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Effects of 3,3′,4,4′,5-pentachlorobiphenyl on *in vitro* oocyte maturation in dusky tripletooth goby, *Tridentiger obscurus*: an implication of estrogenic potency

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Polychlorinated biphenyls (PCBs) are endocrine-disrupting chemicals that mimic estrogen in fish. Among the various PCBs, 3,3′,4,4′,5-pentachlorobiphenyl (PCB126), considered an estrogen antagonist, has been reported to elicit estrogenic activity in fish. We investigated the estrogenic potency of PCB126 in *in vitro* oocyte maturation in dusky tripletooth goby, *Tridentiger obscurus*. In this study, we quantified steroid metabolites following exposure to PCB126 and estradiol-17β (E_2). Vitellogenic ovarian follicles were incubated, *in vitro*, with 10 and 100 ng/mL PCB126 or E_2 with [^{3}H]17α-hydroxyprogesterone as a precursor. Testosterone (T) and E_2, were identified using thin-layer chromatography, high-performance liquid chromatography, and gas chromatography-mass spectrometry. Both PCB126 and E_2 increased T and E_2 metabolite production. Further, vitellogenic ovarian follicles were exposed to PCB126 (1, 10 and 100 ng/mL) or E_2 (0.01, 0.1 and 1 ng/mL) *in vitro*, and T and E_2 from the incubation media were measured. PCB126 (100 ng/mL) inhibited T production and increased E_2 production. Furthermore, we investigated the effects of PCB126 on the final oocyte maturation process. Germinal vesicle migration ovarian follicles were *in vitro* incubated with 0.1, 1, 10 and 100 ng/mL of PCB126 or E_2. High doses of PCB126 (10 and 100 ng/mL) inhibited germinal vesicle breakdown. These results suggest that PCB126 has an estrogenic potency in oocyte maturation in *T. obscurus*.

KEYWORDS

estrogen, 3,3′,4,4′,5-pentachlorobiphenyl, ovarian follicles, maturation, dusky triple tooth goby

1 Introduction

Considerable investigations have been carried out on various chemicals that can disrupt the endocrine system of humans and other animals (Colborn et al., 1993). These endocrine-disrupting chemicals (EDCs) include diverse chemicals such as pesticides, industrial detergents, sewage effluents, and polychlorinated biphenyls (PCBs). Several types of EDCs have the potential to mimic the estrogens of vertebrates, including fish (Scholz and Mayer, 2008; Diamanti-Kandarakis et al., 2009). Estrogens regulate physiological processes during vertebrate reproduction. In fish, vitellogenin production from the liver controlled by estradiol-17β (E_2), has been a powerful method for detecting the estrogenicity of EDCs (Jobling and Tyler, 2003; Navas and Segner, 2006). After vitellogenesis, progestins such as 17α,20β-dihydroxy-4-pregnen-3-one (17α20βP) and 17α,20β,21-trihydroxy-4-pregnen-3-one, induce final oocyte maturation, including germinal vesicle breakdown (GVBD) and ovulation (Nagahama et al., 1994; Das and Thomas, 1999). E_2 and estrogenic EDCs inhibit *in vitro* progesterone–induced GVBD in amphibian ovarian follicles Baulieu et al., 1978; Lin and Schuetz, 1983; Pickford and Morris, 1999). In addition, inhibition of progestin-induced GVBD by EDCs has been reported in fish oocytes (Ghosh and Thomas, 1995; Tokumoto et al., 2005; Hwang et al., 2010).

PCBs, including 209 congeners, have been used extensively in the industry as insulating materials, flame retardants, diluents, and detergents (Safe, 1994). PCBs have been reported to be environmental pollutants because of their high stability and bioaccumulation with lipophilic characteristics (Bonefeld-Jørgensen et al., 2001; Guruge et al., 2001). Among the various PCBs, 3,3',4,4',5-pentachlorobiphenyl (PCB126) is a coplanar congener and is known to be the most toxic PCB congener with dioxin-like properties (Safe, 1994). PCB126 is also an estrogen antagonist in mammals and fish (Lind et al., 1999; Wojtowicz et al., 2000; Muto et al., 2002; Vaccaro et al., 2005). In contrast, PCB 126 has been suggested to elicit estrogenic activity in fish (Gregoraszczuk et al., 2003a; Liu et al., 2006; Matthews et al., 2007; Mortensen and Arukwe, 2008a; Mortensen and Arukwe, 2008b). We reported that PCB126 does not have estrogenic or anti-estrogenic effects on *in vitro* steroid production from oocytes of the red lip mullet, *Chelon haematocheilus* (Baek et al., 2011). Gregoraszczuk et al., (2003b) reported that PCB126 has both estrogenic and anti-estrogenic effects in fish, depending on the time of exposure and developmental stage of follicles.

