Effect of Acid Precipitation on Heterotrophic Nitrification in Canadian Precambrian Shield Lakes

by

Tat-Yee Tam

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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A B S T R A C T

The *in vitro* and *in situ* production of nitrite (NO_2^-) and nitrate (NO_3^-) in waters from an artificially acidified and a nonacidified lake in the Experimental Lakes Area (ELA) were studied. The south basin of Lake 302 (L.302S) has been acidified with sulfuric acid (H_2SO_4) since 1982 whereas Rawson Lake (L.239) was used as a control lake for the present study. Heterotrophic nitrification was identified as the sole bacterial process responsible for the production of NO_2^- and NO_3^- in the two lakes.

The presence of the autotrophic nitrification inhibitor, allylthiourea, did not inhibit NO_2^- and NO_3^- production in the water samples. The addition of ammonium sulfate [$(NH_4)_2SO_4$] did not stimulate and in some cases depressed the production of NO_2^- and NO_3^-. The addition of sodium acetate to give a final C:N ratio of 3:1 stimulated NO_2^- production. *In situ* studies involving ^{15}N labeling of the NH_4^+ pool indicated that NO_2^--N and NO_3^--N were not derived from NH_4^+-N. Furthermore, the production and disappearance of NO_2^- and NO_3^- were always accompanied by increases in pH to as high as 2·0 units. Autotrophic nitrifying bacteria were not found in any water samples from the two lake studied. During the course of *in vitro* heterotrophic nitrification, N_2O was produced.

Heterotrophic nitrification occurred throughout the water column in both lakes during the ice-cover periods. The rates of heterotrophic nitrification in the acidified lake, L.302S, were much greater than those in the control lake, L.239. *In vitro* studies showed that highest

rates of net NO_3^- production occurred in March at the 9-m depth of L.302S and the 30-m depth of L.239, being 128·75 and 19·2 ug NO_3^--N·L^{-1}·d^{-1}, respectively.

Several heterotrophic nitrifying bacteria were isolated from the two lakes. Among them, Pseudomonas fluorescens was the most predominant heterotrophic nitrifying species present. P. fluorescens was capable of growth on Nitrosomonas and Nitrobacter media with alkaline and acidic pH and at low temperatures. The significance of heterotrophic nitrification in the lake acidification process is discussed.

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INTRODUCTION

INTRODUCTION

Acid precipitation could have dramatic effects in lakes and streams (EPA, 1980). Acidification of freshwaters and loss of fish populations of lakes in at least 33,000 km of southern Norway have been reported (Overrein et al., 1980). Approximately 40,000 lakes in eastern Canada have been affected by acid precipitation and over 22% of them received greater than 20 kg $SO_4 \cdot ha^{-1} \cdot yr^{-1}$ (Harvey et al., 1981). The main components of atmospheric pollutants are NOx and SO_2 (Evans et al., 1981; Tamm, 1976). In Canada, it has been estimated that the natural and anthropogenic emissions of SO_2 alone were $2 \cdot 0$ and $2 \cdot 4 \times 10^{12}$ $g \cdot yr^{-1}$, respectively (Harvey et al., 1981). Morling (1981) studied the effects of acidification on several lakes in western Sweden for 14 years and noticed the decrease in pH closely followed the increase in SO_4 concentrations in the lake waters. Acid rain in Bermuda has been reported to be attributed to H_2SO_4 formed from SO_2 pollutants in the precipitation (Jickells et al., 1982).

Most previous studies indicated that HNO_3 did not promote long-term acidification of aquatic ecosystems as compared to H_2SO_4 and that NO_3^- was usually assimilated by biota in both terrestrial and aquatic ecosystems (Driscoll and Likens, 1982; Galloway et al., 1983; Likens et al., 1977). However, the recent projected increase of NOx emissions is a cause for concern if they lead to an excess of NO_3^- over base cations in the precipitation (Galloway and Likens, 1981). The total emissions of NOx and NH_3 in Canada have been estimated to be about 5 and 7×10^{11} g

$\cdot yr^{-1}$, respectively (Harvey et al., 1981). The source of NH_3/NH_4^+ mainly derives from agricultural fertilizers and animal wastes (Gorham et al., 1984). NH_3/NH_4^+ because of its acid-neutralizing effects has not been considered as an air pollutant contributing to acidification in ecosystems. However, autotrophic nitrification, a bacterial process oxidizing reduced nitrogen compounds such as NH_4^+ to NO_2^- and NO_3^-, produces a considerable amount of acidity (Harvey et al., 1981). Van Breeman et al. (1982) reported that NH_4^+ in the atmosphere reacted with SO_2 to form $(NH_4)_2SO_4$ and that the atmospheric $(NH_4)_2SO_4$ in the forest canopy throughfall resulted in soil acidification. They found that the acid inputs to the forest soils were 2-5 times more than that due to acid precipitation with H_2SO_4 and HNO_3 alone. They further noticed that $(NH_4)_2SO_4$ was rapidly oxidized by soil microorganisms to HNO_3 and H_2SO_4 producing extremely low pH values (2·8-3·5) and high concentrations of dissolved aluminium in the noncalcareous soils. In a red Alder ecosystem, acid production during nitrification caused a 15% decline of the soil buffering capacity (Van Miegroet and Cole, 1984). Kelly et al. (1982), based on hypothetical acid depositions for the Lakes 226N, 227 and 223 in the Experimental Lakes Area (ELA), speculated that the H^+ inputs into these noncalcareous Precambrian Shield lakes potentially derived from NH_4^+ oxidation could be substantial. Alexander et al. (1960) have defined nitrification as a biological conversion of the nitrogen in organic or inorganic compounds from a reduced to a more oxidized state. Autotrophic nitrification produces one mole of H^+ per mole of NH_4^+ oxidized whereas heterotrophic nitrification produces alkalinity (Verstraete and Alexander, 1973).

In ELA, accumulation of NO_2^- and NO_3^- following the diffusion of NH_4^+ from anoxic sediments to the water column during the ice-cover periods in both artificially acidified and nonacidified lakes has been observed repeatedly for many years. On the other hand, it is known that autotrophic nitrification is inhibited or at least the rate is very much retarded under acidic conditions (Painter, 1970; Strayer et al., 1981). The present study was conducted to confirm if nitrification was indeed responsible for the production of NO_2^- and NO_3^- in an artificially acidified and a nonacidified lake at ELA. In addition, the types of nitrification processes involved, the potential and the in situ rates of nitrification in the lakes were also determined.

LITERATURE REVIEW

L I T E R A T U R E R E V I E W

The biological oxidation of inorganic ammonium compounds to nitrite and nitrate in soils is termed nitrification (Schlöesing and Müntz, 1877). It involves two sequential overall reactions (Equation 1):

$$\underset{\text{(ammonia)}}{NH_3} \longrightarrow \underset{\text{(nitrite)}}{NO_2^-} \longrightarrow \underset{\text{(nitrate)}}{NO_3^-} \qquad \text{(Equation 1)}$$

oxidation of NH_3 to NO_2^- followed by oxidation of the NO_2^- to NO_3^- (Waring-ton, 1884). Both reactions are zero-order with the oxidation of NH_3 to NO_2^- being the rate-limiting step (Wong-Chong and Loehr, 1975). Nitroso-monas and Nitrobacter, the two representative nitrifying bacteria responsible for the two oxidation steps respectively, were the first nitrifiers isolated from soil by Winogradsky (1890). They are considered to be very important in nitrification and most predominant in both soils and freshwater habitats (Belser, 1979; Schmidt, 1978; Watson and Water-bury, 1971) when compared to other genera of nitrifiers that were sub-sequently isolated and studied. However, there is some recent evidence that in some natural ecosystems Nitrosolobus (Walker, 1978) and Nitro-sospira species (Martikainen and Nurmiaho-Lassila, 1985) are dominant over Nitrosomonas.

Five genera of NH_3-oxidizing bacteria (Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrosospira and Nitrosolobus) and three genera of NO_2^--

oxidizing bacteria (Nitrobacter, Nitrococcus and Nitrospina) are now classified under the family Nitrobacteraceae (Belser, 1979; Bremner and Blackmer, 1981; Watson et al., 1983). All of them are chemoautotrophs. They derive their energy from oxidation of inorganic nitrogen compounds such as NH_3 and NO_2^- using O_2 as electron acceptor and obtain their carbon source from fixation of inorganic carbon such as CO_2, HCO_3^- and $CO_3^=$ (Watson et al., 1983). Nitrosomonas and Nitrobacter, respectively, could fix up to 86 and 23 nmol HCO_3^- per mole substrate oxidized (Belser, 1984). Although some NH_3- and NO_2^--oxidizing bacteria possess certain degree of heterotrophic growth potentials (Bock et al., 1983; Clark and Smith, 1966; 1967a; 1967b; Delwiche and Finstein, 1965; Krümmel and Harms, 1982; Smith and Hoarse, 1968; Watson and Waterbury, 1971), their growth rates and nitrifying activities are mostly insignificant when compared to their autotrophic mode of growth (Bock, 1978; Bock et al., 1983; Krümmel and Harms, 1982).

(1) PATHWAYS AND ENZYMOLOGY OF AUTOTROPHIC NITRIFICATION

(A) Ammonia Oxidation

The pathway of NH_3 oxidation to NO_2^- by Nitrosomonas europaea has been studied extensively (Equation 2). The oxidation of NH_3 to NH_2OH is an endothermic reaction, ΔF=4·7 kcal (Aleem and Nason, 1963). The reaction is catalyzed by the enzyme, NH_3 oxidase, which is a mixed-function oxy-

$$NH_3 \longrightarrow NH_2OH \longrightarrow NOH \longrightarrow NO_2^- \qquad \text{(Equation 2)}$$

(ammonia) (hydroxylamine) (nitroxyl) (nitrite)

genase or monooxygenase (Aleem and Nason, 1963; Suzuki, 1974). This Cu^{++}-containing enzyme is extremely sensitive to metal chelators. N-Serve [nitrapyrin or 2-chloro, 6(trichloromethyl) pyridine] and allylthiourea at 1·0 ppm and 50 uM, respectively, completely inhibit NH_3 oxidation by fresh cells of Ns. europaea (Campbell and Aleem, 1965a; Hofman and Lees, 1953). The involvement of Cu^{++} in NH_3 oxidation has been further evidenced by recent studies using inhibitors, uncouplers and quinacrine fluorescent techniques (Bhandari and Nicholas, 1979).

The oxidation of NH_2OH, an obligate intermediate of NH_3 oxidation by Ns. europaea, is catalyzed by the enzyme, NH_2OH oxidase (Falcone et al., 1963) or NH_2OH-cytochrome c reductase (Aleem and Lees, 1963; Hooper and Nason, 1965) to NOH (Lees, 1955; 1960). The oxidation is completely inhibited by 3 mM hydrazine resulting in accumulation of NH_2OH during NH_3 oxidation by Ns. europaea (Hofman and Lees, 1953). The presence of 100 nM Mn^{++} stimulates the oxidation rates of NH_2OH by 3 fold but inhibits the production of the normal end product, NO_2^- (Hooper, 1978).

Under aerobic conditions, NOH is oxidized mainly to NO_2^- but the enzyme involved is still uncertain (Suzuki, 1974). Whereas under anaerobic conditions, NOH dimerizes to form hyponitrite which chemically decomposes to form N_2O (Equation 3).

$$\underset{\text{(nitroxyl)}}{2NOH} \longrightarrow \underset{\text{(hyponitrite)}}{N_2O_2H_2} \longrightarrow \underset{\text{(nitrous oxide)}}{N_2O} + H_2O \quad \text{(Equation 3)}$$

Anaerobically, NO_2^- is a major source of N_2O produced by Ns. europaea (Ritchie and Nicholas, 1972). Ns. europaea possesses the enzyme, NO_2^- reductase, which reduces NO_2^- to N_2O and NO using NH_2OH as electron donor under anaerobic conditions (Anderson, 1964; Hooper, 1968; Ritchie and Nicholas, 1972; Wallace and Nicholas, 1968). Conversely, oxidation of NH_2OH to NO_2^- could occur under anaerobic conditions, but the NO_2^- formed is rapidly reduced to gaseous form of nitrogen (Yamanaka and Sakano, 1980). The enzyme appears to contain Cu^{++} since it is strongly inhibited by inhibitors specific for NH_3 oxidase (Suzuki, 1974).

The process of NH_3 oxidation (Equation 4) produces H^+ and 66·5 kcal

$$2NH_3 + 3O_2 \longrightarrow 2H^+ + 2NO_2^- + 2H_2O + 66{\cdot}5 \text{ kcal} \quad \text{(Equation 4)}$$

(Baas-Becking and Parks, 1927; Fromageot and Senez, 1960). The oxidation of NH_2OH to NO_2^- is an exothermic reaction which couples to ATP formation (Delwiche et al., 1961). Using $^{18}O_2$ as a tracer, Rees and Nason (1966) found that only one of the 2 oxygen atoms in NO_2^- formed from NH_3 oxidation was derived from molecular oxygen and the other one was from water. For the NH_3/NH_4^+ equilibrium, the pKa for NH_3 is 9·25 at 30°C (Drozd, 1980). Suzuki et al. (1974) reported that NH_3 rather than NH_4^+ was the actual substrate for oxidation by Ns. europaea since the lowest Km values occurred at the optimal pH ranges of 7·7-8·0.

The free energy efficiency of Nitrosomonas cells is about 11-27% (Laudelout et al., 1968) and the efficiency decreases during growth.

This phenomenon usually attributes to energy being consumed for NH_3 transport into the cells, NO_2^- transport out of the cells, respiration and other assimilatory processes (Aleem and Nason, 1963; Drozd, 1980).

(B) Nitrite Oxidation

The oxidation of NO_2^- to NO_3^- by the species Nitrobacter agilis and Nb. winogradskyi has been studied extensively. The reaction is exothermic producing 17·5 kcal (Baas-Becking and Parks, 1927). The enzyme, NO_2^- oxidase, oxidizes NO_2^- to NO_3^- using O_2 as electron acceptors (Suzuki, 1974). The enzyme is sensitive to CN^-, N_3^- and some metal binding agents (Aleem and Nason, 1960a; 1963). N-Serve at 80-175 ppm inhibits up to 60-75% of NO_2^- oxidation by Nb. agilis (Campbell and Aleem, 1965b). Hynes and Knowles (1983) reported that 0·5 mM ClO_3^- caused 58% inhibition of NO_2^- oxidation by Nb. winogradskyi. The pKa for HNO_2/NO_2^- equilibrium is 3·4 (Focht and Verstraete, 1977). The enzyme shows a high affinity for the substrate at lower pH suggesting that HNO_2 rather than NO_2^- is the actual substrate (Suzuki, 1974).

Cobley (1976) reported that a proton motive force was generated during NO_2^- oxidation by Nb. winogradskyi and that the electrical component of which controlled the rate of NO_2^- oxidaton. However, Hollocher et al. (1982) were unable to detect such proton pump in Nb. agilis. Using $^{18}O_2$, Aleem et al. (1965) found that the oxygen atom in NO_2^- was derived from water and not from O_2. The involvement of O_2 is restricted to electron transport for energy generation (Focht and Verstraete, 1977). Aleem (1968) suggested that electron flow from NO_2^- to O_2 (Equation 5) coupled to phosphorylation of ADP (Aleem and Nason, 1960b). However, Chaudhry et

NO_2^- $\longrightarrow$ Cytochrome $\underline{a}_1$ $\longrightarrow$ Cytochrome $\underline{a}_3$ $\longrightarrow$ O_2 (Equation 5)

al. (1980) implicated the possible involvement of cytochrome b in NO_2^- oxidation by Nb. agilis. So far, the exact mechanism of electron transport and oxidative phosphorylation in Nitrobacter is still speculative. Nitrobacter also possesses an enzyme system which reduces NO_3^- through NO_2^- to NH_4^+ (Wallace and Nicholas, 1968). This reductive process does not couple to ATP formation (Faull et al., 1969) and the NH_4^+ thus formed is incorporated into cell biomass.

The free energy efficiency in Nitrobacter is about 15-51% (Laudelout et al., 1968) and is a function of initial substrate concentration and partial pressure of CO_2 (Dessers et al., 1970; Kiesow et al., 1972).

(2) FACTORS AFFECTING AUTOTROPHIC NITRIFICATION

Rate of autotrophic nitrification is primarily governed by factors such as pH, O_2 concentration, temperature, concentration of substrates and products, concentration of specific inhibitors and alternate substrates present, etc.

(A) pH

Neutral to slightly alkaline pH is usually optimal for both growth and metabolism of the autotrophic nitrifying bacteria (Focht and Verstraete, 1977). In pure cultures of Nitrosomonas, NH_3 oxidation appears

to be optimal at pH 7·5-8·8 (Engel and Alexander, 1958; Lees, 1954; Meyerhof, 1916a; 1916b). Suzuki (1974) reported that NH_3 oxidation by both intact cells and cell-free extracts was optimal at pH 7·7. A 80% inhibition of NH_3 oxidation by Ns. europaea when pH was raised from optimal to 8·7 or dropped to 6·0 has been observed (Laudelout et al., 1976).

The oxidation of NO_2^- by Nitrobacter is optimal at pH 8·0 (Aleem and Alexander, 1960). Nitrobacter can grow at pH up to 10·2 when all N sources except NO_2^- are absent (Meyerhof, 1916a; 1916b). Further studies by Prakasam and Loehr (1972) indicated that pH up to 11·2 had no effect on the growth and metabolism of Nitrobacter when free NH_3 present was below 20 ng $N \cdot L^{-1}$. It appears that the toxicity of free NH_3 at alkaline pH and HNO_2 at acid pH affects the growth and metabolism of Nitrobacter (Focht and Verstraete, 1977). Quinlan (1984) concluded that the optimal pH for nitrification was not fixed but was a function of ambient total free NH_3-N concentration.

With initial pH of 5·1, Morrill and Dawson (1967) were unable to detect any nitrifying activity by pure cultures of soil nitrifiers. Painter (1970) and Alexander (1965) stated that nitrification would not occur at pH below 5·0. It is known that Nitrosomonas consumes a large amount of energy to transport NH_3 into the cells and NO_2^- out of the cells. This amount of energy is greatly increased under acidic conditions as the NH_3/NH_4^+ and HNO_2/NO_2^- equilibria shift towards NH_4^+ and HNO_2, respectively. Consequently, Drozd (1980) speculated that Nitrosomonas would not grow well in more acid conditions. It is known that the autotrophic nitrification process would produce a considerable amount of

acidity. Van Breemen et al. (1982) reported that the atmospheric deposition of $(NH_4)_2SO_4$ to the forest soil was rapidly oxidized to HNO_2 and H_2SO_4 with resulting pH values of 2·8-3·5. In a red Alder ecosystem, acid production during nitrification causes a 15% decline of the soil buffering capacity (Van Miegroet and Cole, 1984).

