Fecundity and egg weight in English sole, *Pleuronectes vetulus*, from Puget Sound, Washington: influence of nutritional status and chemical contaminants

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Abstract.–Differences in fecundity and egg weight were evaluated in English sole, *Pleuronectes vetulus*, from four sites in Puget Sound (the Duwamish Waterway, Eagle Harbor, Sinclair Inlet, and Port Susan) with differing concentrations and types of sediment contamination. Duwamish Waterway sediment has high concentrations of both polychlorinated biphenyls (PCB's) and polycyclic aromatic hydrocarbons (PAH's), Eagle Harbor sediment has high concentrations of PAH's, and Sinclair Inlet sediment has low concentrations of PAH's and moderate concentrations of PCB's, whereas sediments at Port Susan, the reference site, are minimally contaminated. Fish from the Duwamish Waterway and Eagle Harbor had significantly higher levels of fluorescent aromatic compounds (FAC's) in bile than sole from Port Susan and Sinclair Inlet, and fish from the Duwamish Waterway had significantly higher concentrations of PCB's in ovary and liver tissue than fish from the other sampling sites. Fecundity and egg weight were compared in fish of equivalent size, age, and reproductive maturity from the four sites; fish from the Duwamish Waterway showed significantly higher relative fecundity and lower egg weight than fish collected from the three other sites. Production of more and smaller eggs in fish from the Duwamish Waterway site was associated with elevated hepatosomatic indices, elevated plasma triglyceride levels, and elevated levels of PCB's in liver and ovarian tissue, and reduced plasma vitellogenin levels (as estimated from alkali-labile protein (ALP) concentrations). Fish from the Duwamish Waterway and Sinclair Inlet also had higher age-specific fecundity than animals from other sites because of their larger size at age. On an individual fish basis, elevated tissue PCB concentrations were significantly correlated with low plasma ALP, reduced egg weight, and increased egg number, whereas elevated biliary FAC's were associated with increased ovarian atresia, increased egg weight, and reduced egg number. The results of this study suggest that English sole exposed to chemical contaminants may experience alterations in egg development; however, nutritional or other environmental factors may also contribute to the observed intersite differences in egg weight and fecundity.

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Reproductive impairment is potentially one of the most damaging effects of aquatic pollution on marine fish and shellfish because of its impact on population growth and consequently on the abundance of marine resources (Hose and Guillette, 1995; Grosse et al., in press). Environmental contaminants exert their effects on reproductive function through a variety of mechanisms: they may have direct toxic effects on germ-cell tissue or may disrupt the endocrine mechanisms that regulate reproduction and early development, causing inhibited or abnormal gonadal development or reduced fertility (Donaldson, 1990; Colburn et al., 1993; Kime, 1995).

Sediments from several areas of Puget Sound, Washington, are polluted with xenobiotic compounds such as polychlorinated biphenyls (PCB's), and polycyclic aromatic hydrocarbons (PAH's) (Malins et al., 1984, 1985; PSWQA[1]). These compounds are known or suspected disrupters of endocrine function (Colburn et al., 1993) and, as such, pose a potential threat to the reproductive health of marine fish that reside in these areas. In previous studies, we examined the effects of these contaminants on several aspects of reproductive function in English sole, *Pleuronectes vetulus*, a commercially important bottomfish species that is widely distributed in Puget Sound (Johnson et al., 1988, 1993; Casillas et al., 1991; Collier et al., 1992). These investigations revealed that sole from two heavily polluted sites, Eagle Harbor and the Duwamish Waterway, exhibited various types of reproductive dysfunction, including inhibited gonadal development (Johnson et al., 1988), depressed plasma estradiol levels and reduced ovarian estradiol production in vitro (Johnson et al., 1988, 1993), and reduced spawning success (Casillas et al., 1991). In contrast, fish from Port Susan and Sinclair Inlet, two sites

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[1] PSWQA (Puget Sound Water Quality Authority). 1994. Puget Sound update: fifth annual report of the Puget Sound ambient monitoring program. Puget Sound Water Quality Authority, Seattle, WA, 122 p.

with low to moderate levels of sediment contamination (Malins et al., 1984), showed little evidence of reproductive dysfunction. Although the causative agents were not definitively identified, aromatic and chlorinated hydrocarbons present in sediments at the Duwamish Waterway and Eagle Harbor sites were shown to be significant risk factors for the development of these reproductive abnormalities (Johnson et al., 1988; Casillas et al., 1991). The present study extends our previous work by examining egg weight and fecundity in English sole from the same four sites in Puget Sound.

Fecundity and egg size are important determinants of reproductive output in fish (Bagenel, 1973). Fecundity provides a measure of the potential number of offspring a female can produce, whereas egg size is an indicator of the nutritional reserves available to developing embryos and may strongly influence the growth and survival of larval fish (Blaxter and Hempel, 1963; Miller et al., 1988). Both egg size and fecundity vary considerably among stocks, species, and individuals. However, in most marine teleosts, fecundity within a stock or species is highly correlated with fish size or weight. Egg size is generally more constant but may vary with factors such as fish age or spawning time or with genetic, nutritional, or environmental factors (Hempel and Blaxter, 1967; Bagenal, 1971; Gall, 1974; Zastrow et al., 1989; Zamaro, 1992).

Teleost fish may have either determinate fecundity, in which egg production is set before the spawning season, or indeterminate fecundity, in which egg production can be increased, by recruiting additional oocytes into vitellogenesis during gonadal development, or reduced through atresia (Hunter and Macewicz, 1985a). In Puget Sound English sole populations, potential fecundity appears to be determined several months before the spawning season because these fish recruit a single clutch of oocytes in late summer or early fall and no additional oocytes enter vitellogenesis prior to spawning in February or March (Johnson et al., 1991). However, the extent to which fecundity declines as a result of atresia of developing oocytes is unclear.

In most fish species, both egg size and fecundity can be influenced by environmental conditions such as water temperature, salinity, and food supply. In herring (*Clupea* sp.), for example, water temperature 60 to 90 days before spawning may be critical in determining the balance between egg size and number. Unusually warm temperature leads to high fecundity and smaller eggs (Tanasichuk and Ware, 1987). Other stressors, such as handling or crowding, may also be associated with alterations in egg size and number; typically, stressed animals produce more and smaller eggs than do controls (Contreras-Sanchez et al., 1995; Short et al., 1995).

Field and laboratory studies have demonstrated that fish exposed to certain chemical contaminants exhibit alterations in both egg size and fecundity. Contaminant-associated declines in egg size, fecundity, or in both, have been noted in several marine fish species collected from urban embayments, including white croaker and kelp bass from the Los Angeles area (Hose et al., 1989), striped bass from San Francisco Bay (Setzler-Hamilton et al., 1988), and winter flounder from Boston Harbor and Long Island Sound (Nelson et al., 1991; Johnson et al., 1994). Similarly, white sucker exposed to pulp mill effluent in a contaminated lake of Ontario, Canada, showed a decrease in egg size and fecundity (McMaster et al., 1991). Reductions in egg size and fecundity have also been observed in a number of other fish species in conjunction with controlled exposure to chlorinated and aromatic hydrocarbons and to other organic pollutants (reviewed in Kime, 1995). These compounds may have direct toxic effects on oocytes and supporting cells (Armstrong, 1986), or alternatively, may disrupt normal hormonal regulation of gonadal growth (Donaldson, 1990; Thomas, 1990).

