the NP of the gonads. MYP mRNA was found in the epithelium of the digestive tract and in the NP of ovaries and testis, but not in germ cells (Unuma et al. 2010).

The role of the MYP seems to be much broader than a simple source of nutrients for the gametes. In *P. lividus*, Cervello & Matranga (1989) identified both the coelomic and gonad

[4] Actually in Echinozoa, which includes Echinoidea and Holoturioidea (Prowse & Byrne 2012).
[5] Both denominations have been published but notice that, since MYP is present in the mature egg, and Walker et al. (op. cit.) report that vitellogenin is a precursor of MYP present in the coelom, the term Mayor Yolk Precursor seems misleading and thus should not be used.
[6] Somatic cells of the gonads that supply nutrients to the developing germ cells.
[7] MYP reaches 80% of the protein content in the gonads of both sexes (Unuma et al. 2003).
[8] However, unlike transferrin, MYP is ironless and binds calcium (Noll et al. 2007).
[9] nutritive phagocytes contain both types.
[10] A *S. purpuratus* Vtg gene of ca. 19 Kb was described by Shyu et al. (1987).

forms of MYP - formerly termed sea-urchin Vtg -, as presurcors of a large oligomeric glycoprotein isolated from blastula embryos termed **toposome**, so called because it expressed positional information essential for cell adhesion during sea urchin embryogenesis (see also Gratwohl et al. 1991). According to Dev & Robinson (2014), the MYP of sea-urchins has a complex tertiary structure (a hexamer[11]) whose changes enable this protein to drive membrane-membrane interactions essential for egg cleavage and **embryo development**. Differences between the egg and coelomic fluid forms of the MYP may reflect differing functional responsibilities.

MYP seems to play a role in **larval development** also, since MYP mRNA was detected using Real Time PCR starting in the early 8-arm pluteus stage and its expression increased until metamorphosis, with a subsequent decrease but persistence of expression in juveniles (Unuma et al. 2009). Immunohistochemistry located the larval MYP synthesis in the larval digestive tract. According to its molecular weight (180 kDa), larval MYP belongs to the coelomic type (see above). Unuma et al. (2009) suggest that a major role of larval MYP is **Zn transportation** from the digestive tract to tissues[12].

The coelomic form of the MYP has been speculated also to play **defensive role**, in a similar way to insect transferrins whose expression is up-regulated after bacterial challenge (see Discussion in Yokota et al. 2003).

In females, MYP is present in the eggs even after fertilization, while in males it can no longer be recognized in the testis after gametogenesis is completed (Walker et al. 2013). Unuma et al. (2003) reported a gradual decrease in *Pseudocentrotus depressus* gonad MYP along gonad development, particularly remarkable in male individuals, which lack MYP at full maturity (Figure 3). A marked decrease in *P. lividus* toposome along gonad development has been reported for both sexes, but particularly dramatic in male sea-urchins (Ghisaura et al. 2016). They found a drop occurring in toposome abundance in gonadal stage I and III, with complete disappearance of toposome in stage IV males (see Figure 3).

[11] Toposome, isolated from the sea urchin *Tripneustus gratilla* or *Paracentrotus lividus* appears to be a glycoprotein composed of six identical polypeptides of 160–170 kDa in size, depending on the species […] proteolytically processed after fertilization into smaller molecular-mass species [Noll et al., (1985) cited by Hayley et al. (2008)].

[12] The same role would be played in adults also, explaining elevated levels of coelomic MYP in females during early gametogenesis (Unuma et al. 2007), due to the high demand of Zn demanded by oogenesis.

Another subset of yolk proteins, the **YP30** family, unlike the MYP, are synthetized in situ by the oocyte. In *S. purpuratus* YP30 protein forms belonging to 4 clades have been described, with clades 1 and 2 expressed mainly in oocyte and clade 3 in the larva (Song et al. 2006).

