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Gonadal maturation in turn with ovulatory stimulation response to dietary inclusion of diverse seaweeds classes for *strombus cornis*

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Abstract

The global requirement for fish protein in growing, meeting challenges within the aquaculture industry, and addressing consumer demands becomes imperious. Enhancing fish reproduction and in turn promoting overall growth are among these challenges. To achieve such goals and benefit the aquaculture projection, various researches were conducted to explore a protein replacer of high nutritive value and achieve the desirable purposes. Owing to their lower costs and availability, seaweeds have attracted more attention as a good source of bioactive nutrients. Algae succeeded as a novel substitute of protein-based sources with potential nutritional benefits in aquaculture purposes. Thus, a rise in protein content of seaweed is duly contributory in improved ovary morphology and fish reproduction. Gastropods and other molluscs species are among the principal group of structurally unique natural output in Red Sea, Egypt. From the histological aspect, the outline of this plan was to estimate the potential effect of marine algae in the enhancement of reproductive performance of marine fish. Overall, 45 *Strombus cornis* conchs within 100 ± 20 g evenly classified into three experimental groups (n=15) incorporated with three successive seaweeds classes including *racemosa, J. rubens,* and *D. ciliolate*. Female gonads specimens were collected from control sea conches and experimental groups for histological monitoring. In comparison with the standard sea samples, seaweeds enhanced the reproductive histomorphology in *Strombus* species, promoting follicle maturity, oocytes sizes, and epithelial development. Seaweeds are an emerging tool with potential positive influence utilized to replace the protein in the feed formulation for aquaculture practices.

Keywords: Seaweeds, *Strombus cornis*, Reproduction, Gonad, Histology.

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Introduction

Growing in the world population encourages the desire for fish products. The aquaculture sector serves a crucial effect in boosting urgent demands (Fry et al., 2016). Thence, global aquaculture resumes growing higher than other agricultural projects (FAO, 2018). The nutritional viability of various foods constitutes a challenge for researchers, particularly related with uses of agro-industrial byproducts and co-products (Ayoub and Abdullah, 2012).

Accordingly, bloodstock feeding obtained more regard from both production and scientific fields (Bombardelli et al., 2017) because of the helpful effect of broodstock nutrition on reproduction and embryo efficiency (Ciji and Akhtar, 2023). Dietary nourishment in particular is essential for females during vitellogenesis (Bombardelli et al., 2021) reflecting gonadal maturity and vitellus efficacy. Moreover, nutrition modulates the reproductive pattern to favor the powerful reproductive function of fish (de Lima et al., 2020), and identify the quality of the offspring (Ng and Wang, 2011) where offspring and developmental larvae use vitellus for nutrition (Sarih et al., 2019). Nonetheless, data concerning the impact of feeding on aquatic reproduction are still deficient (Bobe and Labbé, 2010). This cause favor encourage the establishment of researches on phosphorus (Carvalho et al., 2018), and protein requirements (de Oliveira et al., 2014); energy-protein percent (Bombardelli et al., 2017); and a utilization of variable lipid sources (Ng and Wang, 2011).

Latterly, the aquafeed industry has improved feed quality through using alternative sustainable proteins to conventional terrestrial feed sources (Jones et al., 2020). Among these promising alternatives, natural feed supplements are applied than other antibiotics, hormones, prebiotics, and probiotics (Ljubic et al., 2020). Microalgae are used in aquaculture for varying purposes (Siddik et al., 2023). Marine microalgae are utilized as feed additives to improve reproductivity, productivity, prolificacy, and elevate immune responses of the animal (Ljubic et al., 2020).

Based on their pigmentation, marine algae are broadly categorized into three basic taxonomic grouped algae such as; red (*Rhodophyta*), green (*Chlorophyta*) and brown (*Phaeophyta*) (Øverland et al., 2019). The benefits of algae utilization occurred in the amelioration of the reproductive performance of fish, distinguished by increased gonad development, larvae performance, and egg yield (Cardona et al., 2022). Earlier studies confirmed the advantages of dietary algae inclusion for fish reproduction in many fish species (Carneiro et al., 2020). Dietary algae are rich sources in essential nutrients including amino acids, fatty acids, carotenoids, minerals, and vitamins (Shah et al., 2018) which are major constituents for reproduction comprising fertilization, fertility, improved egg quality, and larvae development (Salze et al., 2005). The incidence of high long-chained polyunsaturated fatty acids concentrations in algae also influences maturation and metabolism (Fernández-Palacios et al., 1995) favoring reproductive performance and upgrading egg quality (Cardona et al., 2022).