Egg production is strongly influenced by the nutritional status of fish; if this status is extremely poor, animals may not reproduce at all (e.g. Burton and Idler, 1987), or fecundity may be reduced (Penzak, 1985; Springate et al., 1985; Chappaz et al. 1987; Rozas and Odum, 1988). Studies also suggest that egg size and number may change seasonally or as environmental conditions vary, thus maximizing the larvae's probability of survival as food availability changes (Buckley et al., 1991). Interactive effects of toxicant exposure and nutrition may also occur. For example, toxicants may influence reproductive output indirectly, by reducing food quality, food abundance, or the ability of animals to digest food or to forage effectively. In some studies where fish were exposed to oil or other organic compounds, declines in fecundity were associated with reduced food intake and weight loss; thus the contaminants were likely affecting reproductive success by reducing the animal's condition (e.g. Ghatak and Konar, 1991). In order to account, more precisely, for possible effects of nutritional status on egg weight and fecundity in English sole from contaminated Puget Sound sites, we measured several indicators of nutritional status (i.e. condition, plasma glucose and triglyceride levels, and hepatosomatic index [HSI]) in sampled fish, as well as parameters associated with reproductive development and contaminant exposure. Our objective in this paper is to describe age and size-specific

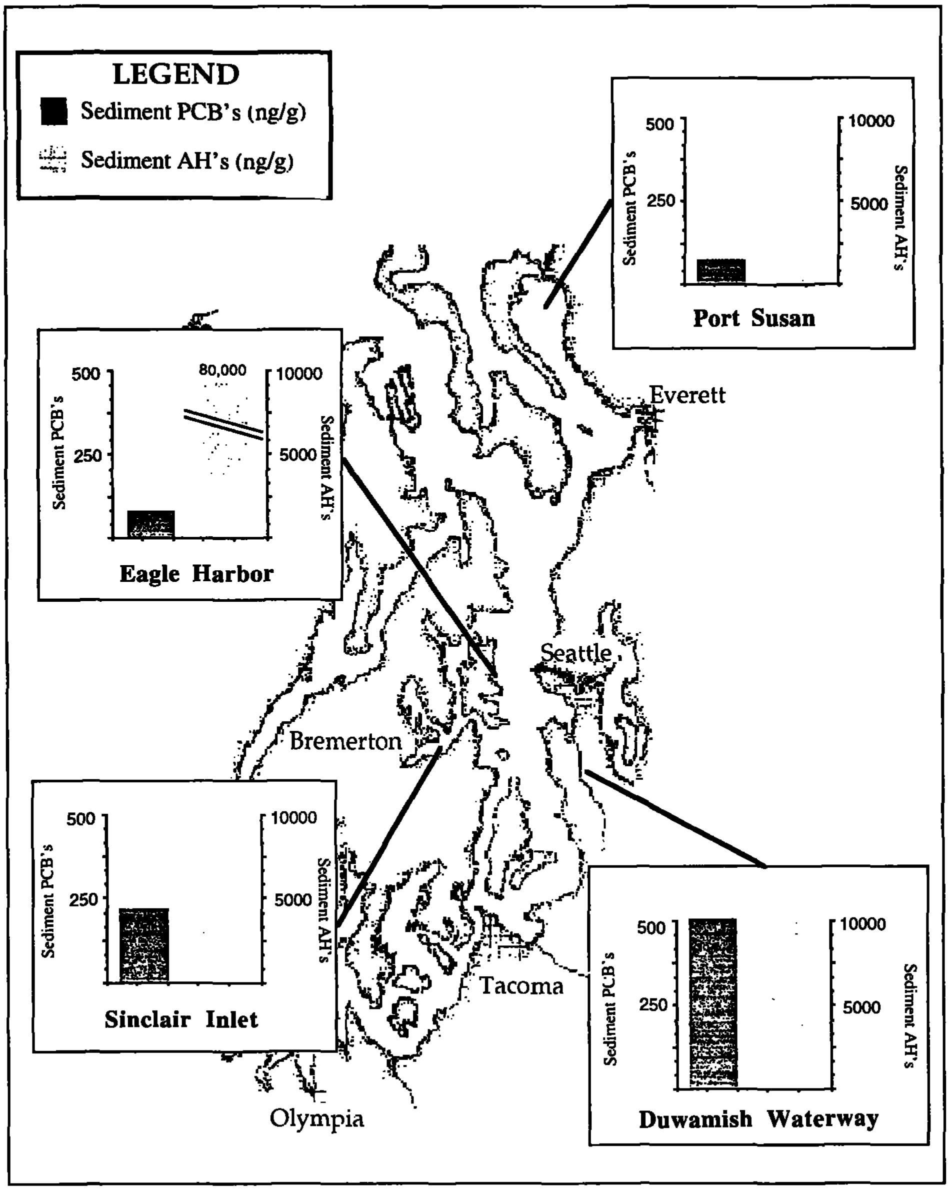

Figure 1

Chart of Puget Sound, showing locations of sampling sites and concentrations of aromatic hydrocarbons (AH's) (ng/g dry weight) and polychlorinated biphenyls (PCB's) (ng/g dry weight) in sediments collected from these areas. Data from Malins et al. (1984, 1985); contaminant levels in the same range have been observed in more recent samplings (see Footnote 1 in the main text).

patterns of egg production in Puget Sound English sole and to examine how these patterns vary in relationship to collection site, chemical contaminant exposure, and nutritional status.

Materials and methods

Collection of samples

Vitellogenic female English sole (greater than 250 mm total length [TL]) were collected by otter trawl from Eagle Harbor, Sinclair Inlet, Port Susan, and the Duwamish Waterway in Puget Sound, Washington (Fig. 1). Sampling was conducted during the winters of 1986–87 and 1989–90 in mid-December and from mid to late January, to coincide with the period in which vitellogenesis normally occurs in this species, before substantial migration to spawning areas has taken place (Johnson et al., 1991). Aside from a relatively brief spawning migration, sole are relatively territorial and reside at these sites throughout the year (Day, 1976). It should be noted that because these animals were not actively spawning, fecundity determinations estimated potential rather than actual fecundity. However, we chose to collect animals at this earlier stage of development to ensure that specimens came from resident subpopulations at sites with known sediment contaminant concentrations. This selection would not have been possible if animals had been collected on their spawning grounds.

Fecundity determinations were carried out on 5 to 10 animals from each site at each sampling time. Fish sampled in 1986–87 were collected as part of a study on gonadal development in English sole (Johnson et al., 1988); ovary samples were preserved for fecundity determination and archived, but not analyzed, at that time. Fish collected in 1989–90 were sampled specifically for fecundity determination.

Fish were caught by otter trawl in 5-min tows and held in aerated saltwater in holding tanks on the deck of the research vessel until they could be processed. Within an hour of capture, fish were weighed and measured. From each animal, a 1-mL blood sample was collected with a heparinized syringe from the caudal vessel. Blood samples for measurement of plasma estradiol concentrations were centrifuged at 3,000 ×g, and the plasma was stored at –20°C. Fish were sacrificed by severing the spinal cord. Ovaries from vitellogenic females were removed and weighed; one ovary was slit longitudinally and preserved in modified Gilson's fluid (Simpson, 1951) for later determination of fecundity and egg weight. Ovarian tissues for histological examination were preserved in Davidsons' fixative (Mahoney, 1973). Additionally, tissue samples for determination of PCB concentrations were collected from the liver and ovary and stored at –20°C. Bile for measurement of fluorescent aromatic compounds (FAC's) was collected and stored at –0°C.

Analysis of samples

Ovaries collected for histology were embedded in paraffin, stained with hematoxylin and eosin (Luna, 1968), and examined microscopically to confirm their developmental stage and to record ovarian atresia and related lesions by using criteria outlined in Hunter and Macewicz (1985b) and Johnson et al. (1991). Ovarian lesion severity was ranked on a subjective scale of 1 to 7, with 1 being minimal and 7 being severe.

Fecundity was determined by using the gravimetric method described by Bagenal and Braum (1971). Ovaries were preserved in Gilson's fluid for at least 3 months to allow eggs to harden and ovarian connective tissue to disintegrate. Preserved eggs were washed with water, filtered to separate them from residual ovarian connective tissue fragments, and dried at 60°C for 24 hours. All eggs were weighed, and then 3 subsamples of 200 eggs each were weighed. Fecundity, relative fecundity, and reproductive rate were subsequently determined by using the formulas below:

$$\mathit{Fecundity} = 2\frac{[(\text{total weight of eggs})(\#\text{of eggs in subsample})]}{(\text{mean weight of eggs in subsample})},$$

Relative fecundity = [Fecundity/gutted body weight (g)]; and

Reproductive rate (g of eggs/year) = [Fecundity × egg weight in (g)].

