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THE EFFECT OF CHLORINE, HEAT AND PHYSICAL STRESS ON ENTRAINED PLANKTON AT KOEBERG NUCLEAR POWER STATION.

by

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SCENARIO

"The year class will come to order." The portly striped bass with scarred gill plates and half his dorsal fin missing flexed his back once and snapped his jaws. The fry fluttered their tails and gave the professor the kind of nervous attention that is partly respect but principally a suspicion that they may be dinner.

"This course is Advanced Survival, S 303," the professor continued.

"Its prerequisites are Elementary Survival, S 101, and Intermediate Survival, S 202. In S 101 you have learned of the natural hazards of inner space, that bounded by the bottom and the water surface. In S 202 you have learned the more common dangers arising from the bug-eyed monsters who inhabit outer space: bloodworms which conceal barbed hooks, nets, weirs, and similar devices that descend on us through the surface. Formerly, the work on survival terminated here. However, a new and more atrocious invasion, the power plant cooling system, is multiplying and, in the opinion of the faculty, you should not be allowed to continue in your customary ignorance. Since, in my youth, I was once entrained in a cooling system - and lived - I have been appointed as your instructor.

"The most insidious thing about entrainment is that you will not know that it is happening. Everything looks and feels normal. Temperature is normal. However, off in the distance there is a bit more noise than usual. But that's all. If at the instant you detect the noise, you swim vigorously with the noise to one side of you - not away from it - you fry may just escape. However, such evasive action in response to every slight increase in noise is impractical. For a long time there is nothing much out of the way to be seen, but you will notice that predators seem to be getting unusually plentiful. Appropriate action in the face of predators was covered in S 101. Remember the school motto: 'Edere Non Ederi'.

"In the last few seconds as you approach the screen the noise level goes up sharply. The meshes of the screen are large enough for you to slip through quite easily, but small enough to hang up that white perch who regards you as food. There is considerable satisfaction in seeing your pursuer gasping his life out flattened against the screen. But you had better look sharp! You won't be able to hang around and your troubles have really begun.

"As you enter the conduit the most disorienting sensation will be the reports from your lateral lines. As you know, these give directional pressure signals which permit you to turn away from an attacker. In the conduit you will feel yourself completely surrounded by predator. The walls go by with a rush and you will feel yourself twisted and rolled by the current. You will have the sensation that you have surfaced from 20 metres in only a few seconds. There will be thousands and thousands of others with you and those with vacuoles will explode. Then there is the burning biocide; not always but too often for comfort. If you are caught directly in one of these gas attacks, you and everyone around you has bought it. In my own experience, I was

lucky enough to go through just behind a gas attack but the burns were, and still are, painful.

"The experiences of the next second pass belief. You are wrenched and twisted and bounced about until you feel you will break. Around you you will see many of your fellows with eyes dangling out, with heads cut off, and with snapped spines. Quite a few of them will be smashed to death against the impeller blades.

"The lot of you - the dead, the dying, and the living - will be spat out into comparative quiet. At least it will be no worse than it was before - although *you* will be.

"But, once again, you are carried along with a rush and the twisting and churning increases. You are spun and dropped. And the worst is yet to come.

"Ahead is hell! Dozens of small openings lie before you and into one or another of them you go willy nilly. The twisting, rolling and accelerations become even more intense. But the worst is the heat. It is worse than anything you can imagine or that you will ever experience in this world. Breathing! All you can do is gasp and wonder where your next breath is coming from - if you should live long enough to take it. And it goes on and on and on. About the only solace I have to offer is that the burning from the gas attack is easing off just a little. However, you won't find that much comfort since you will begin to taste copper, lead and zinc in the water.

"Again you will find yourself rushed from depth to surface and then flung into outer space. The sound of exploding bodies reaches a drumfire. Fragments and broken bodies are all around you. You will be fortunate if you are alive to see it, although it may not strike you that way. The shambles is worse than Pickerel's charge at Gettysburg.

"Then follows the long, long trek through the desert. The water is running more smoothly, but the heat and the suffocation go on for what seems like forever. The burning from the gas attack has definitely begun to abate.

"At long last you're out - but not yet in the clear. All around you is the senseless carnage wrought by the monsters from outer space and you are feeling none too lively. Further, the brotherhood of fish is not a doctrine that applies at mealtimes. Many of your relatives and 'friends' will be gathered around to welcome you with gently smiling jaws.

"What practical advice can course S 303 offer you? Like most theoretical courses, very little beyond, 'Don't get entrained.' However, when the bomb goes off it's always nice to know how the thing works."

(from "Purgatorio - Two rather different views of the same event" by Robert E. Ulanowicz and Blair Kinsman)

ABSTRACT

The large volume of seawater used for cooling at Koeberg Nuclear Power Station contains many planktonic organisms which are exposed to heat, chlorine and physical stress during their passage through the system. Phytoplankton biomass, measured as chlorophyll *a*, was reduced by an average of 55.32% due to entrainment, and productivity was decreased by 38.30% on average, mainly due to chlorination. Zooplankton mortality averaged 22.34% for all species and 30.52% for copepods, the dominant group. The copepod *Paracartia africana* was used in laboratory experiments designed to simulate entrainment. Latent mortality was monitored up to 60 hours after a 30-minute application of stress factors (physical stress was not simulated), and approximately 75% of the total mortality occurred within the 30-minute period. Male *Paracartia* experienced higher mortalities than females. Extrapolation of these results predicts an overall entrainment mortality (including latent mortality) of 40% for copepods and 29.04% for total zooplankton, although the latter cannot be substantiated. Plankton entrainment at Koeberg was not considered to be overly detrimental to the marine environment because of the very localised area affected, rapid dispersion of heat and chlorine, rapid regeneration times of phytoplankton and some zooplankton, low abundance of commercially important species and potential recruitment from the surrounding productive Benguela upwelling region.

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1. INTRODUCTION

Many fossil fuel and nuclear power plants use large quantities of natural water for the "once-through" method of condenser tube cooling. Water from a lake, a river or the sea is drawn into the power station, receives excess heat from the condensers, and is discharged back into the environment.

A consequence of using natural waters with their varied biota for cooling is biological fouling of condenser tubes (Fox and Moyer, 1975). All water passing through a plant contains bacteria and algae as well as many other species capable of passing through the intake screens (Mattice and Zittel, 1976).

Many pass through without settling, but a large variety of sessile organisms adapted to living in tidal waters and strong currents find the concrete walls of the culverts an ideal habitat, free from tides, exposure to air, direct solar radiation and many predators (Schaal, 1973). Bacteria, algae, protozoans, sponges, fungi, bryozoans, tube worms and amphipods, hydroids, coelenterates, tunicates, mussels, barnacles and clams are all potential fouling candidates. Bacterial and algal slimes decrease the transfer of heat in the condensers by adding an insulation layer to the pipes. Barnacle larvae (cyprids) can settle in high flow rates (Newell, 1970; Schaal, 1973) and, in so doing, roughen the surface, facilitating the settlement of other organisms such as mussels. Barnacles and mussels can clog pipes and decrease water flow through the plant to such an extent that they necessitate plant shut-downs for their removal (Mattice and Zittel, 1976). Growth on

condenser tube walls also causes pitting which, unless controlled, can cause leakage of cooling water into the distilled water steam system used to drive the turbines (Fox and Moyer, 1975).

To minimise biological fouling and its expensive repurcussions, many power stations routinely chlorinate intake water, using chlorine gas, or sodium or calcium hypochlorite. Chlorination has proved to be the cheapest, most reliable and most effective method for controlling both marine fouling and bacterial slimes in power station cooling circuits (Coughlan and Whitehouse, 1977).

Continuous low-level chlorination has been adopted as standard anti-fouling practice in the United Kingdom (Schaal, 1973). Research has shown that chlorination suppresses mussel fouling by discouraging larval settlement, suppressing the growth of settled mussels and promoting the removal of established mussels. Chlorination reduces foot activity in larval mussels so that larvae are less able to stop their movement past a settlement surface (Holmes, cited in Schaal, 1973). The growth rate of chlorinated mussels has been shown to decline markedly over the first few weeks until it is reduced to insignificant values. The "condition" of the mussels deteriorates and their ability to remain attached is reduced. Intermittent attempts to feed use up their energy resources; they lose weight and become debilitated (Beauchamp, cited in Schaal, 1973).

Chlorine also interferes with the mussels' attachment system, weakening attachment strength by suppressing the process of thread production and thread hardening of the byssus system. The average attachment force of chlorinated mussels has been measured to be one tenth or less than that

of unchlorinated mussels. They are thus more easily swept away in rapidly moving water because chlorine prevents them from securely attaching themselves to the culvert wall (Holmes, cited in Schaal, 1973).

The use of chlorine has evoked concern about the combined effects of temperature and chlorine on marine animals. Because of their relatively immobile, free-floating character, planktonic organisms are often "entrained" or passively drawn into the cooling water condenser systems of power plants (Marcy *et al.*, 1978). Plankton species entrained include phytoplankton, zooplankton - both permanent and larval organisms - and juvenile fish. These organisms, because they are the most easily entrained species and because of their small and fragile nature, experience the most severe stresses that result during power plant operation (Goldman *et al.*, 1978). Besides exposure to heat and chlorine, entrained organisms experience physical stresses which include mechanical buffeting, acceleration, velocity shear forces and changes in hydrostatic pressure. The simultaneous application of these stresses (thermal, chemical and mechanical) may result in antagonistic, additive or possibly synergistic effects on entrained biota (Poje *et al.*, 1982).

The effects of entrainment are plant specific, since the combinations of site, biota, receiving water characteristics and plant operating conditions are almost limitless (Beck and the Committee on Entrainment, 1978). The environmental impact of entrainment is related to the composition and abundance of affected organisms, the numbers of organisms in adjacent waters, survival rates during entrainment as related to natural survival, the ecological roles of entrained organisms, and their reproductive strategies (Marcy *et al.*, 1978). It

is apparent from the numerous field studies conducted at power plants around the world that many organisms do not survive the entrainment process.

Koeberg Nuclear Power Station, situated approximately 35 km north of Cape Town on the west coast of South Africa, came into operation in 1984. Seawater, used as a coolant, is pumped through the system at a rate of 42.6 cubic metres per second for each turbo-generator unit, of which there are two. Thus, when the power station is operating at full load, over seven million cubic metres of seawater pass through Koeberg during a 24-hour period.

The seawater is drawn from a basin created by specially constructed breakwaters and enters the pump station situated on the sea front. Here chlorine, as sodium hypochlorite, is produced by electrolysis of the water to obtain a chlorine residual of 0.2 ppm. Raking screens and rotating drum screens prevent debris and large marine organisms such as fish from entering the conduits leading to the condensers. The water is pumped through four conduits - 3 m diameter concrete pipes - to the condensers in the turbine building. In the process of cooling the exhausted steam in the condensers the temperature of the seawater is increased by approximately 10°C, and the warmer water is then discharged back to the ocean at the outfall works.

The outfall is a long channel situated some 200 metres south of the intake basin. It is designed to jet the warmer water beyond the breakers into deep water to achieve good mixing in the cool ocean, thus minimizing the effects of thermal pollution in the area (ESCOM publication, 1982). The passage of water takes between 10 and 30

minutes from intake to outfall, the exact time in the discharge canal depending on tides and wave action. The velocity of the discharged water at the mouth of the channel is approximately 2 $m.s^{-1}$, and also depends on the tidal state. An investigation of the dissipation, path and progress of the discharged water (referred to as the "plume") has been fully documented by Rattey and Potgieter (1987).

This study was undertaken to determine the effects of plant entrainment on the plankton passing through the cooling system of Koeberg Nuclear Power Station.

2. ENTRAINMENT STRESSES

Environmental studies of power plants have recently shifted their emphasis from examination of the effects of heated discharges to studies of the impacts of entire cooling systems (Marcy *et al.*, 1978). Plankton, small fishes and invertebrates which pass through the intake screens intact are subjected to various and simultaneous stresses which often lead to mortality. Power plants can be represented as large predators that not only reduce the abundance of vulnerable organisms but may also disrupt the community structure through selective mortality and enhancement of the growth of some of the surviving species. Entrainment survival is determined by:

- size, life-stage and relative susceptibility to injury of the species involved,
- ambient temperature and the quality of the withdrawn and receiving water,
- amplitude of the temperature rise (ΔT) as the water passes through the condenser cooling system,
- duration of exposure to elevated temperatures,
- pressure changes resulting from turbulence (shear forces) and acceleration, as well as physical abrasion during passage through the system,
- exposure to biocides used for fouling control, and
- gas bubble disease (the formation of air embolisms) possibly caused by pressure and temperature changes in the cooling system (Marcy *et al.*, 1978).

Certain entrainment studies are now beginning to separate the effects of chemical, thermal and physical stresses and to describe their

synergistic effects. Several case-studies will be cited for each category, but there will be more discussion of these in the following chapters.

2.1 Chemical Stress (Chlorination)

When any form of chlorine is added to water, initial reactions occur with organic matter, dissolved gases and inorganic salts; this is especially true in seawater. Only after this demand is met is there any chlorine residual. The chemistry of chlorine in seawater is particularly complex, partially because of halides released by the action of the chlorine. Other halogens, especially bromine, then form amines and other derivatives (Morgan and Carpenter, 1978).

When considering the chemistry of chlorine in water, four components must be defined : free residual, combined residual, total residual and chlorine demand. Free residual chlorine is the hypochlorous acid (HOCl) or hypochlorite ion (OCl^-) remaining after all chlorine demand is fulfilled. Combined residual chlorine is the chlorine which represents reactions with either ammonia or nitrogenous compounds after chlorine demand is fulfilled. Total residual chlorine is the summation of the combined residual and free residual chlorine. These components determine toxicity of the chlorinated water to organisms. An important component of chlorine chemistry in aqueous solutions is chlorine demand. In most cases, chlorine demand is the difference between added chlorine and total residual chlorine for a given temperature and elapsed time, or more simply, the amount of chlorine needed to oxidize all reducing substances in the water (Morgan and Carpenter, 1978).

In unpolluted freshwater (salinity < $0.03^{o}/_{oo}$), chlorine chemistry is simple and is primarily governed by temperature, pH and ammonia-nitrogen concentrations. In marine waters, chlorine chemistry becomes complicated because of the bromide ions present (approximately 0.08 mM

in concentration). The rapid formation of hypochlorite in seawater induces a series of displacement reactions which produce hypobromite and chlorite ions (Farkas and Lewin, 1947). At a pH of 8.0 or lower, this reaction is rapid; excess ammonia in the water causes the formation of hypobromites (Johannesson, 1958). When organic material is present in the water, both chlorinated and brominated organics can be formed. Therefore, in any discussion of estuarine or marine chlorine chemistry, toxicity is related to total residual chloro-bromo reactions. In estuarine or marine waters, the constituents of chlorine demand may be toxic (Morgan and Carpenter, 1978).

Fox and Moyer (1975) found that primary production was decreased by 57% when the cooling water at a coastal plant in Florida was chlorinated (0.1 to 1.0 ppm residual), but by only 13% when there was no chlorine - just a ΔT of 4.4 to 5.5°C. Gentile *et al.* (1976) found that a total residual chlorine greater than 1.0 ppm was responsible for complete mortality of all entrained phytoplankton.

In a New England coastal power plant, Carpenter *et al.* (1972) varied the dosage of chlorine and observed the effect on entrained phytoplankton. Chlorine, applied as a gas, was added at concentrations of 0.1 to 1.2 ppm. At the lowest dosage of 0.1 ppm, which resulted in no detectable chlorine residual at the discharge, productivity was depressed by an average of 79%.

The duration of exposure of a phytoplankter to chlorine is critical in determining whether it will be affected. Gentile *et al.* (1976) found no measurable mortality for a 0.5 min exposure of the marine diatom *Skeletonema costatum* to 0.4 ppm chlorine, but measured 25 to 50%

inhibition of growth for a 2-10 min exposure. Similar results were obtained for *Thalassiosira pseudonana*.

The ultimate recovery of phytoplankton populations exposed to chlorine appears to be variable. Brooks and Seegert (1977) found that phytoplankton subjected to chlorine concentrations below 0.1 ppm showed an initial decrease in ^{14}C-uptake, but recovery of the stressed phytoplankton population was evident. Eppley *et al.* (1976), however, noted that there was no recovery of photosynthetic activity in samples incubated for 2-4 hr at 0.1 ppm, even after residual chlorine had fallen to undetectable levels.

Variations in recovery at different sites may be related to differences in species composition. Gentile *et al.* (1976) and Hirayama and Hirano (1970) found algal species to vary in sensitivity to chlorine, and Kott (1969) noted that some freshwater algal species, most notably *Cosmarium*, are resistant to chlorine (see Chapter 3).

Chlorine also has an adverse effect on many zooplankton species. Davies and Jensen (1975) observed an overall mortality of approximately 50% for entrained zooplankton exposed to a 0.25 to 0.75 ppm chlorine residual at an estuarine power plant in Delaware.

As with phytoplankton, the period of exposure to chlorine is critical in determining zooplankton mortality. Laboratory studies conducted by Gentile *et al.* (1976) on the copepod *Acartia tonsa*, which appears to be particularly sensitive to chlorine, showed that if exposure times are short (less than 5 min) and chlorine concentrations are below 1.0 ppm, mortality will be low. Seegert *et al.* (1977) also found that the

freshwater copepods *Cyclops bicuspidatus* and *Limnocalanus macrurus* sustained low (<1-5%) mortalities if chlorine did not exceed about 1.0 ppm for 30 min. However, the effect of chlorine may be magnified by temperature and other stresses in a power plant (Morgan and Carpenter, 1978).

Capuzzo (1977) found that acute exposure to either free chlorine or chloramine resulted in subsequent reductions in growth and metabolic activity of larval lobsters (*Homarus americanus*). Significantly lower increases in dry weight and significant reductions in standard respiration rates were measured among lobsters exposed to 1.0 ppm free chlorine or chloramine for 60 min, compared to control organisms. Capuzzo *et al.* (1976) also demonstrated the synergistic effect of temperature on the toxicity of both free chlorine and chloramine. Significant larval mortality was noted with both 30 and 60 min exposures to >0.1 ppm free chlorine (at 25 and 30°C), >0.5 ppm chloramine (at 20 and 25°C) and >0.25 ppm chloramine (at 30°C).

Extensive research has been conducted on the effects of entrainment and chlorination on fish eggs and larvae, especially those of the striped bass *Morone saxatilis* (Flemer *et al.*, 1971; Lanza *et al.*, 1975; Middaugh *et al.*, 1977a; Koo and Johnston, 1978; Burton *et al.*, 1979; Hall *et al.*, 1981b). Experiments have demonstrated different susceptibilities for eggs and larvae, with eggs generally being more resistant to entrainment stresses. Age-related effects have also been observed in both egg and larval stages of species subjected to a range of residual chlorine levels (Morgan and Prince, 1977). Alderson (1974) found that sensitivity of plaice (*Pleuronectes platessa*) larvae decreased with increasing age, before metamorphosis.

Capuzzo *et al.* (1977) reported on the differential effects chloramine and free chlorine have on juveniles of three species of fish, namely killifish (*Fundulus heteroclitus*), scup (*Stenotomus versicolor*) and winter flounder (*Pseudopleuronectes americanus*). Below lethal levels, behavioural changes such as gill distention and abnormal swimming behaviour were common. Middaugh *et al.* (1977a) found that gills and pseudobranchs were damaged when striped bass larvae were exposed to 0.21 to 2.4 ppm residual chlorine.

Avoidance responses to chlorine are also important in the overall reaction of organisms to biocides. White (1972) and Sprague and Drury (1969) reported avoidance behaviour for fishes in discharge plumes containing residual chlorine concentrations of 0.01 to 1.0 ppm (a preference response occurred at 0.1 ppm). Middaugh *et al.* (1977a) noted reproducible avoidance reactions by 24-day-old striped bass larvae to total residual chlorine concentrations of 0.79 to 0.82 ppm and 0.29 to 0.32 ppm. No measurable avoidance was observed between 0.16 and 0.18 ppm.

Lanza *et al.* (1975) worked with estuarine gammarids *Gammarus daiberi*, *G. tigrinus* and *Leptocheirus plumlosus* in a series of temperature-chlorine avoidance studies. From the experimental data it appears that *Gammarus* were able to "sense" chlorinated effluent and to avoid high residual chlorine concentration. Ginn and O'Connor (1978) found that *G. daiberi* avoided chlorinated discharge water at test temperatures of 26.4-26.6°C and 15.3-15.7°C.

From both field and laboratory studies, it is apparent that chlorine

added to aquatic systems seriously affects the growth and survival of entrained organisms. There is species-to-species variation, but the majority of species within a group appear to be affected at relatively low concentrations of residual chlorine (less than 0.5 ppm) (Morgan and Carpenter, 1978). In some cases, such as with phytoplankton, the action of chlorine may be irreversible. Apart from causing immediate or delayed mortality, the effect of chlorine may be manifested by abnormalities in growth or reproduction and by other sublethal expressions.

2.2 Thermal stress

Strictly speaking, increased temperature should be regarded as a physical stress of entrainment. However, heating of the cooling water is a variable process, determined by the operational status of the power station concerned, and is independent of the conventionally termed "physical" stresses experienced by organisms during entrainment. These may include pressure changes, shear forces, buffeting, collision and abrasion, and remain constant for a particular power plant design. As the precedent for this terminology has been well-established in the literature, and facilitates comparisons with other studies, it has been decided to adhere to this format.

Some researchers have found it impossible to separate the effects of thermal and mechanical stress on entrained organisms, and this should be kept in mind with regard to the following sections. Repetition has, however, been avoided as far as possible.

The organisms that pass through a plant are subjected initially to a very rapid rise in temperature, approximately equivalent to the temperature rise across the condensers. They are exposed to this maximum temperature during passage through the plant and to the point of discharge, and then to decreasing temperatures as they are carried down the plume (Schubel *et al.*, 1978).

The thermal stresses experienced during passage through the cooling system, and subsequently in the discharge waters, may result in physiological damage, debilitation and/or death (Schubel *et al.*, 1978). Temperature has been shown to affect survival, growth, metabolism,

morphology, reproduction and behaviour in aquatic poikilotherms (Goss and Bunting, 1976). The degree to which these biological processes are affected is dependent upon several factors, including the increment (ΔT) above ambient water temperature, the absolute value of the maximum temperature, the duration of the thermal stress (exposure time), the developmental stage of the organism, and interaction of temperature with other environmental parameters (Schubel *et al.*, 1978).

Temperature may act synergistically with other environmental stresses in limiting the tolerance zone of marine animals (Capuzzo, 1979). Cairns *et al.* (1975) suggested that if a chemical toxicant (such as chlorine) affected cellular enzymes involved in energy metabolism, or caused a change in respiratory activity, then toxicant exposure combined with an increase in temperature may result in increased uptake and enhanced toxic effects in aquatic organisms. In studies at the Crystal River Plant, Florida, Alden *et al.* (1976) concluded that an interaction existed between temperature and mechanical injuries which caused higher mortalities than would result from temperature alone.

Although much has been published on the effects of temperature on a wide variety of aquatic organisms, relatively little of this research is of direct use in predicting the effects of thermal increments during entrainment (Schubel *et al.*, 1978). The classical 24-, 48- and 96-hr exposure times used to calculate LT_{50}'s are often inappropriate to power plant entrainment assessments. A resistance pattern can be obtained that identifies both time and temperature variables, but not in the range of exposure times (usually a few minutes to a few hours at most) characteristic of entrainment. Data are needed for shorter exposure times.

A case in point is provided by Lauer *et al.* (1974). These workers concluded that for most species in their Hudson River study, use of 24-, 48- and 96-hr tolerance data would have led to predictions of 100% mortalities, but bioassay results for the exposure times (5-60 min) characteristic of the Indian Point plant, New York, as well as field observations at that plant, indicated that nearly 100% of the entrained organisms survived.

The Critical Thermal Maxima (or CTM) theory is based on the concept that loss of equilibrium is an important endpoint for ecological survival, and that during temperature rise or fall a temperature endpoint can be observed "at which locomotory activity becomes disorganised and the animal loses its ability to escape from conditions that will probably lead to its death" (Cowles and Bogert, 1944). Schubel *et al.* (1978) state that thermal tolerance information in this form can not be used as a predictive tool for entrainment; it is mostly useful for screening organisms for relative thermal sensitivity (Coutant, 1970; Hair, 1971).

Despite reservations about the CTM theory, pre-death debilitation may be an important consideration for the more mobile members of the plankton, such as fish larvae. It is apparent that organisms go through progressive debilitation under high temperature stress prior to actual death, and that this debilitation can have important consequences for an organism's survival in a natural ecosystem. Abrupt thermal shocks may disturb normal processes in the development of early life stages of aquatic organisms, or result in death of either young or adults (Kinne, 1970). Yocum and Edsall (1974) found that brief heat shocks increased the vulnerability of whitefish (*Coregonus clupeaformis*) fry; more were

captured per attack by predators (yellow perch, *Perca flavescens*) than in control groups. Debilitation sufficient to significantly increase predation on juvenile trout and salmon occurs at temperatures about 2°C lower than temperatures necessary for death at the same exposure time (Coutant, 1973).

Goss and Bunting (1976) reported freshwater cladocerans (*Daphnia pulex* and *D. magna*) to frequently be initially stunned or disorientated in their behaviour at ΔT's of 15°C or more, and it was several minutes before apparently normal swimming was resumed. Bradley (1975) also reported initial stunning and disorientation of the copepod *Eurytemora affinis* when shocked to a temperature of 34.5°C. Several minutes were required for recovery, the period increasing with the size of the ΔT.

Acclimation temperature is also an important factor to consider when investigating thermal effects of entrainment. Fish have a discrete temperature tolerance range which varies with acclimation temperature and is characteristic for each species (e.g Fry, 1971; Brett, 1960). Temperatures which exceed this range induce mortality which is time-dependent; the higher the temperature above the tolerance limit, the more rapid is death. Organisms which are acclimated at higher temperatures and are exposed to a particular temperature increment appear to experience higher mortalities than those acclimated at lower temperatures. Larval white perch (*Morone americana*) showed higher mortality after applied chlorine and heat stress when acclimated to 23°C than when they were acclimated to 15°C (Hall *et al.*, 1981a), presumably because the higher temperature was closer to their upper thermal tolerance limit.

Sublethal effects of temperature exposure are difficult to quantify, but may be of considerable importance when assessing the impact of power plant entrainment. Eggs and larvae are highly sensitive life stages undergoing rapid changes in the processes of cell and tissue determination and differentiation; some stages are much more sensitive to temperature than others (Needham, 1942; Frank, 1973). Temperature elevation at such times might cause the shift of a determinative process and throw it out of coordination with other developmental processes, leading to abnormalities in later life. It has been shown that gross deformities may develop in response to thermal shocks lasting only minutes during egg stages (eg. Bergan, 1960; Hopkins and Dean, 1975).

As is the case with chlorine, temperature acts differentially on organisms of different life-stage, size and sex. Kennedy *et al.* (1974) subjected embryos and larvae of the hard clam *Mercenaria mercenaria* to different ΔT's for different exposure periods. They showed clearly that the early cleavage stages were the most sensitive, that thermal tolerance increased in trocophore larvae, and that "straight-hinge" stage larvae of the hard clam were even more tolerant. Sprague (1963) found that for the amphipod *Gammarus fasciatus*, the males appeared less temperature sensitive than the females. Size was shown to be a modifying factor of mortality in the isopod *Asellus intermedius* with a general tendency for the larger animals to have shorter survival times.

Alden *et al.* (1976) made detailed analyses of the response patterns of the most abundant copepods near the Crystal River steam generating plant, Florida, namely *Oithona* sp., *Acartia tonsa*, *Paracalanus crassirostris* and *Euterpina acutifrons*. Comparing the thermal sensitivity of the four species, the coastal marine copepods *Paracalanus*

and *Euterpina* were the most sensitive. Even at lower temperatures they showed some tendency for a gradual increase in mortality with temperature. *Oithona* and *Acartia*, estuarine species adapted for life in fluctuating environments, showed little mortality with increasing temperatures until the thermal threshold was approached. The estuarine species appeared pre-adapted to tolerate many stresses imposed by power plants, being more tolerant in this respect than the open coastal species. Usually the juveniles of a species showed lower mortalities than adults, and adult females were usually less sensitive to entrainment effects than were males.

Schubel *et al.* (1978) constructed thermal resistance curves for zooplankton, macroinvertebrates, ichthyoplankton and juvenile fishes using thermal tolerance data with the following criteria: (1) that exposure to the full ΔT was applied almost instantaneously so that no thermal adaptation could occur, (2) that mortalities were reported as functions of both temperature and exposure time, and (3) that mortalities were reported for a range of exposure times of a few minutes up to about two hours.

The curves indicated that thermal death is a dose response, but that maximum temperature of exposure is the primary cause of death. After about 20-30 min, mortality is a function of temperature alone. For power plants with exposure times of more than about 20 min, thermal death can be predicted solely on the basis of exposure temperature.

The results also indicated that fish eggs and larvae are usually significantly more sensitive to temperature stress than zooplankton and macroinvertebrates found in the same environment. If thermal criteria

are set to protect ichthyoplankton, most zooplankton will be adequately safeguarded against thermally induced mortalities.

2.3 Physical Stress

Entrained organisms are stressed by mechanical buffeting, acceleration, velocity shear forces and changes in hydrostatic pressure (Marcy *et al.*, 1978). Inner-plant physical stresses arise from:

- contact with fixed or moving equipment, such as screens, pumps and piping,
- pressure changes, especially the negative pressures or vacuums within the pumps,
- shear forces in the areas of extreme turbulence or boundary proximity,
- accelerative forces resulting from changes in velocity and direction,
- buffeting and collision with the particles (e.g. organic load) passing along with the organisms, damage depending on load density and size, and
- cavitation in regimes of partial vacuum.

The rapid pressure changes that occur in power plants may have the greatest potential for damaging entrained organisms, especially fishes (American Nuclear Society, 1974). Pressure changes encountered in plant passage may be sufficient to produce air embolism in fry. These embolisms, even if not lethal in themselves, may buoy fry to the surface, keep them in the warmest part of the plume, subject them to increased thermal shock, and cause them to become more vulnerable to predation (Marcy *et al.*, 1978).

Changes in hydrostatic pressure should induce minimal physical strain upon an aquatic organism containing no gas vacuoles (Marcy *et al.*,

1978). Large positive pressure changes are usually benign unless the organism possesses a natural gas space, in which case the cavity may implode. Negative pressure changes, by contrast, have a high potential for inducing physical damage, especially during decompression. In organisms possessing a gas cavity, it may explode if the organism is unable to equilibrate the pressure across the membrane wall fast enough. Also, the solubility of dissolved gases drops as the ambient pressure drops. The water then becomes supersaturated with dissolved gases and, in the presence of living tissue, which serves as a form of catalyst, the gas may come out of solution and the consequent bubbles may cause physical trauma (gas bubble disease) (Marcy *et al.*, 1978).

Acceleration forces, which result from changes in the velocities of flowing waters as they pass from the intake to the discharge area, may cause high mortality of entrained organisms (Ulanowicz, 1975). The lowest forces include accelerations due to changes in the bulk speed of water flow; they commonly range from very low to near that of gravitational force. These forces probably cause little damage. The next range includes those forces resulting from turbulent eddies. These forces are of such magnitude, usually several times that of gravity, as to possibly cause immediate or latent damage to entrained fish larvae. The highest range of acceleration forces is that of the short duration, high magnitude forces that result from impact with solid surfaces. Forces in this range are many times the force of gravity and, combined with damage from mechanical abrasion on impact, are probably immediately lethal (Marcy *et al.*, 1978).

Shear forces, expressed as units of force per unit area (dynes/cm^2), develop when spatial differences in velocity exist in a moving fluid,

for example, at the edges in a turbulent flow regime or when water flows across a solid surface (Ulanowicz, 1975). The greatest shear stresses occur in close association with solid surfaces, such as pipe walls, pump impellers, travelling screens and water boxes. Shear stress has two major components: rotation and deformation. When a fish egg is caught in a changing velocity field, the rotational effect of shear disturbs the internal order of the egg while the deformation effect stresses the outer membrane, leading to possible break-up of the egg if the shear forces are high enough (Ulanowicz, 1975).