Owing to the economic concern of the gastropods, Strombidae family encompasses popular conches as crucial resource of protein for the domestic shoredwelling people (Rusmore-villaume, 2008). Herein, strombus species are used, among the family Strombidae recorded in Egypt. The histological method was devoted for an assessment of different feed impact on fish. This method confers additional information about status of the organs used; is valuable in laboratory and field experiment. It can exhibit signs of malnutrition, or negative influence of the feed on the tissue's morphology. Nutritional pathology can describe lower productivity resulted from inadequate feed in fish. It is also capable of explain an impact of the subacute environmental pollution on a delicate histostructure, most often in fishponds (Raškovi et al., 2011). Therefore, in this study; the histological technique was used to determine the probable capability of different groups seaweeds on the promotion of the reproductive status of *strombus cornis*.

Materials and Methods

Ethical statement

This trial procedure approved by Local Committee of the National Institute of Oceanography and Fisheries in Egypt according to Aquatic Animal Scientific Procedures Act with approval No (**NIOFAQ3I23R035**).

Experimental fish

Marine *Strombus tricornis* mussels within the initial body weights of 100±20 g were obtained from Abu Sadaf area, Red Sea, Egypt. The project was set up at the National Institute of Oceanography and Fisheries, Hurghada, Red Sea, Egypt. Fish allowed for adaptation at the laboratory conditions for 2 weeks at a minimum. A total of 45 *Strombus tricornis* were stocked into 9 glass aquariums (50×40×30 cm) at a stocking density of five fish aquaria each, where each treated group was graded in three treatment triplicate each. The water exchange occurred via underwater pumping system to transport saline water directly from the sea to the aquatic lab.

Marine algae or seaweeds

Three variant types of seaweeds were utilized in this study, were collected from Hurghada, Red Sea, Egypt. Seaweeds were well washed, cleaned, and air-dried then the resultant dried seaweeds were preserved in plastic containers for future uses (Ashour et al., 2020).

Formulated diets

The randomized-controlled design was established with three seaweed diets on the recirculating aquaculture system. Three experimental diets were formulated to be contained 10.2, 20.3 and 15.31g/kg crude protein and 3.51, 1.21 and 0.451 g/kg crude lipid for *racemosa, D. ciliolate,* and *J. rubens* marine algae, respectively. Meanwhile, in three algae treated groups, diets were formulated to contain green *racemosa* seaweed, red *J. rubens* seaweed, and brown *D. ciliolate* seaweed, respectively depending on their morphological taxonomy as recorded according to Sahoo (2001). The treatment period was prolonged for 12 weeks. During seaweeds exposure, *Srombus cornis* conches were carefully inspected for any clinical changes, also measurements of total length and width were taken.

Histological examination

The female oviduct and ovary specimens from the treated *strombus* conches and sea control samples were carefully extracted and fixed in neutral buffered formalin (NBF) of 10% concentration, accompanied by dehydration in successive series of absolute methanol to be ready for the clearness in xylene. The cleared specimens were softened in paraffin wax blocks. The prepared paraffin sections (5-μm thick) were stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2002), finally the sections were microscopically imaged using a microscopic camera. Besides this, three slides of the stained sections were randomly selected from each group for histopathological scoring as (-) not detectable, (+) mild, (++) moderate, and (+++) severe. While, measurements of the epithelium, and mature oocytes diameters were done on tissue images.

Statistical analysis

Statistics were run with SPSS Program by using one-way ANOVA. The significant differences between the means were carried out using Scheffe test when P<0.05. Illustrated numerical results were as the means ± SD (standard deviation of the mean).