Despite the above contradictory studies, there are few studies with endocrine disrupting profiling of PCB126 on the maturation process of fish oocytes. Therefore, in the present study, we aimed to investigate the estrogenic potency of PCB126 on *in vitro* steroidogenesis in vitellogenic ovarian follicles of the dusky tripletooth goby, *Tridentiger obscurus*. Furthermore, we compared the estrogenic potency of PCB126 with that of exogenous E_2 treatment. We conducted a GVBD assay, liquid chromatography, and steroid hormone quantification to confirm the direct estrogenic potency of PCB126. Gobiid fish are appropriate subjects for investigating the effects of EDCs due to their small size, ease of handling, and strong tolerance even in the extreme environmental condition (Robinson et al., 2007). Therefore, we used the dusky tripletooth goby *Tridentiger obscurus*, an ideal model organism (Kaneko and Hanyu, 1985; Hwang et al., 2010), in the present study.

2 Materials and methods

2.1 Chemicals

Authentic steroids were purchased from Sigma Chemical (St. Louis, Missouri, USA) or Steraloids, Inc. (Wilton, NH, USA). Stock solutions (mg/mL) were prepared by dissolving the authentic steroids in pure ethanol. PCB126 (Aldrich Chemical, Milwaukee, WI, USA) was prepared as a stock solution (mg/mL) by diluting in ethanol. The ethanol concentration in the incubation medium was maintained at less than 0.1%. Testosterone (T) and E_2 were purchased from Sigma Chemical (St. Louis, MO, USA) or Steraloids, Inc. (Wilton, NH, USA). The antiserum for T was purchased from Sigma Chemical, and that for E_2 was kindly donated by Dr. Alexis Fostier (INRAE, Paris, France). Radioactive [^{3}H]-17α-hydroxyprogesterone ([^{3}H]-17αP), [^{3}H]-T, and [^{3}H]-E_2 were obtained from Amersham Life Sciences (London, England).

2.2 Experimental fish and histological observation of ovarian follicles

The dusky tripletooth goby used in this study were captured in coastal waters off Jeju Island, South Korea, during the spawning season (May–June). We isolated ovaries from 10 fishes. The ovaries were dissected manually into individual ovarian follicles using fine forceps. The ovarian follicles were separated in accordance with oocyte diameter of 0.53 and 0.78 mm from three different fish, respectively. And then we used these ovarian follicles for separated incubation experiment with duplicate (steroid metabolites analysis) or triplicate (RIA and GVBD assay). To determine the developmental stage of ovarian follicles, we conducted histological analysis. Gonadal fragments were processed by Midway and Scharf (2012). The paraffin-embedded samples were cut at 5-6 μm and sections were stained using Mayer's hematoxylin and eosin and observed under a light microscope (BX50, Olympus, Japan).

2.3 Cell culture

Three separate experiments were performed. Prior to culture, ovarian follicles were separated in an ice-cold balanced salt solution (132.96 mM NaCl, 3.09 mM KCl, 0.28 mM $MgSO_4{\cdot}7H_2O$, 0.98 mM $MgCl_2{\cdot}6H_2O$, 3.40 mM $CaCl_2{\cdot}6H_2O$, and 3.65 mM HEPES). Twenty ovarian follicles were incubated in 24-well culture plates containing 1 ml of Leibovitz L15 medium (Gibco, Grand Island, NY, USA).

To assess estrogenic potency of PCB126 on steroidogenic metabolism of ovarian follicles, we quantified steroid metabolites following exposure to PCB126 and exogenous E_2 in the presence of precursor. We incubated vitellogenic ovarian follicles with 10 and 100 ng/mL PCB126 or E_2 in the presence of [^{3}H]-17αP (55 kBq) as a precursor in two separate experiments. To evaluate estrogenic

potency of PCB126 on steroid production from vitellogenic ovarian follicles, we quantified T and E_2 levels following exposure to PCB126 and exogenous E_2. The vitellogenic ovarian follicles were incubated with only 1, 10 and 100 ng/mL of PCB126 or 0.01, 0.1 and 1 ng/mL E_2 in three separate experiments. For evaluation of estrogenic potency of PCB126 on final maturation process, we conducted GVBD assay. The fully vitellogenic ovarian follicles were separated and incubated with 0.1, 1, 10 and 100 ng/mL of PCB126 or E_2 in three separate experiments.

