

**Abstract.**—Gonadal development, and associated changes in reproductive parameters were investigated in adult female and male English sole (*Pleuronectes vetulus*) in Puget Sound to provide baseline data for future research on effects of contaminants on reproductive endocrine function in this species. Changes in gonadal histology, gonadosomatic index (GSI), and plasma reproductive steroids (female: 17 $\beta$ -estradiol [E2], testosterone [T]; male: T, 11-ketotestosterone [11KT]) were monitored throughout the spawning season. Female sole sampled July through early September were primarily regressed or previtellogenic and had low GSI and plasma steroid levels. GSI and plasma steroid levels were also low in male sole sampled during this time, but the majority had already entered spermatogenesis and, in some fish, production of mature sperm was observed. Fish sampled in October were in the early stages of vitellogenesis and spermiogenesis and showed increases in GSI and plasma steroid levels. By November, about 50% of female fish had entered vitellogenesis and about 30% of males were producing sperm. Proportions of vitellogenic females and sperm-producing males continued to increase through January, with significant numbers of spawning females and males present in February. By late March, the majority of both sexes were spent. Vitellogenic female sole had the highest plasma E2 levels, vitellogenic sole with hydrated oocytes had the highest GSI, and spawning female sole had the highest plasma T levels. Plasma T, 11KT, and GSI were highest in spawning male sole. Reproductive parameters returned to baseline levels in spawned out female and male sole. A potent maturation-inducing steroid (MIS) in many species of teleosts, 17 $\alpha$ , 20 $\beta$ -dihydroxy-4-pregnen-3-one, was not detected in spawning English sole. Additional research is needed to identify the MIS in English sole and to understand better the hormonal regulation of early gonadal development in male sole.

## Gonadal development and associated changes in plasma reproductive steroids in English sole, *Pleuronectes vetulus*, from Puget Sound, Washington

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English sole (*Pleuronectes vetulus*) is a commercially important flatfish species indigenous to the west coast of North America. It is also a primary sentinel species for a number of environmental monitoring programs on the west coast of the United States, including the National Benthic Surveillance Project (Myers et al., 1994) and the Puget Sound Ambient Monitoring Program (PSWQA<sup>1</sup>). Studies suggest that female English sole are quite sensitive to environmental contaminants. For example, female sole from several sites within Puget Sound, WA, with high levels of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (in sediment), exhibit various types of reproductive dysfunction, including depressed sex hormone levels, altered or inhibited reproductive development, and reduced egg and larval viability (Johnson et al., 1988, 1993; Casillas et al., 1991; Collier et al., in press).

Male English sole may also be at risk for reproductive injury. Recent studies suggest that exposure to certain chemical compounds (e.g.

alkylphenols, phthalates, and some PCBs and chlorinated pesticides) can reduce testicular growth and sperm production in male teleosts (Jobling et al., 1996; Nimrod and Benson, 1996), and alterations in reproductive steroids have been reported in male fish exposed to oil and other pollutants (Truscott et al., 1992a; Idler et al., 1995). However, no comprehensive studies have been conducted on the effects of environmental contaminants on reproductive function in male English sole. Before such studies can be carried out effectively, the normal reproductive endocrine cycle of male English sole must be characterized.

The general life history of English sole is well known (Ketchen, 1947; Harry, 1959; Garrison and Miller, 1982), and its cycle of oocyte development and related changes in estradiol-17 $\beta$  (E2) and other plasma parameters have been described

<sup>1</sup> PSWQA (Puget Sound Water Quality Authority). 1995. 1994 Puget Sound update: fifth annual report of the Puget Sound Ambient Monitoring Program. Puget Sound Water Quality Authority, Olympia, WA, 122 p.

(Johnson and Casillas, 1991; Fargo and Tyler, 1994). However, no information exists on other reproductive steroids involved in oocyte development and spawning in female English sole, or on changes in reproductive parameters in male English sole during gonadal recrudescence. This study was designed to gain a better understanding of reproductive processes in both female and male English sole. This information can be used to assess the impact of environmental degradation on their reproductive development and to develop effective techniques for flatfish mariculture. It may also be useful in evaluating the reproductive status of English sole stocks that are a fisheries resource off the northwest coast of the United States.

Wild populations of female and male English sole were sampled during the 1992–93 and 1994–95 spawning cycles at two residential sites as well as at a known spawning ground in Puget Sound, Washington. Several reproductive parameters, including gonadosomatic index (GSI), histologically determined stages of gonadal development, and steroid hormones (female: plasma testosterone [T] and E2; male: T and 11-ketotestosterone [11KT]) were monitored throughout the spawning season. Additionally,  $17\alpha, 20\beta$ -dihydroxy-4-pregnen-3-one ( $17\alpha, 20\beta$ -P), a potent maturation inducing steroid (MIS) in many teleosts (see review by Scott and Canario, 1987), was tested as the potential MIS in English sole.

## Materials and methods

### Chemicals

Tritium-labeled steroids were purchased from DuPont NEN (USA), and Amersham (UK). The antibodies for E2 and T were purchased from G. Niswender (Colorado State University, United States). The 11KT antibody was a gift from Y. Nagahama (Japan), and the  $17\alpha, 20\beta$ -P antibody was a gift from A. P. Scott (United Kingdom). The standards for the steroids were purchased from Steraloids, Inc. (USA).

### Collection of samples

The sampling times for fish were chosen to coincide with the period of gonadal recrudescence and spawning in English sole (Ketchen, 1947; Harry, 1959; Johnson et al., 1991). Maturing female (>200 mm total length (TL)) and male sole (>190 mm TL) were collected from Tulalip Bay (October through January) and University Point (January through March) by otter trawl during the 1992–93 (October–

March) and 1993–94 (October) spawning cycles. Additional sole were collected monthly at Pilot Point from February 1994 to December 1995. Tulalip Bay and Pilot Point (residential sites) were chosen for their large populations of English sole, and University Point was chosen because sole migrate to this area for spawning (Johnson et al., 1991; Collier et al., 1992).