Results

Effect on growth parameters

As illustrated in Table 1, the total length of female *strombus cornis* was gradually increased with significant differences among seaweed-treated groups. Additionally, the average of width significantly increased in-between green and brown supplemented *strombus cornis*.

Table 1 shows effect of different seaweeds diets on growth parameters of female *strombus cornis*.

Groups Parameters	Initial day	Green *racemosa*	Red *J. rubens*	Brown *D. ciliolate*
Length (cm)	9.2±0. 09	10.4±0.08*	10.32±0.55*	10.32±0.27*
Width (cm)	6.6±0.1	7.6±0.25*	7.8±0.60*	7.4±0.15

*** → referring to significant difference (*P*<0.05) when compared with control.**

Histological findings

Morphometric measurements

Based on the histological findings of the microscopic examination, the average diameters of the epithelial lining, and mature oocytes of the female oviduct-supplemented seaweeds indicated substantial differences with increases in their diameters expressed mostly developed gonads with vitellogenic oocytes in comparison with those of control findings at the same age and weights which still at the inactive premature stage as detailed in Table 2.

Table 2: shows effect of different seaweeds diets on average diameters in oviduct (µm). (X±SD)

Groups Lesions	Control	Green *racemosa*	Red *J. rubens*	Brown *D. ciliolate*
Ciliated epithelial cells	62.9±16.1	92.23±34.9*	61.3±36.1	82.8±43.38*
Secretory epithelial cells	37.64±5.05	64.46±19.0*	166.94±9.74*	117.35±24.18*
Vitellogenic oocyte	2.36±.0.69	4.087±1.7	6.26±1.91*	4.54±0.34*

*** → referring to significant difference (*P*<0.05) when compared with control.**

Histological lesions

As descriptive in Table 3, Figure 1; the oviduct histology of the control female *strombus* within the same weights and age, lack maturation distinctive by immature previtellogenic oocyte; and manifested decreased oocyte sizes and abundant connective tissue distribution. In addition, a variety of histological lesions were detected in the form of inflammation; characterized by interstitial lymphocyte infiltration, severely congested and dilated blood vessels, and besides this, the epithelial lining was sloughed and desquamated. Contrary to the marine algae treated groups, histology of the female *strombus cornis* oviduct exhibited vitellogenic and maturation stages. No histological changes were discovered between these groups compared to those of the control. Accordingly, treatment with *racemosa*, *J. rubens* and *D. ciliolate* seaweeds showed fully developed and reached complete maturation, where follicle supply ducts were expanded and filled with mature oocytes. Similarly, the ovary of marine algae treated conches showed maturation with diverse mature follicles packed with mature vitellogenic oocytes (Figure 2b, c, d) comparable to the immature control ovary (Figure 2 a).

Table 3 shows semi-quantitative histological lesions scoring of the female *strombus cornis* reproductive gonads.

Groups Lesions	Control	Green *racemosa*	Red *J. rubens*	Brown *D. ciliolate*
Desquamated epithelial lining	++	-	-	-
Interstitial inflammation	+	-	-	-
Inflammatory cells infiltration	++	-	-	-
Congested blood vessels	++	-		-
Connective tissues	++	+++	+++	+++

-, +, ++, and +++ referring to not detectable, mild, moderate and severe, respectively.