Additionally, gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor were calculated as follows:

$$GSI = \left[\frac{\text{ovary weight (g)}}{\text{gutted body weight (g)}}\right] \times 100$$

$$HSI = \left[\frac{\text{liver weight (g)}}{\text{gutted body weight (g)}}\right] \times 100$$

Condition factor = gutted body weight (g)/length^3 (cm).

Levels of fluorescent aromatic compounds (FAC's) in bile were measured according to the method of Krahn et al. (1987), which provides a semiquantitative determination of the concentrations of metabolites of PAH's (Krahn et al., 1993). Bile sampled from fish was injected directly into a Spectra-Physics Model 8800 high performance liquid chromatograph (HPLC) equipped with a Phenomenex reversed-phase C18 analytical column. The polar analytes (primarily metabolites of AH's) in bile were eluted with a linear gradient from 100% water containing 5 mL of acetic acid/L to 100% methanol and monitored by two fluorescence detectors connected in series. Fluorescence of metabolites was measured at two wavelengths: 290/335 nm, where metabolites of naphthalene (NPH) and related two-ring aromatic compounds from petroleum fuels fluoresce; and 380/480 nm, where metabolites of benzo[a]pyrene (BaP) and related multi-ring AH's from combustion sources fluoresce (Krahn et al., 1987). Levels of biliary FAC's were reported as equivalents of known concentrations of BaP or NPH standards on the basis of biliary protein because recent studies (Collier and Varanasi, 1991) have shown that such normalization can account for variation in FAC levels associated with the feeding status of sampled fish. Concentrations of biliary protein were determined by the method of Lowry et al. (1951) with bovine serum albumin (BSA) as the standard.

Liver and ovary tissue were analyzed for PCB's by following the method described by MacLeod et al. (1985) and modifications later described in Stein et al. (1987). Tissue samples (approximately 2 g) were ground with 10 g of silica and then added to a column (270 × 23 mm) containing 3 g of activated silica gel (Amicon Corp., Danvers, MA) held in place by a glass wool plug. PCB's were eluted with pentane:methylene chloride (90:10, V/V). The first 50 mL of eluant were collected, concentrated, and exchanged with 1 mL of hexane prior to analysis by gas chromatography with electron capture detection (GC/ECD) (MacLeod et al., 1985). Selected samples of ovary tissue required the removal of lipids by size-exclusion HPLC (Krahn et al., 1988) prior to analysis with GC/ECD.

Plasma estradiol-17β concentrations were determined by radioimmunoassay as described by Sower and Schreck (1982). Plasma glucose and triglycerides were determined as described by Casillas et al. (1983) and Casillas and Ames (1986), respectively.

Fish age was estimated from length by using site-specific age-length curves calculated from length and age data collected from female English sole sampled during previous studies in Puget Sound. Fish ages were determined from otolith analysis (Chilton and Beamish, 1982). Site-specific growth relationships were fitted by using the von Bertalanffy growth curve (Ricker, 1987), and age was then estimated with the formula

$$age = t - \left(\left(\ln(1 - length / L_\infty)\right)\right) / K,$$

where t = time at which length = 0;
L_∞ = asymptotic length; and
K = Brody's growth coefficient.

Substituting site-specific values for t, L_∞, and K into the general formula, age-length equations for female sole from the specific sites were as follows:

$$age_{\text{Port Susan}} = -3.41 - ((\ln(1 - length/487)))/0.096;$$

$$age_{\text{Sinclair Inlet}} = -1.82 - ((\ln(1 - length/445)))/0.200;$$

$$age_{\text{Duwamish Waterway}} = -2.87 - ((\ln(1 - length/586)))/0.085;$$

$$age_{\text{Eagle Harbor}} = -2.41 - ((\ln(1 - length/394)))/0.209.$$

Statistical analyses

Data were initially analyzed to identify the major biological factors affecting fecundity and egg weight so that potential confounding factors could be adjusted before evaluating the impacts of sampling site and contaminant exposure on these endpoints. Analysis of variance (ANOVA), and Fisher's protected least-significant difference multiple-comparison test (Fisher's PLSD) were used to examine the effects of site, year, and month of capture on fecundity and egg weight. Intersite differences in ovarian atresia severity, an ordinal variable, were compared by using the nonparametric Kruskall-Wallis test. Linear regression analysis was used to examine the relationships of fecundity and egg weight with biological variables (i.e. fish size, condition, and gonadosomatic index (GSI). Stepwise multiple-regression analysis was subsequently used to assess the relationships of fecundity and egg weight with indicators of contaminant exposure (e.g. tissue PCB levels, biliary FAC's, and site of capture) after adjusting for relevant biological factors identified in initial regression analyses. Data were log-transformed as necessary prior to statistical analyses to normalize data and reduce heteroscedasticity. These standard statistical analyses are described in detail in Sokal and Rohlf (1981) and Dowdy and Wearden (1991). For all statistical tests, α was set at 0.05.

Results

Biological factors affecting egg production

The number of eggs produced by sole from our sampling sites ranged from approximately 120,000 in a 28-cm TL fish to approximately 1.2 million in a 43-cm TL fish. In multiple regression analysis (Table 1), fish length was the strongest predictor of fecundity, but GSI (an indicator of the level of gonadal development) also showed significant associations with fecundity. Fish length explained the highest proportion (48%) of variation in fecundity; GSI accounted for 8% of variation in fecundity. Fish age was also positively correlated with fecundity (r^2=0.41, P=0.0001, n=47), but the association was not as strong as the association between fecundity and length because of the high variability in size, and consequently, in egg production, among fish of the same age class. After the influences of fish length and GSI had been accounted for, fish age and sampling time had weak but significant negative relationships with fecundity, a finding that suggests a tendency for fecundity to decline in older animals and at the end of the sampling season. Sampling time and age accounted for approximately 3% and 4% of variation in fecundity, respectively.

In contrast to fecundity, egg weight was not highly correlated with either fish size or age but was re-

Table 1

Results of multiple regression analysis examining effects of biological factors (length, age, GSI, sampling year, and sampling time in Julian day) on egg weight and fecundity. Factors that did not contribute significantly to the model are not included in the table.

	Regression results				
Model variables	df	*F*-test	r^2	*t*-value	*p*-value
Fecundity					
Overall model	98	42.25	0.63	—	<0.0001
length			0.48	8.31	<0.0001
+GSI			0.56	5.98	<0.0001
+sampling time			0.59	–3.41	0.0010
+age			0.63	–3.13	0.0023
Egg weight					
Overall model	98	154.23	0.76	—	<0.0001
GSI			0.73	8.674	<0.0001
+sampling time			0.76	3.164	0.0004

lated primarily to the degree of gonadal development (i.e. GSI), and increased as vitellogenesis progressed. In vitellogenic sole sampled in December, mean egg weight was 4.6 ± 0.4 µg (n=66), whereas in January, it was 13.0 ± 0.9 µg (n=34) (P<0.0001, 1-way ANOVA). In multiple regression analysis, the best predictors of egg weight were GSI (73% of variation) and sampling time (3% of variation), both of which were positively correlated with egg weight (Table 1).

Because of their strong influence on fecundity and egg size and because of their high degree of individual variability, the basic parameters included in Table 1 were adjusted for in subsequent analyses to assess the effects of contaminant exposure, nutritional factors, and site of capture on fecundity and egg size.

Site-specific patterns of egg production

Mean fecundity, relative fecundity, egg weight, and reproductive output for English sole from Port Susan, Sinclair Inlet, the Duwamish Waterway, and Eagle Harbor are shown in Table 2, along with results of 2-way ANOVA examining the effects of site and sampling time on these parameters. Overall, fecundity did not change significantly with sampling time, and no significant site-month interactions were seen for fish from Port Susan, the Duwamish Waterway, or Eagle Harbor. Fish from Sinclair Inlet, however, had significantly higher fecundity in December than in January (t=2.613, P=0.0105). Site of capture had a significant effect on fecundity; fish from the Duwamish Waterway exhibited higher fecundity than fish from the Port Susan reference site (t=2.016, P=0.0467). Like fecundity, relative fecundity did not show any consistent overall change with sampling time, but significant site-month interactions were seen for fish from Sinclair Inlet, Port Susan, and Eagle Harbor. Relative fecundity of fish from Sinclair Inlet was significantly higher in the December than in the January sampling (t=2.829, P=0.0058), whereas in fish from Eagle Harbor (t=–2.747, P=0.0072) and Port Susan (t=–4.232, P=0.0001), relative fecundity was significantly lower in December than in January. There was also a significant effect of site on relative fecundity, which was lower in Sinclair Inlet than in Port Susan sole (t =–3.46, P=0.0006).

