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The Development and Evaluation of Methods to Assess the Impact of Chronic Toxicity on Ichthyoplankton - A Pilot Study

Leanne Gunthorpe, Geoff Nicholson and Greg Jenkins

Project No. 97/217

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September 2000

ISBN: 0 7311 4723 5

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OBJECTIVES

To assess the impacts of chronic pollution in ichthyoplankton by:

1. Developing methods for using imaging analysis as a tool for rapid and objective identification of fish eggs, teratogenic abnormalities and chromosome aberrations.

2. Evaluating the applicability of the 'fish egg abnormality' technique for temperate species and evaluate its use in Port Phillip Bay.

NON-TECHNICAL SUMMARY

The upper 50 μm of the sea-surface at the air-sea interface is known as the surface microlayer. The surface microlayer is an important habitat for the eggs and larvae of many fish and invertebrates, as these eggs will float near or rise to the surface microlayer at salinities of around 30 or greater. The sea surface microlayer is also a concentration point for many anthropogenic hydrophobic contaminants such as petroleum hydrocarbons, polyaromatic hydrocarbons and chlorinated hydrocarbons. Fish eggs are sensitive to environmental toxicants. A number of researchers have noted abnormalities in fish embryos and larvae after exposure to petroleum hydrocarbons.

For this study, fish eggs were collected from summer and winter tows in Port Phillip Bay at 4 locations and preserved in 95% ethanol. While the fish eggs collected for this study were unidentified, apart form the distinctly ovoid shaped anchovy eggs, they were most likely from gobies, snapper, whiting, flathead and flounder. The locations were Corio Bay, Hobsons Bay, St Leonards and Rosebud. Corio Bay and Hobsons Bay sites were in proximity of the industrialised cities of Geelong and Melbourne, respectively, St Leonards was in proximity of agricultural activities and Rosebud was nominated as the 'clean' area.

The fish eggs were to be viewed microscopically, sized, categorised into 5 stages of incubation ranging from early cell division to full embryo development and perceived malformations noted, as per studies by other researchers elsewhere. However, the other studies viewed live eggs whereas in this study, due to logistical constraints, the eggs were preserved. Unfortunately, the preservation technique and the length of time between capture and viewing severely compromised the structural integrity of the fish eggs, not withstanding pilot studies suggesting that the technique would be suitable. Osmotic pressures appeared to drive ethanol within the eggs and the egg lipids out of the eggs. The only malformations able to be discerned were bent spines on embryos in what appeared to be the most developed stage of egg incubation and it was assumed that these malformations were due to the effects of eggs coming in contact with anthropogenic contaminants. Researchers elsewhere, investigating live eggs, had noted that the greatest proportion of malformations occurred in eggs at the earliest stages of embryonic development and the smallest proportion of malformations occurred in eggs occurred at the latest stages of embryonic development.

This study found the proportion of mean malformations in what was considered the most developed embryonic stage to be less than 10%. This agreed in general with what other researchers found among the most developed stage of fish eggs of cod, dab, flounder, plaice and sole form the Baltic and North seas.

KEYWORDS: Port Phillip Bay, bay and coastal fisheries, fish eggs

BACKGROUND

Many fish species will spend some period of their lifecycle in shallow coastal waters and most of these are important to recreational and/or commercial interests. Reproduction of many commercial North Sea fish, for example, takes place in coastal waters (Cameron *et al*, 1992). However, the environmental integrity of many coastal waters is compromised due to inflows transporting persistent and bioaccumulating deleterious compounds from adjoining urban, industrial and agricultural regions.

Water quality criteria, while in place to control such emissions, are based on the concentrations of single chemical constituents and do not provide an integrated measure of chronic exposure to a toxicant load that may comprise known and unknown constituents (Klumpp and Westernhagen, 1995). The recently completed Port Phillip Bay Environmental Study (Harris *et al*, 1996) recognised this and recommended that investigations should be conducted into the "long-term chronic effects of low-level toxicants on the biota" within the bay.

This approach requires that integrated measures of environmental quality are developed. The monitoring of biological effects, which are manifest, as a result of chronic exposures are the tools used internationally to measure the environmental health of ecosystems and populations.

A number of fisheries in Australia are either over-exploited or at the limit of sustainability and it would be beneficial to the fisheries to enhance yields from these resources. Fisheries managers can no longer afford to simply consider harvesting as the only impact on fisheries stock, with the more enlightened attempting to discriminate between harvesting and pollutant impacts on fish numbers. Chronic exposure to pollutants can reduce habitat, reduce stock quality, reduce stock yield and tonnage, increase stress and reduce the reproductive viability of a fishery.

Incidences of reproductive impairment considered due to the affects of pollutants on natural populations in shallow aquatic habitats appears to have increased in the last decade. These have been manifested as elevated levels of oestrogen (Lye *et al*, 1997), decreased levels of male sex hormones, reductions in penis size, masculinisation of female individuals, biased sex ratio towards females and reductions in egg hatching success (Westernhagen *et al*, 1988).

Developing fish eggs and larvae are generally considered the most sensitive stages of the life cycle of a fish (Klumpp and Westernhagen, 1995). Exposure of fish eggs and larvae to sub-lethal concentrations of pollutants has been shown to significantly reduce the viability of eggs which in turn may be manifest as a reduction in recruitment. The occurrence of developmental (teratogenic) abnormalities in the ichthyoplankton has been correlated with toxicant perturbation in the North Sea and Puget Sound (Cross *et al*, 1987; Hardy *et al*, 1987; Westernhagen *et al*, 1988; Cameron *et al*, 1992).

NEED

A pilot program is needed for developing methods of determining the impacts of chronic toxicity on fish eggs and larvae. This approach will allow the measurement of the entire pollutant load on an ecosystem and the successful application of this technique will allow fisheries managers to quantify the total toxicant loadings in habitats and to evaluate the potential impacts these toxicant loads have on fishery stocks.

METHODS

Exploratory cruises

Six exploratory cruises were undertaken between February and July 1998, at various locations in Port Phillip Bay according to the protocols described in Cameron *et al.*, (1992) and von Westernhagen and Klumpp (1995). A plankton net with a 0.8 m x 0.8 m opening and 500 μm mesh size was towed at <1 knot for 15 minutes at a sampling depth of < 1m below the water surface. Two hauls were conducted at each site. Eggs were collected on dusk. Eggs were examined (live) within two hours of collection. Sites/stations containing < 100 eggs were to be discarded.

In January 1999, the collecting methodology was modified. Cruises were undertaken during the day and areas further offshore were investigated. A decision was taken to preserve the eggs as it was no longer feasible to examine the eggs within two hours of collection. Following each haul (20 minute duration), the sample was carefully drained of as much water before being washed into a 1 L jar with absolute ethanol (~ 95%) as preservative, sealed tightly, labelled and delivered to the laboratory for later egg extraction and analysis.

Field sampling

Fish eggs were collected with six hauls per site in Port Phillip Bay during winter 1999 and summer 1999/2000. Port Phillip Bay is an almost land-locked marine embayment of 1950 km^2 area, having limited water exchange with Bass Strait through a 3 km wide entrance. It has a 9800 km^2 catchment area and is bounded by the cities of Melbourne (pop. 3,000,000) and Geelong (pop. 200,000). Both cities have extensive port facilities and support manufacturing and petrochemical industries. Petroleum and organochlorine hydrocarbons historically entered Port Phillip Bay from discrete licensed discharges, diffuse sources such as urban street runoff and atmospheric transport and from the sewage complex at the Werribee treatment plant (WTP) (Fig. 1).