Interestingly, autotrophic nitrifying bacteria have been found in acid soils (pH 4·0-4·5) from tea estates in Sri Lanka and Bangladesh (Walker and Wickramasinghe, 1979). Walker and Wickramasinghe (1979) isolated Nitrosospira sp., Nitrosolobus sp. and Nitrosovibrio sp. from soil samples of Sri Lanka but only Nitrosospira spp. were present in soil samples from Bangladesh. They also reported that pure cultures of the Nitrosospira isolates were able to nitrify at pH 4·1. In an acid forest soil ecosystem (pH 3·9-4·4), Hankinson and Schmidt (1984) found that Nitrosospira was the predominant autotrophic NH_3-oxidizing bacterium present. They also noticed that autotrophic NO_2^--oxidizing bacteria were 10 to 1000 times more numerous than the autotrophic NH_3-oxidizing bacteria in the ecosystem. They proposed that the autotrophic NH_3 oxidizers were probably restricted to circumneutral microsites in the acid soils whereas the autotrophic NO_2^- oxidizers were not limited to these sites. Interestingly, an extensive study by Boylen et al. (1983) on 5 acid lakes (pH 4·3-4·9) and 3 near neutral lakes (pH >6·0) of various trophic levels in the Adirondack mountain regions of New York revealed that no significant difference in number of nitrifiers was due to the effect of pH.

(B) Oxygen Concentration

Production of NO_2^- by Nitrosomonas cells is most rapid at atmospheric O_2 level (Gundersen et al., 1966). Nitrification rate appears to be unaffected when O_2 is above 2 $mg \cdot L^{-1}$ but very much reduced at less than 1 mg $O_2 \cdot L^{-1}$ (Wuhrmann, 1963). In some estuarine sediments, reduction in O_2 concentration could reduce nitrification rates up to 2 orders of magnitude (Jenkins and Kemp, 1984). Because of high O_2 diffusion rate required for nitrification, Chen et al. (1972) suggested that nitrification only occurred at the sediment-water interface in the oxygenated water. Henriksen et al. (1981) found that nitrifiers were present in anoxic sediment layer but activity was restricted only to the surficial O_2 penetration zone (1·5-5·5 mm) of the sediments in the Danish waters. Indeed, Vincent et al. (1981) observed that active nitrification in Lake Vanda, Antarctica, by a narrow band of autotrophic nitrifiers was lying well above the oxycline. Ward (1984) reported that NH_3-oxidizing activity was negatively correlated with O_2 concentration in the seawater of the northeast Pacific Ocean.

Decrease in O_2 concentration usually reduces the production of NO_2^- and the growth of nitrifiers but increases the evolution of N_2O (Goreau et al., 1980). Anaerobic conditions result in 5-fold increase in N_2O production by Ns. europaea when compared to that under aerobic conditions (Hynes and Knowles, 1984). Goreau et al. (1980) suggested that oceanic N_2O was a product of nitrification and not denitrification. Accumulation of N_2O usually associates with O_2 minima in water columns (Cohen and Gordons, 1978; Elkins et al., 1978; Kaplan et al., 1978). The

production of N_2O by nitrification in coastal lagoon sediment from Kysing Fjord, Denmark, reached the maximum at 0·17 mg $O_2 \cdot L^{-1}$ with the rate being 50 umol $N_2O \cdot L^{-1} \cdot h^{-1}$ which represented 25% of the total nitrification activity in the sediment (Jørgensen et al., 1984).

Interestingly, Carlucci and McNally (1966) observed more inorganic C uptake by nitrifiers under low than high O_2 concentrations. They also found that marine autotrophic nitrifiers could oxidize NH_3 and NO_2^- in liquid and solid media at 0·14 and 0·07 mg $O_2 \cdot L^{-1}$, respectively. Recently, Macfarlene and Herbert (1984) isolated an estuarine strain Nitrosomonas capable of nitrification in liquid media at 0·1 mg $O_2 \cdot L^{-1}$. The occurrence of nitrification in the bottom water of Lake St. George, Ontario, during the winter when dissolved O_2 was less than 1 ug $\cdot L^{-1}$ has also been reported (Lean and Knowles, 1982).

The Km of O_2 consumption in Nitrosomonas and Nitrobacter are 0·51 and 1·98 mg $O_2 \cdot L^{-1}$, respectively (Laudelout et al., 1974; 1975; 1976). The lower Km values for O_2 in Nitrosomonas suggests that it may still be able to nitrify at reduced O_2 concentrations where Nitrobacter cannot oxidize NO_2^- to NO_3^-. In a study of mixed cultures of Ns. europaea and Nb. winogradskyi, Laudelout et al. (1976) observed that upon O_2 depletion in the culture media, added NH_4^+ was oxidized to NO_2^- only with no NO_3^- production resulting in a temporal shift to NO_2^- accumulation. This could explain the accumulation of high NO_2^- concentrations in the deeper, O_2-poor water of some oceans such as those off the west coasts of Costa Rica and Peru in the Pacific Ocean and in some areas of the Indian Ocean where O_2 concentrations were about 0·14 mg $\cdot L^{-1}$ (Carlucci and McNally,

1969). Lardner and Larsson (1972) observed the constant presence of high concentrations of NO_2^- (0·5-2·3 mg $N \cdot L^{-1}$) for several years in the bottom water of the Bay of Köping, westernmost part of Lake Mälaren, Sweden. They also noticed that the bottom-water samples nitrified 75 to 100% of the added NH_4^+ to NO_2^- only. Similar observations with the NO_2^- peaks in the water column during destratification in Chesapeake Bay has been reported (McCarthy et al., 1984).

During nitrification, O_2 is being consumed by both NH_3 and NO_2^- oxidizers. In Grand River, nitrification accounted for 75% of the total BOD (Courchaine, 1968). Lean and Knowles (1982) also attributed most of the observed O_2 loss in the water column under the ice cover in Lake St. George to nitrification. In the mesotrophic Lake Grasmere, nitrification was found to be responsible for 15-20% of the total O_2 depletion of the hypolimnion (Hall and Jeffries, 1984).

(C) Temperature

Temperature affects the biological rate processes. In the temperature range of 15-35°C, the effect of temperature on the rates of nitrification could possibly be described by the Arrhenius equation (Focht and Verstraete, 1977). In pure-culture studies, 25-35°C appears to be the optimal temperatures for autotrophic nitrification (Buswell et al., 1954; Deppe and Angel, 1960; Meikeljohn, 1954). The temperature quotient (Q_{10}) for Nitrosomonas and Nitrobacter are 3·0 and 1·7, respectively (Belser, 1979). Therefore, higher incubation temperatures (within the range of 15-35°C) would increase rate of NH_3 oxidation much more than

that with NO_2^- oxidation. Belser (1979) predicted that higher but not lower temperature would cause a temporal accumulation of NO_2^-. In Two-Mile Creek, a heated stream, NH_4^+ oxidation rate (0·5 mg NH_4^+-N·L^{-1}·h^{-1}) indeed exceeded the NO_2^- oxidation rate (0·29 mg NO_2^--N·L^{-1}·h^{-1}) resulting in NO_2^- accumulation in the stream water (White et al., 1977). Ward (1984) reported that the NH_3-oxidizing activity was positively correlated with the temperature in oceans. At optimal pH, changes in temperature appear to have more pronounced effect on NH_3 than NO_2^- oxidation whereas the reverse is true under more acid or alkaline pH (Focht and Verstrate, 1977). For instance, Wong-Chong and Loehr (1975) found that NO_2^- oxidation at pH 8·5 or 6·5 was optimal at 25°C but shifted to 34°C when the pH was adjusted to 7·5. It appears that both the energy of activation and the optimal temperature are strongly pH dependent. Wong-Chong and Loehr (1975; 1978) proposed that both the effects of temperature and pH, not alone, on nitrification should be considered.

Yoshida and Alexander (1970) found that high temperatures (above 45°C) inhibited NH_3 oxidation by pure cultures of Nitrosomonas cells. Focht and Verstraete (1977) concluded that NO_2^- and NO_3^- were seldom detected when the temperature reached above 40°C in field soils. Keeney and Bremner (1967) observed that at 40°C incubation temperature soil nitrifying activity was completely inhibited. Interestingly, a Nitrosomonas strain isolated from hot springs of Kamchatka and Tadzhk, USSR, has been reported to grow optimally at 50°C and had pH and temperature ranges of 6·3-7·5 and 50-86°C, respectively (Golovacheva, 1975).

Lower incubation temperatures usually result in decrease in nitrifying activity. An estuarine strain of Nitrosomonas growing in a chemostat at a dilution rate of 0·025 h^{-1} was washed out when the incubation temperature was reduced below 15°C (Macfarlene and Herbert, 1984). However, Focht and Verstraete (1977) pointed that the effect of lower temperatures (0-15°C) on in situ nitrification were far more relevant than the high temperature range mentioned previously since lower temperature ranges were more common in many aquatic and terrestrial ecosystems. They also commented on the great many difficulties inherent in assessing the temperature effects on in situ nitrification. One other fact that makes the assessment more complex is that autotrophic nitrifying bacteria apparently become acclimated to the temperature regime of their habitats and do not appear to vary their adaptability to low temperatures. Mahendrappa et al. (1966) noted that nitrification rates were faster at 20 and 25°C incubation than at 35 and 45°C with Western soils and the reverse was true with soils from the warmer Southern climate. Similarly, Anderson et al. (1971) found that acid soils from the colder mountains area of Georgia had a much higher nitrification activity than those from the warmer coastal plain when all samples were incubated at 6°C.

(D) Concentration of Substrates and Products

Under optimal pH and temperature conditions, the optimal concentration of substrates for Nitrosomonas and Nitrobacter are both in the range of 30-50 mM NH_4^+-N and NO_2^--N, respectively (Engel and Alexander, 1958; Lees, 1954; Meyerhof, 1916a; 1916b). Nitrite, the end product of

NH_3 oxidation and substrate for NO_3^- production is of particular interest. It has been reported that NO_2^- inhibits proton-dependent active transport, O_2 uptake, oxidative phosphorylation and activities of certain metabolic enzymes in a wide variety of microorganisms (Yarbrough et al., 1980). Drozd (1980) pointed out that it was the undissociated weak acid acting as an uncoupler of oxidative phosphorylation. While 10 mM NO_2^- inhibits growth of the denitrifying bacterium, Paracoccus denitrificans (Van Verseveld et al., 1977), up to 20 mM NO_2^- appears to have no effect on NH_3 oxidation by Ns. europaea (Ritchie and Nicholas, 1972). Concentration of NO_2^- above 20 mM inhibits NH_3 oxidation (Anthonisen et al., 1976). At 100 and 336 mM NO_2^-, O_2 uptake by NH_3 oxidizers is inhibited by 36 and 100%, respectively (Meyerhof, 1916a; 1916b). No growth of NH_3 oxidizers was observed in the presence of 500 mM NO_2^- (Drozd, 1980). However, Bock (1978) found that 21·34 mM NO_2^- completely inhibited growth of Nitrosomonas. Watson et al. (1971) reported that 100 mM NO_2^- inhibited growth of Nitrosolobus. On the other hand, the end product of NO_2^- oxidation, NO_3^-, usually does not interfere with NO_2^- oxidation (Aleem and Alexander, 1960). However, NO_2^- oxidation would be inhibited if NO_3^- concentration reached very high levels (Boon and Laudelout, 1962).

Free nitrous acid (0·2-2·8 mg $N \cdot L^{-1}$) formed under acidic conditions completely inhibits NO_2^- oxidation in a noncompetitive manner (Anthonisen et al., 1976; Boon and Laudelout, 1962). Under alkaline conditions, free NH_3 in the range of 0·1-1·0 and at 150 mg NH_3-$N \cdot L^{-1}$ inhibits NO_2^- and NH_3 oxidations, respectively (Anthonisen et al., 1967). Belser (1979) demonstrated the inhibition of NO_2^- oxidation by total NH_3-N present being 200 mg N at pH 7 but was 3 mg N at pH 9. Quinlan (1984) concluded that

the combined effects of pH, temperature and ambient free NH_3 concentration, not alone, on nitrification should be considered.

The Km for substrates at 20-30°C for Nitrosomonas and Nitrobacter are, respectively, 1-10 mg NH_4^+-N·L^{-1} (Hofman and Lees, 1953; Loveless and Painter, 1968; Ulken, 1963) and 5-9 mg NO_2^--N·L^{-1} (Gould and Lees, 1960; Laudelout and Van Tichelen, 1960; Lees and Simpson, 1957; Ulken, 1963). Alexander (1965; 1977) concluded that the populations and in situ activities of nitrifiers were usually limited by the production rates of NH_4^+ (i.e. ammonification) because the potential rates of NH_3 oxidation greatly exceeded the rates of ammonification in soils. In addition, the maintainance requirements for NH_3 and NO_2^- oxidizers were reported to be 0·023 pmol NH_3·$cell^{-1}$·h^{-1} (Belser, 1979) and 0·002 pmol NO_2^-·$cell^{-1}$·h^{-1} (Chiang, 1969), respectively. These requirements further limit the sizes that the nitrifier population can reach in a system where ammonification is the only source of substrate (Belser, 1979). This is particularly relevant to lacustrine ecosystems where NH_4^+ is mainly derived from deamination of proteins, amino acids and urea (Jones et al., 1982). Indeed, Belser (1979) observed a rapid increase in growth of nitrifier population in many soils following the additions of NH_4^+ fertilizers.

(E) Specific Inhibitors and Alternate Substrates

There are several specific inhibitors and alternate substrates for NH_3 oxidation but very few specific for NO_2^- oxidation are known. Ammonia oxidation by Ns. europaea is inhibited by 1·0 ppm N-Serve (Campbell and Aleem, 1965a), 1·0 ppm thiourea (Hofman and Lees, 1953; Malhi et al.,

1979), 5-10 uM allylthiourea (Campbell and Aleem, 1965a; Hofman and Lees, 1953), low levels of visible light, e.g. 0·3 uE·cm^{-2} at 410 nm (Bock, 1978; Hooper, 1978), 10^{-5} atm C_2H_2 (Hynes and Knowles, 1978), 10% N_2O (Hooper, 1978; Hynes and Knowles, 1982) and 5 uM ClO_2^- (Hynes and Knowles, 1983). Some other inhibitors of NH_3 oxidation have been listed by Hooper and Terry (1973). Sjwerinski (1977) suggested that some compounds excreted by the spring phytoplankton were inhibitory to nitrification since nitrification in Kiel Fjord ceased at a time when phytoplankton bloom started. Suzuki et al. (1976) reported that CH_4, CH_3OH and CO were competitive inhibitors of NH_3 oxidation in Ns. europaea. Recently, CO, CH_4 (Jones and Morita, 1983a; 1983b), C_2H_4 and C_2H_4O (Hyman and Wood, 1984) were shown to be alternate substrates for the enzyme NH_3 oxidase.

Very often, much higher concentrations of inhibitors are required to confer the same degree of inhibition in natural samples as achieved in pure culture studies. It has been reported that C_2H_4 at concentrations of 10^{-4} atm (Berg et al., 1982) to 10^{-1} atm (Mosier, 1980) were required to completely inhibit NH_3 oxidation in soils. Bremner and Bundy (1974) found that 5 ug $CS_2 \cdot g^{-1}$ soil inhibited 97-99% of the soil NH_3 oxidation. In lake sediment cores and enrichment cultures of nitrifying bacteria, 10 mg·L^{-1} allylthiourea appeared to be sufficient to halt NH_3 oxidation (Hall, 1984).

NO_2^- oxidation by Nb. winogradskyi is inhibited by 10 mM ClO_3^- (Hynes and Knowles, 1983). ClO_3^- is a competitive inhibitor of NO_3^- reductase in Nitrobacter (Faull et al., 1969) and can be reduced by the enzyme to

form ClO_2^-. It has been reported that 500 uM ClO_2^- inhibited NO_2^- oxidation by 58% in Nb. winogradskyi and that ClO_3^- was reduced to ClO_2^- by the cells (Hynes and Knowles, 1983). With sediment, soil slurries and pure cultures of Nb. winogradskyi, 10 mM ClO_3^- was found inhibitory to NO_2^- oxidation (Belser and Mays, 1980). In addition, visible light was shown to be lethal to the cells of Nitrobacter (Bock, 1978).

(3) METHODOLOGY IN ASSAYING NITRIFICATION

There are several methods currently being used in measuring in situ autotrophic nitrification rates.

(a) Belser and Mays (1980) proposed a sensitive and simple technique by using ClO_3^- inhibition of NO_2^- oxidation and measured the amount of NO_2^- accumulated against time of incubation. This technique assumed that the oxidation of NH_3 but not NO_2^- was the rate-limiting step (Wong-Chong and Loehr, 1976) and that ClO_3^- or its byproducts had no effect on NH_3 oxidation to NO_2^-. However, Hynes and Knowles (1983) reported that ClO_3^- was rapidly reduced to ClO_2^- by NO_2^--oxidizing bacteria and the amount of ClO_2^- produced was sufficient to inhibit NH_3 oxidation. Their findings indicated the limited usefulness of the method.

(b) Billen (1976) reported the successful use of dark incorporation of $H^{14}CO_3^-$ in the presence and absence of N-Serve. The method was thought to be simple and sensitive for measuring autotrophic nitrification rates. This technique assumed the ratio of ^{14}C uptake to actual nitrogen oxidized to be 9·01:1. There are, however, several

drawbacks. Firstly, the C:N ratio varies under different conditions (Gundersen, 1966). Secondly, there are considerable variations in sensitivity towards N-Serve among strains of NH_3 oxidizers (Belser and Schmidt, 1981). N-Serve affects carbon assimilation of other bacteria and phytoplankton and is a potential inhibitor of denitrification (Henninger and Bollag, 1976), methylotrophy (Topp and Knowles, 1982) and methanogenesis (Salvas and Taylor, 1980). Thirdly, methylotrophs can nitrify (Hutton and ZoBell, 1953; O'Neill and Wilkinson, 1977; Malashenko et al., 1980).

(c) Unlabeled substrates are used in the mass balance approach (Billen, 1975; Schwert and White, 1974; Webb and Wiebe, 1975). This approach is applicable only when a differential exists between NH_4^+ and NO_2^- oxidation rates or a specific water mass can be traced with time. This method is very insensitive, unable to discriminate the origin of the process involved and subjected to a great deal of sampling error.