Egg weight increased significantly, by 2- to 3-fold, between December and January at all sampling sites. No significant site-month interactions were seen. Site of capture did not have a significant effect on egg weight, although Duwamish Waterway fish tended to exhibit lower egg weights than fish from Port Susan (t =–1.878, P=0.0635). Like egg weight, reproductive rate was significantly higher in January than in December. However, no significant intersite differences or month-site interactions were seen for reproductive rate.

The effects of site of capture on egg weight and fecundity were also evaluated by using multiple regression, after adjusting for the influence of fish length, age, GSI, and sampling time (see Table 1). Results showed that even after fish length, sampling time, and GSI had been accounted for, Duwamish Waterway fish had significantly higher fecundity (t=4.52, P=0.0001) than comparable animals from the Port Susan reference site (see Fig. 2A). Age and fecundity relationships were also analyzed by using multiple regression (excluding fish length from the model); the results showed that Duwamish Water-

Table 2

Mean values (± SE) of fecundity, egg weight, relative fecundity, and reproductive rate in English sole from four sites in Puget Sound, and results of 2-way analysis of variance (ANOVA) assessing effects of site and month of collection on these variables. All variables were normalized by log transformation prior to statistical analysis. EH = Eagle Harbor, DW = Duwamish Waterway, SI = Sinclair Inlet, and PS = Port Susan. ns = not significant.

	Fecundity (egg no. × 10^5)		Egg weight (µg)		Relative fecundity (eggs/g gutted wt)		Reproductive rate (g eggs/yr)	
Site	Dec	Jan	Dec	Jan	Dec	Jan	Dec	Jan
Port Susan	2.34 ± 0.25 (n=19)	2.65 ± 0.28 (n=9)	5.3 ± 0.2 (n=19)	16.7 ± 1.8 (n=10)	770 ± 50 (n=19)	1,250 ± 126 (n=9)	1.4 ± 0.40 (n=19)	4.2 ± 0.70 (n=9)
Sinclair Inlet	5.44 ± 0.70 (n=15)	2.78 ± 0.27 (4)	4.1 ± 0.5 (n=15)	13.9 ± 2.2 (n=4)	1,080 ± 82 (n=15)	670 ± 62 (n=4)	2.4 ± 0.45 (n=15)	3.7 ± 0.27 (n=4)
Duwamish	4.74 ± 0.38 (n=17)	3.92 ± 0.43 (n=10)	4.4 ± 0.7 (n=17)	10.7 ± 1.4 (n=10)	1,190 ± 57 (n=17)	1,220± 106 (n=10)	2.2 ± 0.41 (n=17)	4.0 ± 0.64 (n=10)
Eagle Harbor	3.22 ± 0.32 (n=15)	3.64 ± 0.46 (n=10)	4.6 ± 0.9 (n=15)	11.4 ± 1.2 (n=10)	760 ± 64 (n=15)	1,020 ± 73 (n=10)	1.7 ± 0.44 (n=17)	4.3 ± 0.74 (n=10)
2-way ANOVA results								
Month	ns	ns	$F = 94.42$ $P = 0.0001$ Dec < Jan		ns	ns	$F = 36.57$ $P = 0.0001$ Dec < Jan	
Site	F=8.83 P=0.0001 DW > PS, $t = 2.02$, $P = 0.047$		ns ns DW < PS, $t = -1.88$, $P = 0.064$		F=6.22 P=0.0004 SI < PS , $t = -3.55$ $P = 0.0006$		ns	ns
Month*Site	F=3.23 P=0.026 SI: Dec > Jan, $t = 2.61$, $P = 0.011$		ns	ns	F=8.86 P=0.0001 SI: Dec > Jan, $t = 2.83$, $P = 0.0058$ EH: Dec < Jan, $t = -2.78$, $P = 0.0078$ PS: Dec < Jan, $t = -4.23$, $P = 0.0001$		ns	ns

way (t=6.23, P=0.0001) and Sinclair Inlet sole (t=4.33, P=0.0001) had significantly higher age-specific fecundity than Port Susan reference fish (see Fig. 2B). Mean age-specific fecundity in sole from the four sampling sites is shown in Table 3. Similarly, results of multiple regression analysis indicated that intersite differences in egg weight were only partially explained by variation in the degree of gonadal development (i.e. GSI) or sampling time. Even after adjusting for these factors, Duwamish Waterway fish exhibited significantly lower egg weight (t=–4.070; P=0.0001) than comparable fish from the other sites (Fig. 2C).

Intersite differences in biological and chemical factors

Indicators of contaminant exposure Mean levels (± SE) of biliary FAC's in English sole from Port Susan, Sinclair Inlet, the Duwamish Waterway, and Eagle Harbor are shown in Fig. 3A; mean concentrations of PCB's (± SE) in liver and ovary tissue are shown in Fig. 3B. English sole from Eagle Harbor had significantly higher biliary FAC-BaP levels than fish from any of the other sampling sites, including the Duwamish Waterway, and significantly higher FAC-NPH levels than fish from either Port Susan or Sinclair Inlet. Duwamish Waterway fish had significantly higher biliary FAC-BaP and FAC-NPH levels than Port Susan or Sinclair Inlet fish. No significant differences were found between biliary FAC concentrations in Port Susan and Sinclair Inlet fish. Duwamish Waterway fish had significantly higher liver PCB concentrations than fish from any of the other sampling sites and significantly higher ovarian PCB concentrations than fish from either Port Susan or Eagle Harbor. Concentrations of PCB's in liver and ovary tissues of fish from Sinclair Inlet, although not as high as those observed in Duwamish fish, were significantly elevated in comparison with those found in Port Susan and Eagle Harbor fish. Tissue PCB concentrations in Port Susan and Eagle Harbor fish were not statistically different.

Size and nutritional status Mean values (± SE) of length, age, condition factor, HSI, plasma triglyceride levels, and plasma glucose levels for sole collected from the four sampling sites are shown in Table 4. Port Susan fish were significantly smaller (in length) and Sinclair Inlet fish significantly larger than ani-

