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IMPACT OF AQUATIC HERBICIDES (DIQUAT-PARAQUAT) ON SELECTED ORGANISMS IN OKANAGAN LAKE, BRITISH COLUMBIA

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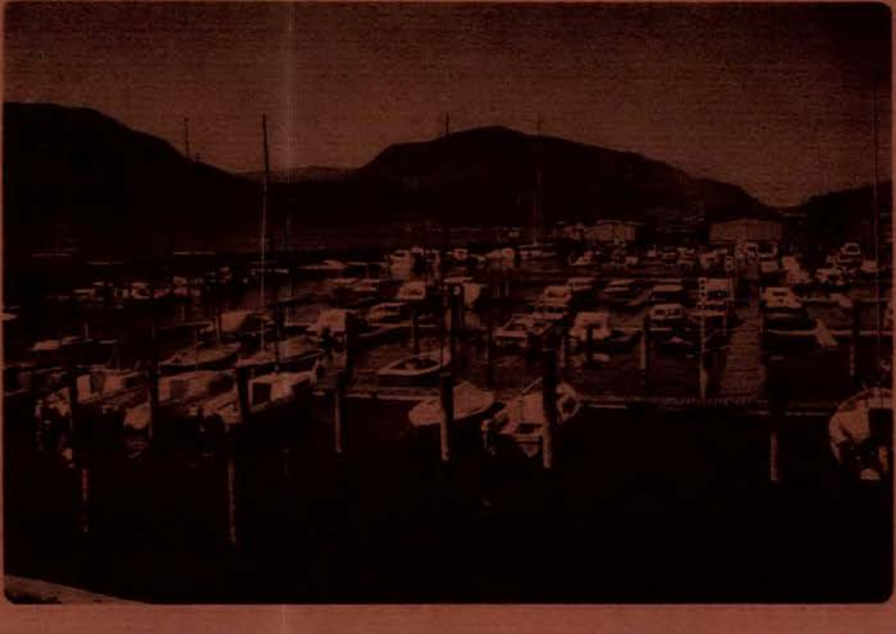

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June 1977

IMPACT OF AQUATIC HERBICIDES (DIQUAT-PARAQUAT) ON SELECTED ORGANISMS IN OKANAGAN LAKE, BRITISH COLUMBIA

by

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SUMMARY

1. There was an immediate depression in periphyton standing crop, expressed in terms of chlorophyll 'a' following herbicide treatment. Recovery, however, was rapid.

2. There was an increase in phytoplankton standing crop three weeks after herbicide treatment.

3. The herbicide application resulted in the death of amphipods (*Hyallela azteca*), held in cages in the marina. Snails (*Lymnaea sp.*) held in cages were not affected by the herbicide application. The results of bioassays with damselfly larvae (*Zoniagrion exclamationis*) were inconclusive.

4. The mortality of fish (redside shiners and prickly sculpins) held in cages in the marina could not be attributed to the herbicide treatment.

5. The number of fish utilizing the marina decreased following the application of herbicides. However, the composition of the fish species (mainly redside shiners, northern squawfish, prickly sculpins and long nose suckers) did not change as a result of the herbicide application.

6. There was a significant reduction in dissolved oxygen levels in the marina surface waters following herbicide treatment.

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1. INTRODUCTION

Increased nutrient additions from sewage effluent discharges and diffuse sources have been blamed for the accelerated growths of aquatic plants in the main lakes of the Okanagan Valley in British Columbia (Stockner *et al*, 1972).

In 1973 the Water Investigations Branch (WIB) of the British Columbia Department of the Environment established an "Interagency Technical Advisory Committee on Control of Aquatic Weeds" (B.C. Aquatic Weed Committee) to bring together all government agencies with an interest in water resources and aquatic plant management. The agencies comprising this committee were to provide technical expertise on the various implications of aquatic plant control.

In recognition of the long term effects of nutrient additions on aquatic weed growth, the Federal and Provincial governments developed a comprehensive management plan for water resources in the Okanagan Basin. A major aspect of this program is concerned with long term measures to control eutrophication of the Okanagan Lakes. A Federal-Provincial agreement (Okanagan Basin Implementation Agreement, 1976) presently outlines control measures which will affect the long term water quality of the Okanagan Lakes.

The WIB has been involved in ecological and management studies to assess the distribution, growth and control of nuisance aquatic plants. Particular attention has been focused on the proliferation of the Eurasian water milfoil (*Myriophyllum spicatum*), a plant which has spread rapidly throughout Okanagan Lake and downstream areas. To date, the emphasis of the WIB has been placed on evaluating various physical and chemical weed control techniques.

On June 3, 1975, the WIB initiated an experiment to assess the effectiveness of a 1:1 mixture of the bi-pyridylium herbicides, Regulone A(1) (diquat) and Gramoxone(2) (paraquat) in controlling Eurasian water milfoil and other aquatic weeds in the Kelowna boat marina in Okanagan Lake. The Habitat Protection Directorate of the Department of Fisheries and the Environment, Fisheries and Marine Service monitored the impact of this experimental herbicide application on selected biota within the marina.

This report documents the effects of the herbicide mixture on phytoplankton, periphyton (attached micro-algae), selected invertebrates and indigenous fish in the Kelowna marina.

2. MATERIALS AND METHODS

2.1 Site Description and Herbicide Application

The Kelowna marina (Figure 1) is enclosed by a breakwater of pilings and is approximately 15,000 m^2 (1.5 hectares) in area. Water depths in the marina varied from approximately 0 to 6 m with an average depth of 2 m.

To facilitate the establishment of control areas, a water movement study using fluorescein dye was carried out prior to herbicide treatment. Fluorescein was injected through a 100 cc syringe at a depth of 0.5 m in several areas of the marina (Figure 2). On the basis of the water movement study, two control areas were established; one north, and one south of the marina (Figure 3). Control areas supported less luxurient growths of macrophytes than the marina.

The herbicide mixture was pre-mixed with fluorescein dye on shore in a mix tank and was applied by boat to the marina water through a

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1. Chipman Chemicals (Canada) Ltd., Ste. 2601-1155 Dorchester Boulevard W., Montreal 2, Quebec.

2. ibid

hose connected to the mix tank. The dye allowed treated areas to be visually identified. The herbicide dye mixture was administered to the marina from 20:30 hours on June 3, 1975 to 02:00 hours on June 4, 1975 at a depth of approximately 0.5 m., and was applied to give a calculated herbicide concentration throughout the water column of the marina of 1.5 mg l^{-1}.

2.2.1 Phytoplankton Standing Crop

A two litre Van Dorn water sampler was used to collect water samples from a depth of 0.3 m at each of the sites shown in Figure 4. Samples were collected 24, 10 and 5 hours before herbicide treatment, and 10 hours, 20 hours, 2 days, 6 days and 19 days after herbicide treatment.

A one litre sub-sample was retained from each sample site for analysis. The sub-samples were immediately frozen and stored in the dark until analyzed at the Fisheries and Marine Service - Environmental Protection Service laboratory at West Vancouver.

Chlorophyll 'a' determinations were used as an index of phytoplankton standing crop. Samples were analyzed using the procedure outlined in Appendix I (Method 1).

2.2.2 Periphyton Standing Crop

The effect of the herbicide on periphyton was examined using artificial plexiglass substrates (18 cm x 7.3 cm x 0.3 cm). The substrates were uniformly scored with steel wool prior to being placed in the marina on May 22, 1975. Eight substrates were attached with brass screws to a wooden frame as shown in Figure 5. One frame was then attached to a permanent structure (piling or pier) at each of three treatment and control sites (Figure 4) at a depth of 0.75 m. Growth surfaces of the substrates were oriented to receive a southern exposure.

After 21 days exposure prior to the herbicide treatment, two artificial substrates were removed from each site. These substrates were replaced with two new artificial substrates which were subsequently removed after a 20 day post treatment exposure. Additional groups of two artificial substrates installed May 12 were removed from each site 1, 7 and 20 days after treatment.

All substrates were immediately frozen upon removal and stored in the dark until analyzed for chlorophyll 'a' using the method given in Appendix I (Method 2).

2.3 Invertebrate Bioassays

The effect of the herbicide on invertebrates was monitored using *in situ* bioassays.

Damselflies (*Zoniagrion exclamationis*), snails (*Lymnaea spp.*), and amphipods (*Hyalella azteca*) were collected by dip-netting in the marina, and held in bioassay cages at the control and treatment sites before the herbicide application. The containers were constructed of gray polyvinyl chloride tubing (internal diameter 8 cm, length 20 cm). Holes (2.5 cm diameter) were drilled in the sides of the tubing and covered with 100 µ mesh screen (Figure 6).

On June 2, 1975, four sites were selected in the treatment area and designated A, B, C, and D (Figure 3). One bioassay container holding 10 damselflies was placed at each of sites A and D. One container with 10 damselflies, one with 6 snails, and one with 10 amphipods was placed at each of sites B and C. Two containers, each with 10 damselflies, were placed in the north control area. One container with 10 amphipods was placed in the north control area on June 5.

2.4 Fish Bioassays

Three expanded aluminum bioassay cages (60 cm x 60 cm x 120 cm) were placed in each treatment and control area four days prior to the herbicide treatment (Figure 4). Sixty redside shiners (*Richardsonius balteatus*) and 15 prickly sculpins (*Cottus asper*) were placed in each of the cages. Ten redside shiners were removed from each cage prior to treatment and 10 were removed at intervals of 2, 6, and 20 days after the herbicide treatment. These fish were immediately frozen and retained for tissue herbicide residue analysis.

Three yellow perch (*Perca flavescens*) which were caught by seine in the marina, were also held in a cage in the marina during treatment (Figure 4).

2.5 Fish Populations

Fish were sampled in the treatment and control areas (Figure 3) with a 15 m beach seine (leading end of 12 mm stretched mesh; bunt end of 6 mm stretched mesh). Collections were made in each area four days prior to and on the day of herbicide treatment. The estimated volume of water fished per seine haul was 95 m^3. Seine hauls were also made 20 days after treatment. All the fish caught were counted, identified and divided into two size classes (greater than or less than 50 mm fork length). Subsamples of 10 fish of each species collected were retained for stomach content analysis.