The plates were then incubated for 24 h at 18°C with gentle shaking. The pH and osmolality of the medium were adjusted to the plasma values of this species, 7.62 and 290 mOsm/kg, respectively.

2.4 Steroid metabolites

At the end of incubation with the radiolabeled precursor, the incubation media, and ovarian follicles were collected and stored at -80°C. Steroids were extracted three times from the medium and ovarian follicles, using 4 mL of dichloromethane. The extracts were concentrated and applied to a thin-layer chromatography (TLC) plate ($60F^{254}$; Merck, Darmstadt, Germany) with non-radioactive standard steroids as carrier steroids and then developed in a mixture of benzene:acetone (4:1) and benzene:ethyl acetate (4:1). Radioactive steroid metabolites were analyzed using a BAS 1500 bio-imaging analyzer (Fuji Film, Tokyo, Japan), and standards E_1 and E_2 were visualized by exposure to iodine vapor. Other standard steroids were detected using UV absorption at 254 nm. The migration zones corresponding to the carrier steroids, T and E_2, were eluted twice from the bands of the silica plates with 5 ml of dichloromethane:methanol (90:10). Following centrifugation at 1000 × *g* for 10 min, the supernatants were vacuum-dried before being dissolved in 20 µl of acetonitrile. The extracts were then analyzed using reverse-phase high-performance liquid chromatography (HPLC; Table 1). After HPLC analysis, metabolites with the same retention time as the standard were recovered and identified using gas chromatography-mass spectrometry (GC-MS, Table 2).

2.5 Radioimmunoassay

After incubation, steroids were extracted twice from aliquots of the medium, using ethyl acetate and cyclohexane. Then, T and E_2 levels were measured by radioimmunoassay (RIA), following Kobayashi et al., 1987. The intra-assay coefficients of variance were 2.5% (n=3) and 3.2% (n=3) for the T and E_2 assays, respectively, and the respective inter-assay coefficients of variance were 12.1% (n=5) and 11.3% (n=5). The minimum detectable limits were 10.5 and 12.2 pg/mL for T and E_2, respectively.

TABLE 1 HPLC instruments and analysis conditions for separation of steroid metabolites.

HPLC instrument	Waters Alliance
Column	Sunfire C_{18}, 4.6×150 mm
Mobile phase	20% methanol: acetonitrile = 60: 40
Flow rate	1 mL/min
UV detector	Waters 2487 Multi-wavelength Absorbance Detector
Injection volume	20 µL
Ending time	15 min

TABLE 2 Analytical conditions of gas chromatography-mass spectrometry for identification of steroid metabolites.

Instrument	GC/MS (Shimadzu 5050A)
Column	DB-5MS (30 m × 0.25 mm × 0.2 µm)
Flow rate (gas)	1 mL/min (He, 99.999%)
Injection mode	Splitless mode
Injector temperature	257.00 °C
Detector temperature	280.00 °C
Oven temperature	80.00°C (1.00 min) → 10.00 °C/min, 250.00 °C (0 min) → 3.00 °C/min, 280.00 °C (0 min) → 5.00 °C/min, 295.00 °C (4 min)

2.6 GVBD assay

For the GVBD assay, the incubated ovarian follicles were fixed with a clearing solution (ethanol:formalin:glacial acetic acid, 6:3:1) after 24 h of incubation. The location of the germinal vesicle (GV = nucleus) was observed under low-power magnification using a dissection microscope. The number of ovarian follicles completing GVBD, that is, the dissolution of the nucleus, was counted in each well and calculated as a percentage.

2.7 Statistics

The data from RIA and GVBD were expressed as mean with a standard error of the mean (SEM) from three separate experiments (n =3). These data were analyzed by Kolmogorov-Smirnov and Levene's test to verify whether the variance fulfilled the conditions of normality and homogeneity, respectively. The results were not satisfactory fit to the normal distribution and were therefore conducted in the non-parametric test. Differences between control and each treatment were analyzed by Kruskal-Wallis test followed by Bonferroni adjustment. Statistical significance was set at $p < 0.05$ using SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL, USA).

3 Results

3.1 Histological observations of the ovarian follicles

In 0.53-mm-diameter ovarian follicles, yolk globules (Yg) were accumulated into the cytoplasm, the oil droplets (Ods) were distributed over the cytoplasm, and the nucleus was present in the center of oocytes (Figure 1A). In 0.78-mm-diameter oocytes, Yg

and Ods continued to accumulate to the cytoplasm (Figure 1B). In these oocytes, one or two large Ods (40–50 μm) were observed around the nucleus, and the nucleus began to migrate.

