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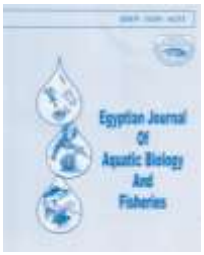

Involvement of thyroid hormone in the intestine and liver development of the common sole (*Solea solea* L.) larvae

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ABSTRACT

The role of thyroid hormone (TH) during fish larval development is well recognized particularly for flatfish as the common sole (*Solea solea*), while the consequences of thyroid homeostasis disruption during the larval-juvenile transitions are indistinct. To investigate these impacts, common sole newly hatched larvae were reared (triplicate) in four groups: the control group, the second is water-treated thyroxine (10 nM), the third and fourth groups were two doses goitrogen (perchlorate 30 and 100mg/L) for 30 days. By the end of exposure period, larval growth, survival, and whole body T4 and T3 were measured. In addition, thyroid follicles, intestine (intestinal fold height and goblet cell count) and liver were histologically examined. Exogenous Thyroxine significantly increased larval total length and number of goblet cells, and displayed larger thyroid follicles with more vacuoles, in addition to an increase in lipid accumulating hepatocytes. For goitrogen-treated fish groups, total weight, total length and survival significantly decreased and the 30 mg/L dose had more suppressive effect. The whole body T4 displayed significant decrease only for the goitrogen high dose, while no change was detected in the T3 content between treatments. Thyrocyte hypertrophy was observed in goitrogen-treated larvae groups and hepatocytes showed less lipid storage. Moreover, vacuoles between hepatocytes were detected for the high dose of goitrogen. These observations confirm the previously reported role of the TH in flatfish larvae development and growth. Moreover, the development and maintenance of both intestinal and hepatic tasks involve an intact thyroid endocrine axis.

INTRODUCTION

Common sole (*Solea solea*) is a widespread and highly valued Mediterranean flatfish, which is appreciated by the market and represents a good candidate for rearing on a commercial scale (Imsland *et al.*, 2003; Claireaux and Davoodi, 2010). As a flatfish, common sole undergoes metamorphosis, which is a vital stage where the symmetrical pelagic larva is converted into an asymmetrical benthic juvenile (Tanaka *et al*., 1996; Sæle *et al*., 2006). Metamorphosis for *S. solea* occurred 9-24 days after hatching (DAH) (Imsland *et al*., 2003; Palazzi *el al*., 2006) and a complete larval cycle. The differentiation of the digestive system in *S. solea* is achieved during the metamorphosis period, between 19 and 22 DAH, and by 30 DAH *S. solea* is able to digesting and absorbing nutrients (El-Dahhar *et al*., 2013).

The significant role of thyroid hormones (TH), thyroxine (T4) and triiodothyronine (T3), during flatfish metamorphosis is well acknowledged in (Schreiber and Specker, 1998; Solbakken *et al*., 1999; Einarsdottir *et al*., 2006; Klaren *et al*., 2008). Yamano (2005) explained clear increase in thyroid hormone at metamorphic peak for many teleost and lampreys larvae, whereas exposure to thyroid hormone inhibitors retards flatfish metamorphosis (Blanton and Specker, 2007; Manchado *et al*., 2008). The TH regulates several morphological, physiological and ecological changes during larval–juvenile conversion in fishes (McMenamin and Parichy, 2013). TH treatment induces early metamorphoses in Atlantic halibut (*Hippoglossus hippoglossus*), while exposure to agents that inhibit TH synthesis delayed or eliminate Japanese flounder metamorphosis (de Jesus *et al*., 1993; Solbakken *et al*., 1999).

Perchlorate is one of the goitrogens that are frequently used to manipulate thyroid state; as it inhibits TH synthesis and is used as experimental goitrogen (Bernhardt *et al*., 2006; Mukhi *et al*., 2007; Schmidt *et al*., 2012). Perchlorate blocks iodide uptake into the thyroid follicle (Wolff, 1998; Carr and Norris, 2006).

Dissimilar to other vertebrates, most teleosts do not have an organized compact thyroid gland; instead, they have dispersed thyroid follicles found individually or in clusters around the basibranchial region (Brown *et al*., 2004; Ortiz Delgado *et al.*, 2006; Geven *et al.*, 2007; Kawakami *et al.*,2008; Hsu *et al.*, 2014; Chalde and Miranda, 2017). For fish larvae, a few small follicles appear at the first stage of larval development, and then the follicles increase gradually in size and number (Miwa and Inui, 1987). These small follicles are able to produce thyroid hormone (Miwa and Inui, 1987; Yamano, 2005).

Follicles are spherical structures formed by epithelial cells and are surrounded by a basement membrane. The lumen enclosed inside the follicles is full of colloid and serves as a reservoir of thyroid hormone synthesis materials, precursors, and the hormone itself (Carr and Patiño, 2011). The TH is synthesized in the thyroid follicles epithelium by combining iodine with residues of the amino acid tyrosine (Solbakken *et al*., 2002) and stored in thyroglobulins in the colloid of the follicles (Yen, 2001). Thyroid hormones are excreted chiefly as T4 into the circulating flow then taken up by peripheral tissues and converted to the potent active form T3, through the enzymatic action of iodothyronine deiodinases (Klaren *et al*., 2005).

The TH is implicated in many processes, which control growth, development, differentiation and metabolism (Blanton and Specker, 2007; McMenamin and Parichy, 2013). Involvement of TH in gastrointestinal development in fish is recognized for summer flounder (*Paralichthys dentatus*) (Miwa *et al*., 1992; Huang *et al*., 1998) and zebrafish (Liu and Chan, 2002). Treatment of newly-hatched zebrafish with goitrogen delayed the intestinal development, while T4 co-treatment with TH brings back its normal development (Liu and Chan, 2002).