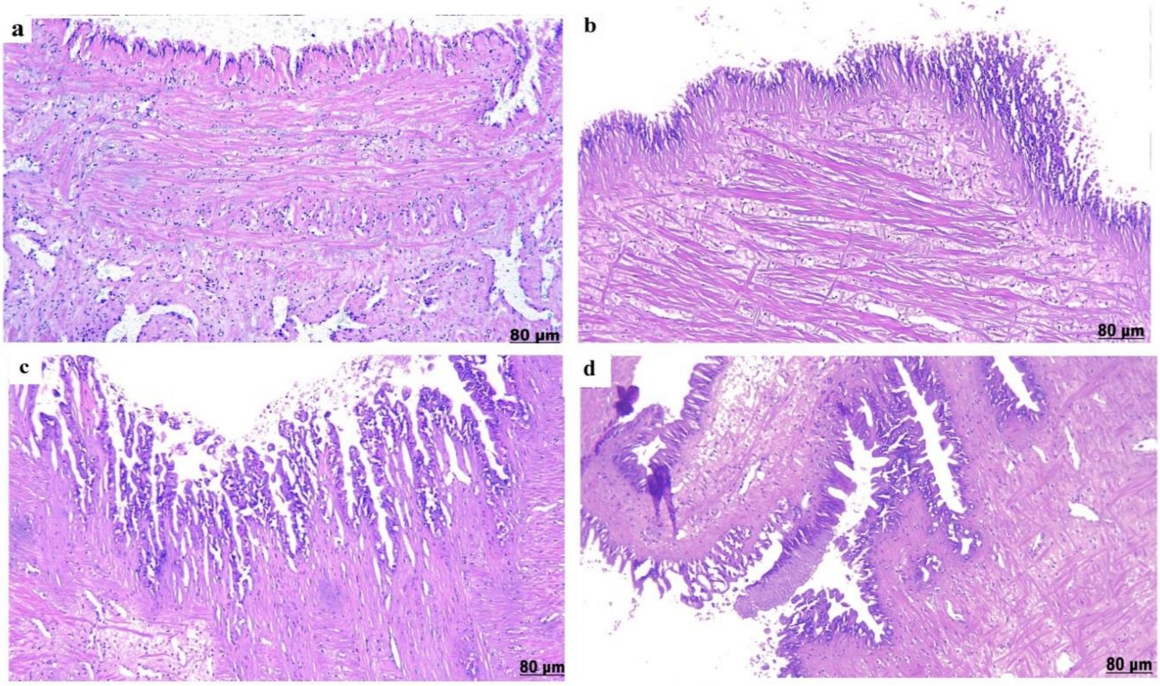

Figure 1: Transverse section stained with H &E of *strombus cornis* oviduct from control untreated **(a)** and seaweeds treated *strombus cornis* **(b, c, d)**: **a)** showing inactive oviduct suffered from remarked inflammation with diffuse mononuclear cell infiltration. **b, c, d)** showing active and distended oviducts with vitellogenic oocytes indicating oogenesis. **Scale bar= 80 µm**