(d) ^{15}N tracer technique (Dugdale and Goering, 1967; Koike and Hattori, 1978; Ohmari et al., 1981; Wada et al., 1977) is still by far the most reliable method. There are, however, some drawbacks such as large sample size, lengthy incubation in order to obtain measurable results, high cost of purchasing a mass spectrometer and the lengthy preparation and analytical time.

(e) Very recently, Jones et al. (1984) suggested the use of the combinations of ^{14}CO and N-Serve to estimate in situ chemolithotrophic NH_3 oxidation. In addition to the problems of using N-Serve, ^{14}CO method cannot distinquish the NH_3-oxidizing activity between NH_3

oxidizers and CH_4 oxidizers since these two groups of oxidizers can oxidize CO.

(f) Ward (1984) reported the successful use of combined $^{14}CO_2$ autoradiography and immunofluorescence for estimation of single cell activity by autotrophic NH_3-oxidizing bacteria in seawater. One major problem when using this method would be to obtain pure cultures of all the autotrophic nitrifiers involved for the preparation of immunofluorescent stains.

In a recent study of NH_3 oxidation, Salvas and Taylor (1984) found that picolinic acid (2-carboxy-pyridine) selectively inhibited NH_3 oxidation by CH_4 oxidizers but had no effect on NH_3 oxidation by autotrophic NH_3 oxidizers. On the other hand, Hall (1984) reported that 10 mg $\cdot L^{-1}$ allylthiourea inhibited NH_3 oxidation <u>in situ</u> but did not have any shortcomings as with N-Serve. Unlike N-Serve, allylthiourea is very soluble in water hence has no solvent effect and its effectiveness lasts much longer (Hall, 1984). Perhaps in the future study, <u>in situ</u> nitrification assay may be possible by using ^{14}CO technique coupled with the specific inhibitors, allylthiourea and picolinic acid.

(4) NITROUS OXIDE AND NOx

Nitric oxide (NO) plays a key role in tropospheric chemistry regulating peroxy (HO_2) and O_2 (Levy, 1973; Logan <u>et al.</u>, 1981) and N_2O is the major source of stratospheric NO (Crutzen, 1971; McElroy and McConnell, 1971; Nicolet and Vergison, 1971,). The stratospheric O_3 layer is important in reducing solar radiation (harmful wavelength region) on Earth.

At high altitudes, N_2O reacts with O_3 catalyzed by light energy to form NO_2 and O_2 resulting in depletion of O_3 layer in the stratosphere (Crutzen and Ehhalt, 1977; McElroy et al., 1977; NAS, 1977; Pratt et al., 1977). Anthropogenic depletion of stratospheric O_3 has been linked to several adverse biological effects such as skin cancer in man (Cutchis, 1974; Johnson et al., 1968).

However, at lower altitudes of some areas particularly agricultural lands, the emission of N_2O increases the concentration of tropospheric O_3. Crutzen and Howard (1978) discovered that under photochemical "smog" conditions in polluted atmosphere some reactive radicals reacted with O_2, which in turn reacted with N_2O to generate NO and O_3. Wang and Sze (1980) and Wang et al. (1976) calculated that an increase in the atmospheric N_2O abundance due to increased application of nitrogen fertilizers alone (not even including industrial pollution of NOx) would affect the troposphere climate. The thickening of the tropospheric O_3 and the increase in atmospheric N_2O (having strong infra-red absorptions) both contribute a "green-house effect" which could increase the temperature of the land surface in the area by as much as 30% (Wang and Sze 1980). This green-house effect could possibly affect the yields of food crop in some agricultural areas.

More recent studies indicate that autotrophic nitrification is a dominant source of atmospheric N_2O. In terrestrial ecosystems, soil nitrification contributes a substantial amount of N_2O emissions (Blackmer et al., 1980; Bremner and Blackmer, 1979). Lipschultz et al. (1981) confirmed nitrification in soils as a potentially significant source of NO

and N_2O. They observed that 0·3-1·0% of the NH_3 oxidized by Ns. europaea was converted to gaseous forms of nitrogen. They further estimated that the nitrification process alone contributed, on a global basis, 15 X 10^6 tonnes NO-N·yr^{-1} and 5-10 X 10^6 tonnes N_2O-N·yr^{-1}. Yoshida and Alexander (1970) found that low-temperature storage of the cells and the presence of phosphate stimulated N_2O production by Ns. europaea during oxidation of NH_3 and NH_2OH. Yamanaka and Sakano (1980) noted that NO_2^- formed from NH_2OH oxidation by purified NH_2OH oxidoreductase lasted for only 5 min and subsequently disappeared in the gaseous form. In addition, purified NO_2^- reductase of Ns. europaea reduces NO_2^- to N_2O with NH_2OH as electron donor and involved the combined action of NH_2OH oxidase and NO_2^- reductase (Hooper, 1968). Although a theoretical ratio of 350:1 for NH_3 oxidized to N_2O produced has been suggested (Bremner and Blackmer, 1978; Elkins et al., 1978; Goreau et al., 1980); Wood et al. (1981) found that 43% of the total NH_3 oxidized by Nitrosomonas cells released as N_2O via the mechanism as described earlier by Hooper (1968). In fact, Ns. europaea produces N_2O during oxidation of NH_3 under both aerobic and anaerobic conditions and anaerobiosis stimulates the amount of N_2O produced by 5-fold (Hynes and Knowles, 1984). The presence of suspending particles also stimulates N_2O production by Ns. europaea in liquid media (Hynes and Knowles, 1984). This increase in the amount of N_2O is probably due to higher nitrification rates promoted by the presence of the particle matrix. Particle size of approximately 0·2 u has been reported to promote the highest in situ nitrification rates (Kholdebarin and Oertli, 1977). The presence of suspending particles also stimulates NO_2^- oxidation. Audic et al. (1984) reported that the specific activity of Nb.

winogradskyi was stimulated by 130% through attachments in granular media.

Elkins et al. (1978) reported that nitrification is the dominant source of N_2O in both freshwater and saltwater ecosystems. They found that 1 mole of N_2O was produced for every 700 moles of NH_4^+ oxidized in the Cheasapeake Bay and the Peruvian Upwelling. Their results suggested that the oxidation of NH_4^+ and amino nitrogen represented a major source of marine N_2O. Yoshinari (1976) found that the concentration of N_2O was negatively correlated with the O_2 profile and that N_2O concentration reached maximum at water depth where dissolved O_2 was about 3 $mL \cdot L^{-1}$ in the oceans. He detected supersaturation of N_2O in the surface waters of the Gulf of St. Lawrence in early June and Carribbean Sea in March. Goreau et al. (1980) reported that decreasing O_2 concentration would increase the N_2O:NO_2^- ratio from 0·3% to 10% by NH_3 oxidizers. They found that neither oceanic NO_2^- oxidizers nor dinoflagellates could produce N_2O. In the warm meromictic Lake Vanda, Antarctica, accumulation of extremely high concentration of N_2O in the saline bottom waters (>2 umol $\cdot L^{-1}$) was attributed to intense nitrification by a narrow band of nitrifiers lying well above the oxycline (Vincent et al., 1981). High concentration of N_2O was also detected in two lakes during the early spring stratification, being 343 and 3 ug $N_2O \cdot L^{-1}$, in the oxygenated NO_3^--containing waters (Knowles et al., 1981). Lemon and Lemon (1981) reported 10 times higher fluxes of N_2O than normal for open oceans. They suggested that shallower and warmer Great Lakes such as Lake Erie served as a net source of N_2O derived from nitrification whereas the cooler and deeper Great Lakes such as Lake Huron served as a sink for N_2O due to

intensive denitrification activity. Seitzinger et al. (1983) detected high benthic N_2O production (152 $nmol \cdot m^{-2} \cdot h^{-1}$) and N_2O fluxes (20 to >900 $nmol \cdot m^{-2} \cdot h^{-1}$) in the Narragansett Bay and that eutrophic area had higher N_2O production than relatively unpolluted area. They suggested that coastal marine sediment was a net source of N_2O. In the oligotrophic Lake Taupo, New Zealand, NO_2^- and N_2O accumulated in the hypolimnion throughout stratification and that the concentrations increased with depths towards sediment (Vincent and Downes, 1981). High nitrification rates for the planktonic and benthic nitrifiers were detected in the epilimnion and the surficial sediment (2·5 mm) whereas the lowest rates were found in the deep hypolimnion of the lake (Vincent and Downes, 1981). In a deep oligotrophic arctic lake, N_2O derived from both nitrification and denitrification could reach 25 ng $N \cdot cm^{-3} \cdot d^{-1}$ and that nitrification only occurred in the sediment at a rate of 49 ng $N \cdot cm^{-3} \cdot d^{-1}$ (Klingesmith and Alexander, 1983). During nitrification in the Chesapeake Bay, McCarthy et al. (1984) detected the accumulations of high concentration of NO_2^- (35 ug $N \cdot L^{-1}$) in the bay water during incubation and the ratio of N_2O produced to NO_2^- formed ranged from 0·2% to 0·7%. They found that the primary source of N_2O and NO_2^- derived from nitrification was in the water and not in the sediment. They also noted that NO_2^- in the pycnocline was mostly in the concentrations of 4·2-9·8 ug $N \cdot L^{-1}$ and that the levels of N_2O and NO_2^- increased if anthropogenic loading of nutrients, thus causing anoxia, was elevated. By analyzing the $^{15}N/^{14}N$ ratio of N_2O in water samples from the eastern tropical Pacific Ocean, Yoshida et al. (1984) confirmed that subsurface waters acted as a source of N_2O whereas extremely O_2-depleted waters acted as a

sink. Marine sediments from a coastal lagoon in Kysing Fjord, Denmark, showed maximum N_2O production at 0·1 kpa O_2 and the amount accounted for 25% of the total nitrifying activity being 50 nmol $N \cdot mL^{-1} \cdot h^{-1}$ of the sediment suspension (Jørgensen et al., 1984).

Denitrification is another process considered to be an important source of N_2O (Knowles, 1982). However, in the artificially fertilized oligotrophic Lake 227 in ELA, N_2O was found to be a minor product of denitrification (Chan and Campbell, 1980). Reduction of NO_3^- by heterotrophic bacteria such as Bacillus subtilis, Escherichia coli and Aerobacter aerogenes and reduction of NO_2^- by fungi such as Aspergillus flavus and Penicillium atrovenetum would produce N_2O (Yoshida and Alexander, 1970). The production of N_2O by green algae belonging to the family Chlorophyceae has been reported and suggested as an important source of N_2O in aquatic systems (Weathers, 1984). Another source of N_2O is the chemical decomposition of oxidized nitrogen. In sandy acid soils, Gerretsen and De Hoop (1957) observed that HNO_2 was decomposed to NO and escaped to the atmosphere before it could be oxidized to NO_3^-. Abel et al. (1931) indicated that HNO_2 became unstable at pH below 5·5-6·0 resulting in formation of NO and NO_2. The formation of NH_4NO_2 under acidic conditions would also result in its decomposition to form N_2O (Allison, 1963). Bollag et al. (1973) observed that at pH 5·0, NO_2^- chemically decomposed to form NO and NO_2 but the products were N_2O and N_2 instead if biological activity was involved. At this pH, the presence of Fe^{++} stimulated the abiological production of N_2O and N_2 (Chalamet, 1973; Nelson and Bremner, 1970). At pH 6·0, the presence of 800 mg $Fe^{++} \cdot L^{-1}$ and 5 mg $Cu^{++} \cdot L^{-1}$ induced the chemical decomposition of 50% of the

NO_2^- to N_2O (Moraghan and Buresh, 1977). In addition, Bremner et al. (1980) found that the intermediate of NH_3 oxidation, NH_2OH, decomposed to N_2O and that the rate was correlated to the presence of exchangeable and oxidized Mn.

(5) HETEROTROPHIC NITRIFICATION

The report on the production of NO_2^- from the oxidation of organic nitrogen by Bacillus (Mishustin, 1926) has since introduced the concept of heterotrophic nitrification. The oxidation of NH_4^+ to NO_2^- by pure cultures of heterotrophs has subsequently been demonstrated (Cutler and Mukerji, 1931; Nelson, 1929). In terrestrial ecosystems, Hirsch et al. (1961) observed the formation of NO_2^- and NO_3^- by pure cultures of actinomycetes and fungi isolated from soils. Using $^{15}NH_4^+$ as a tracer, Schimel et al. (1984) found that NO_2^- formed in an acid soil was not derived from the $^{15}NH_4^+$ added. In aquatic ecosystems, methylotrophs are thought to be the major group of bacteria responsible for heterotrophic nitrification due to their ability to oxidize NH_4^+ to NO_2^- (Hutton and ZoBell, 1953; Malashenko et al., 1980; O'Neill and Wilkinson, 1977) and their frequent association with NO_2^- maxima in stratified lakes (Hanson, 1980).

All known heterotrophic nitrifiers derive their carbon and energy source from oxidation of organic carbon. Cutler and Mukerji (1931) found that 0·1% sucrose stimulated NO_2^- production by the soil heterotrophic isolates. With 1·5% sucrose and 0·6% $(NH_4)_2HPO_4$, NO_2^- production by A. flavus reached 141 ug NO_3^--N·mL^{-1} within 14 days when incubated at 30°C and pH 7·0 (Hirsch et al., 1961). Glucose along with organic N such as

peptone are frequently used to study heterotrophic nitrification (Gowda et al., 1977; Obaton et al., 1968; Odu and Adeoye, 1970). Acetate (Castignetti and Gunner, 1981; Verstraete and Alexander, 1972a; 1973), citrate, pyruvate (Castignetti and Gunner, 1981), yeast extracts (Castignetti and Hollocher, 1984) and some other N-containing organic carbon compounds (Castignetti et al., 1985) have been reported to support heterotrophic nitrification. Production of NH_2OH from NH_4^+ by Arthrobacter sp. is regulated by the C:N ratio. Verstraete and Alexander (1972a) found that C:N ratio of 3 permitted maximum growth of Arthrobacter but minimum NH_2OH production. Effect of carbon source on heterotrophic nitrification varies with types of carbon source and natural soil samples used (Verstraete and Alexander, 1973). Verstraete (1975) stated that NO_2^- production was not affected by the C:N ratio whereas NH_2OH production would decrease 10-fold when the C:N ratio was lower than 3-5. On the other hand, too much carbon source added may not increase NH_2OH and NO_2^- production. Jensen (1951) found that the addition of excessive amount of glucose (resulting C:N was 20:1) inhibited heterotrophic nitrification by stimulating cell biomass synthesis.

Focht and Verstraete (1977) compiled a list of micro and macroorganisms capable of heterotrophic nitrification. The list included both heterotrophic bacteria and fungi which was categorized according to the types of nitrogenous substrates used and products formed. Heterotrophic bacteria capable of transforming organic and inorganic nitrogen into NH_2OH, NO_2^- and NO_3^- that are relevant to the present study are abstracted from the list and briefly described below.

(a) Heterotrophic bacteria producing free NH_2OH and substituted NH_2OH from NH_4^+ or amino-N and oxime-N (RC=NOH): free NH_2OH production from NH_4^+ or amino-N by Arthrobacter sp. (Verstraete and Alexander, 1972a), A. globiformis (Gunner, 1963) and Methylococcus thermophilus (Malashenko et al., 1980).

(b) Heterotrophic bacteria producing NO_2^- and nitro compounds from NH_4^+-N or amino-N, free NH_2OH, hydroxylamino compounds, oximes, hydroxamic acids, aliphatic and aromatic nitro compounds and NO_3^-:

(i) NO_2^- production from NH_4^+-N or amino-N by Pseudomonas sp. (Gowda et al., 1979), Arthrobacter sp. (Tate, 1977), M. thermophilus (Malashenko et al., 1980) and many other heterotrophic bacteria and actinomycetes (Gode and Overbeck, 1972; Hirsch et al., 1961; Odu and Adeoye, 1970).

(ii) NO_2^- production from free NH_2OH by Pseudomonas sp. (Amarger and Alexander, 1968), Arthrobacter sp. (Verstraete and Alexander, 1972a), M. thermophilus (Malashenko et al., 1980), Chromobacberium violaceum, Flavobacterium sp. Pseudomonas denitrificans, P. aureofaciens, P. fluorescens, P. stutzeri (Castignetti and Hollocher, 1984), Alcaligenes sp. (Castignetti and Hollocher, 1982), Proteus sp. and Microbacterium sp. (Castell and Mapplebeck, 1956).

(iii) NO_2^- production from oximes by Pseudomonas aeruginosa (Obaton et al., 1968) and Agrobacterium spp. (Jensen, 1951); and NO_2^- production from pyruvic-oximes by Alcaligenes sp. (Castignetti and Gunner, 1981), A. faecalis, P. denitrificans, P. aeruginosa, P. fluorescens and P. aureofaciens (Castignetti and Hollocher, 1984).

(iv) NO_2^- production from hydroxylamino compounds by many heterotrophic bacteria (Doxtader and Alexander, 1966).

(v) NO_2^- production from aliphatic nitro compounds by Arthrobacter sp. (Verstraete and Alexander, 1972b) and P. aeruginosa (Obaton et al., 1968).

(vi) NO_2^- production from aromatic nitro compounds by Arthrobacter sp., Flavobacterium sp. (Focht and Verstraete, 1977), Pseudomonas sp. and Nocardia sp. (Germanier and Wuhrmann, 1963).

(c) Production of NO_3^- from NH_4^+-N or amino-N, NO_2^-, oximes, aliphatic and aromatic nitro compounds:

(i) NO_3^- production from NH_4^+-N or amino-N by Arthrobacter sp. (Verstraete and Alexander, 1972a) and A. globiformis (Gunner, 1963).

(ii) NO_3^- production from aliphatic nitro compounds by Arthrobacter sp. (Verstraete and Alexander, 1972b)

(iii) NO_3^- production from aromatic nitro compounds by Pseudomonas sp. (Germanier and Wuhrmann, 1963).

(iv) NO_3^- production from pyruvic oximes by Alcaligenes sp. (Castignetti and Gunner, 1981).