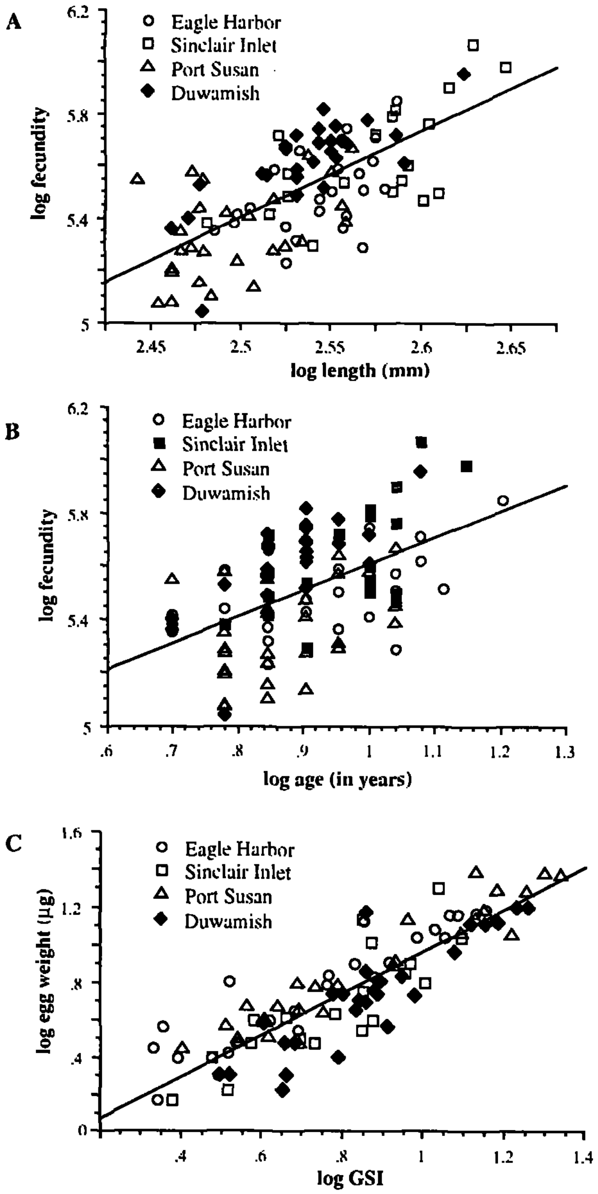

Figure 2

(A) Linear regression of log fecundity vs. log length in gravid English sole from Port Susan, Sinclair Inlet, Eagle Harbor, and the Duwamish Waterway. Multiple regression analysis indicated that fecundity was significantly higher in fish from the Duwamish Waterway than in fish of comparable size from the other sites (t=3.601, P=0.0005). (B) Linear regression of log fecundity vs. log age (in years) in gravid English sole from Port Susan, Sinclair Inlet, Eagle Harbor, and the Duwamish Waterway. Multiple regression analysis indicated that fecundity was significantly higher for a given age in fish from the Duwamish Waterway (t=6.23, P=0.0001) and Sinclair Inlet (t=4.33, P=0.0001) than in animals from other sites. (C) Linear regression of egg weight vs. GSI in gravid English sole from Port Susan, Sinclair Inlet, Eagle Harbor, and the Duwamish Waterway. Multiple regression analysis indicated that egg weight was significantly lower for given GSI in fish from the Duwamish Waterway than in animals from other sites (t=–3.218, P=0.0018).

mals collected from the other sites; fish age (as estimated from length) was significantly lower in fish from the Duwamish Waterway and Port Susan than in fish from Sinclair Inlet and Eagle Harbor. No significant intersite differences were found in either condition factor or length-weight relationship. In contrast, other indicators of nutritional status showed significant intersite differences. Duwamish Waterway fish had significantly higher HSI than fish from other sites, as well as significantly higher triglyceride levels in plasma. Plasma glucose levels, on the other hand, were significantly lower in Eagle Harbor fish than in those from the other sampling sites. Moreover, condition factor and the other proposed indicators of nutritional status were not consistently correlated. A significant positive correlation was found between condition factor and plasma triglyceride concentrations (r=0.312, P=0.014, n=62), but no significant relationship was found between condition factor and either HSI (r=0.093, P=0.356, n=100) or plasma glucose concentrations (r=0.036, P=0.773, n=65).

Reproductive indicators Mean values (± SE) of GSI, plasma 17-β estradiol, and plasma ALP (vitellogenin) for sole collected from the four sampling sites are shown in Table 5, along with results of 2-way ANOVA examining the effects of site and sampling time on GSI and plasma estradiol concentrations, and 1-way ANOVA examining the effects of sampling site on plasma ALP (measured in December only). Both GSI and plasma estradiol concentrations increased significantly between December and January at all sites; no significant month-site interactions were observed. Site of capture also influenced both GSI and plasma estradiol concentrations. Fish from Eagle Harbor exhibited significantly lower GSI (t=–2.566, P=0.0115) and Duwamish Waterway fish exhibited significantly lower plasma estradiol concentrations (t=2.464, P=0.0156) than fish from the Port Susan reference site. Plasma ALP concentrations were significantly lower in sole from Sinclair Inlet (t=–3.004, P=0.0058) and higher in sole from Eagle Harbor (t=2.860, P=0.0039) than in fish from the Port Susan reference site.

Ovarian atresia Ovarian atresia was also assessed in fish sampled for fecundity analysis. The prevalence of atresia of yolked oocytes was significantly higher in sole from Eagle Harbor than in sole from the other sampling sites (G-

Table 3

Age-specific fecundity in thousands of eggs (mean ± SE) for English sole from Port Susan, Duwamish Waterway, Sinclair Inlet, and Eagle Harbor.

	Sampling site			
Fish age (yr)	Port Susan	Sinclair Inlet	Duwamish Waterway	Eagle Harbor
5	351 (1)	—	240 ± 12 (2)	242 ± 11 (3)
6	191 ± 29 (8)	243 (1)	223 ± 113 (2)	331 ± 54 (2)
7	215 ± 31 (7)	368 ± 58 (4)	406 ± 25 (8)	277 ± 51 (5)
8	218 ± 35 (4)	271 ± 74(2)	489 ± 29 (10)	284 ± 16 (2)
9	301 ± 60 (4)	531 (1)	542 ± 58 (2)	364 ± 59 (4)
10	377 (1)	468 ± 71 (1)	464 ± 58 (2)	410 ± 153 (2)
11	330 ± 70 (3)	499 ± 122 (4)	—	298 ± 53 (3)
12	—	1,180 (1)	901 (1)	467 ± 49 (2)
13	—	—	—	—
14	—	970(1)	—	—
15	—	—	—	—
16	—	—	—	708 (1)

Table 4

Mean values (± SE) for length (mm), age, condition factor, hepatoxomatic index (HSI), plasma triglyceride, and plasma glucose levels in English sole from four sites in Puget Sound. Plasma triglyceride and glucose concentrations were normalized by log-transformation prior to statistical analysis; other variables were already normally distributed. Values with different superscripts are significantly different (Fisher's PLSD multiple range test, $P \leq 0.05$).

Site	Length (mm)	Age (yr)	Condition	HSI	Triglycerides	Glucose
Port Susan	314 ± 28^{a} (n=29)	7.6 ± 0.3^{a} (n=28)	0.0083 ± 0.0013 (n=29)	1.82 ± 0.07^{a} (n=18)	74 ± 9^{a,b} (n=18)	52 ±9^{a} (n=18)
Sinclair Inlet	376 ± 37^{b} (n=19)	9.4 ± 0.5^{b} (n=19)	0.0088 ± 0.0007 (n=19)	2.01 ± 0.09^{a,b} (n=15)	101 ± 11^{b} (n=15)	42 ± 3^{a} (n=15)
Duwamish	346 ± 29^{c} (n=27)	7.7 ± 0.3^{a} (n=27)	0.0088 ± 0.0013 (n=27)	3.06 ± 0.07^{c} (n=17)	187 ± 17^{c} (n=17)	53 ± 5^{a} (n=17)
Eagle Harbor	350 ± 22^{c} (n=25)	8.8 ± 0.6^{b} (n=25)	0.0091 ± 0.0010 (n=25)	2.22 ± 0.10^{b} (n=15)	73 ± 21^{a} (n=15)	25 ± 41^{b} (n=15)
	P=0.0001	P=0.0076	P=0.1016	P=0.0001	P=0.0001	P=0.0005

statistic, $P<0.05$). At Eagle Harbor, 43% of sampled fish exhibited atresia, in comparison with 28%, 21%, and 17% of fish at Sinclair Inlet, Port Susan, and the Duwamish Waterway, respectively. Atresia severity also tended to be greatest at Eagle Harbor (average rankings were 1.33 at Eagle Harbor, 1.00 at Sinclair Inlet, 0.759 at Port Susan, and 0.542 at the Duwamish Waterway), but intersite differences in severity were not statistically significant (P=0.2659 in the nonparametric Kruskall-Wallis test).

Chemical and nutritional parameters as predictors of egg production patterns

Results of multiple regression analysis examining associations of egg weight, fecundity, and relative fecundity with biomarkers of contaminant exposure and nutritional factors are shown in Table 6 (reproductive rate was not included in this analysis because no significant intersite differences were seen in this variable). As noted above, potentially confounding biological factors (i.e. fish length, age, sampling time, and GSI in the case of fecundity, and GSI and sampling time in the case of egg weight; see Table 1) were incorporated into the regression analyses along with bioindicators of exposure in order to adjust for their contribution to the observed variation in fecundity and egg weight.