2.6 Dissolved Oxygen and Temperature

Vertical temperature and dissolved oxygen profiles were taken in the water column at sites shown in Figure 4. Determinations were made every 4 hours for 2 days preceding treatment to determine the diurnal variation in dissolved oxygen and temperature. Similarly, vertical dissolved oxygen and temperature profiles were determined for several days following treatment to determine if there was any change in dissolved oxygen following treatment. Dissolved oxygen and temperature determinations were made at the surface and at 0.75 m intervals to the marina bottom using an IBC Model No. 480-051 dissolved oxygen and temperature meter.[1]

[1] International Bio-physical Corporation, 2700 Dupont Drive, Irving, California 92664.

3. RESULTS

On the morning after herbicide treatment (June 4, 1975), fluorescein was visible in the near shore waters of the control area to the north of the marina indicating that water movements had resulted in the possible contamination of the control area with "treated" marina water. However, water samples collected by the Water Investigation Branch from the control areas on June 4 did not contain detectable levels of diquat or paraquat. Plant samples collected 36 hours after treatment from the north control area contained paraquat (mean of 0.4 mg l^{-1}), indicating that the area was contaminated with herbicide (P. Newroth, pers. comm.).

3.1 Phytoplankton Standing Crop

The chlorophyll 'a' values obtained from analysis of phytoplankton samples are presented in Table 1. At all stations, values before treatment were at or below 0.4μg l^{-1} (detection limit 0.1 μgl^{-1}). Chlorophyll 'a' values for both the control and treatment stations remained less than 0.5μg l^{-1} until 19 days after treatment at which time chlorophyll 'a' in the control and treatment areas had increased to means of 0.60 and 0.85μg l^{-1} respectively.

3.2 Periphyton Standing Crop

The chlorophyll 'a' values obtained from analysis of artificial substrates are presented in Figure 7. After a 21 day exposure before herbicide treatment, mean chlorophyll 'a' values for the control and treatment stations were 0.36 and 0.29μg cm^{-2} respectively. One day after treatment, the chlorophyll 'a' levels decreased sharply in both the control and treatment areas. The mean chlorophyll 'a' levels recorded were 0.16 and 0.08μg cm^{-2} in the control and treatment areas respectively. Seven days after treatment, the mean chlorophyll 'a' values increased in the control and treatment areas to 0.31 to 0.44μg cm^{-2} respectively, declining thereafter by the 20th day to 0.16 and 0.03μg cm^{-2} respectively. Substrates placed in the control and treatment areas (C_1, T_1, Figure 7) at the time of treatment (June 3, 1975) and exposed for 20 days had mean chlorophyll 'a' values of 0.06 and 0.03μg cm^{-2} respectively.

3.3 Invertebrate Bioassays

The results of the *in situ* invertebrate bioassays are presented in Table 2.

All amphipods (*Hyalella azteca*) held in the treatment area died within 12 hours of the herbicide application, while 30% of those placed in the control area the day after treatment died within 5 days. There was no measurable effect of the herbicide treatment on the snails (*Lymnaea spp*) held in the marina. All were alive 4 days after treatment. The mortality of damselfly larvae (*Zoniagrion exclamationis*) held in cages varied between 0 and 70% in the treated area and from 30 to 50% in the control area.

3.4 Fish Bioassays

Because fish were sampled from the cages during the bioassay experiment no attempt was made to assess fish mortality. Dead fish were recorded from both control and marina cages.

The three yellow perch caught in the marina and held during the treatment showed no adverse effects from the herbicide treatment. All the perch were alive 20 days after treatment.

All the fish samples from the bioassay cages were accidently destroyed so tissue residue analysis could not be carried out.

3.5 Fish Populations

The data from seine hauls are summarized in Table 3. The fish collected mainly consisted of juvenile redside shiners (*Richardsonius balteatus*) and juvenile northern squawfish (*Ptychocheilus oregonensis*), the former being the most abundant species. Prickly sculpins (*Cottus asper*) and long nose suckers (*Catastomus catastomus*) were the next most abundant species with carp (*Cyprinus carpio*) and yellow perch (*Perca flavescens*) present in small numbers.

The "occurrence" method described by Hynes (1950) was used to demonstrate what organisms were being fed upon. Stomach contents were examined and individual food organisms sorted and identified. The number of stomachs in which each item occurred was recorded and expressed as a percentage of the total number of stomachs examined. This method indicates the percent of fish feeding on a particular organism but it gives no information on quantities or numbers of organisms (Windell, 1968). The number of stomachs analyzed and the percentage of stomachs containing food are presented in Table 4. The organisms comprising the stomach contents of the fish are presented in Table 5.

3.6 Dissolved Oxygen and Temperature

Average dissolved oxygen and temperature profiles are presented in Appendices II and III respectively. Average surface dissolved oxygen values from the control and treatment areas are depicted in Figure 8.

To test whether significant differences occurred between dissolved oxygen concentrations before treatment and after treatment in the control and marina areas, the surface data were divided into four groups:

1) before treatment in the marina,
2) after treatment in the marina,
3) before treatment in the control areas, and
4) after treatment in the control areas.

One-way analysis of variance followed by Newman-Keuls multiple range test for unequal sample size (Zar, 1974), revealed that there was not a significant difference between the control groups before and after treatment, however, the before treatment marina group was signifcantly greater than all other groups and the after treatment marina group was significantly less than all other groups ($p < 0.05$) (Table 6).

There was a slight reduction in water temperatures in the marina and control areas over the period of this study, however, by 20 days after herbicide treatment temperatures had recovered to before treatment levels.

4. DISCUSSION

Due to water movements out of the marina, which may have contaminated the control areas, there is no indication whether the results observed were caused by the herbicide or were the result of natural processes. Accordingly, any interpretation of the data and comparison of results between control and treatment sites is very difficult.

4.1 Phytoplankton Standing Crop

The low phytoplankton standing crop in the marina prior to treatment and the subsequent increase in phytoplankton standing crop 19 days after herbicide treatment is similar to observations made by other investigators studying heavily weed-infested areas (Forsberg, 1965_a; Fish, 1966; Newman and Way, 1966; Brooker and Edwards, 1973; Newbold, 1975). Fish (1966) reported an absence of phytoplankton in areas of a lake heavily infested with weeds prior to treatment with 0.5 mg l^{-1} diquat. Following treatment there was a "greatly increased population of ciliates and photosynthetic algae". Brooker and Edwards (1973) reported chlorophyll 'a' values which were low throughout the season of macrophyte growth followed by a 15 fold increase after *Myriophyllum spicatum* died in the fall.

There is some evidence that aquatic macrophytes can absorb nutrients through their stems and leaves from the surrounding water and in fact, may absorb and retain nutrients beyond their immediate needs (Littlefield and Forsberg, 1965; Forsberg, 1965_b; Fish and Will, 1966). *M. spicatum* growing in extensive patches may absorb and store dissolved nutrients making them unavailable for phytoplankton production. This could explain the greatly reduced phytoplankton levels in weeded areas prior to herbicide treatment.

Williams (1973) reported a seasonal average chlorophyll 'a' value of 5 $\mu g\ l^{-1}$ for the main body of Okanagan Lake. This value, however, can not be directly compared with chlorophyll 'a' values obtained from this study because there are undoubtedly temporal and spatial effects associated with Williams' "seasonal average".

Elevated phytoplankton chlorophyll 'a' values recorded 19 days after herbicide treatment may have been the result of nutrients being released from decaying macrophytes and/or a reduction in nutrient utilization by these macrophytes. Newroth (pers. comm.) reported that ammonia-N levels and orthophosphate levels increased dramatically shortly after the diquat/paraquat application to the Kelowna marina. High ammonia levels persisted for about 12 days while high phosphate levels persisted for only 2 days. Other investigators (Boyd, 1970; Jewell, 1970) have also reported a release of nutrients following death and decay of macrophytes.

Higher mean phytoplankton chlorophyll 'a' values recorded 19 days after treatment in the marina (0.85 as compared to 0.6$\mu g\ l^{-1}$ in the control) could be the result of more nutrients being made available in the marina following the decay of the dense growths of macrophytes. The data, however, are not extensive enough to support this hypothesis.

4.2 Periphyton Standing Crop

Periphyton chlorophyll 'a' values of 0.36 and 0.29 $\mu g\ cm^{-2}$, in control and treatment areas respectively, are comparable to those reported by Stockner *et al* in 1972 (<1.0$\mu g\ cm^{-2}$). They measured periphyton chlorophyll 'a' from the same general area of Okanagan Lake and at the same time of year as this study but not in dense weed beds.

Stockner *et al* (1972) described a seasonal periphyton "growth peak" (above average periphyton growth) in areas of Okanagan Lake receiving high nutrient inputs. Periphyton in other areas however, showed little seasonal variation. In the area of the Kelowna marina they observed increasing periphyton growth beginning in early May, peaking in late May ($\simeq$2.0μg cm^{-2}) and then falling off to <1.0μg cm^{-2} by mid-June. That peak follows the same general pattern as the one observed for this study (Figure 7) with the exception that in this study there was an immediate drop in periphyton chlorophyll 'a' the day after herbicide treatment. Way *et al* (1971) in describing the algal community (free floating, epiphytic and attached algae) of a small lake noted "protoplasmic disorganization" and death, in most species observed, the day following treatment with 0.5 mg l^{-1} paraquat. This observation suggests that the drop in periphyton chlorophyll 'a' following herbicide treatment in this study could be a response to the herbicide. The reasons for the subsequent drop observed 20 days after herbicide treatment are unknown.

4.3 Invertebrates

Several investigators have observed toxic effects of diquat and paraquat on invertebrates.

