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Exposure to Sewage Plumes and the Incidence of Deformities in Larval Fishes

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Point-source impacts such as sewage plumes can cause significant degradation of larval habitat. Data on larval abundance, current speed and the shape of surface and subsurface sewage plumes off the coast of Sydney, Australia, indicated that long-shore currents can transport large numbers of larvae through plumes that can potentially affect the health of larvae. Deformities to the notochord, eyes and head were found in samples of preserved wild larvae. Some deformities (e.g. Lateral curl of the notochord) were probably caused by preservation and 'packing effects' (shaped by organisms and objects in the sample), while others (e.g. half-pigmented eyes and corrugated notochord) were unlikely to have been caused by sampling artefacts. Deformed larvae were found in waters around Sydney that are influenced by multiple sewage outfalls, and at locations up to 250 km from Sydney. It was concluded that deformities were caused by natural and potentially anthropogenic factors. Cyto- and histopathological studies of larvae are required. Moreover, relationships between oceanography and concentrations of pollutants in plumes are needed to further assess impacts of pollutants on assemblages of vulnerable planktonic animals. © 1997 Elsevier Science Ltd

Fishes are particularly vulnerable to pollutants during the early stages of their development (Westernhagen, 1988). Laboratory studies have demonstrated that even brief exposures to pollutants can affect eggs or larvae and result in death, or sublethal effects such as deformities and changes in physiological function and behaviour (Rosenthal and Alderdice, 1976; Fuiman, 1993). Most of the evidence on the vulnerability of larvae to pollutants is from laboratory studies (but see Raimondi and Schmitt, 1992) and in general it has been difficult to translate the findings from these studies to field situations. Some of the problems include: *1.* concentrations of pollutants used in the laboratory are often much higher than those measured in the field (Westernhagen, 1988); *2.* it is difficult to 'scale up' the results from laboratory experiments to wild cohorts and populations of fish (Underwood and Peterson, 1988); *3.* it is difficult to determine concentrations of pollutants and regimes of exposure in the field because of variable conditions of oceanography where pollutants will disperse from point-sources by dispersion, but may concentrate at convergences (e.g. Cross *et al.*, 1987; Shanks, 1987; Tanabe *et al.*, 1991; Kingsford and Gray, 1996), the output of pollutants from point sources may also vary; and *4.* larvae are small, often transparent, and can be transported great distances by currents (Leis, 1991), and as a result it is potentially difficult to detect impacts (*cf.* territorial adult fish).

Although larvae are small and inconspicuous, there are concerns that the degradation of larval habitat could affect survivorship of larvae and subsequent year class strength, particularly if the species are of commercial or recreational importance. Major numerical impacts on the larvae of specific groups of fishes, therefore, could affect the ecology of local marine assemblages (e.g. Nisbet *et al.*, 1996). Kingsford and Gray (1996) discussed the potential for point sources of pollution to influence larvae that had resulted from spawnings over a broad area. Unlike the 'halo' of influence from point sources of pollution for adult and juvenile organisms that are territorial, sedentary or sessile (e.g. McLean *et al.*, 1991), larvae can be swept into pollution plumes from great distances. A combination of longshore currents (e.g. Griffin and Middleton, 1991) nearshore distributions of larvae (e.g. Kingsford and Choat, 1989) and surface or subsurface pollution plumes could affect a significant proportion of offspring from local populations. There are, however, no calculations of potential exposure based on a knowledge of current velocity and abundance of larvae in different depth strata.

Impacts on the abundance of plankters in polluted waters have been detected (e.g. Karas *et al.*, 1991). However, comprehensive sampling of ichthyoplankton (adequate replication in space and time) in dynamic coastal waters (Griffin and Middleton, 1991) has failed to detect predictable relationships between the abundance (or absence) of larvae and the proximity of pollution plumes (Gray *et al.*, 1992; Gray, 1995). The challenge, therefore, is how can the degradation of larval habitat be measured? The study of deformities (so called 'terata') in fish eggs and larvae is potentially informative (Longwell *et al.*, 1992; Bodammer, 1993). In the case of larvae they must survive long enough to be collected once they have been exposed to pollutants before they are lost from the system. A variety of deformities have been found in fishes that were exposed to pollutants in laboratory studies (reviews: Westernhagen, 1988; Kingsford and Gray, 1996) and links between polluted waters and the incidence of deformities have been established (Karas *et al.*, 1991). This has to be tempered, however, with the knowledge that some deformities may occur naturally (e.g. Purcell *et al.*, 1990).

Our study was done in coastal water off New South Wales, Australia. A broad spectrum of pollutants including industrial waste, sewage, insecticides and storm water are released into coastal waters off Sydney (e.g. Fagan *et al.*, 1992) through cliff-face and three deep ocean outfalls; the latter replaced cliff-face outfalls (Kingsford and Gray, 1996). Deep ocean outfalls are located at North Head, Bondi and Malabar (Fig. 1). These outfalls have been operational since 1991 and release sewage 2.5–3.5 km from shore in 60–80 m of water (Waterboard, 1991). A total of 1040–5300 megalitres of sewage is released per day from these outfalls (Waterboard, 1991). Sewage exits through a series of diffusers over the last 700 m of each outfall and rises as a 'curtain' into the water column. Pollutants (e.g. Cd, Cr, Zn, Hg, Mn; Beder, 1989; Rendell and Espey, 1993) reach the surface, particularly when the water column is not stratified by a thermocline (e.g. in winter), but the area in which plumes surface varies according to the strength and direction of currents.

A study on broad-scale (10s–100s of kilometres) patterns of the abundance of deformed fish was designed based on the findings from an earlier study. Kingsford *et al.* (1991) studied deformities in fish larvae that were found in a sewage plume, the front of the plume and control sites (1–10 km from the plume). Although the percentage of deformed fish in collection were often highest in the plume and associated front, deformed fish were also found in controls. Given the complicated oceanography of the study area it was possible that fish captured in control areas had at one or multiple times been exposed to pollution in plumes. Moreover, the relative contribution of 'natural' (e.g. genetic, temperature, availability of food etc.) and anthropogenic processes in influencing the representation of deformities remained unresolved. Sampling was required at greater distances from the potentially polluted 'pool' of Sydney water (Beder, 1989; Rendell and Espey, 1993), so that larvae from locations with sewage plumes could be compared with areas of low pollution.