3.2 Effects of PCB126 and E_2 on steroid metabolites from [^{3}H]-17αP

When vitellogenic ovarian follicles (0.53 mm in diameter) were incubated with [^{3}H]-17αP, the two major metabolites were separated and identified as T and E_2 by TLC, HPLC, and GC/MS (Figures 2, 3). Fractions of two progestins, co-migrated with standard 17α20βP and 17α,20α-dihydroxy-4-pregnen-3-one (17α20αP), were not identified because of their low levels. Steroid metabolites produced from [^{3}H]-17αP in the presence of PCB126 and E_2 were compared with the values obtained from photo-stimulated luminescence (PSL) autoradiography (Figure 4).

In T metabolites (Table 3), both 10 and 100 ng/ml PCB126 stimulated T synthesis (11.05, 13.74 and 10.82, 15.95 (PSL-BG)/mm^2, respectively), compared with the control (10.44 and 11.80 (PSL-BG)/mm^2). Exogenous E_2 treatment with 10 and 100 ng/ml also stimulated T synthesis (14.79, 15.52 and 12.50, 16.90 (PSL-BG)/mm^2, respectively) compared with the control.

In E_2 metabolites (Table 4), both 10 and 100 ng/ml PCB126 stimulated E_2 synthesis (23.31, 32.96 and 18.90, 31.95 (PSL-BG)/mm^2, respectively) compared with the control (18.76 and 25.89 (PSL-BG)/mm^2). Exogenous E_2 treatment with 100 ng/ml also stimulated E_2 synthesis (23.55 and 34.73 (PSL-BG)/mm^2); however, the second value in the treatment with 10 ng/ml (25.44 (PSL-BG)/mm^2) was decreased slightly compared to controls.

3.3 Effects of PCB126 and E_2 on steroid production

PCB126 treatment with 100 ng/mL decreased T production (152.60 ± 16.49 pg/mL) compared to the control (212.93 ± 7.31 pg/mL, $p < 0.05$), although there were no significant effects at 1 and 10 ng/mL of PCB126 treatment (Figure 5). In the exogenous E_2 treatment, E_2 decreased T production in a dose-dependent manner; however, the effect was not significant. E_2 treatment (1 ng/mL) increased E_2 production (3353.85 ± 385.93 pg/mL) compared with the control. (1228.91 ± 136.15 pg/mL, $p < 0.05$). Lower concentrations of E_2 (0.01 and 0.1 ng/mL) increased E_2 production slightly, although there was no significant effect.

In the ratio of E_2/T, as a sensitive biomarker of sex-steroid concentrations (Bevans et al., 1996; Folmar et al., 1996), PCB126 increased E_2/T significantly at the highest dose (12.07 ± 1.28) compared to the control (5.22 ± 0.68, $p < 0.05$). Exogenous E_2 treatment also increased E_2/T significantly at the highest dose (18.27 ± 3.44) compared to the control (4.52 ± 0.80, $p < 0.05$).

3.4 Effects of PCB126 and E_2 on GVBD

We incubated germinal vesicle migratory (GVM) ovarian follicles (0.78 mm diameter) with PCB126 or exogenous E_2. PCB126 decreased GVBD in all treatments, and 10 and 100 ng/mL PCB126 resulted in a significant decrease in GVBD (0.94 ± 0.77 and 0%) compared with the control (12.12 ± 1.52%, $p < 0.05$). In the exogenous E_2 treatment, 0.1, 10, and 100 ng/mL E_2 resulted in a decrease in GVBD, although there were no significant differences (Figure 6).