Abrasion can occur when two surfaces move in contact past one another or when a smaller suspended particle with a different velocity impinges upon an organism's surface (Marcy *et al.*, 1978). They state that abrasion is a difficult stress to qualify, since it is highly dependent upon the various natures of the contacting surfaces or colliding particles. It acts to decrease the lethal shear threshold of an organism. Although no quantitative data exist on abrasion as a factor in entrainment mortality, Emadi (1973) and Marcy (1976) mention abrasion as a possible factor in ichthyoplankton entrainment mortality.

A high silt and detritus load passing through the cooling system with the organisms may cause an increased abrasion/collision mortality (Marcy, 1973, 1975, 1976; Coutant and Kedl, 1975). A high organic load during plant passage may have caused the physical damage mortality of phytoplankton and zooplankton at four Florida fossil-fuel plants (Weiss, cited in Marcy *et al.*, 1978).

Physical damage is perhaps most severe in the pumps (Lauer *et al.*, 1973). Once the entrained organisms reach the pumps, they are exposed

to sudden pressure fluctuations, velocity shear forces, and physical buffeting and abrasion. Rapid positive and negative changes in hydrostatic pressure occur. In a few seconds, velocity shear forces fluctuate widely, along with severe buffeting and possible contact with pump walls or impeller blades. Gentile and Lackie (cited in Marcy *et al.*, 1978) demonstrated that the major cause of mortality to entrained phytoplankton and zooplankton was physical damage, due mainly to pumping effects. Their experiments showed that before the pumps the average mortality was 10%; after the pumps, 50%; and after condenser passage, 60%.

Certain power stations have been reported to produce near total destruction of meroplankton while others cause much less damage (Jensen, 1977). Jensen postulates that these differences may be related to excessive turbulence and pressure from cavitation in inefficiently operated circulating pumps.

Few data are available concerning only physical effects on phytoplankton. Adverse impact has been demonstrated in several cases, but the contribution of physical stress to total mortality is not known (Marcy *et al.*, 1978). Some stimulation of photosynthesis from the physical effects of condenser passage occurred at the Allen generating plant on Lake Wylie, North Carolina (Gurtz and Weiss, 1972). Williams (1971) found no significant physical effects and Smith and Brooks (1971) found that some stimulation (17.5 to 30%) in productivity could occur from physical effects. On the other hand, Flemer *et al.* (1971) found a 13% reduction in productivity which they attributed to physical effects. Passage of diatoms through the Millstone Point plant without chlorination or thermal addition resulted in mortalities as high as 72%

for five species (AEC, 1974).

Percentages of total mortality caused by physical stress as reported in the literature are in many cases approximations (Marcy *et al.*, 1978), but more recent studies where direct measurement of physical effects has been possible have indicated that the associated zooplankton mortalities may be high. Carpenter *et al.* (1974b) report that 70% of the copepods entering the cooling water system of the Millstone Point plant at Niantic, Connecticut were killed by the physical or hydraulic stresses of passage and that total mortality ranged from 69 to 83%. Beck and Miller (1974, cited in Marcy *et al.*,) noted that physical damage caused mortality of 50% of zooplankton and that the primary cause of the mortality was pumping effects. A 5 to 30% loss in mobility of zooplankton as the result of passage through just the pumps and condensers was observed at many plants (Marcy *et al.*, 1978).

Many zooplankton have intricate and delicate appendages covered with fine hairs which are involved in a variety of functions including filter-feeding, sensing, swimming and reproduction. Thermal, and particularly mechanical, damage to these delicate appendages may lead to an inability of the animal to function normally. Subtle mechanical damage to zooplankton during entrainment may be a serious cause of delayed mortality; mortality which has often not been observed because it may not occur until a number of days after entrainment (Schubel *et al.*,1978).

Maturo *et al.* (1974) found that the extent of physical damage to zooplankton was related to size of the organism at the Crystal River, Florida, plant where *Oithona* suffered little mortality but *Labidocera*, a

larger copepod, was affected throughout the year. In later studies at this plant, Alden *et al.* (1976) confirmed these results. Their research indicated that damage was lowest for the smallest zooplankton (*Oithona* sp.), highest for the largest (*Labidocera* sp.) and intermediate for intermediate sizes.

Further examples of size-related mortality come from the Waukegan plant (Lake Michigan), where zooplankton larger than 0.9 mm suffered 17% mortality while sizes smaller than 0.9 mm suffered only 4% mortality; zooplankton larger than 2.0 mm had 21% immobility while those around 0.4 mm had only 4% (Industrial Bio-Test Laboratories, Inc., 1971, 1972). At the Morgantown nuclear plant in Maryland, mortality of zooplankton at sizes between 0.05 and 0.5 mm was low and mortality of *Acartia tonsa* in the 0.5 to 1.5 mm range was 18%, but both mysid and polychaete larvae (1.5mm to 5.0 mm) suffered severe physical damage (Gentile and Lackie, cited in Marcy *et al.*, 1978).

Physical damage caused 80% of the 100% mortality of the young of nine fish species (2.6 to 40 mm) entrained at the Connecticut Yankee plant (Marcy, 1971, 1973). Almost all (99.7%) of the striped bass (*Morone saxatilis*) eggs which passed through the Vienna, Maryland generating station were killed and a high percentage of the eggs disintegrated (Flemer *et al.*, 1971). Passage through the Northport, New York plant caused 27 to 54% maceration mortality of four species of juvenile marine fish (Austin *et al.*, 1973). Nawrocki (1977) found that damage to 17 larval marine fishes by physical stresses averaged 22.8% and that several species of clupeid larvae sustained 62.5% physical damage.

Increased physical injury with increased size during plant passage has

also been reported for a few species of fish. Marcy (1973), for example, found that the greatest physical damage occurred at night when larger fish (20 to 40 mm) were available for entrainment. During the day, the majority of entrained fish were less than 15 mm long.

Generally, the highest mortality of entrained organisms from physical stress is found with the relatively large (>15 mm) fragile fish larvae (Marcy *et al.*, 1978).

Size alone is only a partial measure of potential mortality of entrained organisms. Different species and life-history stages show different susceptibilities to physical damage. It seems that once larvae are beyond a critical developmental period they can withstand a greater degree of physical abuse (Marcy *et al.*, 1978).

The high percentage of physical damage (80%) noted in the Connecticut Yankee study was linked to the fact that 97.5% of the entrained fish were in the critical post-yolk sac stage and 97% of the larvae were fragile clupeids (Marcy, 1973). High physical damage of the fragile anchovy, as compared to nine other species entrained, was noted at the Brunswick nuclear plant (Copeland *et al.*, 1975) and yolk and post-yolk sac stages were the most vulnerable to damage at the Millstone Point station (Nawrocki, 1977). Of the physical damage mortality of zooplankton entrained at the Connecticut Yankee plant, those species killed were considered fragile and had larger respiratory apparatus (Massengill, 1976).

Marcy (1975) and Adams (1968) indicate that physical stresses may have a much greater impact on fish eggs and larvae than does temperature.

Schubel (1973) came to the same conclusion, noting that ΔT's up to 10°C were not detrimental to the egg development or hatching success of five estuarine fish, and that physical damage may have a much greater impact on development and hatching success.

2.4 Combined Effects

Observations at operating power plants show that many organisms that pass through their cooling systems do not survive. The effects of the physical, chemical and thermal stresses act in concert on the entrained biota. Because the combinations of site, biota, receiving water characteristics and plant operating conditions are almost limitless, the effects are plant specific. At a plant the dominant source of damage may change seasonally and with variations in the mode of plant operation (Beck and the Committee on Entrainment, 1978). Thermal stresses may control mortality of entrained organisms during periods of high ambient temperature, while chemical stresses may dominate during periods of heavy chlorination. Physical stresses, however, including pressure changes, shear, turbulence, impact and abrasion, are experienced continuously whenever cooling water is being pumped, and in many cases are the principal causes for mortality of entrained organisms.

In those cases where mortality of entrained ichthyoplankton and juvenile fishes has been apportioned among the several kinds of stresses, physical stresses usually dominate. In every study of entrained ichthyoplankton and juvenile fishes where the causes of mortalities have been identified, physical stresses had a greater effect on mortality than did temperature (Beck and the Committee on Entrainment, 1978). In all cases but one, physical stresses were more important than chemical stresses in producing mortality.

The relative importance of chlorination in causing mortality may be greater for zooplankton than for larval and juvenile fishes. The effects of chlorination on mortality were greater than either the

thermal or physical stresses in more than 75% of those studies in which it was possible to apportion the mortality among the various classes of stress (Beck and the Committee on Entrainment, 1978). Physical stresses usually had a greater impact on mortality than did temperature.

Available data indicate that mortality of entrained species is at least partially related to several factors: size and life stage, tolerance of the individual species and life stage, and differences in power plant cooling system designs and operational characteristics. Every receiving water is unique in that the physical oceanographic features which affect the movement, dissipation and distribution of the effluent water are different from one another. These features, in turn, vary in time, both diurnally and seasonally, for any discharge location. For this reason it is important that each plant be considered separately and that the results, other than wide generalizations, not be extrapolated to other sites (Mitchell and North, 1971).

The effects of plant passage on entrained organisms can cause changes in community structure through changes in diversity caused by elimination of less tolerant species and life stages, and size selectivity because of damage or mortality to various life stages of species (Marcy *et al.*, 1978).

Organisms may die during the actual entrainment process (*entrainment mortality*), or else hours or even days later as a result of stresses encountered during plant passage (*latent mortality*). These terms will be referred to in the following chapters.

3. PHYTOPLANKTON

3.1 Introduction

Phytoplankton communities are highly dynamic, with cells which have the ability to divide rapidly under the appropriate conditions (Sakshaug, 1980). The impact of power plant entrainment on these communities is usually assessed by determining changes in cell biomass and growth rate, or productivity, of the phytoplankton.

Mitchell and North (1971) noted that damage to phytoplankton from excessive temperature, sudden temperature change, violent agitation or chlorination could be reflected as a decrease in chlorophyll concentration from its degradation to phaeophytin, bleaching or release from ruptured cells. Production by phytoplankton, measured in terms of carbon fixation rate, is probably the most sensitive measure of physiological effect : cell condition determined visually by microscope or chemically by chlorophyll content does not reflect the physiological characteristics of plant cells as well as the cell's performance as measured by carbon fixation (Mitchell and North, 1971).

The study site lies within the southern Benguela upwelling region, an area where intense upwelling frequently occurs, particularly in spring and summer (Shannon, 1966) when south east winds predominate. Upwelling areas are distinguished by high primary productivity and fish yields (Brown, 1986). Newly upwelled water is poor in chlorophyll *a*, but rich in nutrients, so although phytoplankton levels are low there is a high potential for growth. When upwelling conditions subside, the older, more mature upwelled water, which floods back towards the coast during

downwelling, is rich in chlorophyll *a* (Brown, 1984).

Phytoplankton standing stocks during the summer upwelling season are high, on average, but variable. During winter, however, turbulence, reduced light and reduced upwelling produce a relatively uniform distribution of phytoplankton biomass in the area (Brown, 1984).

3.2 Methods

Field samples were collected in the intake basin and at the discharge canal of Koeberg Nuclear Power Station. Cell biomass was measured in terms of chlorophyll *a*, which can be regarded as the key photosynthetic pigment (Leftley *et al.*, 1983), and has been used extensively for estimating phytoplankton biomass (Sakshaug, 1980). The productivity of pre- and post-entrainment water was measured as photosynthetic carbon fixation in bottle experiments.

3.2.1 Chlorophyll *a*

Analysis of chlorophyll *a* was conducted following the method of Parsons *et al.* (1984), with extraction of chlorophyll in acetone and its concentration determined by spectro-photometric analysis.

A ten litre water sample was taken from the surface at four sites just outside the intake pumphouse in the harbour (Fig. 1). Sampling here was conducted from a ski-boat with the assistance of staff from the Koeberg meteorological station. At the outfall area a bucket on a rope was lowered from the "bridge" above the outlet to remove water from two sites (see Fig. 2), and a further site was chosen several metres down from the bridge on each side of the canal. Water samples were taken to

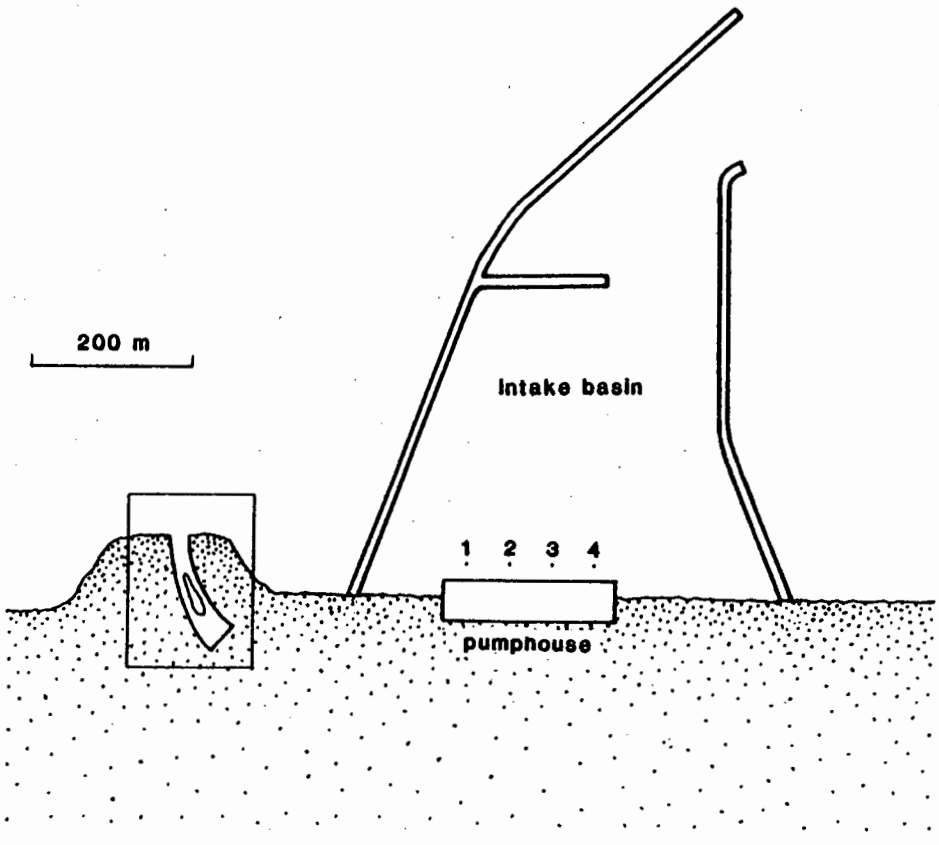


Fig. 1: Diagram showing sampling sites in the intake basin.

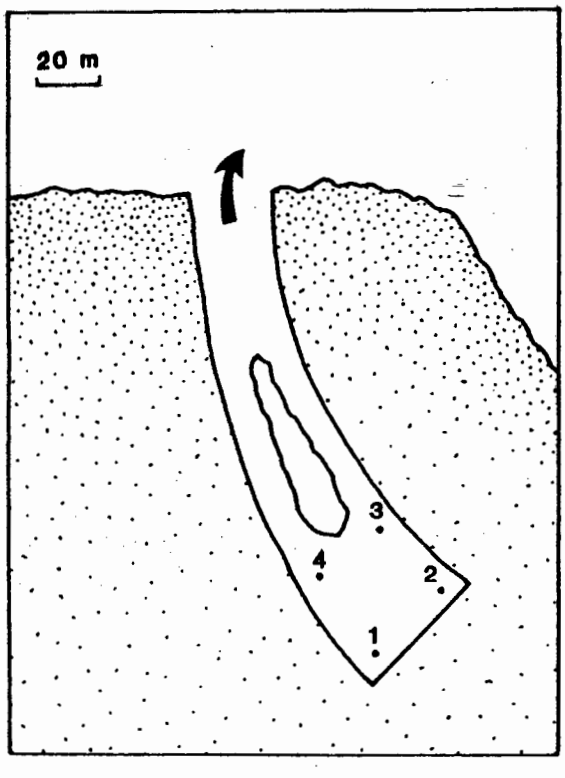


Fig. 2: Diagram showing sampling sites at the outfall canal.

the laboratory for processing and analysis.

Four samples of 0.5 or 1.0 litres of seawater (depending on the amount of organic matter present) for each site were filtered onto 0.5 µm, 47 mm glass microfibre filter papers, under negative vacuum. Three to five drops of $MgCO_3$ solution were added to the seawater during filtration to prevent acidity on the filters. The filter papers were folded, wrapped in tinfoil and stored frozen until the extraction process was undertaken. The filter papers were then thawed and placed in centrifuge tubes. 2 ml of 90% acetone was added to each and a glass rod was used to grind the contents thoroughly. A further volume of 10 ml acetone was added to each tube, care being taken to expose the pigments to a minimum amount of light. The tubes were then centrifuged at 9000 rpm for 15 mins at 15°C.

A 5 ml aliquot from each sample was pipetted into a test-tube. Tubes were sealed with parafilm and kept on ice in the dark. Optical density of each sample was determined using a Beckman DU-40 Spectrophotometer at wavelengths of 750, 664, 647 and 630 nm. The concentration of chlorophyll *a* was calculated using the Jeffrey and Humphrey (1975) equation:

$$(Ca)\ \text{Chlorophyll } a = 11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630}$$

In this equation E is the absorbance at different wavelengths (corrected by the 750 nm reading) and Ca is the amount of chlorophyll *a* (in µg/ml if a 1 cm light path cuvette is used); then

$$\text{mg chlorophyll}/m^3 = \frac{C \times v}{V \times 10}$$

where v is the volume of acetone in ml (12 ml), V is the volume of seawater in litres and Ca is the chlorophyll which is substituted for C in the above equation.

3.2.2 Photosynthesis

^{14}C-incubations were carried out following the basic method of Parsons *et al.* (1984), but this was adapted to incorporate the acid-bubbling technique of determining total primary production as described by Schindler *et al.* (1972) and modified by Theodorsson and Bjarnason (1975).

For each of the sites described above, 200 ml seawater was decanted into 1 dark and 2 light bottles. Five μCi of $NaH^{14}CO_3$ was added to each bottle, which was then sealed and shaken. The bottles were placed in a net and immersed in the harbour water. They were incubated near the surface for three to four hours over the midday period, between approximately 11 a.m. and 3 p.m. After incubation, a 3 ml sample from each bottle was placed into a scintillation vial, acidified with 40 μl 1N HCl and kept in a cool-box. Five 1 ml ^{14}C standards were made by diluting the 5 $\mu Ci.ml^{-1}$ solution with pH 9 double-distilled water to obtain a final concentration of 0.5 $\mu Ci.ml^{-1}$.

In the laboratory the samples were purged of excess sodium ^{14}C bicarbonate by bubbling air into the acidified samples via hypodermic needles. The air flow was adjusted to give vigorous bubbling but no splattering. Purging was conducted in a fume-cupboard for 20 minutes. 3.6 ml of Insta-Gel (scintillation cocktail) was added to the sample vials, which produced a stable gel of 45.5% sample load (without acid). 10 ml Insta-Gel was added to each of the standard vials, which produced

a clear liquid of 9% sample load. The vials were then counted on a Packard Tri-Carb model 460 scintillation counter. A set of 10 quenched standards manufactured by Packard were counted to produce a quench curve. The external standard method was used to determine counting efficiency and thus convert cpm (counts per minute) to dpm (disintegrations per minute) values.

The final dpm values were used in the following equation from Parsons *et al.* (1984) to calculate radiocarbon measured photosynthesis:

$$\text{photosynthesis } (mgC.m^{-3}.h^{-1}) = \frac{(R_S - R_B) \times W}{R \times N}$$

In the previous equation:

R_S = sample count (dpm)

R_B = dark bottle (blank) count (dpm)

W = weight of total CO_2 present in $mgC.m^{-3}$

R = total activity of bicarbonate added (dpm)

N = no. of hours of incubation

As only 1.5% of the total activity was measured (3 ml of the original 200 ml), the value of R in the above equation was taken to be 1.5% of the activity of the added bicarbonate.

The formula used to calculate W was also obtained from Parsons *et al.* (1984), as follows:

W = 12 000 x TC (total carbon dioxide)

An approximation of total CO_2 is made for oceanic waters where:

Total alkalinity = salinity ($S^o/_{oo}$) x 0.067 meq/l

Carbonate alkalinity = total alkalinity - 0.05

Total carbon dioxide = 0.96 x carbonate alkalinity

For a salinity of $35^{o}/_{oo}$, W was calculated to be 26 438 $mgC.m^{-3}$.

Considerable difficulties were encountered with photosynthesis measurements before satisfactory results were obtained. Initially a constant temperature cabinet was used for the incubations, but unsatisfactory results and eventual mechanical failure of the cabinet led to the decision to perform the incubations in the harbour. A new scintillation cocktail manufactured by Beckman, called Hionic-Fluor and purported to be ideal for seawater owing to its tolerance of highly concentrated salt solutions, did not form a homogeneous solution as indicated, and was ultimately replaced with the more tried and tested cocktail Insta-Gel.

3.2.3. The acid-bubbling method

Many researchers who conduct productivity experiments have used the full method of Parsons *et al.* (1984), whereby the contents of the incubation bottles are filtered onto filter papers and counted to obtain estimates of particulate production, and a sample of the filtrate is acidified, purged of excess sodium ^{14}C bicarbonate, and counted to measure excreted production. Assuming reassimilation (refixaton of respired CO_2) occurs, the final value is considered to lie somewhere between net photosynthesis (complete reassimilation of respired $^{12}CO_2$) and gross photosynthesis (no reassimilation) (Harris, 1980; Brown, 1986).

As the research aims at Koeberg were primarily directed at comparisons between the productivity of pre- and post-entrainment water, it was decided to use the less time-consuming acidification and bubbling method

of measuring total production, as mentioned by Henry (unpubl.) and described by Schindler *et al.* (1972) and Theodorsson and Bjarnason (1975). This technique has been used to avoid assumed cellular rupture by filtration (Theodorsson and Bjarnason, 1975; Lean and Burnison, 1979), and may therefore be superior to membrane-filtration for post-incubation treatment of ^{14}C-productivity samples (Schindler *et al.*, 1972).

Cautionary comments by several authors concerning this method were taken into consideration. Inorganic carbon content is considerably higher in salt waters than in most fresh waters, and may therefore be harder to remove completely (Sharp, 1977). Thomas (1971), Sharp (1977) and Lean and Burnison (1979) have stressed the importance of complete removal of unused (inorganic) ^{14}C during purging, especially where low rates of photosynthesis are involved. The ^{14}C used when estimating excreted production should be chemically pure, as the presence of small amounts of labelled organic contaminants may give spuriously high results when low excretion rates are encountered in the field (Williams *et al.*, 1972; Williams and Yentsch, 1976; Sharp, 1977). Excretion of organic carbon was usually found to be low in experiments conducted in the southern Benguela upwelling area (Brown, 1986), but since the above warning is directed primarily at measurements of excreted production alone (from the filtrate of incubation bottles) and not at estimates of total production, it was not considered to be a major hazard for the present study. It is possible, however, that the results obtained may overestimate production to some extent.

Brown (1986) noted that rates of organic productivity estimated from bottle experiments are sometimes regarded as an index of primary

productivity and not as an absolute value. It is suggested that the production values obtained from the acid-bubbling technique in this study should be treated as such.

3.3 Results

3.3.1 Chlorophyll *a*

The average concentrations of chlorophyll *a* (± sample standard deviation) measured in $mg.m^{-3}$ for the intake and outfall sites are summarised in Table 1. On each occasion there was a significant reduction in chlorophyll *a* concentration after entrainment. Values ranged from a 42.71% to a 70.29% drop in biomass, and on average there was 55.32% less chlorophyll *a* in the outfall compared to the intake water. The chlorophyll levels before and after entrainment are also illustrated graphically in Figure 3.

A two-sample, two-tailed *t* test (Zar, 1984) was conducted to test the difference between the intake and outfall means of chlorophyll *a* for each sampling date. In each case, the means were found to be significantly different ($P < 0.001$). Simple linear regression analysis of the data (Zar, 1984) did not indicate any correlation between ΔT or discharge temperature and the corresponding reductions in chlorophyll *a*.

3.3.2 Photosynthesis

The results of the incubation experiments and photosynthesis calculations are presented in Table 2. They show significant reductions in photosynthesis for post-entrainment water-samples, with decreases in productivity ranging from 22.94% to 59.63% and averaging 38.30% overall. These results are also depicted graphically in Figure 4. Reduction of

TABLE 1: Summary of chlorophyll *a* results, where TEMP is the temperature increment, CHL *a* IN and OUT refer to the concentrations of chlorophyll *a* (± standard deviation) measured in the intake basin and outfall canal respectively, and CHL *a* is the percentage reduction in chlorophyll *a* due to entrainment. The final column shows the results of the t-test conducted for each site.

DATE	TEMP (C)	CHL *a* IN ($mg.m^{-3}$) ±S.D.	CHL *a* OUT ($mg.m^{-3}$) ±S.D.	CHL *a*	t-test
27.09.85	9.5	7.03 ±0.98	3.48 ±0.79	-48.47%	P < 0.001
12.11.85	8.6	3.67 ±0.59	2.10 ±0.79	-42.61%	P < 0.001
06.05.86	9.0	3.23 ±0.49	1.73 ±0.16	-46.64%	P < 0.001
05.11.86	10.0	32.72 ±2.95	10.57 ±2.01	-67.71%	P < 0.001
14.01.87	8.0	10.48 ±1.75	3.11 ±1.16	-70.28%	P < 0.001
25.03.87	8.0	3.89 ±0.80	1.79 ±0.27	-54.08%	P < 0.001

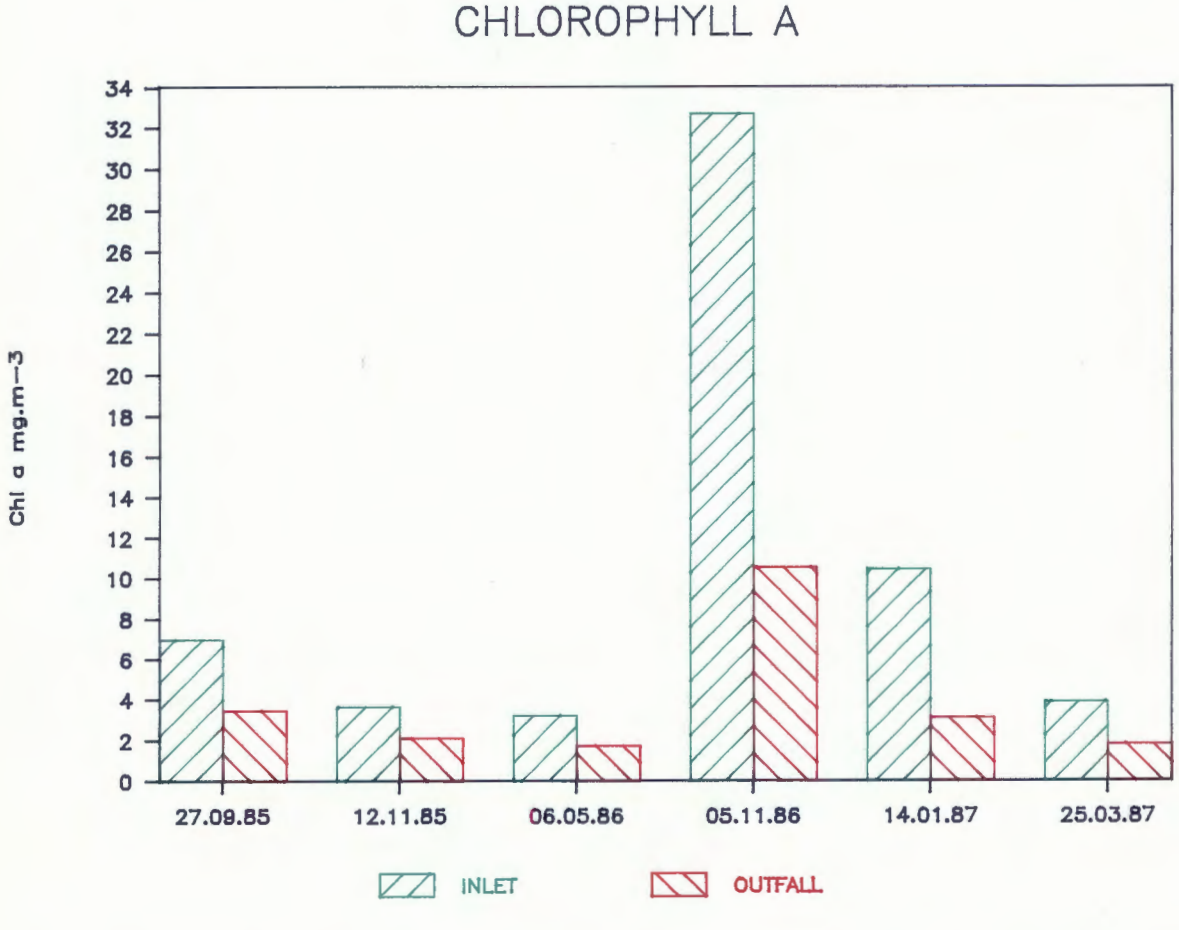


Fig. 3: Comparison of chlorophyll *a* concentrations ($mg.m^{-3}$) measured at sites in the intake basin (INLET) and outfall canal (OUTFALL) on six sampling occasions.

TABLE 2: Summary of photosynthesis results, where TEMP is the temperature increment, PHOTO IN and PHOTO OUT refer to the levels of photosynthesis (± standard deviation) measured at the intake basin and outfall canal respectively, and PHOTO is the percentage reduction in photosynthesis due to entrainment. The final column shows the results of the t-test conducted for each site.

DATE	TEMP (C)	PHOTO IN ($mgC.m^{-3}.h^{-1}$) ±S.D.	PHOTO OUT ($mgC.m^{-3}.h^{-1}$) ±S.D.	PHOTO	t-test
05.11.86	10.0	136.70 ±10.30	89.73 ±22.52	-34.39%	P < 0.001
25.03.87	8.0	66.11 ± 8.47	26.69 ± 9.08	-60.00%	P < 0.001
17.02.88	7.9	65.86 ±11.19	50.75 ± 9.25	-31.13%	P < 0.1
25.02.88	10.0	127.84 ±16.52	81.53 ±20.56	-33.03%	P < 0.002

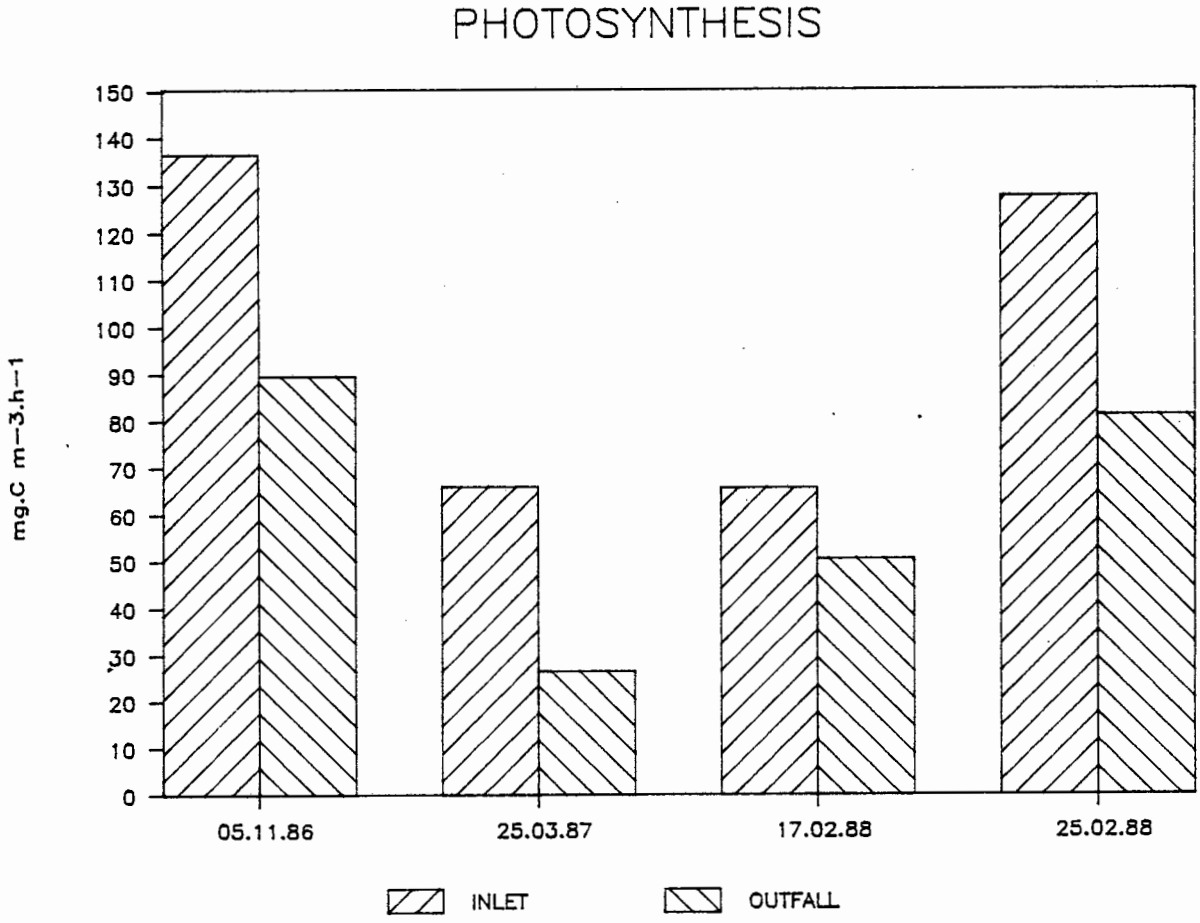


Fig. 4: Comparison of photosynthesis ($mgC.m^{-3}.h^{-1}$) calculated from incubations of seawater collected from the intake basin (INLET) and the outfall canal (OUTFALL) on four sampling occasions.

photosynthesis also did not appear to be directly correlated to ΔT or discharge temperature. Overall decreases in primary production are probably determined by a more complicated relationship between ΔT, discharge temperature, level of chlorination and reactor status, as well as the physiological state of the entrained phytoplankton.