The sites chosen were selected for both good geographic coverage of the Bay and proximity to industry. The site in Hobsons Bay was selected to it's proximity to Melbourne and the mouth of the Yarra River, which drains about 65% of the Melbourne catchment. The Corio Bay site was selected because of the proximity to Geelong, whereas St Leonards could be considered to be impacted by overland run-off

from farming practices. The site at Rosebud, although there is urban settlement all along the local shoreline, also is in the region where daily tidal influence from Bass Strait is felt and thus this site was selected as the 'clean' site in the Bay (Fig. 1).

A plankton net with a 0.8 m x 0.8 m opening and 500 μm mesh size was towed at a speed of two knots for 20 minutes at a sampling depth of < 1m below the water surface. The water temperature, dissolved oxygen (DO) and salinity was measured and recorded prior to each haul. Mean values of tow speed, tow time, temperature, salinity and DO for each site and date during summer and winter sampling periods are shown in Appendices 1a and 1b respectively. Following each haul, the sample was carefully drained of as much water as possible through a 350 μm mesh filter before being washed into a 1 L jar with absolute ethanol, sealed tightly, labelled and delivered to the laboratory for later egg extraction and analysis.

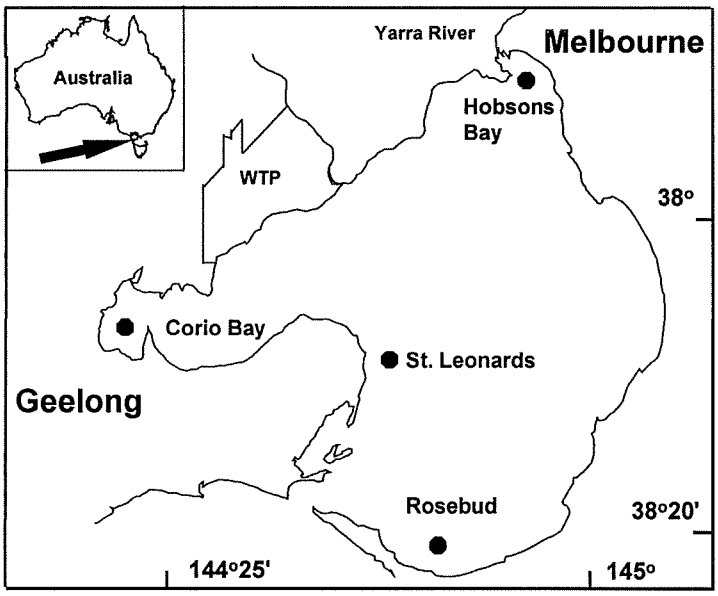

Figure 1: Locations of all 4 sites (Corio Bay, Hobsons Bay, Rosebud and St Leonards) in Port Phillip Bay where six hauls per site were conducted during winter 1999 and summer 1999/2000.

Analytical procedures

A minimum of at least 100 eggs when possible were subsampled from each haul's field sampling jar and placed in a small labelled vial under fresh absolute ethanol. It was anticipated that each egg from each haul would be viewed microscopically for morphological aberrations from normal development and the stage of embryonic development as indicated in Westernhagen *et al* (1988), where five different stages of embryonic development were classified (Figure 2). These were:

1a. Early cleavages until the building of the blastodisc;

1b. Epibloxy, the building of the embryonic shield;
2. Differentiation of the embryo, formation of the head and growth around the yolk up to 180°.
3. Embryo between 180° and 270° around the yolk with further differentiation, further expression of sense organs, primordial fins present.
4. Embryo between 270° and 360° around yolk, tail free, eye pigmentation.

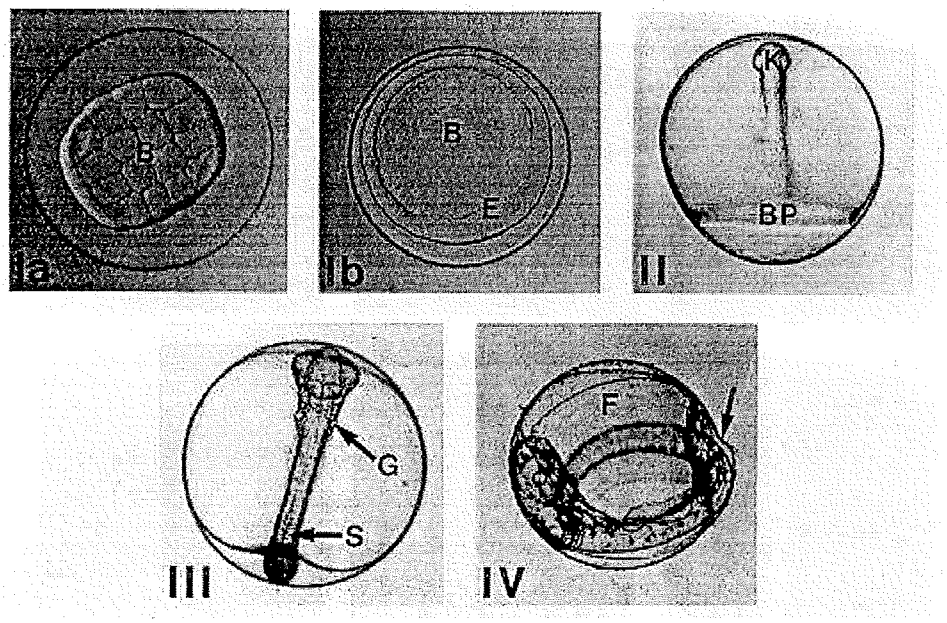

Figure 2: Photographs of living eggs from Cameron *et al* (1992). The legend to each picture within this figure is as follows: 1a. Early cleavages until the building of the blastodisc. 1b. Epibloxy, the building of the embryonic shield. 2. Differentiation of the embryo, formation of the head and growth around the yolk up to 180°. 3. Embryo between 180° and 270° around the yolk with further differentiation, further expression of sense organs, primordial fins present. 4. Embryo between 270° and 360° around yolk, tail free, eye pigmentation.

Expected morphological aberrations to be viewed included irregular cleavage of developing cells, underdeveloped or malformed early differentiation procedures, malformations in older embryos and blister formation in all stages (Cameron *et al*, 1992).

However, this procedure was modified after viewing the subsampled eggs and making the following observations:

1. The egg walls seemed to have taken on an opacity which made viewing through the egg difficult.
2. The interior structure of the egg seemed convoluted and in some instances embryos when found did not appear to be in good condition.

3. Eggs that were stored in field jars along with seagrass and/or algae under absolute ethanol took on irreversible colouring from the liberated chlorophyll.
4. The poor internal egg quality and the reduced transparency of the egg membrane made determination of internal subtle malformations such as blistering unattainable.

The storage of samples under absolute ethanol appeared to be the cause for reducing the analytical quality of the eggs. In an aqueous environment, the egg membrane is sufficiently impermeable to keep water from entering the egg and internal oils from escaping to the environment. However, when placed in absolute ethanol the osmotic pressures appear to be great enough to drive fish oils from within the egg through the membrane to the ethanol and to drive ethanol from outside of the egg through the egg membrane to the inside of the egg. This was undoubtedly the cause for irreversible colouring of the egg, reduced transparency of the egg walls and convoluted internal structure.