The association between TH and liver functions are well-established in vertebrates, thyroid hormones regulating liver metabolism, as well lipid metabolism and iodothyronine deiodinase activities (Malik and Hodgson, 2002; Blanton and Specker, 2007; Carr and Patiño, 2011). While the hepatic deiodinases contributing to the regulation of T3 creation and metabolism and consequently TH homeostasis (Darras *et al*., 1998; Malik and Hodgson, 2002; Brown *et al*., 2004).

Therefore, the objective of this study is to identify the roles of thyroid hormone in intestine and liver development during the entire period of *S. solea* larval progress, and to identify the consequence of thyroid activity disruption. This study examined histological impacts of thyroid endocrine disruption to the intestine and liver by

experimentally manipulating hyperthyroid and hypothyroid conditions. Thyroid hormone production was disrupted by goitrogen perchlorate; control (untreated) and T4 treatments were applied from first day of hatching to 30 DAH. Growth, total body T4 and T3 and tissue histological analyses were carried out on specimens by the end of exposure.

MATERIALS AND METHODS

General husbandry

Induced spawning:

Broodstock *Solea solea* were obtained from Damietta and maintained at the marine hatchery of the National Institute of Oceanography and Fisheries. Fish of 294± 30 g were kept in 1 m^3 flow-through sea water fiberglass rectangular tanks in a sex ratio of 1 female: 2 male. Water temperature was 16±1 ºC, salinity was 37±2 ‰ and fish were fed warms and shrimp. For spawning induction, priming dose of carp pituitary homogenate (CPH) (Argent chemical laboratories, Redmond) of 200 µg/kg fish was injected, second dose (resolving) of 200 µg/kg (LHRHa) (Argent chemical laboratories, Redmond) was injected twenty four hours later. 48 hours after injection fish were spawned average 118460 ± 2300 egg/fish, with 91 % fertilization rate and 89±4 % hatching rate.

Larvae maintenance

At the first day of hatching (DAH), groups of 300 larvae from same spawning batch were placed in 10 liter circular tanks (3 tanks for each experimental treatment) at 16±2 °C and SNP photoperiod throughout the study. After three days of the experiment start (3 DAH), green water was added and larvae were fed on Rotifer 10 ind/ml until 12 DAH, then from 10 DAH, larvae were fed on *Artemia* 4 ind/ml. Larvae from day 20 DAH till the end of the experiment were fed on newly hatched *Artemia* 8 ind/ml. Uneaten food and debris were removed and one third of the rearing water was replaced with appropriate treated water on daily basis. Water quality was checked regularly.

Experimental design

Sodium perchlorate monohydrate (JPH7630, Wako, Japan) was diluted in distilled water to prepare stock solution and then diluted to the required treatment concentrations. Treatment doses were selected according to that relevant to environmentally found concentrations (Crane *et al*., 2005; Furin *et al*., 2015). Sodium Thyroxine anhydrous slat (Eltroxine® tablets, Aspen bad Oldesloe, GmbH Germany). Thyroxine dose was selected according to Sharma and Patino (2013).

There were a total of four treatment groups: control, 10 nM thyroxine, and 30 and 100 mg/L perchlorate. Each treatment was included in a triplicate assembly (three tanks). Treatment exposures began at first day of hatching and ended after 30 DAH. By the end of the experiment, larvae were sampled and measured for total length (1 mm), and pooled (5 larvae) for total weight (0.001g). Whole larvae were placed in 4% buffered formalin solution for 48 hours and stored in 70% ethanol until histological processing.

Histological methods

Samples were dehydrated, cleared and embedded in paraffin blocks and frontal sections were cut at 7µm starting from the ventral side of the body. For thyroid follicles examination, sections were stained with hematoxylin and eosin. For goblet cells identification, sections were first stained with Alcian blue, followed by hematoxylin and eosin. Goblet cells were counted in mid and posterior regions of

intestine; also intestinal fold height was measured for 3 different individuals for each treatment tank. Height was regarded as the distance from the base of the fold to its apex. Microscopical observations were made with a compound microscope and microphotographs were taken with a Leica digital camera.

Thyroid hormones extraction and measurement

By the end of 30 days of exposure, 50 larvae from each rearing tank were collected, washed with distilled water and kept in -80 °C till extraction. Extraction of larvae thyroid contents (T4 and T3) was preformed according to Coa *et al*. (2016). Frozen larvae were homogenized in 0.5 mL 0.01 M PBS (pH = 7.0–7.2), then by centrifugation (8000 xg; 4 ºC) for 20 min, the supernatant was isolated and used for T3 and T4 measurement. Hormones were assessed by automatic immunodiagnostic analyzer (Sorin Biomedica, Model: 0-2730, S/N = 0654, Chemila SP.A., Italy), using T3 ELISA kit (EIAab, no E0453f, USA) and T4 ELISA kit (MBS701162, Biosource, USA) following the manufacturer's instructions.

Statistical analysis

Data are expressed as mean ± standard division. Significant differences between treated groups and control were tested using one-way analysis of variance (ANOVA) followed by Tukey –HSD test for multiple comparisons. Probability of a significant difference was set at $p<0.05$ .The analysis was carried-out using the SPSS® version 22.0 package (SPSS 1998).