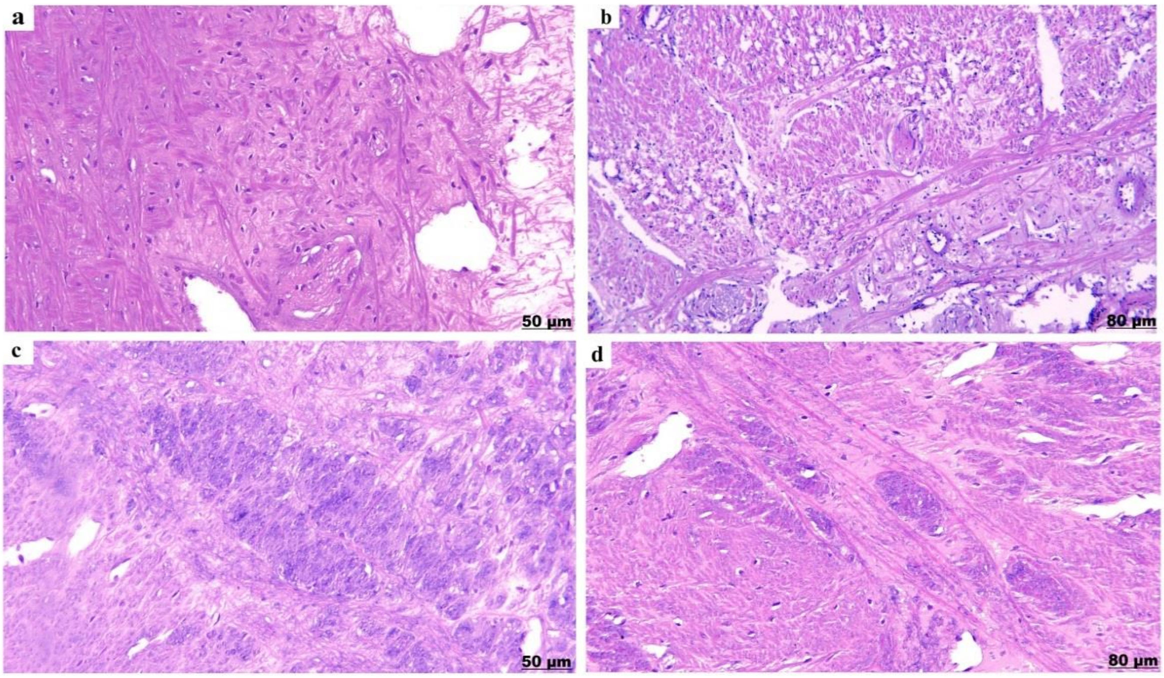

Figure 2: Transverse section stained with H &E of *strombus cornis* ovary from control untreated **(a)** and seaweeds treated *strombus cornis* **(b, c, d)**: **a)** showing inactive ovary with immature follicles. **b, c, d)** showing mature ovary with mature follicles filled with vitellogenic oocytes. **Scale bar= 50 & 80 µm**

Discussion

Utilizing novel compounds in aquatic feeding are needed for continual increase of aquaculture activity (Siddik et al., 2023). Global expulsion dominated by marine algae, grown in brackish and sea water. Seaweeds are relatively novel ingredients gained attractive consideration due to their essential bioactive contents in the aquatic nutrition (Patel et al., 2018). Reproductive biology is among of the major regards in formulation a real management for a fishery practice (Kao et al., 1999). The histological ovarian examination is a proper feature to assess ovarian changes (El Leithy et al., 2022).

Herein, dietary supplementation with seaweeds enhanced growth indices and increase the total length and width of fish. Several investigations illustrated the close link between fecundity and fish length in different fish species. Generally, an increase in fish length is accompanied by increased fecundity (Bahuguna and Khatri, 2009). Accordingly, a positive association was demonstrated between the total length and absolute fecundity of female *Cyprinus carpio* (Mohamad et al., 2018). Various studies have investigated the microalgae in aquaculture could alter the protein sources while upholding sustainability standards (Sarker et al., 2020). Diverse microalgae have been enclosed in aquafeeds formulation, as a major origin of vitamins, protein, lipids, and carotenoids (Shah et al., 2018). They are plentiful with trace elements; such as calcium, phosphorous, iron, magnesium, zinc, and iodine, and abundant in polyunsaturated fatty acids. Such health-promoting ingredients make algae meal favorable as natural supplements in aquafeeds (Siddik et al., 2023).

Usage of different types of marine algae was able to improve and enhance reproductive response of female *strombus cornis* in the current design. Overall, of 350 red species, 25 green species, and 90 brown species seaweeds inhabitant in the world seas are commercially important (Santhanam et al., 1990). Ultimately, gonad tissues exhibited intact architecture with complete development and maturity. Green, red, and brown marine seaweeds are abundant in variable bioactive constituents possessing beneficial biomedical and pharmaceutical potentials that are assigned as health promoter components in animal feeding. Bioactive compounds present in marine algae based on species, as well as some environmental factors included; season, geographic place, and harvest time are dependent (Eom et al., 2012). Macroalgae species poses to cover considerable quantity of tocopherols with powerful antioxidant roles. The dietary incorporation of red *Pterocladia capillacea* and green *Ulva lactuca* had a significant effect for Nile tilapia (*Oreochromis niloticus*) (Khalafalla and El-Hais, 2015). Correspondingly, brown algae contain high levels of α, β, γ, and δ-tocopherols, meanwhile green and red algae contain large amount of α-tocopherol, and with scarce amounts of the other tocopherols (Belghit et al., 2017).

Similar results obtained through Chamorro-Cevallos et al. (2008) who mentioned that blue-green *Spirulina* provoke protective effect on gonads. It induced possible antioxidant and antiapoptotic properties on the ovarian histomorphology, attributed to own multiple components included B-complex β-carotene, vitamins, chlorophyll, superoxide dismutase, and many minerals (Belay, 2002). *Spirulina* diminished lipid peroxidation and inhibit target tissues injury (Chamorro-Cevallos et al., 2008). Other authors reported that *spirulina* treated group revealed a prominent recovery of ovarian tissues resulted in improved corpora lutea and increased follicular number; moreover, decreased cystic and atretic follicles number. The more corpora lutea presence could be linked to changing of the estrous cycle to normal functioning (Rezvanfar et al., 2012). *SP* attenuate the ovarian histological alterations enhanced by drugs-associated ovarian toxicity (Yener et al., 2013).