Our specific objectives were as follows: *1.* determine the potential of point source(s) of pollution to degrade larval habitat, this was based on a knowledge of the area of surface and subsurface sewage plumes facing mainstream currents, current velocity and densities of larvae; *2.* describe morphological deformities in fish larvae that could be used for measuring deleterious effects on larval habitat; and *3.* determine whether there is a higher proportion of deformed larval fish in the neuston off Sydney (i.e. exposed to multiple sources of pollution) than off less urbanized locations to the north and south of Sydney?

All fish larvae in this study were sampled from the neuston. Surface waters are ideal for examining the potential effects of sewage on larval fish. Greases and other hydrocarbons which float to the surface (e.g. from deep ocean outfalls) often bind with other pollutants (e.g. Balls, 1990) that may affect larval fish. Trace metals and chlorinated hydrocarbons have been found in the surface microlayer of the ocean in New South Wales (Rendell and Espey, 1993) and other parts of the world (Kingsford and Gray, 1996) and the 'enrichment factor' at the surface can be 1.5–30× that of subsurface water (Rendell and Espey, 1993). Moreover, convergence zones in which pollutants may accumulate (e.g. Tanabe *et al.*, 1991) are conspicuous in coastal waters (review Kingsford, 1990). Fish larvae are abundant in surface waters along the coast of NSW (e.g. Gray *et al.*, 1992; Kingsford and Suthers, 1996). This depth stratum, therefore, was appropriate for the study of pollutants and larval fish.

Methods

Exposure of larval fish to pollution plumes

Calculations of the potential exposure of fishes to surface and subsurface plumes was based on estimates of concentrations of larvae collected near the surface (i.e. potentially affected by surface sewage plumes) and those collected at depth (30 m and potentially affected by subsurface pollution plumes) over a period of four years (Gray, 1995). The size of surface plumes varied greatly according to currents, wind and the volume of effluent released (Kingsford and Gray, 1996), thus we calculated numbers of larvae that would pass through a 1 m^2 window of a plume (Fig. 2). Subsurface plumes rise as a 'curtain' of sewage from the diffusers. The plume is often trapped below the surface when the water column is stratified, but when the water column is unstratified the plume rises to the surface. Calculations are, therefore, given for total numbers of larvae exposed to the plume per unit time in both of these situations. In this part of the study, larvae were collected using a 0.5 mm mesh net (mouth area 0.5 m^2). The mesh was organized into cylinder and cone sections, and efficiency was calculated at 1:7. Volume of water sampled was measured with a General Oceanics 2030 flowmetre. The range of current speeds was based on data collected by the Sydney Waters Ocean Reference station.

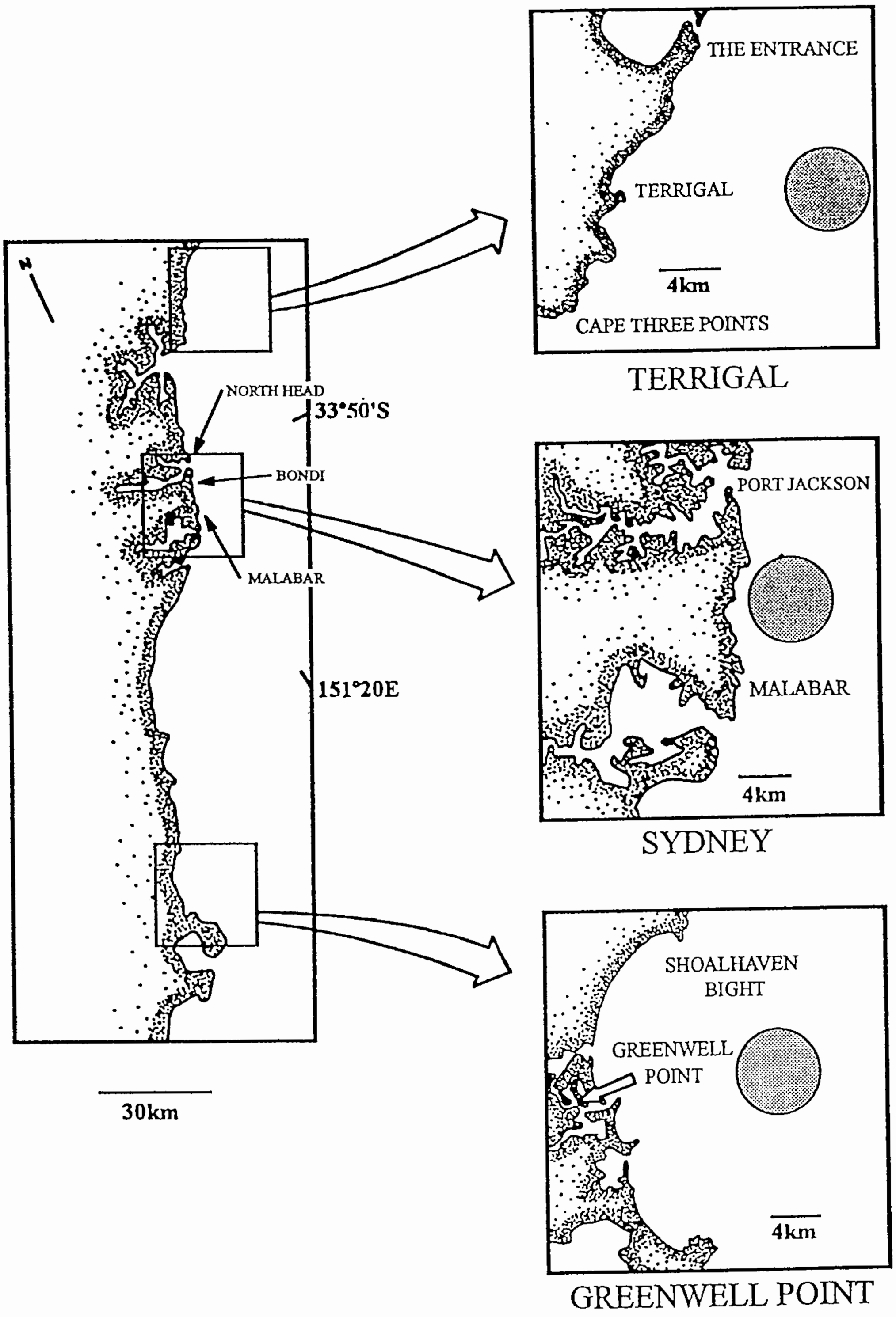
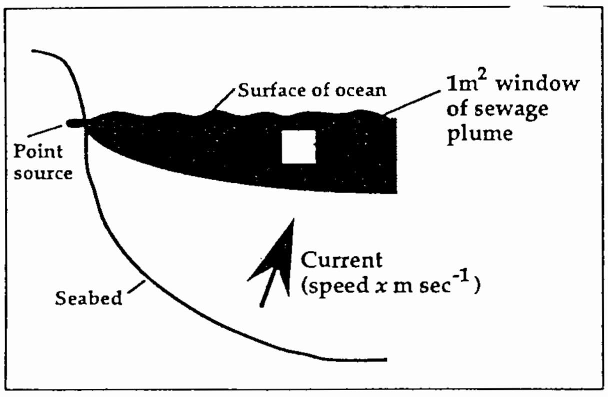