RESULTS

Growth and Survival

Total larval weight for the P30 treatment group was significantly less than the control and T4 groups (1-way ANOVA and Tukey's MCT, $F=8.799$, $p<0.05$), while larval weight of T4 group was significantly higher than the P30 and the P100 groups but not significant different from control (Fig. 1a). Mean larval total length for both the P30 and P100 treatment groups was smaller relative to the control and the 10 nMT4 groups (1-way ANOVA and Tukey's MCT, $F=32.240$, $p<0.05$), while thyroxin raised the larval total length comparing to the control (Fig. 1b). Moreover, larvae of the P30 group were significantly smaller than the P100 group. Mean survival during the experimental period was near 90% in most treatments except in 30 mg/L perchlorate treatment, in which significant decrease in survival percentage was detected (78.22±1.17 %; Fig. 1c). Most mortality occurred in the first 10 days of the experiment.

Thyroid follicle changes

The thyroid follicles of control fish had oval thyroid follicles of variable size filled with colloid with peripheral vacuoles. The follicles were lined with squamous epithelium cells (thyrocyte) (Fig. 2a). The treatment with T4 for 30 days increased the size of thyroid follicles and the peripheral vacuoles but did not influence thyrocyte height (Fig. 2b). For goitrogen-treated groups, a mild colloid depletion in fish exposed to 30 mg/L perchlorate and maximum thickening of the follicle cell (hypertrophy) were detected, as well many small follicles with hypertrophy thyrocyte, less vacuoles and increase in number of follicles (hyperplasia) were observed comparing to control (Fig. 2c). For larvae exposed to 100 mg/L goitrogen, colloids were more sensitive than thyrocytes. Colloid depletion with cellular inclusions and accompanying partial or complete follicular collapse, follicular structure was often irregular and difficult to define. Thyroid tissue is very dense, characteristic of thyroid hyperplasia (increase in number) and hypertrophy (goiter) (Fig. 2d).

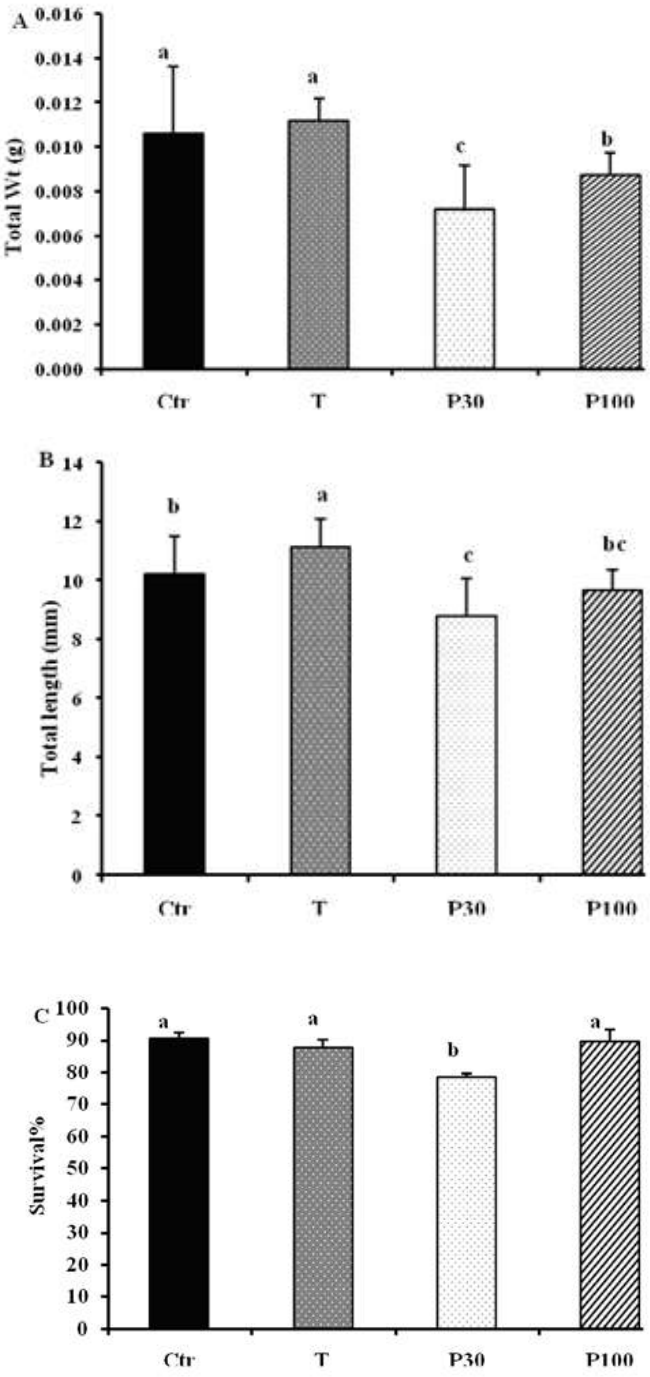

Fig. 1: Total weight (A), total length (B) and survival (C) of common sole larvae after 30 days of treatment for control (Ctr), exposed to 10 nM thyroxin (T), 30 mg/L perchlorate (P30), or 100 mg/L perchlorate (P100). Values represent mean ± SEM (n=6 for total weight), (n= 12 for total length), (n= 3 for survival), different letters indicate significant differences between exposure groups and the corresponding control group ($p< 0.05$).