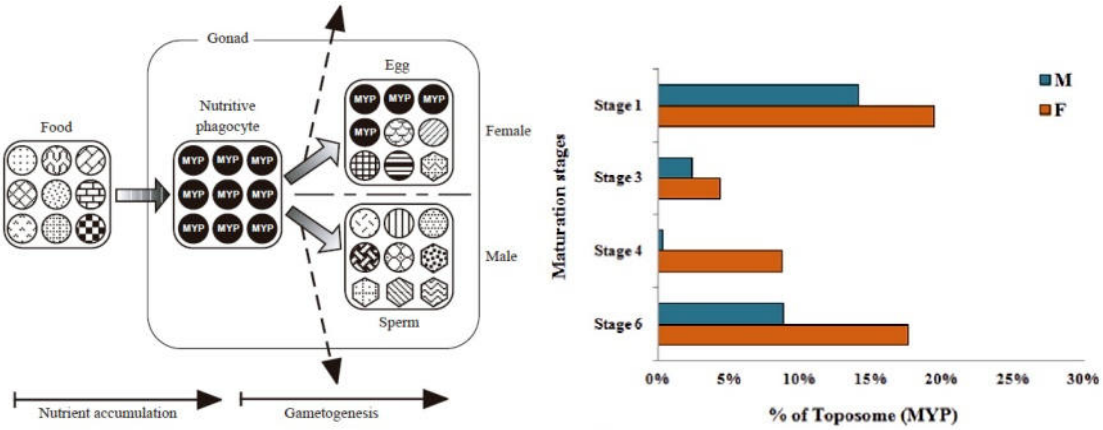


***Figure 3**. Diagram of the nutritional role of major yolk protein (MYP) in sea urchin reproduction (left) and histogram illustrating the relative percentage of toposome (right) in different maturation stages for both sexes. F, females; M, males. Figures from Unuma et al. 2003 and Ghisaura et al. 2016.*

5. Endocrine disrupting xenobiotics in echinoderms.

In a similar way to what is reported for vertebrates, the EDCs in echinoderms not only affect the reproductive biology, but also the immune system, as shown by some studies in asteroids and echinoids with PCBs and TBT (Coteur et al. 2001; Matranga et al. 2005). Endocrine disruption by androgenic and anti-androgenic xenobiotics has been reported in echinoderms (Sugni et al. 2007). TBT and the androgenic xenobiotic TPT caused P450-aromatase inhibition in *P. lividus* and increased testosterone levels in *A. mediterranea* (but not in *P. lividus*) (Lavado et al. 2006ab). TPT at nominal 100-200 ng/L caused oogenesis inhibition, a reduction in egg diameter and a concomitant decrease in estradiol concentration in *P. lividus* (Sugni et al. 2007). Other authors reported evident anomalies in sea urchin spermatogenesis and reduced sperm motility after long-term exposure to phenols (Au et al. 2003). In addition, results on crinoids demonstrated that PCBs and 4-nonylphenol can affect regenerative development by inducing marked abnormalities in growth rate, cell proliferation and migration, cell / tissue rearrangement and cytological disorders (Carnevali et al. 2001; Carnevali et al. 2003).

In our laboratory, we corroborated no clear patterns in the physiological endpoint, gonad index, after a waterborne exposure to different concentrations of tris-chloroisopropyl-

phosphate (TCPP) (a flame retardant used in plastics), the synthetic hormone ethinyl-estradiol (EE2) and the esterified hormone estradiol dipropionate (EDP), with individuals exposed during 7 and 28 days (Figure 4). No significant differences were found between groups of treatment individuals and solvent control (CTRL).