Fig. 1 Map showing the study area and locations of the three deep-ocean outfalls in Sydney, North Head, Bondi and Malabar which exit in 60–80 m of water 2.5–3.5 km offshore. Within each of the locations marked with a box, a circle indicates the area in which samples were taken at three sites that were separated by 2–3 km.

Distribution patterns of deformed fish larvae

Our approach was to sample fish larvae in the neuston to the north of, within and to the south of the Sydney area (Fig. 1). It was assumed that a predominantly south flowing current would make it unlikely that larvae exposed to pollutants in the vicinity of Sydney would be advected as far north as Terrigal. Terrigal and Jervis Bay were considered to be clean locations. Although these locations have sewage outfalls, they are of very low volume (*cf.* outputs around a). Surface plume from cliff-face outfall



b). Submerged plume from deepwater outfall

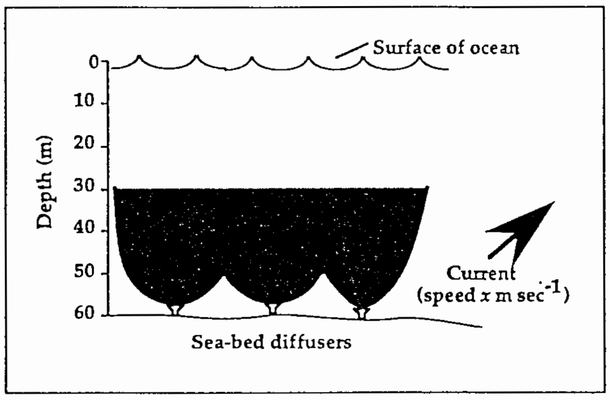


Fig. 2 Diagrammatic representation of surface and subsurface sewage plumes off Sydney, Australia. (a) Cross-section of a surface sewage plume 2–2.5 m deep showing a 1 m^2 window. The number of fish larvae that could potentially pass through this window per hour would be dependent on the abundance of larvae and speed of the current. (b) Subsurface plume that is held at a fixed depth by a thermocline. The plume is generated by the release of effluent through diffusers.

Sydney) and with little or no industrial waste. Moreover, Jervis Bay is sufficiently far from Sydney that deformed larvae are unlikely to be advected, or last (i.e. high natural mortality or pollution induced mortality), over such a distance. Most larvae were late preflexion of early post flexion larvae (*sensu* Leis and Trnski, 1989), we would have expected the majority of these larvae to be less than 15 days old, further minimizing the likelihood of advection from Sydney to other locations. If deformed larval fish were found north and south of Sydney, their condition would be assumed to be due to 'natural causes'. The term 'fish larvae' to mean developmental stages from egg hatching to metamorphosis into a juvenile (after Ahlstrom, see Kingsford, 1988).

Samples were collected at three random sites at each of the three locations along the coast of New South Wales (Fig. 1). Fish were collected over waters of a similar depth range (20–30 m). Sites were separated by 2–3 km. All sampling was done during the day between July 19th and August 20th 1993, when the water column was generally unstratified (CDM Report, 1989) and plumes from the deep ocean outfalls would be expected to surface. Many species of fish spawn at this time of year including members of the Scorpididae, Cheilodactylidae, Labridae; Odacidae and Gobiidae. The unstratified water column was relevant because sewage plumes would reach surface waters where fishes were sampled (Wilson *et al.*, 1996). Sampling was done at four times at each location, when conditions allowed. It is important to note that time x at one location does not relate to time x from another location (i.e. time was nested and not orthogonal with location), because it was impossible to sample all locations on the same day or time block due to variation in local conditions. In the analyses, location was treated as a fixed factor (*sensu* Underwood, 1981), while the factors 'time' and 'site' were treated as random factors. 'Location' was treated as a fixed factor because Terrigal is to the north and generally 'upcurrent' of Sydney, Sydney is in the area affected by deep ocean outfalls and Jervis Bay was to the south, down current and out of the polluted Sydney waters.

A square neuston net was used for all samples collected. The net had a mouth area of 0.56 m^2 (when the upper edge of the net was out of the water) and a mesh size of 500 µm. The mesh was organized as a 2.2 m box and a 2 m pyramid. The filtration efficiency of the net (area of mouth:open area of mesh) was 1:7. The net was towed to the side of the vessel at 3–4 knots for approximately 3 min filtering an average of 200 m^3. A General Oceanics flowmetre was positioned 17 cm from the edge of the frame to record average flow.

Samples were generally preserved in a final concentration of 2–5% formalin and fish larvae were sorted and identified under a dissection microscope. All fish were sorted from other plankton for total counts and the description of deformities (as above). In some samples 1000s of larvae were collected (15/144 samples) and in these cases a subsample of fish were examined for deformities (minimum of 200 fish). No sewage derived fibrous material was observed in the collections (*cf.* Kingsford and Gray, 1996).