The absolute figures of carbon production are fairly high (66.11 to 136.70 $mgC.m^{-3}.h^{-1}$ at intake sites) possibly indicating fairly recent upwelling in the area (the experiments were conducted during summer, from November to March). It should also be remembered that these figures represent total production without any correction for excreted production or other losses due, for example, to sinking out of the water column. Brown (1986) followed a patch of upwelled water and obtained maximum production rates (considered to lie somewhere between gross and nett production) between 82.3 and 155.2 $mgC.m^{-3}.h^{-1}$ during the course of five different cruises. These values were found relatively near the surface (2 - 5m) at light penetration depths of 10 - 50%. Considering the discussion of the method used to measure photosynthesis in this study, it is possible that the values obtained may slightly overestimate "real production" at the particular sites, but they are still valuable in providing a comparison of the "condition" of the seawater before and after the entrainment process.

3.4 Discussion

An average decrease in chlorophyll *a* concentration of 55.32% and an overall 38.30% reduction of photosynthesis as a result of entrainment, appear to be consistent with, although often lower than, the findings of similar studies in the literature. At a power plant at Crystal River,

Florida, Fox and Moyer (1975) found an average decrease in primary production of 57% due to plant passage and chlorination. In the absence of chlorine, the average decrease was 13%. Bienfang and Johnson (1980) found that primary production of marine phytoplankton decreased by ca. 30% after a 5-6°C thermal shock associated with power plant passage (chlorination was not employed). After ambient temperatures were reattained, however, ^{14}C-fixation rates recovered with time. No significant effects on chlorophyll *a* or ATP levels were observed in their study.

The effects of heating alone on phytoplankton (i.e no chlorination) appear to vary considerably, depending on the site, ambient temperature, magnitude of temperature increase and length of exposure to the thermal stress. Briand (1975) indicated that two interacting factors, intake water-temperatures and magnitude of temperature increase, appeared to control the severity of the impact. While some researchers have reported reductions in primary productivity as a result of the thermal effects of entrainment (e.g. Fox and Moyer (1975) - 13% decrease; Bienfang and Johnson (1980) - 30% decrease; Fox and Moyer (1973) - 25.9% decrease; Takesue and Tsuruta (1978) - 71-77% decrease in summer and 31-46% decrease in winter), others found that production was stimulated by the rise in temperature, eg. Hamilton *et al.* (1970) and Eiler and Delfino (1974). Brook and Baker (1972) found that nonchlorinated condenser discharge water (32 to 36°C) showed a depression in photosynthesis of 5 to 15% and a stimulation of respiration up to 50%, as compared with cooler water upstream (23 to 25°C). They noted, however, that much of the respiration effect was probably related to bacterial, not merely algal, metabolism.

Mitchell and North (1971) investigated temperature-time effects on marine plankton passing through the cooling water system at the San Onofre generating station (California). Analysis of ^{14}C and chlorophyll studies indicated little, if any, negative effect on phytoplankton productivity, and possibly some enhancement (ΔT=10-11°C).

The thermal effects of a power plant cooling system in Japan on marine phytoplankton photosynthesis was investigated by Takesue and Tsuruta (1978). After passage in August with high temperatures (25-27°C), chlorophyll *a* levels were halved and phaeo-pigments doubled, but chlorophyll *a* and phaeo-pigment content changed little in January with low ambient temperature (16°C). The authors concluded that the content of chlorophyll *a* is affected very little at temperatures below 20°C.

In general, it seems that reductions in primary productivity due to thermal stress are observed when ambient temperatures are warm, while stimulation is common in winter months (Sellner *et al.*, 1984). In marine and estuarine localities unicellular algae grow prolifically where there is local warming in areas which are well supplied with nutrients, often resulting in "red tide" and other forms of algal blooms. In Britain it has been shown that heated effluents result in increased total plankton production and that the spring outburst of phytoplankton may occur earlier than in unheated areas (Naylor, 1965).

Fox and Moyer (1973) found that effects from increased water temperature were most profound immediately following heat exposure, the severity being proportional to the increase in temperature of the water. Primary productivity dropped by an average of 25.9%, ATP and bacterial populations generally increased, and chlorophyll *a* showed wide

fluctuations. The authors concluded that some organisms, such as phytoplankton, may be killed (or at least hindered in their ability to assimilate carbon) whereas other organisms, such as bacteria, survive condenser tube passage and may even increase in numbers as a result of prolonged exposure to increased heat.

The process of chlorination, in addition to heating, appears to be most detrimental to phytoplankton. Brook and Baker (1972) found that chlorination depressed river phytoplankton photosynthesis and respiration rates to a much greater extent than did heating, and Flemer and Sherk (1977), in their investigations on entrained estuarine phytoplankton, found that in most cases, chlorine rather than heat was implicated as the most important factor in the reduction of the rate of carbon assimilation. Studies by Hamilton *et al.* (1970) demonstrated a 91% reduction of primary production as a result of chlorination, as well as reductions in bacterial densities and chlorophyll *a* concentrations. Eppley *et al.* (1976) observed a 70 to 80% depression of photosynthesis from a 1.0 ppm chlorine injection producing 0.04 to 0.02 ppm total residual in the outfall. Without chlorine there was no inhibition of photosynthesis. Gentile *et al.* (1976) found that a total residual chlorine concentration of greater than 1.0 ppm was responsible for complete mortality of all entrained phytoplankton.

Brooks and Seegert (1977) investigated the effects of intermittent chlorination on phytoplankton. In many cases, chlorination resulted in chlorophyll *a* reductions and phaeophytin *a* increases. The effect was generally pronounced at chlorine concentrations above 1 ppm. ^{14}C uptake rates showed drastic reductions following a 30-min chlorine exposure to 0.5 ppm residual chlorine. At chlorine concentrations below 0.1 ppm,

there was an initial decrease in ^{14}C uptake (~20%) but recovery of the stressed phytoplankton population was evident. Chlorine concentrations less than 0.1 ppm did not significantly stress the phytoplankton populations.

It appears, according to Morgan and Carpenter (1978), that phytoplankton affected by chlorine subsequently die. Gentile *et al.* (1976) noted that chains of the diatoms *Detonula confervacea* and *Skeletonema costatum* fragmented and that chlorophyll *a* decreased by an average of 63% as a result of chlorination. At one site, Gentile *et al.* (1976) noted almost complete destruction of phytoplankton ATP. Free residual chlorine was 0.5-0.32 ppm at the point of dosage, and 0.2-0.1 ppm in the discharge canal. Since ATP is rapidly lost in cell death, these results indicate irreversible loss of living biomass.

Eppley *et al.* (1976) also noted that there was no recovery of photosynthetic activity in samples even after residual chlorine had fallen to undetectable levels. Thus, rather than merely inhibiting growth, chlorine, as applied in these power plants, apparently acts irreversibly on exposed phytoplankton.

Studies have shown that algal species differ in their sensitivity to both heat and chlorine, and the composition of entrained phytoplankton communities may be altered by the species specific interactions of factors affecting vulnerability and entrainment loss (Jordan *et al.*, 1983). The chlorine concentration needed to reduce growth of 11 marine species by 50% in a 24-hr exposure ranged from 0.075 to 0.33 ppm, a factor of 4.4 (Gentile *et al.*, 1976). Kott (1969) states that some freshwater algal species, most notably *Cosmarium*, are resistant to

chlorine. Similarly, Hirayama and Hirano (1970) observed that *Skeletonema costatum* was killed after exposure to 1.5-2.3 ppm chlorine for 5 to 10 min whereas *Chlamydomonas* was affected only at 20 ppm. Sanders *et al.* (1981) found that thermal stress led to a reduction in centric diatoms, especially *Chaetoceros* spp., and predominance of microflagellates.

Sellner *et al.* (1984), however, noted that when reductions in cell numbers were experienced as a result of thermal stress, the loss was most commonly in the flagellated species, including dinoflagellates and microflagellates. Laboratory studies have also indicated that flagellates may be the most thermally susceptible phytoplankton group. In their study at the Calvert Cliffs Nuclear Power Plant, the passage of bay water through the plant was accompanied by a decline in the photosynthetic activity of the micro-flagellate *Cryptomonas acutea.* Photosynthetic rates of the diatom *Thalassionema* and the dinoflagellate *Prorocentrum* were not significantly altered by passage through the power plant.

Lassus and Maggi (1980) found that the development of the flagellate *Dunaliella tertiolecta* (Butcher) was only slightly affected by thermal shock, since the most extreme conditions tested (17°C increment above an acclimation temperature of 24°C) resulted in only a slight retardation of growth. However, exposure to 0.5 ppm chlorine caused a significant reduction in growth, inhibition being total when combined with thermal shock. Larger protozoa, *Ceratium* sp., tintinnids and ciliates displayed low, if any, mortality due to thermal stress at a power plant in California (Mitchell and North, 1971).

Entrainment effects appeared very disruptive, in changing the structure of phytoplankton communities and in constantly reducing species diversity, at two Californian power plants (Briand, 1975). Passage affected algal species differentially, killing diatoms in greater numbers (45.7%) than dinoflagellates (32.8%), and reinforcing the dominance of the two major species that were the most tolerant. Briand indicated that only productive cells survived entrainment.

Differing results, such as those described above, make it difficult, if not impossible, to generalise with respect to the vulnerability of the various algal types, and conclusions concerning heat and chlorine susceptibility of phytoplankton should remain confined to species only.

The duration of exposure of a phytoplankter to chlorine is also important. Gentile *et al.* (1976) found no measurable mortality for a 0.5 min exposure of *Skeletonema costatum* to 0.4 ppm chlorine, but 25 to 50% inhibition of growth after a 2-10 min exposure. Similar results were obtained for *Thalassiosira pseudonana.*

The actual mechanisms of chlorine inhibition of phytoplankton growth are still not known, but chlorine and its by-products appear to impair cell function and the ability to take up nutrients such as nitrate, ammonia and phosphate. Toetz *et al.* (1977) found that chlorine at an initial concentration of 0.028 ppm depressed nitrate uptake by 50%, and suggested that small quantities of chlorine or chloramine may destroy or inactivate enzymes in the cell membrane that are responsible for the uptake of nitrate.

A study by Videau *et al.* (1980) showed that small amounts of chlorine,

although inducing no mortality, significantly reduced phosphate uptake in *Dunaliella primolecta*, a unicellular marine alga. Higher chlorine concentrations caused a leakage of PO_4^{3-} ions but, after a time depending on chlorine concentration, the phosphate absorption rate in the surviving chlorinated cells and in their respective control cells was similar. One hour after chlorination, which induced a 50% mortality, the intracellular ATP content was reduced by 70% and the formation of other phosphorylated compounds was completely inhibited. Twenty-four hours later, however, the surviving cells showed no apparent metabolic alteration.

Although it is often not stated, phytoplankton entrainment necessarily includes physical, or mechanical, effects of passage. Jordan *et al.* (1983) detected shortening of entrained *Skeletonema costatum* chains in both summer and winter, indicating a mechanical effect of turbulence. However, physical stresses during entrainment are considered to be minor compared to the thermal and chemical stresses on phytoplankton, and are unlikely to contribute directly to phytoplankton mortality.

In attempting to extrapolate the results of specific studies to assess the overall effects that power plant chlorination may have on marine phytoplankton populations, the general conclusions appear to be that chlorination effects are of minor importance, despite the considerable mortalities that have been documented at some power stations. The rationale for this conclusion stems from the fact that entrained phytoplankton exposed to chlorine represents only a small fraction of the standing crop and the portion of the phytoplankton not killed during entrainment is capable of rapid recovery and subsequent replacement of the fraction lost through chlorination. Goldman *et al.* (1978) state

that short-term heat rise either alone or combined with chlorine dosage has virtually no effect on the recoverability potential of surviving cells. Goldman and Davidson (1977) showed that the growth rates of cells that had recovered from chlorine exposure were not altered. Phytoplankton possess rapid generation periods, and unless complete destruction is accomplished during entrainment, the surviving organisms are capable of renewed growth once returned to the receiving water.

In a ten year programme in the vicinity of the Calvert Cliffs Nuclear Power Plant, Sellner *et al.* (1984) found no continuous or seasonally detectable impact on phytoplankton standing crop or productivity in the immediate vicinity of the installation. Recovery of the entrained flagellate populations on mixing with bay waters ensured maintenance of the overall productivity of the bay.

Koeberg Nuclear Power Station relies on turbulent dispersion to mix and dilute the thermal effluent water. Most of the dispersion is due to natural processes in the coastal environment, although the discharged plume also generates its own forces. Breaker zone currents and longshore wind-induced currents determine the path of the discharged water (Rattey and Potgieter, 1987).

The schematic representation of plume dispersal at the power station in Figure 5 is typical of an offshore south-easterly wind regime. The surf zone mixes and dilutes the warm water from +10°C to +5°C within the first 500m from the outfall (ESCOM Databank, 1986), and the flow of warm water inside the surf zone appears to be southwards along the beach. The temperature of the diluted water outside the surf zone is further reduced by wind-blown currents. The south-east wind drives the plume

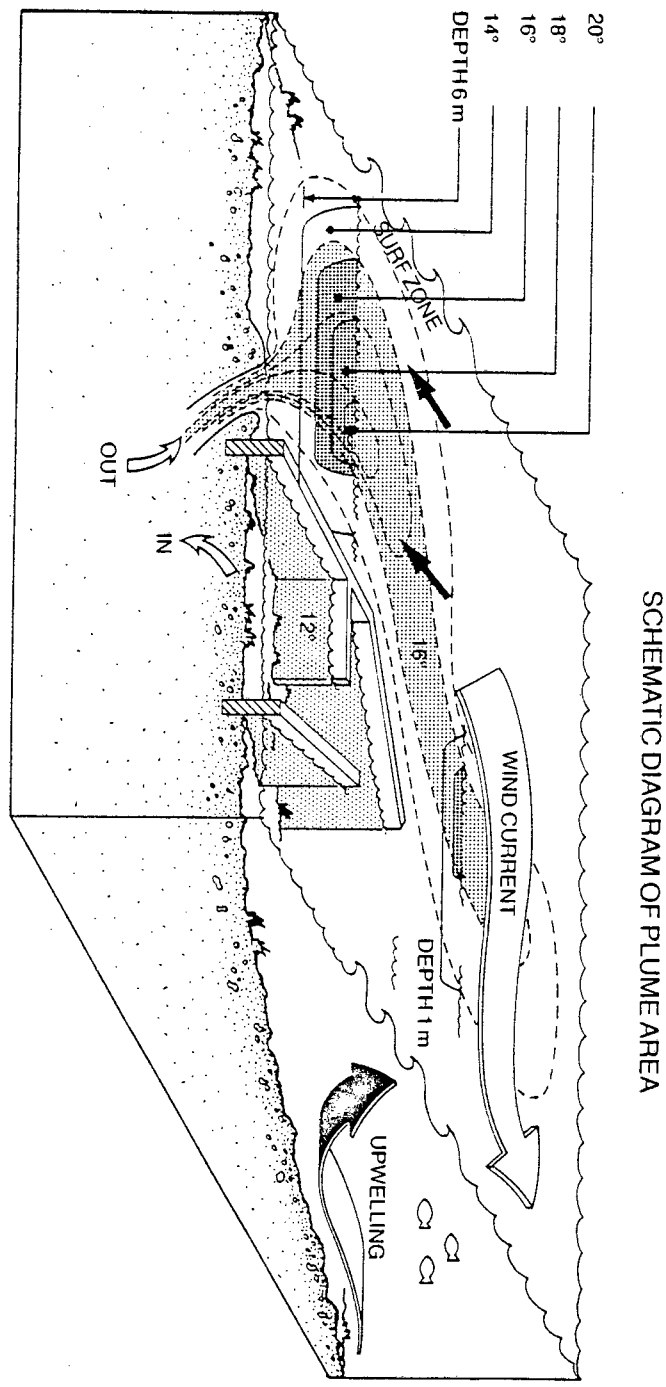


Fig. 5: Schematic diagram of plume area, showing typical conditions for a south-easterly wind direction (taken from ESCOM Databank, 1986).

around the breakwater to extend mainly offshore to a maximum distance of 7km to the west. North-west or onshore winds keep the plume close to the coast for about 3km in a southerly direction before it extends offshore (ESCOM Databank, 1986; Rattey and Potgieter, 1987).

The warm water extends to a maximum depth of about 6m. Below this depth there is no measurable increase in sea temperature (ESCOM Databank, 1986; Rattey and Potgieter, 1987).

The overall picture suggested from these data is that of a very localised warming effect in the vicinity of the power station. The low levels of chlorine used for anti-fouling purposes have in most cases completely dispersed by the time the effluent water meets the surrounding ocean. The plume should therefore have a fairly low impact on the sea near Koeberg.

Approximately half of the phytoplankton biomass, on average, survives the entrainment process at Koeberg Nuclear Power Station, and the associated reduction in productivity is not unduly high. In the light of metabolic recovery of surviving cells from similar studies, it is likely that surviving phytoplankton cells at Koeberg will regain their photosynthetic potential and regenerate lost biomass.

Even if this were not so, the southern Benguela upwelling region is characterised by high primary productivity, and a localised loss of biomass near Koeberg would be unlikely to have a devastating impact on the environment as a whole, since entrained phytoplankton represent only a small fraction of the standing crop.

4. ZOOPLANKTON

4.1 Introduction

Large amounts of zooplankton are entrained into power stations with the water used for cooling purposes. Samples were collected from the intake basin and the outfall canal at Koeberg Nuclear Power Station to assess zooplankton entrainment mortality.

4.2 Methods

For quantitative zooplankton estimates, 80 litres of seawater were collected at two sites in the intake basin, using buckets. The water was filtered through a mysid net with a mesh size of 80 μm and a mouth area of 0.2 m^2. To obtain a larger and more diverse zooplankton sample (i.e a qualitative estimate), the net was towed in the harbour at low speed for several minutes. From the outfall canal, the quantitative samples were taken at a site on either side of the canal, and the qualitative samples were obtained by holding the net "face" into the current for several minutes.

After collection, the zooplankton samples were placed in 500 ml plastic containers, and 5 ml of 0.1% w/v neutral red stain was added to each sample, following the method of Crippen and Perrier, 1974). These samples were left to stain for eight hours. Neutral red is a "vital" stain, i.e. only living cells are stained.

After staining, the samples were fixed in 40% formalin, and then passed through a 72 μm sieve, rinsed and placed in 50 ml fresh water. A

further 5 ml of 40% formalin was added to each, together with 2 ml of a 1N HAc-NaAc (acetic acid-sodium acetate) solution. The latter solution enhances both the intensity of the stain in organisms that were alive prior to preservation, and especially the differences between living and dead individuals (Crippen and Perrier, 1974). The samples were stored at 2-3°C until live-dead counts were undertaken. The organisms were identified to species where possible, and the numbers of live and dead individuals were recorded.

4.3 Results

The high velocity and volume of the discharged water in the outfall canal (2 $m.s^{-1}$ and up to 80 $m^3.s^{-1}$) made it difficult to keep the sampling net in the correct position, and may have resulted in less water being sampled at outfall sites than at inlet sites. To test this possibility, total numbers of zooplankton collected at the intake and outfall sites using the net-tow method were compared, and are listed in Table 3. Total numbers of zooplankton collected per m^3 using buckets are also given.

The results indicate that much higher numbers of zooplankton were collected from intake sites using the net, mainly due to large swarms of *Evadne*, *Podon* and copepods in the harbour. Netted intake samples also contained a higher abundance of medusae, barnacle nauplii, cyprids, isopods, euphausiid larvae, echinoderm larvae and fish eggs, while outfall samples had higher densities of polychaete larvae, chaetognaths, mysids and crab zoeae.

Qualitative (bucketed) samples from the outfall contained more

TABLE 3: Comparison of total numbers of zooplankton collected at the inlet and outfall sites for 8 bucketed samples (number.m^{-3}) and 8 netted samples (total number).

ZOOPLANKTON TYPE	BUCKETED SAMPLES (number.m^{-3})		NETTED SAMPLES (numbers)	
	Inlet	Outfall	Inlet	Outfall
Medusae	0	1.26	387	43
Ctenophores	0	2.50	27	36
Nematodes	0.38	2.64	1	1
Polychaete larvae	28.76	36.14	3	44
Chaetognaths	0.63	0.38	5	49
Evadne	48.15	224.38	5031	623
Podon	136.26	1734.39	40491	634
Penilia	0	0	2	0
Barnacle nauplii	45.52	71.12	512	201
Cyprids	64.78	137.52	717	208
Mysids	6.38	123.14	5	75
Cumaceans	0	0.63	1	12
Isopods	7.14	0.63	159	23
Amphipods	1.01	0.63	20	32
Euphausiid larvae	3.13	13.76	452	181
Zoeae (type a)	10.53	3.14	39	124
Zoeae (type b)	3.75	5.63	10	145
Copepods	257.41	1516.81	23098	6653
Harpacticoids	0	2.64	2	0
Copepod larvae	40.27	41.27	64	59
Gastropod larvae	3.76	4.14	97	28
Bivalves	0	0	4	0
Echinoderm larvae	1.25	1.25	216	0
Appendicularia	0.63	2.26	0	1
Fish eggs	93.90	317.64	681	278
TOTAL	753.64	4243.80	72024	9450
SAMPLE MEAN (÷8)	94.21	530.48	9003	1181.25

zooplankton than intake samples. This was also mostly due to larger numbers of cladocerans and copepods in the outfall, in particular on 14.01.87, when zooplankton density was very high.

Overall it would appear that zooplankton samples collected by both methods reflected the natural variation and patchiness of zooplankton populations, and it is not felt that qualitative (net-tow) sampling was strongly biased towards the intake sites.

Tables 1 to 8 in Appendix I list the numbers of live and dead zooplankton (per m^3) collected using buckets, and any mortality difference between intake and outfall samples. Tables 9 to 16 list the same details for net-tow samples. Considerable variation in mortality was found for the different types of zooplankton, although in many cases only one or a few individuals of a certain group were present per sample. To smooth out this variation, the total numbers of live and dead zooplankton collected over the whole study were pooled, and these figures were used to calculate an overall mortality for each group (see Table 4). Entrainment mortality was thus considered to be the difference between intake mortality (due to natural causes and sampling) and outfall mortality (due to natural causes, sampling and entrainment). The entrainment mortality for each zooplankton type is depicted graphically in Figure 6.

The results reveal a range of entrainment mortality values for the different groups or species, from 0% for ctenophores, echinoderm larvae and appendicularia to 80.0% for harpacticoid copepods. It should be noted, however, that even with pooled results, very few (less than 10) individuals were collected from some groups, e.g. nematodes, *Penilia*,

TABLE 4: Total numbers of live and dead zooplankton collected from the intake basin and outfall canal, with percent change in mortality, for the entire study period (September 1985 to January 1987).

ZOOPLANKTON TYPE	INLET			OUTFALL			% Change in Mortality
	Alive	Dead	% Dead	Alive	Dead	% Dead	
Medusae	387	0	0.0	44	1	2.22	+2.22
Ctenophores	28	0	0.0	40	0	0.00	0.00
Nematodes	3	0	0.0	4	1	20.0	+20.00
Polychaete larvae	95	0	0.0	130	7	5.11	+5.11
Chaetognaths	4	0	0.0	43	6	12.24	+12.24
Evadne	5059	57	1.11	663	337	33.7	+32.59
Podon	40706	3	0.01	3472	34	0.97	+0.96
Penilia	2	0	0.00	-	-	-	-
Barnacle nauplii	591	3	0.51	261	60	18.69	+18.18
Cyprids	726	97	11.79	269	173	39.14	+27.35
Mysids	23	2	8.0	61	18	22.78	+14.78
Cumaceans	1	0	0.0	8	6	42.86	+42.86
Isopods	166	1	0.6	23	3	11.54	+10.94
Amphipods	23	1	4.17	25	7	21.88	+17.71
Euphausiid larvae	457	0	0.0	195	8	3.94	+3.94
Zoeae (type a)	33	1	2.94	83	46	35.66	+32.72
Zoeae (type b)	36	2	5.26	105	58	35.58	+30.32
Copepods	23488	217	0.92	6454	2959	31.44	+30.52
Harpacticoids	2	0	0.0	1	4	80.0	+80.00
Copepod larvae	172	0	0.0	156	28	15.22	+15.22
Gastropod larvae	108	0	0.0	30	6	16.67	+16.67
Bivalves	1	0	0.0	-	-	-	-
Echinoderm larvae	218	0	0.0	2	0	0.0	0.0
Appendicularia	1	0	0.0	7	0	0.0	0.0
Fish eggs	888	30	3.27	770	54	6.55	+3.28
TOTAL	73218	414	0.56	12846	3816	22.90	+22.34

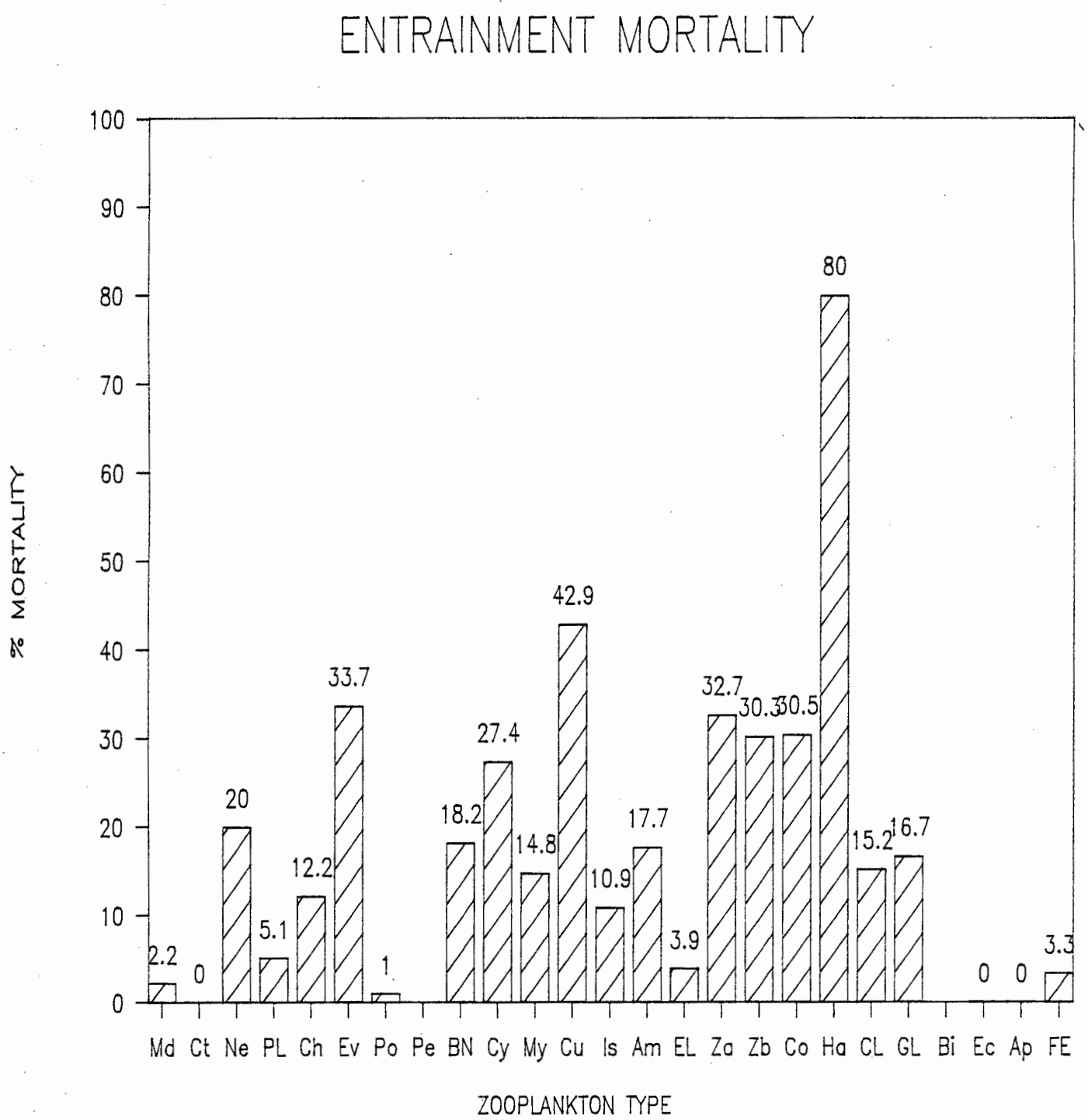


Fig. 6: Overall percentage zooplankton mortality due to entrainment. Md = Medusae, Ct = Ctenophores, Ne = Nematodes, PL = Polychaete larvae, Ch = Chaetognaths, Ev = *Evadne* sp., Po = *Podon* sp., Pe = *Penilia* sp., BN = Barnacle nauplii, Cy = Cyprids, My = Mysids, Cu = Cumaceans, Is = Isopods, Am = Amphipods, EL = Euphausiid larvae, Za = Crab zoeae (type a), Zb = Crab zoeae (type b), Co = Copepods, Ha = Harpacticoid copepods, CL = Copepod larvae, GL = Gastropod larvae, Bi = Bivalves, Ec = Echinoderm larvae, Ap = Appendicularians and FE = Fish eggs.

harpacticoid copepods, bivalves and appendicularians, and undue significance should not be placed on the entrainment mortalities calculated for these zooplankton. Much of the mortality of the more abundant zooplankton groups was dominated by crustacean forms, such as cladocerans, cirripede larvae, crab larvae and copepods.

Except for a large swarm of cladocerans (*Podon polyphemoides)* on one particular sampling day (14.01.87), copepods were the most abundant organisms found. This group showed an overall increase in mortality of 30.52%. The average mortality increase for all the species combined was 22.34%.

An alternative method of calculating entrainment mortality was used to compare with the pooled data method. The average entrainment mortality of each group or species was calculated using data from samples containing at least 6 animals per m^3 of seawater (bucketed samples) or at least 10 individuals (netted samples) at both intake and outfall sites. The results are presented in Table 17 in Appendix I. Lack of sufficient data resulted in entrainment mortality estimates for only 10 of the 25 zooplankton groups recorded, compared to 23 groups using the pooled data method. For many groups, mean mortality was the average of only one or two data points.