Eggs and embryos were expected to be normal unless otherwise indicated. As no subtle malformation effects were able to be observed, coarse criteria with respect to four internal physiological states were selected. These were:

1. Deformed: This in all instances related to the observed structure of the embryo and was used when the spine of the embryo showed a distinct sharp bend.
2. Peculiar: This was used to describe situations where the yolk and embryo may have both shrivelled up together, with the embryo usually ending up against the egg wall and presumably very much reduced in size.
3. Disintegrating: Some embryos appeared to crumble or partially disintegrate.
4. Blastodisc/Unfertilised: This criterion was used to describe those eggs that were either too opaque to determine any internal features, or else those eggs that appeared to have a dark zone along the internal surface of the egg membrane which was assumed to be blastodermal cells or the formation of the embryonic shield.

Images of the above four criteria plus an egg considered as examples of normal development are shown in Figure 3. After viewing a number of hauls from the same site and collection date and realising the consistently poor quality of the eggs, it was felt that nothing would be gained by the effort of continuing to inspect every egg subsampled from every haul in view of the above selected criteria. Instead, one haul per site per collection date was considered as representative of that site during that collection date. The results of analytical observations are shown in Appendix 2.

Analysis of chromosome aberrations is undertaken using cells at the blastodisc stage of development. Because of the quality of the eggs, it was not possible to distinguish reliably between unfertilised and blastodisc eggs, chromosome analysis was not undertaken.

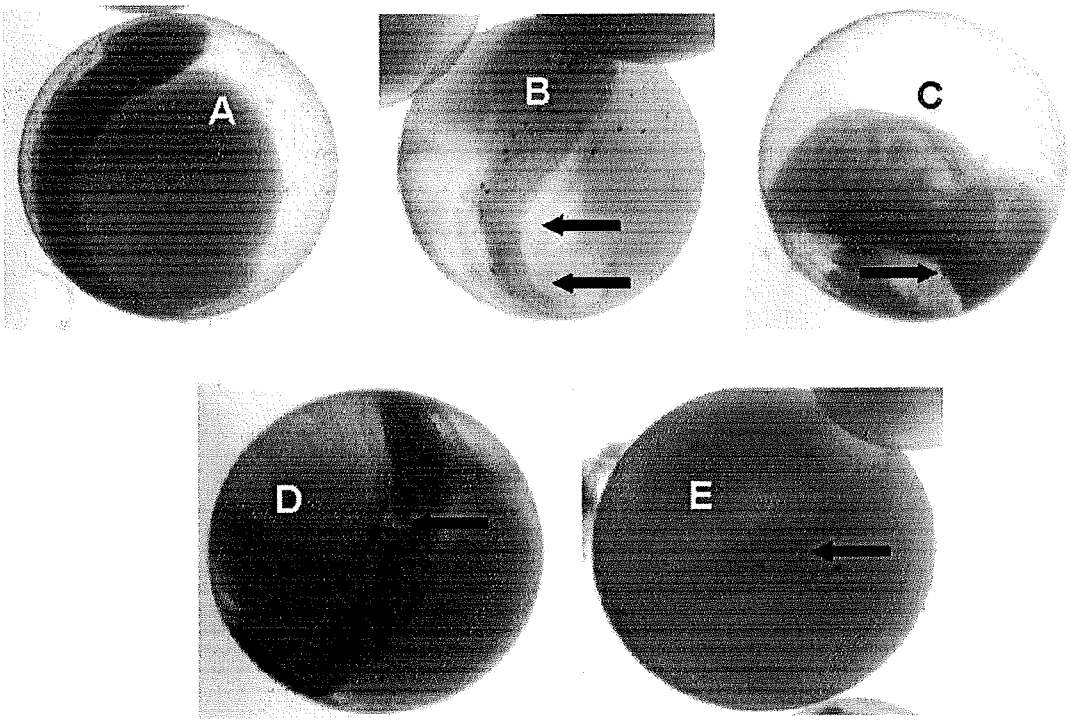

Figure 3: Five images of various states of development and malformations are shown in the above figure. The labelled images are categorised as : **(A)** Normal; **(B)** Deformed, with two spinal bends indicated by arrows; **(C)** Peculiar, with arrow indicating shrivelled embryo; **(D)** Disintegrating, with arrow showing a break or disintegration of the embryo; **(E)** Blastodisc/unfertilised, with arrow showing area of darker shading which was considered indicative of blastodermal cells or embryonic shield.

Data analysis

Each sub-sample of eggs from each haul was viewed microscopically. The diameter of each egg was optically measured from the viewing scale within the microscope and marked down in one of the following 6 size gradations: > 1.5 mm, 1.5 - 1.2 mm, 1.19 - 1 mm, 0.99 - 0.8 mm, 0.79 - 0.6 mm and < 0.6 mm (Appendix 2). Anchovy eggs were distinctive by their ovoid shape and were not classified with respect to the above category but were simply acknowledged as being present (Fig. 4). Each egg was then inspected for physiological state and malformation and marked down in the appropriate category as in Appendix 2. The data in Appendix 2 was then used to draw Figures 4 - 10.

RESULTS

Exploratory cruises

The number of eggs and larvae detected in the six exploratory cruises undertaken between February and July 1998 and January 1999 are shown in Table 1 and 2 respectively.

Table 1: Summary of fish eggs collected February to July 1998.

Site	No eggs	No larvae	Comments
Hobsons Bay	0	2	Species 1
PPB central	0	0	
PPB south	0	0	
Popes eye	1	1	Species 2
Popes eye	0	0	
Popes eye	0	2	Species 2
Swan Bay entrance	0	0	
Swan Bay entrance	0	0	
Corio Key	19	1	Species 3
St Helens	14	0	

Table 2: Summary of fish eggs collected January 1999.

Site	No species	No eggs	Site	No species	No eggs
Portarlington	Sp 1	860	Clifton Springs	Sp 1	1014
	Sp 2	493		Sp 2	476
	Sp 3	91		Sp 3	27
Portarlington	Sp 1	812	Clifton Springs	Sp 1	1027
	Sp 2	1105		Sp 2	138
Portarlington	Sp 1	1065		Sp 3	7
	Sp 2	569	Western Channel Marker	Sp 1	257
	Sp 3	38		Sp 2	1019
Clifton Springs	Sp 1	1177	Western Channel Marker	Sp 1	414
	Sp 2	467		Sp 2	1404
	Sp 3	15	Western Channel Marker	Sp 1	429
				Sp 2	1117

Extending the towing period and sampling areas further offshore increased the number of eggs collected.

Egg size frequency

The size frequency of eggs from each site during summer months and winter months is presented in Figure 4. Winter samples of eggs from the Hobsons Bay site were considered as being too low in numbers to provide meaningful data.