Types of deformities

Collections of fish collected from 1990 to 1993 were used to identify different types of deformities (Kingsford *et al.*, 1991; Kingsford *et al.*, 1994). The types of deformities we checked for included those that had been documented in other studies (review: Kingsford and Gray, 1996), and some different types were identified. Each larvae was examined for the presence/absence of the following: gross deformities; conspicuous infection; abnormal mouth; myomere haemorrhaging; bent notochord (conditions shown in Fig. 3); abnormal or crooked fins; protruding eyes; bubble eye (Fig. 4); unevenly pigmented eye (Fig. 4); white eye; deformed eye and deformed gut (misshapen, misaligned or growth in gut). We were careful to differentiate between deformed fish and ones that had been damaged in

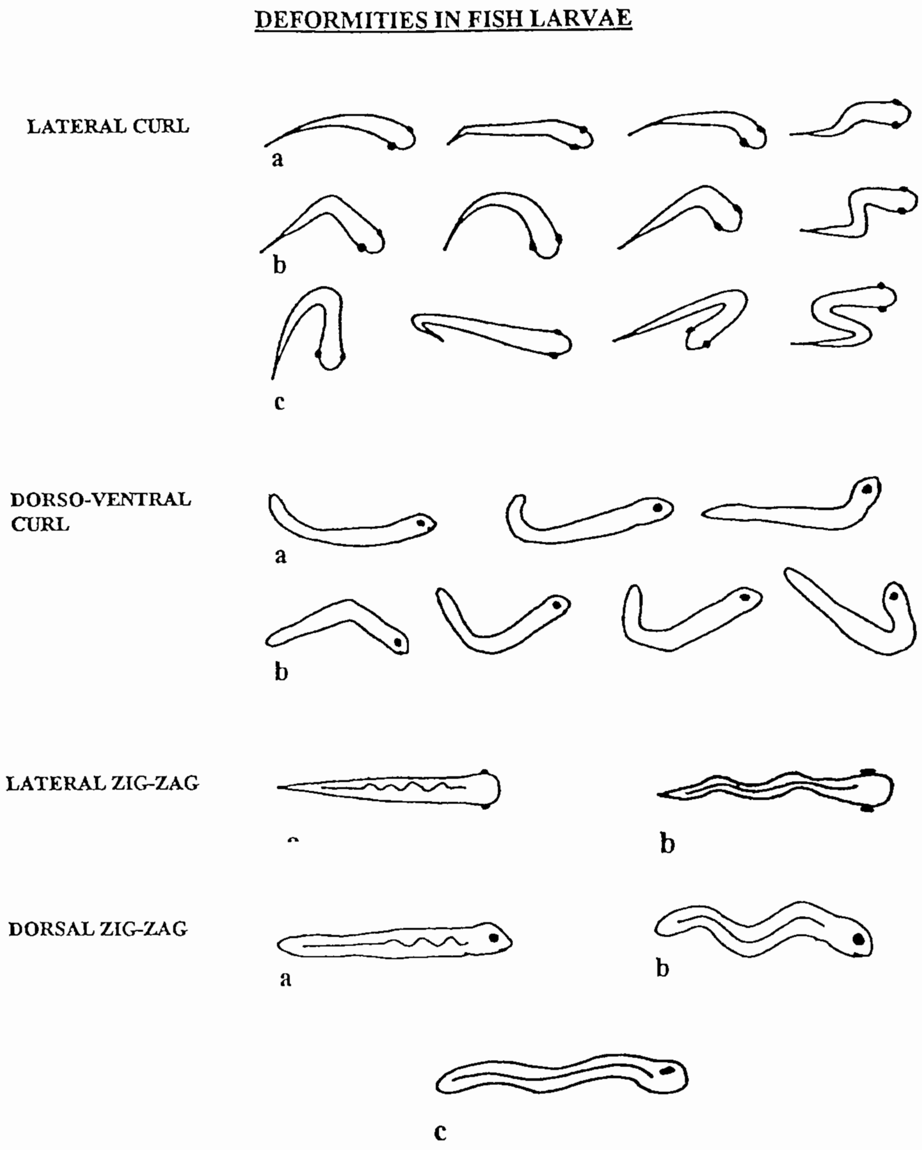


Fig. 3 Types of notochord deformities. Categories 'lateral curl' a,b,c and dorso-ventral curl a were considered to be 'non-serious deformities', while dorso-ventral curl b, dorsal zig-zag and lateral zig-zag were treated as 'serious deformities'.

nets. The frequency of 'lateral curl' and dorso-ventral curl A of the notochord was recorded, but based on the experimental findings of Kingsford and Gray (1996) were not considered to be 'serious deformities'. Kingsford and Gray (1996) demonstrated that the occurrence of lateral curl can be influenced by the quantity of fibrous material in nets (e.g. toilet paper). For this reason, no detailed analyses of this type of deformity were done. Deformities were considered serious if it was likely they would impair swimming, result in death through disease (e.g. conspicuous infections) or compromise the ability of larvae to digest food (gut conditions), see food and predators (eye deformities).

Oceanographic data

Oceanographic data from the Ocean Reference Station located 3 km off Bondi, and computer modelling results for the deepwater outfalls were available for the sampling period (R. Lee EPA, pers. comm.). Temperature stratification was weak (0.5–1°C) at the four times of sampling off Sydney and current measurements indicated a weak and variable field (flows of 0.1–0.2 m s^{-1} with predominant diurnal reversals in direction). Modelling suggested that diluted sewage effluent from the deepwater outfalls should have been surfacing for greater than 90% of the sampling period. Dilutions of 150–3000 were predicted. Only at time 4 off Sydney was it predicted that the plume would have been trapped beneath the thermocline at 30 m. The oceanographic information suggested that the transport of potentially deformed larvae from the Sydney region would have been slow. That is, it was unlikely that larvae captured at distant locations (e.g. Greenwell Point, Jervis Bay) had been bathed in pollutants from