Fish were kept in holding tanks aboard the research vessel for approximately one hour before sampling was carried out. The sole were weighed (to the nearest g) and measured (to the nearest mm), and blood (1 mL) was collected from the caudal vein with a heparinized syringe. Blood samples were centrifuged for 10 minutes at  $800 \times g$ , and plasma was collected and stored in triplicate bullet vials (250  $\mu$ L each vial) for subsequent analysis of reproductive steroids (females: E2, T, and  $17\alpha, 20\beta$ -P; males: 11KT, and T). Immediately following blood collection, the fish were sacrificed by severing the spinal cord. The gonads were removed and weighed, and samples of gonadal tissue were collected and preserved in Dietrich's fluid (Gray, 1954) for histological examination. The abdominal contents were removed and the gutted carcass was weighed. Plasma samples were frozen and stored at  $-80^\circ\text{C}$  until steroid analyses could be performed.

### Sample analyses

Plasma T, E2, and 11KT levels were determined by radioimmunoassay (RIA) as described by Sower and Schreck (1982), and  $17\alpha, 20\beta$ -P levels were determined by RIA as described by Scott et al. (1982). Each RIA (E2, 11KT, and T, and  $17\alpha, 20\beta$ -P) consisted of duplicates of standards (ranging from 0 to 2.0 ng/mL), samples, and quality control samples (pooled plasma from male or female English sole). Detection limits of the assays (20–80% binding range of the standard curve) were 0.006–0.3 ng/mL plasma for E2; 0.009–0.4 ng/mL for 11KT; 0.002–0.13 ng/mL for T; and 0.003–0.08 ng/mL plasma for  $17\alpha, 20\beta$ -P. For each assay, samples with steroid concentrations outside the detection limits of the assay were retested by adjusting the sample volume. All samples were corrected for dilution factor. Mean ( $\pm$ SE) of quality control samples were  $4.4 \pm 0.55$  ng/mL for E2 ( $n=15$ );  $0.89 \pm 0.12$  ng/mL for 11KT ( $n=6$ ); and  $0.29 \pm 0.04$  ng/mL for T ( $n=16$ ). The steroid  $17\alpha, 20\beta$ -P was not detected in quality control samples.

Gonadal tissues collected for histological examination were embedded in paraffin, sectioned, stained with Harris hematoxylin and eosin-phloxine (Luna, 1968) and examined by light microscopy. Ovarian developmental stage (Table 1) was classified accord-

**Table 1**

Classification scheme for female ovarian developmental stages, modified from histological criteria outlined in Johnson et al. (1991).

Stage	Features
Regressed	Primary and secondary oocytes
Previtellogenic	Vacuolated secondary oocytes with clear peripheral vacuoles; zonal radiata present
Vitellogenic	Yolked oocytes present
Vitellogenic with hydrated oocytes	Yolked globules coalescing; hydrated oocytes present, no postovulatory follicles (POFs)
Spawning	Hydrated oocytes with POFs
Spawned out	Many POFs visible, yolked oocytes undergoing resorption, and inflammatory infiltrate, beta or gamma atretic follicles or macrophage aggregates (or both) generally present

ing to the criteria modified from Johnson et al. (1991). Testicular developmental stage was classified as modified from Billard (1992). GSI was calculated by using the formula  $GSI = (\text{gonad weight (g)} / \text{guttated weight (g)}) \times 100$ .

### Statistical analysis

Mean ( $\pm$ SE) GSI and plasma reproductive steroid levels were calculated by month and gonadal maturation stage. Analysis of variance (ANOVA) and subsequent multiple comparison testing by Fisher's protected least significant difference (PLSD) test (95% CI), were used to test for changes in these factors during the reproductive cycle (Dowdy and Wearden, 1991). The data was normalized by log transformation prior to statistical comparisons.

## Results

### Classification of testis

The testicular developmental stages for male English sole are shown in Figure 1, A–F. The testis consists of tubules of reproductive cells in various stages of development. Testicular development is categorized into six stages: regressed (Fig. 1A), early recrudescence (Fig. 1B), late recrudescence (Fig. 1C), early spermiogenesis (Fig. 1D), spawning (Fig. 1E), and spawned out (Fig. 1F). Each stage is characterized as follows: in the regressed stage, tubules contain

only spermatogonia; in early recrudescence, primary and secondary spermatocytes begin to appear; in the late recrudescence stage spermatogonia, spermatocytes (primary and secondary), and spermatids are evident, but no spermatozoa; in early spermiogenesis primarily spermatocytes (primary and secondary), spermatids, and early sperm production are evident; in the spawning stage predominately mature sperm fill the tubular space and sperm ducts; in the spawned-out testis, the tubular space and sperm ducts are largely empty and few sperm remain.