Entrainment mortalities from pooled data were higher for *Evadne*, copepods, fish eggs and total zooplankton, but lower for barnacle nauplii and copepod larvae, compared to the above method. Mortalities calculated for medusae, polychaete larvae, *Podon*, cyprids and euphausiid larvae were similar (<5% difference). It was felt, however, that the pooled data method of calculating entrainment mortality was more

realistic than that of averaging separate sample mortalities, especially when considering the paucity of data for the latter method.

Entrainment mortality of copepods and total zooplankton from each sample did not show any correlation with ΔT or outfall temperature when linear regression analysis (Zar, 1984) was applied.

Figs 7a-7x illustrate the pooled overall pre- and post-entrainment mortality for each group or species taken separately (note the different scales). These will be discussed in taxonomic order.

4.4 Discussion

On the whole, staining of the zooplankton samples was successful, although some organisms did not absorb the dye as well as others. All the crustaceans showed good absorption of the dye, but some of the gelatinous plankton (especially the medusae) were only coloured lightly. Consistent staining of fish eggs was doubtful in some samples.

Medusae and Ctenophores (Figs. 7a and 7b).

Hydromedusae were the dominant cnidarians in the vicinity of Koeberg, the most common being *Obelia* spp. and *Bougainvillia ramosa*. A few siphonophorans (*Muggiaea* sp.) were also found. The much lower number of medusae collected in outfall samples is probably because many were too large to pass through the screens at the intake pumphouse. The increase in mortality due to entrainment was low for medusae (2.22%), resulting from only one dead post-entrainment specimen.

Ctenophores collected in the harbour were mostly *Pleurobrachia pileus*.

Fig. 7a MEDUSAE

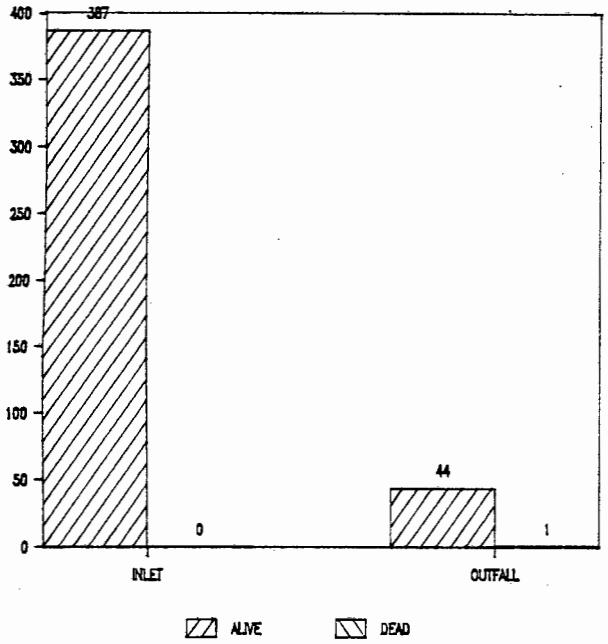


Fig. 7b CTENOPHORES

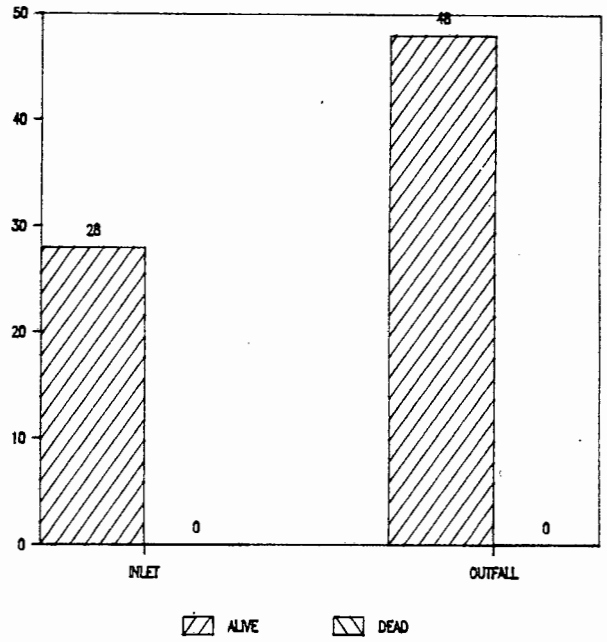


Fig. 7c NEMATODES

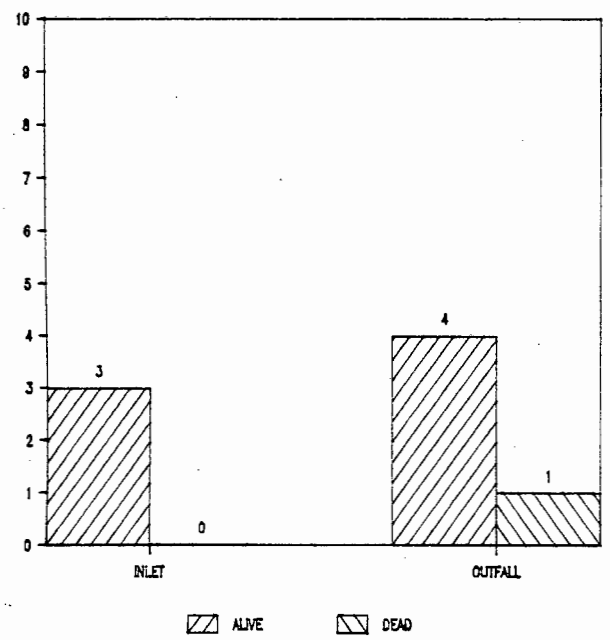
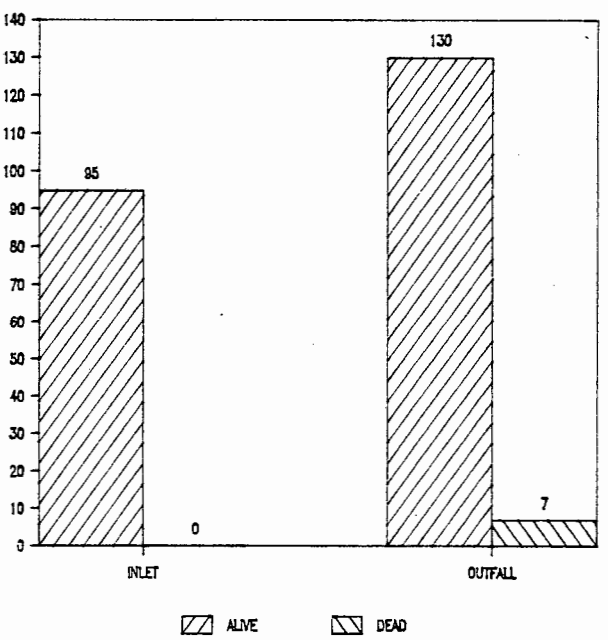


Fig. 7d POLYCHAETE LARVAE



Figs. 7a-7d: Total numbers of live and dead medusae (7a), ctenophores (7b), nematodes (7c) and polychaete larvae (7d) collected from inlet and outfall sites over the sampling period.

All the specimens found in the outfall samples survived the entrainment process, and as with the medusae, the larger ones were not entrained.

Sandine (1973) showed that physical "abuse" in the cooling system injured larger macrozooplankters such as coelenterates, ctenophores and arrow-worms, whilst Mihursky and Dorsey (1973) indicate that large ctenophores suffered greater mortality from physical effects than small ones. No references have been found regarding the effects of thermal or chlorine stress on these organisms; the nearest taxonomic species mentioned was *Bimaria franciscana*, a colonial hydroid, which experienced four and a half days of chlorination to 4.5 ppm with no effects (Davis and Middaugh, 1978). Icanberry and Adams (1974) found other soft-bodied invertebrates to be resistant to higher temperatures and longer temperature exposure effects, and Mitchell and North (1971) also found that soft-bodied animals (including medusae) showed little, if any, effect from condenser passage. Those medusae and ctenophores small enough to be entrained thus appear to be remarkably tolerant to the associated stresses.

Nematoda (Fig 7c)

Only 8 nematodes were collected in samples, and these were not identified to species. Little significance should be placed on the entrainment mortality figure of 20%, since this results from the presence of one dead specimen in an outfall sample.

Polychaete larvae (Fig. 7d)

Polychaete larvae were mostly at the nectochaete or a more advanced stage. They were not identified to species, but it is likely that most belonged to the family Nereidae. The increase in mortality due to

entrainment was estimated to be 5.11%.

Icanberry and Adams (1974) and Mitchell and North (1971) include polychaete larvae in the category of soft-bodied invertebrates which appear to be resistant to entrainment-associated stresses. The low mortality of the larvae in Koeberg samples would seem consistent with this, although the larger larvae may be prone to mechanical damage. Williams (1971) found polychaete mortality ranged from 0-50% and averaged 11.9%. Other workers have found higher larval mortality resulting from entrainment. Beck and the Committee on Entrainment (1978) cite an incident where combined mechanical and thermal effects caused 92% mortality for polychaete larvae. Prager *et al.* (1970) report 66.7 to 100% mortality for polychaete larvae where the power plant flow-through time was only 1-2 min. Temperature and chlorine data, however, were not provided for these cases.

Chaetognatha (Fig. 7e)

The chaetognaths collected at Koeberg were *Sagitta friderici*. These members of the holoplankton (permanent plankton) are a good indicator species of cold, inshore water (Lazarus, 1969). 12.24% of the arrow-worms were killed by entrainment in this study.

Larger arrow-worms may be prone to greater mortality from the physical effects of passage through power stations (Sandine, 1973). Results from this study suggest a higher tolerance to heat and chlorine during passage. The only other reference to chaetognaths is by Prager *et al.* (1970) who reported 100% mortality for *Sagitta* sp. after a 1-2 minute entrainment (data based on <100 individuals).

Fig. 7e CHAETOGNATHS

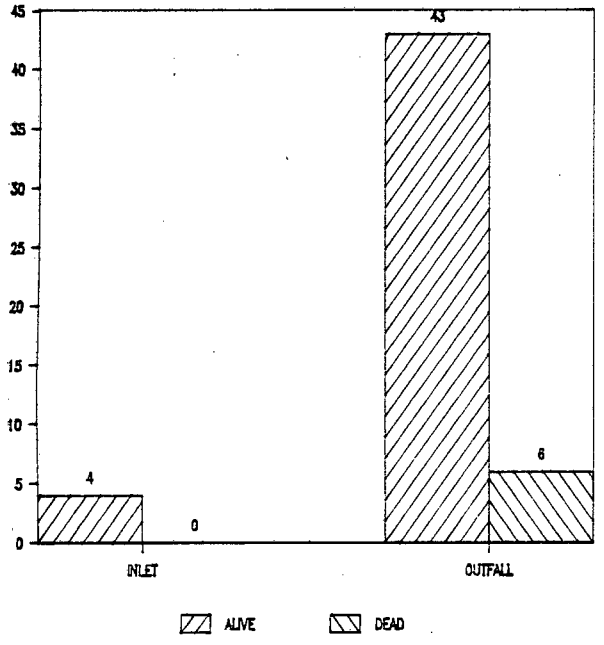


Fig. 7f EVADNE

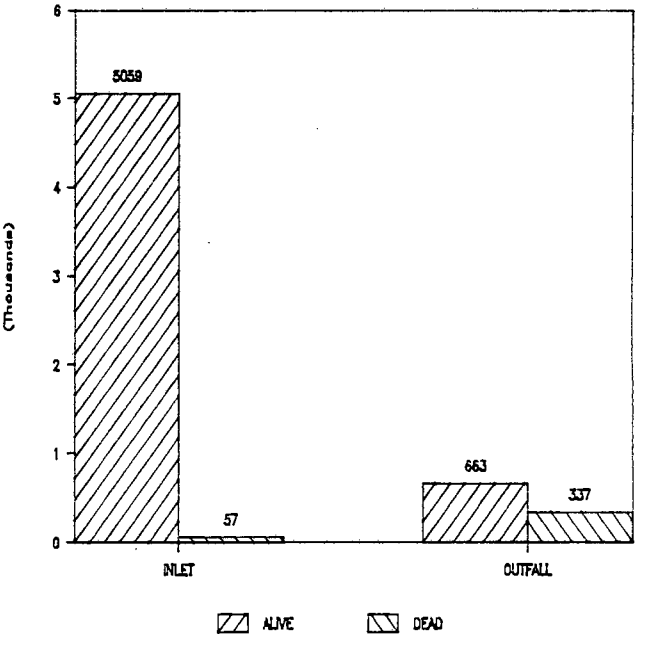


Fig. 7g PODON

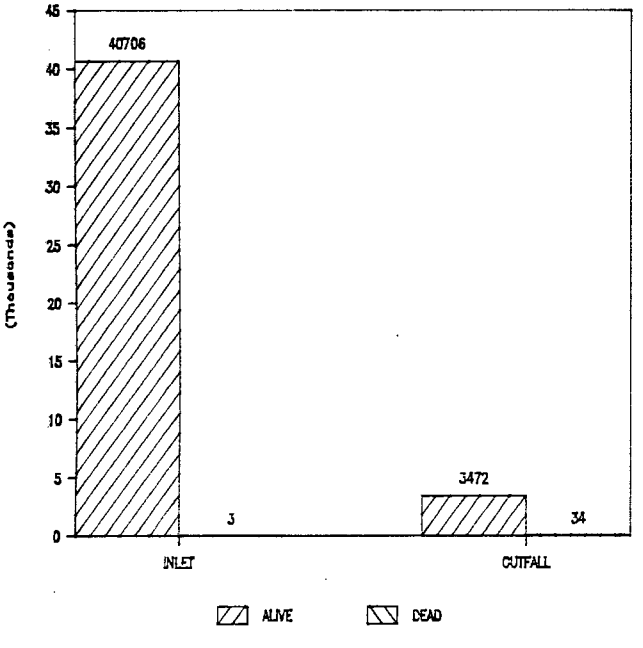
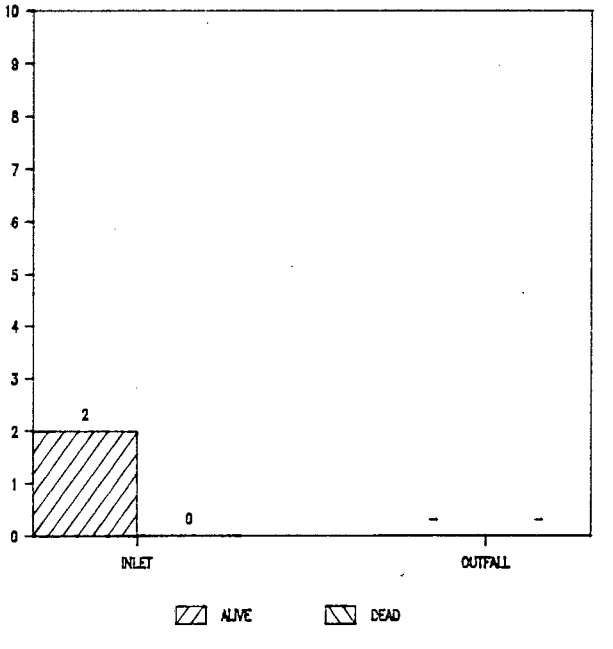


Fig. 7h PENILIA



Figs. 7e-7h: Total numbers of live and dead chaetognaths (7e), Evadne sp. (7f), Podon sp. (7g) and Penilia sp. (7h) collected from inlet and outfall sites over the sampling period.

Cladocera (Figs. 7f, 7g and 7h)

This sub-order of the Crustacea was represented by very large numbers of *Evadne nordmanni* and a substantial quantity of *Podon polyphemoides*. These populations consisted mainly of adult females and females with brood pouches containing ova and embryos in various stages of development. Some *Podon* juveniles were present. A few specimens of *Penilia avirostris* were also found in the samples. These are apparently rarely found off the west coast (Lazarus, 1969), being most common in the warmer Agulhas Bank waters (Shannon and Pillar, 1986).

Evadne displayed an increase in mortality of 32.59% due to entrainment. *Podon* specimens underwent a much lower increase overall of 0.96%, but this was mainly due to a vast swarm collected on 14.01.87 which passed largely unharmed through the cooling system. The majority of these specimens were not in a reproductive state. Without the latter data, the increase in mortality would have averaged 20.65%. This result suggests that new swarms or populations are more resistant to environmental or other stresses, but lose this tolerance as the population ages and disperses. Only two *Penilia* specimens were found in harbour samples, and none were collected from the outfall.

Studies of cladoceran mortality are fairly numerous, although most are for freshwater species, notably *Daphnia* spp. De Nie (1982) investigated entrainment mortality in a shallow, eutrophic lake with ΔT's averaging 4.5 to 5.5°C and maximum temperature not exceeding 30°C. The most abundant species (*Bosmina coregoni*, *B. longirostris*, *Daphnia hyalina* and *Chydorus sphaericus*) had low mortalities of 2-3%, but three less common species experienced 19 to 25% mortality. Goss and Bunting (1976) list upper lethal temperatures ranging from 30 to 50°C for 17 species of

Cladocera, and noted the importance of acclimation temperature; *Daphnia pulex* and *D. magna* succumbed more rapidly upon instant immersion at 35°C as the temperature at which they were acclimated decreased. Cairns *et al.* (1978, cited in Talmage and Coutant, 1979) found that chlorine toxicity to *Daphnia pulex* increased with temperature (tested over the range 5-25°C).

Barnacle larvae (Cirripedia) (Figs. 7i and 7j)

These were predominantly naupliar stages and cypris larvae of *Balanus algicola*, which is the most common barnacle occurring around the Cape (Sandison, 1954). The nauplii experienced an overall increase in mortality of 18.18% due to entrainment, and cyprid mortality rose by 27.35%.

In a study by Williams (1971) *Balanus* sp. larvae experienced 0 to 19.8% mortality from an entrainment of less than 10 minutes. When larvae of *Balanus improvisus* were exposed to a chlorine concentration of 2.5 ppm for 5 mins, an 80% mortality was found three hours after chlorination (Rosenberger, 1972). Nauplii of the barnacle *Elminius modestus* are killed at concentrations of 0.5 ppm (Waugh, 1964), and McLean (1973) found that when barnacle larvae were exposed to 2.5 ppm free chlorine, 75% died in 5 mins. Lack of temperature details for some of these cases makes conclusions difficult, but barnacle mortality at Koeberg (18.18%) seems low compared to the above data. This is most likely due to the lower chlorination levels experienced (±0.2 ppm).

Mysidacea (Fig. 7k)

The mysid species collected at Koeberg was *Mysidopsis major*, which often swarms in large numbers in the spring and summer months (Lazarus, 1969).

Fig. 7i BARNACLE NAUPLII

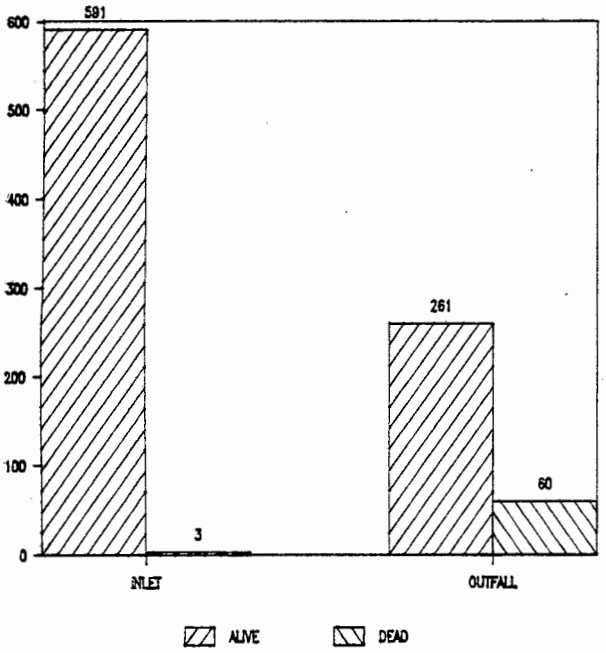


Fig. 7j BARNACLE CYPRIDS

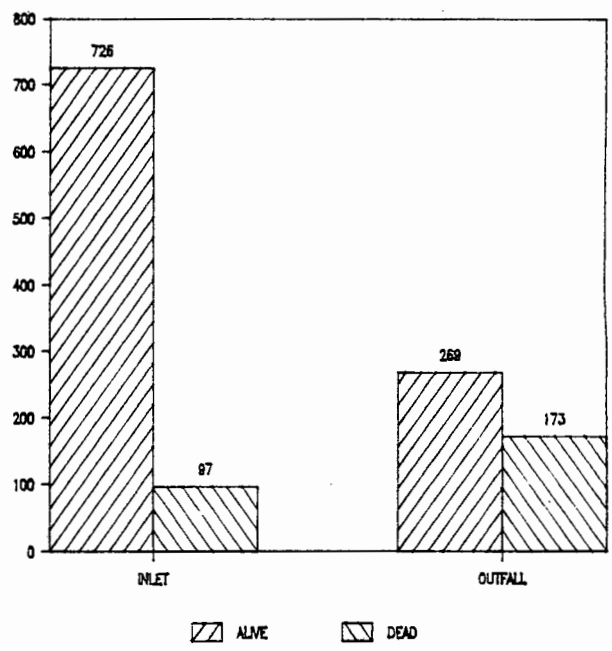


Fig. 7k MYSIDS

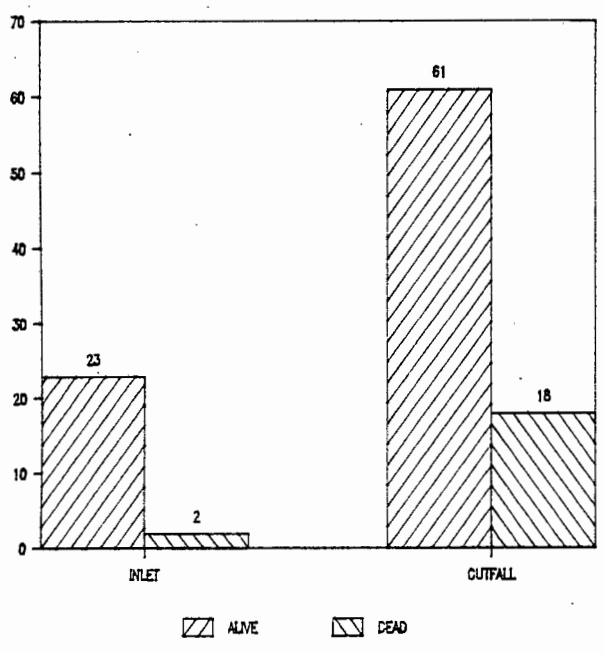
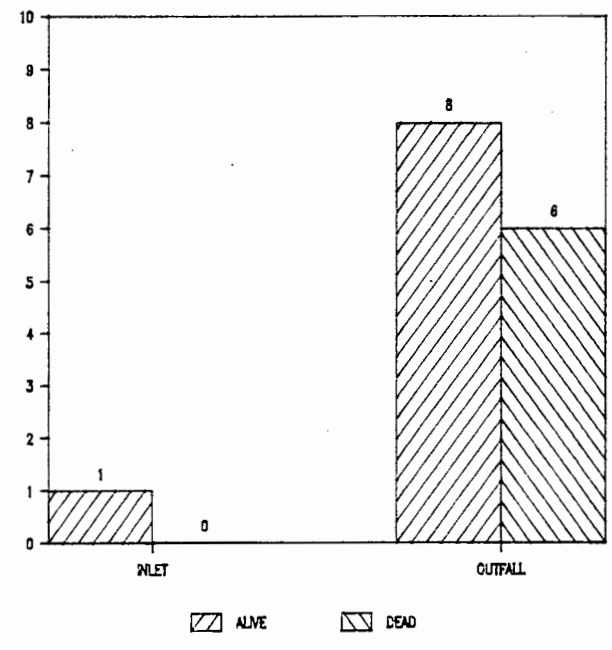


Fig. 7l CUMACEANS



Figs. 7i-7l: Total numbers of live and dead barnacle nauplii (7i), cyprids (7j), mysids (7k) and cumaceans (7l) collected from inlet and outfall sites over the sampling period.

Mostly mature and immature females were collected, and the increase in mortality due to entrainment was estimated to be 14.78%. Many of the larger specimens collected in outfall samples were found broken in two, with severed head and carapace sections nearly or totally detached from the abdomens.

References in Talmage and Coutant (1979) cite the mysids *Mysis oculata* and *Neomysis americana* as having high tolerance to temperature and thermal shock. Hair (1971) noted that thermal tolerance of the adult opossum shrimp *Neomysis awatschensis* to rapid temperature increases decreased with increasing acclimation temperature. ΔT's greater than 14°C caused significant mortalities, even though a summer maximum tolerance temperature (31°C) was not reached. Thatcher (1978) states that a mysid, *Neomysis* sp., had a 96-hour LC_{50} value of 0.162 ppm, which indicates a fairly high chlorine tolerance.

Gentile and Lackie (cited by Beck and the Committee on Entrainment, 1978) found that mysid larvae (1.5 to 5.0 mm) suffered severe physical damage as a result of entrainment at a nuclear plant in Maryland. Mitchell and North (1971) observed mutilated mysids "on numerous occasions" in the field analysis of discharge samples. Similar observations from mysids found in outfall samples collected at Koeberg suggest that physical or mechanical stress experienced during entrainment is the major cause of mortality in this group.

Cumacea (Fig. 71)

Cumaceans are generally benthic in habitat, but coastal species, especially the more active males, frequently swim upwards from the bottom, especially at night, and may be captured close to the surface

(Lazarus, 1969). At certain times of the year, females also make these nightly migrations, probably to find mates.

Fifteen specimens of *Pseudocuma longicornis* were captured in samples at Koeberg (collected during the day), and these experienced an increase in mortality of 42.86%.

No references to cumacean mortality were found, probably because of their predominantly benthic existence.

Isopoda (Fig. 7m)

Isopoda are not truly planktonic forms, being more terrestrial and common in large swarms on sandy beaches. The most common species found at Koeberg was *Eurydice longicornis*, which, along with the white sand mussel *Donax serra*, is the most abundant species of macrofauna on the nearby sandy beaches (Cook, unpubl.). Specimens of *Cyathura carinata*, *Exosphaeroma kraussi* and *Paridotea ungulata* were also collected.

The only relevant reference found was to the isopod *Asellus aquaticus*, occurring in Norway, which has a high temperature tolerance (Salin and Granger, 1978, cited in Talmage and Coutant, 1979). Isopods at Koeberg had a fairly low entrainment mortality of 10.94% (from only 3 dead specimens) and the swimming ability of those that survived entrainment did not appear to have been affected in any way.

Amphipoda (Fig. 7n)

Amphipods were not numerous, but the gammarids *Paramoera capensis* and *Ampelisca palmata* were amongst those present. Several specimens of the hyperiid amphipod, *Parathemisto gaudichaudi*, were also collected.

Fig. 7m ISOPODS

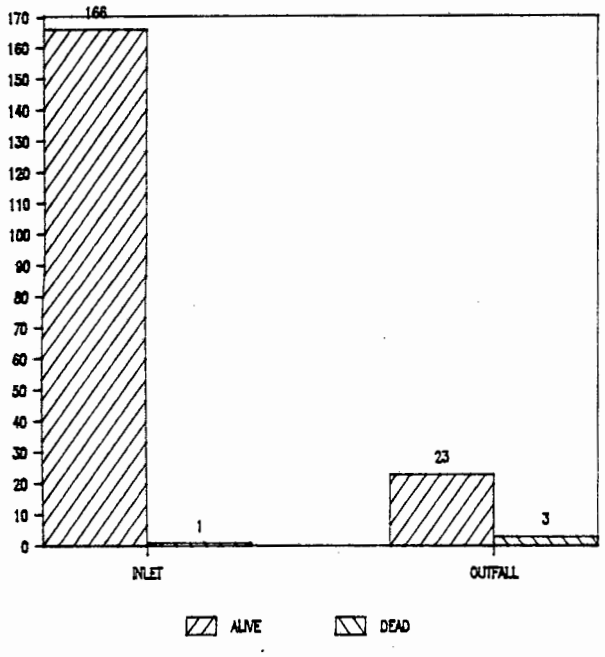


Fig. 7n AMPHIPODS

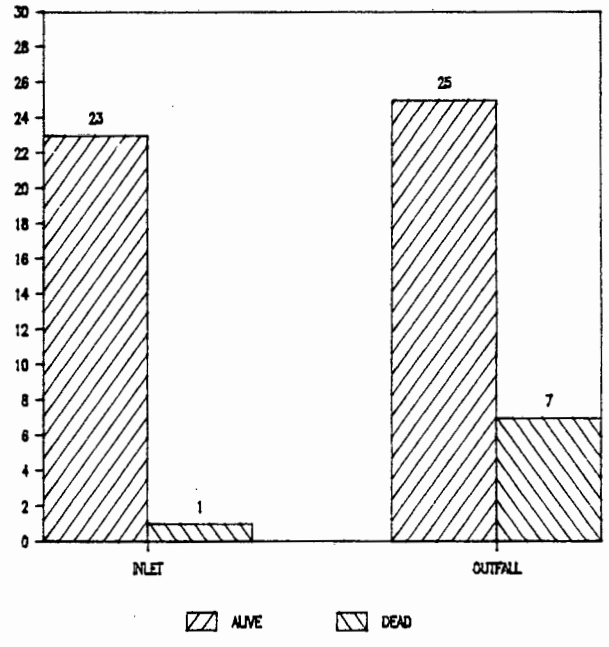


Fig. 7o ZOEAE (TYPE A)

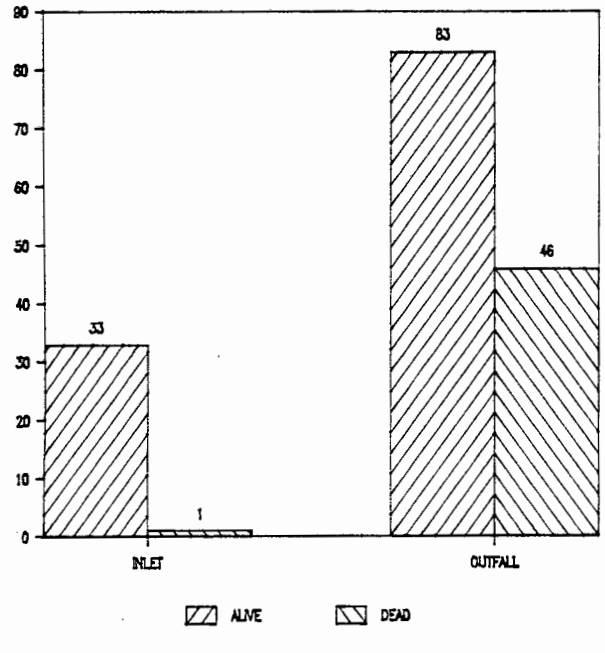
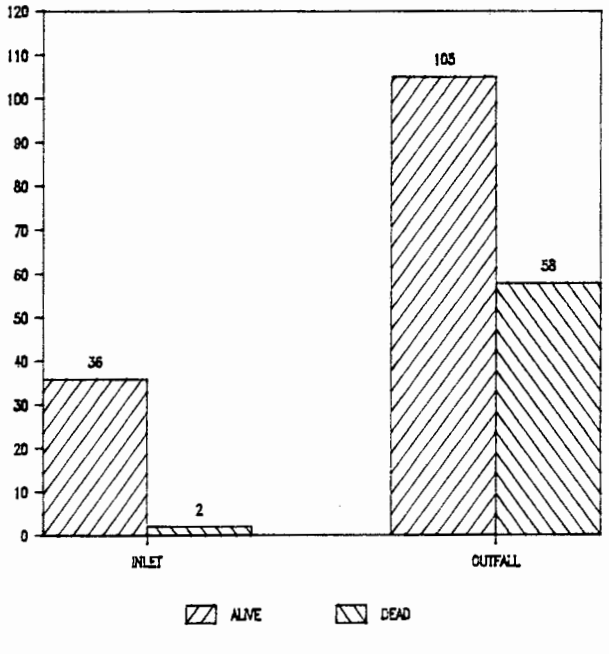


Fig. 7p ZOEAE (TYPE B)



Figs. 7m-7p: Total numbers of live and dead isopods (7m), amphipods (7n), and crab zoeae (7o and 7p) collected from inlet and outfall sites over the sampling period.

Entrainment mortality was measured to be 17.71%.