Crosby and Tucker (1966) reported median immobilization concentrations of 7.1 mg l^{-1} diquat for *Daphnia magna* and Gilderhaus (1967) found that 1.0 mg l^{-1} diquat prevented the formation of the adult stage of *Daphnia pulex*. Wilson and Bond (1969) conducted controlled laboratory bioassays on selected invertebrates and showed that at concentrations of diquat of 100 mg l^{-1} dragonfly and damselfly naiads and midge larvae were not affected after a 96 hour exposure. Amphipods, however, were very susceptable (96 hour LC_{50} = 0.48 mg l^{-1}), while mayfly nymphs were slightly more resistant (96 hour LC_{50} = 16.4 mg l^{-1}).

Paraquat concentrations of 0.6 mg l^{-1} in a small lake did not result in significant mortality to gastropods *Lymnaea peregra* or megalopteran insects *Sialis spp.* (Way *et al*, 1971). The authors did however report some mortality of crustacean larvae *Asellus spp.*

It is likely that with a calculated concentration of 1.5 mg l^{-1} diquat/paraquat in the marina, that the amphipod mortalities in this study were the result of the direct toxicity of diquat and paraquat. This is suggested by the low LC_{50} value of 0.48 mg l^{-1} reported for amphipods by Wilson and Bond (1969).

In other studies (Newman and Way, 1966; Hilsenhoff, 1966) secondary effects were reported after the application of paraquat and diquat. These included habitat destruction, the forced migration of invertebrates associated with macrophytes and mortalities caused by deoxygenation. In addition, the difficulties with sampling and the interpretation of results because of the change in habitat from heavily weeded to almost clear areas following herbicide treatment were discussed.

4.4 Fish

Gilderhaus (1967) and Yeo (1967) examined the acute toxicity of diquat to fish in controlled experiments. Gilderhaus reported 96 hour LC_{50} values of paraquat for rainbow trout (*Salmo gairdneri*) and adult bluegills (*Lepomis macrochirus*) of 11.2 mg l^{-1} and 35 mg l^{-1} respectively. In an examination of the adult bluegills, he failed to discover any histopathological changes in selected tissues after repeated exposures to 1 and 3 mg l^{-1} diquat. Similarly, an examination of hematocrit and hemoglobin levels in the bluegills did not reveal changes that could be attributed to the herbicide treatment. Yeo (1967) noted some mortality of mosquito fish (*Gambusia affinis*) after long term exposure to 1 and 4 mg l^{-1} diquat, however, mortalities were also reported in the controls. Green sunfish (*Lepomis cyanellus*) and small mouth bass (*Micropterus dolomieu*) exposed to the same concentrations were not harmed. In this study no significant mortalities occurred in the bioassay fish that could be attributed to the herbicide.

The reduction in the number of fish utilizing the marina and control areas after herbicide treatment may be the result of the destruction of invertebrate habitat. The three fish most predominant before herbicide treatment, redside shiners, northern squawfish, and long nose suckers, are all typical of nearshore waters where there is an abundance of food organisms associated with heavy growths of rooted vegetation (Scott and Crossman, 1973). After herbicide treatment and macrophyte destruction there were probably not enough invertebrates to sustain the large populations of rearing fish that were present before treatment. The majority of these fish were probably displaced and forced to compete for food in areas occupied by other populations.

An examination of northern squawfish and long nose sucker stomachs revealed a slight increase in the number of stomachs containing chironomid larvae. This is consistent with the increase in populations of benthic chironomids after herbicide treatment reported by other investigators (Fish, 1966; Hilsenhoff, 1966; Newman and Way, 1966; and Newman, 1967). Such increases in abundance are thought to be related to the increased availability of food provided by the decaying plants. Chironomids are primarily herbivorous and feed on algae, higher aquatic plants and organic detritus (Pennak, 1953). Stomach content data were too incomplete to allow any further interpretation of the feeding habits of fish before and after herbicide treatment.

Yellow perch have not previously been described this far north in the Okanagan system. Northcote, Halsey and MacDonald (1973) conducted an extensive capture program in the Okanagan Valley Lakes and collected this species only as far north as Vaseux Lake. Carl, Clemens and Lindsay (1973) also only describe yellow perch from Osoyoos and Vaseux lakes. Yellow perch are a very adaptable fish and are able to use a wide variety of warm to cooler eutrophic habitats in ponds, lakes, and slow flowing streams. They are most abundant in lakes with moderate vegetation (Scott and Crossman, 1973). The presence of this highly adaptable fish this far north in the Okanagan lakes may be an indication of the modification of the littoral areas of Okanagan Lake to habitats more suitable to fish such as yellow perch.

4.5 Dissolved Oxygen and Temperature

Several investigators (Newman and Way, 1966; Hilsenhoff, 1967; Newman, 1967) have reported depressed dissolved oxygen levels following herbicide treatment. Newman and Way (1966) reported depressed oxygen levels and fish rising to the surface in distress four days after the addition of paraquat at 0.5 mg l^{-1} to a one acre shallow lake. Dissolved oxygen levels remained depressed for up to 32 days after treatment.

The high dissolved oxygen levels recorded in the Kelowna marina prior to treatment could be due to the greater photosynthetic activity of the rooted macrophytes. Control areas, although containing weeds, were not as heavily infested as the marina and consequently dissolved oxygen levels were not as high. Following treatment, when submerged weeds were killed, the reduction in photosynthesis combined with the increased oxygen demand of the decaying vegetation may explain the recorded depression in dissolved oxygen levels in the marina.

The slight decrease in water temperature recorded after treatment was probably the result of cooler weather as well as rising lake levels due to snow melt and spring runoff. This temperature reduction would have reduced the rate of oxygen demand of the decaying vegetation and therefore would have helped minimize any oxygen depression resulting from the herbicide treatment.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Boyd, C.E., 1970. Losses of mineral nutrients during decomposition of *Typha latifolia*. Arch. Hydrobio. 66, 511-517.

Brooker, M.P. and Edwards, R.W., 1973. Effects of the herbicide paraquat on the ecology of a reservoir. I. Botanical and chemical aspects. Freshwat. Biol. 3, 157-175.

Carl, G.C., Clemens, W.A., and Lindsey, C.C., 1973. The freshwater fishes of British Columbia. British Columbia Provincial Museum Handbook No. 5.

Crosby, D.G., and Tucker, R.K., 1966. Toxicity of aquatic herbicides to *Daphnia magna*. Science 154: 289-290.

Culling, C.F.A., 1957. Handbook of histopathological techniques. Butterworth and Company Limited, London, England, 446 p.

Fish, G.R., 1966. Some effects of the destruction of aquatic weeds in Lake Rotoiti, New Zealand. Weed Res., 6, 350-358.

Fish, G.R., and Will, G.M., 1966. Fluctuations in the chemical composition of two lake weeds from New Zealand. Weed Res., 6, 346-349.

Forsberg, C., 1965a. Environmental conditions of Swedish charophytes. Symb. bot. upsal, 18(4), 1-67.

Forsberg, C., 1965b. Nutritional studies of chara in axenic cultures. Physiologia Pl., 18, 275-290.

Gilderhaus, P.A., 1967. Effects of diquat on bluegills and their food organisms. Prog. Fish. Cult. 29, 67-74.

Hilsenoff, W., 1966. Effect of diquat on aquatic insects and related animals. J. econ. Ent. 59, 1520-1521.

Hynes, H.B.N., 1950. The food of freshwater sticklebacks *Gasterosteus aculeatus* and *Pygosteus pungitius* with a review of methods used in studies of the food of fishes. Jr. anim. Ecol. 19,1, 36-58.

Jewell, W.J., 1970. Aquatic weed decay, dissolved oxygen utilization and nitrogen and phosphorous regeneration. J. Wat. Poll. Control, Fed. 43, 1467-1475.

Littlefield, L., and Forsberg, C., 1965. Absorption and translocation of phosphorous - 32 by *Chara globularis* Thuill. Physiologia Pl. 18, 291-296.

Newbold, C., 1975. Herbicides in aquatic systems. Biol. Conserv. (7) 1975.

Newman, J.F., 1967. The ecological effects of paraquat and diquat when used to control aquatic weeds. Proc. Br. Insect. Fungic. Conf. 4th, 45-47.

Newman, J.F. and Way, J.M., 1966. Some ecological observations on the use of paraquat and diquat as aquatic herbicides. Proc. Br. Weed Control, Conf. 8th, 582-585.

Northcote, T.G., Halsey, T.G., and MacDonald, S.J., 1972. Fish as indicators of water quality in the Okanagan basin lakes, British Columbia. Okanagan Basin Study Committee Prelim. Rep. No. 22.

Okanagan Basin Implementation Agreement 1976: agreement between the Government of Canada and Government of British Columbia, signed February 9, 1976.

Pennak, R.W., 1953. Fresh-water invertebrates of the United States. The Ronald Press Company, New York, 769 pp.

Scott, W.B. and Crossman, E.J., 1973. Freshwater fishes of Canada, Bulletin 184, Fisheries Research Board of Canada, Ottawa, 1973.

Stockner, J.G., Pomeroy, M., Carney, W. and Finlay, D., 1972. Studies of periphyton in lakes of the Okanagan Valley, British Columbia. Okanagan Basin Study Committee Prelim. Rept. No. 33, 23 pp.

Way, J.M., Newman, J.F., Moore, N.W., and Knaggs, F.W., 1971. Some ecological effects of the use of paraquat for the control of weeds in small lakes. J. appl. Ecol. 8: 509-532.

Williams, D.J., 1973. General limnology of the Okanagan mainstem lakes. Okanagan Basin Study Committee Prelim. Rep. No. 19: 14 pp.

Wilson, D.C., and Bond, C.E., 1969. The effects of the herbicides diquat and dichlobenil (Carson) on pond invertebrates. Part 1. Acute toxicity. Trans. Amer. Fish. Soc., 1969, No. 3, 438-443.

Windell, J.T., 1968. Food analysis and rate of digestion, in Methods for assessment of fish production in fresh waters, International Biological Programme Handbook No. 3. W.E. Ricker, ed. Blackwell Scientific Publications, Oxford and Edinburgh.