Brown algae (*Colpomenia sp*.) excited an ameliorative effect in reproductive organs related to their antioxidant and immunostimulatory profit (Ramadan et al., 2013). Brown algae *Colpomenia sp.* profusely applied for medicinal and feed purposes returned to broad range of bioactive substances like essential amino acids, vitamins, minerals, indigestible carbohydrates (Chojnacka et al., 2012). Also, *Colpomenia sp.* is abundant with structural and biological polysaccharides (Shilpa et al., 2003). Brown seaweeds have much bioactive ingredients than those in green or red seaweeds (Seafood plus, 2008). They are served as antioxidant, antiinflammatory, antimicrobial, antiviral, and antitumoral (Bhagavathy et al., 2011).

Conclusion

Studying the relationship between feed quality and the reproductive potential of the fish is necessary to identify which success can be applied. Because of their lowered feed costs, high nutritive contents, and availability, seaweeds are prospective replacer for protein origin applied in cultured fish. The aforementioned discussed findings confirm the advantageous yield with the dietary incorporation of seaweeds in fish diets in the stimulation of gonads development and fish fecundity. In turn seaweeds in fish feeds cover favorable effectiveness on the reproductive performance of fishes.

Conflicts of interest

The authors emphasize no conflict of interest.

References

Ashour M, Mabrouk MM, Ayoub HF, El-Feky MMM, Sharawy ZZ, Hoseinifar SH, Rossi W, Van Doan H, El-Haroun E, Goda AMS (2020). Effect of dietary seaweed extract supplementation on growth, feed utilization, hematological indices, and non-specific immunity of Nile Tilapia, *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. J. Appl. Phycol., 32: 3467-3479.

Ayoub M, Abdullah AZ (2012). Critical review on the current scenario and significance of crude glycerol resulting from biodiesel industry towards more sustainable renewable energy industry. Renewable and Sustainable Energy Reviews, 16(5): 2671-2686.

Bahuguna SN, Khatri S (2009). Studies on fecundity of hill stream Loach *Noemacheilus montanus* (McClelland) in relation to total length, total weight, ovary length and ovary weight. Our Nature, 7:116-121.

Bancroft JD, Gamble M (2002). Theory and Practice of Histological Technique. (5th edition), Churchill Livingstone, Edinburg, London.

Belay A (2002). The potential application of *Spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement in health management. Journal of the American Nutraceutical Association, 5(2): 27-49.

Belghit I, Rasinger JD, Heesch S, Biancarosa I, Liland N, Torstensen B, Bruckner CG (2017). In-depth metabolic profiling of marine macroalgae confirms strong biochemical differences between brown, red and green algae. Algal research, 26: 240-249.

Bhagavathy S, Sumathi P, Bell IJB (2011). Green algae *Chlorococcum humicola*. A new source of bioactive compounds with antimicrobial activity. Asian Pac. J. Trop. Biomed., 1: 1-7.

Bobe J, Labbé C (2010). Egg and sperm quality in fish. General and comparative endocrinology, 165(3): 535-548.

Bombardelli RA, dos Reis Goes ES, de Negreiros Sousa SM, Syperreck MA, Goes MD, de Oliveira Pedreira AC, Meurer F (2017). Growth and reproduction of female Nile tilapia fed diets containing different levels of protein and energy. Aquaculture, 479: 817-823.

Bombardelli RA, Mewes JK, Buzzi AH, de Oliveira Pedreira AC, Syperreck MA, Dalmaso ACS, Meurer F (2021). Diets containing crude glycerin modify the ovary histology, cause reproductive harm on Nile tilapia females and impair the offspring quality. Aquaculture, 533: 736098.