Both fecundity and relative fecundity were found to be significantly and positively associated with PCB concentrations in liver. Egg weight showed a significant positive association with biliary FAC's-BaP; the relationship with FAC's-NPH was also positive, but not statistically significant. Additionally, a near-sig-

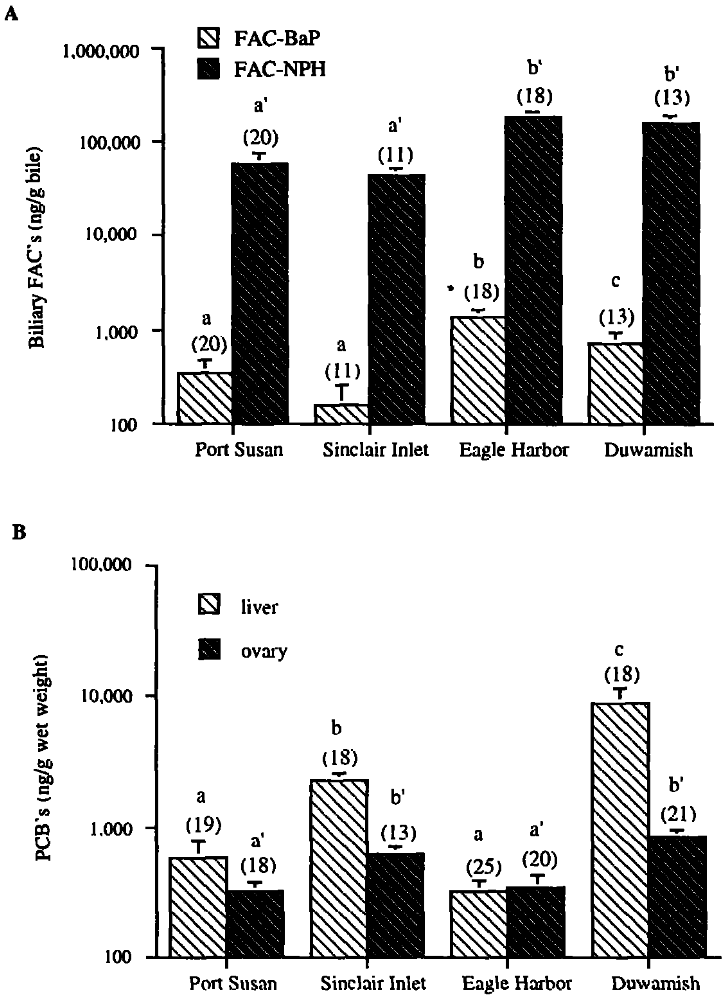

Figure 3

(**A**) Mean levels (± SE) of aromatic compounds (ng/mL) fluorescing at benzo[a]pyrene (FAC-BaP) and napthalene (FAC-NPH) wave lengths in bile of gravid female English sole collected from Eagle Harbor, Sinclair Inlet, Port Susan, and the Duwamish Waterway. (**B**) Mean (± SE) concentrations of PCB's (ng/g wet weight) in liver and ovary of gravid female English sole collected from Eagle Harbor, Sinclair Inlet, Port Susan, and the Duwamish Waterway. Numbers of samples analyzed per treatment are indicated in parentheses. Significant differences in site means (as determined by ANOVA and Fisher's PLSD, $P < 0.05$) are indicated by letter superscripts. Series a–c is used for FAC-BaP and liver PCB concentrations; series a'–c' is used for FAC-NPH and ovarian PCB concentrations.

nificant negative association (P=0.0617) was found between liver PCB concentrations and egg weight. A significant negative association was also observed between fecundity and atresia severity of yolked oocytes, and a positive association between atresia and egg weight. Vitellogenin concentration showed significant or near-significant negative associations with fecundity (P=0.0579) and relative fecundity (P=0.0164) and was significantly positively correlated with egg weight (P=0.0164). Plasma estradiol concentration showed a similar relationship with fecundity, relative fecundity, and egg weight, but the associations were not statistically significant at $\alpha = 0.05$ ($0.06 < P < 0.18$).

In addition to bioindicators of contaminant exposure and reproductive condition, fecundity and rela-

Table 5

Mean values (± SE) of gonadosomatic index (GSI), plama estradiol concentration and plasma vitellogenin concentrations (as estimated from plasma alkali-labile phosphate (ALP)) in English sole from four sites in Puget Sound, and results of 2-way analysis of variance (ANOVA) assessing effects of site and month of collection on these variables. All variables were normalized by log-transformation prior to statistical analysis. No significant month-site interactions were observed for either GSI or plasma estradiol concentration, so the interaction term was suppressed in the final model. EH=Eagle Harbor, DW=Duwamish Waterway, SI=Sinclair Inlet, and PS=Port Susan. nd=not determined.

	GSI		Plasma estradiol 17-β (pg/mL)		Plasma ALP (vitellogenin) (mg/mL)	
Site	Dec	Jan	Dec	Jan	Dec	Jan
Port Susan	5.5 ± 0.7 (*n*=19)	15.0 ± 1.4 (*n*=9)	5000 ± 600 (*n*=19)	12000 ± 1700 (*n* =10)	35 ± 3 (*n*=19)	nd
Sinclair Inlet	5.8 ± 0.6 (*n*=15)	9.4 ± 0.1.3 (*n*=4)	5300 ± 400 (*n*=15)	11000 ± 2100 (*n*=4)	24 ± 8 (*n*=19)	nd
Duwamish Waterway	6.4 ± 0.7 (*n*=17)	11.4 ± 1.3 (*n*=10)	3300 ± 400 (*n* =17)	9000 ± 900 (*n* =10)	29 ± 2 (*n*=17)	nd
Eagle Harbor	4.5 ± 0.6 (*n*=15)	9.6 ± 1.1 (*n*=10)	4900 ± 700 (*n* =13)	8500 ± 800 (*n* =10)	49 ± 3 (*n*=15)	nd
2-way ANOVA Results						
Month	*F*=65.9	*P*=0.0001	*F*=49.4	*P*=0.0001	nd	nd
	Dec < Jan		Dec < Jan			
Site	*F*=3.05	*P*=0.032	*F*=3.57	*P*=0.016	*F*=11.25	*P*=0.0001
	EH< PS, t=–2.57, *P*=0.012		DW < PS, t =–2.46, *P*=0.016		EH > PS, *t*=2.86, *P*=0.006 SI < PS, *t*=–3.00, *P*=0.004	

tive fecundity were significantly related to several indicators of nutritional status. Fecundity was positively associated with condition factor (P=0.0006) and showed a near-significant tendency (P=0.0603) to increase with increasing plasma triglyceride levels. Relative fecundity was significantly positively associated with plasma triglyceride levels. These relationships were also observed when only sole from the least contaminated sites (Port Susan and Sinclair Inlet) were examined (e.g. for fecundity vs. condition factor, t=3.177, P=0.0028, n=47; for fecundity vs. plasma triglyceride concentration, t=1.923, P=0.0647, n=32). In addition, significant positive associations were observed between both fecundity and relative fecundity and HSI (P=0.0011) when fish from all sites were included in the analysis. These associations were not apparent when only reference fish were examined (e.g. for fecundity vs. HSI, t=0.776, P=0.4423, n=47). None of the nutritional factors examined were significantly associated with egg weight.

Significant correlations were found between indicators of contaminant exposure and several of the factors related to fish nutritional status (Table 7). Hepatosomatic index showed strong positive correlations with both biliary FAC and tissue PCB concentrations ($0.0001 < P < 0.0002$), and plasma triglyceride and glucose levels were significantly positively correlated with PCB concentrations in the liver. Plasma triglyceride and glucose levels also tended to increase as ovarian PCB concentrations increased and to decrease as biliary FAC levels increased ($0.05 < P < 0.07$), but the correlation was not statistically significant at $\alpha = 0.05$. Condition factor was not significantly correlated with any biomarker of contaminant exposure. No significant correlations were seen between either GSI or plasma estradiol concentrations and either tissue PCB levels or biliary FAC's. However, significant negative correlations were found between plasma vitellogenin (ALP) concentrations and both hepatic and ovarian PCB concentrations.