Yeo, R.R., 1967. Dissipation of diquat and paraquat, and effects on aquatic weeds and fish. Weeds 15, 42-46.

Zar, J.H., 1974. Bio statistical analysis. Prentice Hall, Englewood Cliffs, New Jersey. 620 pp.

Figure 1 KELOWNA BOAT MARINA ON OKANAGAN LAKE

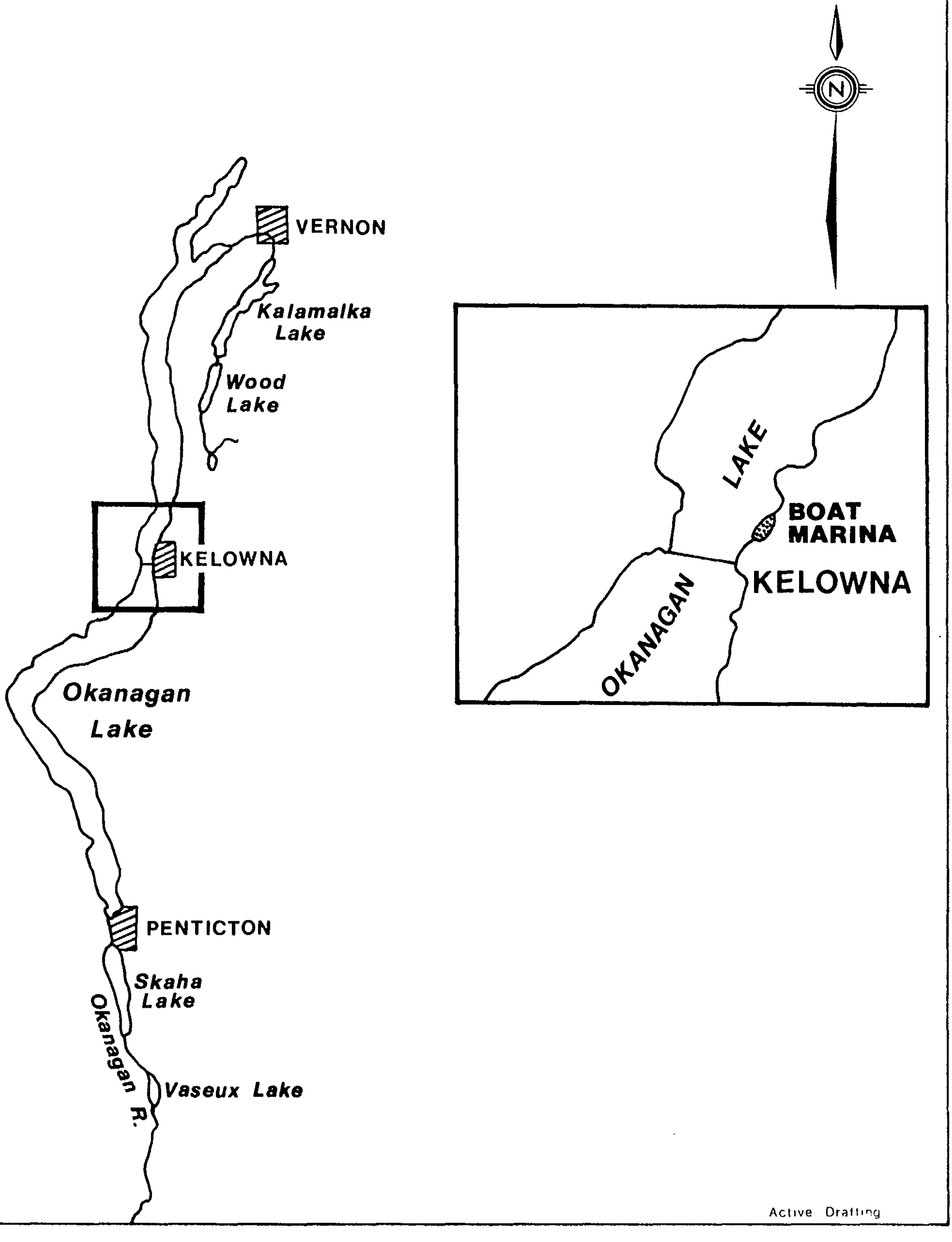

Figure 2 DIAGRAM OF THE KELOWNA BOAT MARINA INDICATING POSITION OF DYE INJECTION FOR WATER MOVEMENT STUDY

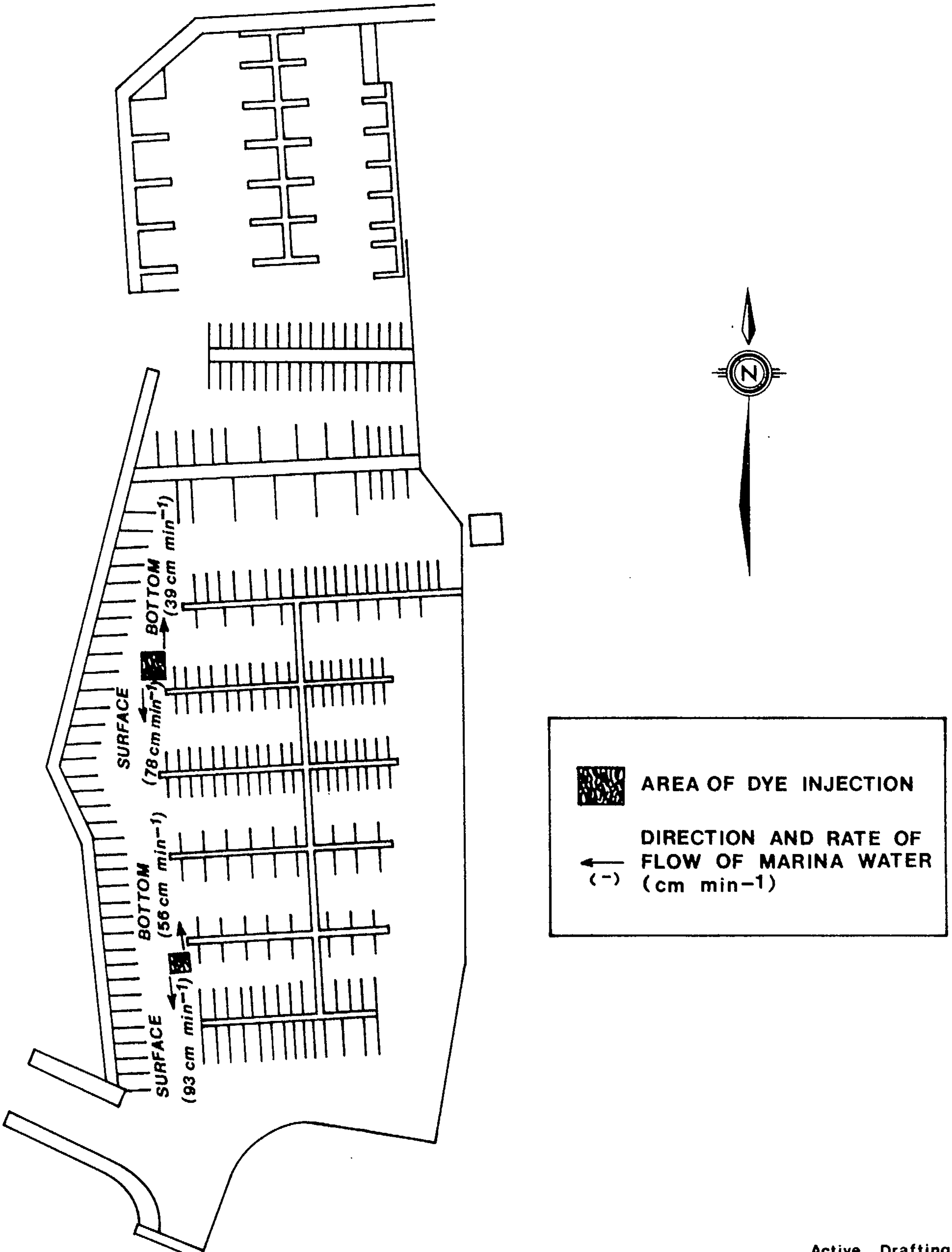

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Figure 3 DIAGRAM OF THE KELOWNA BOAT MARINA SHOWING CONTROL AREAS, INVERTEBRATE BIOASSAY AND SEINING SITES