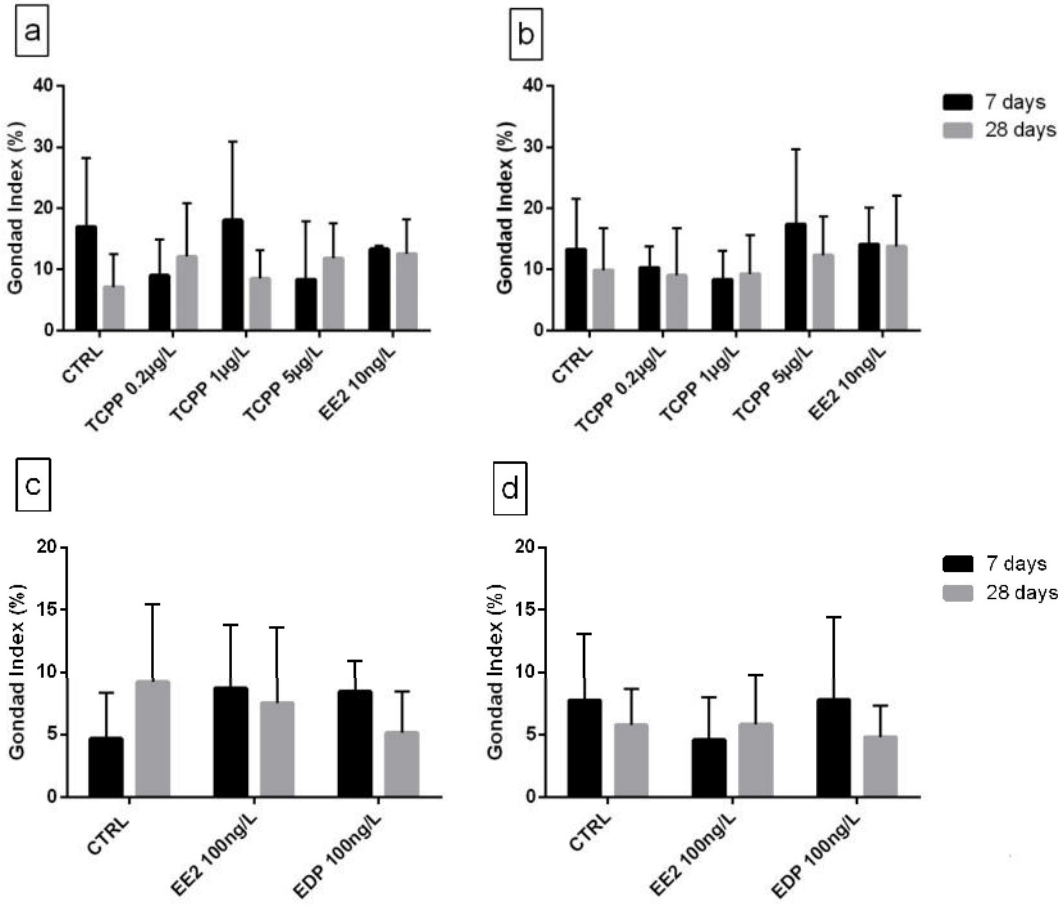


Figure 4. *Gonad Index (mean ± SD, n = 20) of female (a-c) and male (b-d) P. lividus sea-urchins exposed for 7 days (black bars) and 28 days (grey bars) to TCPP and EE2 (a-b) and EE2 and EDP (c-d). No significant data (P < 0.05) differences to control (CTRL).*

In sea-urchins, the size of the gonads, and thus the **gonad index** (gonad weight divided by body weight or diameter) **does not** necessarily **relate to the progress of gametogenesis**, and one must resort to observation of the relative size and abundance of germinal and somatic cells in histological sections in order to determine the stage of gametogenesis (Walker et al. 2013). Therefore, **changes in gonad size are not considered as a suitable endpoint** to study endocrine disruption, and gonad index does not correlate either with estrogen levels (Barbaglio et al. 2007).

Histological observation is time and labor consuming. Several scales to classify male and female gonad developmental stages are available (Fuji, 1960; Byrne, 1990). Differences among discrete stages are somewhat observer dependent and reading is not automatable. To overcome these limitations Mantilla-Aldana et al. (2019) developed a Pixelar Index based on image analysis of hematoxylin and eosin stained sections of the gonads (see figure 5). This stain makes a difference in the color pattern between germinal or gametogenic cells (which are basophilic and show violet color) and the nutritive phagocytes and non-germinal accessory cells (which are eosinophilic and show pink color). In order to consider the very different anatomy of the male and female gonads, two PI were calculated, the second taking into account not only the proportion of germinal vs somatic cells but also the empty spaces created in the gonads after spawning.

Pixelar Indices PI_1 and PI_2 are quantitative, objective and observer-independent tools to monitor the gametogenic cycle of female and male sea urchins. Both indices show a bell-shaped relationship with gametogenic stage and fit well to Gaussian models. However, in females PI values markedly increase until stage IV, whereas in males the shape of the curve is flatter and more symmetrical, and similar PI values are recorder for stages II, III and IV. This is consistent with a lower energy investment and a more sustained spawning period in male sea urchins. Research with data obtained from other populations and spanning all year round will be necessary to choose the best PI.