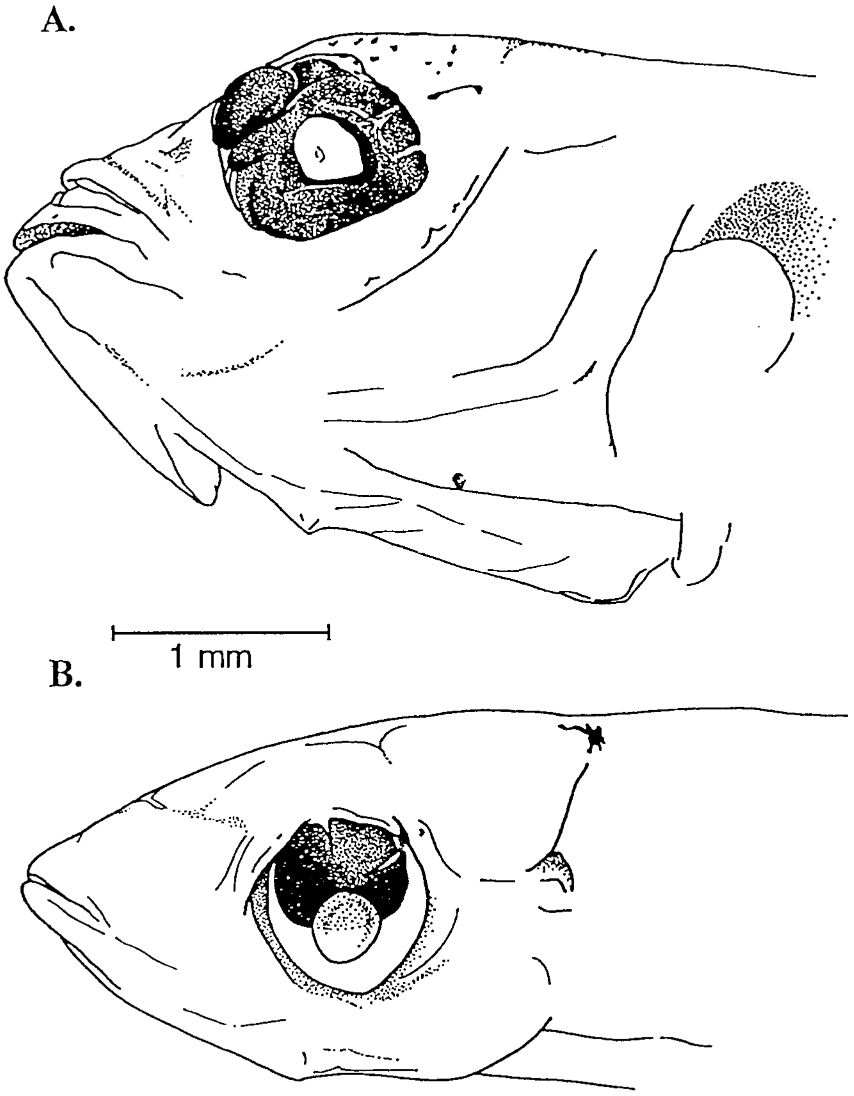


Fig. 4 Eye deformities. Illustrations of (A) 'Bubble eye', and (B) unevenly pigmented eye deformities.

the Sydney region. This conclusion was also based on the likely young age of larvae (i.e. estimated at < 15 days) and high mortality rates in the plankton.

Statistical analyses

Densities of fish larvae were expressed as number per 200 m^3. Homogeneity of the data was tested using Cochran's tests. If raw data were heterogeneous they were transformed using Loge(x + 1) and the Cochran's test repeated. Percentage data were arcsine ($\sqrt{}$proportion) transformed. If data remained heterogeneous after transformation an analysis of variance (ANOVA) was still used, as the analysis is robust to heterogeneity of variance (Underwood, 1981); in this situation factors were tested at $p = 0.01$. Data were analysed using 3-way nested analyses of variance (ANOVA).

Results

Exposure of fish larvae to pollution plumes

Large numbers of fish larvae are transported into surface sewage plumes, by longshore currents, particularly since plumes and/or diffusers are generally orientated perpendicular to these currents (e.g. Fagan *et al.*, 1992; Kingsford and Gray, 1996). The number transported toward a plume depends on abundances of larvae and current velocity and shape of the plume. Abundance of larvae ranged from 0–1500 per 300 m^3 and current speed between 0.1 and 0.8 m s^{-1}. When abundances of larvae were low (e.g. 20 per 300 m^3) between 24 and 240 fish larvae could be transported into a 1 m^2 window of the plume front each hour (depending on current speed, Fig. 5). When abundances were great (e.g. 1500 per 300 m^3) the number of larvae affected could be 1800–18 000. The number of larvae that could potentially be transported into the effluent field of

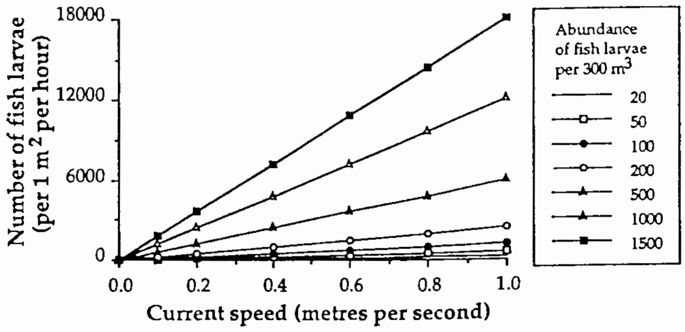


Fig. 5 The potential number of fish larvae (per hour) that could be transported into a 1 m^2 window of a surface sewage plume over a range of larval abundances and current speeds. Fish larvae were treated as passive particles. The range of larval abundances and current speeds were based on those measured in the field.

deepwater outfalls was also high (Table 1). Separate calculations were done for surfacing (sea-bed to surface) and submerged (sea-bed to 30 m) plume conditions. These calculations indicated that between 16 and 205×10^5 fish larvae could be transported into plumes each hour, depending on ambient abundances of larvae, current speed and plume characteristics. Hence, very large numbers of larvae are exposed to plumes with potential lethal or sublethal effects. The percentage of larvae in mainstream currents that would intercept surface or subsurface plumes would depend on the depth and cross-shelf distribution patterns of individual taxa. Larvae from local spawnings would have a high probability of exposure.

TABLE 1

Examples of the number of fish larvae that potentially could be transported into the effluent field at each of the three deepwater sewage outfalls in a 1 h period. Larvae were treated as passive particles, and abundances were averaged throughout the water column. Examples of three levels of larval abundance and three current speeds are given. The area of the initial effluent field (i.e. 'wall' of effluent) at North Head, Bondi and Malabar was calculated to be 45 600 m^2, 34 200 m^2 and 57 600 m^2, respectively. This was calculated from the width of the diffuser section and the depth of water at each outfall. Surfacing plumes were calculated between the seabed and the surface, whereas submerged plumes were calculated between the seabed to 30 m (see Fig. 2)