The occurrence of large population of heterotrophic nitrifying bacteria in ecosystems with low number of autotrophic nitrifiers has been reported (Alexander et al., 1960; Gode and Overbeck, 1972; Laurent, 1971; Odu and Adeoye, 1970; Remacle and Froment, 1972). However, it is still speculative on the occurrence and significance of heterotrophic nitrification in terrestrial and aquatic ecosystems. Many workers are still doubtful, despite the demonstration of heterotrophic nitrification

in pure cultures, whether this potential of N oxidation can occur *in situ* with any significance. Their doubts were based on the facts that heterotrophic nitrification rates were generally 10^3 to 10^4 times lower than the autotrophic nitrification rates (Focht and Verstraete, 1977). Odu and Adeoye (1970) suggested that the production of NO_2^- and NO_3^- by heterotrophic nitrifiers may be of ecological significance in environments where the inefficiency of heterotrophic nitrification could possibly be compensated for by their large numbers. Verstraete (1975) pointed out that nitrification by heterotrophs was not associated with their cell growth nor proportional to their overall cellular biomass. Focht and Verstraete (1977) speculated that heterotrophic nitrification may be significant both qualitatively and quantitatively in two types of environments where autotrophic nitrifiers were absent, namely the acid soils and the highly alkaline, nitrogen-rich aqueous environments.

(A) Pathways of Heterotrophic Nitrification

The pathways and the mechanisms involved in heterotrophic nitrification are still unknown. Verstraete and Alexander (1972b) proposed pathways of heterotrophic nitrification, based on the results of their previous works on *Arthrobacter* sp., which included both an inorganic and an organic pathway (Fig. 1). The inorganic pathway is identical to the one for the autotrophic nitrification. In the organic pathway, NH_4^+ is thought to be converted to an amide which is then oxidized to yield acetohydroxamic acid. The latter is rapidly converted by a fast, reversible reaction to free NH_2OH, or further oxidized to 1-nitroso-1-oxoethane.

Reduction of the latter gives rise to 1-nitrosoethanol. The enzymatic hydrolysis of 1-nitroso-1-oxoethane results in formation of NO_2^- and NO_3^-. Using $^{18}O_2$ as a tracer, Verstraete and Alexander (1972b) showed the incorporation of $^{18}O_2$ during NH_4^+ oxidation to NH_2OH by the Arthrobacter cells suggesting that the oxidation of NH_4^+ would involve the oxygenase system. They proposed that the oxidation of N was NADPH-dependent since an organic C source was required to provide a continuous supply of reduced pyridine nucleotides for the oxidation of NH_4^+ to NH_2OH.

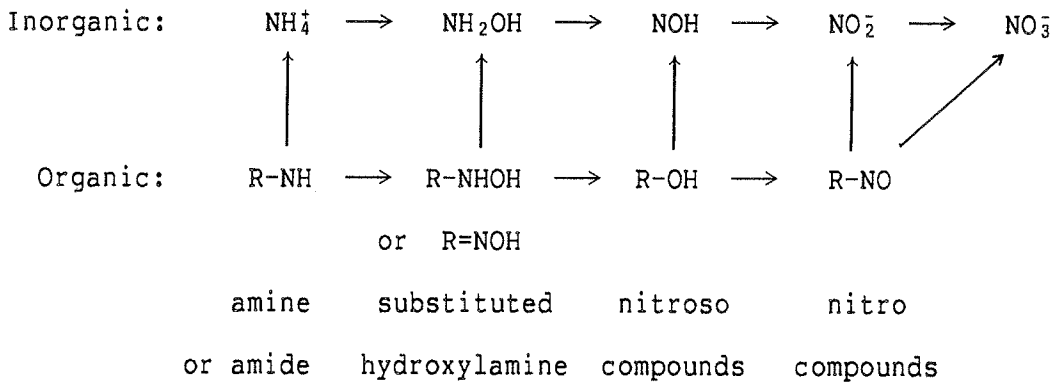


Fig. (1) Hypothetical pathways of heterotrophic nitrification in Arthrobacter sp.

Information on the inorganic pathway is scarce. Amarger and Alexander (1968) reported that P. aeruginosa produced NO_2^- from NH_2OH and several oximes and that NADP was a cofactor for the nitrifying enzymes. Verstraete and Alexander (1972b) found that enzymes excreted by Arthrobacter sp. converted NH_2OH to NO_2^- in culture medium but neither NAD, NADP nor cytochrome c, alone or in combination, were required. They further

postulated that a peroxidase or catalase enzyme might be responsible for the extra-cellular oxidation of NH_2OH to NO_2^-. Study with the oxidation of pyruvic oxime by cells of Alcaligenes sp. indicated that the pathway did not involve the initial hydrolysis of the substrate to pyruvate but probably involved the oxidation of N and/or C before C-N bond breakage (Castignetti et al., 1983). There appears to be no other information regarding this aspect of the inorganic metabolism.

(B) pH

Ishaque and Cornfield (1972) reported that NO_2^- production in an acid Pakistan tea soil was optimal at pH 4·5 and that the addition of lime inhibited nitrifying activity. Focht and Verstraete (1977) found the similar optimal pH values (4·5-5·0) for NO_2^- production in some acid forest soils. Acid forest soils from the Adirondack mountains (pH 5·6 and 6·3) produced NO_3^- when amended with simulated acid rain (pH 3·2) in the presence of N-Serve (Strayer et al., 1981). The production of NO_3^- occurred when Bangladesh tea soil was supplemented with urea or oxamide but not $(NH_4)_2SO_4$ (Ishaque and Cornfield, 1974) suggesting that heterotrophic nitrification was responsible. It has been reported that the addition of peptone to some acid soils increased NO_3^- production by 2-fold whereas the addition of $(NH_4)_2SO_4$ had no effect (Focht and Verstraete, 1977; Weber and Gainey, 1962). Van de Dijk and Troelstra (1980) obtained similar results with an acid soil (pH 4·3) following the above treatments. Cooper (1975) also found manure-N was nitrified much faster than NH_4^+-N in an acid soil. Very recently, Schimel et al. (1984) using ^{15}N

tracer technique discovered that the NO_2^--N produced in an acid Sierran forest soil (pH 5·8) was not from the added $(^{15}NH_4)_2SO_4$. Other evidences supporting heterotrophic nitrification are:

(a) No autotrophic nitrifiers can be isolated from the acid soils (pH <4·5) that are producing NO_2^- and NO_3^- (Ayanaba and Omayuili, 1975; Herlihy, 1973; Ishaque and Cornfield, 1974; 1976; Lemee, 1976; Overrein, 1971; Focht and Verstraete, 1977). Alluvial soil (pH 6·0) in Cuttack, India, produced NO_2^- from NH_4^+ and an nonfluorescent Pseudomonas sp. isolated from the soil also produced NO_2^- from NH_4^+ when supplemented with glucose (Odu and Adeoye, 1970). Nitrate was found to be the predominant inorganic-N accumulated in acid forest soils (pH 3·5) and the pattern remained unchanged when pH was adjusted to 5·6 (Klein et al., 1984).

(b) NO_3^- production was positively correlated to the amount of organic-N present in the acid soils (pH 4·5) and also that the addition of $(NH_4)_2SO_4$ has no effect on NO_3^- production. Klein et al. (1983) noted that the NO_3^- accumulation in acid forest soils from the Adirondacks was 10 fold higher in the organic horizon than in the mineral horizon.

All these studies suggested that heterotrophic nitrification proceeds optimally at acidic pH values. Baxter et al. (1973) detected a trace amount of NH_2OH in water samples from an Ethiopian lake which was aerobic and highly alkaline. However, no definite proof of heterotrophic nitrification was present in their report.

Verstraete and Alexander (1973) noted the occurrence of heterotrophic nitrification in some water, sewage and soil samples at neutral or alkaline pH. They also noticed that during heterotrophic nitrification in some sewage samples, pH was increased from 7·4 to 9·0. Many other studies with pure cultures also reported that heterotrophic nitrification only proceeded at neutral or alkaline pH (Gode and Overbeck, 1972; Hirsch et al., 1961; Verstraete, 1975; Verstraete and Alexander, 1972a). Verstraete and Alexander (1972a) reported that the growth of Arthrobacter sp. ceased when the pH of the media was at 6·0 or below and at 10·0 or above. They also noticed that the production of NH_2OH was independent of pH values. Amargar and Alexander (1968) found that NO_2^- production from oximes and NH_2OH by P. aeruginosa was greatly reduced at pH 6·0 and almost negligible at pH 5·0. Castignetti and Gunner (1981) reported that Alcaligenes sp. produced NO_2^- at both acidic and alkaline pH but NO_2^- was subsequently assimilated under acidic conditions. Using citrate as a carbon source, they also noticed an increase in pH from 5·4 to 6·7 during the nitrification process. They further observed that at pH 5·2-5·4, heterotrophic nitrification by the Alcaligenes sp. was greatly reduced and growth was negligible under shaking incubation conditions but the pH effect was not apparent when the culture was incubated statically. They speculated that in the static culture, pH of micro-niche surrounding the cells was altered to allow heterotrophic nitrification to occur.

(C) O_2 Concentration

Heterotrophs have a much higher affinity for O_2 (Km=1 uM) than autotrophic nitrifiers (Greenwood, 1961; Painter, 1970; Wimpenny, 1969). It is conceivable that heterotrophs are better competitors for O_2 at low concentrations than autotrophic nitrifiers. When a metabolizable carbon source is added , the respiration rates of heterotrophs are usually greatly increased. However, under anaerobic conditions, heterotrophic nitrification would not occur (Castignetti and Hollocher, 1982). Interestingly, Castignetti and Hollocher (1984) found that 8 out of 12 representatives of denitrifying bacteria were capable of heterotrophic nitrification under aerobic conditions. The well-studied heterotrophic nitrifier, Alcaligenes sp., could denitrify under anaerobic conditions using NO_3^-, NO_2^-, NO and N_2O as electron acceptors (Castignetti and Hollocher, 1981; 1982) . and couples denitrification to oxidative phosphorylation (Castignetti and Hollocher, 1983).

(D) Temperature

The Q_{10} for heterotrophs varies depending on the concentration of organic carbon substrates present (Novak, 1974). Focht and Chang (1975) suggested Q_{10} = 5·0-16·0 at 0-15°C the temperature range which was more applicable to aquatic ecosystems. The effect of the higher temperature range (15-55°C) on the rate of heterotrophic nitrification could be described by the Arrhenius equation (Focht and Verstraete, 1977). Ishaque and Cornfield (1974) found that heterotrophic nitrification in an acid Bangladesh tea soil was rapid at 40°C and the process continued

but with a decrease in rate at 50-60°C. Soils from arid and semi-arid areas of Israel showed production of NO_3^- from inherent soil-N being more rapid at 37-40°C than at 28°C (Etinger-Tulczynska, 1969). Myers (1975) also observed NO_3^- production in tropical soils incubated at 60°C and that the rate of NO_3^- production was greater at 50°C than at 20°C. These results suggested that heterotrophic nitrifiers in terrestrial ecosystems were thermophiles and most likely used organic-N as initial substrates. Verstraete (1975) found that heterotrophic nitrification by pure cultures was optimal at around 30°C. The rate of heterotrophic nitrification by Arthrobacter sp. was found negligible at below 12°C (Laurent, 1971).

(E) Concentration of Substrates and Products

Nothing is known concerning the effect of the concentration of nitrogenous substrates and products on the heterotrophic nitrification process except that a C:N ratio of 3-5 would promote the highest rates of heterotrophic nitrification (Focht and Verstraete, 1977; Verstraete, 1975). Under favourable incubation conditions Arthrobacter sp. could accumulate as much as 60 ug NH_2OH-$N \cdot L^{-1}$ (Verstraete and Alexander, 1972a) whereas Alcaligenes sp. produced up to 1867 mg NO_2^--$N \cdot L^{-1}$ and 42 mg NO_3^--$N \cdot L^{-1}$ (Castignetti and Gunner, 1981). Whether these were the maximum concentration of substrates, intermediates and end products that did not affect heterotrophic nitrification rates and the growth of the heterotrophic nitrifiers is still not known.

(F) Inhibitors

All known specific inhibitors for autotrophic NH_3 and NO_2^- oxidation have no effect on heterotrophic nitrification (Verstraete, 1975). C_2H_2, up to 0·06 and 0·1 atm had no effect on heterotrophic nitrification by Arthrobacter sp. (Hynes and Knowles, 1982) and by an acid Sierran forest soil (Schimel et al., 1984), respectively. Gowda et al. (1977) found that neither 5000 ppm benomyl [methyl 1-(butylcarbamoyl)-2-benzimid azolecarbamate], 10 ppm N-Serve nor 10 ppm AM (2-amino-4-chloro-6-methyl pyrimidine) could prevent NO_3^- formation in a flooded alluvial soil. Chlorate at 10 mM could not inhibit NO_3^- production by soils from deserts (Etinger-Tulczynska, 1969) and forests (Schimel et al., 1984). Rho (1983) reported that 0·1-0·5 ppm Cu or 10 ppm Cd inhibited NH_2OH production by Arthrobacter sp. and 0·5-1·0 ppm Cu or 100 ppm Cd inhibited its growth. They found that other heavy metals such as Fe, Pb and Zn up to 100 ppm had no such effects.

(G) N_2O

Verstraete and Alexander (1972a) detected no N_2O, NO or NO_2 during heterotrophic nitrification by pure cultures of Arthrobacter sp. However, recent reports indicated that during heterotrophic nitrification by methylotrophs, NO (Verstraete, 1981) and N_2O (Topp and Knowles, 1982) were produced. Heterotrophic nitrifiers other than methylotrophs could possibly produce N_2O and NO indirectly as NO_2^- and NO_3^- produced under aerobic conditions would be reduced to N_2O and NO under anaerobic conditions. This is likely since most of the known heterotrophic nitrifiers are also denitrifiers (Castignetti and Hollocher, 1981; 1982; 1984).

(H) Techniques

No study on heterotrophic nitrification in aquatic ecosystems has been reported. Consequently, any method available in the literature would be from those used in studying heterotrophic nitrification in acid soils. However, these techniques may not be applicable to assay in situ heterotrophic nitrification in lakes and oceans.

(6) ACID PRECIPITATION

Acidification is defined as the process of creating an excess of hydrogen ions, H^+ (Harvey et al., 1981). Henriksen (1982) viewed lake acidification as a large scale acid-base titration in which a bicarbonate was titrated by a strong acid. Acidification of both terrestrial and aquatic ecosystems can be attributed to either local air and water pollution or acidic precipitation (acid rain) originated from long-range transport of air pollutants through the atmosphere, or both of these processes (Carlson and Rodhe, 1982; Gorham, 1976). Jickells et al. (1982) attributed the occurrence of acid rain in Bermuda to long-range transport of air pollutants from 1,000 km east of the Atlantic seaboards of the United States. Oxides of sulfur (e.g. SO_2) and nitrogen (e.g. NOx, N_2O) and some reduced compounds of sulfur (e.g. H_2S) and nitrogen (e.g. NH_3) are considered to be the main components of air pollutants (Evans et al., 1981; Tamm, 1976).

Atmospheric precipitation in equilibrium with CO_2 in the air usually has a pH value of about 5·6. Precipitation having pH values lower than

5·6 is generally considered as acid rain (Krug and Frink, 1983). However, there is evidence that in some pristine areas without atmospheric pollution influence, precipitation could have pH values below 5·0 and therefore the arbitrary value of pH 5·6 as the reference point to indicate anthropogenic pollution may not always be appropriate (Carlson and Rodhe, 1982).

Morling (1981) studied two lakes in western Sweden from 1966 to 1980. In 1969 to 1975 he observed that the pH of the lake water decreased as SO_4 concentration increased. From 1975 to 1980 when SO_4 cocentration was kept unchanged, he found that the pH of the water also levelled off. However, in Lake Unden, SNV (1979) reported that while SO_4 concentration (180 ueq·L^{-1}) remained unchanged from 1965 to 1979, the increase in NO_3^- concentration (ca. 14 ueq·L^{-1}) was accompanied with a decrease in alkalinity (ca. 17 ueq·L^{-1}) during the same period. However, Gorham et al. (1984) stated that there was a better correlation between the acidity in precipitation and its SO_4 contents ($r = 0·923$) than its NO_3^- or H^+ contents. Galloway et al. (1984) also suggested the use of SO_4 concentration in precipitation as an indicator of anthropogenic influences. Hydrogen ion concentration alone cannot be used as a reliable tracer because pristine areas have a different mixture of acids and bases in the atmosphere (Galloway et al., 1982; Keene et al., 1983; Keene and Galloway, 1984). Nitrate as well cannot be used because it is rapidly assimilated by biota in both terrestrial and aquatic ecosystems and itself does not promote long-term acidification of aquatic ecosystems as much as SO_4 (Driscoll and Likens, 1982; Galloway et al., 1983; Likens et al., 1977).

Nonetheless, the recent projected increases of NOx emissions are a cause for concern if they lead to an excess of NO_3^- over base cations (NH_4^+, Ca^{++}) in the precipitations (Galloway and Likens, 1981). The magnitudes of natural and anthropogenic emissions of $NO_2 + N_2O$ and NH_3 in Canada have been estimated to be about 7 and 13 X 10^{11} g $N \cdot yr^{-1}$, respectively (Harvey et al., 1981). Ammonia is mainly derived from agricultural fertilizers and products of animal wastes (Gorham et al., 1984).

Henriksen (1982) defined an acid lake as having pH below 5·3 and that pH became a function of the concentration of strong acids and aluminium in that lake. Acidification of surface water in lakes would result in elevated concentration of many metals existing as positive ions (e.g. Al^{+++}, Fe^{++}) some of which can be toxic to fish and other aquatic organisms (Krug and Frink, 1983; Norton, 1982; Schindler and Turner, 1982). Evans et al. (1981) recommended the maximum permissible concentration of H^+ in the precipitation to be less than 25 $ueq \cdot L^{-1}$ in order to protect the most sensitive areas from permanent lake acidification.

There are several reports indicating the dramatic effects of acid rain in streams and lakes by killing eggs of fish, salamanders and frogs (EPA, 1980) and the loss of fish population (Overrein et al., 1980). McKinley and Vestal (1982) studied the effects of acid precipitation on litter decomposition in an Arctic lake. They found that at pH 5·0 aquatic fungal population disappeared and that at pH 4·0 and below diatoms were extinct and bacterial population decreased markedly. However, there are some recent reports suggesting that the effects of acid precipitation may not be that drastic in every aquatic ecosystem. In a study of

artificial acidification of L.223 at ELA with H_2SO_4, with the pH being lowered from 6·7 to 5·1, Schindler (1980) did not observe any inhibition of algal photosynthesis. Kelly et al. (1984) found no significant decrease in rates of in situ organic matter decompositions in the lake sediments. Kollig and Hall (1982) studied the effects of acids on some biological processes in microcosms by continuously flowing HCl (pH 4·6-5·0) through the systems for an extended period of time. They found no measurable impact of acid on community function, uptake or release of major nutrients by microorganisms, biodegradation rate of a plasticizer, algal community structure and total biomass. Boylen et al. (1983) surveyed several lakes having different pH values and trophic levels in the Adirondacks and found no significant differences in microbial population due to the pH effect in all of the lakes studied. They also noticed that most of the microorganisms isolated from lakes with neutral pH could grow at pH 5·0 suggesting possible adaptation or tolerance of some bacteria to lower pH.