Cardona E, Segret E, Cachelou Y (2022). Effect of micro-algae *Schizochytrium* sp. supplementation in plant diet on reproduction of female rainbow trout (*Oncorhynchus mykiss*): maternal programming impact of progeny. J Anim Sci Biotechnol., 13(1):1-18.

Carneiro WF, Castro TFD, Orlando TM, Meurer F, de Jesus Paula DA, Virote BDCR, Murgas LDS (2020). Replacing fish meal by Chlorella sp. meal: effects on zebrafish growth, reproductive performance, biochemical parameters and digestive enzymes. Aquaculture, 528: 735612.

Carvalho PLPF, Koch JFA, Cintra FT, Júnior ACF, Sartori MMP, Barros MM, Pezzato LE (2018). Available phosphorus as a reproductive performance enhancer for female Nile tilapia. Aquaculture, 486: 202-209.

Chamorro-Cevallos G, Gardu˜no-Siciliano L, Barr´on BL, Madrigal-Bujaidar E, Cruz-Vega DE, Pages N (2008). Chemoprotective effect of *Spirulina* (*Arthrospira*) against cyclophosphamide-induced mutagenicity in mice," Food and Chemical Toxicology, 46(2): 567-574.

Chojnacka K, Saeid A, Witkowska Z, Tuhy L (2012). Biologically active compounds in seaweed extracts: the prospects for the application. Open Conf. Proc. J., 3(Suppl 1-M4): 20-28.

Ciji A, Akhtar MS (2023). Impact of Climate Change on Fish Reproduction and Climate-Resilient Broodstock Management. In Outlook of Climate Change and Fish Nutrition, pp. 397-406. Singapore: Springer Nature Singapore.

de Lima SA, de Oliveira Pedreira AC, de Freitas JMA, Dalmaso ACS, Chiella R. J, Meurer F, Bombardelli RA (2020). Diets containing purified nucleotides reduce oxidative stress, interfere with reproduction, and promote growth in Nile tilapia females. Aquaculture, 528: 735509.

de Oliveira MM, Ribeiro T, Orlando TM, de Oliveira DGS, Drumond MM, de Freitas RTF, Rosa PV (2014). Effects crude protein levels on female Nile tilapia (*Oreochromis niloticus*) reproductive performance parameters. Animal Reproduction Science, 150(1-2): 62-69.

El Leithy AA, Al-Karmalawy AA, Youssif OM, Ebrahim YA, Khalifa, AS, Elkaeed EB, Abo-Zeid F S (2022). *Spirulina* therapeutic potentiality in polycystic ovarian syndrome management using DHEA-induced rat model. European Review for Medical & Pharmacological Sciences, 26(8).

Eom SH, Kim YM, Kim SK (2012). Antimicrobial effect of phlorotannins from marine brown algae. Food Chem Toxicol., 50:3251-3255.

Fernández-Palacios H, Izquierdo MS, Robaina L, Valencia A, Salhi M, Vergara J (1995). Effect of n- 3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata L.*). Aquaculture, 132(3-4): 325-337.

Food and Agriculture Organization (FAO) (2018). The state of world fisheries and aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

Fry JP, Love DC, MacDonald GK, West PC, Engstrom PM, Nachman KE, Lawrence RS (2016).

Environmental health impacts of feeding crops to farmed fish. Environ Int., 91:201-214.

Jones SW, Karpol A, Friedman S, Maru BT, Tracy BP (2020). Recent advances in single cell protein use as a feed ingredient in aquaculture. Curr Opin Biotechnol., 61:189-197.

Kao HC, Chan TY, Yu H P (1999). Ovary development of the deep-water shrimp *Aristaeomorpha foliacea* (Risso, 1826) (*Crustacea*: Decapoda: Aristeidae) from Taiwan. Zoological Studies Taipei, 38(4): 373-378.

Khalafalla MM, El-Hais AMA (2015). Evaluation of Seaweeds *Ulva rigida* and *Pterocladia capillaceaas* Dietary Supplements in Nile Tilapia Fingerlings. J. Aquac. Res. Development, 6: 312.

Ljubic A, Jacobsen C, Holdt SL, Jakobsen J (2020). Microalgae *Nannochloropsis oceanica* as a future new natural source of vitamin D3. Food Chem., 320: 126627.