	Current speed (m s^{-1})	Abundance of fish larvae per 30 m^3 20	100	500
		No. of fish larvae per hour × 10^5		
Surfacing plume				
North Head	0.2	22	109	547
	0.6	66	328	1638
	1.0	109	547	2736
Bondi	0.2	16	82	410
	0.6	49	246	1229
	1.0	82	410	2052
Malabar	0.2	28	138	691
	0.6	83	414	2069
	1.0	138	691	3456
Subsurface plume (restricted to 30 m)				
North Head	0.2	11	55	274
	0.6	33	164	819
	1.0	55	274	1368
Bondi	0.2	8	41	205
	0.6	25	123	614
	1.0	41	205	1026
Malabar	0.2	17	86	432
	0.6	52	259	1293
	1.0	86	432	2160

Abundance of larval fish

Great numbers of larval fish were collected from the neuston off the coast of New South Wales (Fig. 6). Significant differences in total numbers of fish were found between times and sites, but no consistent differences in abundances were found among locations (Table 2). Great differences in abundance were found among times at each location and emphasized the importance of sampling at multiple times (given the vagaries of the supply of larvae, based on variation in spawning times, mortality rates etc.) if an adequate description of frequency of larval deformities were to be obtained. Although differences in abundance were found between sites (nested within times), indicating spatial variation in larval abundance on a scale of 2–3 km, these differences were small compared to the differences in abundance found between random times (i.e. time was significant, Table 2).

Forty-six different types of fish were caught. Three types of fish, however, were by far the most abundant in samples: Scorpididae, *Sardinops neopilchardus* and Cheilodactylidae. Exceptionally high numbers of scorpidids were captured at Greenwell Point at Time 1 (> 12 000 per 200 m^3). All of these species showed significant differences among sites and times, but no consistent differences in concentrations were found among locations.

Deformities in wild fish larvae

Fish larvae with serious deformities were found in this study (Tables 3 and 4). 269 fish were found with serious deformities, which represented 0.3% of the total number of fish caught (99 636 fish larvae). Serious

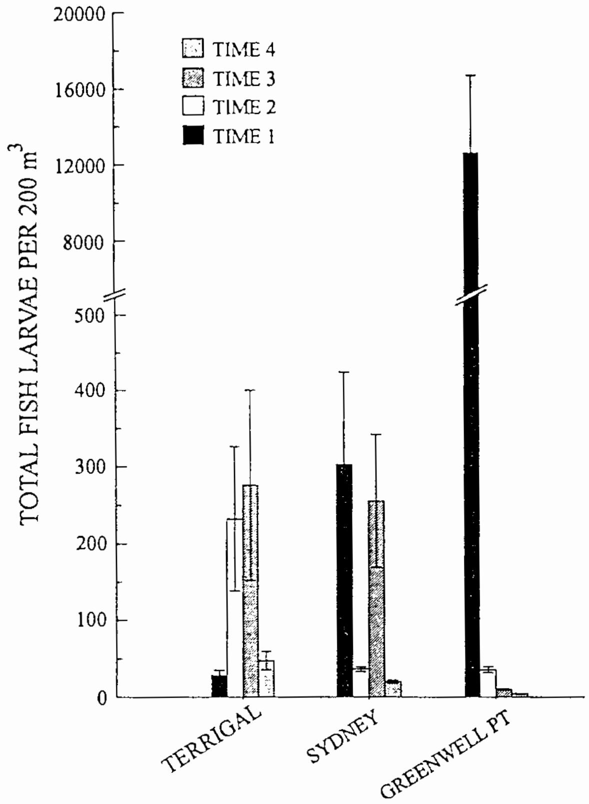


Fig. 6 Total number of fish larvae 200 m^{-3} collected at three locations at four times in winter 1993 along the coast of NSW. Data are mean values of hauls taken at three sites and $n = 3$ hauls at each site.

deformities were primarily to the notochord and eyes (Table 3). Most of the notochord deformities were 'dorso-ventral-curl', 'dorsal zig-zag' and 'lateral zig-zag', whilst eye deformities were generally 'bubble eye' or 'deformed eye'. Some fish had a combination of serious deformities (generally a notochord deformity plus 1 other). In general, this was a 'non-serious' 'lateral-curl' type notochord deformity and a relatively rare serious category (e.g. 'body infection').

Fish larvae with serious deformities were found at all locations (i.e. Terrigal, Sydney and Greenwell Point). There was no evidence that the presence/absence, abundance or proportional representation of deformed larvae were higher in the vicinity of the relatively polluted waters of Sydney. 73 to 100 fish larvae with serious deformities were found at each location and this represented 0.11–1.9% of total fish caught at each location (Table 4). The number and percentage of fish larvae with serious deformities that were caught at each location varied among times (Fig. 7). ANOVA detected significant differences in percentage representation of fish with deformities among times and sites, but not among locations. These patterns were also true for 'deformed eyes' which made up a substantial proportion of 'total serious deformities'.

Comparisons of numbers and proportional representation of fish larvae with deformities for individual groups of fish suffered from low numbers of deformities. In some cases very low numbers of fish were captured at particular locations. The results, therefore, should be treated with caution. Seventy percent of fish larvae with serious deformities belonged to the families Clupeidae, Cheilodactylidae and Scorpididae. Most deformed *S. neopilchardus* and cheilodactylids were found at Terrigal (Mean Number per 200 m^3 for 4 times; Terrigal, Sydney, Greenwell Point): *S. neopilchardus* (0.1, 0,0); cheilodactylids (2.2, 0.09, 0.1). The highest proportional representation of fish that were deformed was also found at Terrigal for these two groups. This pattern did not appear to relate to a higher load of pollutants in seawater around Sydney. Deformed scorpidids were found at all locations (as above; 1.2, 1.25, 3.8) and greatest numbers of deformed fish were found at Greenwell Point at one time, thus the representation of deformed fish showed no relationship with the assumed gradient of pollution.

Discussion

Point-source impacts have the potential to cause significant biological degradation of larval habitat. In contrast to the 'halo' of influence that a point source-impact (e.g. a sewage outfall) has for many juvenile and adult organisms, surface and subsurface pollution plumes can affect any planktonic organisms that drift into them. Plumes essentially create a 'barrier'

TABLE 2

ANOVA of total numbers of larvae caught, serious deformities and percentage serious deformities per 200 m^3. All analyses were done using transformed data, *number* transformed to $\log_e(x = 1)$, *percentage* transformed to arcsin($\sqrt{}$proportion).