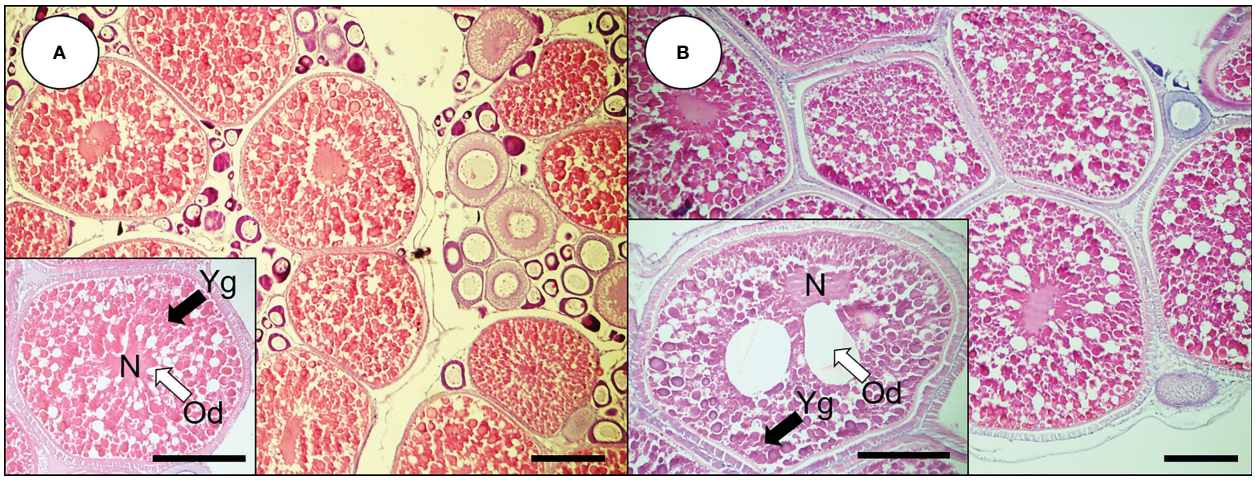

FIGURE 1
Histological observations of oocytes from dusky tripletooth goby. **(A)** oocytes of 0.53 mm; **(B)** oocytes of 0.76 mm. Scale bars indicate 200 μm. N, nucleus; Od, oil droplet; Yg, yolk granule.

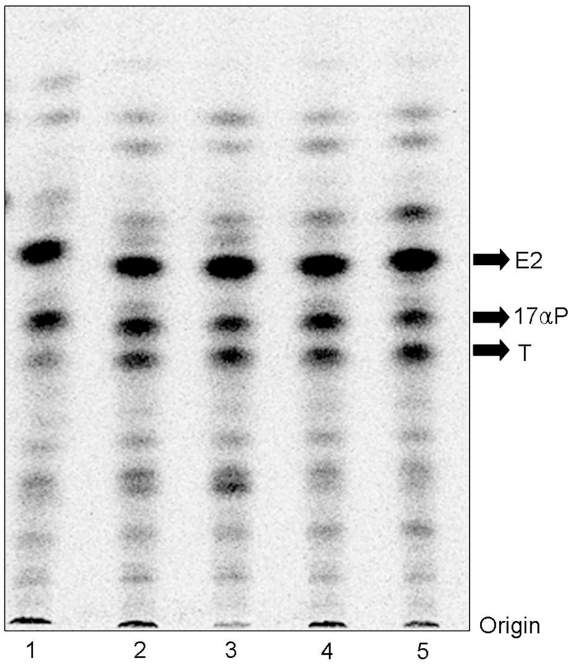

FIGURE 2
Autoradiograms of steroid metabolites incubated with [^{3}H]-17αP from dusky tripletooth goby oocytes. T and E_2 were separated by thin layer chromatography developed with a benzene: acetone (4: 1) and benzene: ethyl acetate (4: 1) mixture. 1, Control; 2, E_2 10 ng/mL; 3, E_2 100 ng/mL; 4, PCB126 10 ng/mL; 5, PCB126 100 ng/mL.

4 Discussion

In the present study, we investigated the estrogenic potency of PCB126 on *in vitro* steroid synthesis in vitellogenic ovarian follicles with an exogenously labeled precursor. In addition, we compared estrogenic potency in the final oocyte maturation process with that in GVM ovarian follicles. There have been controversies regarding PCB126 whether its effects are estrogenic or anti-estrogenic, although it is the most toxic PCB congener (Safe, 1994; Lind et al., 1999; Wojtowicz et al., 2000; Gregoraszczuk et al., 2003a, b; Vaccaro et al., 2005; Mortensen and Arukwe, 2008a, b; Gregoraszczuk et al., 2008; Sechman et al., 2016). The different effects may be caused by the diverse actions of the aryl hydrocarbon receptor (AhR) (Calo et al., 2010). In our previous study, PCB126 had no significant effect on *in vitro* E_2 production from the ovarian follicles of red lip mullet (Baek et al., 2011). Coupled with prior studies, Gjernes et al. (2012) hypothesized that PCB126 has estrogenicity *via* estrogen receptor (ER)-hijacking (modulating estrogenic responses with activated AhR in the absence of an ER agonist). Recent studies have also reported that PCB126 exhibits estrogenic activity (Pinto et al., 2018; Licata et al., 2019).