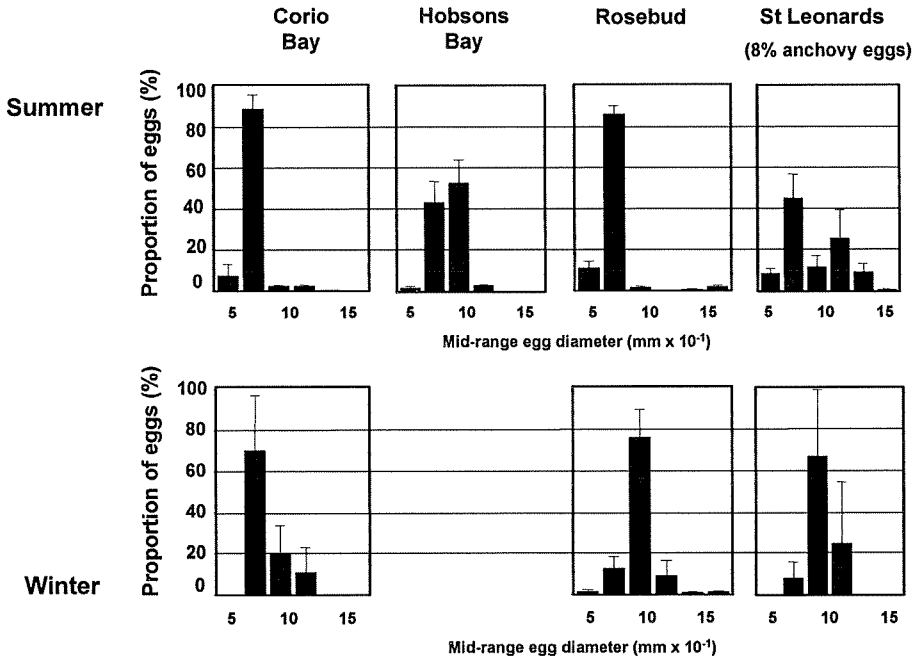

Figure 4: Size frequency of eggs (diameter in mm x 10^{-1}) from all sites and all dates for summer and winter months. Approximately 8% of the eggs collected from St Leonards during summer were anchovy eggs. Error bars are $\pm$ one standard error.

It is likely that egg diameter is species specific. It appears that different species preferentially spawn during either summer or winter, with an apparent trend of smaller eggs being found during summer at respective sites. It also appears as if there was a very similar species spawning composition at the Corio Bay and Rosebud sites during summer and slightly less so at the Rosebud and St Leonards sites during winter. The only anchovy eggs found during the sampling program were during summer at the St Leonards site.

Presentation of physiological criteria

Presentation of results in the form of a block map for the four physiological criteria selected and for normal development of eggs as a percentage of the subsampled eggs counted for that site and date are shown in Figures 5 - 9. Winter samples of eggs from the Hobsons Bay site were not in sufficient numbers to be considered as providing meaningful data.

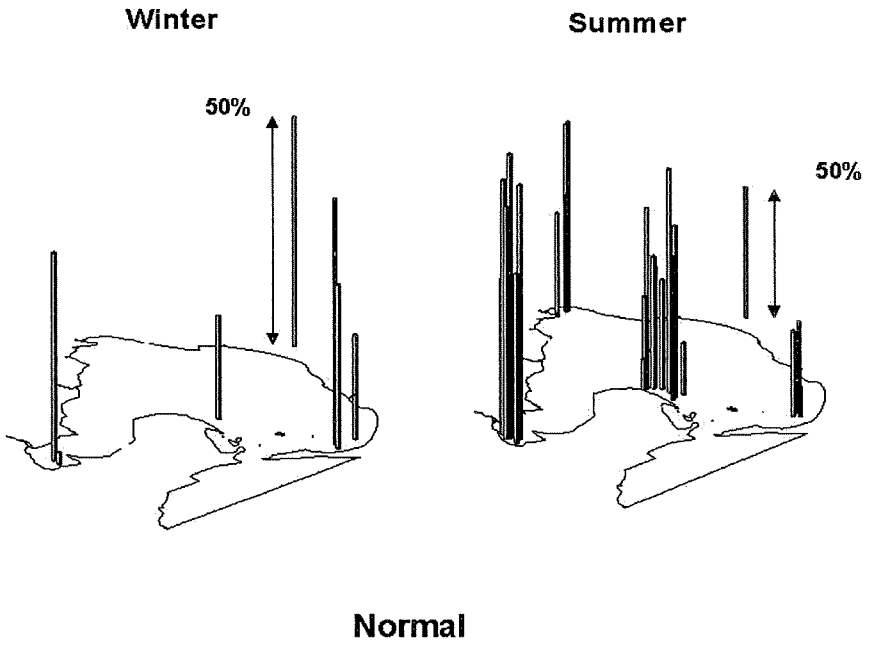

Figure 5: Diagrammatic representation of the percentage of the subsampled eggs from each haul per site and date for winter and summer months investigated and considered as having normal development. A block of height equivalent to 50% is included as a scaling factor for both seasonal diagrams.

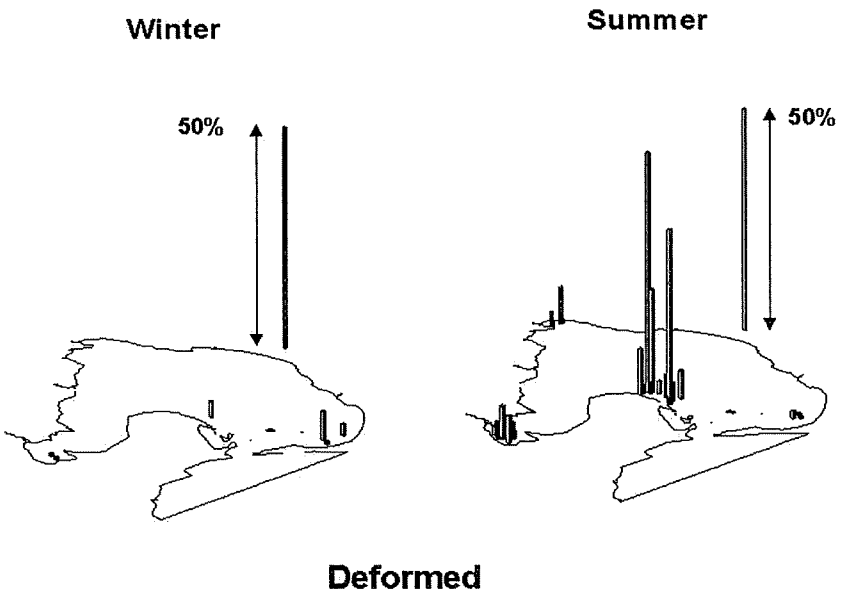

Figure 6: Diagrammatic representation of the percentage of the subsampled eggs from each haul per site and date for winter and summer months investigated and considered as having deformed embryonic development. A block of height equivalent to 50% is included as a scaling factor for both seasonal diagrams.

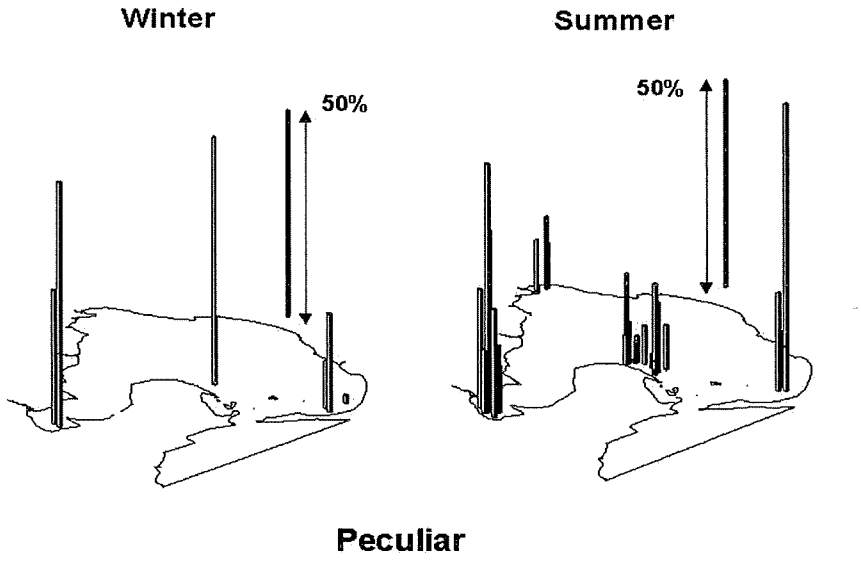

Figure 7: Diagrammatic representation of the percentage of the subsampled eggs from each haul per site and date for winter and summer months investigated and considered as having peculiar internal structures. A block of height equivalent to 50% is included as a scaling factor for both seasonal diagrams.