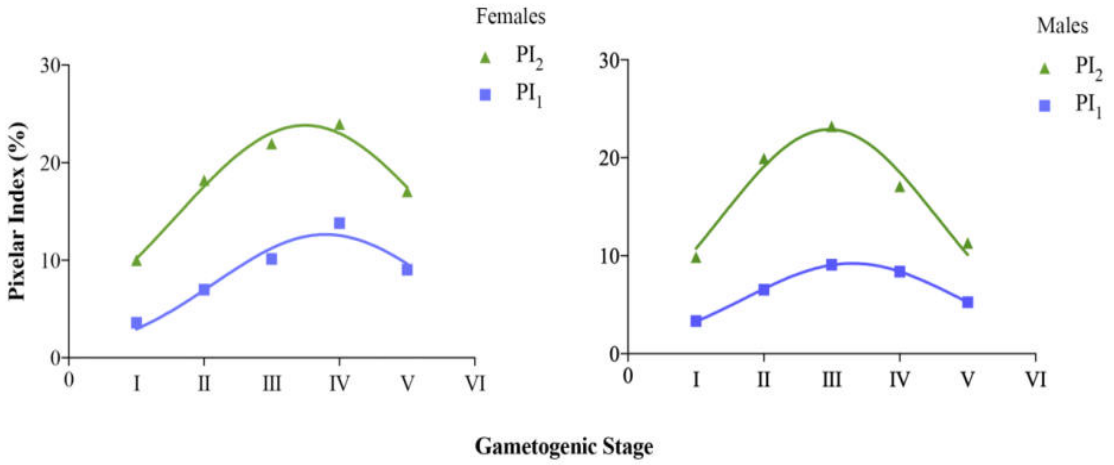


Figure 5. *Pixelar Indices, PI_1 (squares) and PI_2 (triangles) by sex depicted as a function of the gametogenic stage in P. lividus sea urchins. Data were correlated to bell-shaped Gaussian models with r^2 ranging 0.937-0.999.*

In our studies, Pixelar Index 1 was used for the assessment of the stage of development in male and female gonads after TCPP, EE2 and EDP exposure. In Figure 6, PI values of experimental studies carried out in our laboratory are shown. In females exposed during 28 days, significant data were achieved in EE2 (10ng/L) and TCPP (1ug/L) respect to solvent control. However, in a second experiment with an increase nominal concentration of EE2 and also EDP (100ng/L), no significant effects were achieved in any sex and time-point (see Figures 6c and 6d). For this reason, anatomical changes in the gonads demand long term exposures. Alternative tools for the observation of endocrine disruption related to gametogenesis and gonad development may be based on the distribution and abundance of proteins with functions related to gametogenesis and differential roles among sexes.

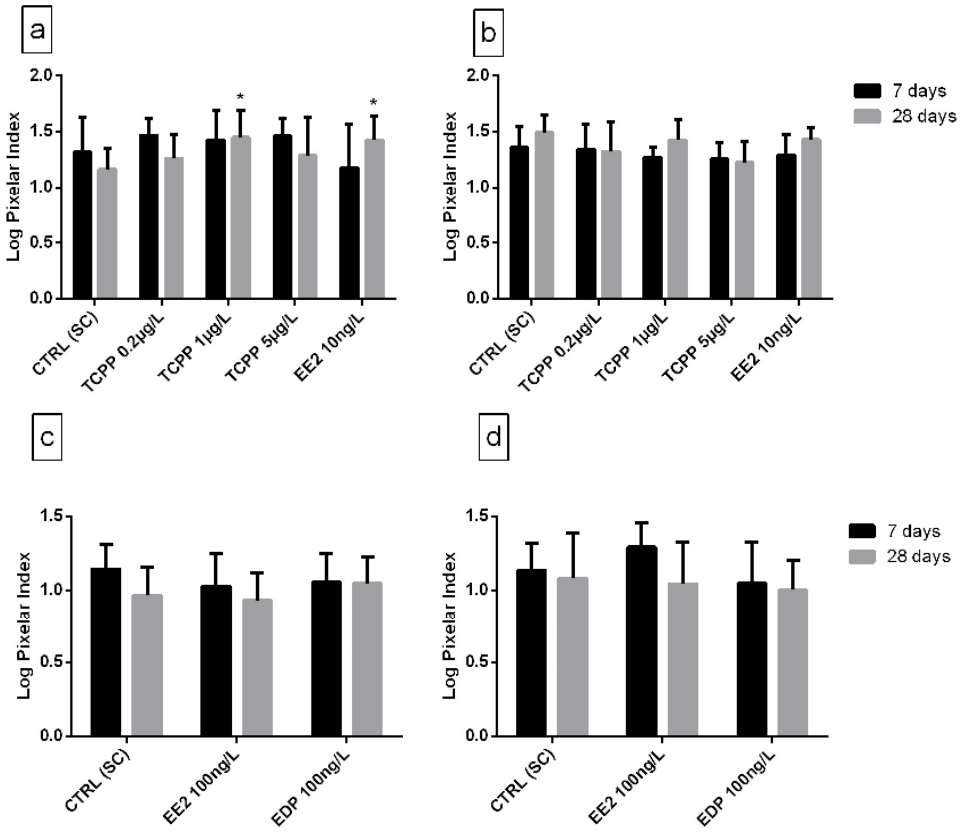


Figure 6. *Log Pixelar Index 1 (mean ± SD, n= 20) of female (a-c) and male (b-d), P. lividus sea-urchins exposed for 7 d (black bars) and 28 d (grey bars) to to TCPP and EE2 (a-b) and EE2 and EDP (c-d). Asterisks indicate significant (P < 0.05) differences to control (CTRL).*