In the present study, we separated and identified two major metabolites, T and E2. The metabolites were scanned and the data were converted as a PSL value. According to Kamarainen et al. (2006), PSL provides high sensitivity and resolution and superior linearity compared with the other methods such as radioactivity scanning and film autoradiography. Our results indicate that PCB126 has estrogenic

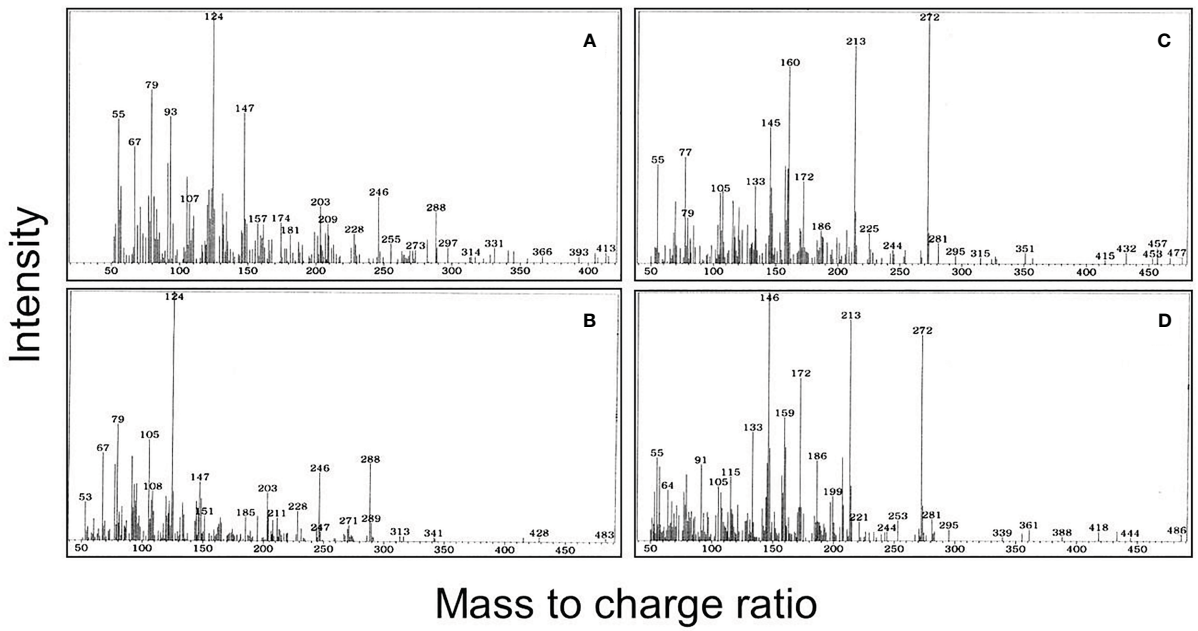

FIGURE 3
Mass spectra of steroid metabolized from dusky tripletooth goby oocytes were identified as T and E_2. **(A)** authentic T; **(B)** metabolized T; **(C)** authentic E_2; **(D)** metabolized E_2.

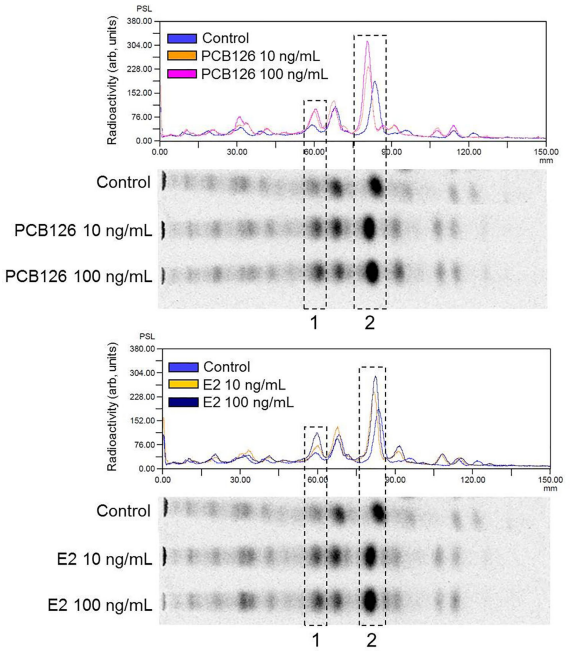

FIGURE 4
Comparison of radioactivity distribution on the TLC plate by digital photo-stimulated luminescence (PSL) autoradiography. The chromatograms are normalized to the maximum peak intensity in each chromatogram. 1, fraction of T metabolite; 2, fraction of E_2 metabolite.