Talmage and Coutant (1979) noted the following amphipods as having a high temperature tolerance: *Gammarus* spp., *Monoculodes edwardsi*, *Pontogammarus robustroides* and *P. crastus*. Ginn *et al.* (1974) found that *Gammarus* sp. experienced a significant difference in survival after a ΔT = 7.1-8.3°C. Thermal tolerance was found to be dependent on exposure time and ambient temperature. The lower the acclimation temperature, the greater the ΔT they could survive. The authors also observed initial and latent mortalities during chlorination versus entrainment without chlorination. Organisms entrained into the plume (i.e without having passed through the plant) did not show the higher mortalities associated with entrainment.

Ginn and O'Connor (1978) drifted individuals of *Gammarus daiberi* through a discharge plume during chlorination. The amphipods did not display increased immediate or latent mortalities. At ambient temperatures of 26.4-26.6°C, ($\Delta T > 3.3$°C), the amphipods avoided unchlorinated effluent; at lower temperatures of 15.3-15.7°C (ΔT = 7.1°C), there was no avoidance. Chlorinated discharge was avoided at both temperature situations.

When combined with the above data, the mortality of 17.71% due to entrainment at Koeberg indicates that amphipods are fairly resistant to low chlorination and medium temperature increments.

Euphausiacea (Fig. 7q)

Nauplii, calyptopis larvae and a few furcilia larvae and juvenile forms of *Nyctiphanes capensis* were found in the zooplankton samples. No

adults were collected, but the sampling methods utilised would tend to exclude capture of faster-moving organisms such as fish larvae, euphausiids and planktonic shrimps. Entrainment resulted in a low mortality of 3.94% overall.

No effects of entrainment on euphausiids have been found in the literature. The low overall mortality experienced at Koeberg suggests that they are fairly tolerant of heat and chlorine, yet not large enough to sustain physical damage from condenser passage. It is probable, however, that adult euphausiids would experience considerable physical damage due to entrainment.

Decapoda (Figs. 7o and 7p)

Two types of crab zoea larvae were found fairly consistently at Koeberg. Little work has been done locally on the hatching and rearing of known species, so identification was not possible, and the two recognisable zoeal forms were noted as "type a" and "type b". It is likely that they are larvae of *Plagusia chabrus*, *Cyclograpsus punctatus* or *Ovalipes punctatus*, which are abundant in the study area (Beviss-Challinor, 1983; pers. obs. this study). Entrainment mortalities of type a and b zoeae were 32.72% and 30.32% respectively, placing them amongst the more sensitive organisms encountered.

Roberts (1978) assessed the acute and subacute effects of chlorinated seawater on *Panopeus herbstii* and *Pagurus longicarpus* (crab species) eggs and larvae. *P. herbstii* eggs were found to be more tolerant of chlorine-induced oxidants than larvae. Roberts explained that crab eggs have thick impermeable membranes, whereas zoeal stages have a relatively poor calcified exoskeleton which may be more permeable than the egg

Fig. 7q EUPHAUSID LARVAE

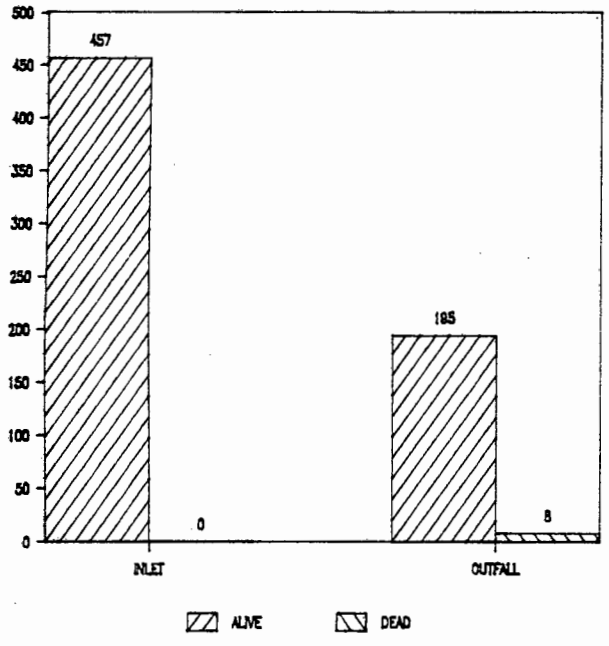


Fig. 7r COPEPODS

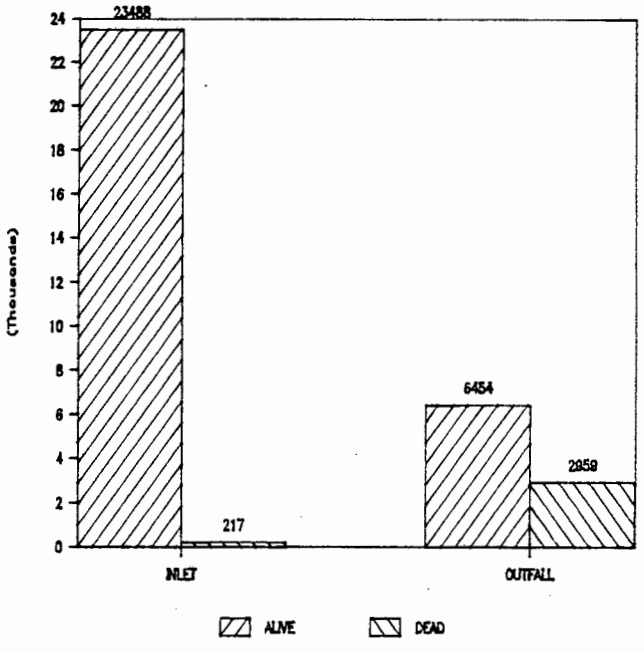


Fig. 7s HARPACTICOID COPEPODS

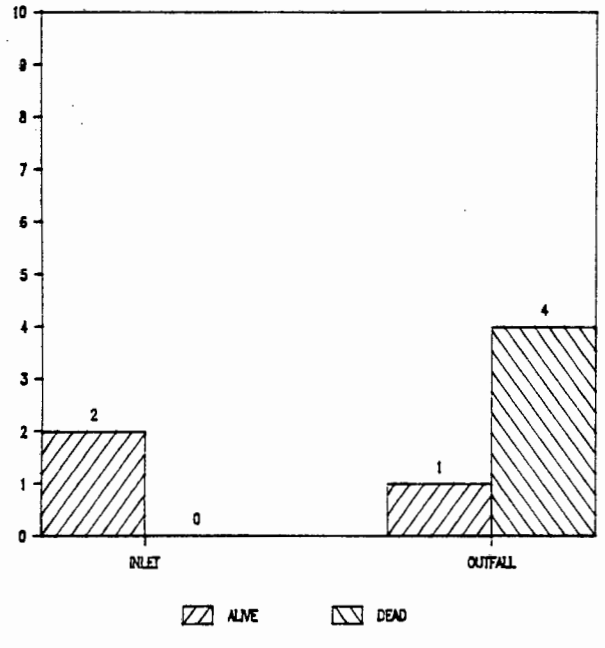
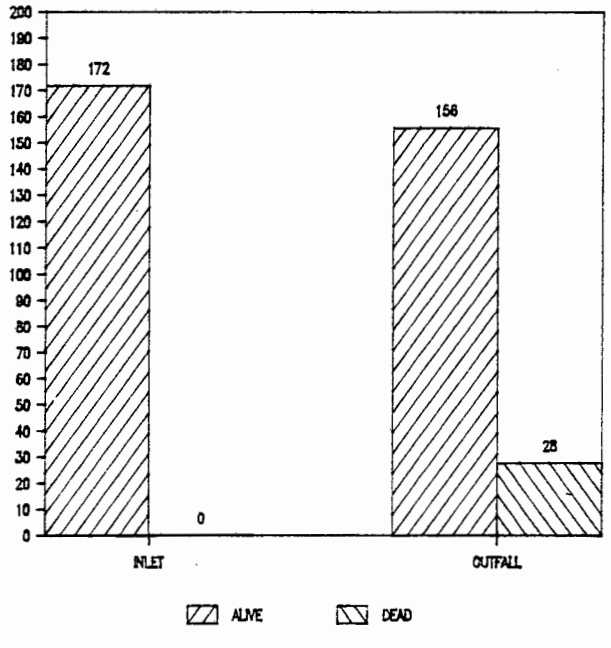


Fig. 7t COPEPOD LARVAE



Figs. 7q-7t: Total numbers of live and dead euphausiid larvae (7q), copepods (7r), harpacticoid copepods (7s) and copepod larvae (7t) collected from inlet and outfall sites over the sampling period.

membrane. Further, during the moulting process the exoskeleton is shed and water rapidly taken in before the new exoskeleton hardens. During this period larvae would be especially sensitive to any toxicant.

The author also found an apparent seasonal change in the acutely toxic dose for *P. herbstii* zoeae, with larvae produced late in the breeding season being more sensitive than those produced during the rest of the breeding season. Survival of larvae hatched from eggs exposed to 0.05 ppm was markedly less than for control larvae. *P. longicarpus* larvae exposed to doses of 0.05 ppm and above exhibited reduced survival relative to the control animals.

A few other decapod larvae were collected but not identified. One of the initial objectives of this study was to investigate the effect of entrainment on larvae of *Jasus lalandii*, the Cape rock lobster, and to assess any implications thereof for the local adult population. However, no phyllosoma were found during the entire study period, although puerulus larvae have been found on mussel mats on the floor of the intake basin. Lazarus (1969), in his survey of inshore zooplankton of the western Cape, found very few *Jasus* larvae. Only 18 phyllosoma and 22 puerulus larvae were captured over the 3 year period of investigation, for which monthly blanket-net samples were collected. He suggested that scarcity of *Jasus* larvae may be due to the fact that the larvae hatch at that time of year when upwelling is on the increase or at a maximum, which may account for the larvae being passively carried further offshore and thus being rare near the coast. Pollock and Goosen (1983, cited in Shannon and Pillar, 1986) confirmed the offshore distribution of the late stage phyllosoma larvae, although relatively few individuals were caught.

Copepoda (Fig. 7r)

Except for one large swarm of the cladoceran *Podon polyphemoides*, copepods were by far the most abundant zooplanktonic organisms found near Koeberg. Calanoid and cyclopoid copepods, which predominated, are listed together as "copepods" in the mortality tables and figures, with their nauplii and copepodites forming the "copepod larvae" columns. Few (seven) harpacticoid copepods were found, and these are listed separately since they are strictly benthic. [Note: it is possible that early stage euphausiid nauplii have been counted with copepod nauplii, since they are difficult to distinguish when small].

Copepods found include the calanoids *Centropages brachiatus* (dominant species), *Calanoides carinatus, Paracalanus parvus, Paracalanus scotti* (previously *P. crassirostris*), *Paracartia africana, Metridia lucens* and *Rhincalanus nasutus*, the cyclopoids *Oithona* spp., *Oncaea* sp. and *Corycaeus* sp., and the harpacticoid *Alteutha depressar*.

Copepod entrainment mortality was 30.52% overall; copepod larvae experienced a lesser mortality of 15.22%, and the seven harpacticoids collected reflected a high (80%) mortality.

Much research has been conducted with respect to entrainment effects on copepod survival. Results seem to vary considerably amongst the different species.

Gaudy (1977) found that *Acartia clausi* populations exhibited comparatively reduced metabolism after passage through heated effluent. He noted that this was similar to the respiratory modifications of warm-

acclimated copepods. Heinle (1969) studied mortality of the copepods *Acartia tonsa* and *Eurytemora affinis*. The upper limits of thermal tolerance for both copepods were found to be near ambient summer temperatures, and *Eurytemora* had a slightly higher thermal tolerance than *Acartia*. In another study, Heinle (1976) found that elevated temperatures and pumping caused little mortality to estuarine copepods, while use of chlorine caused extensive mortalities. *Scottolana canadensis*, *Eurytemora affinis* and *Acartia tonsa* all had different sensitivities.

Carpenter *et al.* (1974b) discovered that 70% of entrained copepods were not returned to the original water body in the effluent. The authors noted that the copepods sank rapidly after passage, with approximately 50% dying within 3.5 days and approximately 70% dying within 5 days, compared to 10% from the intake. This mortality was considered to have resulted from mechanical or hydraulic stresses. Mitchell and North (1971) observed mutilation of larger copepods passing through the San Onofre generating station on numerous occasions.

Laboratory studies conducted by Gentile *et al.* (1976) on *Acartia tonsa* showed that if exposure times are short (less than 5 min) and chlorine concentrations are below 1.0 ppm, mortality will be low.

Latimer et al (1975) conducted laboratory bioassays to determine the toxicity of residual chlorine exposures of 30-min to the copepods *Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi*. The 30-min TL_{50} was 1.54 ppm for *L. macrurus* at both 5 and 10°C. For *C. b. thomasi*, the TL_{50} values for exposures at 10, 15 and 20°C were 14.68, 15.61 and 5.76 ppm respectively. Predicted "safe" concentrations (30-min TL_5)

were 0.9 ppm for *L macrurus* and 0.5 ppm for *C. b. thomasi*. It was apparent for *Cyclops* that a temperature increase from 15 to 20°C markedly increased its sensitivity (Morgan and Carpenter, 1978).

Lanza *et al.* (1975) reported substantial mortalities for *Eurytemora affinis* and *Acartia tonsa* when exposed to a simulated plume with a ΔT of 5.1°C and a condenser residual chlorine of 0.44 ppm. The simple application of temperature did not produce significant mortalities.

Chlorine appears to be the most serious stress for the copepods. The entrainment mortality of 30.52% for species found at Koeberg indicates that they are more sensitive to chlorination than the freshwater or estuarine species overseas (see "safe" concentrations for *L. macrurus* and *C. b. thomasi* above, Latimer *et al.*, 1975).

Harpacticoid copepods (Fig. 7s)

Prager *et al.* (1970) recorded 47.9% mortality for adult harpacticoids entrained for 1-2 mins. In a study by this author in 1971 no mortality was observed, but apparent sinking of killed copepods caused discrepancies in the observed mortalities. An entrainment mortality of 80% was obtained for harpacticoids at Koeberg, but this was for only seven specimens. It does appear, however, that harpacticoid copepods are more sensitive to entrainment than calanoid and cyclopoid copepods.

Copepod larvae (Fig. 7t)

Heinle and Beaven (1977) conducted LC_{50}'s of total residual chlorine for adult and immature copepodids (combined) of *Acartia tonsa*. Preliminary results suggested that the nauplii had lower LC_{50}'s than the adults (i.e. were less resistant). However, results at Koeberg indicate the

opposite, with copepod larvae mortality averaging 15.22% compared to 30.52% for adult copepods. Alden *et al.* (1976) also found that juvenile copepods experienced lower entrainment mortality than the adults.

Acartia tonsa eggs and early nauplii had mortalities ranging from 0 to 93.7%, which Heinle (1969) reported as being within expected natural variation. The same was true for *Canuella canadensis* eggs and early nauplii (0-58.6% mortality). Mortalities for *Oithona brevicornis* eggs and early nauplii were 43.2 to 72.3%, and Prager *et al.* (1970) noted 20 to 77.4% mortality for copepod nauplii after a 1-2 min passage. Larval copepod mortality at Koeberg (15.22%) is thus low in comparison with these figures.

Mollusca (Fig. 7u)

Very few gastropod veliger larvae were encountered. Tiny juvenile gastropods (not identified) were more common. An entrainment mortality of 16.67% was measured for these organisms.

Prager *et al.* (1970) recorded 0-85% mortality for a 1-2 min passage for gastropod larvae. A local mortality of 16.67% is low in this range.

A few small bivalve larvae were found in preliminary samples - probably *Choromytilus meridionalis* or *Donax serra* larvae - but only one was collected in routine sampling.

Bivalve larvae also appear to be sensitive to chlorine but there is some disagreement in the literature. Waugh (1964), for example, states that larvae of the European oyster *Ostrea edulis* are unharmed by as much as 2.5 to 5.0 ppm chlorine for 10 min at 30°C and are capable of growing to

Fig. 7u GASTROPOD LARVAE

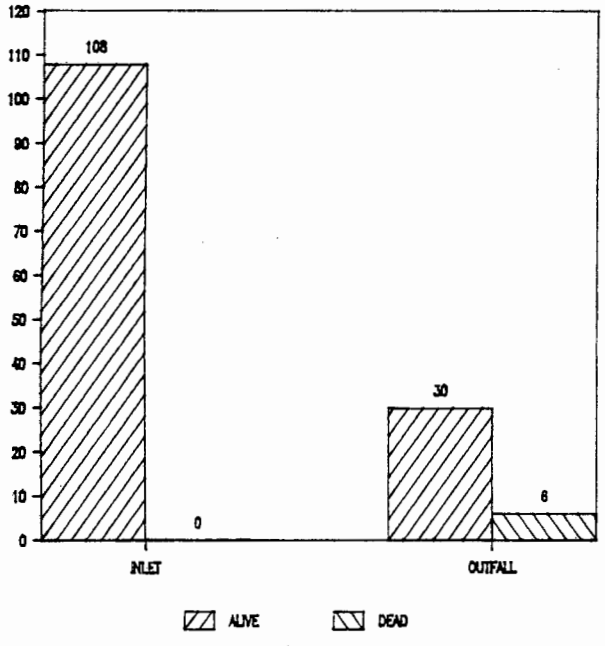


Fig. 7v ECHINODERM LARVAE

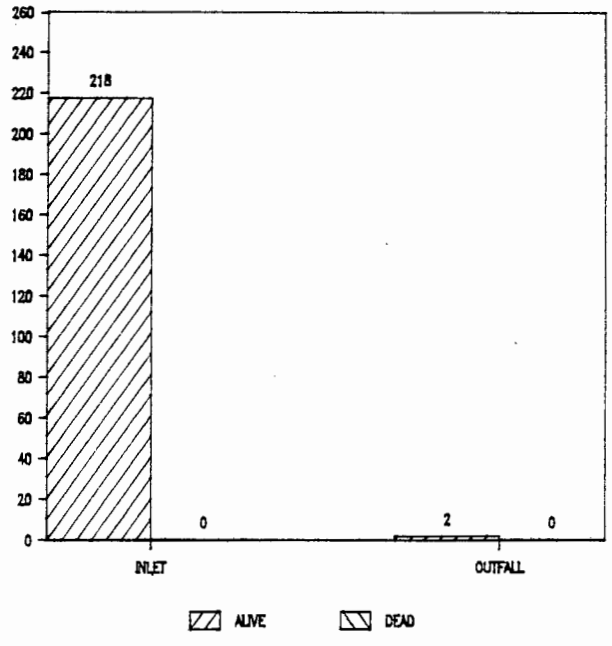


Fig. 7w APPENDICULARIANS

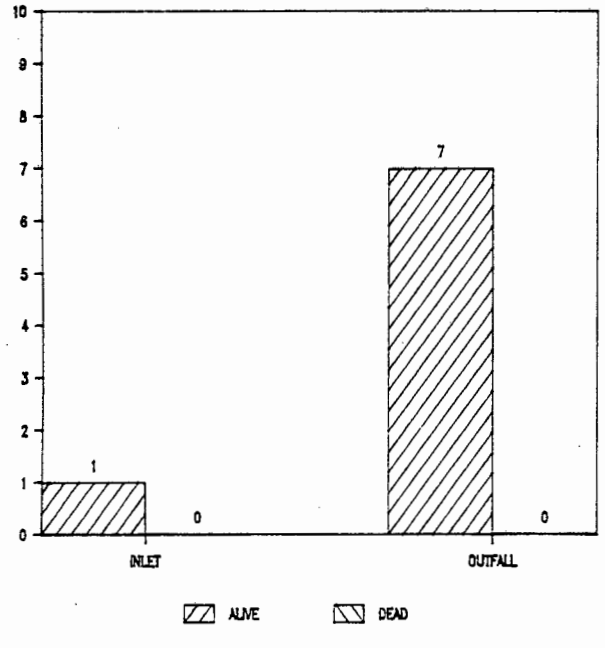
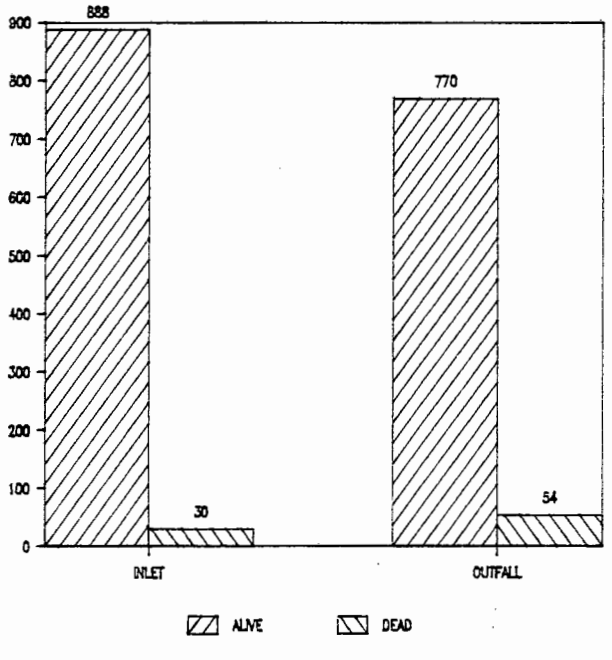


Fig. 7x FISH EGGS



Figs. 7u-7x: Total numbers of live and dead gastropod larvae (7u), echinoderm larvae (7v), appendicularians (7w) and fish eggs (7x) collected from inlet and outfall sites over the sampling period.

the pre-settlement stage. Oyster larvae that had passed through a power station employing chlorination revealed few differences in growth rates or mortality from the control larvae (Coughlan and Whitehouse, 1977).

Roberts *et al.* (1975), however, found the larvae of the American oyster *Crassostrea virginica* to be very sensitive to chlorine. They obtained an LC_{50} concentration of less than 0.005 ppm for residual chlorine in a continuous 48 hr exposure. It is possible that the age of the larvae is a major factor affecting sensitivity to chlorine and that this is the reason for the differing results observed by Waugh (1964) and Roberts *et al.* (1975). Kennedy *et al.* (1974) found that different embryonic and larval stages of the hard clam *Mercenaria mercenaria* had differing thermal sensitivities. They showed clearly that the early cleavage stages were the most sensitive, that thermal tolerance increased in trocophore larvae, and that "straight-hinge" stage larvae of the hard clam were even more tolerant. These differences may also apply to chemical stress.

Straight-hinge bivalve larvae were found at Koeberg, but their absence in routine sampling did not allow the assessment of entrainment mortality.

Two small *Octopus* specimens, which survived entrainment, were also found in preliminary sampling.

Echinodermata (Fig. 7v)

Echinopluteus and ophiopluteus larvae were collected in later samples. No entrainment mortality was observed.

No data has been found on larval entrainment for echinoderms. Mortality due to entrainment at Koeberg is not known; only two specimens were recovered from the outfall, and these had survived entrainment.

Appendicularia (Larvacea) (Fig. 7w)

Species collected were *Oikopleura dioica* and *Fritillaria pellucida*. Numbers were low and no entrainment mortality was observed. Mitchell and North (1971) included larvacea in their category of soft-bodied animals which showed little or no effect from entrainment.

Pisces (Fig. 7x)

Large numbers of clupeid fish ova were collected. These displayed a low entrainment mortality of 3.28%. Only one fish larva was found during preliminary investigations; this had been damaged and was not identified, but was probably damaged by mechanical stresses encountered during entrainment.

Prolarvae and larvae usually experience higher mortality than eggs after condenser entrainment, Hall *et al.* (1981b) and Hall *et al.* (1983). Koo and Johnston (1978), however, noted that egg mortality and hatchability were not necessarily good criteria in assessing temperature effects, for even when there was no significant difference in mortality and hatchability between experimental and control samples, adverse effects were manifested in the deformity of hatched larvae. The severity and incidence of deformity were related to temperature dose, i.e the product of the elevated temperature and exposure time. No immediate heavier mortality was observed in deformed larvae than in normal larvae, but the lack of normal swimming ability of deformed larvae suggest their poor eventual survivability.

Morgan and Prince (1977) also found that abnormal larvae issued from blueback herring eggs (*Alosa aestivalis*) exposed to low chlorine concentrations. Other examples of fish egg and larval sensitivity to entrainment were given in Chapter 2.

General

There seem to be very few field studies recorded in the literature which investigate the effects of entrainment on entire zooplankton populations, whereas laboratory studies on single species are plentiful. Grouping all entrained zooplankton species together, Davies and Jensen (1975) observed about 50% zooplankton mortality for a 0.25 to 0.75 ppm chlorine residual at an estuarine plant in Delaware.

Mitchell and North (1971) found average zooplankton mortality to be 12.7% at the San Onofre generating station, California for a temperature increment of 10 to 11°C. Estimates of individual species mortality in each sample indicated that the larger microcrustacea were affected the most. The copepods *Acartia tonsa, Euterpe acutifrons, Corycaeus affinis* and *Oithona helgolandica*, and the Mysidacea represented nearly all the mortalities. Soft-bodied animals (polychaete larvae, *Sagitta*, larvacea, medusae etc.) and protozoa showed little, if any, effect from their passage. Rank analysis of abundance indicated that zooplankton structure in both intake and discharge water was similar. Affected species, however, decreased in rank abundance in the discharge and were replaced by less affected species.

Thermal tolerance studies and chlorine bioassays have been conducted for a wide range of species, e.g. Arthur and Eaton (1971), Capuzzo (1977 and

1979), Ginn and O'Connor (1978), Ginn *et al.* (1974), Heinle (1976), Latimer *et al.* (1975), Roberts (1978), Roberts *et al.* (1975), Scott and Middaugh (1978), Thatcher (1978). In the U.S.A. particular emphasis has been placed on ichthyoplankton, e.g. Buckley (1977), Capuzzo *et al.* (1977), Heath (1977), Koo and Johnston (1978), Mattice *et al.* (1981), Middaugh *et al.* (1977a and b), Morgan and Prince (1977) and Roberts *et al.* (1975) amongst others.

As was stated earlier, many of these are of little value in assessing entrainment effects, as they are generally used to calculate TL_{50}'s, LC_{50}'s or similar values which involve the application of stresses for long periods of time (e.g. 2- to 96-hr experiments). This is often unrealistic with respect to operating power stations, where flow-through times usually range from 2 to 30 minutes. Nevertheless, they may be helpful in gauging the general sensitivity of an organism to temperature and/or chlorine induced stress.

Useful reviews which tabulate the results of relevant studies are provided by Beck and the Committee on Entrainment (1978) - entrainment effects on zooplankton and ichthyoplankton, Davis and Middaugh (1978) - toxic effects of chlorinated wastes and water on marine invertebrates, Goss and Bunting (1976) - upper temperature tolerance of zooplankton, Mattice and Zittel (1976) - chlorine toxicity to marine organisms, and Talmage and Coutant (1979) - thermal effects.

Vital staining of entrained organisms has been conducted by de Nie (1982) and Heinle (1976).

Whitehouse (1975) concluded that all groups of organisms were

susceptible to some combination of chlorine concentration, exposure time and water temperature. The loss of these animals may be serious, because zooplankton constitute a vital link in the trophic food web of composite ecosystems (Davies and Jensen, 1975).

Davies and Jensen (1975) also stressed the importance of the amplitude of thermal rise above seasonal ambient temperatures to which zooplankton populations have been seasonally acclimatised. Large elevations in winter months seem to be more damaging than smaller elevations during warmer periods that result in similar discharge canal water temperatures.

The synergistic role of temperature increase and chlorine toxicity is complex (Goldman *et al.*, 1978). At lower temperatures the relative toxicity of chlorine and chloramine is least pronounced, but it increases with increasing temperature. It appears that as long as an organism is not subjected to a temperature shock great enough so that the upper thermal tolerance limit is reached, the effect is hardly measurable. When the thermal limit is approached the synergism becomes readily apparent.

It seems that the degree of halogen toxicity is related to the size of the organisms, the smallest organisms being the most susceptible, and their responses requiring a shorter exposure time for any given temperature and dose rate. This suggests a surface/volume effect (Goldman *et al.*, 1978; Whitehouse, 1975), but may simply reflect a response related to a relative increase in metabolic rate with decreasing size (Goldman *et al.*, 1978).

Size-related mortality effects in zooplankton have also been noted by Alden *et al.* (1976), Beck and Lackie (1974) and Gentile and Lackie (both cited in Marcy *et al.*, 1978), Industrial Bio-Test Laboratories (1971 and 1972), Mihurskey and Dorsey (1973), Sandine (1973).

Sensitivity depending on developmental stage has been noted by Alden *et al.* (1976), Kennedy *et al.* (1974) and Roberts *et al.* (1978).

In some instances, entrained organisms may not be killed by passage but may incur sublethal effects. Examples of such effects are decreased productivity, decreased growth, decreased sperm motility, decreased fertilization success, decreased activity, behavioural signs of distress or irritation, and decreased reproduction. Although the organism's immediate survival is not affected, the sublethal effects may lower its reproductive and feeding "success", and affect the whole population's chances of survival.

From an overall perspective, however, zooplankton entrainment may not be too serious for the population as a whole. Factors such as widescale oceanic mixing, limited area affected, fast regeneration times and gradual post-entrainment recovery all help to minimise local environmental impact. With reference to permanent plankton (both phytoplankton and zooplankton - organisms with relatively rapid generation periods), Goldman and Quinby (1979) noted that unless complete destruction is accomplished during entrainment, the surviving organisms are capable of renewed growth once returned to the receiving water.

Ginn (1977), found that plant operation had no adverse impact on local

gammarid amphipod populations and that the impact on Atlantic coast populations of *N. americana* was minimal because of the exposure of a limited portion of the coastal population to the plant.

Gore *et al.* (1977) estimated mortality of the local surface zooplankton as a result of entrainment at the Millstone Nuclear Power Plant (Connecticut) to be between 0.08 and 0.27%, based on surface flow from Long Island Sound, the flow through the Millstone stations, zooplankton densities, and a 70% entrainment mortality estimate. Because zooplankton reproduce rapidly, such mortality rates were thought to be easily tolerated by the population.

At the Marsden "A" Power Plant in New Zealand, the observed damage to entrained marine plankton, which occurred in the absence of chlorine and lethal temperatures, was attributed to mechanical stress (Bradford and Burns, 1977). Because of continual replenishment of plankton by water flow and the probability that the damaged plankton constituted useful food detritus, the authors suggested that losses were probably not important when the whole area was considered.

Evans *et al.* (1978) showed that damage to zooplankton populations at the D.C. Cook Power Station was minimized by, amongst other factors, locating the plant on a large, well-mixed body of water (Lake Michigan).

These conclusions, combined with the relatively low zooplankton mortalities found in this study, have fairly positive implications for Koeberg Nuclear Power Station. Rapid mixing and dispersion of the plume at Koeberg, and the limited extent of warmed water, do not suggest a serious impact on local zooplankton populations. The survival of a

large percentage of entrained zooplankton (allowing for latent mortality) means that a substantial recovery of population standing stocks is likely. It should be remembered, however, that a huge volume of seawater - over seven million cubic metres - is entrained on a daily basis. The intake basin has been designed to minimise recirculation of effluent water, but if organisms were entrained more than once within a short time period, much higher mortalities could be anticipated. Some reduction in local zooplankton biomass is therefore inevitable.

From the available data and discussions in the literature, it appears that chlorination is the most important factor determining the ultimate survival or mortality of the majority of entrained zooplankton. Temperature and physical stress play lesser roles, although both are of obvious significance. Thermal stress becomes important when ambient temperatures are high, and the upper thermal tolerance limit of an organism is approached. Physical stress is most significant for the larger species of zooplankton, and is most damaging to ichthyoplankton, in particular to the larval types. Beck and the Committee on Entrainment (1978) concluded that, when the stress is identified, the impact of physical stresses on mortality of ichthyoplankton outweighs thermal and chemical impacts and that the relative importance of chlorine stress may be greater for zooplankton than for larval and juvenile fish.

5. LABORATORY EXPERIMENTS

5.1 Introduction

Field sampling and subsequent comparisons of intake and outfall mortalities are adequate for assessing initial plankton mortality due to entrainment (sometimes referred to as *immediate* mortality), but give no indication of any latent mortality that may occur. A laboratory experiment was therefore designed to investigate delayed mortality following heat and chlorine treatment.