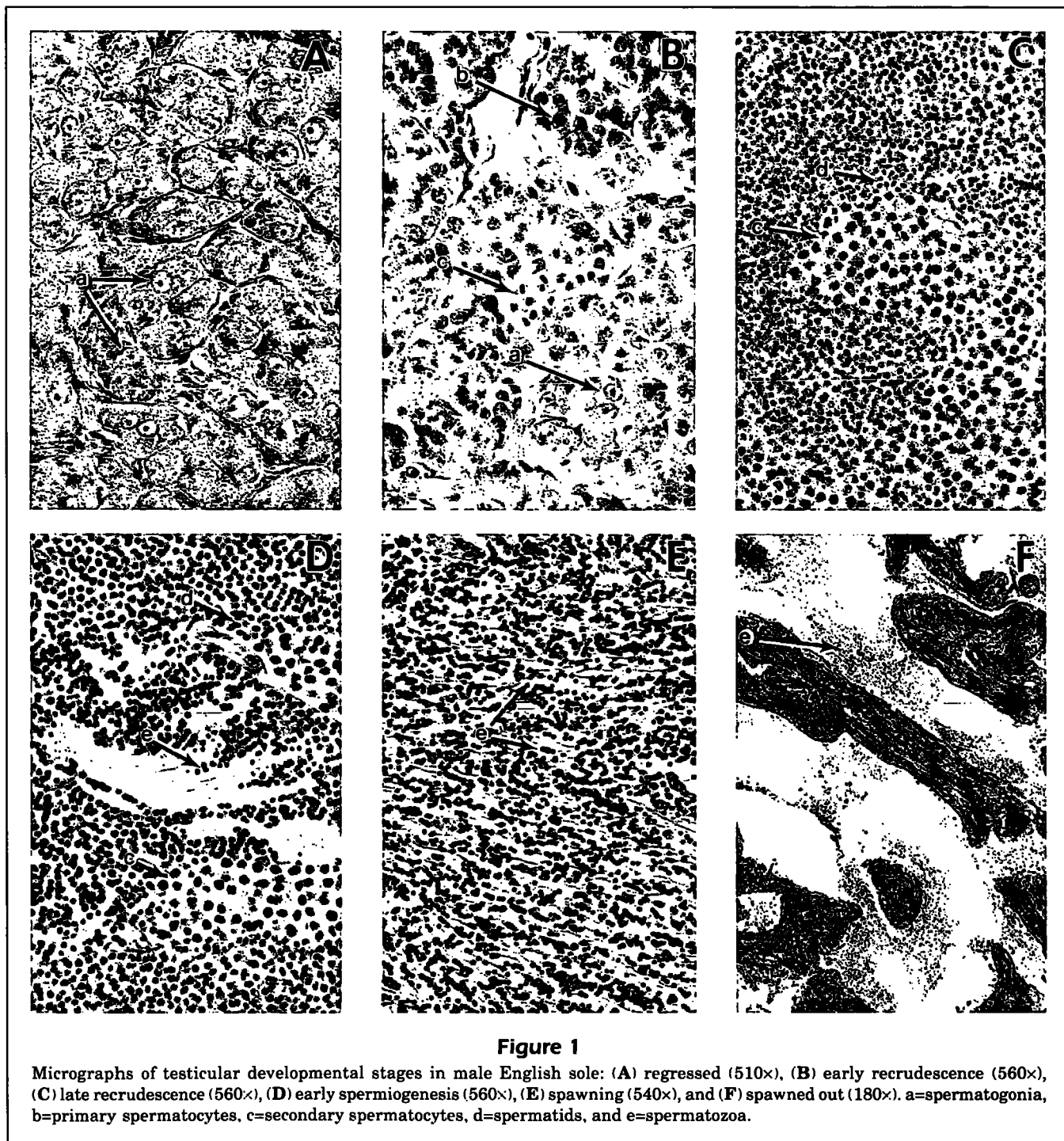
### Size at sexual maturation

The size of English sole captured in this study ranged from 212 to 455 mm for females and from 195 to 345 mm total length for males. Histological analyses of the gonads showed that a majority of the female sole <260 mm TL were immature (regressed–previtellogenic), and failed to reach vitellogenesis (Table 2). Male sole <230 mm reached spermatogenesis but most did not fully mature and spawn (Table 3). Female sole <260 mm were excluded from further statistical analyses, but no size limit was used for male sole.

### Proportions of fish undergoing gonadal maturation and spawning

**Female** Figure 2 shows the proportion of female sole at different ovarian developmental stages by month. Even among adult sole (>260 mm), regressed females were found all year, and the mean proportion of the regressed animals each month was 24% ( $\pm$ 11%, SD). Developing females were found as early as July; 14% of the female sole sampled in July were vitellogenic. The proportion of vitellogenic sole generally increased until January (78%), then decreased in the following months; by March only 11% of the sole sampled were vitellogenic. Spawned out females were found as early as October (1%), and the proportion increased through March (31%).

**Male** Figure 3 shows the proportion of male sole at different testicular developmental stages categorized by month of capture. Animals collected in July consisted of regressed males (12%) and early developing males (25% each were at early and late recrudescence), and males in early spermiogenesis (38%). Spawning males were found as early as October (16%), and the proportion generally increased until February (91%). By March, 41% were spawning and 36% were spawned out. Unlike females, in which a certain proportion of fish did not develop during the reproductive cycle, almost all males collected did



enter spermiogenesis. However, only a small proportion of males <230 mm were actually producing sperm, and none in this size range were collected on the spawning ground. The smallest male caught at University Point measured 238 mm, suggesting that males <230 mm may not migrate to the spawning ground (University Point) (Table 3).

#### Changes in reproductive parameters during gonadal recrudescence

**Female** Figure 4, A and B, shows reproductive parameters in female sole at each developmental stage. Generally, the immature (regressed and previtellogenic) sole had low GSI ( $1.4 \pm 0.1$ ,  $n=85$ ), and low

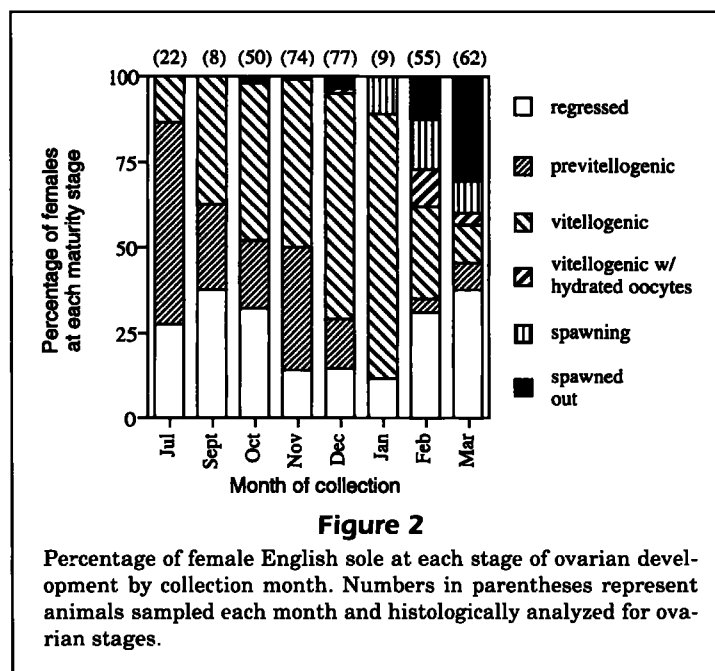
**Table 2**

Length maturation relationship in female English sole collected during months of gonadal recrudescence, October–March. Numbers represent percentage of the animals in the length class at each ovarian stage.