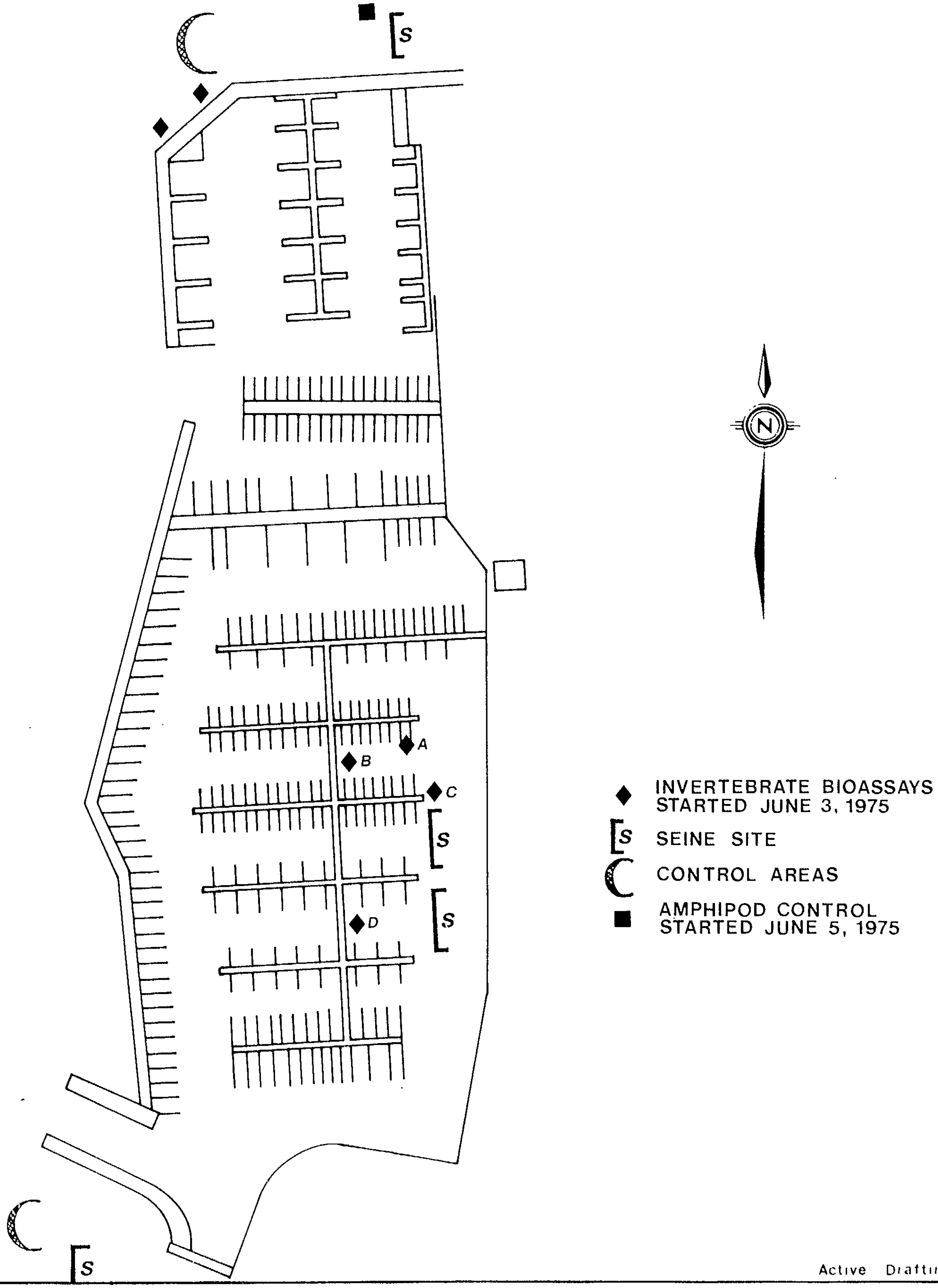

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Figure 4 DIAGRAM OF THE KELOWNA BOAT MARINA SHOWING FISH BIOASSAY, DISSOLVED OXYGEN, PERIPHYTON AND PHYTOPLANKTON SITES

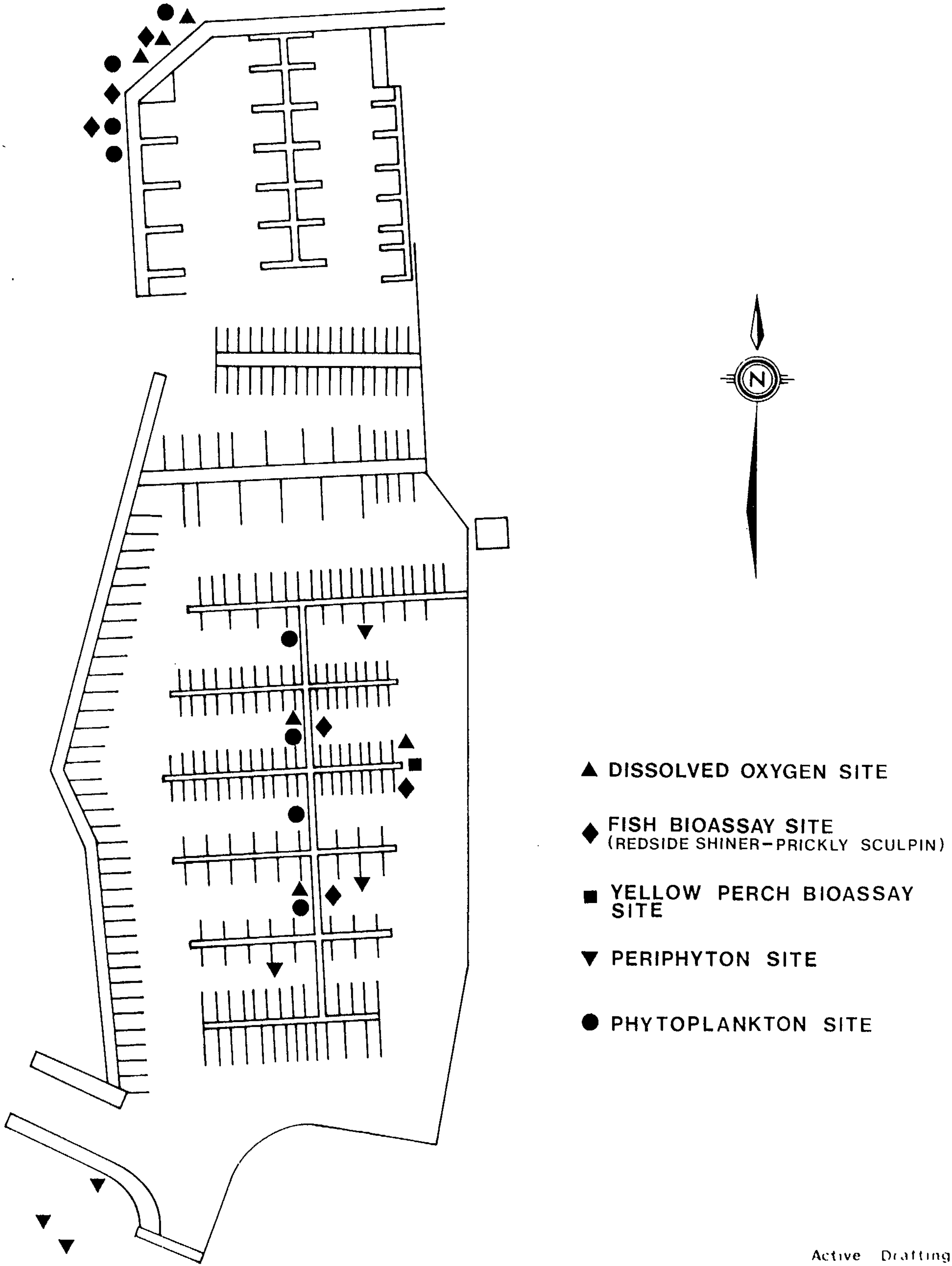

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Figure 5 - Artificial substrates used for periphyton growth analysis