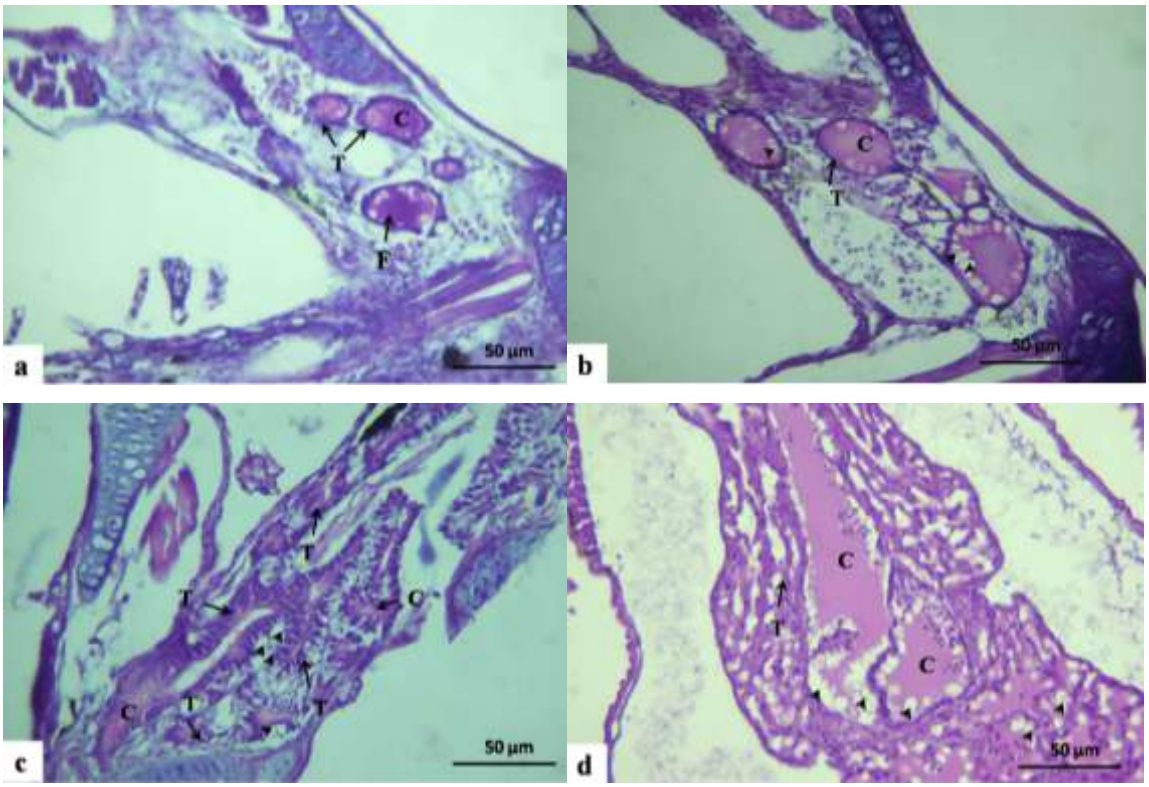

Fig. 2: photomicrograph of thyroid follicles of common sole larvae after 30 days of treatment, **a.** control untreated larvae displaying thyroid follicle (F), thyrocyte (T), colloid (c). **b.** T4 treated larvae with larger thyroid follicle and more large vacuoles (arrowheads). **c**. 30 mg/L perchlorate treated larvae with Hypertrophic thyrocytes (T), depleted colloid (arrowheads).**d**. 100 mg/L perchlorate treated larvae with disorganized or collapsed follicular tissue, E&H.

By the end of the experiment, the mean thyrocyte height was significantly larger in both P30 and P100 (goitrogen-treated groups) compared to both the control and the T4 treated groups (1-way ANOVA and Tukey's HSD, $F = 41.649$, $p<0.05$) (Fig. 3). The thyrocyte height for P30 treatment group was significantly higher than P100 treatment group.

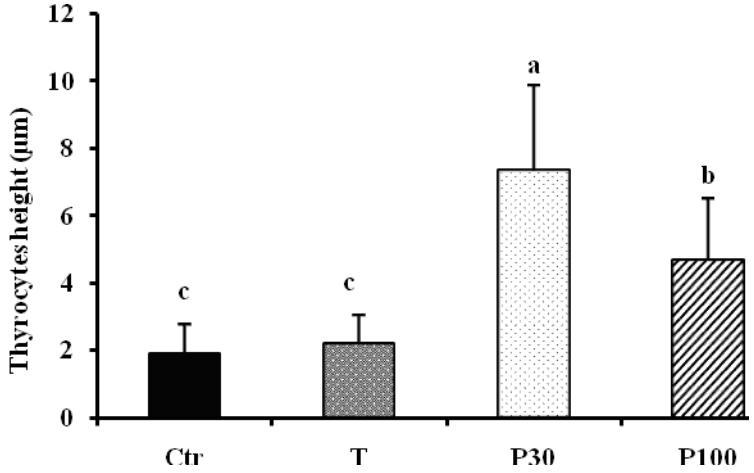

Fig. 3: Thyrocyte height (mean + SEM) of common sole larvae after 30 days of treatment for control (Ctr), exposed to 10 nM thyroxin (T), 30 mg/L perchlorate (P30), or 100 mg/L perchlorate (P100). Values represent mean ± SEM (n=9), different letters indicate significant differences between exposure groups and the corresponding control group ($p< 0.05$).

Whole-body T4 and T3 contents

Whole-body T4 was significantly lower in fish exposed to 100 mg/L perchlorate than in either fish exposed to 30 mg/L, thyroxine (T4) or control fish (F = 18.724, p<0.05; Fig. 4a). While, there was no significant difference between the whole-body T3 content of control group and that of any larvae group exposed to goitrogen or thyeoxine (Fig. 4b).