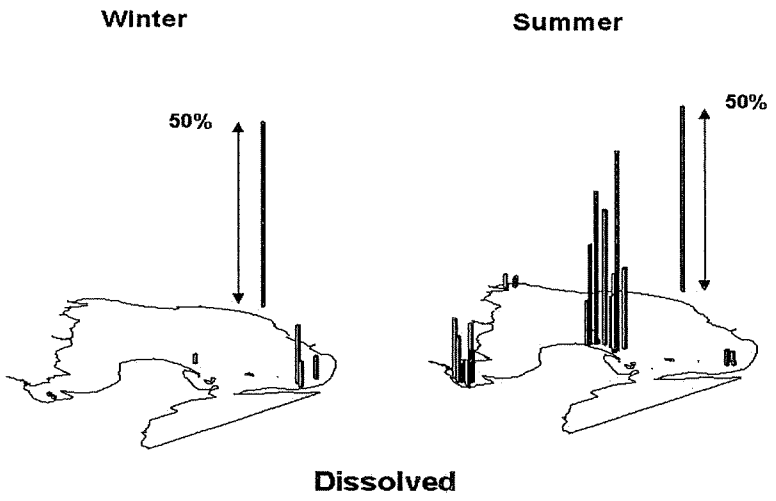

Figure 8: Diagrammatic representation of the percentage of the subsampled eggs from each haul per site and date for winter and summer months investigated and considered as having fragmented or dissolved embryos. A block of height equivalent to 50% is included as a scaling factor for both seasonal diagrams.

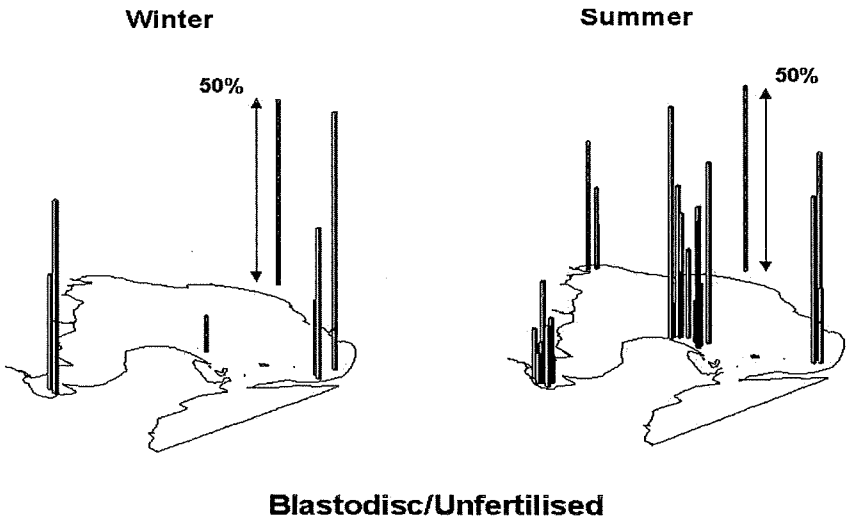

Figure 9: Diagrammatic representation of the percentage of the subsampled eggs from each haul per site and date for winter and summer months investigated and considered as being either unfertilised or at the blastodisc/blastodermal stage of development. A block of height equivalent to 50% is included as a scaling factor for both seasonal diagrams.

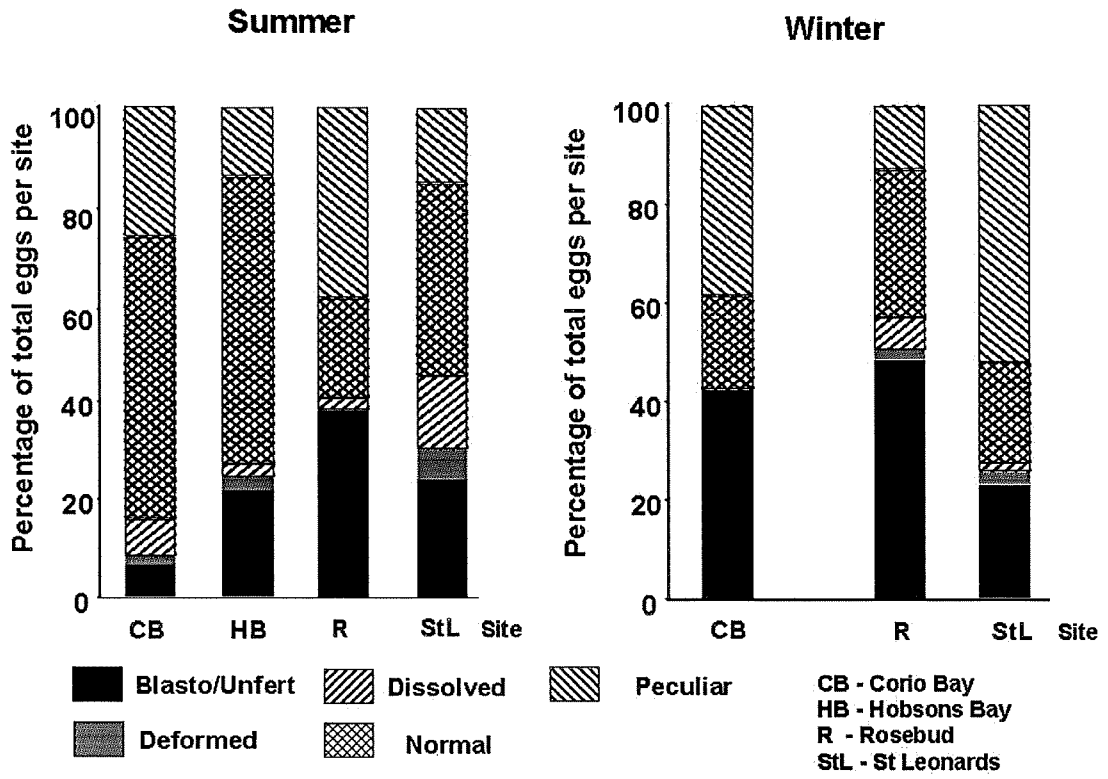

Figure 10: Presentation of data to illustrate the contribution of each of the 5 physiological criteria (Blastodisc/unfertilised; deformed embryos; dissolved embryos; normal developed embryos; peculiar internal egg formations) as a percentage of subsampled eggs counted per site per season.

Figures 5 to 9 present a view of the individual percentage contributions of each of the 5 physiological criteria able to be identified within each haul per site per season, whereas Figure 10 presents an overall view of the percentage contribution of each of the 5 physiological criteria able to be identified per site per season.