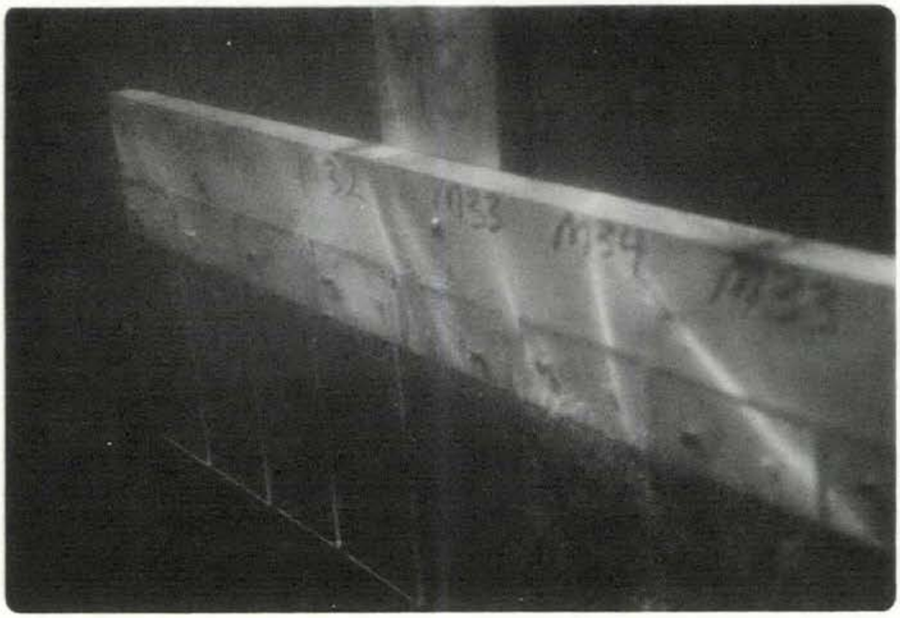


Figure 6 - Invertebrate bioassay container

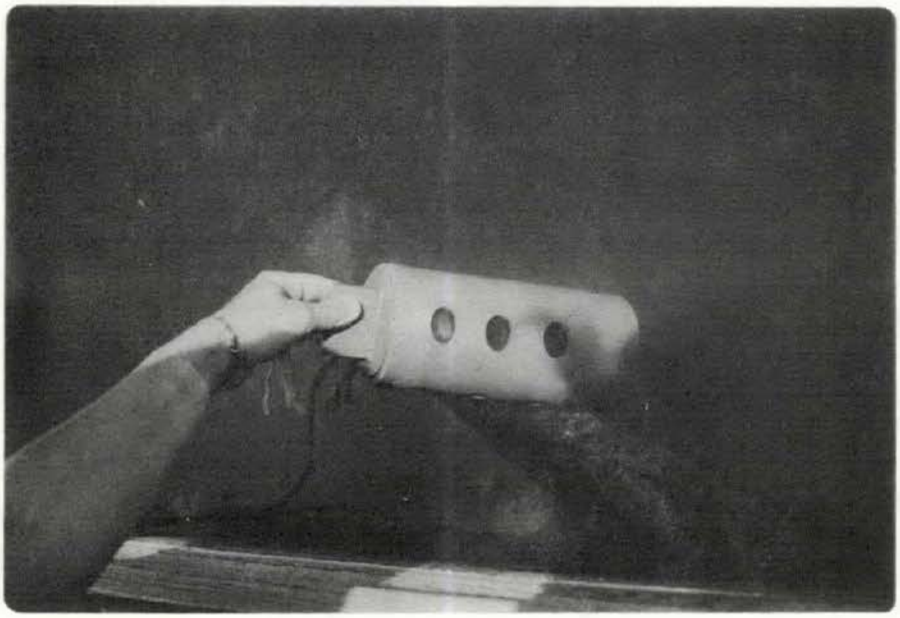


Figure 7 MEAN (n=6) PERIPHYTON CHLOROPHYLL 'a' ($\mu g\ cm^{-2}$) FROM ARTIFICIAL SUBSTRATES, BEFORE AND AFTER TREATMENT

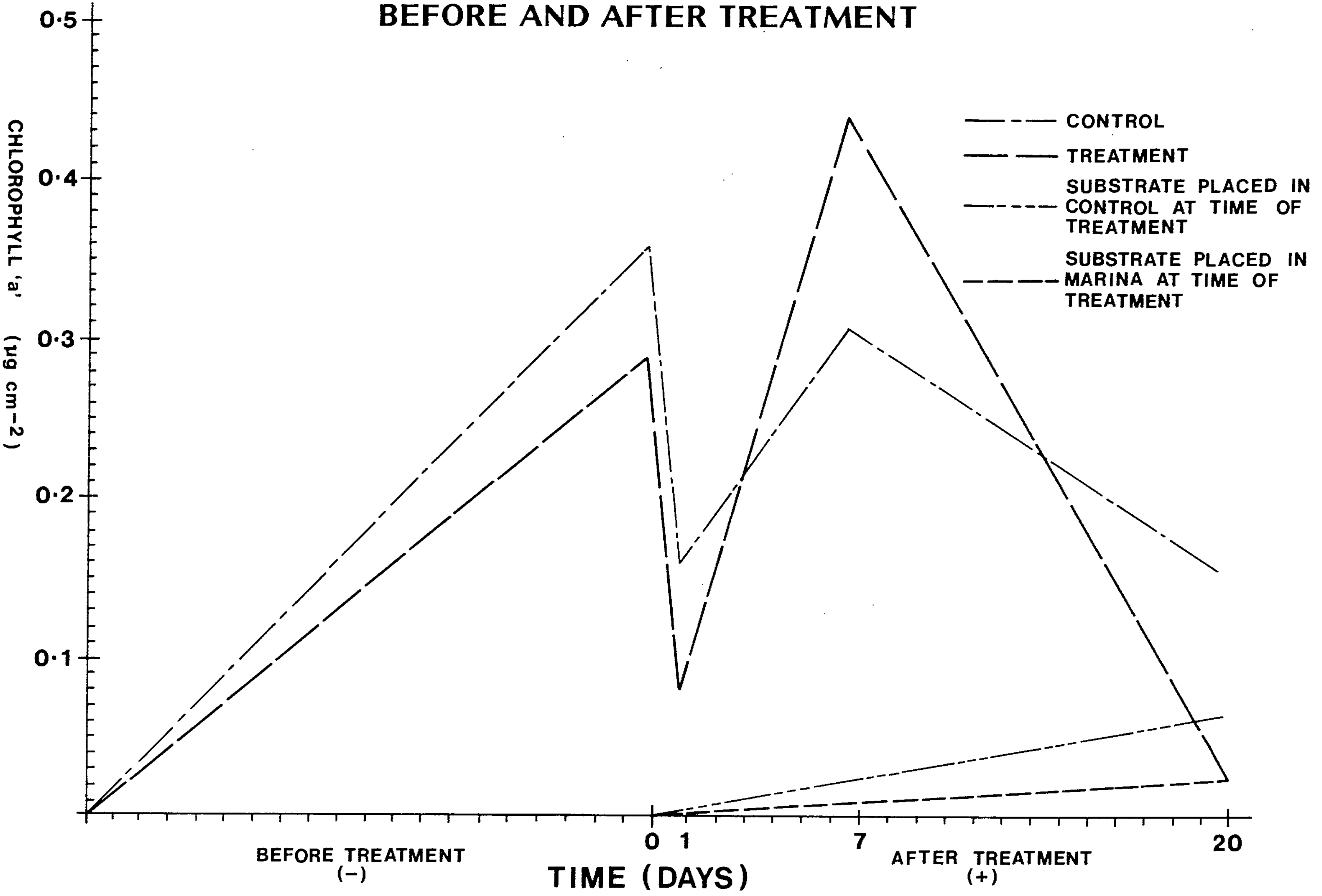


Figure 8 MEAN SURFACE DISSOLVED OXYGEN (PERCENT SATURATION) VS. TIME (DAYS) FROM TREATMENT AND CONTROL AREAS

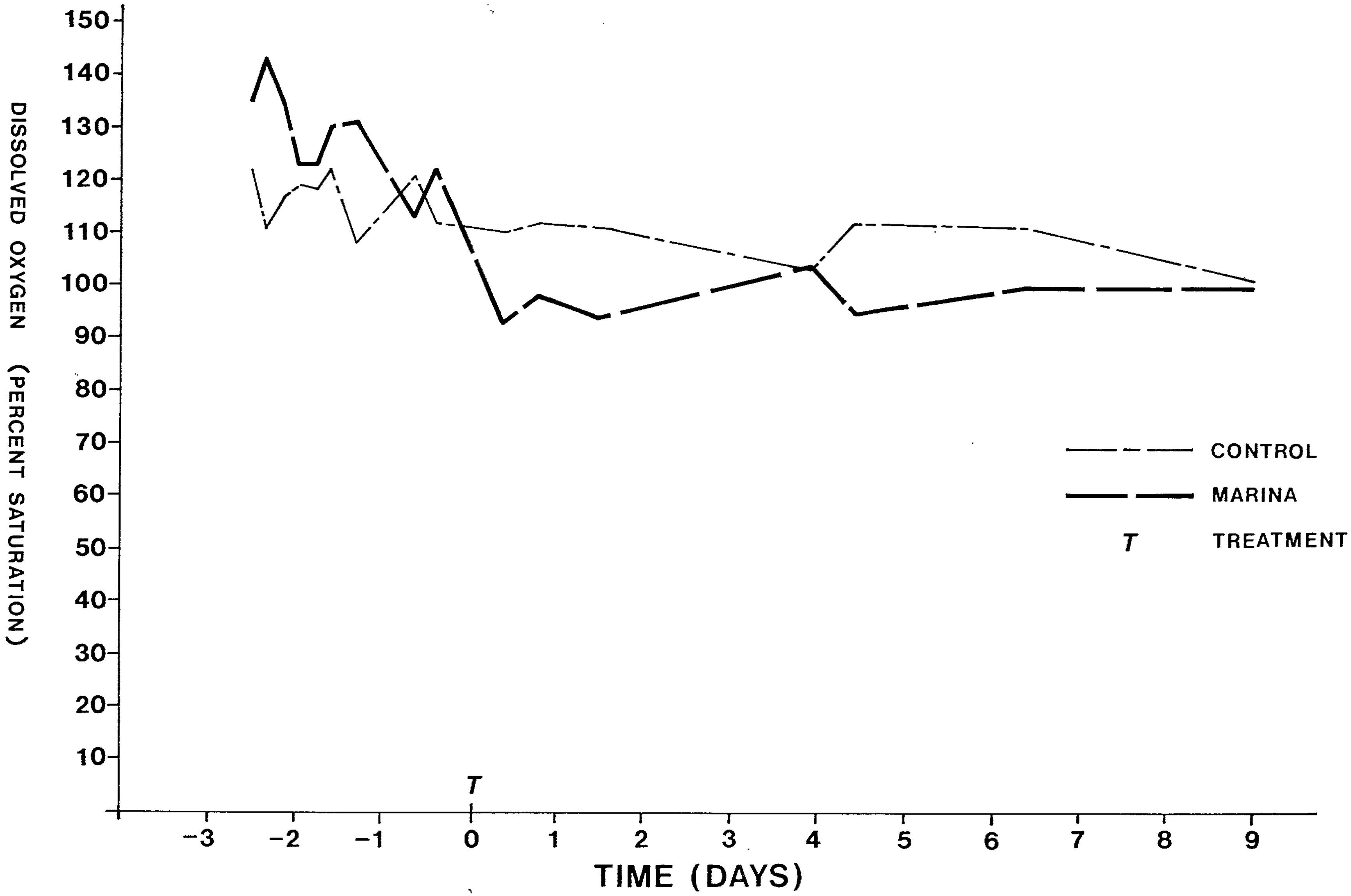


Table 1: Mean phytoplankton chlorophyll 'a' values ($\mu g\ l^{-1}$) from marina and control sites before (-) and after (+) herbicide treatment.

	Marina					Control				
Time	1	2	3	4	$\bar{x}$	1	2	3	4	$\bar{x}$
-24 hours	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
-10 hours	<0.1	0.3	<0.1	<0.1	<0.1	<0.1	0.4	<0.1	<0.1	<0.1
-5 hours	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
+10 hours	<0.1	<0.1	<0.1	0.5	<0.1	<0.1	<0.1	0.5	<0.1	<0.1
+20 hours	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
+ 2 days	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
+ 6 days	*	*	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
+19 days	*	*	0.8	0.9	0.9	0.7	0.6	0.5	0.6	0.6

*sample was not analyzed

Table 2: Mortality of invertebrates in bioassay experiments one day after herbicide treatment (June 4) and 5 days after herbicide treatment.

	SITE	INVERTEBRATES	INITIAL NUMBER OF INVERTEBRATES	% MORTALITY	
				June 4	June 8
MARINA	A	*Zoniagrion exclamationis*	10	0	30
	B	*Hyalella azteca*	10	100	100
		Zoniagrion exclamationis	10	0	0
		Lymnaea spp.	6	0	0
	C	*Hyalella azteca*	10	100	100
		Zoniagrion exclamationis	10	0	10
		Lymnaea spp.	6	0	0
	D	*Zoniagrion exclamationis*	10	0	70
CONTROL	I	*Zoniagrion exclamationis*	10	0	30
	II	*Zoniagrion exclamationis*	10	0	50
	III*	*Hyalella azteca*	10	JUNE 5	JUNE 10
				0	30

* Control III started June 5, 1975

Table 3: Total number of fish caught before and after herbicide treatment at control and marina sites.