Entrained organisms are exposed to a sudden and relatively short-term exposure to the combined effects of chlorine, increased temperature and mechanical damage; thus any meaningful bioassay must simulate these stresses to some degree (Goldman *et al.*, 1978). The bioassays of these workers incorporated short-term toxicant and/or heat rise, rapid elimination of these stress conditions, and subsequent long term observation of the test organisms, so as to simulate, as far as possible, the entire history of test species as they might pass through a plant entrainment and enter receiving water. These criteria wre kept in mind when designing the present study. As with the experiments of Goldman *et al.* (1978) no attempt was made to simulate mechanical damage during entrainment, although the major importance of this effect was recognised.

A copepod species was used for this investigation, as copepods dominated the zooplankton samples collected earlier in the study, and are important as prey items for many larger organisms in marine food webs. With few exceptions copepods are the dominant constituent of the

plankton in every sea-area, usually comprising at least 70% of the plankton fauna (Raymont, 1984). The copepod selected, *Paracartia africana*, is a local coastal calanoid species and was common in field samples collected at Koeberg. It is a neritic form with its occurrence possibly restricted to the cool waters of the west coast of Southern Africa (Unterüberbacher, 1964). Individuals used in experiments were obtained from the Sea Fisheries Research Institute at Sea Point, Cape Town.

5.2 Methods

Four 15 litre glass aquaria were set up in a constant temperature room as follows: Tank 1 was used as a control, and was maintained at ambient temperature without chlorination; Tank 2 was heated 10°C above ambient, and not chlorinated; Tank 3 was heated 10°C above ambient and chlorinated to 0.2 ppm; Tank 4 was maintained at ambient temperature and chlorinated to 0.2 ppm (see Plate 1). Tank 3 most closely approximated the conditions prevailing in the condenser pipes at Koeberg, except for mechanical stresses, which could not be simulated in the experiment.

Two drip bags were filled with a concentrated calcium hypochlorite stock solution and used to drip chlorine into Tanks 3 and 4 at a sufficient rate to maintain concentrations of 0.2 ppm, a faster rate being necessary for Tank 3 with its higher temperature. A PVC pipe perforated along its length, stoppered with a rubber bung at one end and connected to an air-lift pump at the other, was placed in each tank to cause water circulation. 0.2 µm filtered sea water was used in the tanks, and Tanks 2 and 3 were heated by standard combination heater/thermostat devices.

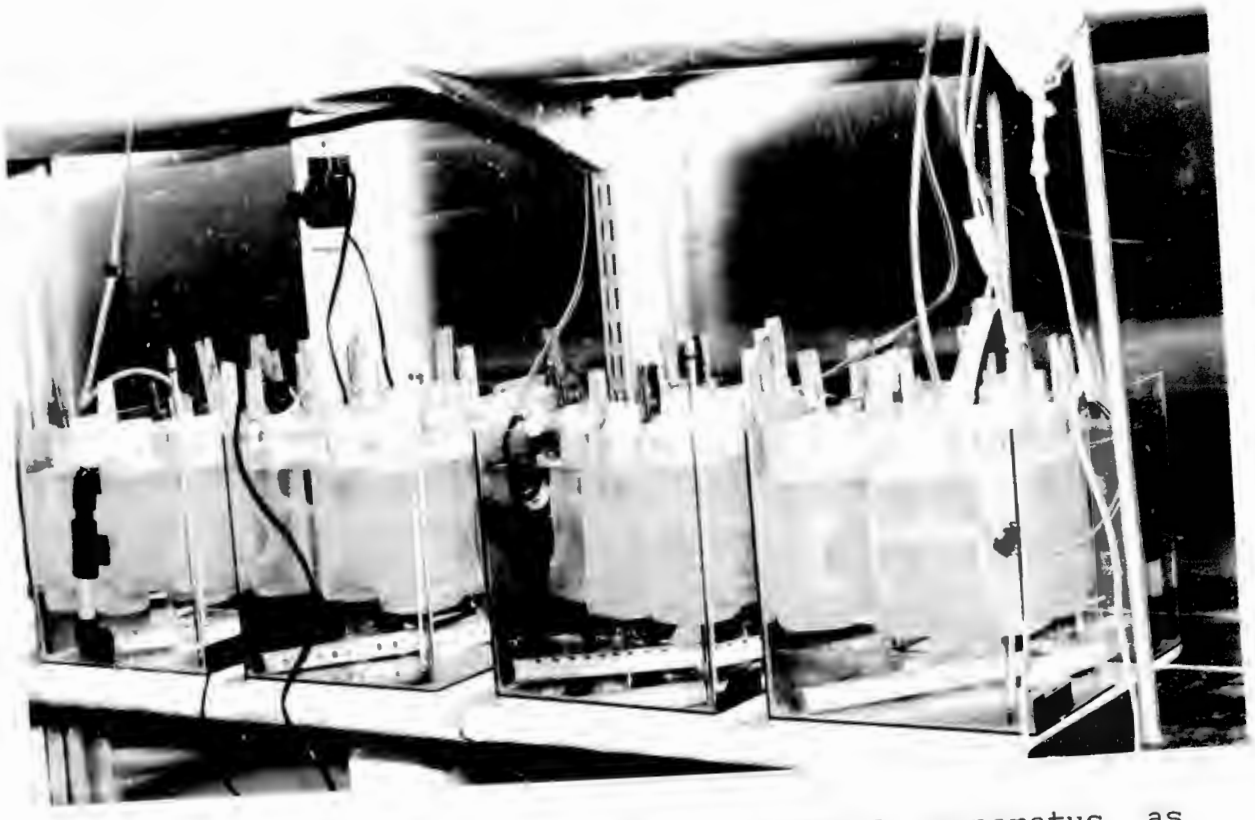


Plate 1: Photograph showing experimental apparatus as assembled in the constant temperature cell. Tanks 1-4 are numbered from left to right.

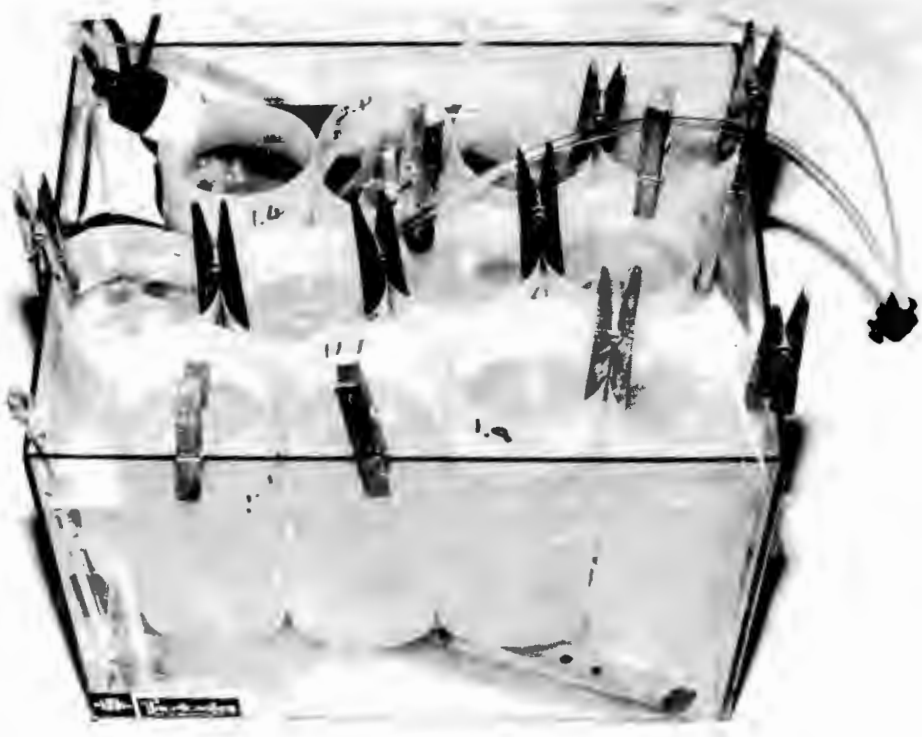


Plate 2: A close-up view of one of the tanks used in the experiment, showing the containers used to hold the copepods.

The experiment was conducted at two different ambient temperatures - 12°C, which represents the average sea temperature at Koeberg (based on temperatures recorded in the harbour during sampling), and 19°C, which is the highest sea temperature recorded at Koeberg. The temperature increment from the cooling process at Koeberg usually ranges from 8 - 10°C, so a 10° rise above a 19°C ambient sea temperature (i.e. 29°C), combined with chlorination, is likely to provide the most extreme conditions to which entrained organisms at Koeberg would be subjected.

Experimental chambers to hold copepods were made from plastic containers approximately 160 mm in length and 60 mm in diameter, with four 40 mm diameter "windows" covered by 300 μm nylon mesh (see Plate 2). Thirteen such containers were placed into each experimental tank and 20 or more copepods were added to each. The containers were maintained in upright positions in the tanks.

The maximum retention time for entrained organisms at Koeberg, from the intake to the end of the discharge canal, is approximately thirty minutes. Thus, thirty minutes after initiating the experiment, the heating and chlorination of Tanks 2, 3 and 4 was terminated, and the water in these tanks was flushed and replaced with fresh filtered sea water. The exchange process for each tank took 5 - 10 minutes, which is similar to the time taken for most of the excess heat to disperse upon mixing of the effluent and the receiving water at the end of the discharge canal. There is some mixing with the sea in the canal prior to the water reaching the sea, but measurements taken in the canal show that there is little dissipation of heat at this stage. Residence time in the canal varies depending on the tides and wave action. High tides

tend to bring sea water quite far into the canal where it mixes with the effluent water. At low tide there is a steadier seaward movement of discharged water and more of a delay in the mixing process.

Once the experiment was initiated, one container per tank was removed at the following time intervals: 0h, 0.25h, 0.5h, 1h, 2h, 4h, 8h, 12h, 16h, 24h, 36h, 48h and 60h. Upon removal, each container was rinsed with fresh sea water from a wash bottle to detach any copepods from the mesh windows. Nine ml of a 0.1% w/v stock solution of neutral red stain was added to the remaining water at the bottom of the containers holding the copepods, and the contents were left to stain for at least one hour. The copepods were then preserved in 40% formalin, rinsed with fresh water, concentrated in a 70 µm sieve and placed in 50 ml fresh water. Two ml of a 1N HAc-NaAc (acetic acid - sodium acetate) solution and a further 5 ml of formalin was added to the samples, which were then refrigerated until analysis. Microscopic examination determined the sex of each copepod, and whether it was alive at time of staining. Only adult sexually mature copepods, which dominated the samples in a fairly even sex ratio, were counted. The copepods were not fed during the experiment.

5.3 Results

The percentage of dead copepods in each of the containers was divided by the corresponding time-period to obtain hourly mortality rates. These were then plotted cumulatively to give an index of the mortality rate in each tank. The resulting curves for the experiments conducted at ambient temperatures of 12 and 19°C are shown in Figures 8 and 9 respectively. The original data are tabulated in Appendix II.

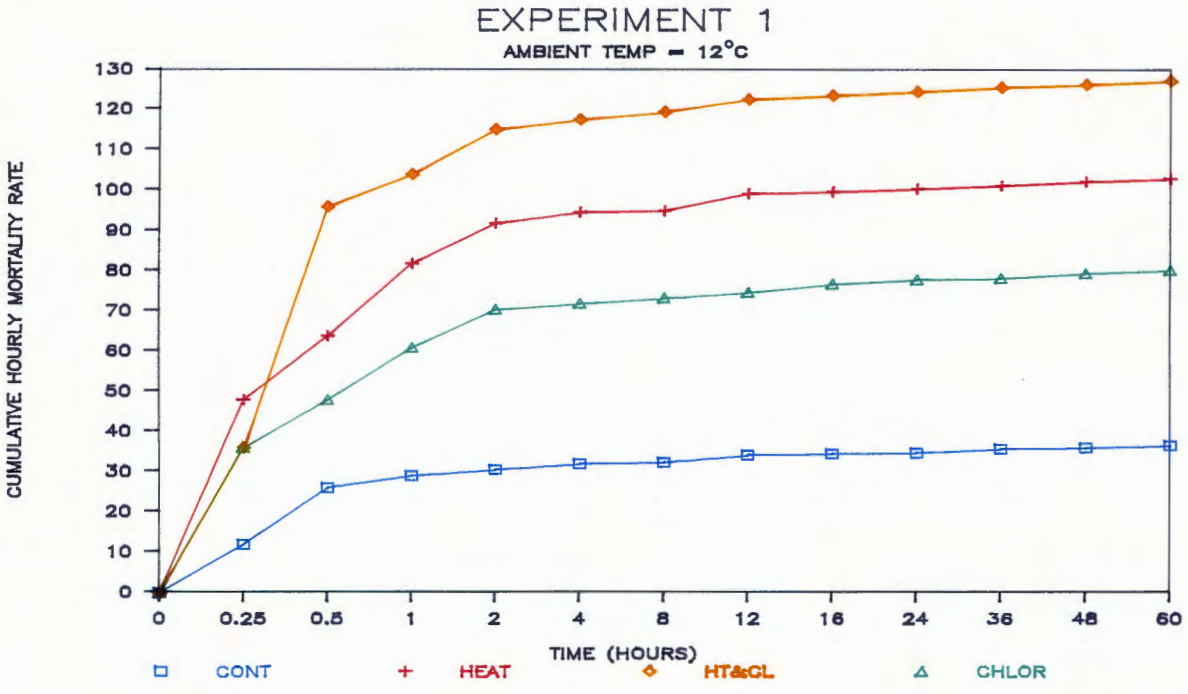


Fig. 8: Cumulative hourly mortality rate of *Paracartia africana* after a 30-min exposure to thermal and/or chlorine treatment at an ambient temperature of 12°C. CONT = control tank, HEAT = heated tank, HT&CL = heated and chlorinated tank and CHLOR = chlorinated tank.

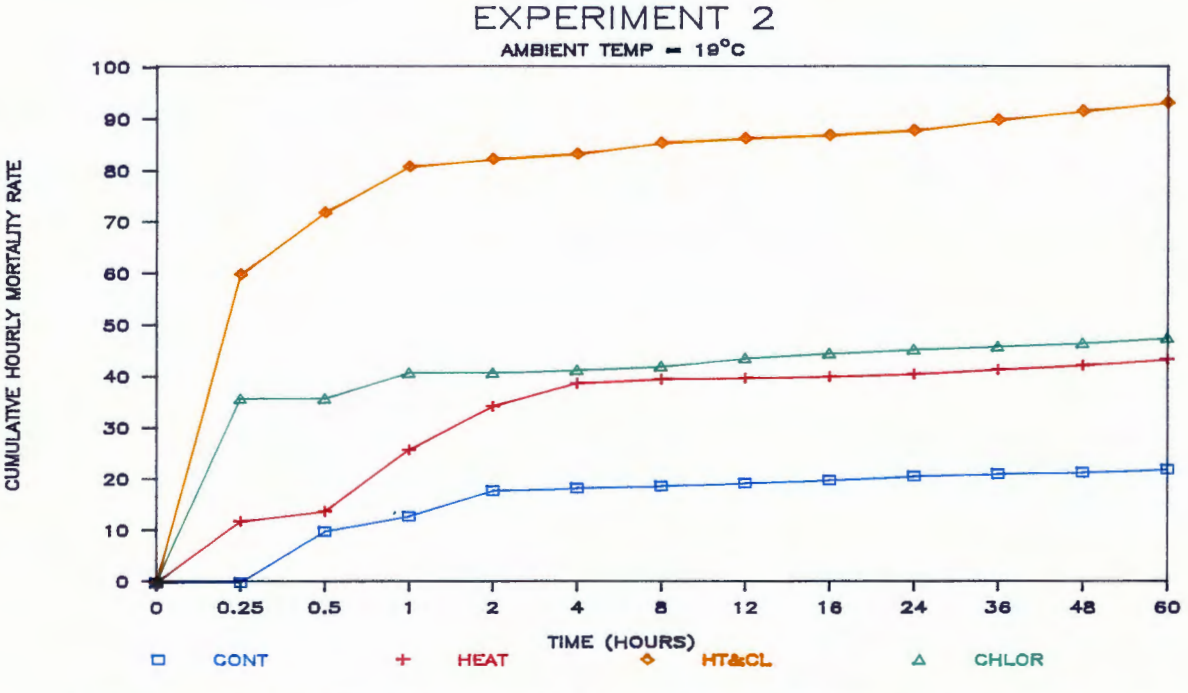


Fig. 9: Cumulative hourly mortality rate of *Paracartia africana* after a 30-min exposure to thermal and/or chlorine treatment at an ambient temperature of 19°C. CONT = control tank, HEAT = heated tank, HT&CL = heated and chlorinated tank and CHLOR = chlorinated tank.

The curves indicate high initial rates of mortality upon application of stress compared to the control tanks for both experiments, with the highest mortality rates occurring for copepods exposed to both heat and chlorine. Approximately two hours after removal of the stresses the mortality rates declined, and were similar in all the tanks, although the latent mortality rate up to the end of the 60-hour period was still slightly higher in the experimental tanks compared to the control tanks. It can also be seen from the graphs that most of the mortality (i.e. the highest mortality rates) occurred during the 30-minute treatment period. The Kolmogorov Smirnov goodness-of-fit test for continuous data was used to compare the four curves from each experiment (Zar,1984). All curves were shown to be significantly different from the control curve when tested at the 0.1% level of significance, except for the mortality curve due to heat stress only (Tank 2) at the higher ambient temperature (19°C), which was significantly different only at the 10% level.

Table 5 shows the overall 60-hour copepod mortality and stress mortality (overall mortality minus control mortality) in each tank for Experiments 1 and 2. In each experiment the mortality due to heat and chlorine combined was greater than that due to either heat or chlorine alone. In the experiment conducted at 12°C the mortality due to heat and chlorine combined was greater than the sum of the mortalities due to heat only and chlorine only (16.5% compared to 15%), possibly indicating a synergistic effect between heat and chlorine. However, this was not the case for the higher ambient temperature (19°C), where mortalities were 7.8% compared to 8.2%. Although overall mortalities in the control tanks for the two experiments were similar, exposure to stress at 19°C resulted in lower mortalities than at 12°C, contrary to expectation.

TABLE 5: Overall copepod mortality and stress mortality (individual tank mortality minus control mortality) in each tank during Experiments 1 and 2.

TANK	EXPERIMENT 1: 12°C		EXPERIMENT 2: 19°C	
	OVERALL MORTALITY	STRESS MORTALITY	OVERALL MORTALITY	STRESS MORTALITY
Control	9.5%	-	9.6%	-
Heat	15.4%	5.9%	12.7%	3.1%
Heat & Chlorine	26.0%	16.5%	17.4%	7.8%
Chlorine	18.6%	9.1%	14.7%	5.1%

TABLE 6: Results of X^2-tests used to compare mortality in each tank for both experiments. Results are presented as X^2-values for each comparison and corresponding level of significance (P). Cont = Control, Ht & Cl = Heat and Chlorine, Chlor = Chlorine

TANKS COMPARED	EXPERIMENT 1: 12°C		EXPERIMENT 2: 19°C	
	X^2	P	X^2	P
Control vs. Heat	15.25	$P < 0.001$	8.96	$P < 0.005$
Cont. vs. Ht & Cl	155.33	$P < 0.001$	48.65	$P < 0.001$
Cont vs. Chlorine	44.77	$P < 0.001$	12.16	$P < 0.001$
Heat vs. Ht & Cl	42.43	$P < 0.001$	13.30	$P < 0.001$
Chlor vs. Ht & Cl	17.68	$P < 0.001$	3.90	$P < 0.05$
Heat vs. Chlorine	3.70	$P < 0.10$*	1.38	$P < 0.25$*

* = not significant ($P > 0.05$)

Chi-squared (X^2) tests (Zar, 1984) were used to compare the overall mortalities in Tanks 2-4 against control mortality. For both experiments, there was a significantly higher mortality ($P < 0.05$) in each tank compared to the control (see Table 6). Results of X^2-tests also indicated significant differences in mortality ($P < 0.05$) for heating only vs. heat and chlorine, and for chlorine only vs. heat and chlorine in both experiments. Mortality due to chlorine was slightly higher than heat related mortality at both ambient temperatures, but this difference was not significant.

A combination of heat and chlorine was thus most detrimental to the study animals, followed by chlorine only, and then heat only, with possible evidence for synergism between heat and chlorine at the lower temperature only.

The curves in Figures 8 and 9 were used to calculate the percentage of total mortality after 30 mins (i.e. the end of the simulated entrainment period) from the cumulative hourly mortality rates. These results are shown in Table 7. Except for the control and heated tanks in Experiment 2, over 50% of the total mortality occurred during the 30-minute period. 75.50% of the total copepod mortality in the heated and chlorinated tank in Experiment 1 had occurred by the end of the 30-minute stress period with a similar figure, 77.16%, for the same tank in Experiment 2. Assuming that the heated and chlorinated tank at the lower ambient temperature (Tank 3 in Experiment 1) is representative of average entrainment conditions at Koeberg, and that the effect of the associated stresses on *Paracartia africana* is similar to the effect of heat and chlorine on most entrained copepods, then an average immediate mortality

TABLE 7: Mortality in each tank after 30 minutes as a percentage of total (60-hour) mortality.

TANK	30-MINUTE MORTALITY (% OF TOTAL MORTALITY)	
	EXPERIMENT 1: 12°C	EXPERIMENT 2: 19°C
Control	71.08%	45.19%
Heat	62.09%	32.13%
Heat & Chlorine	75.50%	77.16%
Chlorine	59.72%	75.39%

TABLE 8: Results of X^2-tests used to compare male and female copepod mortality in each tank for experiments 1 and 2. Results are presented as X^2-values with the corresponding level of significance (P).

TANK	EXPERIMENT 1: 12°C		EXPERIMENT 2: 19°C	
	X^2	P	X^2	P
Control	0.68	$P < 0.5$*	4.62	$P < 0.05$
Heat	12.04	$P < 0.001$	0.03	$P < 0.9$*
Heat & Chlorine	22.46	$P < 0.001$	8.70	$P < 0.005$
Chlorine	6.43	$P < 0.025$	4.88	$P < 0.05$

* = not significant ($P > 0.05$)

of 30.52% of all copepods passing through Koeberg (from Table 4, Chapter 4) would result in an overall mortality (immediate plus latent deaths) of approximately 40% for these organisms. This conclusion also assumes that the latent copepod mortality rate due to physical effects, which was not simulated in the experiment, is the same as the latent mortality rates resulting from heating and chlorination.

The *Paracartia* specimens used in the experiments displayed a sexual dimorphism in response to the treatments, with males experiencing a higher mortality than females in each of the tanks (see Figs. 10 and 11). X^2-tests (Table 8) showed significant differences ($P < 0.05$) in six of the tanks, with slightly less significance ($P < 0.5$) for the control tank in Experiment 1, and no significant difference ($P < 0.9$) for Tank 2 (heat only) in Experiment 2. Male and female mortalities averaged 21.25 and 12.43% respectively for Experiment 1 (12°C) and 15.5 and 11.35% respectively for Experiment 2 (19°C).

5.4 Discussion

Exposure of *Paracartia africana* to heat and chlorine stresses similar to those experienced during entrainment resulted in latent mortality up to 60 hours after application of the stresses. Most of the mortality, however, occurred during the 30-minute entrainment simulation period and the following two to four hours, as indicated by the very high mortality rates during this interval. After 8 to 12 hours the mortality rates were not very different from those of the controls. As was anticipated, the combination of heating and chlorination was most stressful to the copepods, and although some degree of synergism between these two factors was indicated, the evidence was not sufficient to prove this.

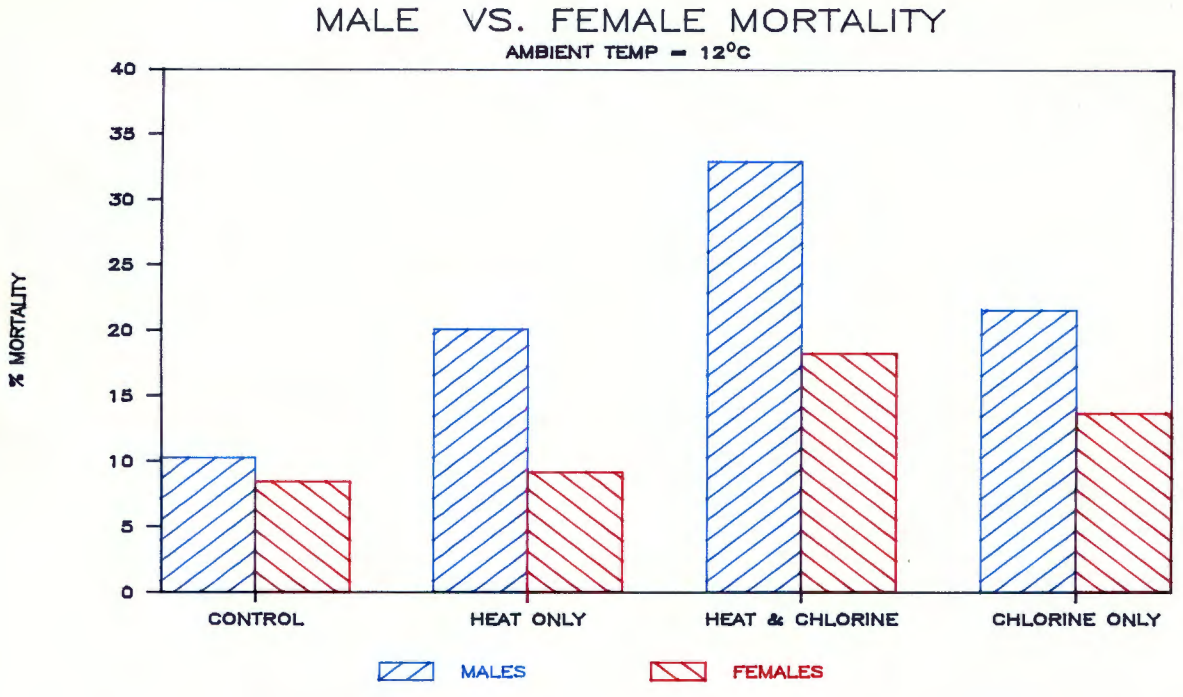


Fig. 10: Comparison of overall male and female *Paracartia africana* mortality during the experiment conducted at an ambient temperature of 12°C.

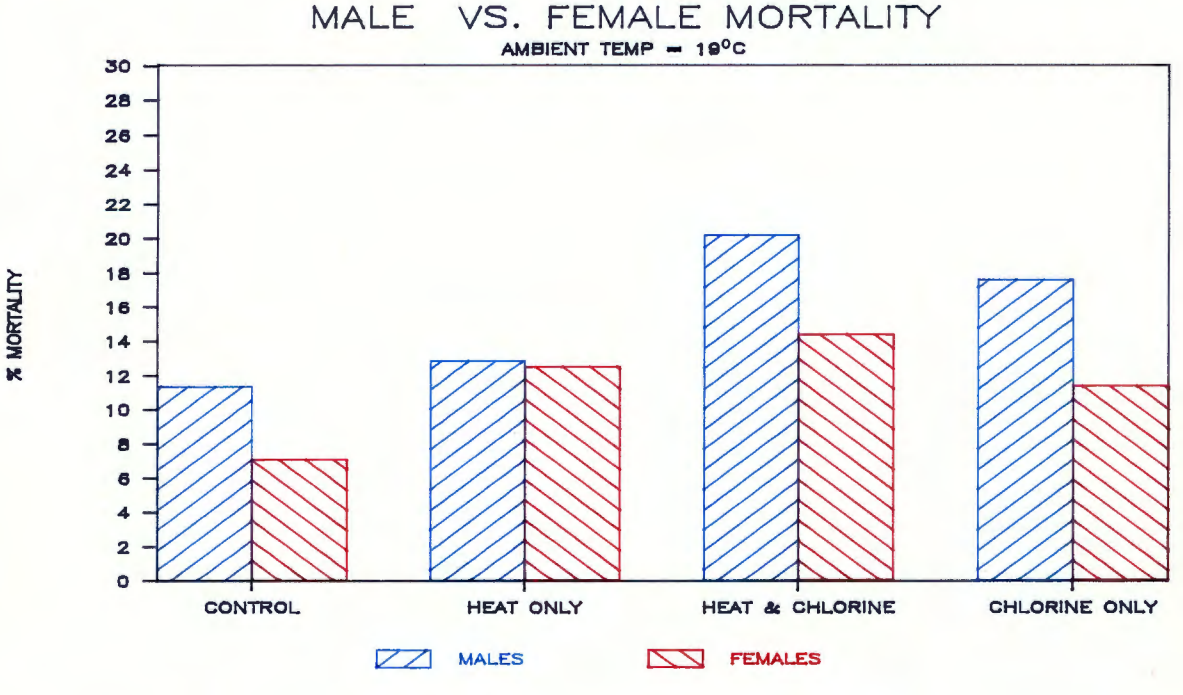


Fig. 11: Comparison of overall male and female *Paracartia africana* mortality during the experiment conducted at an ambient temperature of 19°C.

Synergism, if manifested, would be expected to be more pronounced at higher temperatures but this was not the case in the present study. The copepods used in the first experiment (12°C) were not acclimated to ambient temperature for as long as those used in the second experiment (19°C), which may explain why mortalities were higher. In addition, the copepods used in each experiment were collected on different occasions; the copepods used in the second experiment may have been in a better physiological state than those used in the first experiment.

This study monitored copepod mortality up to 60 hours after stress removal, but other workers have noted that latent effects such as increased respiration or mortality may occur up to 5 days after entrainment (Carpenter *et al.*, 1974; Latimer *et al.*, 1975). Carpenter *et al.* (1974) found that approximately half of the live copepods collected at a discharge (following entrainment) and held in situ died within 3.5 days, and 70% died within 5 days, whereas only 10% of those from the intake died within 5 days.

Davis and Coughlan (1978) observed that the immediate mortality (one hour after collection) of adult copepods, copepod nauplii and barnacle nauplii after entrainment was low at chlorine concentrations of 0 to more than 1 ppm (total residual) but rose 48 hours after collection, particularly when chlorine levels were above 0.5 ppm. Goldman *et al.* (1978) also found that adverse effects of chlorine addition can be manifested long after the entrainment period - acute exposure to either free or combined halogen resulted in decreased standard respiration rates and reduced growth rates during larval development of lobsters.

Latimer *et al.* (1975) conducted laboratory bioassays to determine the

toxicity of short (30-min) exposures of residual chlorine at different temperatures to two copepod species, *Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi*. During mortality estimates 24 hours after exposure, copepods were noted as being actually dead, sluggish or actively swimming. The intermediate category consisted of individuals that appeared to be partially or completely paralyzed, and that displayed responses to probing ranging from active swimming to weak twitches. These copepods were apparently suffering from some sublethal effects of exposure to chlorine. To determine the fate of the sluggish individuals, a further bioassay was conducted in which control and experimental groups were examined 24, 48 and 120 hours after the end of the exposure period. While the percent of the individuals of each experimental group actively swimming remained relatively constant, the percent sluggish decreased and the percent dead increased at 120 h. This indicated that most of the sluggish organisms died between two and five days after the exposure to chlorine. The authors noted that in the natural environment, sluggish individuals would be susceptible to predation and would face increased competition for food, therefore having a lower survival rate than normal individuals.

Blaich (1986) also investigated the effects of heat and chlorine on zooplankton entrained at Koeberg, and conducted laboratory bioassays on the copepod *Calanus finmarchicus*. However, exposure to stress conditions was maintained for a full 60-hour period, and not for a time-period similar to the duration of entrainment at the power station.

Blaich (1986) tested copepods at 14 and 22°C, the latter temperature corresponding to the increased temperature due to entrainment, and chlorination levels of 0.1 and 0.2 ppm. Copepod mortality was monitored

after the same time intervals as were used in the present study. She found that although cumulative mortality was higher at 22°C than 14°C in both chlorinated and unchlorinated tanks, the difference was not significant. However, mortality over the 60-hour period was significantly higher when copepods were exposed to 0.1 and 0.2 ppm compared to non-chlorinated tanks at both temperatures, and mortality resulting from exposure to 0.2 ppm was significantly higher than mortality due to exposure to 0.1 ppm. Mortality differences due to exposure to the various combinations of temperature and chlorine concentration only became apparent after two hours or even longer exposure. After approximately four hours the responses diverged rapidly. Thus *Calanus* individuals took longer to be affected by heat and chlorine than *Paracartia*, even though exposure to these stresses was continuous instead of limited to 30 minutes.