Length (mm)	Regressed	Previtellogenic	Vitellogenic	Vitellogenic with hydrated oocytes	Spawning	Spawned out	n
<220	50	50					2
221–230	100						1
231–240	100						11
241–250	78	11	11				9
251–260	69	15	15				13
261–270	31	23	31	7.7		7.7	13
271–280	32	16	37			16	19
281–290	44	11	44				18
291–300	25.7	23	40	2.9		8.6	35
301–310	34	13	44		3.1	6.3	32
311–320	33	12	45		3	6.1	33
>320	26.9	16	47	2.9	7	11	172

plasma reproductive steroid concentrations (E2:  $650 \pm 110$  pg/mL plasma,  $n=66$ ; T:  $80 \pm 12$  pg/mL plasma,  $n=66$ ). Increases in GSI and reproductive steroids were observed in animals at the previtellogenic stage, and peak levels of GSI ( $20.0 \pm 4.8$ ,  $n=9$ ) and plasma E2 levels ( $7000 \pm 4700$ ,  $n=4$ ) were found in vitellogenic sole with hydrated oocytes, whereas peak plasma T levels ( $2300 \pm 620$ ,  $n=9$ ) were found in spawning females. All reproductive parameters were reduced in spawned out sole (GSI:  $2.8 \pm 0.5$ ,  $n=31$ ; E2:  $280 \pm 70$ ,  $n=21$ ; T:  $90 \pm 50$ ,  $n=21$ ).  $17\alpha$ ,  $20\beta$ -P was not detected in female sole.

Figure 5, A–C, shows the mean levels of reproductive parameters in female sole at each month of collection. The sole sampled in July had low GSI ( $2.14 \pm 0.45$ ,  $n=23$ ) and plasma reproductive steroid levels (E2:  $710 \pm 160$ ,  $n=26$ ; T:  $100 \pm 30$ ,  $n=27$ ), which remained low until the onset of vitellogenesis (September–October for the majority of fish). The highest GSI ( $12.0 \pm 2.9$ ,  $n=10$ ) and plasma steroid levels (E2:  $12000 \pm 5000$ ,  $n=3$ ; T:  $2400 \pm 1200$ ,  $n=4$ ) were observed in January. By March, GSI and plasma reproductive steroid levels were reduced to levels comparable to those found in sole sampled in July. In general, fish from Tulalip Bay and Pilot Point had similar levels of reproductive parameters, which were lower than levels in fish from the University Point. The fish at University Point were either in final maturation or were actively spawning, whereas fish from Tulalip Bay and Pilot Point were migrating to spawning areas to undergo final maturation.



**Male** Figure 6, A and B, shows the reproductive parameters in male sole at each developmental stage. The changes in reproductive parameters were similar to those observed in female sole. Generally, GSI ( $0.60 \pm 0.08$ ,  $n=6$ ) and plasma steroid levels (11KT:  $1900 \pm 920$  pg/mL plasma,  $n=4$ ; T:  $160 \pm 60$  pg/mL plasma,  $n=4$ ) were low in regressed animals. Increases in GSI and reproductive steroids were observed in animals at the early recrudescence stage and peaked in spawning fish (GSI:  $1.6 \pm 0.1$ ,  $n=91$ ; 11KT:  $5300 \pm 610$ ,  $n=57$ ; T:  $830 \pm 130$ ,  $n=64$ ). Repro-

Table 3

Length maturation relationship in male English sole collected during months of gonadal recrudescence, October–March. Numbers represent percentage of the animals in the length class at each testicular stage.

Length (mm)	Regressed	Early recrudescence	Late recrudescence	Early spermiogenesis	Spawning	Spawned out	n
<220				85	15		13
221–230	6.7	13	6.7	60	13		15
231–240	13	6.7	13	33	27	6.7	15
241–250		6.7	3.3	27	60	3.3	30
251–260	5.6	5.6	0	47	39	2.8	36
261–270		2.9	2.9	49	40	5.7	35
271–280		4.8		43	48	4.8	21
281–290		6.7		40	53		15
291–300				36	64		14
301–310				38	50	13	8
311–320				25	50	25	4
>320	10		10	10	70		10

ductive parameters were reduced in spawned out fish (GSI:  $0.8 \pm 0.1$ ,  $n=8$ ; 11KT:  $83 \pm 5$ ,  $n=8$ ; T:  $24 \pm 7$ ,  $n=7$ ), and plasma steroid levels in spawned out fish were significantly lower than levels observed in fish at other stages of testicular development.

Figure 7, A–C, shows the mean levels of reproductive parameters in male sole at each month of collection. GSI in sole sampled during July was low ( $0.89 \pm 0.18$ ,  $n=6$ ), increased to a peak in January ( $2.6 \pm 0.7$ ,  $n=4$ ), and returned to baseline levels in sole sampled in March ( $0.8 \pm 0.8$ ,  $n=22$ ). Plasma steroid levels were low in sole sampled in July (11KT:  $890 \pm 140$ ,  $n=13$ ; T:  $120 \pm 30$ ,  $n=12$ ) even though a few fish expressed milt. The levels increased to a peak in February (11KT:  $13000 \pm 2800$ ,  $n=6$ ; T:  $1800 \pm 340$ ,  $n=14$ ), and decreased in March (11KT:  $1300 \pm 950$ ,  $n=18$ ; T:  $400 \pm 310$ ,  $n=15$ ). Highest levels of plasma T and 11KT were found in fish from University Point where fish had migrated to spawn. A few fish with comparable levels of 11KT were found in March from the residential site at Tulalip Bay.