Three microbial processes are of immediate concern regarding the acidification of lucustrine ecosystems, namely, sulfate reduction, denitrification and nitrification (Harvey et al., 1981). The first two processes have been proven to be very important sources of aquatic alkalinity against lake acidification (Kelly et al., 1982). The third process is considered to be quite undesirable since most researchers believed that this process would generate substantial amount of acidity (Harvey et al., 1981; Kelly et al., 1982; Schindler, 1985). Theoretically, per molecule NH_4^+ oxidized, one H^+ molecule is generated (Equation 6). Other microbial processes such as the assimilation of NH_4^+ and NO_3^- by phyto-

$$NH_3 + 2O_2 \longrightarrow NO_3^- + H_2O + H^+ \qquad \text{(Equation 6)}$$

plankton would produce equivalent amounts of H^+ and OH^-, respectively, and hence no net production of acidity or alkalinity (Schindler, 1985). With regards to the substrate of nitrification, NH_4^+, some of which could be derived from runoff and N_2 fixation, is quite often derived from decomposition of organic matter in anoxic sediments (Schindler, 1985). In ELA, NH_4^+ in runoff is usually retained by watersheds (Kelly et al., 1982) to as much as 90% (D.W. Schindler, unpublished data) thereby reducing the amounts of acidity via autotrophic nitrification that would otherwise be added to the lake H^+ budgets.

MATERIALS AND METHODS

MATERIALS AND METHODS

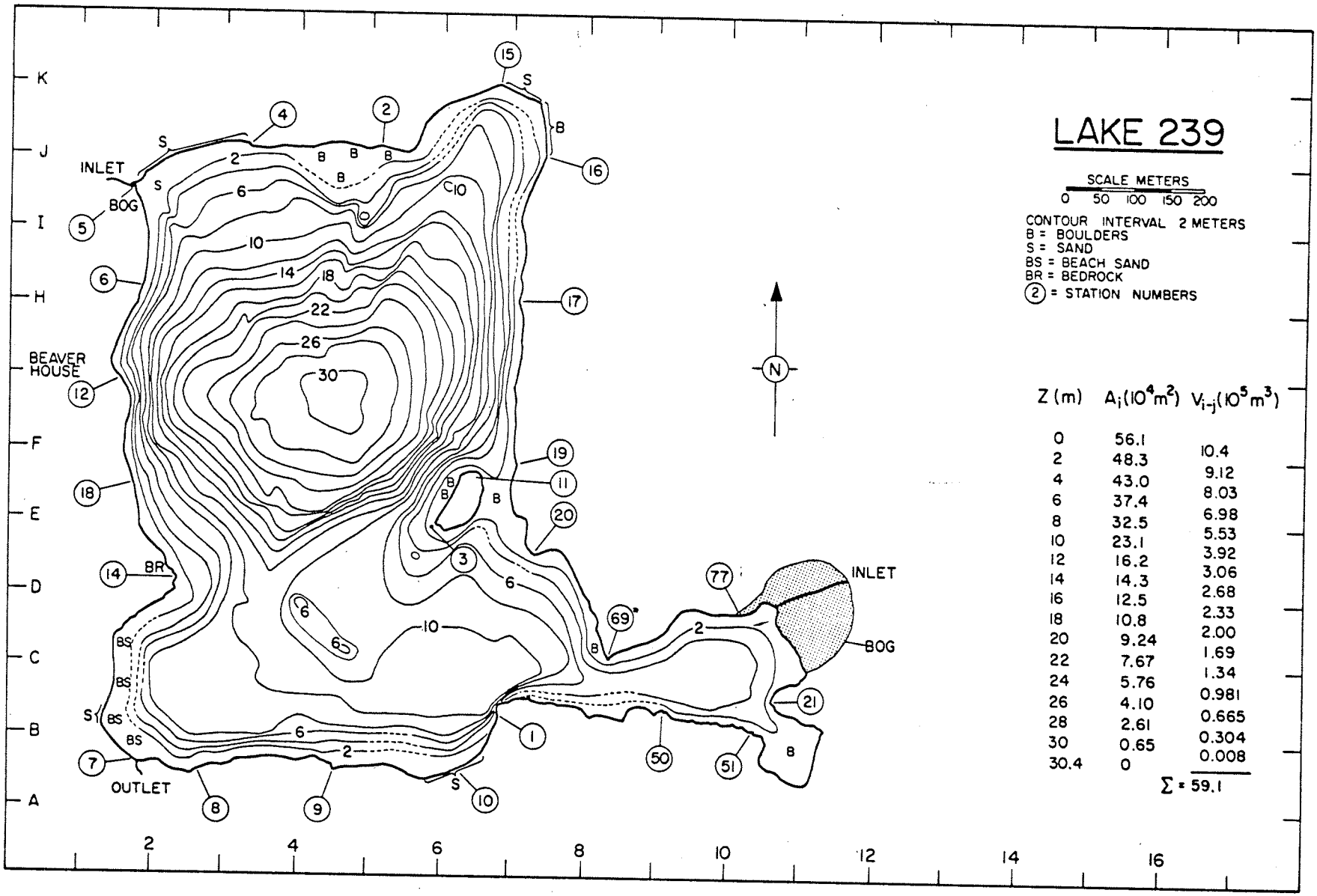
(1) SAMPLING SITES

There are about 46 Precambrian Shield lakes of the Experimental Lakes Area (ELA) southeast of Kenora, Ontario, Canada, being used for whole-lake experiments (Johnson and Vallentyne, 1971; Schindler et al., 1980). These Shield lakes are representatives of most lakes located in the northern and eastern Canada including the Great Lakes. Access to ELA is regulated by the Federal Department of Fisheries and Oceans. These lakes were previously undisturbed and acid inputs due to acid precipitation were minimal. Hydrological, meteorological and associated chemical, biological and physical measurements of these lakes and their watersheds and stream segments have been collected for six years prior to experimental perturbation studies. Hence, comparisons could be made among the measurements of the previously mentioned parameters collected before, during and after perturbations of the lakes. Consequently, the relationship between experimental perturbations and the lake responses can be quantified. Two of the lakes, Lake 239 (Rawson Lake) and Lake 302S, were selected for the present study on the effect of whole-lake acidification on nitrification. Lake 239 has been (and is still being) used as a control lake for many whole-lake experiments including eutrophication and acidification studies. Lake 302S has been used for acidification studies since 1982 by constantly adding H_2SO_4 to the lake to attain target pH values of the lake water.

Lake 239 (Fig. 2) is an oligotrophic lake with a near neutral pH. It has a surface area of 56·1 X 10^4 m^2 and a volume of 59·1 X 10^4 m^3 with average and maximum water depths of 10·5 m and 30·4 m, respectively (Brunskill and Schindler, 1971). In 1982, the highest measurements recorded during the summer for dissolved organic carbon (DOC), suspended carbon (SC), chlorophyll a (Chl a) and biomass of phytoplankton (BP) were no more than 254 umol·L^{-1}, 900 ug·L^{-1}, 14·3 ug·L^{-1} and 2·88 g·m^{-3}, respectively (DeBruyn et al., 1984). The depths of the epilimnion and thermocline (average) were, respectively, 3·25 m and 2·63 m from the surface (Cruikshank, 1984a). Ice completely covered the lake surface on 1 December, 1983 and was melted on 24 April, 1984 (K.G. Beaty, personal communication). Temperatures in the epilimnion and hypolimnion during the ice-cover period were about 1°C and 4°C, respectively (Cruikshank, 1984b). Complete turnovers occurred in May and October.

Lake 302 (Fig. 3) is a small double-basin (divided into North and South Basins) lake. The North and the South basins are of similar size separated by two shallow narrows. The lake was made eutrophic by adding NH_4Cl, sucrose and phosphoric acid at the deepest point (near the center) in the North basin of the lake from 1972-1978. In June 1981, a reinforced plastic "sea curtain" was installed in the narrows to separate the waters of both basins. The South basin (L.302S) has a surface area of 10·9 X 10^4 m^2 and water volume of 5·54 X 10^3 m^3 with mean and maximum water depths of 5.0 m and 10·6 m, respectively (Cruikshank, 1984a). Since 1982, 36N H_2SO_4 (electrolytic grade, SG = 1·835 g·L^{-1}) was added constantly to attain its target pH (5.00). Prior to acidification, the pH of the epilimetic water was 6·75. The total amount of H_2SO_4 added

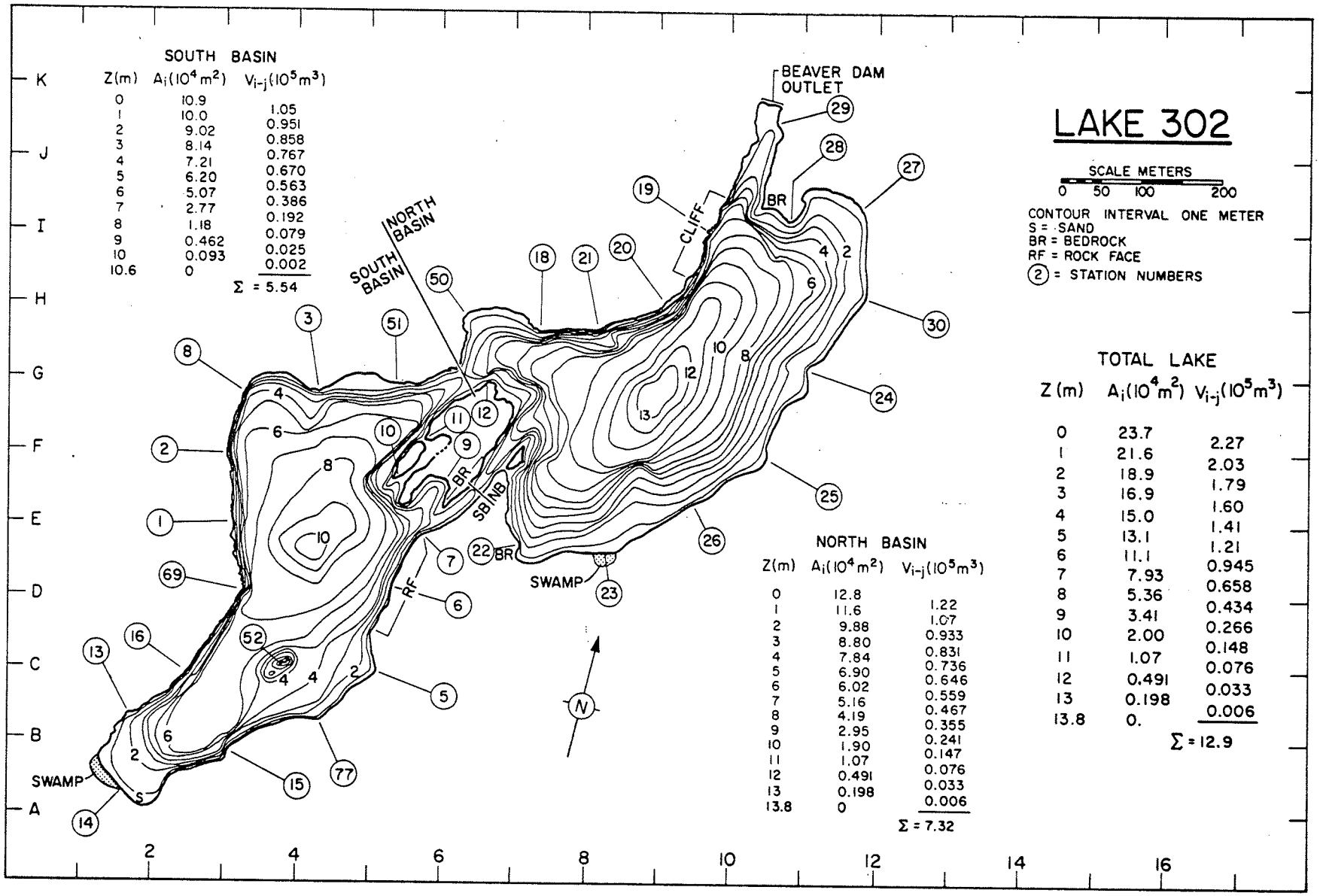
Fig. 2. Bathymetric chart of L.239, Experimental Lakes Area.



Z (m)	$A_i (10^4 m^2)$	$V_{i-j} (10^5 m^3)$
0	56.1	
		10.4
2	48.3	
		9.12
4	43.0	
		8.03
6	37.4	
		6.98
8	32.5	
		5.53
10	23.1	
		3.92
12	16.2	
		3.06
14	14.3	
		2.68
16	12.5	
		2.33
18	10.8	
		2.00
20	9.24	
		1.69
22	7.67	
		1.34
24	5.76	
		0.981
26	4.10	
		0.665
28	2.61	
		0.304
30	0.65	
		0.008
30.4	0	

$\Sigma = 59.1$

Fig. 3. Bathymetric chart of L.302, Experimental Lakes Area.


LAKE 302
SCALE METERS
0 50 100 200
CONTOUR INTERVAL ONE METER
S = SAND
BR = BEDROCK
RF = ROCK FACE
(2) = STATION NUMBERS
SOUTH BASIN
Z(m) | Ai(10^4 m^2) | Vi-j(10^5 m^3)
0 | 10.9 | 1.05
1 | 10.0 | 0.951
2 | 9.02 | 0.858
3 | 8.14 | 0.767
4 | 7.21 | 0.670
5 | 6.20 | 0.563
6 | 5.07 | 0.386
7 | 2.77 | 0.192
8 | 1.18 | 0.079
9 | 0.462 | 0.025
10 | 0.093 | 0.002
10.6 | 0 |
Σ = 5.54
NORTH BASIN
Z(m) | Ai(10^4 m^2) | Vi-j(10^5 m^3)
0 | 12.8 | 1.22
1 | 11.6 | 1.07
2 | 9.88 | 0.933
3 | 8.80 | 0.831
4 | 7.84 | 0.736
5 | 6.90 | 0.646
6 | 6.02 | 0.559
7 | 5.16 | 0.467
8 | 4.19 | 0.355
9 | 2.95 | 0.241
10 | 1.90 | 0.147
11 | 1.07 | 0.076
12 | 0.491 | 0.033
13 | 0.198 | 0.006
13.8 | 0
Σ = 7.32
TOTAL LAKE
Z (m) | Ai(10^4 m^2) | Vi-j(10^5 m^3)
0 | 23.7 | 2.27
1 | 21.6 | 2.03
2 | 18.9 | 1.79
3 | 16.9 | 1.60
4 | 15.0 | 1.41
5 | 13.1 | 1.21
6 | 11.1 | 0.945
7 | 7.93 | 0.658
8 | 5.36 | 0.434
9 | 3.41 | 0.266
10 | 2.00 | 0.148
11 | 1.07 | 0.076
12 | 0.491 | 0.033
13 | 0.198 | 0.006
13.8 | 0.
Σ = 12.9
BEAVER DAM OUTLET
NORTH BASIN
SOUTH BASIN
CLIFF
BR
SBNB
RF
SWAMP
SWAMP
S
N
A
B
C
D
E
F
G
H
I
J
K
2
4
6
8
10
12
14
16

in 1982 and 1983 were respectively 1228·5 L and 1107·7 L with resulting epilimnetic pH of 6·25 and 5·86 (Cruikshank, 1984b). A total of 1077·3 L of H_2SO_4 was added in 1984 and the time-weighted mean epilimnion pH was found to be 5·60 (D.R. Cruikshank, personal communication). In 1982, the maximum concentration recorded during the summer for DOC, SC, Chl a and BP were 352 umol·L^{-1}, 1520 ug·L^{-1}, 35.0 ug·L^{-1} and 5·42 g·m^{-3}, respectively (DeBruyn et al., 1984). The epilimnion and mean thermocline depths were 3·67 m and 5·03 m, respectively (Cruikshank, 1984a). The ice-cover and the turnover periods in 1983-1984 were identical to those in L.239 (K.G. Beaty, personal communication).

(2) COLLECTION OF SAMPLES

Lake water was sampled once a month from December 1983 to October 1984. In the winter months, holes were cut through the ice layer and water was sampled from each selected depth. A peristaltic pump equipped with a thick-walled Tygon tube calibrated at 0·5-m depth was used. Clean 542-mL opaque Nalgene plastic bottles (HDPE type, Nalge Co., Rochester, N.Y.) were rinsed twice with the lake water samples before filling. During filling, the dispensing tube was placed at the bottom of the bottle and approximately 2 volumes of the lake water were allowed to overfill the bottle before the tube was slowly withdrawn to avoid the formation of air bubbles. One parallel set of samples was kept in acid-washed, deionized distilled water-rinsed, 250-mL glass BOD bottles for chemical analyses. Another set of water samples was taken in 20-mL glass syringes then transferred to 14-mL Vacutainer® vials (Becton Dickinson & Co.,

Rutherford, N.J.) each containing 0·5 mL of saturated $HgCl_2$. These samples were subsequently used for dissolved N_2O analysis. In the winter months, all samples were kept in wooden chests equipped with foam-lining and packed with hot-water packs to avoid freezing of samples during their transportation by snowmobiles from the lakes back to the laboratory on shore. Most of the sample processing and analyses were done as soon as the samples were brought into the laboratory. Results are means of duplicate samples.

(3) IN VITRO INCUBATION

To each 1-L Erlenmeyer flask, 100 mL of water sample was added. Duplicate flasks were used for each treatment. Where appropriate, 0·5 mL of $(NH_4)_2SO_4$ and 0·5 mL of 1-allyl-2-thiourea (ATU) (Eastman Kodak, Rochester, N.Y.) solutions were added, alone or in combination, to final concentrations of 2 mg NH_4^+-N·L^{-1} and 10 mg·L^{-1} of lake water, respectively. N-Serve was used for similar experiments done in 1981-1983 but was replaced by ATU because of the high solubility of the latter in the water (no solvent effect) and also due to its effectiveness as an autotrophic nitrification inhibitor (Campbell and Aleem, 1965a). The superior efficacy of ATU over N-Serve for inhibiting autotrophic nitrification in both in vitro and in situ studies of lake nitrification has recently been validated (Hall, 1984). Starting in June 1984, additional parallel sets of flasks in duplicates were amended with 0·5 mL of appropriate concentration of sodium acetate (NaAc) to give a final ratio of acetate-C to total dissolved nitrogen (TDN-N) of 3:1 as recommended by

Verstraete and Alexander (1972a). All flasks were covered with parafilm and incubated statically in the dark at 15°C for up to one month. At appropriate intervals, water samples were withdrawn from all flasks and analyzed for NH_4^+, NO_2^- and NO_3^-. Data are means of duplicate flasks and reported as ug N per litre of lake water.