The percentage of deformed embryos when investigated on an individual haul basis appear to be relatively high, particularly at St Leonards (Fig. 6). Yet in Figure 10 it can be seen that the overall mean proportion of deformed embryos as a physiological classification within this study is relatively minor and, except for possibly one site, less than 5% and in all instances less than 10%. This is because there were 14 hauls taken at St Leonards, with the bulk of these having somewhere between 2 - 5 % classed as deformed and lowering the mean value of this classification. The mean deformity present at Rosebud, the 'clean' site, was less than at the other sites during summer. During winter, Corio Bay had the least mean deformity. However, Rosebud had the greatest mean blasto/unfertilised eggs. Whether this is an indication of the healthier condition of eggs at earlier stages of development can only be surmised, in the absence of directly observed malformations.

The sensitivity of the identification of morphological deformities, as mentioned earlier, was compromised by preservative effects on fish eggs. We cannot with confidence state that what we have seen in this report is an approximate reflection of malformation rates within fish eggs. We do not know what sort of fish eggs we have looked at, apart from the distinctively ovoid-shaped anchovy eggs. Larvae belonging to 24 teleost fish species were identified in samples collected from Port Phillip Bay between September 1995 and February 1996. Larvae of gobies were the most abundant taxa found in the bay during the 1996 survey and are typically most abundant in larval assemblages in estuaries and protected bays elsewhere in temperate Australia (Neira and Tait, 1996). Consequently, by inference, it is likely that the bulk of the eggs captured during this study were gobi eggs. Snapper, flounder, whiting and flathead eggs probably figured prominently among those captured as well.

All presumed deformities noted in this study are essentially gross abnormalities only in eggs with embryos well developed and similar to classification 4 in Westernhagen *et al* (1988) and Cameron *et al* (1992). However, it should be noted that Westernhagen *et al* (1988) determined the amount of defective embryos among pelagic fish eggs captured in the western Baltic Sea was about 20% of the total in 1983 and 30% of the total in 1984. These higher values were based on malformations at all stages of development. Earlier stages showed higher rates of developmental failure than later stages, with up to 51% of flounder embryos in the earliest designated Stage 1a (Westernhagen *et al,* 1988). Throughout development a large percentage of the abnormal embryos in Stage 1a die off before reaching a later stage and thus the rate of visibly affected embryos reduces as development proceeds. Mean malformation rates among eggs of cod, dab, flounder, plaice and sole within the Baltic and North seas at the Stage 4 of development were, except for one instance, always less than 10% of the total (Westernhagen *et al*, 1988; Cameron *et al*, 1992) and similar to what is presented here. Of course, the partial agreement, although with generally lower values, we have with the work elsewhere and referred to above could simply be co-incidental, as we really have no idea how much capture and preservation affected the rate of angular bending of embryos considered as deformed.

DISCUSSION

The upper 50 μm of the sea-surface at the air-sea interface, known as the surface microlayer, is a concentration point for many anthropogenic hydrophobic contaminants such as petroleum hydrocarbons, polyaromatic hydrocarbons and chlorinated hydrocarbons. The surface microlayer is also an important habitat for the eggs and larvae of many fish and invertebrates, as most marine fish eggs, because of their lipid content, will float near or rise to the surface microlayer at salinities of around 30 or greater (Hardy *et al*, 1987; Cameron and Westernhagen, 1997).

Fish eggs are sensitive to environmental toxicants. A number of researchers have been referred to (in Hardy *et al*, 1987) as noting abnormalities in fish embryos and larvae after exposure to petroleum hydrocarbons.

A laboratory based toxicity study in the U.S. exposed fish eggs and larvae to samples of contaminants collected from the surface microlayer around the greater Los Angeles coastline. It was found that when larvae were exposed to samples from Los Angeles harbour, where the concentration of trace metals, chlorinated hydrocarbons and polyaromatic hydrocarbons was 3 to 4 orders of magnitude higher than at other locations, there was 100% mortality (Cross *et al*, 1987).

The incubation periods of fish eggs in Port Phillip Bay are markedly less than fish eggs from either the Baltic Sea or the North Sea, where a number of overseas studies cited in this report have occurred. Snapper and flathead eggs in Port Phillip Bay hatch after about 2 days during summer, and flounder hatch after about 4 days during winter (pers. com. Greg Jenkins, MAFRI). Anchovy eggs in temperate Australian waters have incubation periods of about 30 hours (Arnott and McKinnon, 1985). Four of the nine species of fish investigated for embryonic malformations in Cameron *et al* (1992) from the southern North Sea (*Limanda limanda, Microstomus kitt, Pleuronectes platessa* and *Solea solea*) had incubation periods ranging from 2 days to 37 days depending on water temperature, although most would be presumed to incubate for about 7 - 8 days (Russell, 1976). Klumpp and Westernhagen (1995) considered fish egg development stages 1a to 2 (up to differentiation of the embryo and the formation of the head and growth around the yolk up to 180°) as critical in reflecting not only the impacts of toxicants on eggs within the water column but also the condition of the parent ovary. They noted that it takes about 2 days for fertilised eggs to incubate past stage 2 in the colder waters of the northern hemisphere at latitudes > 54° N, whereas it may take only 2 hours to pass this stage in tropical Queensland waters. It is also likely that the incubation period for fertilised fish eggs to pass stage 2 in Port Phillip Bay would be in the terms of hours rather than days.

The mean salinity at each of the sites sampled for this work during times of collection was 34 and above, which is enough to float fish eggs to the sea surface (Appendix 2) and come in contact with the surface microlayer. As the contaminants found in the surface microlayer are hydrophobic, there would have been solubility pressures for the contaminants to move into the less-polar lipids present within the fish eggs. For those species that have eggs with longer incubation periods, there is the likelihood that more hydrophobic/lipophilic compounds will enter the eggs and have greater detrimental consequences on development than species with eggs of shorter incubation periods. Kuhnhold (1977) noted that eggs at early stages of development accumulate petroleum hydrocarbons even when the petroleum hydrocarbon concentration in seawater is very low. Up to 85% of all cod, plaice and flounder eggs captured during 1983 and 1984 in the western Baltic Sea were at the developmental stage 2 or less in Westernhagen *et al* (1988) and of these, a mean 86% had deformities. Fish eggs undergo a longer incubation period due to colder waters at this latitude (> 54° N) and consequently would have had more contact time with water-borne contaminants. Port Phillip Bay eggs, by virtue of their faster incubation periods, have less contact with water-borne contaminants. The Baltic Sea historically has been severely polluted by organochlorins, with pollutants entering the Baltic from riverine runoff and airborne fallout. The amount of polychlorinated biphenyls (PCBs), an anthropogenic organochlorine contaminant, in muscle tissue from fish caught in the Baltic Sea are commonly greater than 0.5 mg kg^{-1} (Bignert *et al*, 1998), which is the maximum

residue limit for PCBs in seafood (Food Standards Code, 1995). By contrast, PCB levels within muscle tissue from Port Phillip Bay fish were below 0.5 mg kg^{-1} (Nicholson *et al*, 1994). The subsequent implication is that Port Phillip Bay is less contaminated by PCBs (and presumably other contaminants as well) than the Baltic Sea. Consequently, fish eggs in Port Phillip Bay because of their overall faster incubation rate would spend less time in lower concentrations of contaminants than would fish eggs from the Baltic Sea and exhibit lower deformities than eggs from the Baltic. This, to a certain extent, is what we are appearing to see.