## Discussion

Typical size-at-maturity for English sole is 260 and 210 mm TL for female and male sole, respectively (Garrison and Miller, 1982). Histological analyses of gonads collected in our study showed the smallest group of vitellogenic females to be 240–249 mm TL, but generally fish less than 260 mm TL were immature. Also, among females >260 mm, which were presumably old enough to undergo sexual maturation,

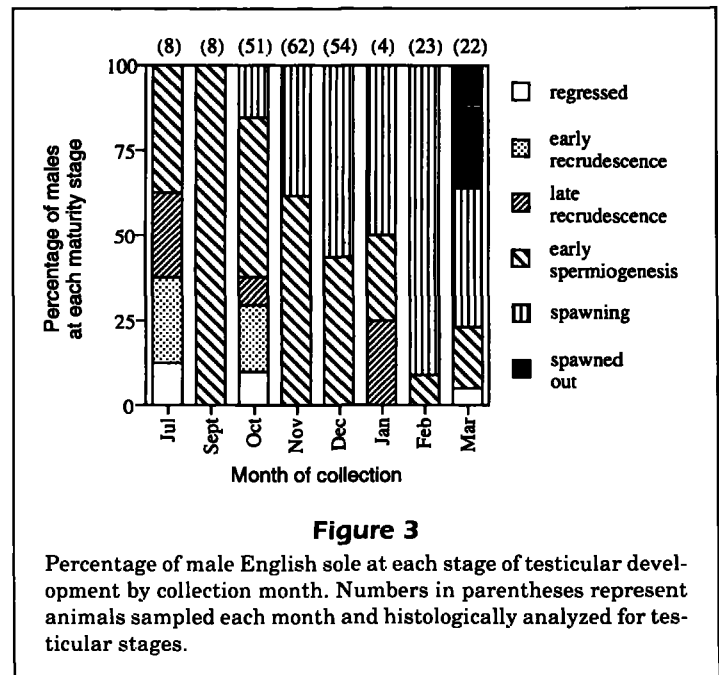


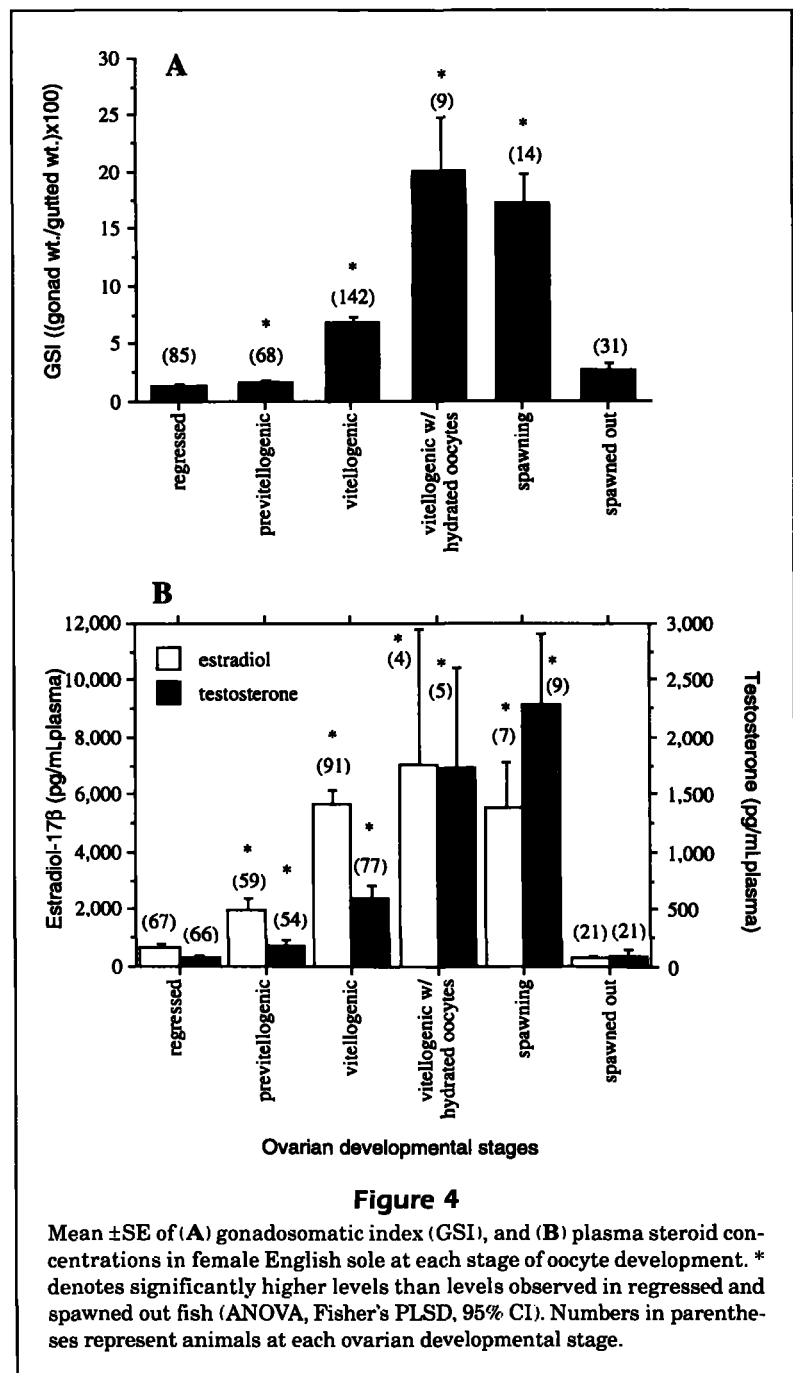
Figure 3

Percentage of male English sole at each stage of testicular development by collection month. Numbers in parentheses represent animals sampled each month and histologically analyzed for testicular stages.

approximately 24% were found in the regressed stage even during the peak of the reproductive season. This finding is consistent with findings by Johnson et al. (1988), in which the percentage of vitellogenic females at reference areas during peak recrudescence was about 80%. In contrast, males at much smaller length (i.e. 195 mm) were maturing, but no spawned out males were found at sizes <230 mm, suggesting that males <230 mm may not migrate to the spawning area. Although fish age was not determined in this study, length-at-age relationships determined for Puget Sound English sole (Myers et al., 1993) showed

that females at 260 mm were three years of age, and males at 195 mm were two years of age. Other studies have shown that females first mature at three years of age, whereas males mature at two years of age (Smith, 1936; Harry, 1959).