		Total larvae caught C = 0.325*		Total no. serious deformities C = 0.510*		Total % serious deformities C = 0.173 ns	
Source of variation	DF	MS	F	MS	F	MS	F
Location	2	0.239	0.006 ns	1.690	0.583 ns	0.051	2.429 ns
Time (Location)	9	37.049	11.129***	2.901	3.164*	0.021	3.500**
Site (Time (Location))	24	3.329	5.259***	0.917	2.310**	0.006	1.000 ns
Residual	72	0.633		0.397		0.006	

C = Cochran's test (df = 2, variances = 36), DF = degrees of freedom, MS = mean square, F = F ratio, ns = not significant. *$P < 0.05$, **$P < 0.01$, ***$p < 0.001$.

TABLE 3

Total numbers of the different types of deformity in fish larvae. (Data pooled for times, locations and sites.) The total number of fish caught was 99 636.

Type of deformity	Number
Serious notochord deformities	
Dorso-ventral curl b	28
Dorsal zig-zag a	0
Dorsal zig-zag b	3
Dorsal zig-zag c	18
Lateral zig-zag a	0
Latral zig-zag b	18
Other serious deformities	
Protruding eye	2
Bubble eye	25
Unpigmented eye	3
White eye	3
Deformed eye	154
Infected eye	4
Deformed gut	4
Notched back	1
Deformed head	4
Body infection	2
Abnormal mouth	0
Myomere haemorrhaging	0
Abnormal fins	0
Crooked rays	0
Summary	
Total eye deformities	191 (or 0.2%)
Total serious deformities	269 (or 0.3%)

perpendicular to longshore currents. Longshore currents are analogous to a conveyor belt shunting larvae into plumes. To our knowledge, the values we have provided are the first estimates of rates at which wild larvae are exposed to pollution plumes (also see Nisbet *et al.*, 1996, re: power plant intakes). It could be argued that larvae that are advected toward the plume can behave in such a way that they do not enter the plume. This is possible, but even if fish only enter the frontal region this is an area where greatest concentrations of pollutants may be encountered (Tanabe *et al.*, 1991; Kingsford and Gray, 1996). Surface pollution plumes are generally of low density and are only a few metres deep (Gray *et al.*, 1992; Kingsford and Gray, 1996). It would be expected that the impact of these plumes would be greatest in surface waters, unless mixing during storms was great. Deep-ocean outfalls, however, can potentially influence large numbers of larvae throughout the entire water column. Exposure to different types of plumes (i.e. surface and subsurface) may change with time of day as larvae undergo diel migrations (e.g. Heath, 1992). It is also difficult to know residence times of larvae in pollution plumes given that larvae may behave in such a way (e.g. for a trophic advantage; Kingsford, 1990) that they would stay in the plume for a longer period than would passive particles.

TABLE 4

Total numbers of fish larvae with deformities at each location (percentage of total)

Deformity	Terrigal	Sydney	Greenwell Pt.
Total eye deformities	28 (0.54%)	65 (0.96%)	98 (0.11%)
Total serious deformities	96 (1.9%)	73 (1.1%)	100 (0.11%)
Total fish caught	5163	6762	87 747

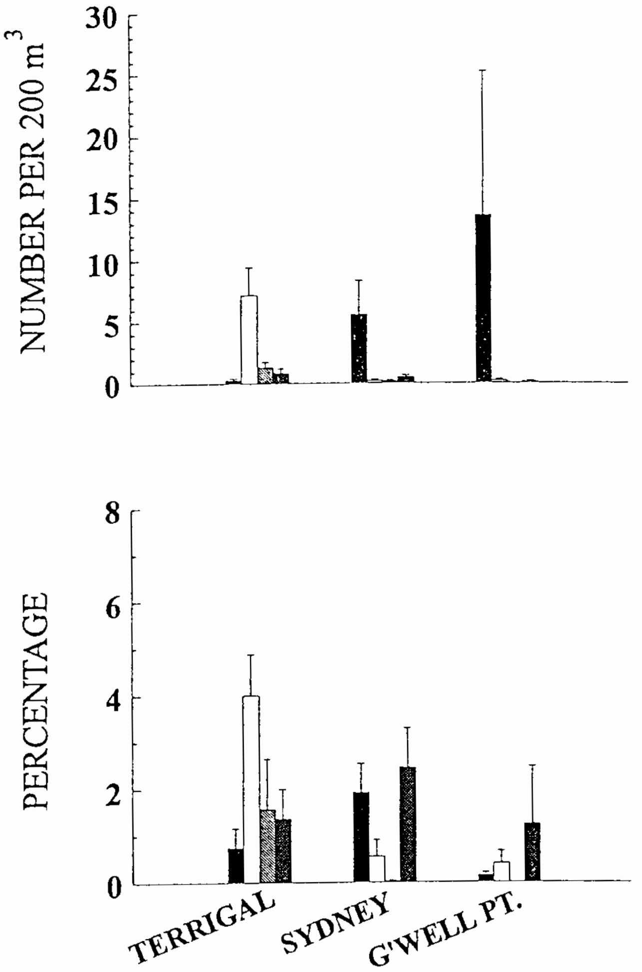


Fig. 7 Number of fish larvae 200 m^{-3} and percentage of total fish (*cf.* Fig. 6) with serious deformities. Mean values as for Fig. 6.