6. Omics tools for the study of EDCs.

The omics tools provide global knowledge of all the macromolecules in a cell, tissue or organism at a given time, and allow exploring any change in mRNA or protein levels associated

with chemical exposure, including xenoestrogens. The complete genome sequence of *Strongylocentrotus purpuratus* has been determined (Sodergren et al. 2006), and information on the genomes of this and other echinoderms is publically available at the open Echinoderm genomic database (http://www.echinobase.org). This triggered omics studies on gene expression and protein synthesis searching for specific genes or proteins (e.g. MYP) that may serve as markers of the gametogenesis and gonad maturation in echinoderms (e.g. Song et al. 2006; Ghisaura et al. 2016).

Estrogen control of MYP expression has been suggested by several studies (Harrington and Ozaki, 1986; Shyu et al. 1987; Kiyomoto et al. 2008). In vertebrates, estrogens regulate the expression of both vitellogenin and transferrin genes. The hormone first binds to the estrogen receptor (ER) and then the resulting complex attaches to short DNA sequences known as estrogen responsive elements (EREs) located upstream of the modulated genes (Prowse and Byrne, 2012). A palindromic sequence, present in vertebrate EREs and essential for estrogen control, has been found upstream MYP gene, strongly suggesting an estrogen involvement in the protein expression (Shyu et al. 1987).

Information available in literature and the echinobase information system now includes genomic information for eight echinoderm species: *S. purpuratus, Strongylocentrotus fransciscanus, Allocentrotus fragilis, Lytechinus variegatus, Patiria miniata, Parastichopus parvimensis, Ophiothrix spiculata, Eucidaris tribuloides*. In recent years, deeper insights into the embryonic development of several sea urchin species have been obtained by using high-throughput transcriptome sequencing as in *H. erythrogramma* and *P. lividus* (Byrne et al. 2015; Gildor et al. 2016). Nevertheless, transcriptome studies on the molecular mechanisms underlying gonadal maturity of sea urchin, especially on the development of ovary and testis and the potential effects of EDCs, have been missing.

However, several lines of evidence have provided insights into transgenerational effects in which parental sea urchin exposure to environmental stressors modelled the phenotype of the offspring. In this scenario, it has been shown that parental exposure of *Strongylocentrotus intermedius* to warming affected hatching and larval morphology (Zhao et al. 2018). Similarly, progeny of metal conditioned *P. lividus* showed fertilization problems and development abnormalities (Migliaccio et al. 2014, 2015). Efforts were also carried out to define, both at specific gene level and transcriptome, the transcriptional profile of the offspring, and it has been observed that adult conditioning affects the gene expression patterns of the progeny during embryo development (Migliaccio et al. 2015; Wong et al. 2018). Noteworthy, the tissue

specific response of adult members of Echinodermata has been addressed (Matranga et al. 2012) and RNAseq studies characterized the transcriptome of different adult tissues including ovary and testis from the sea urchin species *Arbacia lixula* (Perez-Portela et al. 2016), *Loxechinus albus* (Gaitián-Espitia et al. 2016) and *Strongylocentrotus nudus* (Jia et al. 2017).

Although a number of issues still need to be addressed, in recent years, next-generation sequencing (NGS) technologies have emerged as important tools for obtaining genomic or transcriptomic data and gene regulatory networks (GRNs) (Lowe et al. 2017), which describe interactions for development process, where perturbation experiments represent one of the key methods that are going to help to understand issues in endocrine systems of echinoderms and the potential effects of xenoestrogens.

References

Alqaisi, K. M., Lamare, M. D., Grattan, D. R., Damsteegt, E. L., Schneider, W. J., & Lokman, P. M. (2016). A comparative study of vitellogenesis in Echinodermata: Lessons from the sea star. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 198, 72-86.