Water temperatures at the times of sampling within Port Phillip Bay were typical and DO saturation of the water column was always medium to high (Appendix 1), so it is likely that neither of these parameters were abnormal enough to enhance developmental malformations.

The other region in the marine environment where there can be elevated concentrations of metals and hydrophobic contaminants is at the sediment-water interface where many contaminants can be found adsorbed to sedimented particulates. Bottom-dwelling fish, such as flounder and sand flathead in Port Phillip Bay, can accumulate contaminants (Fabris *et al*, 1992; Nicholson *et al*, 1994) which eventually will be transferred to reproductive tissues and can also have negative effects on egg development. It has been suggested that early stages of embryo development also reflect most closely the condition of the parent ovary (Klumpp and Westernhagen, 1995). Consequently, it may be that bottom dwelling fish are already under greater pressure from contaminants than pelagic fish and this may reflect in the proportion of malformations in incubating eggs. The condition of the female ovary is most likely a major determinant of egg viability, as males contribute less than 1% to the variability in fertilisation success provided the sperm is motile (Spies and Rice, 1988).

If the rates of natural egg mortality are as high in Port Phillip Bay as for other marine fish species (> 95%) such as the North Sea sole or the Pacific sardine (Westernhagen *et al*, 1988), then increases in toxicant induced mortalities may be too small in terms of biological significance to cause detectable impacts on other than localised recruitment. Differences of several orders of magnitude are required in egg and larvae numbers to cause detectible impacts on recruitment (Westernhagen *et al*, 1988).

There are other issues concerning surface microlayer contaminants that have an impact on larval mortality that do not relate to malformations. The sea surface is also inhabited by microneuston such as bacteria, tintinnids, small ciliates and microalgae which may be food resources for larval fish (Hardy *et al*, 1987). Any large-scale detrimental perturbation in the numbers of these organisms can remove possibly the only food source available for fish larvae and add an extra stressor to the viability of the larval population and consequent fishery recruitment.

Management implications

Fish embryos and larvae have been used for decades in toxicity testing because the development stages are well studied and the most sensitive to toxicants (Klumpp and Westernhagen, 1995).

Assessment of malformations in embryos and larvae may be more conclusive than chemical analyses for contaminants in the surface microlayer when determining the negative impacts of toxicants on fish species. Results from chemical analyses of surface microlayer contaminants were found to be inadequate and in fact opposite to that expected of predictions of embryo malformations (Cross *et al*, 1987).

Managers may have to reconsider whether assessing levels of contaminants within the water (and also sediments) alone are good enough predictors of negative impacts on fish eggs and larvae.

Another issue fishery managers may have to address is to determine which fish species have floating eggs of longer incubation periods and whether these species are more particularly impacted by surface microlayer contaminants.

Benefits

The most immediate benefit will be obtained by those interests exploiting fish resources for commercial and/or recreational benefit. Fisheries modellers may be able to use this type of data as an extra parameter when designing models to predict fishery yields. The potentially more sophisticated model available could lead to optimal yields from relevant fisheries while preserving the fisheries resource, or at least provide earlier indications of a fishery under stress.

Further development

If this investigation is intended to operate as a laboratory based study of stored fish eggs, then an improved method of preservation which does not negatively impact on the quality of the fish eggs as much as the current situation does is required. The literature tends to indicate that alcohol or alcohol/acetic acid are suitable preservatives for shorter term storages, whereas perhaps solutions of 5-8% buffered formalin may be more suitable for longer term storages (Longwell and Hughes, 1980; Daniel and Graves, 1994). Consequently, one suggested laboratory study is to expose fish eggs to an acknowledged contaminant to induce abnormalities and then compare results for eggs stored in assorted preservatives over various storage times. Hopefully the quality of the samples under an improved preservation technique will permit analytical investigation on eggs at earlier stages of development.

Identification of eggs to species help us determine the incubation period of the eggs with respect to time spent in contact with the surface microlayer, as well as the behaviour of the adult fish with respect to the likelihood of accumulating contaminants and affecting eggs during gestation. However, we will first need to determine the incubation period of the eggs of various fish species in the laboratory, as the incubation period is not known for most species.

A second laboratory based study would be to capture sedentary fish from a range of contaminated to pristine areas, bring them back to the laboratory live, strip them of

gametes, fertilise the eggs and note the level of deformities on living eggs. This would give crucial information on the condition of the parent ovary.

CONCLUSION

The concept of this pilot study in Port Phillip Bay is a good one. The investigation of developmental deformities in fish eggs has been used successfully by various researchers at overseas locations, the major difference being that they scrutinized living eggs within a few hours of capture. The integrity of our study was compromised by the inadequacy of the egg preservation technique and the length of time between capture and inspection (up to 8 months). We attempted to follow the technique of the other researchers in classifying the incubation of eggs to 5 development stages, ranging from initial cell division (Stage 1a) to embryonic maturity immediately prior to hatching (Stage 4). With respect to defining embryonic malformations, the only stage of development we were able to comment on with any type of veracity was those eggs at Stage 4. The mean percentage malformation we obtained from Stage 4 eggs was generally lower and consequently agreed well with Stage 4 eggs from other studies where the level of contaminants in the waters was greater than in Port Phillip Bay waters.

The most realistic improvement to the techniques used in this pilot study would be to develop a means of egg preservation that would last for some months whereby the egg contents would remain in the same condition as when captured and the egg case would remain transparent. To pursue the method as used with overseas researchers where they were able to view live eggs is sometimes difficult logistically if not unrealistic. In some instances in Port Phillip Bay during winter tows, insufficient numbers of eggs were caught in shorter tow times. In these instances, being able to run tows of a long enough duration to collect enough eggs and having a method to suitably preserve eggs would enable samples collected at all times of the year to numerically qualify for examination.

ACKNOWLEDGMENTS

Fisheries Victoria (Department of Natural Resources and Environment) is acknowledged for its support for the program. Tony Sheehan, Brett Abbott, John Turnbull and Wayne Clay conducted egg tows and recorded field data. Fisheries Victoria and FRDC are thanked for their financial support of this project.

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APPENDIX 1: PHYSIO-CEHMICAL DATA AND TOW SPEEDS

Appendix 1a: Mean values for winter ichthyoplankton tows of tow speed, tow time, salinity and DO for each of the 4 sites and dates of tow.

Site	Date	Mean values				
		Tow speed (knots)	Tow time (min)	Temp (C°)	Salinity	DO (% sat'n)
Corio Bay	14-Jul-99	2	20	11.1	37.8	98.4
Rosebud	16-Jul-99	2	20	11.7	37.2	86.9
St Leonards	21-Jul-99	2	20	11.0	37.4	84.4
Hobsons Bay	22-Jul-99	2	20	11.1	37.3	84.8
Corio Bay	23-Jul-99	2	20	11.1	39.8	87.5
St Leonards	26-Jul-99	2	20	11.3	34.0	NA
Hobsons Bay	28-Jul-99	2	20	11.0	36.4	89.6
Rosebud	30-Jul-99	2	20	11.1	36.7	88.3
Rosebud	02-Aug-99	2	20	11.4	36.6	90.2
Hobsons Bay	03-Aug-99	2	20	11.3	35.6	89.3
St Leonards	04-Aug-99	2	20	11.3	36.8	92.1
Corio Bay	05-Aug-99	2	20	10.9	37.3	90.5

Appendix 1b: Mean values for summer ichthyoplankton tows of tow speed, tow time, salinity and DO for each of the 4 sites and dates of tow.