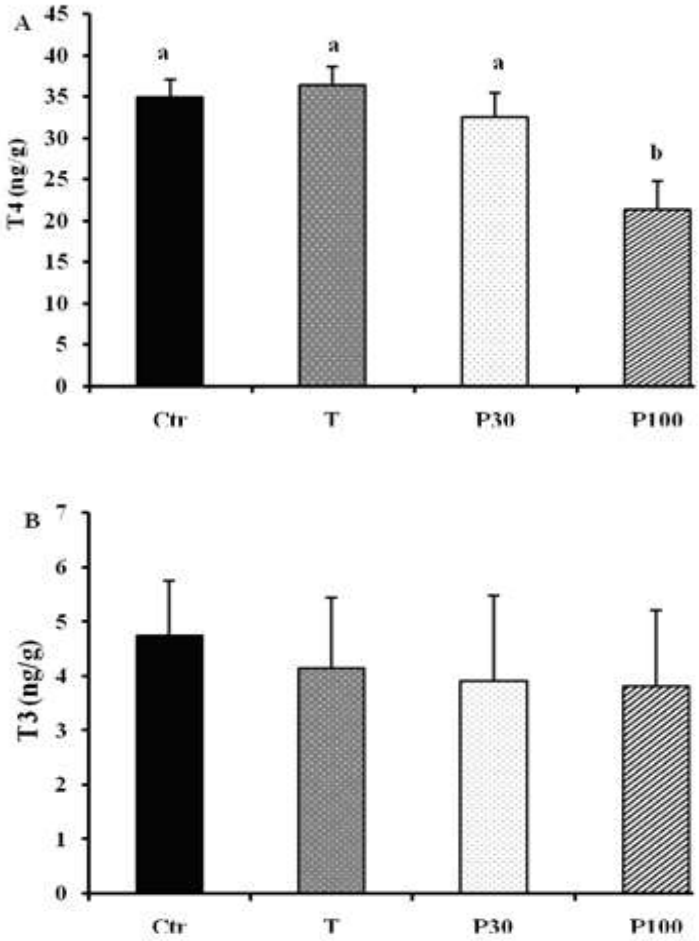

Fig. 4: Whole body contents of thyroid hormone levels A(T4) and B(T3)in common sole larvae after 30 days of treatment for control (Ctr), exposed to 10 nM thyroxin (T), 30 mg/L perchlorate (P30), or 100 mg/L perchlorate (P100). Values represent mean ± SEM (n=3), different letters indicate significant differences between exposure groups and the corresponding control group (p< 0.05).

Changes in the intestine and goblet cell

Intestinal folds were consistently longer in the anterior than the mid intestine; therefore anterior intestine was selected for comparison between different treatments. Treatment by goitrogen or T4 did not affect significantly the anterior intestine folds height (F=18.46, p=0.585).Some goblet cells were observed in the anterior intestine for the control and T4, while no goblet cell detected in P30 and P100 groups (Fig. 5).The P100 treatment group anterior intestine displayed less number of intestinal folds with some vacuoles (Fig.5d).The goblet cell count in the posterior intestine in the goitrogen (P30) treatment group showed significant suppression in goblet cell numbers (F = 3.502, p<0.05), while the T4 induced significant increase in goblet cell numbers comparative to untreated larval intestine (Fig. 6).

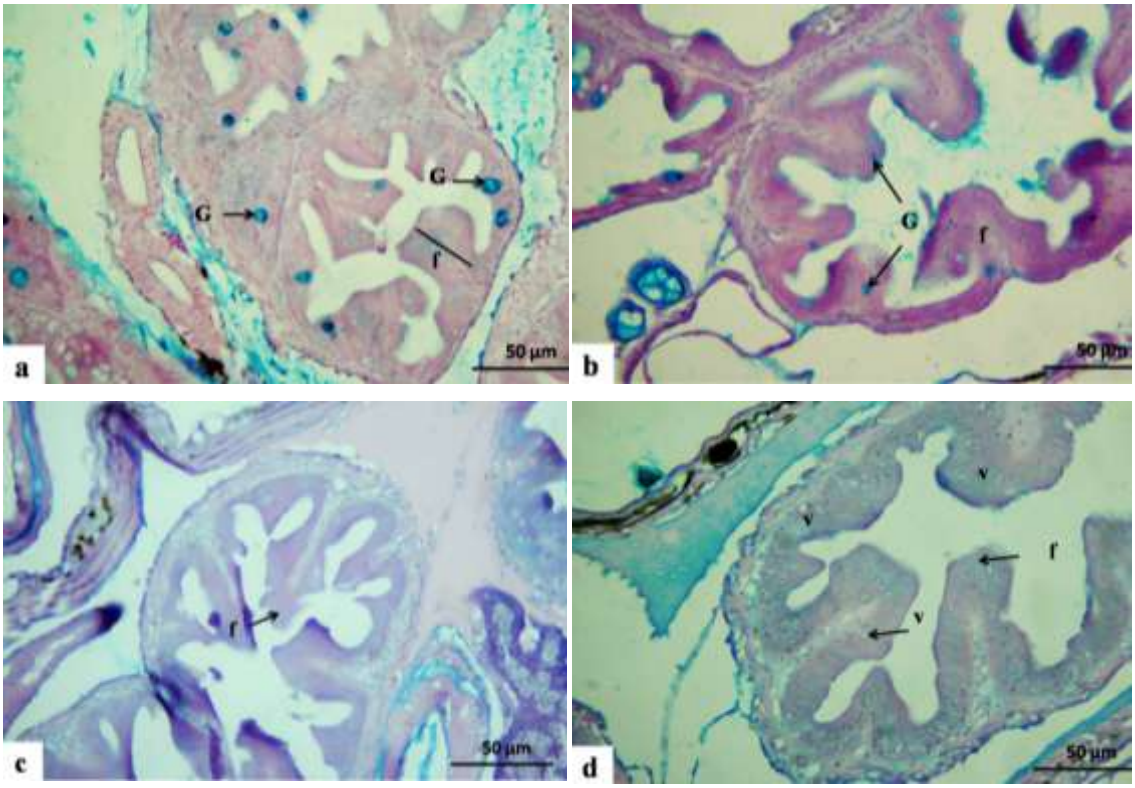

Fig. 5: phtomicrograph of a section of the anterior intestine of common sole larvae after 30 days of treatment for control (a), exposed to 10 nM thyroxin (b), 30 mg/L perchlorate (c), or 100 mg/L perchlorate (d),showing intestinal fold f, goblet cells G with blue coloration, vacuole v. Stained with Alcian blue, hematoxylin-eosin