Barbaglio, A., Sugni, M., Di Benedetto, C., Bonasoro, F., Schnell, S., Lavado, R., ... & Carnevali, D. M. C. (2007). Gametogenesis correlated with steroid levels during the gonadal cycle of the sea urchin Paracentrotus lividus (Echinodermata: Echinoidea). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147(2), 466-474.

Barker MF, Xu RA (1993) Effects of estrogens on gametogenesis and steroid levels in the ovaries and pyloric caeca of *Sclerasterias mollis* (Echinodermata: Asteroidea). Invertebr Reprod Dev 24:53–58.7

Beiras, R. (2018). Marine pollution. Sources, Fate and Effects of Pollutants in Coastal Ecosystems. Elsevier, Amsterdam.

Botticelli CR, Hisaw FL, Wotiz HH (1961) Estrogens and progesterone in the sea urchin (*Strongylocentrotus franciscanus*) and Pecten (*Pecten hericius*) P.S.E.B.M. 106: 887-889.

Brooks, J. M., & Wessel, G. M. (2002). The major yolk protein in sea urchins is a transferrin-like, iron binding protein. Developmental biology, 245(1), 1-12.

Byrne, M. (1990). Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Marine Biology, 104(2), 275-289.

Byrne, M., Koop, D., Cisternas, P., Strbenac, D., Yang, J.Y., Wray, G.A., 2015. Transcriptomic analysis of Nodal- and BMP-associated genes during juvenile development of the sea urchin *Heliocidaris erythrogramma*. Marine genomics 24 Pt 1, 41-45.

Carnevali, M. C., Bonasoro, F. F., P. and S. Galassi. 2003. Regenerative potential and effects of exposure to pseudo-estrogenic contaminants (4-nonylphenol) in the crinoid Antedon mediterranea. Echinoderm Research 2001. Balkema Rotterdam, Netherlands: pp. 201-207.

Carnevali, M. D. C., F. Bonasoro, M. Patruno, M. C. Thorndyke and S. Galassi. 2001. PCB exposure and regeneration in crinoids (Echinodermata). Marine Ecology Progress Series, 215: 155-167.

Cervello, M., & Matranga, V. (1989). Evidence of a precursor-product relationship between vitellogenin and toposome, a glycoprotein complex mediating cell adhesion. Cell differentiation and development, 26(1), 67-76.

Coteur, G., B. Danis, S. W. Fowler, J. L. Teyssié, P. Dubois and M. Warnau. 2001. Effects of PCBs on Reactive Oxygen Species (ROS) Production by the Immune Cells of *Paracentrotus lividus* (Echinodermata). Marine Pollution Bulletin, 42 (8): 667-672.

Dev, S., & Robinson, J. J. (2014). Comparative biochemical analysis of the major yolk protein in the sea urchin egg and coelomic fluid. Development, growth & differentiation, 56(6), 480-490.

Dieleman, S.J., Schoenmakers, H.N.J., 1979. Radioimmunoassay to determine the presence of progesterone and estrone in starfish *Asterias rubens*. Gen. Comp. Endocrinol. 39, 534–542.

Fuji, A. (1960). Superficial and histological gonadal changes in gametogenic process of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius*. Bull. Fac. Fish. Hokkaido Univ, 11, 1-14.

Gaitán-Espitia, J. D., Sánchez, R., Bruning, P., & Cárdenas, L. (2016). Functional insights into the testis transcriptome of the edible sea urchin *Loxechinus albus*. Scientific reports, 6, 36516.

Ghisaura, S., B. Loi, G. Biosa, M. Baroli, D. Pagnozzi, T. Roggio, S. Uzzau, R. Anedda and M. F. Addis. 2016a. Proteomic changes occurring along gonad maturation in the edible sea urchin *Paracentrotus lividus*. Journal of Proteomics, 144: 63-72.

Gildor, T., Malik, A., Sher, N., Avraham, L., Ben-Tabou de-Leon, S., 2016. Quantitative developmental transcriptomes of the Mediterranean sea urchin Paracentrotus lividus. Marine genomics 25, 89-94.

Gratwohl, E. K. M., Kellenberger, E., Lorand, L., & Noll, H. (1991). Storage, ultrastructural targeting and function of toposomes and hyalin in sea urchin embryogenesis. Mechanisms of development, 33(2), 127-138.

Harrington, F. E., & Easton, D. P. (1982). A putative precursor to the major yolk protein of the sea urchin. Developmental biology, 94(2), 505-508.

Harrington, F. E., & Ozaki, H. (1986). The major yolk glycoprotein precursor in echinoids is secreted by coelomocytes into the coelomic plasma. Cell differentiation, 19(1), 51-57.