The larger size of *Calanus* adults compared to adult *Paracartia* specimens may account for the differences in time-related responses. *Calanus* females range in size from 2.77 to 2.98 mm and males from 2.73 to 2.82 mm (De Decker, 1964). *Paracartia* are approximately half the size, females ranging from 1.20 to 1.45 mm and males from 1.12 to 1.30 mm (Unterüberbacher, 1964). As was stated earlier in this report, there is evidence to indicate that smaller copepods, with higher metabolic and respiratory rates, are more sensitive to halogen toxicity than larger species (e.g Goldman *et al.*, 1978).

With reference to *Paracartia* mortality rates being representative of average copepod entrainment mortality, *Paracartia* probably corresponds to the average size-range of the most abundant copepods inhabiting the near-shore environment at Koeberg. It is slightly smaller than

Centropages brachiatus (females 1.70 - 1.91 mm, males 1.58 - 1.75 mm) which is often the most abundant species in the area (Blaich, 1986; pers. obs. this study) and is one of the principal food organisms for fish in the Benguela Current area (De Decker, 1964). Many of the other species commonly found are smaller than *Paracartia*, such as *Paracalanus parvus* (females 0.90 - 0.95 mm) *Oithona* spp. (e.g. *O. nana*, females 0.62 mm) and *Oncaea* spp. (e.g. *O. subtilis*, females 0.58 - 0.59 mm). The largest copepod species collected in this study was *Rhincalanus nasutus* (females 3.60 - 4.52 mm, males 3.45 - 3.67 mm), but this species was not abundant near Koeberg. All size-ranges listed here are taken from De Decker (1964).

Blaich (1986) also investigated the effects of shock-dosing - the addition of chlorine at extremely high concentrations over a short period of time - on *Calanus*. Shock-dosing was sometimes employed in the past at Koeberg in an attempt to kill organisms which had settled and persisted in the intake piping despite continuous low-level chlorination. This practice has been discontinued, however, since it has proved to be ineffectual in removing fouling, and is extremely detrimental to entrained organisms. Blaich exposed copepods to concentrations of 5, 10 and 20 ppm for 15-minute periods. Exposure to 10 and 20 ppm killed all individuals immediately. At 5 ppm mortality immediately following exposure was greater than 70%, and all survivors showed signs of extreme stress which was followed by latent mortality occurring within 36 hours after exposure.

The higher resistance of female *Paracartia* to the various stresses is also worthy of note. Alden *et al.* (1976) investigated the effect of temperature and thermal shock on the copepods *Oithona* sp., *Acartia*

tonsa, *Paracalanus crassirostris* and *Euterpina acutifrons*, and found that adult females were usually less sensitive to entrainment effects than the males.

The reason for this discrepancy is not certain, although it has been suggested that it may be associated with metabolic rate and/or body size. Gill and Crisp (1985) found that *Temora longicornis* males were less tolerant to extremes of temperature than the females. During investigations into the effect of temperature on limb beat frequency, they observed that significantly more males (47%) than females (7%) stopped swimming at 25°C. When differences in metabolic rate were previously recorded between the sexes, it was always the males which had the lower rate (Gill and Crisp, 1985). Male *Eurytemora affinis* and *Calanus finmarchicus* were also less tolerant to changes in temperature than females (Marshall *et al.*, 1935; Bradley, 1978), and male *Calanus finmarchicus* and *Euterpina acutifrons* have lower metabolic rates (Marshall *et al.*, 1934; Vernberg and Moreira, 1974). Gilfillan (1976) found that coastal euphausiids which usually have a higher respiratory rate (compared to deeply migrating species) appear better able to adapt to changes in environmental conditions, in this instance temperature.

This theory, at first thought, appears contradictory to the earlier premise that smaller copepod species are more sensitive to halogen toxicity (i.e. chlorination) than larger species because of their higher metabolic and respiratory rates (Goldman *et al.*, 1978). It seems, however, that a higher metabolic rate results in a greater susceptibility to chemical stress (with a higher respiratory rate there would be a faster uptake of the toxicant), but a lower susceptibility to thermal stress.

From this point of view, the generally smaller male copepods should be more tolerant of chlorination than the females, but less tolerant of thermal stress. As male copepods had higher mortalities in all the tanks compared to females, however, it would seem that their lower fitness with respect to stressful conditions is related to some other inherent physiological, biochemical or anatomical difference as opposed to simply a lower metabolic rate. Male copepods often have shorter life expectancies than the females, as is the case for *Calanus finmarchicus* (Marshall and Orr, 1955) and *Pseudocalanus* spp. (Urry, 1964; Corkett and McLaren, 1978), and the reason behind this may provide the key to their lower fitness. In most species the number of adult females exceeds males mainly because males have a shorter life span (Davis, 1984). Davis also noted that food, pollutants or other environmental factors may selectively alter the survival of one sex or perhaps cause sex reversals.

The reason why male copepods are more susceptible to stress, however, is not the main issue. What is of greater importance with respect to assessing the impact of entrainment is the fact that more females than males are likely to survive the entrainment process. As many adult females store sperm to fertilize eggs for the rest of their lives from one mating, especially in species where the male is short-lived (Davis, 1984), the higher surviveability of the females means that the reproductive potential of copepods surviving entrainment will be better than the mortality figures suggest.

If the assumption from the laboratory experiments that immediate entrainment mortality represents approximately 75% of the total

mortality, is applied to the rest of the zooplankton species entering Koeberg, then the predicted total zooplankton entrainment mortality would be 29.04%, due to an immediate mortality of 22.34% (from vital staining techniques applied during fieldwork) and a latent mortality of 6.70%. Unfortunately records of latent mortality for zooplankton species other than copepods is rare in the scientific literature, so without further information or experimentation the above prediction cannot be substantiated.

6. CONCLUSIONS

Field studies and observations at operating power plants have shown that many organisms do not survive entrainment. It is almost always impossible to unequivocably separate mortalities due to physical, thermal and chemical effects (Schubel *et al.*, 1978). However, studies conducted at power stations which do not employ chlorination procedures or which may temporarily withhold chlorination have, together with laboratory experiments, contributed towards a better understanding of the relative importance of these stresses to the plankton.

It seems that in general chlorination is the most important cause of entrainment-induced mortality, especially with respect to phytoplankton and the smaller species of zooplankton. Larger zooplankton such as mysids and fish larvae are most prone to the physical and mechanical stresses of entrainment. The thermal increment is significant in that it enhances the toxicity of chlorine. It becomes most important when ambient temperatures are high, and the raised temperature of the cooling water approaches the upper thermal tolerance limits of the entrained organisms. In these cases, synergism between temperature and chlorine results in very high entrainment mortalities. Laboratory experiments indicate that immediate and latent, or delayed, mortalities in these situations may reach 100% for some species.

Phytoplankton entrainment at Koeberg Nuclear Power Station may decrease primary productivity by approximately 30 to 60%, and up to 70% of the cell biomass may be destroyed. It seems likely that this is mostly due to chlorination, which reduces the ability of cells to take up nutrients such as nitrate and phosphate (Toetz *et al.*, 1977; Videau *et al.*, 1980),

and may affect the cells irreparably. However, cells which survive entrainment are able to recover and reach normal growth rates again. The high regeneration capacity of phytoplankton, combined with the high standing stocks and productivity associated with West coast waters (Brown, 1986), suggests that the marine environment near Koeberg is unlikely to experience a severe depletion of phytoplankton biomass.

The average residual chlorine concentration of 0.2 ppm at Koeberg is lower than chlorine levels used at many overseas power stations, and this results in comparatively low zooplankton mortalities. A considerable range in mortalities was found, averaging 22.34% overall, but all the species found in some abundance had entrainment mortalities of less than 50%. Chlorine appears to be the major cause of this mortality, although thermal and mechanical stress are also of significance. Many of the larger mysids captured in the outfall canal at Koeberg were severely damaged, with the cephalothorax often being completely severed from the abdomen. Soft-bodied zooplankton such as polychaete larvae, appendicularia and the smaller gelatinous zooplankton were more resistant to physical stress, and displayed fairly low overall mortalities. Of the more abundant taxa, crustacean zooplankton such as copepods, cladocerans and crab larvae experienced the highest mortalities.

Experimental work indicated that latent effects of entrainment may be important. The copepod *Paracartia africana* displayed latent mortality up to 60 hours after the removal of stresses asscoiated with entrainment. However, soon after removal of the stresses the mortality rate declined substantially, and from two to four hours onwards the rate of mortality was not significantly higher than that of the control. Of

the total mortality observed during the 60-hour period, 75% occurred during the 30-minutes of heat and chlorine treatment, i.e. latent mortality was equal to one third of the immediate or entrainment mortality. Using entrainment mortalities measured during field experiments, total zooplankton mortality at Koeberg (including latent effects) was estimated to be 29.04%, and overall copepod mortality to be approximately 40%.

Entrainment may also have sublethal effects on zooplankton. Such effects include decreased growth, decreased sperm motility, decreased fertilisation success, decreased reproduction, decreased activity, behavioural signs of distress or irritation, damage to delicate appendages used for filter-feeding, sensing, swimming and reproduction, and abnormal development during egg and larval stages.

Many workers have concluded that the most serious, immediate threat of power plant entrainment is not on the permanent plankton (holoplankton), which have relatively fast regeneration times, but rather on the larval stages of meroplanktonic species, which spawn intermittently. Reductions in larval populations resulting from entrainment stresses can lead to catastrophic reductions in adult numbers.

From this point of view, Koeberg does not appear to pose a real threat. The larvae of polychaetes, barnacles, crabs, molluscs and echinoderms do not experience very high entrainment mortalities, and the adults of these species are abundant in the area around Koeberg. The phyllosoma larvae of *Jasus lalandii*, the Cape crayfish, appear to move away from the near-shore environment, and none were captured in either the harbour, the outfall canal or the waters beyond the harbour area.

Commercially important fish species are not abundant near Koeberg, and entrained fish eggs showed excellent survival (over 96% on average), although it is possible that the eggs could develop into abnormal larvae.

The main effect of the discharge plume on the receiving waters is that of heating the surface water by several degrees within a 2-3 km radius. Much of the heat disperses upon entering the sea, due to turbulent mixing and wave action. Any residual chlorine is dissipated during this process by the high chlorine demand of the sea, as well as during passage in the outfall canal. This is aided by the turbulent flow of the discharge water. The orientation of the long, south breakwater arm prevents recirculation of discharged water into the power station.

In conclusion, rapid dissipation of chlorine and heat upon entering the receiving water, low entrainment mortality of zooplankton, substantial regeneration capacity of surviving phytoplankton, and replenishment of cell biomass and zooplankton species from the highly productive surrounding upwelling area, all contribute towards minimising the impact of Koeberg Nuclear Power Station on the local ocean environment.

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APPENDIX 1

TABLE 2: Numbers of live and dead zooplankton per cubic metre collected on 16.10.85

16.10.85	INLET			OUTFALL			
Zooplankton type	No. alive per m3	No. dead per m3	% Dead	No. alive per m3	No. dead per m3	% Dead	% Mortality Difference
Medusae	-	-	-	-	-	-	-
Ctenophores	-	-	-	-	-	-	-
Nematodes	-	-	-	0.63	0.38	37.5	-
Polychaete larvae	15.0	0.0	0.0	4.75	0.0	0.0	0.0
Chaetognaths	-	-	-	-	-	-	-
Evadne	-	-	-	-	-	-	-
Podon	-	-	-	-	-	-	-
Penilia	-	-	-	-	-	-	-
Barnacle nauplii	1.63	0.0	0.0	2.5	0.0	0.0	0.0
Cyprids	0.63	0.0	0.0	1.88	1.0	34.78	+34.78
Mysids	-	-	-	-	-	-	-
Cumaceans	-	-	-	-	-	-	-
Isopods	0.38	0.0	0.0	-	-	-	-
Amphipods	-	-	-	-	-	-	-
Euphausid larvae	-	-	-	-	-	-	-
Zoeae (type a)	0.38	0.0	0.0	-	-	-	-
Zoeae (type b)	-	-	-	-	-	-	-
Copepods	6.0	0.0	0.0	4.38	0.63	12.5	+12.5
Harpacticoids	-	-	-	-	-	-	-
Copepod larvae	0.38	0.0	0.0	-	-	-	-
Gastropod larvae	-	-	-	-	-	-	-
Bivalves	-	-	-	-	-	-	-
Echinoderm larvae	-	-	-	-	-	-	-
Appendicularia	-	-	-	-	-	-	-
Fish eggs	-	-	-	-	-	-	-
TOTAL	24.4	0.0	0.0	14.14	2.01	12.45	+12.45

* denotes that there were sufficient numbers (>6 per m3) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 3: Numbers of live and dead zooplankton per cubic metre collected on 24.01.86

24.01.86	INLET			OUTFALL			
Zooplankton type	No. alive per m3	No. dead per m3	% Dead	No. alive per m3	No. dead per m3	% Dead	% Mortality Difference
Medusae	-	-	-	-	-	-	-
Ctenophores	-	-	-	-	-	-	-
Nematodes	-	-	-	-	-	-	-
Polychaete larvae	-	-	-	-	-	-	-
Chaetognaths	-	-	-	-	-	-	-
Evadne	-	-	-	-	-	-	-
Podon	-	-	-	-	-	-	-
Penilia	-	-	-	-	-	-	-
Barnacle nauplii	-	-	-	-	-	-	-
Cyprids	0.38	0.0	0.0	-	-	-	-
Mysids	5.38	0.0	0.0	112.5	8.13**	6.74**	+6.74**
Cumaceans	-	-	-	-	-	-	-
Isopods	0.38	0.0	0.0	-	-	-	-
Amphipods	-	-	-	-	-	-	-
Euphausid larvae	-	-	-	-	-	-	-
Zoeae (type a)	0.38	0.0	0.0	0.63	0.0	0.0	0.0
Zoeae (type b)	-	-	-	-	-	-	-
Copepods	35.38	0.0	0.0	58.13	9.13	13.57	+13.57 *
Harpacticoids	-	-	-	0.38	0.0	0.0	-
Copepod larvae	0.38	0.0	0.0	-	-	-	-
Gastropod larvae	-	-	-	-	-	-	-
Bivalves	-	-	-	-	-	-	-
Echinoderm larvae	-	-	-	-	-	-	-
Appendicularia	-	-	-	-	-	-	-
Fish eggs	2.25	0.0	0.0	3.75	0.0	0.0	0.0
TOTAL	44.53	0.0	0.0	175.77	17.26	8.94	+8.94

* denotes that there were sufficient numbers (>6 per m3) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

** probably underestimates mortality

TABLE 5: Numbers of live and dead zooplankton per cubic metre collected on 23.04.86

23.04.86	INLET			OUTFALL			
Zooplankton type	No. alive per m3	No. dead per m3	% Dead	No. alive per m3	No. dead per m3	% Dead	% Mortality Differenc
Medusae	-	-	-	-	-	-	-
Ctenophores	-	-	-	-	-	-	-
Nematodes	-	-	-	-	-	-	-
Polychaete larvae	-	-	-	1.88	0.0	0.0	-
Chaetognaths	-	-	-	-	-	-	-
Evadne	-	-	-	-	-	-	-
Podon	-	-	-	-	-	-	-
Penilia	-	-	-	-	-	-	-
Barnacle nauplii	2.5	0.0	0.0	4.38	0.63	12.5	+12.5
Cyprids	-	-	-	-	-	-	-
Mysids	-	-	-	0.63	0.0	0.0	-
Cumaceans	-	-	-	-	-	-	-
Isopods	1.25	0.0	0.0	-	-	-	-
Amphipods	-	-	-	-	-	-	-
Euphausid larvae	-	-	-	-	-	-	-
Zoeae (type a)	0.0	0.63	100.00	-	-	-	-
Zoeae (type b)	1.25	0.0	-	-	-	-	-
Copepods	5.63	0.63	10.06	25.63	3.13	10.87	+0.81
Harpacticoids	-	-	-	-	-	-	-
Copepod larvae	1.25	0.0	0.0	0.63	0.0	0.0	0.0
Gastropod larvae	-	-	-	-	-	-	-
Bivalves	-	-	-	-	-	-	-
Echinoderm larvae	-	-	-	-	-	-	-
Appendicularia	-	-	-	-	-	-	-
Fish eggs	1.25	1.88	60.00	-	-	-	-
TOTAL	13.13	3.14	19.30	33.15	3.76	10.17	-9.13

* denotes that there were sufficient numbers (>6 per m3) in both intake and outfall samples for use in mean mortalit calculations (see TABLE 17).

TABLE 7: Numbers of live and dead zooplankton per cubic metre collected on 18.09.86

18.09.86	INLET			OUTFALL			
Zooplankton type	No. alive per m3	No. dead per m3	% Dead	No. alive per m3	No. dead per m3	% Dead	% Mortality Difference
Medusae	-	-	-	-	-	-	-
Ctenophores	-	-	-	-	-	-	-
Nematodes	-	-	-	-	-	-	-
Polychaete larvae	0.63	0.0	0.0	2.5	0.0	0.0	0.0
Chaetognaths	-	-	-	-	-	-	-
Evadne	5.0	1.88	27.27	130.63	43.75	25.09	-2.18 *
Podon	0.63	0.0	0.0	15.63	6.88	30.56	+30.56
Penilia	-	-	-	-	-	-	-
Barnacle nauplii	10.63	1.25	10.53	4.38	0.0	0.0	-10.53
Cyprids	-	-	-	0.0	1.25	100.00	-
Mysids	-	-	-	-	-	-	-
Cumaceans	-	-	-	-	-	-	-
Isopods	-	-	-	-	-	-	-
Amphipods	-	-	-	-	-	-	-
Euphausid larvae	-	-	-	-	-	-	-
Zoeae (type a)	-	-	-	0.63	0.0	0.0	-
Zoeae (type b)	-	-	-	2.5	1.25	33.33	-
Copepods	21.25	1.88	8.11	72.50	47.50	39.58	+31.47 *
Harpacticoids	-	-	-	-	-	-	-
Copepod larvae	-	-	-	-	-	-	-
Gastropod larvae	-	-	-	0.63	0.0	-	-
Bivalves	-	-	-	-	-	-	-
Echinoderm larvae	-	-	-	-	-	-	-
Appendicularia	0.63	0.0	0.0	0.63	0.0	0.0	0.0
Fish eggs	-	-	-	0.63	0.0	0.0	-
TOTAL	38.77	5.01	11.44	230.66	100.63	30.38	+18.94

* denotes that there were sufficient numbers (>6 per m3) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 8: Numbers of live and dead zooplankton per cubic metre collected on 14.01.87

14.01.87	INLET			OUTFALL				
Zooplankton type	No. Alive per m3	No. Dead per m3	% Dead	No. Alive per m3	No. Dead per m3	% Dead	% Mortality Difference	
Medusae	-	-	-	0.63	0.63	50.00	-	
Ctenophores	-	-	-	2.50	0.0	0.0	-	
Nematodes	-	-	-	-	-	-	-	
Polychaete larvae	-	-	-	15.63	0.0	0.0	-	
Chaetognaths	-	-	-	-	-	-	-	
Evadne	18.13	0.63	3.36	50.00	0.0	0.0	-3.36	*
Podon	135.63	0.0	0.0	1711.25	0.0	0.0	0.0	*
Penilia	-	-	-	-	-	-	-	
Barnacle nauplii	1.25	0.0	0.0	57.50	0.0	0.0	0.0	
Cyprids	34.38	0.63	1.8	108.13	23.75	18.01	+16.21	*
Mysids	-	-	-	-	-	-	-	
Cumaceans	-	-	-	-	-	-	-	
Isopods	-	-	-	-	-	-	-	
Amphipods	-	-	-	-	-	-	-	
Euphausid larvae	3.13	0.0	0.0	11.88	1.88	13.66	+13.66	
Zoeae (type a)	-	-	-	1.25	0.0	0.0	-	
Zoeae (type b)	-	-	-	1.88	0.0	0.0	-	
Copepods	101.88	7.5	6.86	801.88	350.00	30.39	+23.53	*
Harpacticoids	-	-	-	-	-	-	-	
Copepod larvae	17.50	0.0	0.0	8.13	2.5	23.52	+23.52	*
Gastropod larvae	-	-	-	1.88	0.0	0.0	-	
Bivalves	-	-	-	-	-	-	-	
Echinoderm larvae	1.25	0.0	0.0	1.25	0.0	0.0	0.0	
Appendicularia	-	-	-	-	-	-	-	
Fish eggs	30.63	4.38	12.51	289.38	5.0	1.7	-10.81	*
TOTAL	343.78	13.14	3.68	3063.17	383.76	11.13	7.45	

* denotes that there were sufficient numbers (>6 per m3) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 10: Numbers of live and dead zooplankton collected by towing plankton net on 19.03.86

19.03.86	INLET			OUTFALL			
Zooplankton type	Alive	Dead	% Dead	Alive	Dead	% Dead	% Mortality Difference
Medusae	-	-	-	6	0	0.0	-
Ctenophores	-	-	-	-	-	-	-
Nematodes	-	-	-	-	-	-	-
Polychaete larvae	-	-	-	8	0	0.0	-
Chaetognaths	-	-	-	-	-	-	-
Evadne	-	-	-	-	-	-	-
Podon	-	-	-	-	-	-	-
Penilia	-	-	-	-	-	-	-
Barnacle nauplii	-	-	-	1	0	0.0	-
Cyprids	-	-	-	-	-	-	-
Mysids	0	2	100.00	3	2	40.00	-60.00
Cumaceans	-	-	-	-	-	-	-
Isopods	26	0	0.0	3	1	25.00	+25.00
Amphipods	7	0	0.0	1	2	66.67	+66.67
Euphausid larvae	-	-	-	-	-	-	-
Zoeae (type a)	-	-	-	-	-	-	-
Zoeae (type b)	-	-	-	-	-	-	-
Copepods	37	9	19.57	39	51	56.67	+37.10 *
Harpacticoids	-	-	-	-	-	-	-
Copepod larvae	1	0	0.0	46	0	0.0	0.0
Gastropod larvae	-	-	-	-	-	-	-
Bivalves	-	-	-	-	-	-	-
Echinoderm larvae	-	-	-	-	-	-	-
Appendicularia	-	-	-	-	-	-	-
Fish eggs	8	16	66.67	29	21	42.00	-24.67 *
TOTAL	79	27	25.47	144	77	34.84	+9.37

* denotes that there were sufficient numbers (>10) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 11: Numbers of live and dead zooplankton collected by towing plankton net on 23.04.86

23.04.86	INLET			OUTFALL			
Zooplankton type	Alive	Dead	% Dead	Alive	Dead	% Dead	% Mortality Difference
Medusae	5	0	0.0	-	-	-	-
Ctenophores	7	0	0.0	-	-	-	-
Nematodes	1	0	0.0	-	-	-	-
Polychaete larvae	1	0	0.0	2	0	0.0	0.0
Chaetognaths	-	-	-	-	-	-	-
Evadne	-	-	-	-	-	-	-
Podon	-	-	-	-	-	-	-
Penilia	-	-	-	-	-	-	-
Barnacle nauplii	3	0	0.0	-	-	-	-
Cyprids	0	1	100.00	-	-	-	-
Mysids	-	-	-	7	2	22.22	-
Cumaceans	1	0	0.0	3	4	57.14	+57.14
Isopods	21	0	0.0	1	0	0.0	0.0
Amphipods	3	0	0.0	-	-	-	-
Euphausid larvae	-	-	-	-	-	-	-
Zoeae (type a)	3	0	0.0	16	2	11.11	+11.11
Zoeae (type b)	-	-	-	-	-	-	-
Copepods	105	12	10.26	194	66	25.38	+15.12
Harpacticoids	-	-	-	-	-	-	-
Copepod larvae	-	-	-	1	0	0.0	-
Gastropod larvae	4	0	0.0	-	-	-	-
Bivalves	3	0	0.0	-	-	-	-
Echinoderm larvae	-	-	-	-	-	-	-
Appendicularia	-	-	-	-	-	-	-
Fish eggs	-	-	-	-	-	-	-
TOTAL	156	12*	7.14	224	74	24.83	+17.69

* denotes that there were sufficient numbers (>10) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 13: Numbers of live and dead zooplankton collected by towing plankton net on 28.05.86

28.05.86	INLET			OUTFALL				
Zooplankton type	Alive	Dead	% Dead	Alive	Dead	% Dead	% Mortality Difference	
Medusae	11	0	0.0	-	-	-	-	
Ctenophores	-	-	-	-	-	-	-	
Nematodes	-	-	-	-	-	-	-	
Polychaete larvae	-	-	-	-	-	-	-	
Chaetognaths	-	-	-	-	-	-	-	
Evadne	3	21	87.50	1	1	50.00	-37.50	
Podon	1	3	75.00	0	3	100.00	+25.00	
Penilia	2	0	0.0	-	-	-	-	
Barnacle nauplii	4	0	0.0	3	0	0.0	0.0	
Cyprids	1	1	50.00	-	-	-	-	
Mysids	-	-	-	-	-	-	-	
Cumaceans	-	-	-	2	0	0.0	-	
Isopods	18	1	5.26	3	0	0.0	-5.26	
Amphipods	-	-	-	-	-	-	-	
Euphausid larvae	-	-	-	-	-	-	-	
Zoeae (type a)	2	0	0.0	4	0	0.0	0.0	
Zoeae (type b)	0	1	100.00	9	4	30.77	-66.23	
Copepods	21	0	0.0	55	11	16.67	+16.67	*
Harpacticoids	-	-	-	-	-	-	-	
Copepod larvae	2	0	0.0	-	-	-	-	
Gastropod larvae	-	-	-	-	-	-	-	
Bivalves	-	-	-	-	-	-	-	
Echinoderm larvae	-	-	-	-	-	-	-	
Appendicularia	-	-	-	-	-	-	-	
Fish eggs	-	-	-	1	1	50.00	-	
TOTAL	63	27	30.00	78	20	20.41	-9.59	

* denotes that there were sufficient numbers (>10) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 14: Numbers of live and dead zooplankton collected by towing plankton net on 18.09.86

18.09.86	INLET			OUTFALL			%	
Zooplankton type	Alive	Dead	% Dead	Alive	Dead	% Dead	Mortality Difference	
Medusae	-	-	-	-	-	-	-	
Ctenophores	-	-	-	-	-	-	-	
Nematodes	-	-	-	-	-	-	-	
Polychaete larvae	1	0	0.0	2	0	0.0	0.0	
Chaetognaths	2	0	0.0	6	1	14.23	+14.23	
Evadne	303	20	6.19	219	41	15.77	+9.58	*
Podon	81	0	0.0	75	11	12.79	+12.79	*
Penilia	-	-	-	-	-	-	-	
Barnacle nauplii	39	1	2.50	7	0	0.0	-2.50	
Cyprids	1	0	0.0	1	2	66.67	+66.67	
Mysids	-	-	-	-	-	-	-	
Cumaceans	-	-	-	-	-	-	-	
Isopods	7	0	0.0	4	1	20.00	+20.00	
Amphipods	5	1	16.67	0	1	100.00	+83.33	
Euphausid larvae	2	0	0.0	0	1	100.00	+100.00	
Zoeae (type a)	24	0	0.0	-	-	-	-	
Zoeae (type b)	-	-	-	10	1	9.09	-	
Copepods	226	8	3.42	356	198	35.74	+32.32	*
Harpacticoids	-	-	-	-	-	-	-	
Copepod larvae	8	0	0.0	2	0	0.0	0.0	
Gastropod larvae	4	0	0.0	2	0	0.0	0.0	
Bivalves	1	0	0.0	-	-	-	-	
Echinoderm larvae	-	-	-	-	-	-	-	
Appendicularia	-	-	-	1	0	0.0	-	
Fish eggs	1	0	0.0	-	-	-	-	
TOTAL	705	30	4.08	685	257	27.28	+23.20	

* denotes that there were sufficient numbers (>10) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 15: Numbers of live and dead zooplankton collected by towing plankton net on 05.11.86

05.11.86	INLET			OUTFALL			
Zooplankton type	Alive	Dead	% Dead	Alive	Dead	% Dead	% Mortality Difference
Medusae	177	0	0.0	5	0	0.0	0.0
Ctenophores	-	-	-	-	-	-	-
Nematodes	-	-	-	-	-	-	-
Polychaete larvae	-	-	-	-	-	-	-
Chaetognaths	-	-	-	1	0	0.0	-
Evadne	31	13	29.55	7	4	36.36	+6.81 *
Podon	5	0	0.0	1	0	0.0	0.0
Penilia	-	-	-	-	-	-	-
Barnacle nauplii	7	0	0.0	5	0	0.0	0.0
Cyprids	83	92	52.57	11	6	35.29	+17.28 *
Mysids	-	-	-	-	-	-	-
Cumaceans	-	-	-	-	-	-	-
Isopods	13	0	0.0	-	-	-	-
Amphipods	2	0	0.0	6	0	0.0	0.0
Euphausid larvae	2	0	0.0	-	-	-	-
Zoeae (type a)	10	0	0.0	-	-	-	-
Zoeae (type b)	9	0	0.0	5	0	0.0	0.0
Copepods	118	25	17.48	27	44	61.97	+44.49 *
Harpacticoids	-	-	-	-	-	-	-
Copepod larvae	-	-	-	2	0	0.0	-
Gastropod larvae	-	-	-	-	-	-	-
Bivalves	-	-	-	-	-	-	-
Echinoderm larvae	-	-	-	-	-	-	-
Appendicularia	-	-	-	-	-	-	-
Fish eggs	3	1	25.00	-	-	-	-
TOTAL	460	131	22.17	70	54	43.55	+21.38

* denotes that there were sufficient numbers (>10) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 16: Numbers of live and dead zooplankton collected by towing plankton net on 14.01.87

14.01.87	INLET			OUTFALL			%	
Zooplankton type	Alive	Dead	% Dead	Alive	Dead	% Dead	Mortality Difference	
Medusae	193	0	0.0	32	0	0.0	0.0	*
Ctenophores	6	0	0.0	36	0	0.0	0.0	
Nematodes	-	-	-	-	-	-	-	
Polychaete larvae	0	0	0.0	20	0	0.0	0.0	
Chaetognaths	0	0	0.0	4	0	0.0	0.0	
Evadne	4640	0	0.0	120	24	16.67	+16.67	*
Podon	40400	0	0.0	536	0	0.0	0.0	*
Penilia	-	-	-	-	-	-	-	
Barnacle nauplii	440	0	0.0	88	4	4.35	+4.35	*
Cyprids	528	0	0.0	32	12	27.27	+27.27	*
Mysids	-	-	-	12	0	0.0	-	
Cumaceans	-	-	-	-	-	-	-	
Isopods	42	0	0.0	8	0	0.0	0.0	
Amphipods	1	0	0.0	12	0	0.0	0.0	
Euphausid larvae	448	0	0.0	176	4	2.22	+2.22	*
Zoeae (type a)	0	0	-	32	0	0.0	0.0	
Zoeae (type b)	0	0	-	56	0	0.0	-	
Copepods	22320	144	0.64	1804	592	24.71	+24.07	*
Harpacticoids	-	-	-	-	-	-	-	
Copepod larvae	48	0	0.0	4	4	50.00	+50.00	
Gastropod larvae	88	0	0.0	20	0	0.0	0.0	
Bivalves	-	-	-	-	-	-	-	
Echinoderm larvae	216	0	0.0	-	-	-	-	
Appendicularia	-	-	-	-	-	-	-	
Fish eggs	512	0	0.0	84	0	0.0	0.0	*
TOTAL	69882	144	0.21	3076	636	17.13	+16.92	

* denotes that there were sufficient numbers (>10) in both intake and outfall samples for use in mean mortality calculations (see TABLE 17).

TABLE 17: Calculation of mean entrainment mortality, using data from samples containing at least 6 animals per m3 (bucketed samples) or 10 animals in total (netted samples) at both intake and outfall sites. S.D. is the sample standard deviation, and N is the number of data points. The mean entrainment mortality calculated for each group by pooling the data (TABLE 4, Chapter 4) is provided for comparison.