Site	Date	Mean values				
		Tow speed (knots)	Tow time (min)	Temp (C°)	Salinity	DO (% sat'n)
St Leonards	30-Nov-99	2	20	19.7	35.8	89.1
Hobsons Bay	02-Dec-99	2	20	20.2	35.4	91.5
Rosebud	06-Dec-99	2	20	19.3	36.1	89.8
Corio Bay	07-Dec-99	2	20	20.7	34.0	87.8
St Leonards	09-Dec-49	2	20	19.1	36.2	84.3
Rosebud	20-Dec-99	2	20	18.2	36.0	79.9
Corio Bay	21-Dec-99	2	20	19.2	37.0	81.4
Rosebud	22-Dec-99	2	20	18.8	36.1	78.7
Hobsons Bay	23-Dec-99	2	20	20.4	34.7	83.9
St Leonards	24-Dec-99	2	20	19.3	36.2	78.1
Corio Bay	30-Dec-99	2	20	19.2	36.2	77.9
Hobsons Bay	10-Jan-00	2	20	20.0	33.7	92.2
St Leonards	11-Jan-00	2	20	20.3	36.1	80.0
Hobsons Bay	12-Jan-00	2	20	21.7	34.6	92.0

APPENDIX 2: SUMMARISED DATA

Results from examinations of eggs for date, site and haul number. Shown are the counts for subsampled eggs examined, deformed embryos, peculiar eggs/embryos, disintegrating eggs/embryos, eggs presumed to be at blastodisc/embryonic shield stage or unfertilised and egg diameters.

Date	Location	Season	Haul No.	Count					Egg quality (1=good) (5=bad)	Egg diameter (mm)					
				Total	Deformed	Peculiar	Disintegrate	Blasto/unfert		>1.5	1.5-1.2	1.19-1	0.99-0.8	0.79-0.6	<0.6
16-Jul-99	Rosebud	Winter	5	116	2	6	5	80	3	0	0	1	106	5	4
30-Jul-99	Rosebud	Winter	6	104	0	23	5	45	3	2	2	25	50	25	0
2-Aug-99	Rosebud	Winter	3	81	4	10	10	21	3	1	0	1	72	7	0
6-Dec-99	Rosebud	Summer	2	107	0	84	1	15	4	0	1	0	0	100	6
20-Dec-99	Rosebud	Summer	3	110	1	29	4	47	3	3	0	0	1	87	19
22-Dec-99	Rosebud	Summer	5	120	0	18	3	66	3	2	0	0	3	104	11
22-Jul-99	Hobsons Bay	Winter	1	19	0	5	2	4	4	0	0	6	8	5	0
28-Jul-99	Hobsons Bay	Winter	3	16	0	9	2	5	3	0	0	0	12	4	0
28-Jul-99	Hobsons Bay	Winter	4	16	1	5	0	2	3	0	0	0	14	2	0
28-Jul-99	Hobsons Bay	Winter	5	26	1	10	2	5	3	0	0	8	15	2	1
2-Dec-99	Hobsons Bay	Summer	3	112	3	18	4	44	4	0	0	1	96	15	0
23-Dec-99	Hobsons Bay	Summer	1	115	0	17	3	9	3	0	0	5	52	53	5
10-Jan-00	Hobsons Bay	Summer	3	89	7	21	2	19	3	0	0	3	24	61	1
12-Jan-00	Hobsons Bay	Summer	2	109	2	4	2	20	3	0	0	2	58	49	0
21-Jul-99	St Leonards	Winter	1	18	0	2	0	9	4	0	0	4	12	2	0
21-Jul-99	St Leonards	Winter	6	7	0	0	0	2	4	0	0	3	1	3	0
21-Jul-99	St Leonards	Winter	2	10	0	1	0	9	4	0	0	10	0	0	0
26-Jul-99	St Leonards	Winter	1	117	3	61	2	27	4	0	0	1	114	2	0
30-Nov-99	St Leonards	Summer	6	133	3	25	17	14	4	0	0	0	38	82	13
9-Dec-99	St Leonards	Summer	6	109	1	28	9	38	4	2	22	77	0	7	1
24-Dec-99	St Leonards	Summer	1	105	5	13	20	51	3	0	31	26	8	35	5
24-Dec-99	St Leonards	Summer	2	134	1	2	62	8	3	0	13	5	25	33	58

cont'd

APPENDIX 2

Appendix 2 (cont'd): Results from examinations of eggs for date, site and haul number. Shown are the counts for subsampled eggs examined, deformed embryos, peculiar eggs/embryos, disintegrating eggs/embryos, eggs presumed to be at blastodisc/embryonic shield stage or unfertilised and egg diameters.

Date	Location	Season	Haul No.	Count					Egg quality (1=good) (5=bad)	Egg diameter (mm)					
				Total	Deformed	Peculiar	Disintegrate	Blasto/unfert		>1.5	1.5-1.2	1.19-1	0.99-0.8	0.79-0.6	<0.6
24-Dec-99	St Leonards	Summer	3	108	24	26	19	33	3	0	1	1	3	96	7
24-Dec-99	St Leonards	Summer	4	109	22	8	1	34	3	1	16	0	16	76	0
24-Dec-99	St Leonards	Summer	5	104	49	0	3	42	3	0	16	5	13	68	2
24-Dec-99	St Leonards	Summer	6	104	9	8	11	68	3	0	2	63	3	36	0
11-Jan-00	St Leonards	Summer	1	105	1	3	14	38	3	0	10	34	14	17	30
11-Jan-00	St Leonards	Summer	2	98	32	9	11	34	3	0	5	3	18	70	2
11-Jan-00	St Leonards	Summer	3	98	4	3	12	5	3	0	1	0	6	87	4
23-Jul-99	Corio Bay	Winter	1	108	0	30	0	38	3	0	0	22	33	53	0
7-Dec-99	Corio Bay	Summer	1	105	1	16	12	9	4	0	0	6	3	59	37
7-Dec-99	Corio Bay	Summer	2	38	0	29	4	1	5	0	1	0	2	35	0
20-Dec-99	Corio Bay	Summer	2	107	2	50	4	21	3	0	0	2	1	104	0
20-Dec-99	Corio Bay	Summer	5	108	0	17	11	1	3	0	0	2	1	104	1
21-Dec-99	Corio Bay	Summer	3	113	2	7	9	3	3	0	0	0	0	112	1
21-Dec-99	Corio Bay	Summer	6	106	5	30	6	10	3	0	0	1	0	105	0
30-Dec-99	Corio Bay	Summer	1	108	6	71	1	4	4	0	0	1	3	103	1
30-Dec-99	Corio Bay	Summer	3	108	1	34	14	7	4	0	0	4	4	100	0
30-Dec-99	Corio Bay	Summer	4	108	3	6	3	0	3	0	0	1	5	101	1
30-Dec-99	Corio Bay	Summer	6	112	1	13	8	0	3	0	0	0	1	110	1

APPENDIX 3: INTELLECTUAL PROPERTY:

No intellectual property has arisen from the research that is likely to lead to significant commercial benefits, patents or licenses. Intellectual property associated with data produced from the project will be shared equally by the Fisheries Research and Development Corporation and the Victorian Department of Natural Resources and Environment.

APPENDIX 4: STAFF:

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Geoff Nicholson	Project scientist
Greg Jenkins	Project scientist