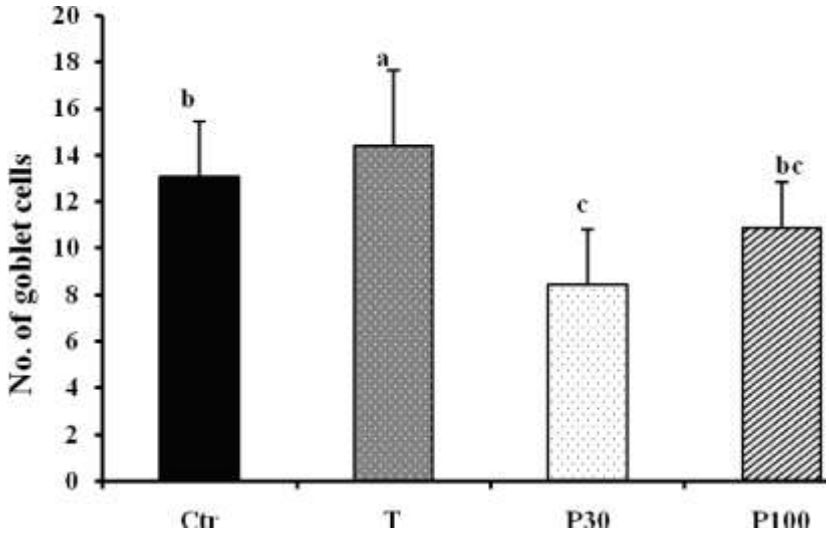

Fig. 6: goblet cell number in posterior intestineof common sole larvae after 30 days of treatment for control (Ctr), exposed to 10 nM thyroxin (T), 30 mg/L perchlorate (P30), or 100 mg/L perchlorate (P100). Values represent mean ± SEM (n=5), different letters indicate significant differences between exposure groups and the corresponding control group ($p< 0.05$).

Liver histology

Comparing liver histology for different treatment groups demonstrated that in both control and T4 groups, the liver displayed vacuoles with lipid accumulation (Fig. 7a&b), the hepatocytes had peripheral nuclei with large lipid vacuoles filling cytoplasm, while T4 induced more lipid accumulation in hepatocytes, which exhibit a severe degree of steatosis (Fig.7b). This accumulated lipids diminished to its minimum for both goitrogen-treated groups (P30 and P100), the hepatocytes cytoplasm was condensed and lacking of lipid inclusions (Fig.7c&d). Moreover, vacuolated liver tissue with degradation of some hepatocytes was observed in higher dose of goitrogen (P100) treated group (Fig. 7d).