To investigate if N_2O was produced during incubation, parallel set of flasks were set up in the same manner except that 50-mL Erlenmeyer flasks were used and the amount of lake water added was 10 mL. All these flasks were capped with serum stoppers (Suba Seal, Barnsley, England). After 30 days of incubation, 0·5 mL of the gas phase from each flask was withdrawn with a 1.0-mL syringe equipped with a mininert valve (Precision Sampling Corp., Baton Rouge, L.A.) and analyzed for N_2O by gas chromatography. Data are means of triplicate flasks and reported as ug N_2O-N per litre of lake water.

(4) IN SITU INCUBATION

For in situ experiments, 1.0 mL of $(^{15}NH_4)_2SO_4$ (99% ^{15}N atom, Merck, Sharp and Dohme, Dorval, Qué) and 1.0 mL of ATU, alone or in combination, were added to the Nalgene bottles containing the water samples to give final concentrations of 2 mg NH_4^+-N·L^{-1} and 5 mg·L^{-1} of water, respectively. When heat-killed controls were required, the water samples were boiled and deionized distilled-water was added to make up the amount of water lost due to boiling. All bottles were tightly capped and placed in nylon bags. A nylon rope, having one end tied to a concrete block and the other end to a large buoy, was used. The nylon bags were

tied to the rope at the appropriate marked locations corresponding to the depths where the water was sampled. The whole assembly was slowly lowered into the water column. The bottles were incubated in situ for 1 day. Bottles were then retrieved and the water samples were filtered through 0·45-um pore size filters (Millipore Canada Ltd., Mississauga, Ont.) and 0·5 mL of chloroform was added to each filtered sample. Filtrates were stored at 4°C in the dark until further processing for ^{15}N content determinations. Data are means of duplicate samples and expressed as delta values in per mil ($\delta^{15}N$).

(5) ANALYSIS OF SAMPLES

(A) Chemical Analyses

All chemical analyses were as described by Stainton et al. (1977). Dissolved oxygen was determined by the Winkler's titrimetric method. For the determinations of DOC, TDN, NH_4^+, NO_2^- and NO_3^-, all samples were first filtered through 0·45-um pore size filters. For DOC analysis, samples were acidified to remove inorganic carbon before the photooxidation of DOC to CO_2 by UV irradiation followed by automated conductimetric measurements. A 3-channel Technicon AutoAnalyzer II (Technicon Corp., Tarrytown, N.Y.) was used for nitrogen analyses. NH_4^+ was determined by the automated phenol-hypochlorite method whereas NO_2^- was measured by the automated diazotization method. For NO_3^- analysis, NO_3^- was first reduced to NO_2^- by reactive cadmium before diazotization. Measurements of TDN was achieved by photocombustion of samples with UV irradiation followed by reduction of NO_3^- to NH_4^+ with zinc powder. The subsequent procedures were the same as with NH_4^+ measurements.

(B) Gas Chromatographic Analysis of N_2O

The multiple-phase equilibrium method (McAuliffe, 1971) was used for the determination of dissolved N_2O in the lake water samples. Ten millilitres of water sample from each Vacutainer® vial was removed with a 30-mL glass Hamilton syringe (Becton-Dickinson & Co., Rutherford, N.J.). All these syringes were previously flushed and filled with 10 mL of Argon (Ar) and were fitted with 3-way valves. Dissolved N_2O was extracted by shaking the syringes with a wrist-action shaker at room temperature for 60 min. A 1.0-mL syringe fitted with a mininert valve was flushed with Ar immediately before withdrawing 0·5 mL of the gas phase from the glass Hamilton syringes via the 3-way valves for N_2O analysis.

For measurements of N_2O, A Pye series 104 gas chromatograph (GC) equipped with a ^{63}Ni type, wide-range electron capture detector (ECD), model 140BN (Valco Instrument Co. Inc., Houston, TX) and a 3·66 m (L), 6·4 mm (OD) glass column packed with 80-100 mesh Porapak Q was used. The column temperature was maintained at 55°C. Using 5% CH_4 in Ar as a carrier gas at a flow rate of 30 $mL \cdot min^{-1}$, a make-up carrier gas at a flow rate of 50 $mL \cdot min^{-1}$ suppling directly to the detector was required in order to stabilize the output signals. In order to remove the impurities that may possibly be present in the carrier gas, a gas-purifier cartridge (to remove O_2 and H_2O) and a hydro-purge cartridge (to remove H_2O, CO_2, H_2S, NO_2 and HCl) were both installed in-line between the gas tank and the carrier gas inlets of the GC. The ECD was operated at 325°C and 340 pA standing current.

(C) Determination of $\delta^{15}N$ Values

Water samples from the in situ experiments were filtered and each sample was divided into two 250-mL portions. The first portion (A) was for $^{15}NO_2^-$-N and the second portion (B) was for $^{15}NO_2^-$-N + $^{15}NO_3^-$-N. The filtrate of the "B" portion was passed through a glass column filled with reactive cadmium to reduce NO_3^-, if any, to NO_2^-. For both "A" and "B" portions, 10-mL portions of the filtrates were withdrawn and analyzed for NH_4^+-N, NO_2^--N and NO_3^--N as previously described. The remaining 240 mL of the filtrate from each portion was transferred to a 250-mL volumetric flask and exactly 0·6 mL of 8·4 mM $Na^{14}NO_2^-$ was added. All flasks were capped with serum stoppers, evacuated, and back-filled with pure helium (He) to 1 atm. A glass syringe was used to remove 10 mL of the gas phase from each flask. Ten millilitres of concentrated sulfamic acid saturated with He was injected into each flask. Sulfamic acid reacted with NO_2^--N in the filtrates vigorously and released N_2 and some NO (amount depending on the pH of the filtrate) to the gas phase of each flask. A 2·5-mL glass syringe equipped with a mininert valve was used to withdraw 2.0 mL of the gas phase for injection into the mass spectrometer to determine the delta values in per mil ($\delta^{15}N$). A Micromass MM602E mass spectrometer (VG Isogas, Cheshire, England) equipped with double collectors for $^{15}N/^{14}N$ ion beams was used. The analyzer was operated at 10^{-10} mbar or below with the source monitor set at 5 mA, 4·5 kV and emission at 200 uA. Samples were analyzed for 29/28 amu in N_2 against the reference N_2 gas (ultra high purity grade, Canadian Liquid Air Ltd., Montréal, Qué.). Similar techniques and calculations have been reported (Koike and Hattori, 1978).

(D) Enumeration of Bacteria

The number of heterotrophs capable of growth at 4 and 15°C on nutrient agar was estimated by plating 0·2-mL portions of decimally diluted water samples onto the agar surface. Plates were incubated for 3-4 weeks in the dark. Numbers of bacteria capable of growth on Nitrosomonas (Suzuki et al., 1981) and Nitrobacter (Cobley, 1976) agar media at pH 5·5 and 7·6 were also estimated by incubating at 4 and 15°C for 4-6 weeks in the dark. The development of colonies was reported as number of bacteria (colony-forming units) per litre of lake water and are means of duplicate plates.

(E) Isolation, Purification and Identification of Bacteria Capable of Growth on Autotrophic Nitrifying Media

Various types of bacteria grown on Nitrosomonas and Nitrobacter agar media were isolated and purified by restreaking single colonies onto fresh agar media. Pure cultures of the isolates were subsequently identified according to the procedures and critieria as described by Gerhardt (1981) and Palleroni (1984). Pure cultures of a heterotrophic nitrifying bacterial isolate were also examined by electron microscopy using shadow casting techniques. A model 801 AEI-EM electron microscope (Associated Electrical Industries Ltd., Harlow, England) was used and pictures were taken at 18,615X magnification.

RESULTS

RESULTS

The results presented are expressed as units per litre of lake water. All significant differences ($p = 0{\cdot}10$) were calculated according to the Student-Newman-Keuls multiple range test.

(1) PROFILES

(A) Some Physico-Chemical Parameters of the Lake Water

(a) Lake 239

The pH of the lake water in L.239 remained close to neutrality throughout the sampling periods (Fig. 4), being slightly alkaline to neutral at the 5-m depth to slightly acidic at the 30-m depth. The concentrations of dissolved oxygen (DO) (Fig. 5) were between 8-14 $mg \cdot L^{-1}$ except the bottom (30-m) where DO could be as low as 1 $mg \cdot L^{-1}$ suggesting that the sediment-water interface was anoxic. The concentrations of dissolved organic carbon (DOC) were very low being $0{\cdot}45$-$0{\cdot}65$ $mmol \cdot L^{-1}$ in most of the sampling periods and slightly higher ($0{\cdot}8$-$0{\cdot}9$ $mmol \cdot L^{-1}$) in April (Fig. 6). The total dissolved nitrogen (TDN) levels was also low and never exceeded 1 mg $N \cdot L^{-1}$ (Fig. 7). The low levels of DOC and TDN reflected the oligotrophic nature of the lake. The concentrations of NO_2^- were below detectability (1 ug $N \cdot L^{-1}$) throughout the year (figure not presented). Both the NH_4^+ (Fig. 8) and NO_3^- (Fig. 9) concentrations were low, even the highest levels sometimes detected at the 25-m and 30-m depths were below 100 and 110 ug $N \cdot L^{-1}$, respectively.

(b) Lake 302S

From December 1983 to October 1984, the pH of the lake water in L.302S was in the range of 5·26-6·10 except the bottom-depth water, 10-m, where pH could reach to as high as 6·43 (Fig. 10) possibly due to sediment denitrification and sulfate reduction producing alkalinity. The concentrations of DO (Fig. 11) were negatively correlated with water depths being lower with deeper depths towards the sediment and became almost anoxic in the bottom water, 8 m to 10 m. The anoxia in the bottom water suggested that denitrification and sulfate reduction could occur at the sediment-water interface resulting in higher pH values of the bottom water. The concentrations of DOC (Fig. 12) were not much higher than those in L.239. TDN concentrations in the lake water (Fig. 13) did not exceed 2 mg $N \cdot L^{-1}$ except at the bottom depth where the levels reached 16·56 mg $N \cdot L^{-1}$ in March. The NH_4^+ concentrations (Fig. 14) were below 1 mg $N \cdot L^{-1}$ throughout the year except the bottom depth where NH_4^+ levels rose to 7 mg $N \cdot L^{-1}$ in March and April suggesting that ammonification occurred in the sediments with consequent decreasing NH_4^+ and TDN gradients up the water column. NO_2^- concentrations in the lake water were below the detection limits in December but started to accumulate in January (Fig. 15). The levels of NO_2^- peaked in March but then gradually decreased and became undetectable after May. The highest concentration of NO_2^- detected was at the 6-m depth in March being 50 ug $N \cdot L^{-1}$ (Fig. 15). The NO_3^- concentrations (Fig. 16) were usually less than 30 ug $N \cdot L^{-1}$ except at the bottom water where the levels were somewhat higher, being 76 ug $N \cdot L^{-1}$ in September. The data suggested that the rates of NO_2^- production were sometimes much greater than those of NO_3^- resulting in accumulation of high concentrations of NO_2^-.

(B) Bacterial Populations in the Lakes

(a) Lake 239

The number of heterotrophic bacteria capable of growth on nutrient agar at 15°C (Fig. 17) were almost twice the number obtained at 4°C (Fig. 18) suggesting that the higher incubation temperatures were more favorable for the growth of heterotrophs on nutrient-rich media. The number of bacteria capable of growth on pH 5·5 Nitrosomonas media at 15°C (Fig. 19) was somewhat higher than that on pH 7·6 media (Fig. 20) suggesting that the growth of some particular groups of bacteria on Nitrosomonas media was more favored at acidic than alkaline pH. However, the pH effect was not very prominent at both pH 5·5 (Fig. 21) and 7·6 (Fig. 22) when the incubation temperature was at 4°C. The pH effect on the number of bacteria capable of growth on Nitrobacter media at 15°C was different from those grown on Nitrosomonas media. The number of bacteria grown at pH 5·5 (Fig. 23) was similar to that at pH 7·6 (Fig. 24). However, the number of bacteria grown on Nitrobacter media at pH 5·5 was lower than that at pH 7·6 when the incubation temperature was lowered to 4°C (Figs. 25 and 26) suggesting that alkaline pH stimulated the growth of some bacteria on Nitrobacter media at low temperatures.

(b) Lake 302S

The effect of incubation temperature on number of heterotrophic bacteria capable of growth on nutrient agar was similar to that observed in L.239, being twice as much for the population growing at 15°C (Fig. 27)

than at 4°C (Fig. 28). The profiles of heterotrophic bacteria showed no definite relationship between the population size and the water depth but at some water depths there appeared to have more bacteria in the warmer months (Figs. 27 and 28). The number of bacteria capable of growth on Nitrosomonas media was 2-3 times higher at 15 than at 4°C incubation (Figs. 29 and 30). The differences were even greater, being 2-4 times, when the media used were at pH 5·5 (Figs. 31 and 32). With Nitrobacter media at pH 7·6, the number of bacteria growing at 15°C (Fig. 33) was up to 15 times higher than that at 4°C (Fig. 34). At pH 5·5, the number of bacteria was only twice as much at 15°C (Fig. 35) than at 4°C (Fig. 36). It seemed that the bacteria of L.302S were probably acid-tolerant rather than acidophilic in nature, and that higher incubation temperatures and alkaline pH appeared to stimulate bacterial growth on autotrophic media. Large bacterial population capable of growth on Nitrobacter was observed at 7 and 8 m (Figs. 33, 34, 35 and 36) suggesting the presence of certain environmental factors favoured their growth at these water depths.

(2) IN VITRO NITRIFICATION

(A) Changes in NO_2^- and NO_3^- Concentrations in Water Samples Obtained from December 1983 to October 1984.

(a) Lake 239

December. There was no NO_2^- accumulated (figure not presented) and the net increases of NO_3^- were negligible (Fig. 37). With the deepest samples, there was a slight decrease in NO_3^- concentration.

January. Accumulation of NO_2^- in small quantities was observed in some of the samples (Fig. 38) but the NO_3^- concentrations remained unchanged (Fig. 39).

February. The fluctuations of NO_2^- occurred in most of the samples (Fig. 40) suggested the most of the produced NO_2^- was probably constantly removed from the system. There were, however, no net increases in NO_3^- concentrations except for the deepest samples where an increase of 154 ug $N \cdot L^{-1}$ occurred within 20 days (Fig. 41).

March. The fluctuation of NO_2^- concentrations (Fig. 42), the lack of net increase of NO_3^- in most samples and the accumulation of NO_3^- in the deepest samples (Fig. 43) were the same as in February. However, the amounts of NO_3^- accumulated in the deepest samples were almost two times higher than those in February.

April. The patterns of NO_2^- accumulation and disappearance (Fig. 44) were the same as with the previous two months except that there were net NO_2^- accumulations in the 15-m and the 30-m samples (Fig. 44). There were no observable net changes in NO_3^- concentrations (Fig. 45).

May. NO_2^- was not detected in the first 15 days of incubation (Fig. 46) in all samples. On day 20, NO_2^- began to accumulate in some of the samples. NO_3^- concentrations remained more or less unchanged (Fig. 47).

June. In samples without NaAc supplements, the patterns of changes in NO_2^- concentrations (Fig. 48) were no different from those observed in May. In NaAc-amended samples, the accumulation and disappear-

ance of NO_2^- in the absence of added NH_4^+ (Fig. 49) were relatively smaller in amounts than those in the presence of added NH_4^+ (Fig. 50). There were no net changes of NO_3^- concentrations in samples without added NaAc (Fig. 51). No net production of NO_3^- was observed with samples amended with NaAc (Fig. 52) or NaAc and NH_4^+ (Fig. 53). NO_3^- concentrations started to decline after day 10 and comletely disappeared in some of the samples at the end of the incubation period.

July. NO_2^- was not detected in samples without NaAc added throughout the incubation periods (figure not presented). The addition of NaAc caused NO_2^- to accumulate in some of the samples (Fig. 54). The presence of added NH_4^+ and NaAc (Fig. 55) also had the similar effect as with NaAc added alone. Very little accumulation but some disappearance of NO_3^- was observed in some of the samples without or with NaAc or NaAc and NH_4^+ added (Figs. 56, 57 and 58).

August. NO_2^- was not detected throughout the incubation period (figure not presented). With the NaAc-amended samples, however, NO_2^- was produced and accumulated (Figs. 59). The addition of NH_4^+ to the NaAc-amended samples resulted in a two-fold increase in NO_2^- accumulation (Fig. 60) as compared to those without NH_4^+ supplements (Fig. 59). NO_3^- concentrations remained unchanged in samples without NaAc added (Fig. 61). There were little or no net increases in NO_3^- in the samples amended with NaAc (Fig. 62) and the NO_3^- present in some of the them was subsequently disappeared. Samples amended with NaAc and NH_4^+ showed disappearance of NO_3^- present (Fig. 63).

September. There were some transformations of NO_2^- in samples without NaAc supplements (Fig. 64) but the amounts were no more than 2 ug NO_2^--N·L^{-1}. The additions of NaAc stimulated of NO_2^- production (Fig. 65) and the amounts were even larger when NH_4^+ was also added (Fig. 66). The concentrations of NO_3^- remained unchanged in samples without added NaAc (Fig. 67) with the exception of the deepest samples. There were only decreases in NO_3^- concentrations in all samples added with either NaAc (Fig. 68) or NaAC and NH_4^+ (Fig. 69).

October. The fluctuations of NO_2^- concentrations were no more than 2 ug N·L^{-1} in samples without added NaAc (Fig. 70). The additions of NaAc stimulated the accumulation of NO_2^- and the effect was more pronounced in the 15-m samples (Fig. 71). When both NaAc and NH_4^+ were added to the samples, net NO_2^- accumulations occurred in all the samples (Fig. 72) but the amounts were much less than those with NaAc added alone. NO_3^- concentrations remained relatively unchanged in most samples without NaAc supplements (Fig. 73). There were, however, small amounts of NO_3^- accumulated in the 25-m and 30-m samples. Results were similar to those amended with NaAc alone (Fig. 74) or NaAc and NH_4^+ (Fig. 75).