MARINA

FISH	fish length	BEFORE TREATMENT				AFTER TREATMENT		
		May 12	May 31			June 23		
Richardsonius balteatus	< 50 mm	89	1080	802	493	0	29	0
	> 50 mm	9	93	73	66	0	56	0
Ptychocheilus oregonensis	< 50 mm	2	198	106	222	0	0	0
	> 50 mm	0	20	59	51	4	79	1
Cottus asper	< 50 mm	49	78	60	25	2	12	17
	> 50 mm	3	5	0	0	0	2	0
Catostomus catostomus	< 50 mm	3	48	123	84	0	2	0
	> 50 mm	3	10	19	11	0	4	0
Cyprinus carpio	-	0	1	0	1	0	0	0
Perca flavescens	-	0	0	0	3	0	0	0

CONTROL

FISH	fish length	BEFORE TREATMENT		AFTER TREATMENT			
		June 3		June 24			
		NORTH CONTROL	SOUTH CONTROL	NORTH CONTROL		SOUTH CONTROL	
Richardsonius balteatus	< 50 mm	16	143	0	1	33	34
	> 50 mm	48	3172	0	0	58	393
Ptychocheilus oregonensis	< 50 mm	4	26	0	0	1	0
	> 50 mm	42	78	0	0	6	18
Cottus asper	< 50 mm	21	0	5	3	0	6
	> 50 mm	1	0	1	0	0	11
Catostomus catostomus	< 50 mm	1	0	0	0	0	0
	> 50 mm	6	13	0	0	0	0
Cyprinus carpio	-	0	0	0	0	0	0
Perca flavescens	-	0	0	0	0	0	0

Table 4: Total number (No.) of fish stomachs analyzed and percentage of stomachs which contained food. Fish were caught before the herbicide treatment on June 3, and after the herbicide treatment on June 23.

	BEFORE TREATMENT				AFTER TREATMENT			
	CONTROL		MARINA		CONTROL		MARINA	
	No.	%	No.	%	No.	%	No.	%
Cottus asper	10	100	0	-	6	100	39	100
Ptychocheilus oregonensis	18	83	30	63	11	63	18	100
Richardsonius balteatus	34	88	10	60	10	100	10	100
Catostomus catostomus	0	-	12	75	0	-	5	100

Table 5: Stomach content of fish caught in the marina and control areas before (June 3) and after (June 23) herbicide treatment. Values represent the percentage of fish stomachs containing each food item.

	Richardsonius balteatus				*Ptychocheilus oregonensis*				*Cottus asper*				*Catostomus catostomus*			
	n= 10 A	n= 10 B	n= 34 C	n= 10 D	n= 30 A	n= 18 B	n= 18 C	n= 11 D	n= 0 A*	n= 39 B	n= 10 C	n= 6 D	n= 12 A	n= 5 B	n= 0 C*	n= 0 D*
ALGAE																
EGGS		10														
NEMATODA		10								5				60		
ANNELIDA																
Oligocheata											20					
MOLLUSCA																
Gastropoda										5	10	17				
ARTHROPODA																
Crustacea																
Branchiopoda																
Cladocera		20		40	30	28				10		17	41	20		
Ostracoda										3				80		
Copepoda																
Cyclopoida				10	20	11				26	40	17	17			
Harpacticoida														40		
Malacostraca																
Cumacea																
Amphipoda					13					10	10	33				
Insecta			6													
Pterygota																
Ephemeroptera										10	10					
Odonata			3			6	17			46	20	17				
Trichoptera							6			3						
Diptera							6									
Chironomidae					3	22				90	70	83		40		
Arachnida																
Acarina				10		6	22			5						
Completely digested	60	30	73	40	30	39	28	63			10		50			

A Before Treatment (marina) B After Treatment (marina) * no stomachs
C Before Treatment (control) D After Treatment (control) analyzed

Table 6: Analysis of Variance comparison of mean surface dissolved oxygen (percent saturation) between the marina and control, before and after treatment and results of Newman-Keuls multiple range test.

Analysis of Variance

	Mean	Std. Dev.	Sample Size	F-Value
1. Before Treatment Marina	12.454	0.895	28	71.412^{***}
2. After Treatment Marina	9.719	0.495	21	
3. Before Treatment Control	11.248	0.526	25	
4. After Treatment Control	10.932	0.518	19	

Significant level $^{***}p < 0.001$

Newman-Keuls Multiple Range Test
for Unequal Sample Size ($p < 0.05$)

	2	4	3	1
Mean	9.719	10.932	11.248	12.454

Solid lines under groups indicate homogenous subsets ($p < 0.05$)

APPENDIX I - METHOD 1

CHLOROPHYLL a, b, c & CAROTENOIDS

REAGENTS

Acetone

Shake 900 mls good grade reagent acetone with a little granular anhydrous sodium carbonate and decant directly.

90% acetone

900 mls acetone treated as above and made up to 1 liter with distilled water.

PROCEDURE

KEEP SAMPLES OUT OF SUNLIGHT AND DO AS MUCH AS POSSIBLE IN SUBDUED LIGHT. GLASSWARE MUST BE ACID FREE.

1. Place sample membrane filter into a 15 ml grad. centrifuge tube.
2. Add approx. 8 mls of 90% acetone, stopper and shake vigorously until the membrane filter dissolves.
3. Place tube in refrigerator in complete darkness for about 20 hours. (Use reagent refrigerator as it is not opened often.) After 2 or 3 hours, shake once more.
4. After 20 hours, take out and warm to room temperature in a dark place.
5. Make up extract to exactly 10 mls and centrifuge for 10 minutes at 3,000 - 4,000 RPM.
6. Decant the clear supernatant into 5 cm cell and run on scan spec. reading at λ7500, 6650, 6450, and 5300 A.

CALCULATIONS

The Parsons and Strickland equation is based on a 10 cm light path. This is corrected for a 5 cm light path in the computer program. The absorbance reading at λ7500 is for turbidity correction.

e.g. E_{6650} = absorb. at λ6650 - λ7500 A

Chlorophyll a = $11.6E_{6650} - 1.31E_{6450} - 0.14E_{6300}$

Chlorophyll b = $20.7E_{6450} - 4.34E_{6650} - 4.42E_{6300}$

Chlorophyll c = $55E_{6300} - 4.64E_{6650} - 16.3E_{6450}$

Carotenoids = $4.0E_{4800_3}$ (predominantly Chlorophyta or Cyanophyta in fresh water)

Results reported as mg/m^3 if 1 liter sample used.

If sample volumes vary

$$mg/m^3 = \frac{\text{value calculated as above}}{\text{volume of sample filtered in liters}}$$

As there are several equations it is important to indicate in the results that the Parsons and Strickland equation was used.

APPENDIX I - METHOD 2

CHLOROPHYLL a, b, c & CAROTENOIDS

Extraction Using a Tissue Grinder

REAGENTS

Same as Method 1.

PROCEDURE

Apparatus

Tissue grinder with approx. 20 ml capacity. Wrap black tape around the exterior surface area. Wrap a large wad of masking tape around handle to prevent blisters.

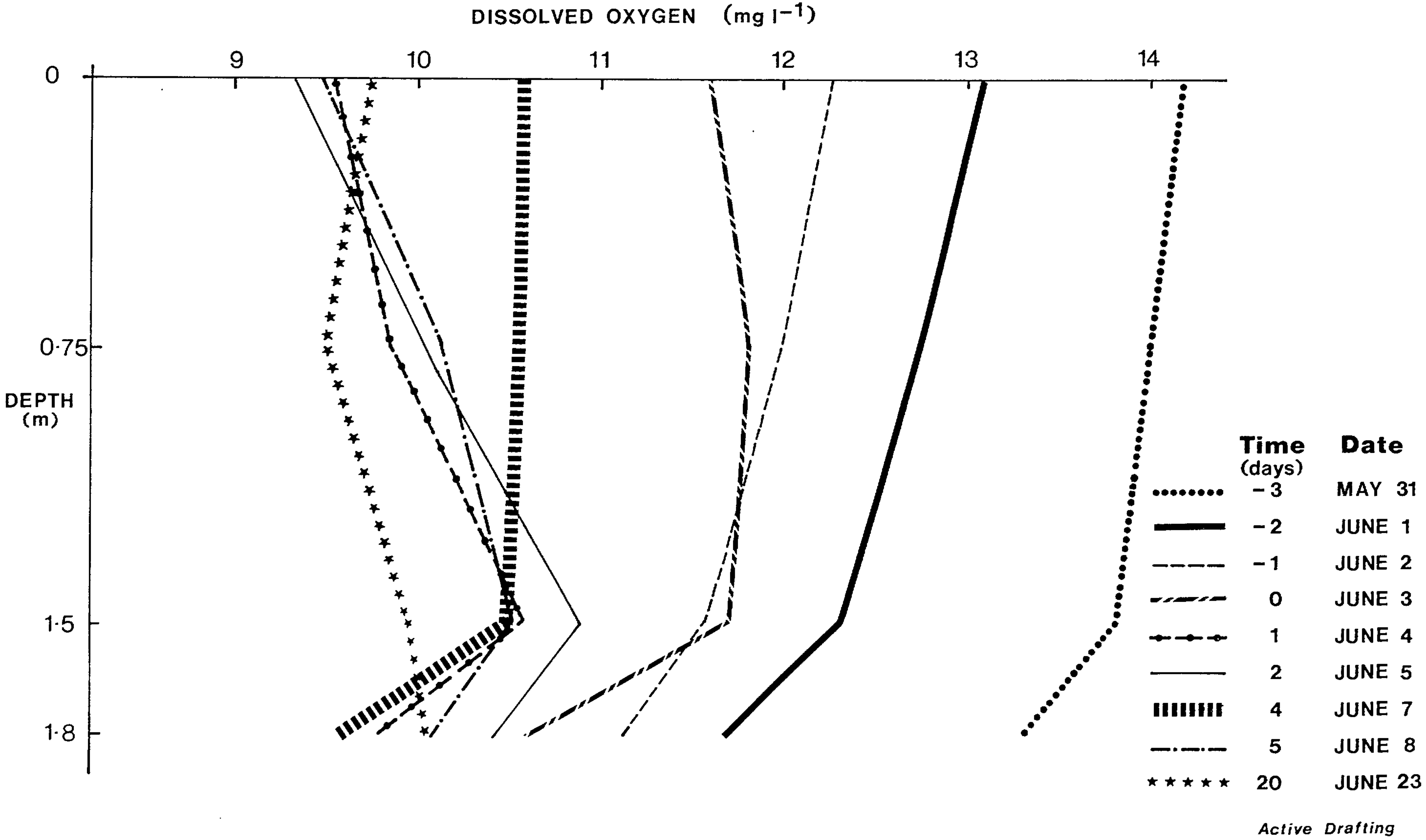
KEEP SAMPLE OUT OF DIRECT SUNLIGHT AND DO AS MUCH AS POSSIBLE IN SUBDUED LIGHT. GLASSWARE MUST BE ACID FREE.