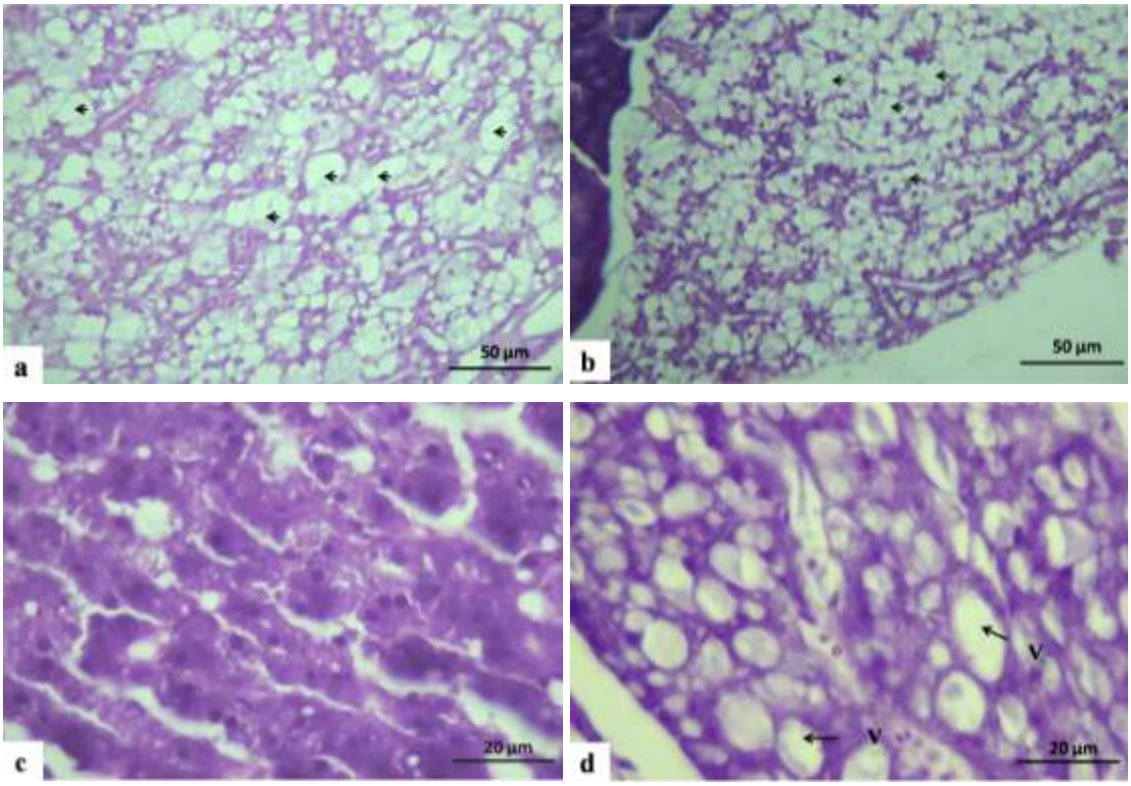

Fig. 7: phtomicrograph of liver sections of common sole larvae after 30 days of treatmentfor control (a), exposed to 10 nM thyroxin (b), 30 mg/L perchlorate (c), or 100 mg/L perchlorate (d). Note the relative increase in hepatocyte lipid accumulation in control and T4treated fish (arrowheads) (a&b), and the complete reversal of these conditions for P30 treatment (c), and vacuoles (v) in the hepatic tissue for P100 treatment (d), hematoxylin-eosin.

DISCUSSION

The results of the present study highlighted the importance of thyroid hormone homeostasis in flat fish larvae *S. solea*. The administration of exogenous T4 induced significant increase in larval growth, while thyroid hormone disruption by using goitrogen (perchlorate) at environmentally related concentrations affected the thyroid follicles structure and growth. Body size, weight, and survival were also greatly affected by thyroid disruption. Moreover, hepatic and intestinal development displayed marked histological changes.

In the present work using exogenous thyroxin stimulates and accelerate the growth of *S. solea* (total length), while goitrogen greatly suppresses the growth (total length and weight). The up-regulating role of exogenous thyroxin on the growth and

metamorphosis of flatfish is well-recognized (Yoo *et al*., 2000; Yamano, 2005). Whereas, the larval growth suppression was reported for some fish larvae under the goitrogenic effect (Goleman *et al*., 2002; Crane *et al*., 2005; Yamano, 2005; Sharma *et al*., 2016).Thyroid hormones are included in regulating the growth of fish and larvae, by directly or indirectly stimulation of growth hormone or insulin-like growth factors (Deane *et al*., 2003).

The combined analysis of both histological and hormonal levels allowed for a better understanding of the thyroid condition. While negligible effect of T4 treatment on the thyroid follicle cells height, marked increase in colloid size was observed, for larvae exposed to goitrogen (both two doses), the thyroid follicle cells height reported a significant increase than control and T4 treated groups.

The change of the whole body T4 concentration was limited as all treated groups were at the same levels with the control except for the high dose of goitrogen; where significant decline in the whole body T4 content was reported. Consistent with the present results, the T4 levels reported significant decrease only for the highest doses of goitrogen in zebrafish (Schmidt *et al*., 2012). These results may indicate that the whole body T4 is less predictive than thyroid histology as for *Gambusia holbrooki* **(**Bradford *et al*., 2005). The limited decline in T4 levels is possibly correlated to the feedback modulation for pituitary release of thyroid stimulating hormone (TSH) (Eales and Brown, 1993) that can initiate compensatory modulations in the thyroid action and restore the T4 production level. Similar results were reported for *Gambusia holbrooki* (Bradford *et al*., 2005; Mukhi and Patiño, 2007). Whereas goitrogens amplified the T4 concentration in whole body fathead minnows *Pimephales promelas* (Crane *et al*., 2005). These inconsistent observations with T4 could be related to the dose and duration of exposure of goitrogen (Brown *et al*., 2004).

It is notable that the levels of whole body T3 remain unchangeable between control and treatments; displaying more resistant than T4. The T3 homeostasis was reported under reduced T4 conditions caused by goitrogens in teleosts (Brown *et al*., 2004; Mukhi and Patiño, 2007; Mukhi *et al*., 2007; Petersen *et al*., 2015).

Our histological results showed a marked hypertrophy of thyroid follicular epithelial cells after goitrogen exposure (P30 and P100), and a significant increase in number (hyperplasia) of small thyroid follicles with enlarged thyrocytes when compared to control. Therefore, thyroid hyperplasia is very likely to have resulted from a hypothyroidism that take place during study period and hence stimulating release of TSH by negative feedback on the pituitary, followed by thyroid follicles stimulation for T4 production (Eales and Brown 1993). The goitrogen (perchlorate) disrupts the thyroid follicles structure in some studied fish as for fathead minnow, *Pimephales promelas* (Crane *et al*., 2005), zebrafish (Van der Ven *et al*., 2006; Mukhi *et al*., 2007; Sharma *et al*., 2016) and threespine stickleback (Petersen *et al*., 2015). Studies demonstrated that thyrocyte height and thyroid follicle morphology could be consistent markers of goitrogen effects in fish over the contradictory outcome of TH levels (Schmidt and Braunbeck, 2011).

Achievement of *S. solea* gut morphogenesis (conversion from a straight to a folded tube) with surge in intestinal goblet cells number occurs by about 30 DAH (El-Dahhar *et al*., 2013). Results showed that goitergen treatment affect the goblet cell density, the T4 had little effect, while TH changes had no significant effect on the intestinal fold height. Results of the present study suggested a role for the TH in the development of the *S. solea* gut. The negative effect of hypothyroidism on the goblet cell differentiation can be a result of suppressing growth and delaying

metamorphoses or presence of specific relations linking TH condition and intestinal morphogenesis. Some research studies pointed out the association of Thyroid endocrine axis during alimentary tract metamorphosis as for zebrafish (Liu and Chan, 2002; Chang *et al*., 2012; Sharma, 2012), and flatfish summer founder (Miwa *et al*., 1992; Tanaka *et al*., 1995; Huang *et al*., 1998; Soffientino and Specker, 2003)

The liver functions are influenced by TH. Thyroid hormones are potent modulators of liver lipid metabolism and iodothyronine deiodinase activities (Blanton and Specker, 2007; Carr and Patiño, 2011), while the hepatic deiodinases contributes to the regulation of T3 production and metabolism and finally TH homeostasis (Brown *et al*., 2004).