potency in 0.53 mm diameter ovarian follicles; it stimulated not only E_2 metabolites but also E_2 production, *in vitro*. Since aromatase is the key enzyme for converting androgen to estrogen (Nagahama et al., 1994), we considered that PCB126 may stimulate aromatase activity, resulting in a decrease in T production and an increase in E_2 production from RIA results. These were remarkably analogous to the exogenous E_2 treatment. However, PCB126 and exogenous E_2 stimulated T metabolite biosynthesis in the presence of the labeled precursor. This difference may be due to different steroidogenic pathways and/or activities due to the absence or presence of the exogenous precursor, [^{3}H]-17αP.

Coupled with the results of the increase in E_2 metabolites, however, we could not identify two progestins that co-migrated with 17α20αP and 17α20βP in our LC system due to their low activity (data not shown). To date, maturation inducing steroid (MIS) of the dusky tripletooth goby has not yet been identified, although 17α20βP, the representative progestin, has been reported as a MIS in various teleost species (Nagahama and Yamashita, 2008). Previous studies have reported that 17α20αP may act as an MIS in several fish species (Canario and Scott, 1990; Hwang and Baek, 2021). The identification of these progestins, their maturational capacity, and the effects of PCB126 on progestin synthesis would provide more information about the estrogenic potency of PCB126.

PCB126 may act as an estrogen or anti-estrogen depending on the time and dose of exposure and different physiological conditions, even in different stages of ovarian development (Wojtowicz et al., 2000; Gregoraszczuk et al., 2003a; b; Mortensen and Arukwe, 2008b). Therefore, we studied the estrogenic potency of PCB126 in the final

TABLE 3 Effects of PCB126 and E_2 on metabolites of T from radioactive precursor.

Treatments	(PSL-BG)/mm^2 (% of control)	
	1st	2nd
Control	10.44 (100.00)	11.80 (100.00)
PCB126 10 ng/mL	11.05 (105.84)	13.74 (116.44)
PCB126 100 ng/mL	10.82 (103.64)	15.95 (135.17)
E_2 10 ng/mL	14.79 (141.67)	15.52 (131.53)
E_2 100 ng/mL	12.50 (119.74)	16.90 (143.22)

The values indicate digital photo-luminescence (PSL) from duplicate by autoradiography and were expressed as PSL-background (BG) per mm^2 of each fraction area.

TABLE 4 Effects of PCB126 and E_2 on metabolites of E_2 from radioactive precursor.

Treatments	(PSL-BG)/mm^2 (% of control)	
	1st	2nd
Control	18.76 (100.00)	25.89 (100.00)
PCB126 10 ng/mL	23.31 (124.25)	32.96 (127.31)
PCB126 100 ng/mL	18.90 (100.75)	31.95 (123.41)
E_2 10 ng/mL	19.03 (101.44)	25.44 (98.26)
E_2 100 ng/mL	23.55 (125.53)	34.73 (134.14)

The values indicate digital photo-luminescence (PSL) from duplicate by autoradiography and were expressed as PSL-background (BG) per mm^2 of each fraction area.

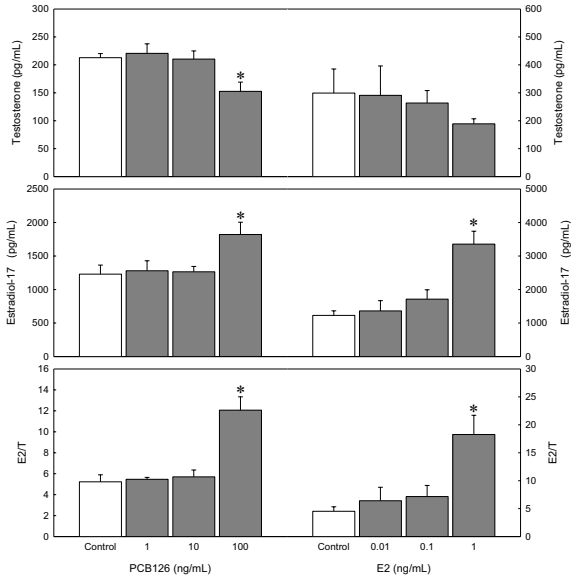

FIGURE 5
Effects of PCB126 on *in vitro* steroid production in dusky tripletooth goby oocytes (oocyte diameter=0.53 mm) after a 24 h incubation. Values are the mean ± SE of each steroid in three replicate wells with 20 oocytes/well. Data were analyzed using the Kruskal–Wallis test followed by the Bonferroni adjustment. Asterisks show significant differences from controls ($p < 0.05$).