Mohamad I, Bhat FA, Balkhi MH, Shah TH, Bhat BA, Wali A (2018). Relationship among body weight, body length, ovary weight and the fecundity of *Cyprinus carpio* Var. communis in Kashmir Himalaya. Journal of Pharmacognosy and Phytochemistry, 7(6).

Ng WK, Wang Y (2011). Inclusion of crude palm oil in the broodstock diets of female Nile tilapia, Oreochromis niloticus, resulted in enhanced reproductive performance compared to broodfish fed diets with added fish oil or linseed oil. Aquaculture, 314(1-4): 122-131.

Øverland M, Mydland LT, Skrede A (2019). Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. Journal of the Science of Food and Agriculture, 99(1): 13-24.

Patel PV, Vyas AA, Chaudhari SH (2018). Effect of seaweed (Ulva sp.) as a feed additive in the diet on growth and survival of *Labeo rohita* fry. Scire Science Multidisciplinary Journal, 2(3): 97-113.

Ramadan MM, Abo-Egla MH, Hussin MH, Ashry NI (2013). The ameliorative role of brown algae (*Colpomenia sp*.) against sodium arsenite induced testicular toxicity in mice. Egypt. J. Exp. Biol. (Zool.), 9(2): 189-198.

Rašković B, Stanković M, Marković Z, Poleksić V (2011). Histological methods in the assessment of different feed effects on liver and intestine of fish. Journal of Agricultural Sciences (Belgrade), 56(1): 87-100.

Rezvanfar MA, Rezvanfar MA, Ahmadi A, Saadi HS, Baeeri M, Abdollahi M (2012). Mechanistic links between oxidative/nitrosative stress and tumor necrosis factor alpha in letrozole-induced murine polycystic ovary: biochemical and pathological evidences for beneficial effect of pioglitazone. Hum Experim Toxicol., 31: 887-897.

Rusmore-Villaume ML (2008). Seashells of the Egyptian Red Sea. The illustrated handbook. The American Univ.

Salze G, Tocher DR, Roy WJ, Robertson DA (2005). Egg quality determinants in cod (*Gadus morhua L.*): egg performance and lipids in eggs from farmed and wild broodstock. Aquac Res., 36(15):1488-1499.

Santhanam RN, Remananthan N, Jagathusan G (1990). Coastal aquaculture in India. CBS Publishers & distributors, pp: 159-162.

Sarih S, Djellata A, Roo J, Hernández-Cruz CM, Fontanillas R, Rosenlund G, Fernández-Palacios H (2019). Effects of increased protein, histidine and taurine dietary levels on egg quality of greater amberjack (*Seriola dumerili, Risso*, 1810). Aquaculture, 499: 72-79.

Sarker PK, Kapuscinski AR, McKuin B, Fitzgerald DS, Nash HM, Greenwood C (2020). Microalgae-blend tilapia feed eliminates fishmeal and fish oil, improves growth, and is cost viable. Sci Rep., 10(1):1-14.

Seafoodplus (2008). http://www.seafoodplus.org/fileadmin/files/news/2004-01-22SFRTD1launchBrussels.

Shah MR, Lutzu GA, Alam A, Sarker P, Kabir Chowdhury MA, Parsaeimehr A, and Daroch M (2018). Microalgae in aquafeeds for a sustainable aquaculture industry. Journal of Applied Phycology, 30: 197-213.

Shilpa A, Agrawal SS, Ray AR (2003). Controlled delivery of drugs from alginate matrix. J. Macromol. Sci. Polym. Rev., 43(2): 187-221.

Siddik MA, Sørensen M Islam SM, Saha N, Rahman MA, Francis DS (2023). Expanded utilisation of microalgae in global aquafeeds. Reviews in Aquaculture. University in Cairo Press-Cairo, New York, pp. 158-294.

Yener NA, Sinanoglu O, Ilter E, Celik A, Sezgin G, Midi A, Aksungar F (2013). Effects of spirulina on cyclophosphamide-induced ovarian toxicity in rats: biochemical and histomorphometric evaluation of the ovary. Biochemistry research international.