(b) Lake 302S

December. NO_2^- accumulations occurred only in some samples of deeper depths (Fig. 76). There were, however, some NO_3^- accumulations in most of the samples. In the 6-m samples, concentrations of NO_3^- accumulated reached 108 ug N·L^{-1} in 5 days of incubation (Fig. 77).

January. There were small amounts of NO_2^- accumulated in most of the samples (Fig. 78). Large amounts of NO_3^- were produced in the 9-m and the 10-m depth samples (Fig. 79). Interestingly, the 10-m samples had accumulated as much as 700 ug NO_3^--N·L^{-1} at the end of the incubation periods.

February. There were only small amounts of NO_2^- accumulated in most of the samples followed by either a gradual or rapid disappearance of the accumulated NO_2^- (Fig. 80). Also, net NO_3^- production ranged from very small amounts to almost none in most of the samples (Fig. 81). In the case of the deepest samples, net NO_3^- production occurred in the first 10 days of incubation, up to 132 ug N·L^{-1}, and was followed by its rapid disappearance.

March. There were little or no net NO_2^- production and the high concentrations of endogenous NO_2^- disappeared very rapidly (Fig. 82). On the other hand, the net production of NO_3^- was very pronounced in the first 5 days of incubation (Fig. 83). Within 4 days, 578 and 236 ug NO_3^--N·L^{-1} were accumulated by the 9-m and the 10-m samples, respectively (Fig. 83).

April. There were no net production of NO_2^- but disappearance of the endogenous NO_2^- in most of the samples (Fig. 84). However, in the 9-m and the 10-m samples, NO_2^- continued to accumulate (Fig. 84). There was some NO_3^- production in most of the samples but the amounts were relatively small (Fig. 85).

May. The NO_2^- concentrations fluctuated between 3 to 6 ug N·L^{-1} in some samples but remained unchanged (about 4 ug N·L^{-1}) in the other (Fig. 86). Net NO_3^- production occurred only after 10-15 days of

incubation in most of the samples and the amounts accumulated were no more than 30 ug $N \cdot L^{-1}$ in the next 15 days of incubations (Fig. 87).

June. The net NO_2^- production was followed by its disappearance and yet the net increases were no more than 3 ug $N \cdot L^{-1}$ (Fig. 88). However, in the presence of added NaAc, these increases reached as high as 21 ug $N \cdot L^{-1}$ (Fig. 89). The stimulatory effects of NaAc and NH_4^+ on net NO_2^- production (Fig. 90) were less compared to that with NaAc added alone. Net NO_3^- production was small in all samples without NaAc added (Fig. 91). In samples amended with NaAc (Fig. 92) or NaAc and NH_4^+ (Fig. 93) some of them showed the NO_3^- disappearance followed by its accumulation.

July. NO_2^- was not detected in most of the samples without NaAc added except the 10-m samples in which small quantity of NO_2^- was accumulated (Fig. 94). Results were similar to those samples amended with NaAc (Fig. 95). In samples amended with both NaAc and NH_4^+, accumulation of small amounts of NO_2^- occurred in some samples (Fig. 96). Net NO_3^- production was small in samples either without NaAc supplements (Fig. 97) or with NaAc added alone (Fig. 98). Similar results were obtained with samples amended with both NaAc and NH_4^+ except for the bottom depth where net production of NO_3^- (5 ug $N \cdot L^{-1}$) occurred after 20 days of incubation (Fig. 99).

August. Little or no NO_2^- accumulated in samples without NaAc added (Fig. 100). There was more net NO_2^- production in samples amended with NaAc alone (Fig. 101) or NaAc and NH_4^+ (Fig. 102). There was no net NO_3^- production in most of the samples except the deepest sam-

ples in which an increase in NO_3^- concentration occurred at 5 days (Fig. 103). Similar results were obtained with samples amended with NaAc alone (Fig. 104). In samples amended with both NaAc and NH_4^+ only the 10-m samples showed the accumulation of NO_3^- (Fig. 105).

September. The amount of net NO_2^- produced was negligibly small in samples either without added NaAc (Fig. 106), with added NaAc alone (Fig. 107) or with both NaAc and NH_4^+ added (Fig. 108). The amounts of NO_3^- produced were small in most of the samples without NaAc supplements except the 9-m samples in which NO_3^- accumulations occurred after a lag period of 15 days (Fig. 109). The accumulation of NO_3^- at 20 days quickly disappeared (Fig. 109). Samples amended with NaAc alone showed similar results (Fig. 110). In samples added with both NaAc and NH_4^+, steady but small increases in NO_3^- concentrations were observed in some of the samples (Fig. 111).

October. In samples without NaAc added, net NO_2^- production only occurred once in the 9-m samples and none in all other samples (Fig. 112). The additions of NaAc or NaAc and NH_4^+ stimulated NO_2^- production, though small in quantity, in some of the samples (Figs. 113 and 114). Net NO_3^- production in samples without NaAc added was small and occurred only in some of the samples (Fig. 115). In the presence of added NaAc, NO_3^- started to accumulate after a 5-day lag period (Fig. 116). However, in samples amended with both NaAc and NH_4^+, the net increase in NO_3^- concentration was negligible (Fig. 117).

(B) pH Changes in the Water Samples During Nitrification

(a) Lake 239

The pH generally increased with time in most of the water samples incubated with or without NaAc and in the presence or absence of added NH_4^+ (Figs. 118, 119 and 120). These increases ranged from 0 to 1·2, 0 to 1·0 and 0·3 to 1·4 pH units in samples without any supplements (Fig. 118), with NaAc added (Fig. 119) and with both NaAc and NH_4^+ added (Fig. 120), respectively. The occurrence of pH increments in some of the samples which showed no net NO_2^- and/or NO_3^- production suggested the rates of their disappearance were probably much faster than that of their production. Also, the pH data indicated that greater production of alkalinity was often associated with samples showing higher concentrations of NO_2^- and/or NO_3^- accumulated.

(b) Lake 302S

The pH increased with time in most of the samples incubated with or without NaAc and NH_4^+ supplements (Figs. 121, 122 and 123). The pH increments were quite similar to those samples of L.239. The increments were in the ranges of 0 to 1·7, 0 to 1·9 and 0·3 to 2·0 pH units in samples without any supplements (Fig. 121), with NaAc added (Fig. 122) and with both NaAc and NH_4^+ added (Fig. 123), respectively. Samples of the deeper depths usually having more NO_2^- and/or NO_3^- accumulation often had greater pH increments.

(C) Transformations of NO_3^-, NO_2^- and NH_4^+ and the Effects of NH_4^+ and Allylthiourea Supplements on NO_3^- Production

The data obtained from the experiments in March with 30 m (L.239) and 9 m (L.302S) samples were used to examine the relationships, if any, among the transformations of NO_3^-, NO_2^- and NH_4^+ and also the effects of NH_4^+ and allylthiourea supplements on NO_3^- production. The net production of NO_3^- and NO_2^- in L.239 (Fig. 124A) and NO_3^- in L.302S (Fig. 125A) occurred at the time when there were little or no changes in NH_4^+ concentrations suggesting that the produced NO_3^- and NO_2^- were not derived from NH_4^+. The data also showed that the disappearance of NO_3^- under aerobic conditions was not accompanied by the production of equivalent amounts of NO_2^- or NH_4^+ (Figs. 124A and 125A) suggesting that the disappearance of NO_3^- was not the result of its reduction to NO_2^- and/or NH_4^+.

The addition of NH_4^+ to the samples from both lakes resulted in decreases in the amounts of NO_3^- produced (Figs. 124B and 125B) suggesting that the presence of NH_4^+ did not stimulate NO_3^- production and, in some cases, depressed its production. The addition of the autotrophic nitrification inhibitor, allylthiourea, to the samples from both lakes did not significantly inhibit NO_3^- production when compared to those samples without the inhibitor added (Figs. 124C and 125C) suggesting that NO_3^- production in these two lakes was not the result of the conventional type of autotrophic nitrification.

(3) IN SITU DISSOLVED N_2O PROFILES AND IN VITRO N_2O PRODUCTION

The dissolved N_2O profiles for both L.239 and L.302S in the month of March (Figs. 126 and 127) were determined since highest nitrifying activities were detected in this month. The concentrations of dissolved N_2O in the surface waters of both lakes (Figs. 126 and 127) appeared to be quite high. The in vitro N_2O production rates were lower in the 30-m samples than samples from the other depths of L.239 (Fig. 126). Whereas for L.302S, 6-, 7- and 10-m samples showed highest rates of N_2O production (Fig. 127). In both in vitro experiments, the results showed that N_2O was produced during heterotrophic nitrification and that higher heterotrophic nitrification rates showed greater rates of N_2O production since L.302S which had higher nitrification rates than L.239 also had greater rates of N_2O production than L.239 (Figs. 126 and 127).

(4) IN SITU NITRIFICATION

In order to further confirm if heterotrophic nitrification was indeed the sole source of in situ NO_2^- and NO_3^- production, ^{15}N tracer techniques were used for the in situ experiments. In December 1984, water samples from the deepest of both L.239 and L.302S, 30 m and 10 m respectively, were amended with $^{15}NH_4^+$ was incubated in situ for 24 hours. Mass spectrometric analyses showed that the samples from L.239 had a $\delta^{15}N$ value of 14·336 ± 1·628% versus 13·286 ± 1·831% obtained from heat-killed controls. Whereas those from L.302S had a $\delta^{15}N$ value of 3·184 ± 0·637% versus 2·500 ± 0·605% obtained from heat-killed controls. The results indicated that the ^{15}N contents in the NO_2^--N and NO_3^--N produced in the

samples from both lakes during the incubation period were not significantly different from their respective sterile controls suggesting that the N atom in the NO_2^--N and NO_3^--N were probably not derived from the N-atoms of the added NH_4^+-N.

(5) ISOLATION, PURIFICATION AND CHARACTERIZATION OF BACTERIA

Lake water samples from all selected depths of L.239 and L.302S were used to inoculate Nitrosomonas and Nitrobacter agar plates. After an appropriate incubation period, various types of bacterial colonies were isolated, purified and characterized. These procedures were repeated with fresh samples obtained monthly. No autotrophic nitrifying bacteria such as Nitrosomonas and Nitrobacter spp. were found. All bacteria capable of growth on Nitrosomonas and Nitrobacter media at pH 5·5 and 7·6 and at 4 or 15°C were heterotrophs. There were five different bacterial species obtained from the L.239 water samples. Among them one particular species was found to be the most predominant and was subsequently identified as Pseudomonas fluorescens (Fig. 128). However, there was consistently only one type of heterotrophic species growing on the autotrophic media inoculated with L.302S water samples. The species was identical to the P. fluorescens isolated from the L.239 water samples.

Pure cultures of P. fluorescens were found capable of growth on Nitrosomonas and Nitrobacter agar media at both pH 5·5 and 7·6 at either 4, 15, 28 or 37°C. It appeared that the bacterium probably obtained its carbon source from some unknown organic compounds present in the agar media as contaminants since the bacterium was not an autotroph. The

effect of pH on growth of the pure cultures in nutrient broth incubated at 28°C was further studied. Growth was most rapid at neutral pH and was slower but had no lag period at acidic pH as low as 4·5. At pH 4·0, however, there was a lag period of 3 weeks before growth commenced. Growth was not observed at pH 3·0 and 3·5 even after 4 weeks of incubation. The results suggested that the heterotrophic nitrifier was probably acid-tolerant rather than acidophilic.

(6) SUMMARY OF RESULTS

Throughout the experimental periods of all water samples from both L.239 and L.302S, the results can be summarized as follows:

(a) Addition of $(NH_4)_2SO_4$ did not stimulate NO_2^- or NO_3^- production and in some cases depressed NO_2^- and NO_3^- production;

(b) The addition of NaAc alone or in combination with NH_4^+, in most cases, stimulated NO_2^- production;

(c) In most of the samples whether having net accumulations of NO_2^- and/or NO_3^- or not, the pH values of the water samples consistently increased;

(d) There were no net changes in the concentrations of endogenous and/or added NH_4^+-N even at end of the incubation periods during which the accumulation and the disappearance of NO_2^- and NO_3^- was occurring;

(e) The disappearance of NO_2^- was not accompanied by the production of NO_3^- under aerobic conditions;

(f) The disappearance of NO_3^- in the presence of NH_4^+ under aerobic conditions was not accompanied by the production of NO_2^- or NH_4^+;

(g) The patterns of changes in concentrations of NO_2^- and NO_3^- in the lake water were identical in the presence or absence of the autotrophic nitrification inhibitor, allylthiourea;

(h) ^{15}N tracer experiments indicated that both NO_2^--N and NO_3^--N were not derived from NH_4^+-N;

(i) Water samples obtained for 11 consecutive months did not contain any culturable autotrophic nitrifiers and all bacteria grown on the autotrophic nitrifying media inoculated with the water samples were heterotrophs which were predominantly Pseudomonas spp.

All the above observations strongly suggested that heterotrophic nitrification was responsible for the production of NO_2^- and NO_3^- in the two lakes.

Fig. 4. The *in situ* pH at various water depths of L.239. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

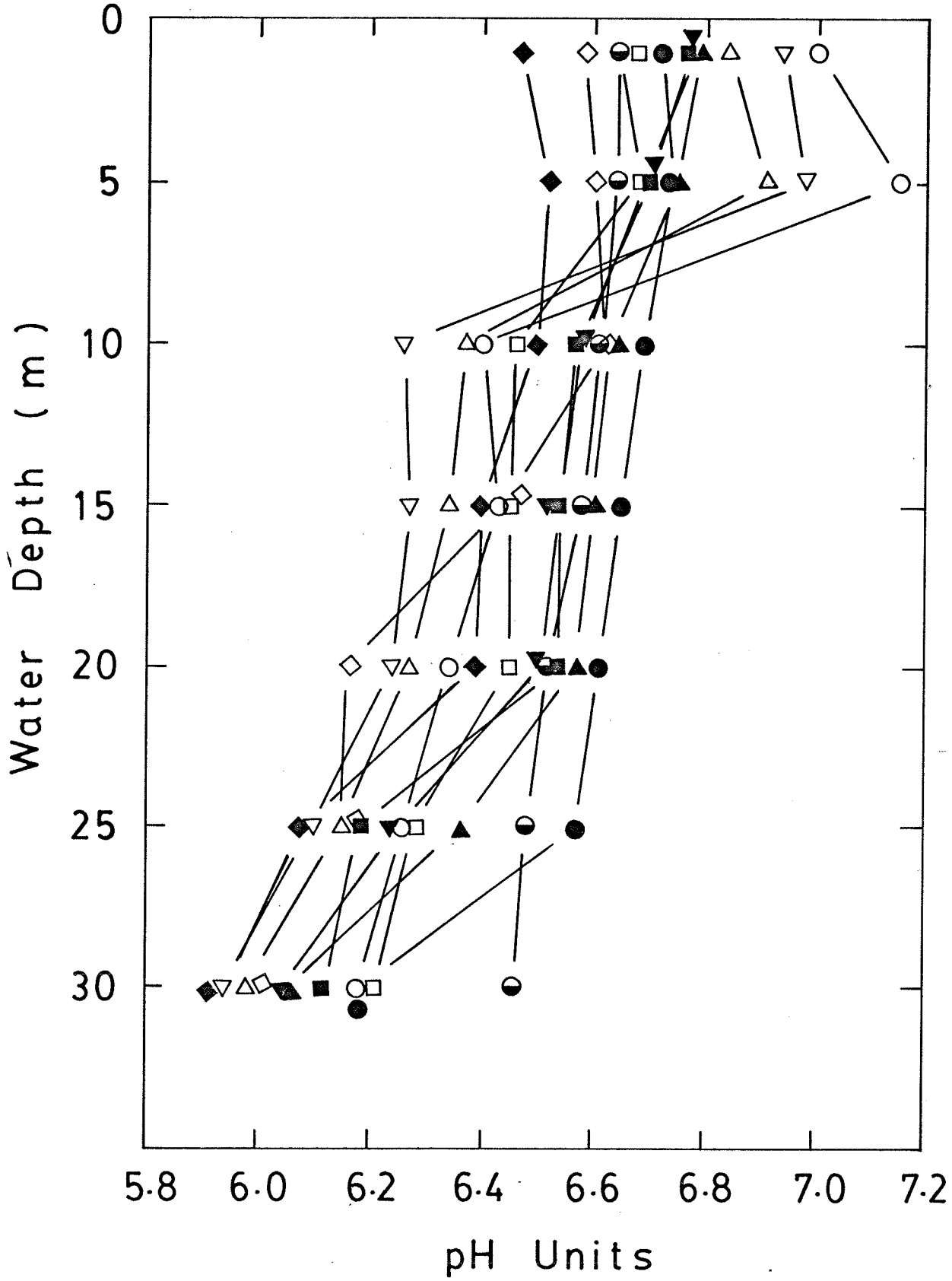

0
5
10
15
20
25
30
Water Depth (m)
5·8
6·0
6·2
6·4
6·6
6·8
7·0
7·2
pH Units

Fig. 5. The *in situ* dissolved oxygen concentration at various water depths of L.239. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May, (○) June and (△) July. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