A variety of types of deformities were identified in fish larvae sampled along the coast of New South Wales. In the broad-scale study deformed fish were found at all locations. It was concluded that deformities were caused by natural processes and potentially by anthropogenic factors. If the cause of fish deformities was solely due to pollutants released from outfalls in the vicinity of Sydney, we would have expected numbers of deformed fish to be zero or relatively low upcurrent (i.e. Terrigal) and 250 km down current (i.e. Greenwell Point, Jervis Bay) of Sydney; this was not the case. Likely scenarios for deformities in fish larvae at great distances from major sources of pollution were: *1.* deformities are found naturally in the early developmental forms of fish. Natural deformities have been found in fish larvae from other parts of the world, causes may include temperature (e.g. Purcell *et al.*, 1990), genetic abnormalities, difficulties at hatching, and quality of the maternal yolk. *2.* Adult fish that have been subjected to pollutants in areas such as Sydney may move to the north and south then spawn eggs that have high concentrations of pollutants in the yolk, as a result, therefore, the longevity of larvae may be greatly affected outside of the Sydney area. Although the movement of some species is potentially substantial (e.g. *Pagrus auratus*, *Pomatomus saltatrix*; 10s to 100s of kilometres), this explanation seems unlikely for most species. *3.* The manifestation of deformities we described are independent of regimes of pollution that larvae experience. The frequency of occurrence of some categories of deformities (e.g. lateral-curl) can be affected by sampling artefacts such as the concentration of sewage derived fibrous material that is collected with the catch (Kingsford and Gray, 1996). We consider it highly unlikely, however, that deformities such as uneven pigmentation of the eye were influenced by methods of collection. It is possible that sewage had no effect on larvae, but in our study there was no way of unconfounding the influence of natural and sewage related effects in the vicinity of Sydney. *4.* The likely scenario of irregular input of dangerous pollutants through the deep-ocean outfalls. The frequency of occurrence of fish larvae that are affected by pollutants probably varies according to these pulses. Loadings of pollutants (e.g. metals; Beder, 1989) that are pulsed into coastal waters through the deep ocean outfalls are unknown over short periods of time (i.e. hours–days). *5.* Larvae were affected by sewage plumes and were advected to controls to the north and south, thus violating our assumptions of the control status of Terrigal and Greenwell Point. We believe this is highly unlikely, based on the following: our knowledge of oceanography, most larvae were probably 1–15 days old, mortality would probably be high for larvae advected over great distances and; there must have been some contribution of local larvae, spawned within 10s, not 100s of kilometres from collection sites.

A small percentage of total fishes had serious deformities (maximum 1.1%). It could be argued that this is insignificant (if this was caused by pollutants), given that the mortality of larvae is normally very high. This interpretation should be treated with caution because affected larvae that exhibit deformities may only last a short while before they are affected fatally and are lost from the system. Because larvae can be quickly affected by pollutants (*cf.* juveniles and adults; Westernhagen, 1988), low numerical percentages of fish larvae with deformities in each catch should not be interpreted as a justification for classifying waters as 'healthy' larval environments. In this study we had no information on the input of pollutants to coastal waters. Moreover, we had no information on fish larvae that were swimming at depths that would have given them greater exposure to effluent from the deep-ocean outfalls.

Kingsford and Gray (1996) found more serious deformities in the cliff face outfalls (5–32%) than we did in the broadscale study. Some caution should be taken in comparing these studies because they were done at different times of the year (winter vs summer), in different water temperatures (which may affect the frequency of deformities; Purcell *et al.*, 1990) and different species were caught.

The decision making of classifying non-serious and serious deformities is difficult (Kingsford and Gray, 1996). Our approach was to distinguish 'non-serious' from 'serious deformities' in an attempt to make our estimates of frequency of deformities in populations of wild fish larvae conservative. We are concerned that larvae may be deleteriously affected by pollutants in ways that do not manifest themselves as deformities. We recommend that qualitative aspects of individual larvae be compared. For example, larvae that have been categorized according to the presence/absence of deformities at different locations along a gradient of pollution could be compared using cytopathological and histopathological techniques (e.g. Bodammer, 1993). Fish that appear 'normal' according to gross morphological characteristics may have irreversible damage to important organs such as the liver. The environmental conditions that fish larvae have experienced may be determined by elemental analysis of the composition of bone. Histology and the analysis of trace elements could be used to further validate the description of deformities as a tool. Kingsford and Gray (1996) commented that fish larvae are so delicate that effects of preservatives and other material in tows may influence the frequency of some deformities. More investigations on artefacts (e.g. changes in pressure?) that may cause some deformities are required. Furthermore, in our study nets of 0.5 mm mesh were used. Hence, very small fish larvae, particularly those with yolk sacs, would have been under sampled. These very young larvae would be particularly susceptible to pollutants and deserve further attentions. *In situ* chambers and controls would be best for field assessments of these delicate fishes (Fairchild and Little, 1993; Hall *et al.*, 1993).

If field studies are to be done for the purposes of detecting pollution related impacts then a number of aspects need to be considered as follows.

- Intensive sampling for larval fish should be spread over a number of times to allow for temporal variation in the presence of small fish. A large amount of sampling effort, and associated expenses can be put into an area with little result if larval fish are not in the area at the time of the investigation.
- The influence of preservative and treatment of the samples on larvae should be examined.
- Use well established approaches to impact studies where multiple samples are taken before and after impacts and at control and impact sites. Although multiple impacts are sometimes found (e.g. sewage

outfalls off Sydney; Gray *et al.*, 1992), investigators often have to deal with one point source impact. In this case the sampling of multiple control sites is recommended (i.e. beyond BACI designs; Underwood, 1992). In many cases managers ask for potential impacts to be measured after the impact has occurred, in which case the impact can only be inferred through spatial differences (i.e. impacted area vs adjacent controls, Green, 1979).

- An understanding of local oceanography (e.g. current velocity), dilution of pollutants and the potential time taken for larvae to be advected over a distance *x* (i.e. from immediate impact), is important for the choice of controls.
- Relationships between oceanography and pollutants that can degrade larval habitat need to be investigated in more detail. For example, larvae often concentrate in convergence zones such as fronts or so called 'Linear Oceanographic Features' (Kingsford *et al.*, 1991; Kingsford and Gray, 1996). LOFs are generated by natural processes such as internal waves (Cross *et al.*, 1987), estuarine fronts (e.g. Kingsford and Suthers, 1994, 1996) and sewage plumes (Gray, 1995; Kingsford and Gray, 1996), these frontal regions should be included in sampling designs (Shanks, 1987). Detailed investigations are required on the chemistry and biology of LOFs.

A knowledge of the distribution and abundance patterns of larvae in areas that are affected by pollution plumes has merit (Kingsford and Gray, 1996), but it is difficult and expensive to detect larval deformities in field samples. Hence a combination of field and laboratory based techniques would be valuable. For example, controlled experiments for testing the effects of pollutants in different water masses on larvae may include: *1.* larval assays done by immersing larvae in water from polluted water masses (e.g. Weis *et al.*, 1989); *2.* immersing larvae *in situ* by using floating chambers (Hall, 1991), so obviating the problems associated with choosing the concentration and composition of pollutants in traditional laboratory studies. Finding suitable water masses for controls can prove difficult, and multiple controls (Underwood, 1992) should be considered to allow for differences in mortality or condition that may result from natural differences in oceanography (e.g. Hoss and Thayer, 1993). Finally, fish larvae and other plankters should be considered in impact studies.

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