Discussion

Significant intersite differences in both egg weight and fecundity were detectable in English sole sampled in this study, even after variation in fish size and sampling time had been taken into account. One notable finding was the tendency for Duwamish Waterway and Sinclair Inlet fish to exhibit higher age-specific fecundity in comparison with fish from Eagle Harbor and Port Susan. This difference appeared to be due, at least in part, to a larger size at age in the Duwamish and Sinclair Inlet fish. Although additional data, particularly on older fish,

Table 6

Associations between bioindicators of contaminant exposure, atresia severity, and nutritional factors in English sole and egg weight, fecundity, and relative fecundity as determined through multiple regression, while adjusting for effects of fish size, GSI, and sampling time. For each independent variable, *t*-value, *P*-value, and sample number (*n*) are shown. The sign of the *t*-value indicates the direction of the association (positive or negative). Statistically significant associations ($P \leq 0.05$) are indicated in bold.

Independent variable	Fecundity[1]	Egg weight[2]	Relative fecundity[2]
Biliary FAC-BaP	–1.379 *P*=0.1733 (*n*=62)	**2.314** ***P*=0.0242** **(*n*=62)**	–1.083 *P*=0.2835 (*n*=62)
Biliary FAC-NPH	0.842 *P*=0.4032 (*n*=61)	1.593 *P*=0.1165 (*n*=62)	–0.029 *P*=0.9767 (*n*=61)
Liver PCB's	**2.402** ***P*=0.0187** **(*n*=79)**	–1.897 *P*=0.0617 (*n*=72)	**2.350** ***P*=0.0214** **(*n*=79)**
Ovary PCB's	0.933 *P*=0.3544 (*n*=72)	–0.105 *P*=0.9166 (*n*=72)	1.641 *P*=0.1054 (*n*=72)
Atresia severity[3] (yolked oocytes)	**–2.162** ***P*=0.0334** **(*n*=91)**	**2.901** ***P*=0.0047** **(*n*=92)**	–1.852 *P*=.0674 (*n*=91)
Plasma estradiol	–1.907 *P*=0.0598 (*n*=96)	1.340 *P*=0.1834 (*n*=97)	–1.775 *P*=0.0792 (*n*=96)
Vitellogenin	–1.934 *P*=0.0579 (*n*=65)	**2.462** ***P*=0.0164** **(*n*=65)**	**–2.468** ***P*=0.0164** **(*n*=65)**
Condition factor	**3.552** ***P*=0.0006** **(*n*=99)**	–1.440 *P*=0.1531 (*n*=100)	0.330 *P*=0.7419 (*n*=99)
HSI	**3.381** ***P*=0.0011** **(*n*=99)**	–1.440 *P*=0.1531 (*n*=100)	**4.350** ***P*=0.0001** **(*n*=99)**
Glucose	1.722 *P*=0.0902 (*n*=65)	0.896 *P*=0.3735 (*n*=65)	1.355 *P*=0.1803 (*n*=65)
Triglycerides	1.917 *P*=0.0603 (*n*=62)	1.680 *P*=0.0983 (*n*=62)	**2.629** ***P*=0.0109** **(*n*=62)**

[1] Adjusted for fish length, age, GSI and sampling time.
[2] Adjusted for GSI and sampling time.
[3] Ranked on a scale of 1–7, where 1 is miminal and 7 is severe.

are needed to confirm that Duwamish and Sinclair Inlet fish do in fact have a higher growth rate or longer period of growth than fish from the other sampling areas, the present results suggest that intersite differences in growth rate may have a significant effect on age-specific egg production in Puget Sound English sole. This problem deserves further investigation because of its potential impact on sole population dynamics.

Another notable finding was the tendency of English sole from the Duwamish Waterway to produce more and smaller eggs than fish of comparable size

Table 7

Associations between nutritional and reproductive factors and biomarkers of contaminant exposure in English sole as determined through multiple regression, while adjusting for effects of sampling time. *T*-value for regression factor, *P*-value, and sample number are shown. The sign of the *t*-value indicates the direction of the association (positive or negative). Statistically significant associations are indicated in bold.

Exposure biomarkers	Nutritional and reproductive factors						
	Condition	HSI	Glucose	Triglycerides	Estradiol	Vitellogenin	GSI
Biliary FAC-BaP	1.701 *P*=0.0942 (*n*=62)	**6.297** ***P*<0.0001** **(*n*=62)**	–1.902 *P*=0.0654 (*n*=37)	–1.880 *P*=0.0699 (*n*=33)	–0.739 *P*=0.4628 (*n*=59)	0.493 *P*=0.6248 (*n*=37)	–0.237 *P*=0.8133 (*n*=62)
Biliary FAC-NPH	1.287 *P*=0.2032 (*n*=62)	**6.660** ***P*<0.0001** **(*n*=62)**	–1.692 *P*=0.0994 (*n*=37)	–0.851 *P*=0.4041 (*n*=33)	–0.636 *P*=0.5274 (*n*=59)	–0.280 *P*=0.7809 (*n*=37)	–0.262 *P*=0.7939 (*n*=62)
Liver PCB's	0.143 *P*=0.8870 (*n*=80)	**3.905** ***P*=0.0002** **(*n*=80)**	**2.357** ***P*=0.0228** **(*n*=47)**	**3.786** ***P*=0.0005** **(*n*=43)**	–1.209 *P*=0.2305 (*n*=77)	**–2.717** ***P*=0.0093** **(*n*=47)**	0.096 *P*=0.9328 (*n*=80)
Ovary PCB's	0.132 *P*=0.8956 (*n*=72)	**5.279** ***P*=0.0001** **(*n*=72)**	1.997 *P*=0.0534 (*n*=38)	1.920 *P*=0.0630 (*n*=34)	0.066 *P*=0.9476 (*n*=69)	**–2.603** ***P*=0.0133** **(*n*=38)**	0.676 0.5010 (*n*=72)

and maturity from other sites. Although this intersite difference in egg production pattern could represent a genetic adaptation of the Duwamish sole stock to its particular habitat, this is not likely on the basis of current knowledge of the population structure of English sole in Puget Sound. Sole populations residing at our sampling sites do not appear to constitute discrete breeding populations but migrate to common breeding areas, such as University Point or Duwamish Head in central Puget Sound, for spawning (Collier et al., 1992). Moreover, their eggs and larvae are pelagic and therefore are transported from the breeding area to nearshore nursery ground settling sites in accordance with current patterns (Lassuy, 1989). Site-specific genetic adaptation would be unlikely in animals with such a breeding system, although some genetic divergence between subpopulations in northern and southern Puget Sound with distinct spawning areas is a possibility. Overall, marine fish show relatively little geographic diversity (Gyllensten, 1985), and their genetic structure is thought to be determined largely by the dispersal potential of the pelagic stages, rather than by adaptation to local environmental conditions (Waples, 1987). Studies of marine flatfish, such as the common sole (*Solea vulgaris*), turbot (*Scopthalmus maximus*), and flounder (*Platichthys flesus*) in the northeastern Atlantic and Mediterranean (Galleguillos and Ward, 1982; Blanquer et al., 1992; Kotoulas et al., 1995), indicate that these species exhibit some geographic isolation or differentiation due to temperature gradients that inhibit larval transport and survival but that they show fairly substantial gene flow on a regional level. In the common sole, for example, a species with a life history strategy similar to that of English sole, the geographic unit of population structure appears to lie within a radius of approximately 100 km (Kotoulas et al., 1995).

Changes in egg weight and number could also be associated with alterations in habitat characteristics, such as water temperature, food supply, and food quality, all of which have been shown to influence egg development in English sole or other fish species. Winters et al. (1993), for example, demonstrated that winter temperature 2 to 3 months before spawning can affect fecundity and egg size in herring from the northwest Atlantic. Temperature can also affect the rate of gonadal development in English sole, and consequently egg size at a particular sampling time (Kruse and Tyler, 1983). In general, however, bottom temperature in Puget Sound is not highly variable over the geographic range encompassed by this study (Collias et al., 1974; Malins et al., 1980, 1982), and water temperatures in the Duwamish Waterway are comparable to those from sites in the main basin (Collier, 1988). Consequently, it is unlikely that temperature is a major contributing factor to the intersite differences in patterns of egg development that we observed in this study.