The pattern and timing of ovarian development observed in our study were similar to previous reports on English sole (Garrison and Miller, 1982; Kruse and Tyler, 1983; Johnson et al., 1991; Fargo and Tyler, 1994). Gonadal recrudescence in Puget Sound English sole began in early fall (September–October), vitellogenesis and spermiogenesis peaked in early winter (December–January), and spawning took place during late winter months (February and March). However, our findings suggest that small proportions of animals did enter vitellogenesis early; vitellogenic sole were found as early as July, whereas spawned out sole were found as early as October. In an earlier study that investigated oocyte development in female English sole (Johnson et al., 1991), the earliest spawning females were found in January. This discrepancy may be due to the fact that the number of fish collected in early fall in the first study (Johnson et al., 1991) was small compared with the present study; therefore, early spawning females may not have been observed. Nevertheless, changes in reproductive parameters (GSI, and plasma sex steroids) were similar to those described by Johnson et al. (1991) and to the reproductive endocrine cycles described for other species of female flatfish (e.g. Liu et al., 1991; Methven and Crim, 1991; Harmin et al., 1995). Generally, regressed fish had low values for the various reproductive parameters. The onset of vitellogenesis was accompanied by an increase in GSI and plasma sex steroid levels. After spawning, these levels declined to levels similar to those observed in regressed animals. Reproductive parameters at the two residential sites (Pilot Point and Tulalip Bay) were similar and supported the findings of Laroche and Richardson (1979) who observed no apparent latitudinal trend in the time of spawning in sole from the Oregon coast. Reproductive parameters in fish from University Point, however, were higher because a majority of sole at this site were undergoing final maturation or spawning (Johnson et al., 1991).



It is well established that E2 stimulates the liver to produce vitellogenin (Ng and Idler, 1983), and in this study, as in Johnson et al. (1991), vitellogenesis in female English sole coincided with a rise in plasma E2 concentrations. Plasma concentrations of T, a biosynthetic precursor to E2 (Kagawa et al., 1982), changed in parallel with the plasma E2 concentrations, although plasma T levels were lower than plasma E2 levels at all stages of gonadal development. Higher plasma T levels were observed in

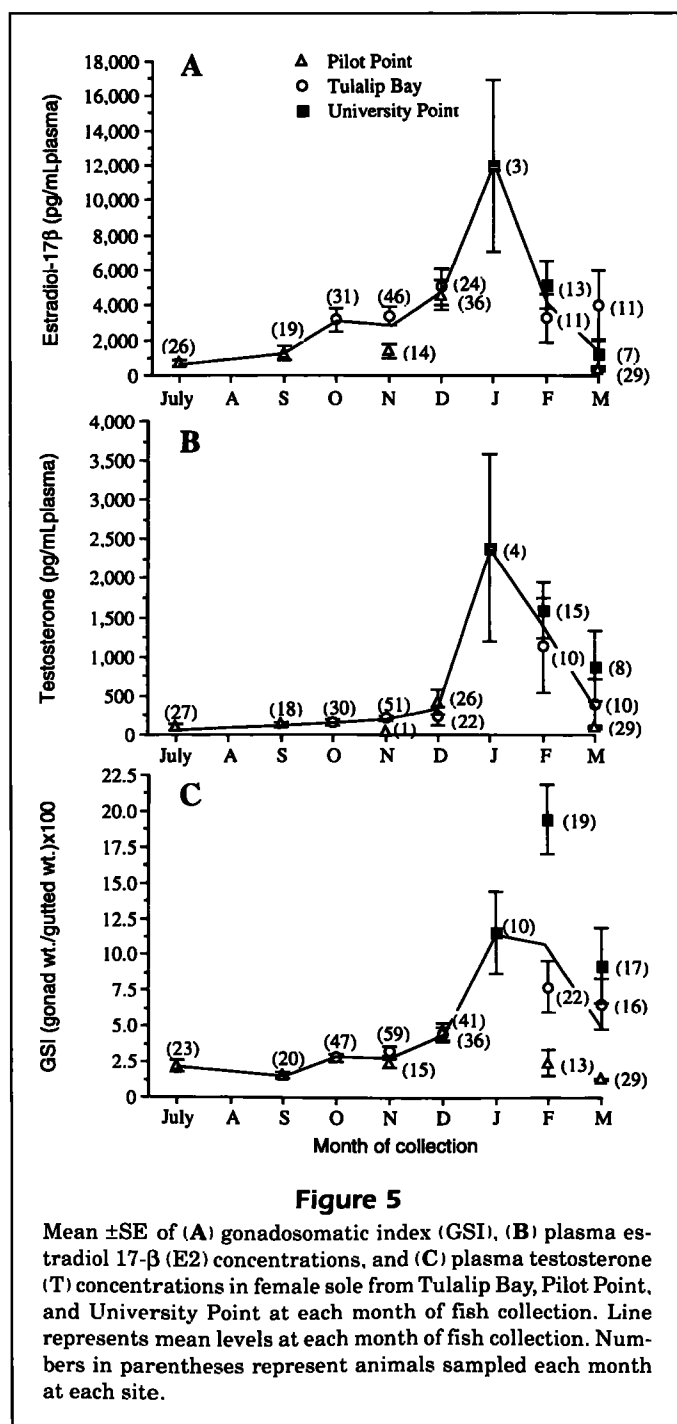
vitellogenic sole compared with regressed sole, and highest plasma T concentrations were observed in spawning sole. Similar observations have been made in winter flounder (*Pleuronectes americanus*), where concentrations of T have been correlated with oocyte stages characterized by germinal vesicle migration (Truscott et al., 1992b). In salmonids, estrogens and androgens such as T appear to play an important role in regulating production of pituitary GTH-II

(Xiong et al., 1993, 1994), which stimulates the production of progestins, steroids involved in inducing final maturation (Swanson, 1991). This would be consistent with the high concentrations of T observed in spawning female English sole and winter flounder.