In the present study, both the control and the T4 treated larvae groups showed lipid storage in hepatocytes with many lipid-containing vacuoles occupying most of the cytoplasm, while both P30 and P100 larvae groups displayed hepatocytes with less accumulated lipid vacuoles. These lipid vacuoles were reported to increase in the liver throughout development when *S. solea* larvae were fed on *Artimia* (Piccinetti *et al*., 2012) and also for *Paralichthys californicus* (Gisbert *et al*., 2004). This lipid accumulation was considered as a sign of gut development, metamorphosis and ability of assimilation of ingested *Artemia* during larval growth (Luizi *et al*., 1999; Piccinetti *et al*., 2012).

Treatment by exogenous T4 enhanced the lipid storage in hepatocytes suggesting being a sign of faster metamorphoses than control. In contrast to the present results, administration of thyroxin caused depletion of liver lipid in *Anabas testudineus* (Varghese and Oommen, 1999). While the goitrogen treatment affect the lipid storage and cause lipid depletion in *S.solea* hepatocytes. Also, in contrast to the current results, administration of goitrogen perchlorate led to increasing of hepatocyte lipid accumulation for zebrafish (Schmidt *et al*., 2012). In the present work, the higher dose for perchlorate (P100) liver histology showed vacuoles between hepatocytes and degeneration in some cells which can be a signs of stress or hormonal disruption (Zhu *et al*., 2014).

In conclusion, results from the present study highlighted the role of TH axis balance on the *S. solea* larval growth, survival, intestinal growth and Hepatic lipid storage. Exogenous T4 increase the larval growth, while goitrogen cause retarded growth after 30 days of treatment. Results suggest that these alterations are caused by hypothyroidism conditions during the early life. Histological studies revealed that thyroid follicular epithelial cell height is a sensitive and appropriate biomarker for goitrogen exposure. T4 and goitrogen *S.solea* treated larvae were able to attain homeostasis of the whole body T3 and T4 levels. Intestinal goblet cells and hepatic lipid storage was noticeably depleted under the hypothyroidism condition. Future studies should include the alterations in the pituitary in response to TH changes to get more knowledge about Thyroid endocrine modulation.

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ARABIC SUMMARY

دور هرمون الثيروید thyroid في نمو الأمعاء والكبد في يرقات أسماك موسى (*Solea solea* L.)

رانيا فهمي خضر إسماعيل
معمل التناسل وتفريخ الأسماك – شعبة تربية الأحياء المائية – المعهد القومي لعلوم البحار والمصايد – الإسكندرية

إن الدور الذي يلعبه هرمون الثيروید thyroid أثناء مراحل نمو وتطور يرقات الأسماك معروف وخاصة في الأسماك المفلطحة مثل أسماك موسى (*Solea solea*) إلا أن آثار اضطراب اتزان الثيروید في المرحلة الانتقالية من اليرقات إلى الإصبعيات مازال غير محدد. للتحقق من هذه الآثار فقد تم تربية يرقات حديثة لأسماك موسى لمدة ٣٠ يوم في أربع مجموعات كل منها في ثلاثة أحواض حيث اشتملت على مجموعة مرجعية ومجموعة ثانية في ماء معالج ب ١٠ نانومول ثيروكسين و ثالثة في مياه بها جويتروجين (مثبط للثيروید) ببركلوريت بتركيز٣٠ مللجرام/لتر أما المجموعة الرابعة والأخيرة فكانت لماء به ببركلوريت بتركيز١٠٠ مللجرام/لتر. في نهاية فترة التجربة تم قياس إعاشة ونمو اليرقات والتركيزات لهرمونى (T4) و(T3). كذلك فقد تم الفحص الهيستولوجي لتجاويف حويصلات الثيروكسين والأمعاء والكبد.

تسببت المعاملة بهرمون الثيروكسين الخارجي في زيادة طول اليرقات وفي عدد خلايا جوبلت وأظهرت حجما أكبر لتجاويف حويصلات الثيروكسين مع المزيد من الفجوات ، بالإضافة إلى زيادة في الدهون المتراكمة فى خلايا الكبد.اما بالنسبة للمجموعات السمكية المعالجة بالجويتروجين، انخفض الوزن الكلي والطول الكلي، وكان للجرعة ٣٠ مللجرام/ لتر تأثير مثبط أكثر.

أظهر T4 في الجسم الكلي انخفاضا كبيرا فقط من الجرعة المرتفعة من الجويتروجين، في حين لم تطرأ أية تغييرات في محتوى T3 بين المعالجات المختلفة. لوحظ وجود تضخم في خلايا الثيروسيت لمجموعات اليرقات المعالجة بالجويتروجين وأظهرت الخلايا الكبدية تخزينا أقل للدهون، كما تم الكشف عن فجوات بين الخلايا الكبدية للجرعة العالية من الجويتروجين.

هذه النتائج تؤكد الدور المثبت لتأثيرهرمونات الثيروید في نمو وتطور يرقات الأسماك المفلطحة. بالإضافة لذلك فإن تطور والحفاظ على اتمام الوظائف المعوية والكبدية ينطوي على محورالاندوكرين للثيروید.