The present findings suggest, on the other hand, that contaminant exposure and nutritional variables, or their interaction, could be important contributing factors to the observed changes in fecundity and egg weight. Although indicators of chemical contaminant exposure were not among the strongest predictors of fecundity and egg weight in the sole examined in this study, some significant associations were observed between tissue PCB and biliary FAC concentrations in individual fish and patterns of egg production. Elevated concentrations of PCB's in liver or ovarian tissue, which were characteristic of fish from the Duwamish Waterway, were associated with reduced plasma ALP (vitellogenin) concentrations, as well as with production of more but smaller eggs. These data suggest that exposure to PCB's might affect egg development, perhaps by inhibiting either the production or uptake of vitellogenin. However, reports of the effects of PCB's on vitellogenin production in fish are somewhat inconsistent. In the larger set of fish sampled in our earlier study (Johnson et al., 1988), a correlation between elevated tissue PCB concentrations and reduced plasma ALP in vitellogenic fish was also observed (n=60, Spearman's ρ=–0.30, P=0.023), as well as a tendency for plasma ALP concentrations to be lower in fish from the Duwamish Waterway and Sinclair Inlet, although intersite differences were less pronounced than in the smaller set of fish for which fecundity and egg weight determinations were performed. In other studies such compounds have proved to be estrogenic and have enhanced vitellogenin production in fish and reptiles (von der Decken et al., 1992; Guillette et al., 1994) or have exerted little effect on plasma vitellogenin concentrations (Monosson et al., 1994). The impact of PCB exposure on egg development might be better clarified through congener-specific analysis of PCB's because the various coplanar and noncoplanar PCB congers present in complex PCB mixtures are known to differ in toxicity (Safe, 1990), as well as in their ability to enhance or inhibit vitellogenin synthesis (Anderson et al., 1996). Exposure to PAH's also appeared to have some influence on egg development because we found that elevated biliary FAC-BaP levels were correlated with both increased atresia of yolked oocytes and a trend toward increased egg weight and lowered fecundity. Interestingly, atresia tended to be most prevalent and of greatest severity at Eagle Harbor, where biliary FAC concentrations in fish were particularly high.

In earlier studies of reproductive function in English sole (Johnson et al., 1988), we observed reduced plasma estradiol concentrations in female fish from both Eagle Harbor and the Duwamish Waterway. These differences were partially associated with inhibited ovarian development in significant proportions of adult fish from these sites, but differences persisted even when only vitellogenic fish were examined. A similar trend was observed in the fish examined in this study, all of which were vitellogenic, although the intersite difference was statistically significant only for fish from the Duwamish Waterway. Depressed plasma estradiol concentrations tended to be associated with increased fecundity but were not strongly correlated with changes in egg production patterns.

Nutritional status appeared to have a significant effect on fecundity in English sole because a strong correlation was found between condition factor and fecundity in fish from minimally to moderately contaminated sites. Similar relationships between fecundity and food supply, condition factor, and other indicators of nutritional status have been observed in other fish species, including winter flounder (Tyler and Dunn, 1976), temperate and tropical clupeids (Hay and Brett, 1988; Milton et al., 1994), plaice (Horwood et al., 1986, 1989), and rainbow trout (Bromage et al., 1992). When sole from the contaminated sites (Eagle Harbor and the Duwamish Waterway) were included in the analyses, additional nutrition-related factors showed correlations with fecundity in English sole. Of these factors, HSI showed a particularly strong relationship with fecundity and was significantly higher in Duwamish Waterway fish than in animals from the other sampling sites. Animals from the Duwamish Waterway also exhibited elevated plasma triglyceride levels, which, like increased HSI, appeared to be associated with production of more and smaller eggs. Milton et al. (1994) also observed production of more, but smaller, eggs in tropical clupeids with increased HSI, and interpreted the alteration in egg size and number as a response to the good nutritional status of the female and a possible adaptation to environmental conditions in which food was abundant. It is possible that elevated HSI and plasma triglyceride levels in Duwamish Waterway fish could be related to favorable feeding conditions. Previous studies have, in fact, shown that benthic invertebrates such as mollusks and polychaetes, which form a significant proportion of the diet of English sole (Varanasi et al., 1989), are relatively abundant in the Duwamish Waterway (Malins et al., 1980, 1982). However, the Duwamish Waterway fish did not have a significantly higher mean condition factor than that of animals from the other sampling sites, and although plasma triglyceride concentrations showed some correlation with condition factor, HSI did not. Both HSI and plasma triglyceride concentrations, however, showed strong correlations with bioindicators of contaminant exposure. Increased HSI in association with exposure to toxicants, particularly agents that induce cell proliferation, is well documented in a number of fish species (Heath, 1987), and toxicant-related increases in serum triglycerides have also been observed in previous studies. For example, English sole showed increased serum triglyceride levels in response to laboratory exposure to model toxicants bromobenzene and *o*-bromophenol (Casillas and Myers, 1989). Consequently, elevated levels of these parameters in Duwamish Waterway fish could be a reflection of toxicant exposure rather than good nutritional status. Moreover, the contaminants may affect fecundity or egg size indirectly through their impact on liver function, lipid disposition, or lipid metabolism. Results of this study suggest the possibility of such interactive effects of contaminants and nutritional factors on egg development.

In addition to PCB's and PAH's, other contaminants, such as heavy metals and organotins, which are present in the Duwamish Waterway and to a lesser extent at Sinclair Inlet (Krone et al., 1989; PSWQA, 1994; Dutch et al.[2]), could affect egg weight or other aspects of gonadal development in English sole. Previous studies have shown that a number of toxic trace elements, including copper, lead, mercury, and cadmium (Kaviraj, 1983; Munkittrick and Dixon, 1988; Dethlefson, 1989), as well as tributyl tin (TBT) (Walker et al., 1990), can affect egg size or gonadal development in fish. Previously, Krone et al. (1989) showed increased tissue concentrations of TBT in English sole from the Duwamish Waterway. However, many of these trace elements in their organic form do not bioaccumulate in English sole (Meador et al., 1994) and indicate low bioavailability.

Increased egg production or production of more but smaller eggs is not the most commonly observed response in fish exposed to environmental contaminants, but such trends have been observed in some previous studies. Slooff and DeZwart (1983) reported increased fecundity in bream exposed to a mixture of chlorinated and aromatic compounds in the Rhine River, and Walker et al. (1990) reported a similar finding for medaka exposed to TBT. Reduced egg size, although not necessarily in conjunction with increased egg production, has been reported in a number of studies in which fish were exposed to PAH's, PCB's, or both (Kime, 1995). Interestingly, since 1900, North sea plaice have also exhibited a trend toward production of more but smaller eggs (Rijnsdorp, 1991). The causes of these changes are unknown, but it is suspected that they are most likely related to changes in environmental conditions, which could include environmental degradation associated with anthropogenic activities.

In summary, the results of this study suggest that egg weight and number in English sole are influenced by a variety of factors, including exposure to organic chemical contaminants such as PCB's and PAH's, nutritional status, and growth rate. Although chemical contaminant exposure did not appear to have a major impact on egg development in English sole, high concentrations of contaminants in tissues or body fluids showed significant associations with certain potentially detrimental changes: elevated PCB concentrations in liver were correlated with reduced plasma vitellogenin levels and reduced egg weight, and high levels of biliary FAC's were associated with increased ovarian atresia and reduced fecundity. The impact of these alterations in egg weight and number on the reproductive fitness of affected fish is not clear. It is likely that smaller eggs will tend to produce smaller larvae, and reduced larval size has been associated with lower growth and survival rates in other flatfish species (Buckley et al., 1991). However, the detrimental effects of reduced egg weight could be offset by increased egg production, or, at least in the case of the Duwamish Waterway fish, by a relatively fast growth rate and high age-specific fecundity. In order to gain a better understanding of the relationships between chemical contaminants, nutritional factors, and alterations in gonadal development and egg production, we are currently investigating the effects of PCB's, AH's, and food supply on egg weight and fecundity in further laboratory studies with English sole.

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