oocyte maturation process in GVM ovarian follicles and hypothesized that PCB126 may also indicate estrogenic potency analogous to exogenous E_2 at this stage. Our results clearly indicate that PCB126 inhibits GVBD, similar to exogenous E_2 treatment. The inhibitory effect of high doses of PCB126 on GVBD was stronger than that of the exogenous E_2 treatment. However, we could not quantify steroid levels from the incubation media of 0.78-mm ovarian follicles due to problems with collecting media. We suspect that PCB126 may disrupt the activity of steroidogenic enzymes, increase E_2 levels and decrease progestogen levels such as 17α20βP, simultaneously. Prior to the maturation stage, there is a dramatic shift in the steroidogenic pattern; an increase in the formation of progestins concurrently with a decline in E_2 level (Nagahama et al., 1994). The shift in the steroidogenic pattern from the synthesis of estrogens and androgens toward the synthesis of progestins involves a decrease in 17-20 lyase activity and a rise in that of 20β-hydroxysteroid dehydrogenase (HSD). An inhibitory mechanism of PCBs on mammalian oocyte maturation was reported; PCBs acted as toxicants and thus inhibited maturation (Pocar et al., 2006). However, we need to consider the estrogenic potency of PCB126: it stimulates E_2 production directly so that inhibition of GVBD by its estrogenic effect rather than toxicity.

The estrogenic effect or estrogenicity defined by previous study (Scholz and Mayer, 2008) refers to (1) mimicking the action of estrogens and activation of responsive genes, or (2) elevation of endogenous plasma estrogen levels caused by competitive binding of EDCs to sex steroid-binding globulins. Although we did not conduct these experiments, our results suggest that PCB126 has an estrogenic potency, stimulates the biosynthesis of E_2 from vitellogenic ovarian follicles, and inhibits GVBD from GVM ovarian follicles with effects analogous to exogenous E_2.

5 Conclusion

In fish, the function of estrogens associated with reproductive characteristics (single or multiple spawning, vitellogenic or maturation stage, and so on) varies, and may regulate many other physiological processes (Nelson and Habibi, 2013). Up to date, PCB126, the most toxic PCB congener may act as an estrogenic agonist or antagonist in various species. The present study demonstrated that PCB126 has an estrogenic potency; (1) increase in E_2 synthesis and (2) decrease in GVBD *in vitro* during the oocyte maturation process of *T. obscurus*. Future studies with authentic steroidogenic pathway, changes in progestin levels and

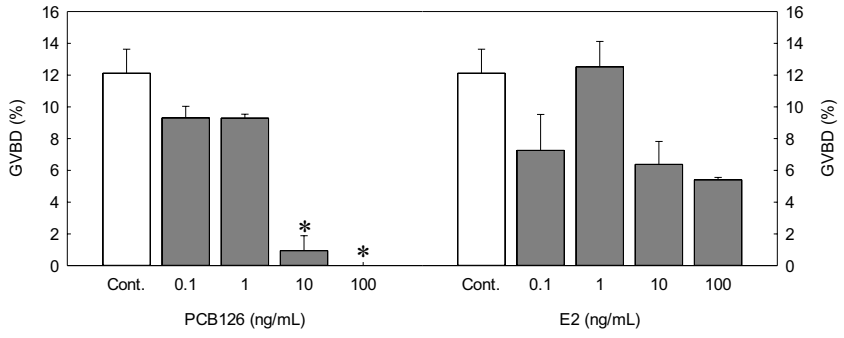

FIGURE 6
Effects of different PCB126 concentrations on *in vitro* GVBD in dusky tripletooth goby oocytes (oocyte diameter = 0.78 mm) after a 24 h incubation. Values are means ± SEM of the percentage of GVBD in three replicate wells with 20 oocytes/well. Controls were incubated with only incubation medium. Data were analyzed using the Kruskal-Wallis test followed by Bonferroni adjustment. Asterisks show significant differences from controls ($P < 0.05$).

the related steroidogenic enzymes during this period would be conducted for more comprehension in estrogenic mechanism of PCB126.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Animal Ethics Committee of Pukyong National University (PKNU; Regulation No. 554).

Author contributions

IH: conceptualization, methodology, formal analysis, investigation, writing – original draft, visualization. HB: conceptualization, writing – review & editing, visualization, supervision, project administration, funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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