Hayley, M., Sun, M., Merschrod S, E. F., Davis, P. J., & Robinson, J. J. (2008). Biochemical analysis of the interaction of calcium with toposome: a major protein component of the sea urchin egg and embryo. Journal of cellular biochemistry, 103(5), 1464-1471.

Jia Z, Wang Q, Wu K, Wei Z, Zhou Z, Liu X (2017) De novo transcriptome sequencing and comparative analysis to discover genes involved in ovarian maturity in Strongylocentrotus nudus Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 23:27-38

Kari, B. E., & Rottmann, W. L. (1985). Analysis of changes in a yolk glycoprotein complex in the developing sea urchin embryo. Developmental biology, 108(1), 18-25.

Kiyomoto, M., Kikuchi, A., Morinaga, S., Unuma, T., & Yokota, Y. (2008). Exogastrulation and interference with the expression of major yolk protein by estrogens administered to sea urchins. Cell biology and toxicology, 24(6), 611.

Lavado R, Sugni M, Candia Carnevali MD, Porte C (2006a) Triphenyltin alters androgen metabolism in the sea urchin Paracentrotus lividus. Aquat Toxicol 79 (2006) 247–256.

Lavado R, Barbaglio A, Candia Carnevali MD, Porte C (2006b) Steroid levels in crinoid echinoderms are altered by exposure to model endocrine disruptors. Steroids 71:489–497.

Lowe, E. K., Cuomo, C., & Arnone, M. I. (2017). Omics approaches to study gene regulatory networks for development in echinoderms. Briefings in functional genomics, 16(5), 299-308.

Mantilla-Aldana L, Pereira-Pinto E, Campoy-López P, Beiras R (2019) Pixelar Index: a new approach quantitative method to the estimation of reproductive condition in the edible sea urchin, *Paracentrotus lividus*, by image analysis. Aquaculture International: under review.

Matranga, V., A. Pinsino, M. Celi, A. Natoli, R. Bonaventura, H. Schröder and W. Müller. 2005. Monitoring chemical and physical stress using sea urchin immune cells. Echinodermata. Springer. Berlin Heidelberg. 85-110.

Matranga V, Pinsino A, Randazzo D, Giallongo A, Dubois P (2012) Long-term environmental exposure to metals (Cu, Cd, Pb, Zn) activates the immune cell stress response in the

common European sea star (*Asterias rubens*) Marine environmental research 76:122-127.

Migliaccio O, Castellano I, Cirino P, Romano G, Palumbo A (2015) Maternal exposure to cadmium and manganese impairs reproduction and progeny fitness in the sea urchin *Paracentrotus lividus* PloS one 10:e0131815.

Migliaccio O, Castellano I, Romano G, Palumbo A (2014) Stress response to cadmium and manganese in *Paracentrotus lividus* developing embryos is mediated by nitric oxide Aquatic Toxicology 156:125-134

Noll, H., Alcedo, J., Daube, M., Frei, E., Schiltz, E., Hunt, J., ... & Lee, H. (2007). The toposome, essential for sea urchin cell adhesion and development, is a modified iron-less calcium-binding transferrin. Developmental biology, 310(1), 54-70.

Pérez-Portela R, Turon X, Riesgo A (2016). Characterization of the transcriptome and gene expression of four different tissues in the ecologically relevant sea urchin *Arbacia lixula* using RNA-seq Molecular ecology resources 16:794-808.

Prowse, T. A., & Byrne, M. (2012). Evolution of yolk protein genes in the Echinodermata. Evolution & development, 14(2), 139-151.

Scott, L. B., & Lennarz, W. J. (1989). Structure of a major yolk glycoprotein and its processing pathway by limited proteolysis are conserved in echinoids. Developmental biology, 132(1), 91-102.

Shyu, A. B., Blumenthal, T., & Raff, R. A. (1987). A single gene encoding vitellogenin in the sea urchin Strongylocentrotus purpuratus: sequence at the 5′ end. Nucleic acids research, 15(24), 10405-10417.

Sodergren et al., 2006. The Genome of the Sea Urchin *Strongylocentrotus purpuratus*. Science, 314 (5801): 941-952.

Song, J. L., J. L. Wong and G. M. Wessel. 2006. Oogenesis: Single cell development and differentiation. Developmental Biology, 300 (1): 385-405.