In male teleosts, 11KT is known to be important in stimulating spermatogenesis, whereas T is known to be important in feedback effects on the pituitary (Borg, 1994). T is a biosynthetic precursor to 11KT (Ozon, 1972). As with E2 in female sole, the levels of T and 11KT covaried in male sole; 11KT levels were significantly higher than T levels at all stages of gonadal development. Similar relations between T and 11KT concentrations have been observed in other flatfish species, such as Pacific halibut (*Hippoglossus stenolepis*) (Liu et al., 1991) and winter flounder (Harmin et al., 1995). These results are consistent with the role of 11-oxygenated androgens as the dominant regulatory androgens in male teleosts, stimulating secondary sexual characters, reproductive behavior, and spermatogenesis (Borg, 1994).

In male winter flounder (Harmin et al., 1995) and plaice (Wingfield and Grimm, 1977), reproductive parameters (GSI, plasma 11KT and T concentrations) increased with the onset of seasonal testicular recrudescence, reached a peak in prespawning and spawning males, then decreased in spawned out males. However, in most male teleosts that have been studied, the levels of plasma 11KT and T peaked during the prespawning period rather than during the spawning period (Borg, 1994). For example, the levels of plasma 11KT and T in Atlantic halibut (*Hippoglossus hippoglossus*) peaked briefly in sperm-producing fish, then declined during the spawning period (Methven and Crim, 1991). The reproductive parameters measured in male English sole were similar to the patterns observed in winter flounder and plaice, where steroid concentrations remained high during the peak spawning period.

Interestingly, the reproductive steroid levels observed in spawned out male English sole were significantly lower than levels observed in regressed males that were collected in summer and early fall. A pattern somewhat like this was also observed in male winter flounder and Atlantic halibut. In both male winter flounder (Harmin et al., 1995) and Atlantic halibut (Methven and Crim, 1991), the plasma steroid levels were low in spent males but began to increase about two months after the peak spawning period. Although this study did not measure reproductive parameters in the months immediately following the peak spawning period (i.e. April through June), similar changes

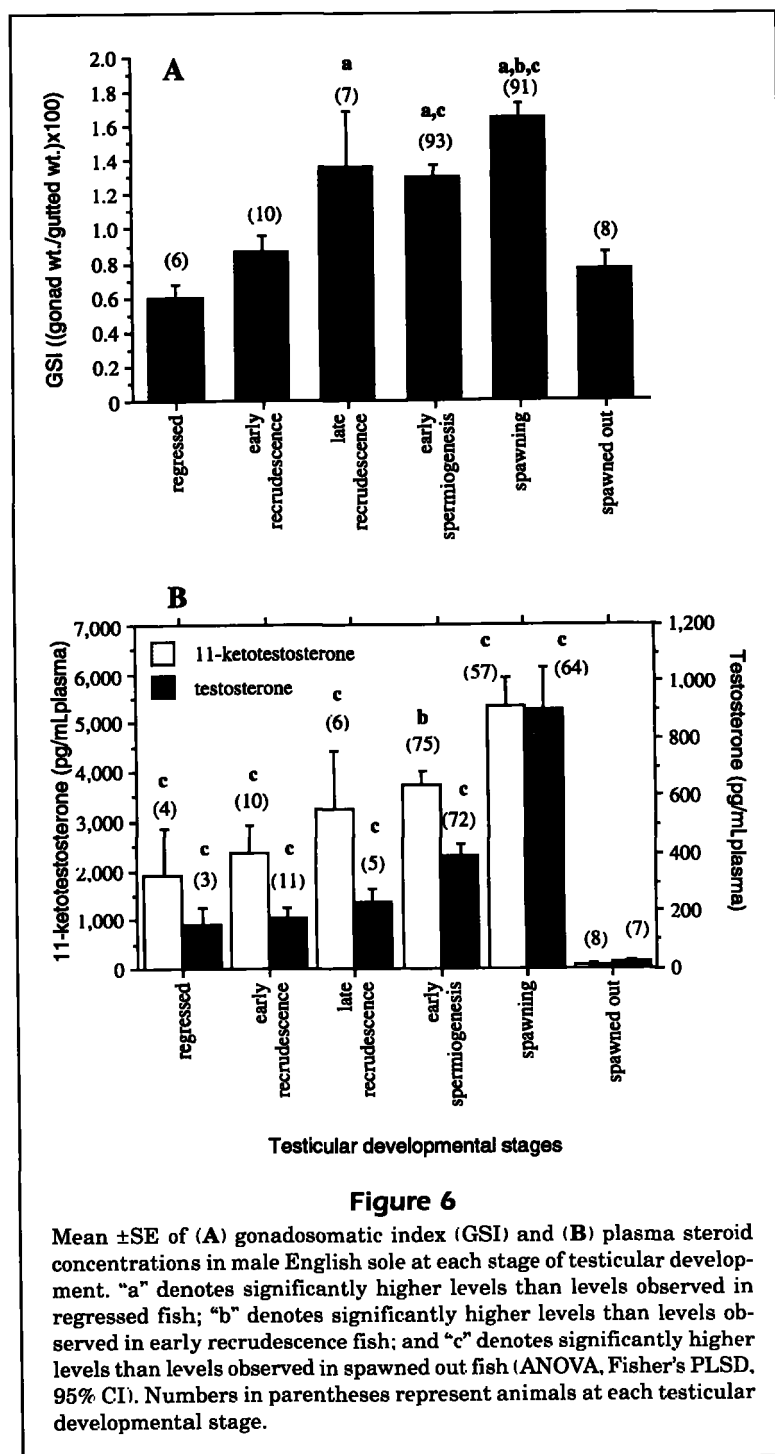




in plasma steroid levels may have occurred in male English sole and would account for the significant difference in plasma steroid levels between spent and regressed males.