ZOOPLANKTON TYPE	MEAN	S.D.	N	POOLED MEAN
Medusae	0	-	1	2.22
Ctenophores	-	-	-	0
Nematodes	-	-	-	20.00
Polychaete larvae	3.34	-	1	5.11
Chaetognaths	-	-	-	12.24
Evadne	5.50	8.38	5	32.59
Podon	4.26	7.38	2	0.96
Penilia	-	-	-	-
Barnacle nauplii	31.75	38.74	2	18.18
Cyprids	31.09	22.24	4	27.35
Mysids	-	-	-	14.78
Cumaceans	-	-	-	42.86
Isopods	-	-	-	10.94
Amphipods	-	-	-	17.71
Euphausid larvae	2.22	-	1	3.94
Zoeae (type a)	-	-	-	32.72
Zoeae (type b)	-	-	-	30.32
Copepods	21.42	13.95	14	30.52
Harpacticoids	-	-	-	80.00
Copepod larvae	23.36	16.67	2	15.22
Gastropod larvae	0	-	1	16.67
Bivalves	-	-	-	-
Echinoderm larvae	-	-	-	0
Appendicularia	-	-	-	0
Fish eggs	-2.72	17.08	5	3.28
TOTAL	16.07	14.90	16	22.34

- indicates insufficient data

APPENDIX 2

EXPERIMENT 1: 12°C

CONTROL TANK

TIME (HOURS)	LIVE MALES	DEAD MALES	LIVE FEMALES	DEAD FEMALES	TOTAL	TOTAL ALIVE	TOTAL DEAD	TOTAL MALE	TOTAL FEMALE	% DEAD MALE	% DEAD FEMALE	% DEAD	HOURLY MORT. RATE	CUM.HRLY MORT. RATE
0	28	2	21	2	53	49	4	30	23	3.77	3.77	7.55	0.00	0.00
0.25	16	0	16	1	33	32	1	16	17	0.00	3.03	3.03	12.12	12.12
0.5	26	2	13	1	42	39	3	28	14	4.76	2.38	7.14	14.29	26.41
1	20	1	10	0	31	30	1	21	10	3.23	0.00	3.23	3.23	29.63
2	20	0	12	1	33	32	1	20	13	0.00	3.03	3.03	1.52	31.15
4	37	3	25	1	66	62	4	40	26	4.55	1.52	6.06	1.52	32.66
8	20	0	14	1	35	34	1	20	15	0.00	2.86	2.86	0.36	33.02
12	13	2	8	4	27	21	6	15	12	7.41	14.81	22.22	1.85	34.87
16	8	1	17	0	26	25	1	9	17	3.85	0.00	3.85	0.24	35.11
24	21	2	8	0	31	29	2	23	8	6.45	0.00	6.45	0.27	35.38
36	4	2	5	3	14	9	5	6	8	14.29	21.43	35.71	0.99	36.37
48	1	1	9	1	12	10	2	2	10	8.33	8.33	16.67	0.35	36.72
60	4	9	15	1	29	19	10	13	16	31.03	3.45	34.48	0.57	37.30
	218	25	173	16	432	391	41	243	189	10.29	8.47	9.49		

HEATED TANK

TIME (HOURS)	LIVE MALES	DEAD MALES	LIVE FEMALES	DEAD FEMALES	TOTAL	TOTAL ALIVE	TOTAL DEAD	TOTAL MALE	TOTAL FEMALE	% DEAD MALE	% DEAD FEMALE	% DEAD	HOURLY MORT. RATE	CUM.HRLY MORT. RATE
0	27	2	15	2	46	42	4	29	17	4.35	4.35	8.70	0.00	0.00
0.25	16	2	13	2	33	29	4	18	15	6.06	6.06	12.12	48.48	48.48
0.5	20	2	22	2	46	42	4	22	24	4.35	4.35	8.70	17.39	65.88
1	21	3	8	3	35	29	6	24	11	8.57	8.57	17.14	17.14	83.02
2	10	3	9	2	24	19	5	13	11	12.50	8.33	20.83	10.42	93.44
4	13	3	19	1	36	32	4	16	20	8.33	2.78	11.11	2.78	96.21
8	26	1	10	0	37	36	1	27	10	2.70	0.00	2.70	0.34	96.55
12	1	2	1	0	4	2	2	3	1	50.00	0.00	50.00	4.17	100.72
16	9	0	17	2	28	26	2	9	19	0.00	7.14	7.14	0.45	101.16
24	12	5	11	0	28	23	5	17	11	17.86	0.00	17.86	0.74	101.91
36	14	12	16	1	43	30	13	26	17	27.91	2.33	30.23	0.84	102.75
48	1	5	4	0	10	5	5	6	4	50.00	0.00	50.00	1.04	103.79
60	1	3	3	0	7	4	3	4	3	42.86	0.00	42.86	0.71	104.50
	171	43	148	15	377	319	58	214	163	20.09	9.20	15.38		

HEATED AND CHLORINATED TANK

TIME (HOURS)	LIVE MALES	DEAD MALES	LIVE FEMALES	DEAD FEMALES	TOTAL	TOTAL ALIVE	TOTAL DEAD	TOTAL MALE	TOTAL FEMALE	% DEAD MALE	% DEAD FEMALE	% DEAD	HOURLY MORT. RATE	CUM.HRLY MORT. RATE
0	29	1	24	5	59	53	6	30	29	1.69	8.47	10.17	0.00	0.00
0.25	16	1	12	2	31	28	3	17	14	3.23	6.45	9.68	38.71	38.71
0.5	16	9	18	6	49	34	15	25	24	18.37	12.24	30.61	61.22	99.93
1	26	3	24	1	54	50	4	29	25	5.56	1.85	7.41	7.41	107.34
2	16	4	16	5	41	32	9	20	21	9.76	12.20	21.95	10.98	118.32
4	12	1	7	1	21	19	2	13	8	4.76	4.76	9.52	2.38	120.70
8	9	4	13	0	26	22	4	13	13	15.38	0.00	15.38	1.92	122.62
12	15	6	10	9	40	25	15	21	19	15.00	22.50	37.50	3.13	125.75
16	7	2	11	1	21	18	3	9	12	9.52	4.76	14.29	0.89	126.64
24	13	4	12	4	33	25	8	17	16	12.12	12.12	24.24	1.01	127.65
36	3	3	10	5	21	13	8	6	15	14.29	23.81	38.10	1.06	128.71
48	2	3	5	1	11	7	4	5	6	27.27	9.09	36.36	0.76	129.46
60	9	44	26	2	81	35	46	53	28	54.32	2.47	56.79	0.95	130.41
	173	85	188	42	488	361	127	258	230	32.95	18.26	26.02		

CHLORINATED TANK

TIME (HOURS)	LIVE MALES	DEAD MALES	LIVE FEMALES	DEAD FEMALES	TOTAL	TOTAL ALIVE	TOTAL DEAD	TOTAL MALE	TOTAL FEMALE	% DEAD MALE	% DEAD FEMALE	% DEAD	HOURLY MORT. RATE	CUM.HRLY MORT. RATE
0	30	1	22	2	55	52	3	31	24	1.82	3.64	5.45	0.00	0.00
0.25	18	3	11	0	32	29	3	21	11	9.38	0.00	9.38	37.50	37.50
0.5	21	0	9	2	32	30	2	21	11	0.00	6.25	6.25	12.50	50.00
1	21	2	12	3	38	33	5	23	15	5.26	7.89	13.16	13.16	63.16
2	22	7	17	2	48	39	9	29	19	14.58	4.17	18.75	9.38	72.53
4	17	1	16	1	35	33	2	18	17	2.86	2.86	5.71	1.43	73.96
8	16	2	8	1	27	24	3	18	9	7.41	3.70	11.11	1.39	75.35
12	19	5	10	1	35	29	6	24	11	14.29	2.86	17.14	1.43	76.78
16	18	9	6	3	36	24	12	27	9	25.00	8.33	33.33	2.08	78.86
24	25	10	16	4	55	41	14	35	20	18.18	7.27	25.45	1.06	79.92
36	15	4	10	0	29	25	4	19	10	13.79	0.00	13.79	0.38	80.31
48	2	13	10	5	30	12	18	15	15	43.33	16.67	60.00	1.25	81.56
60	1	5	4	0	10	5	5	6	4	50.00	0.00	50.00	0.83	82.39
	225	62	151	24	462	376	86	287	175	21.60	13.71	18.61		

EXPERIMENT 2: 19°C

CONTROL TANK

TIME (HOURS)	LIVE MALES	DEAD MALES	LIVE FEMALES	DEAD FEMALES	TOTAL	TOTAL ALIVE	TOTAL DEAD	TOTAL MALE	TOTAL FEMALE	% DEAD MALE	% DEAD FEMALE	% DEAD	HOURLY MORT. RATE	CUM.HRLY MORT. RATE
0	33	0	43	1	77	76	1	33	44	0.00	1.30	1.30	0.00	0.00
0.25	32	0	29	0	61	61	0	32	29	0.00	0.00	0.00	0.00	0.00
0.5	11	1	9	0	21	20	1	12	9	4.76	0.00	4.76	9.52	9.52
1	18	0	10	1	29	28	1	18	11	0.00	3.45	3.45	3.45	12.97
2	15	2	11	1	29	26	3	17	12	6.90	3.45	10.34	5.17	18.14
4	24	1	18	0	43	42	1	25	18	2.33	0.00	2.33	0.58	18.73
8	17	0	19	1	37	36	1	17	20	0.00	2.70	2.70	0.34	19.06
12	9	1	5	0	15	14	1	10	5	6.67	0.00	6.67	0.56	19.62
16	23	1	18	3	45	41	4	24	21	2.22	6.67	8.89	0.56	20.17
24	13	3	5	1	22	18	4	16	6	13.64	4.55	18.18	0.76	20.93
36	35	8	14	2	59	49	10	43	16	13.56	3.39	16.95	0.47	21.40
48	50	9	43	4	106	93	13	59	47	8.49	3.77	12.26	0.26	21.66
60	15	12	11	4	42	26	16	27	15	28.57	9.52	38.10	0.63	22.29
	295	38	235	18	586	530	56	333	253	11.41	7.11	9.56		

HEATED TANK

TIME (HOURS)	LIVE MALES	DEAD MALES	LIVE FEMALES	DEAD FEMALES	TOTAL	TOTAL ALIVE	TOTAL DEAD	TOTAL MALE	TOTAL FEMALE	% DEAD MALE	% DEAD FEMALE	% DEAD	HOURLY MORT. RATE	CUM.HRLY MORT. RATE
0	57	0	32	0	89	89	0	57	32	0.00	0.00	0.00	0.00	0.00
0.25	20	0	17	1	38	37	1	20	18	0.00	2.63	2.63	10.53	10.53
0.5	40	0	31	1	72	71	1	40	32	0.00	1.39	1.39	2.78	13.30
1	10	2	5	0	17	15	2	12	5	11.76	0.00	11.76	11.76	25.07
2	29	3	10	5	47	39	8	32	15	6.38	10.64	17.02	8.51	33.58
4	12	1	15	5	33	27	6	13	20	3.03	15.15	18.18	4.55	38.12
8	35	2	30	2	69	65	4	37	32	2.90	2.90	5.80	0.72	38.85
12	29	0	27	1	57	56	1	29	28	0.00	1.75	1.75	0.15	39.00
16	99	3	44	4	150	143	7	102	48	2.00	2.67	4.67	0.29	39.29
24	25	4	17	2	48	42	6	29	19	8.33	4.17	12.50	0.52	39.81
36	15	11	17	4	47	32	15	26	21	23.40	8.51	31.91	0.89	40.69
48	19	18	19	8	64	38	26	37	27	28.13	12.50	40.63	0.85	41.54
60	9	15	1	5	30	10	20	24	6	50.00	16.67	66.67	1.11	42.65
	399	59	265	38	761	664	97	458	303	12.88	12.54	12.75		

HEATED AND CHLORINATED TANK

TIME (HOURS)	LIVE MALES	DEAD MALES	LIVE FEMALES	DEAD FEMALES	TOTAL	TOTAL ALIVE	TOTAL DEAD	TOTAL MALE	TOTAL FEMALE	% DEAD MALE	% DEAD FEMALE	% DEAD	HOURLY MORT. RATE	CUM.HRLY MORT. RATE
0	53	0	36	0	89	89	0	53	36	0.00	0.00	0.00	0.00	0.00
0.25	11	1	13	3	28	24	4	12	16	3.57	10.71	14.29	57.14	57.14
0.5	34	1	44	4	83	78	5	35	48	1.20	4.82	6.02	12.05	69.19
1	39	7	48	2	96	87	9	46	50	7.29	2.08	9.38	9.38	78.57
2	13	1	16	0	30	29	1	14	16	3.33	0.00	3.33	1.67	80.23
4	34	2	51	2	89	85	4	36	53	2.25	2.25	4.49	1.12	81.36
8	31	9	29	3	72	60	12	40	32	12.50	4.17	16.67	2.08	83.44
12	25	2	13	2	42	38	4	27	15	4.76	4.76	9.52	0.79	84.23
16	20	3	6	0	29	26	3	23	6	10.34	0.00	10.34	0.65	84.88
24	14	7	21	3	45	35	10	21	24	15.56	6.67	22.22	0.93	85.81
36	3	21	8	8	40	11	29	24	16	52.50	20.00	72.50	2.01	87.82
48	2	5	0	3	10	2	8	7	3	50.00	30.00	80.00	1.67	89.49
60	1	12	0	18	31	1	30	13	18	38.71	58.06	96.77	1.61	91.10
	280	71	285	48	684	565	119	351	333	20.23	14.41	17.40		

CHLORINATED TANK

TIME (HOURS)	LIVE MALES	DEAD MALES	LIVE FEMALES	DEAD FEMALES	TOTAL	TOTAL ALIVE	TOTAL DEAD	TOTAL MALE	TOTAL FEMALE	% DEAD MALE	% DEAD FEMALE	% DEAD	HOURLY MORT. RATE	CUM.HRLY MORT. RATE
0	27	3	12	0	42	39	3	30	12	7.14	0.00	7.14	0.00	0.00
0.25	11	2	7	0	20	21	2	13	7	10.00	0.00	10.00	40.00	40.00
0.5	22	0	15	0	37	37	0	22	15	0.00	0.00	0.00	0.00	40.00
1	14	0	5	1	20	19	1	14	6	0.00	5.00	5.00	5.00	45.00
2	11	0	6	0	17	17	0	11	6	0.00	0.00	0.00	0.00	45.00
4	20	0	20	1	41	40	1	20	21	0.00	2.44	2.44	0.61	45.61
8	13	1	18	1	33	31	2	14	19	3.03	3.03	6.06	0.76	46.37
12	7	4	10	0	21	17	4	11	10	19.05	0.00	19.05	1.59	47.95
16	25	3	26	5	59	51	8	28	31	5.08	8.47	13.56	0.85	48.80
24	4	1	9	2	16	13	3	5	11	6.25	12.50	18.75	0.78	49.58
36	3	3	12	1	19	15	4	6	13	15.79	5.26	21.05	0.58	50.17
48	13	11	17	2	43	30	13	24	19	25.58	4.65	30.23	0.63	50.80
60	3	9	6	5	23	9	14	12	11	39.13	21.74	60.87	1.01	51.81
	173	37	163	18	391	339	55	210	181	17.62	9.94	14.07		

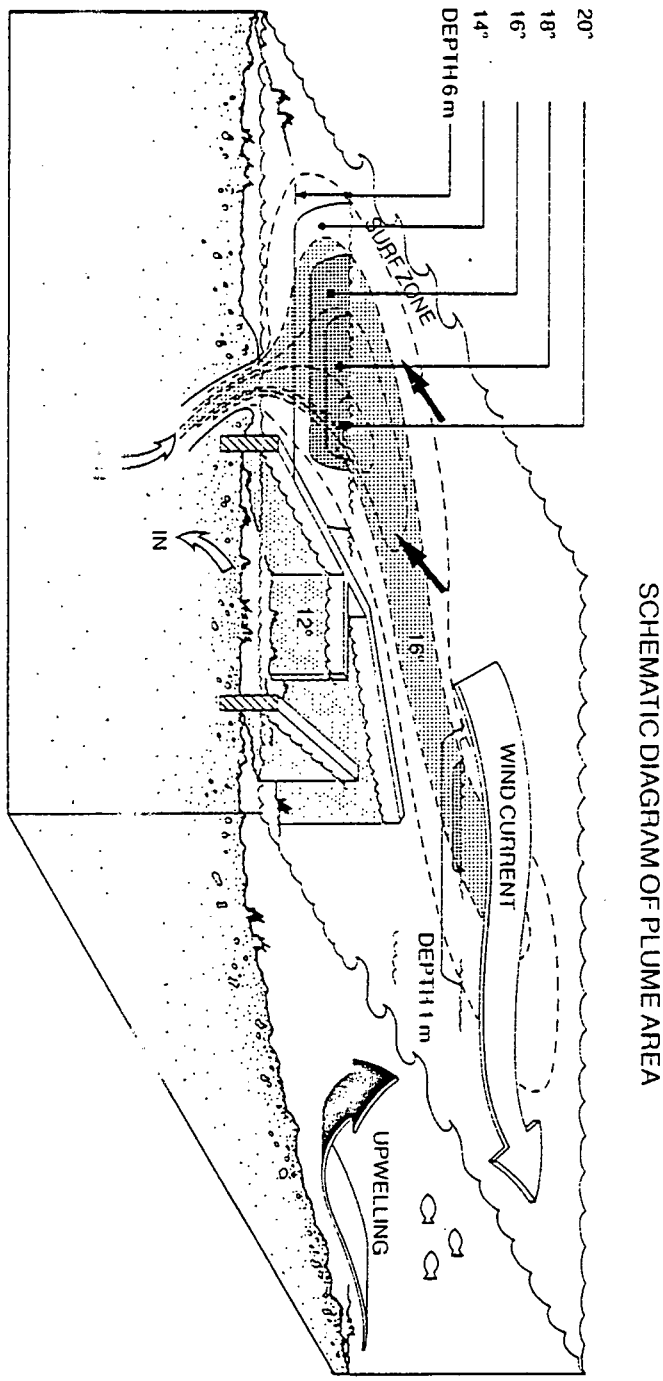
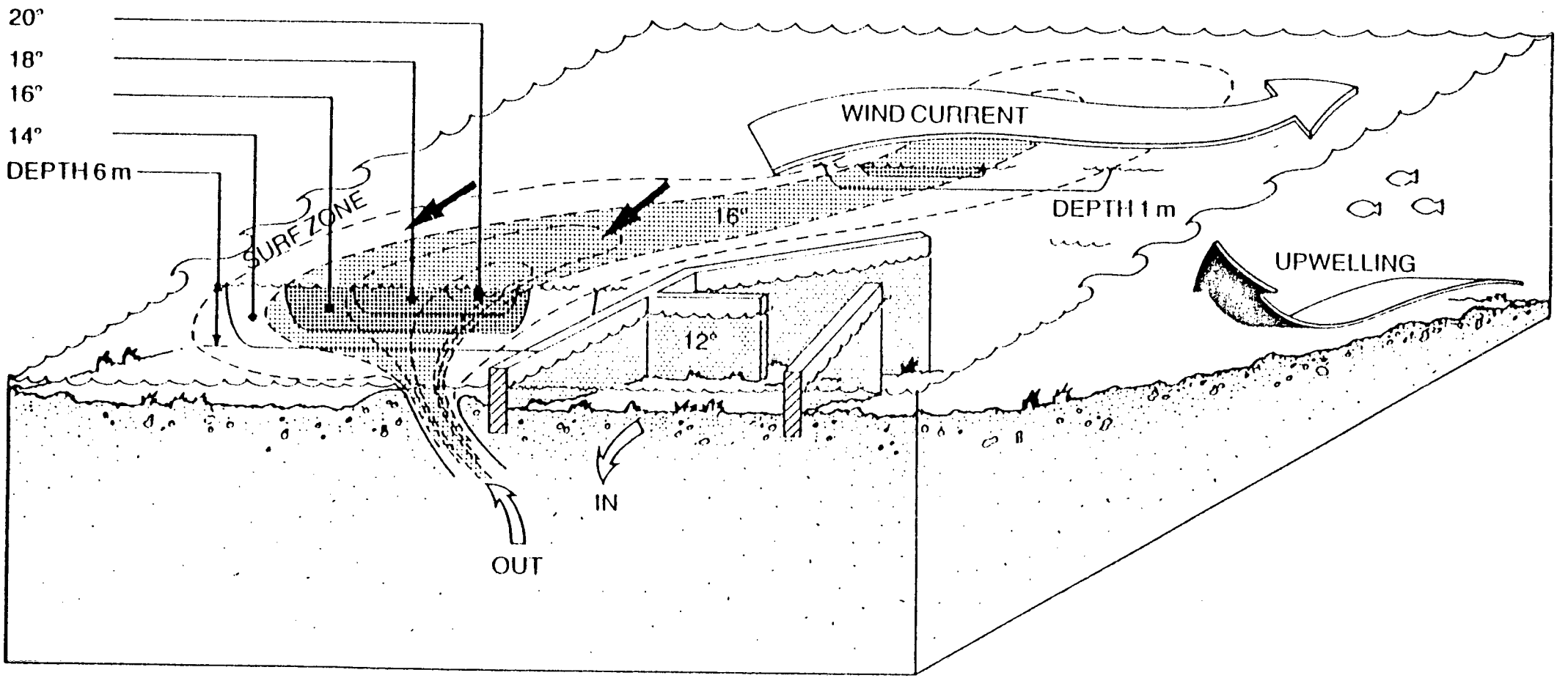
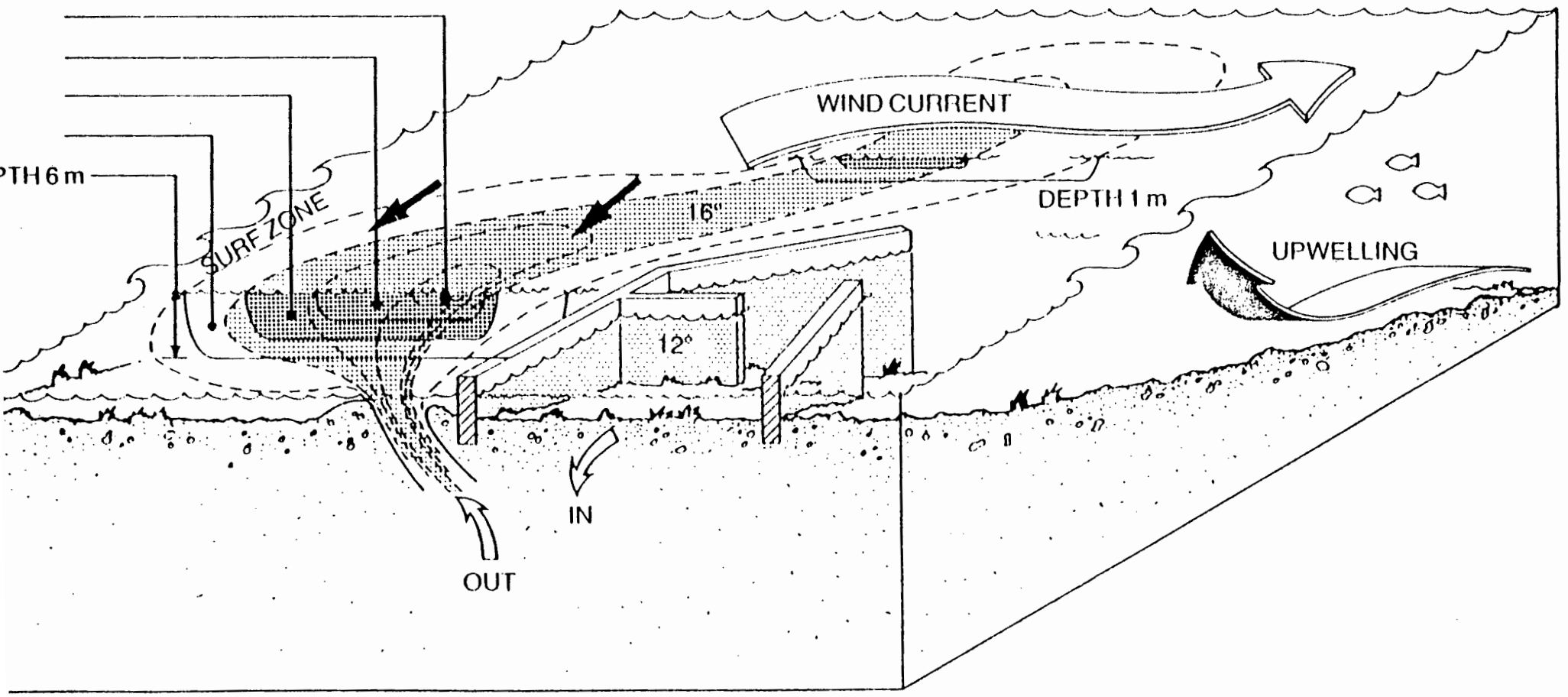


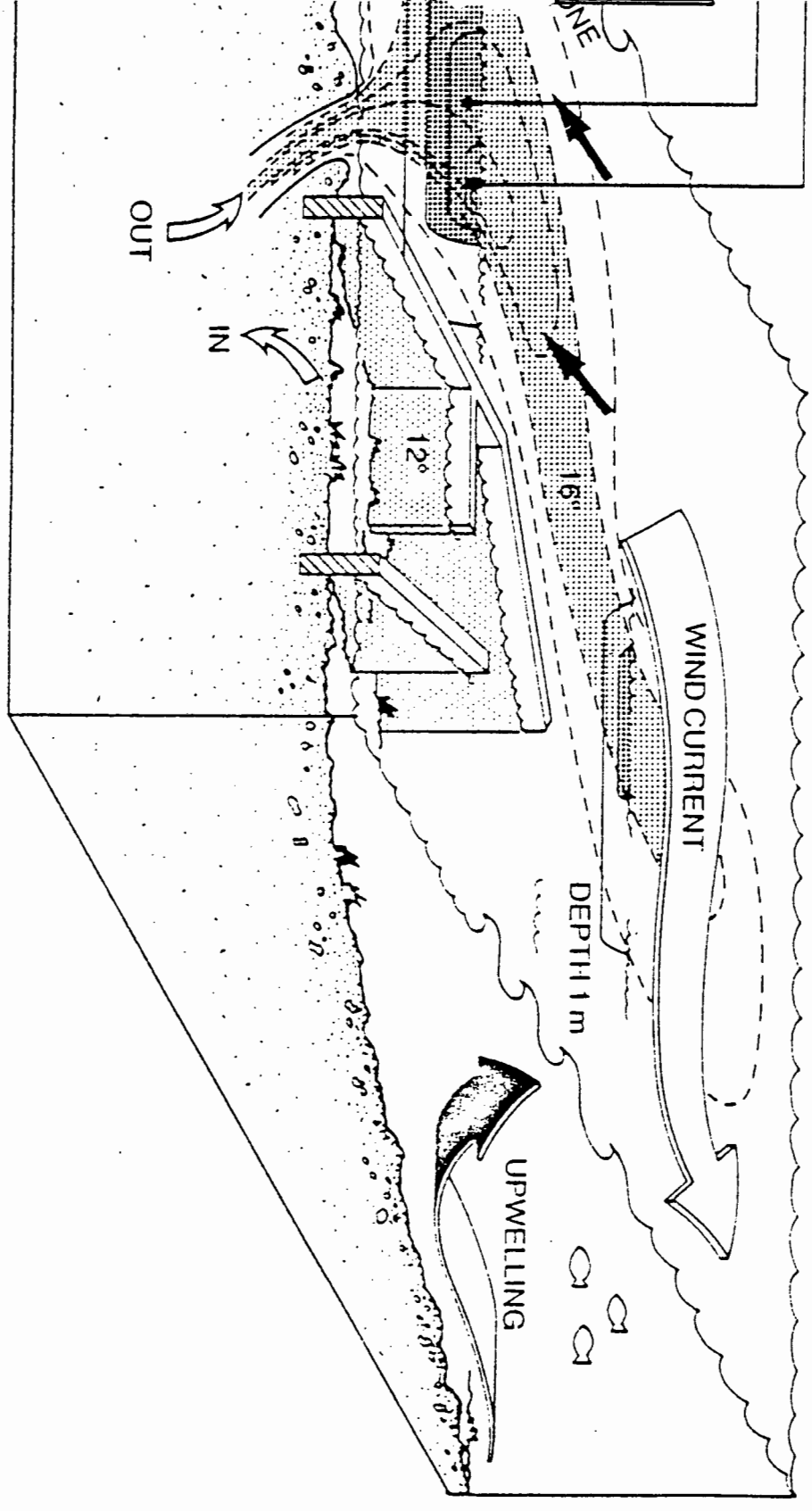
Fig. 5: Schematic diagram of plume area, showing typical conditions for a south-easterly wind direction (taken from ESCOM Databank, 1986).

SCHEMATIC DIAGRAM OF PLUME AREA



SCHEMATIC DIAGRAM OF PLUME AREA

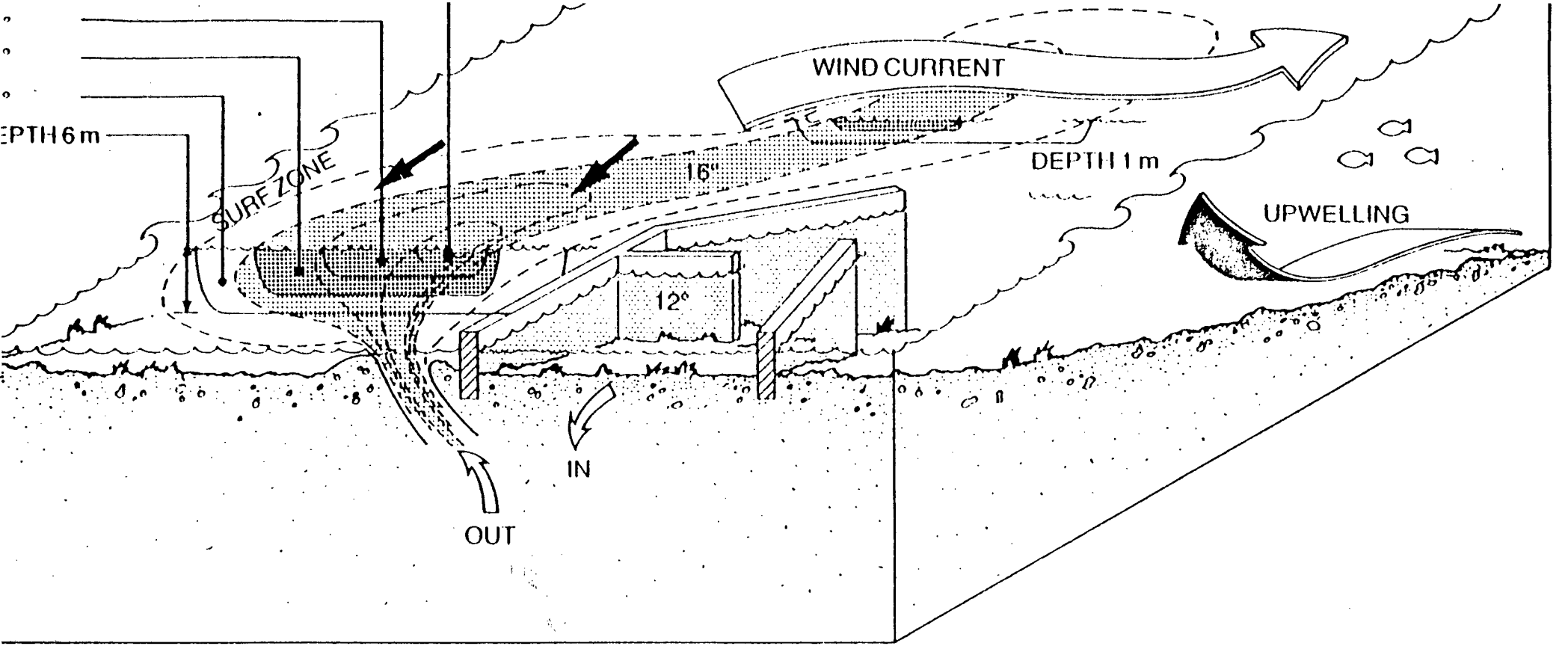




. 5: Schematic diagram of plume area, showing typical conditic
a south-easterly wind direction (taken from ESCOM Databa
1986).

SCHEMATIC DIAGRAM OF PLUME AREA

5. Schematic diagram of plume area, showing typical conditio


EPTH 6 m
SURF ZONE
16°
WIND CURRENT
DEPTH 1 m
UPWELLING
12°
IN
OUT