Sugni et al. (2007) Endocrine disrupting compounds and echinoderms: new ecotoxicological sentinels for the marine ecosystem. Ecotoxicology 16:95–108.

Sugni et al. (2012) Journal of the Marine Biological Association of the United Kingdom, 2012, 92(6), 1419–1426.

Takahashi N (1982a) The relation between injection of steroids and ovarian protein amounts in the starfish Asterina pectinifera. Bull J Soc Sci Fish 48:509–511 (in Jap.)

Takahashi N (1982b) Effect of injection of steroids on the starfish testes Asterina pectinifera. Bull J Soc Sci Fish 48:13–15 (in Jap.)

Unuma, T., Suzuki, T., Kurokawa, T., Yamamoto, T., & Akiyama, T. (1998). A protein identical to the yolk protein is stored in the testis in male red sea urchin, Pseudocentrotus depressus. The Biological Bulletin, 194(1), 92-97.

Unuma, T., Yamamoto, T., Akiyama, T., Shiraishi, M., & Ohta, H. (2003). Quantitative changes in yolk protein and other components in the ovary and testis of the sea urchin *Pseudocentrotus depressus*. Journal of Experimental Biology, 206(2), 365-372.

Unuma, T., Ikeda, K., Yamano, K., Moriyama, A., & Ohta, H. (2007). Zinc-binding property of the major yolk protein in the sea urchin− implications of its role as a zinc transporter for gametogenesis. The FEBS journal, 274(19), 4985-4998.

Unuma, T., Konishi, K., Kiyomoto, M., Matranga, V., Yamano, K., Ohta, H., & Yokota, Y. (2009). The major yolk protein is synthesized in the digestive tract and secreted into the body cavities in sea urchin larvae. Molecular Reproduction and Development: Incorporating Gamete Research, 76(2), 142-150.

Unuma, T., Nakamura, A., Yamano, K., & Yokota, Y. (2010). The sea urchin major yolk protein is synthesized mainly in the gut inner epithelium and the gonadal nutritive phagocytes before and during gametogenesis. Molecular reproduction and development, 77(1), 59-68.

Walker, C. W., M. P. Lesser and T. Unuma. 2013. Sea Urchin Gametogenesis – Structural, Functional and Molecular/Genomic Biology. In: M. L. John. Sea Urchins: Biology and Ecology. Elsevier. 25-43.

Wasson, K. M., Hines, G. A., & Watts, S. A. (1998). Synthesis of Testosterone and 5α-Androstanediols during Nutritionally Stimulated Gonadal Growth in *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea). General and comparative endocrinology, 111(2), 197-206.

Wasson et al. 2000a. Levels of progesterone, testosterone, and estradiol, and androstenedione metabolism in the gonads of *Lytechinus variegatus* (Echinodermata: Echinoidea). Comparative Biochemistry and Physiology Part C 126 (2000) 153 – 165

Wasson et al. 2000b. Progesterone metabolism in the ovaries and testes of the echinoid *Lytechinus variegatus* (Lamarck) Echinodermata Comp Biochem Physiol 127C:263-272.

Wasson KM, Gower BA, Watts SA. 2000c. Responses of ovaries and testes of *Lytechinus variegatus* to dietary administration of estradiol, progesterone and testosterone. Mar Biol 137:245–255.

Wasson & Watts 2013. Endocrine Regulation of Echinoid Reproduction. Chapter 5 in: JM Lawrennce (ed.) Sea Urchins: Biology and Ecology. Pp. 60-67. Elsevier.

Wong JM, Johnson KM, Kelly MW, Hofmann GE (2018). Transcriptomics reveal transgenerational effects in purple sea urchin embryos: Adult acclimation to upwelling

conditions alters the response of their progeny to differential pCO2 levels Molecular ecology 27:1120-1137.

Yamaguchi, A., Ishibashi, H., Kohra, S., Arizono, K., & Tominaga, N. (2005). Short-term effects of endocrine-disrupting chemicals on the expression of estrogen-responsive genes in male medaka (*Oryzias latipes*). Aquatic toxicology, 72(3), 239-249.

Yokota, Y., Unuma, T., Moriyama, A., & Yamano, K. (2003). Cleavage site of a major yolk protein (MYP) determined by cDNA isolation and amino acid sequencing in sea urchin, *Hemicentrotus pulcherrimus*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 135(1), 71-81.

Zhao C et al. (2018). Transgenerational effects of ocean warming on the sea urchin *Strongylocentrotus intermedius.* Ecotoxicology and environmental safety 151:212-219.