A few male English sole sampled in July expressed milt even though reproductive steroid levels were low. Release of milt was also observed in winter flounder (Harmin et al., 1995) and Atlantic halibut (Methven et al., 1991) a few months earlier than vitellogenesis in females. Moreover, in winter flounder (Harmin et al., 1995), milt remained expressible a few months after the spawning season even though steroid levels were low during this time. Histological analyses of English sole testis showed that initiation of spermiogenesis does begin in July in a substantial proportion of sole, in spite of their low plasma steroid concentrations. This phenomenon has also been observed in other male fish, but the mechanism accounting for it is not entirely clear. Borg (1994) suggests that concentrations of T and 11KT in the testis itself may be quite high in early spermatogenesis, sufficient to stimulate spermiogenesis even when plasma concentrations of T and 11KT are low. The early production of mature sperm may be typical of most northern latitude, temperate zone teleosts in which fully developed spermatozoa are formed before winter and stored for discharge in spring (Lofts, 1987).

It is unclear which steroid induces final oocyte maturation in English sole. In all teleosts which have been studied, final maturation is triggered by a surge in plasma concentrations of a maturational gonadotropin (GTH-II). GTH-II stimulates gonadal production of progesterones and related compounds, the C21 steroids, which induce final oocyte maturation and production of sperm (Swanson, 1991). In most teleosts,  $17\alpha$ ,  $20\beta$ -P is believed to be a potent MIS (Scott and Canario, 1987, 1992; Inbaraj et al., 1995). However, in flatfish species that have been studied, low or nondetectable levels of  $17\alpha$ ,  $20\beta$ -P were found (Howell and Scott, 1989; Truscott et al., 1992b; Mugnier et al., 1995). Similarly,  $17\alpha$ ,  $20\beta$ -P was not detected in spawning English sole, and in some flatfish species, other steroids have been suggested as the MIS. For example, in winter flounder  $17\beta$ -hydroxy- $5\beta$ -androst-3-one and T were correlated with oocyte stages



**Figure 6**  
Mean  $\pm$  SE of (A) gonadosomatic index (GSI) and (B) plasma steroid concentrations in male English sole at each stage of testicular development. "a" denotes significantly higher levels than levels observed in regressed fish; "b" denotes significantly higher levels than levels observed in early recrudescence fish; and "c" denotes significantly higher levels than levels observed in spawned out fish (ANOVA, Fisher's PLSD, 95% CI). Numbers in parentheses represent animals at each testicular developmental stage.

characterized by germinal vesicle migration (Truscott et al., 1992b), whereas in turbot (*Scophthalmus maximus* L.)  $17\alpha$ ,  $21\beta$ ,  $21$ -trihydroxy- $4$ -pregnene- $3$ -one- $20$  has been shown to induce final maturation (Mugnier et al., 1995). Recent studies also suggest that in plaice (*Pleuronectes platessa*) and Dover sole (*Solea solea*)  $17\alpha$ ,  $20\beta$ -P actually does induce final maturation but is metabolized before it can be detected in

the bloodstream with conventional RIAs (Scott and Canario, 1992; Inbaraj et al., 1995). Clearly, additional research is needed in this aspect of English sole reproductive biology.

In summary, the reproductive endocrine cycle in female and male English sole from two residential sites and a spawning site in Puget Sound, WA, was characterized by measuring reproductive steroid concentrations and correlating them with collection time

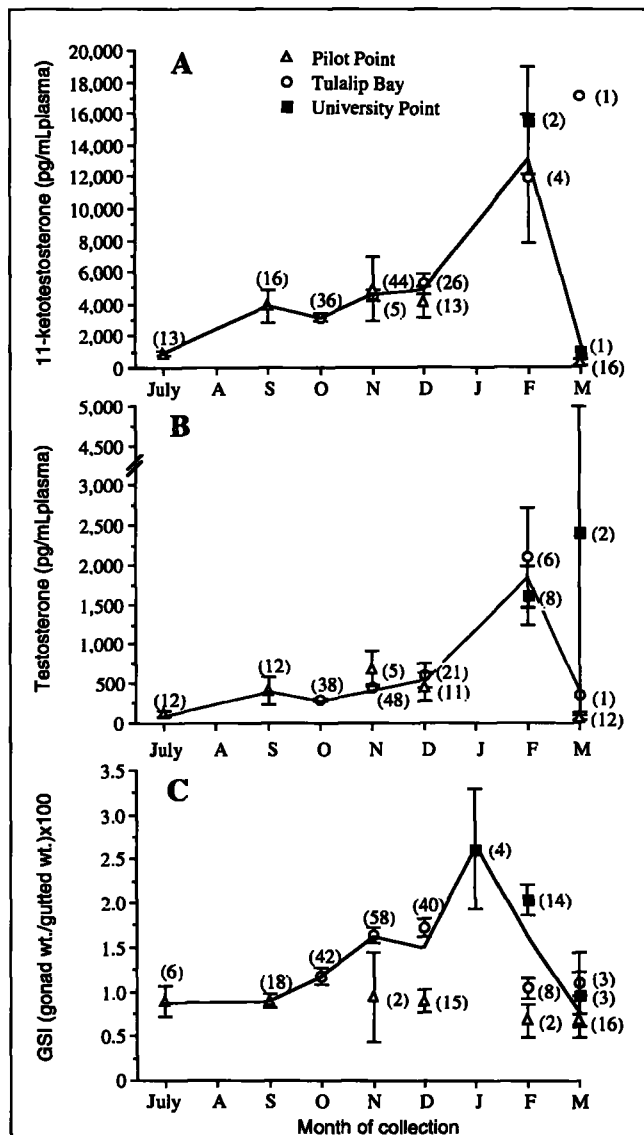
and histological assessment of gonadal stage. This study provides the first description of the reproductive endocrine cycle in male English sole and can be used as a baseline tool in evaluating the effects of contaminants on reproductive endocrine function in male English sole, or in individuals used in mariculture.

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**Figure 7**

Mean  $\pm$  SE of (A) gonadosomatic index (GSI), (B) plasma 11-ketotestosterone (11KT) concentrations, and (C) plasma testosterone (T) concentrations in male sole from Tulalip Bay, Pilot Point, and University Point at each month of fish collection. Line represents mean levels at each month of fish collection. Numbers in parentheses represent animals sampled each month at each site.

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