1. Fold sample filter and place inside tissue grinder.
2. Add 2 - 3 mls 90% acetone.
3. Grind for a few minutes by pushing tube hard against the bottom of the pestle occasionally pushing up and down.
4. Rinse sample into 15 ml graduated centrifuge tube and make up to approx. 8 mls. Stopper.
5. Place sample in the refrigerator for 3 hours.
6. Bring to room temperature and complete as in Method 1.

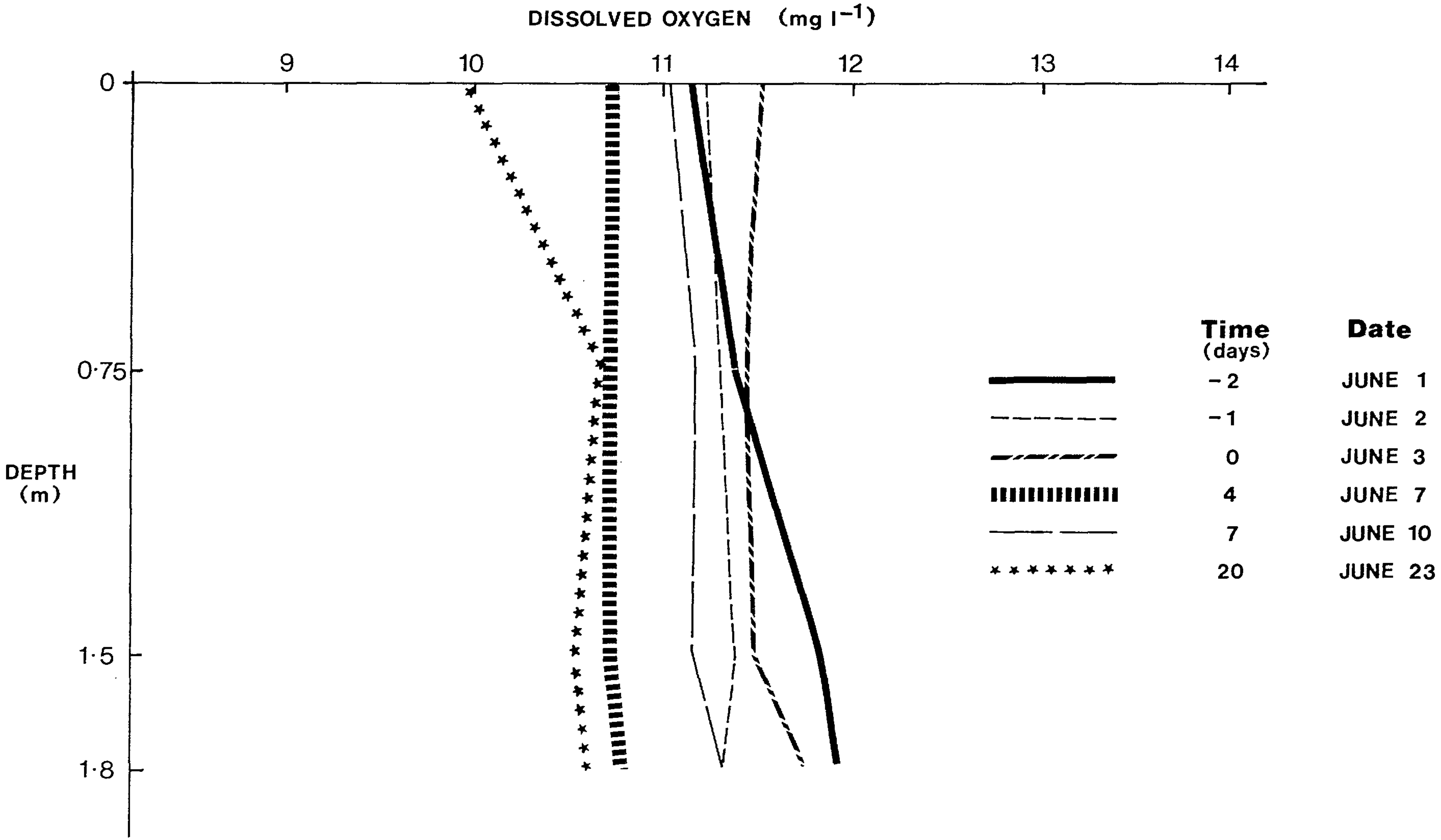
NOTES

1. This method can help obtain better chlorophyll extraction.
2. Extracts may be kept at room temperature for several hours if kept in complete darkness.
3. The disadvantage is the amount of technician time required.

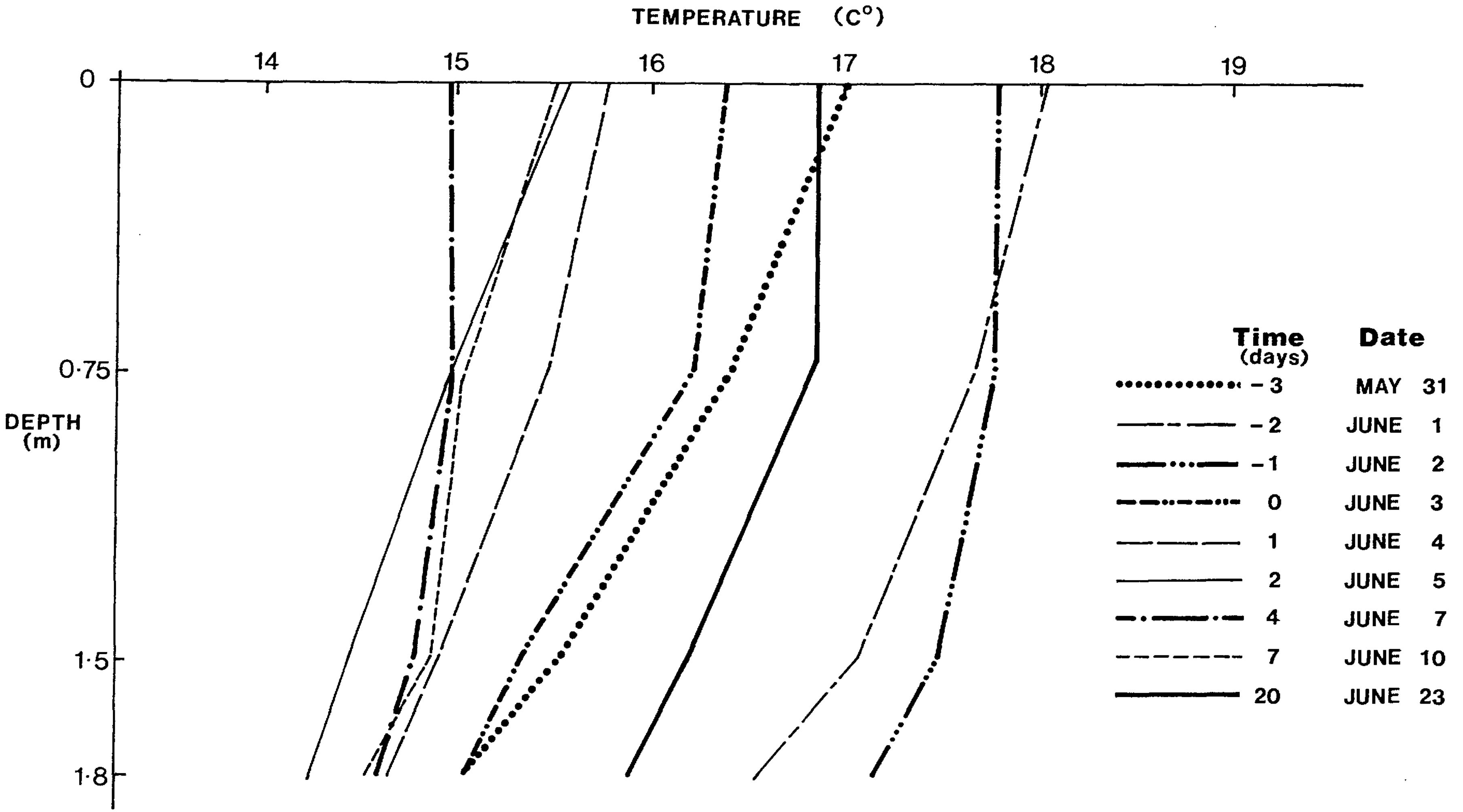
Appendix II A MEAN DISSOLVED OXYGEN (mg l^{-1}) PROFILES FROM MARINA AREAS BEFORE AND AFTER TREATMENT



Appendix II B MEAN DISSOLVED OXYGEN (mg l^{-1}) PROFILES FROM CONTROL AREAS BEFORE AND AFTER TREATMENT



Appendix III A MEAN TEMPERATURE (C°) PROFILES FROM MARINA AREAS BEFORE AND AFTER TREATMENT



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Appendix III B MEAN TEMPERATURE (C°) PROFILES FROM CONTROL AREAS BEFORE AND AFTER TREATMENT