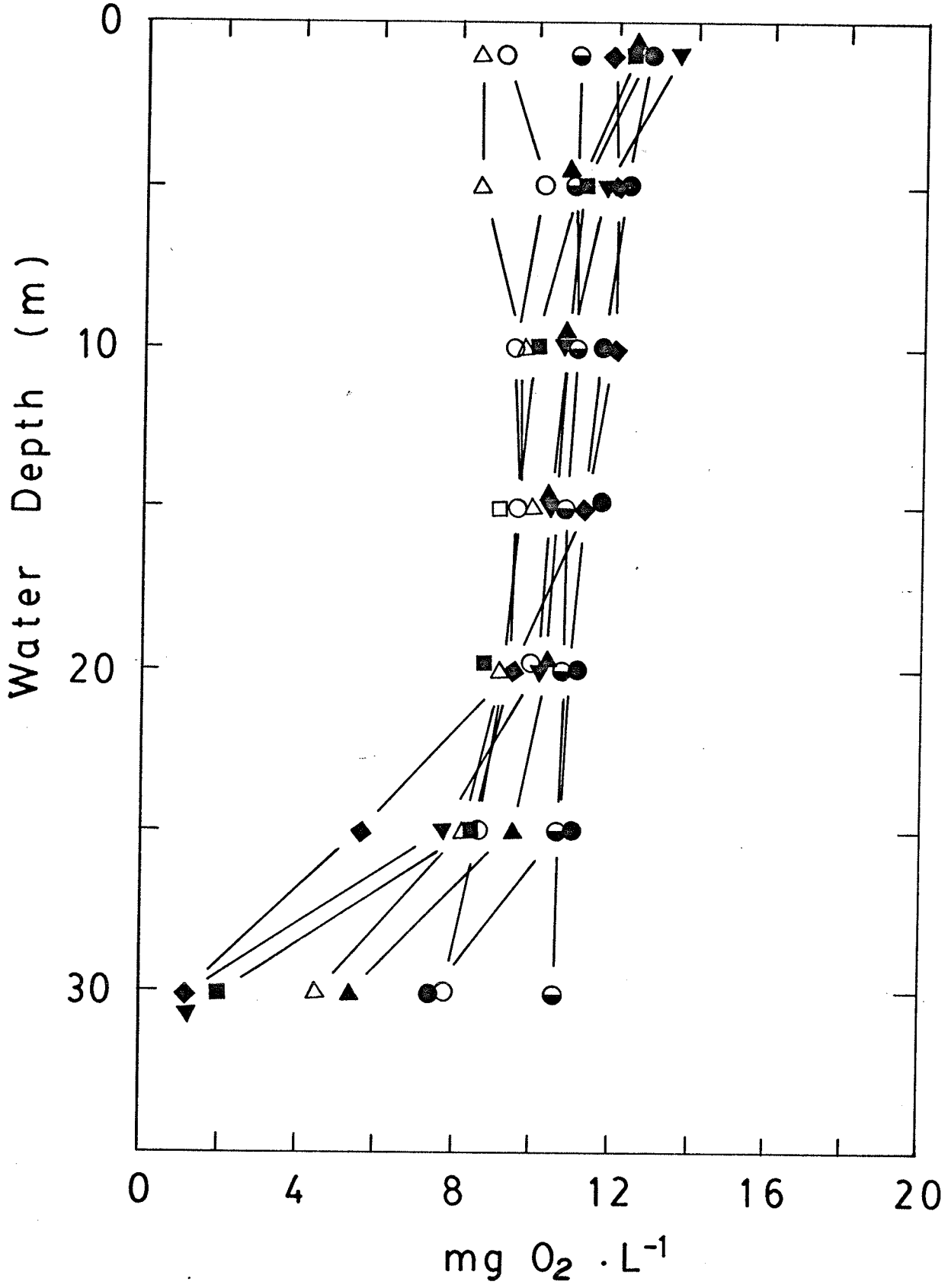

0
10
20
30
Water Depth (m)
0
4
8
12
16
20
mg O_2 · L^{-1}

Fig. 6. The *in situ* dissolved organic carbon concentration at various water depths of L.239. Symbols are : (■) March, (◆) April, (⊖) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

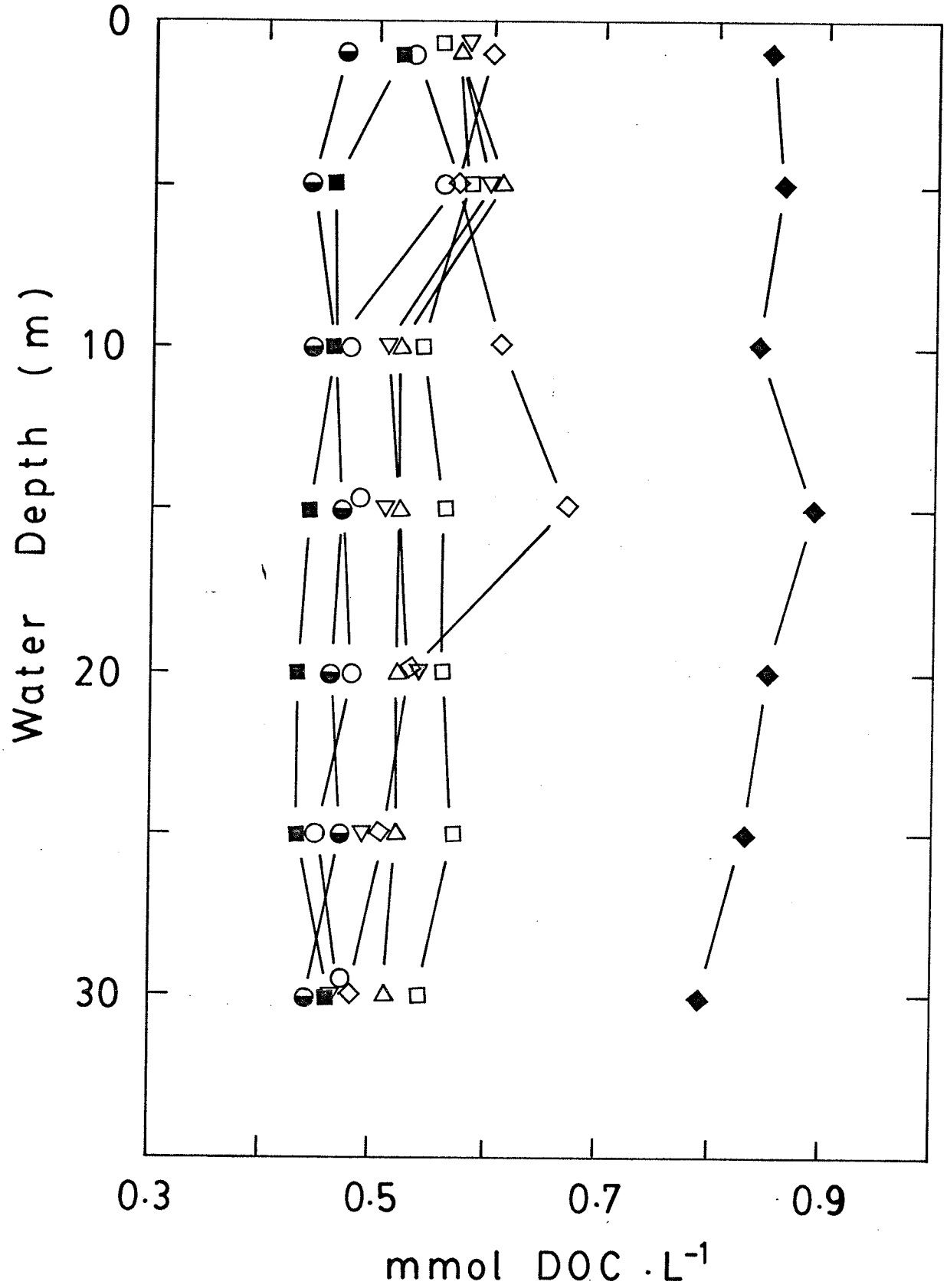

0
10
20
30
Water Depth (m)
0·3
0·5
0·7
0·9
mmol DOC ·L⁻¹

Fig. 7. The *in situ* total dissolved nitrogen concentration at various water depths of L.239. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

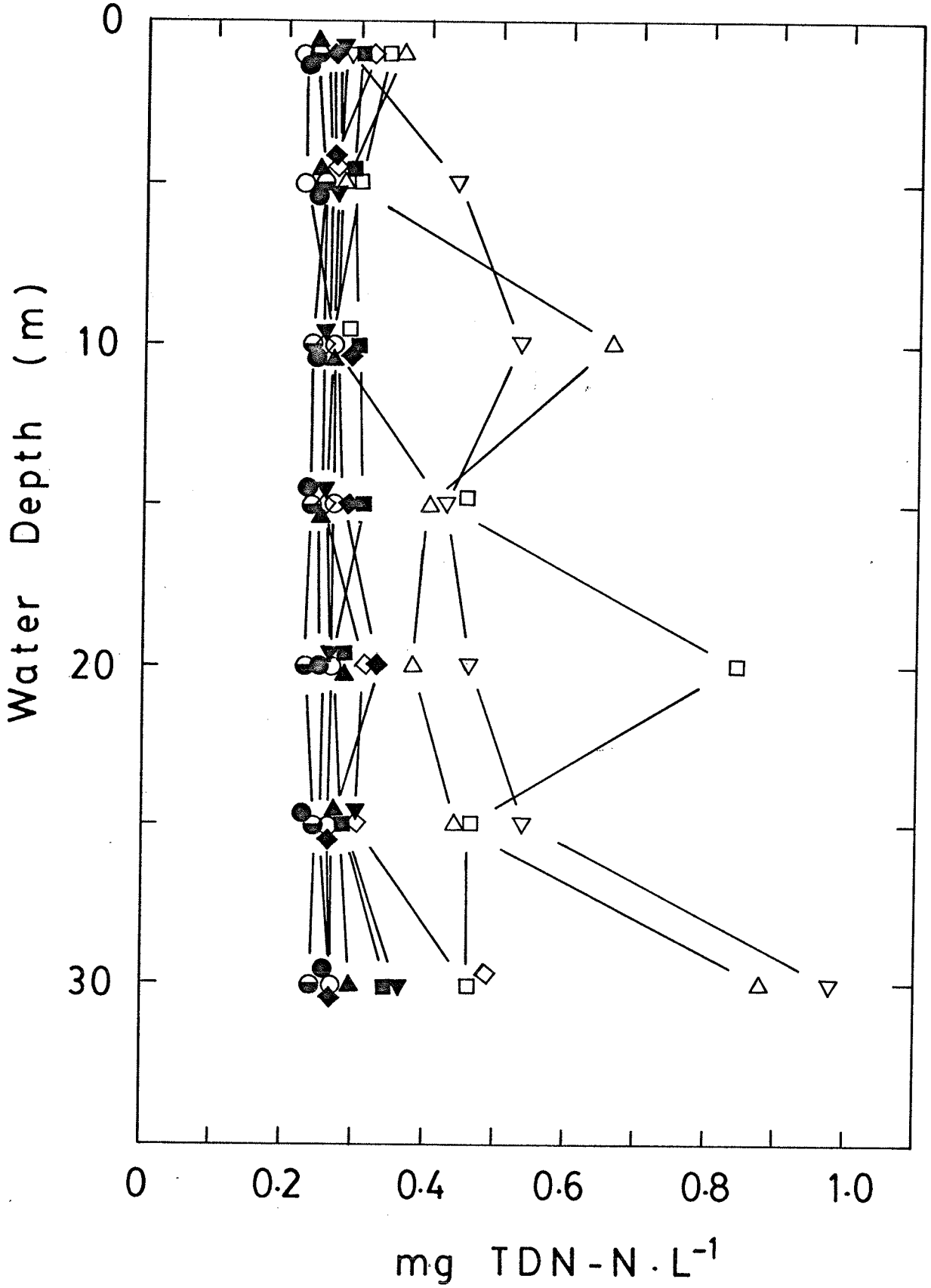

0
10
20
30
Water Depth (m)
0
0·2
0·4
0·6
0·8
1·0
mg TDN-N · L⁻¹

Fig. 8. The *in situ* ammonium-N concentration at various water depths of L.239. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

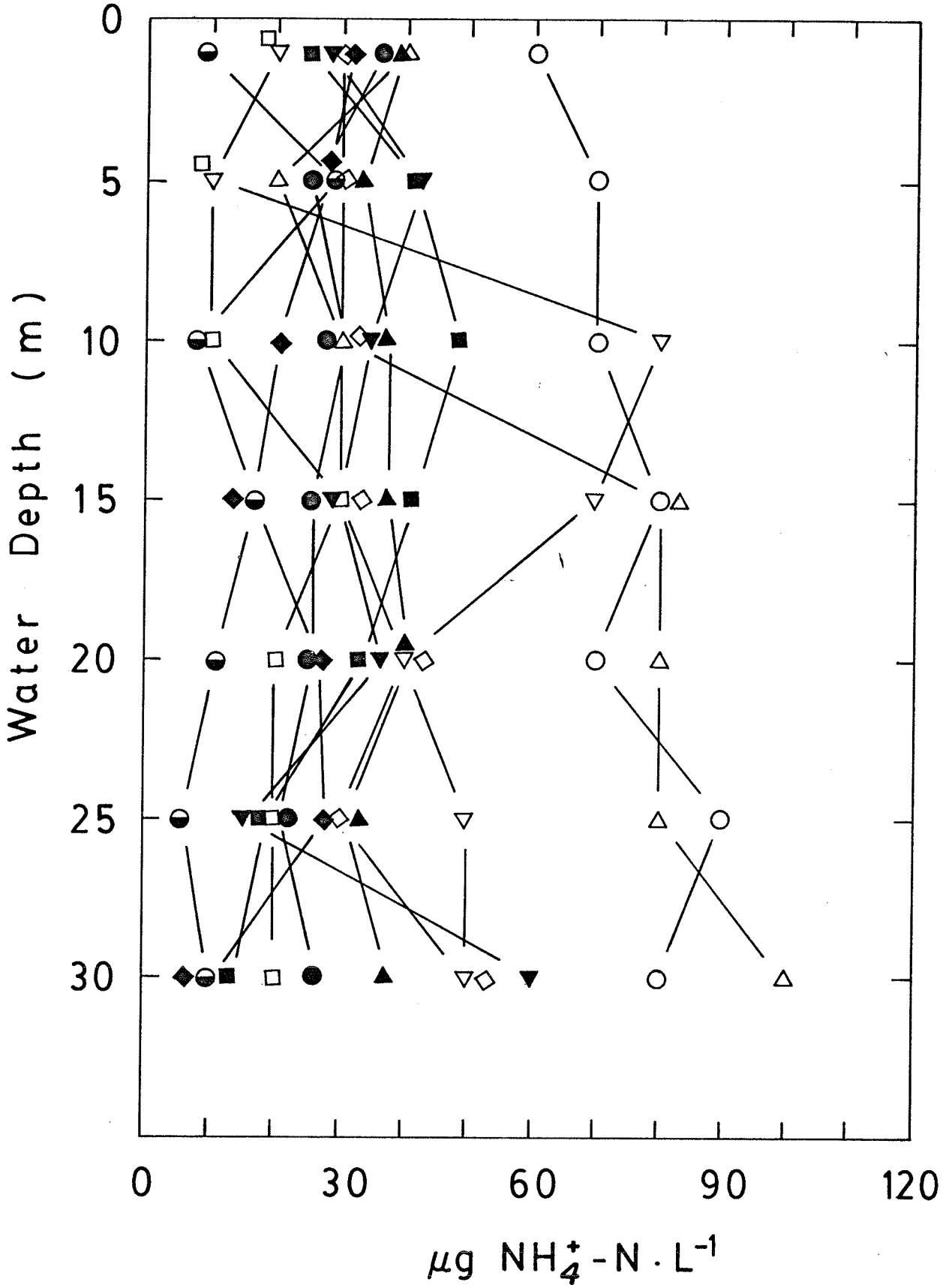

0
5
10
15
20
25
30
Water Depth (m)
0
30
60
90
120
μg NH4+-N · L-1

Fig. 9. The *in situ* nitrate-N concentration at various water depths of L.239. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

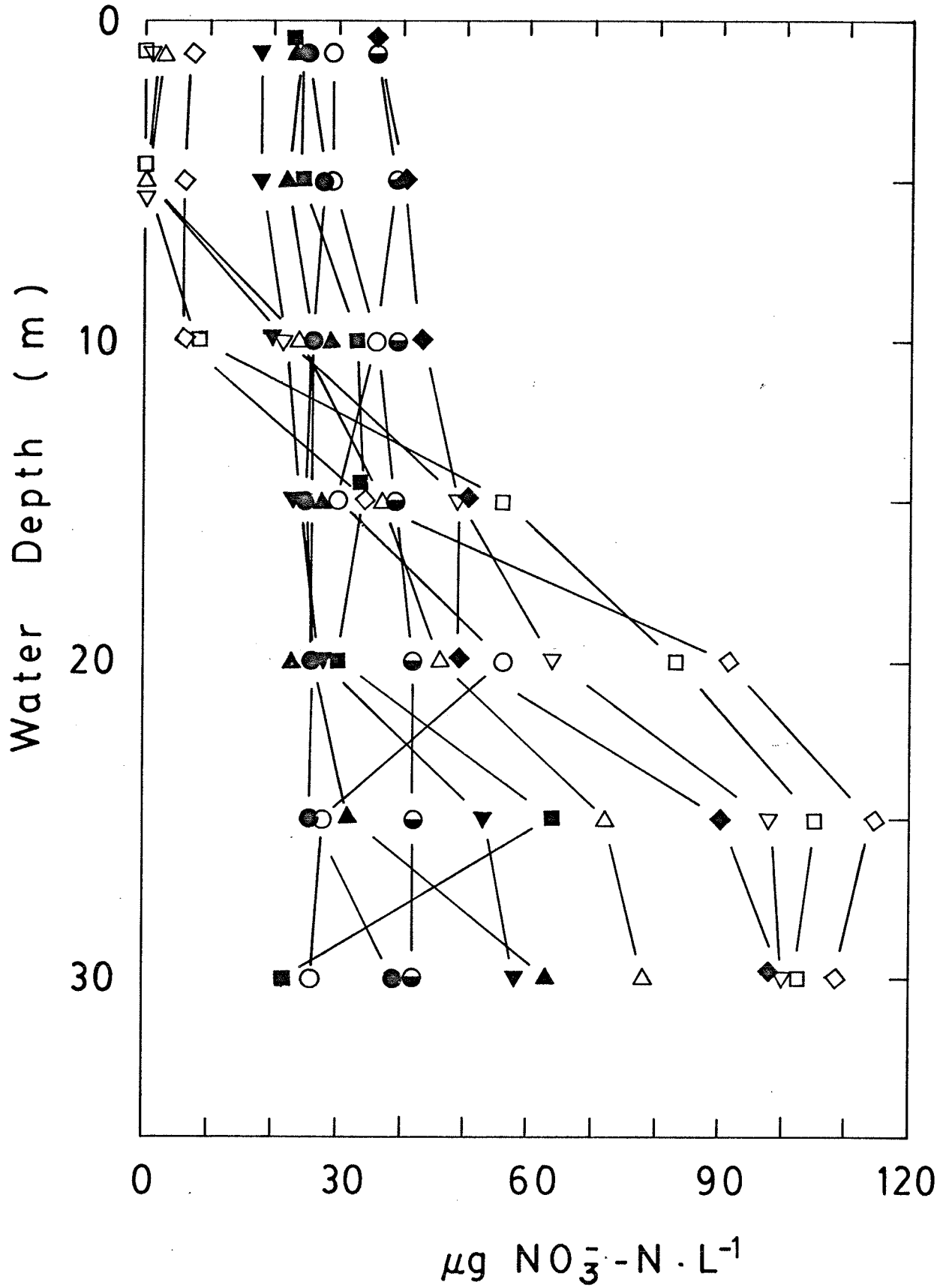

0
10
20
30
Water Depth (m)
0
30
60
90
120
μg NO3- - N · L-1

Fig. 10. The *in situ* pH at various water depths of L.302S. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

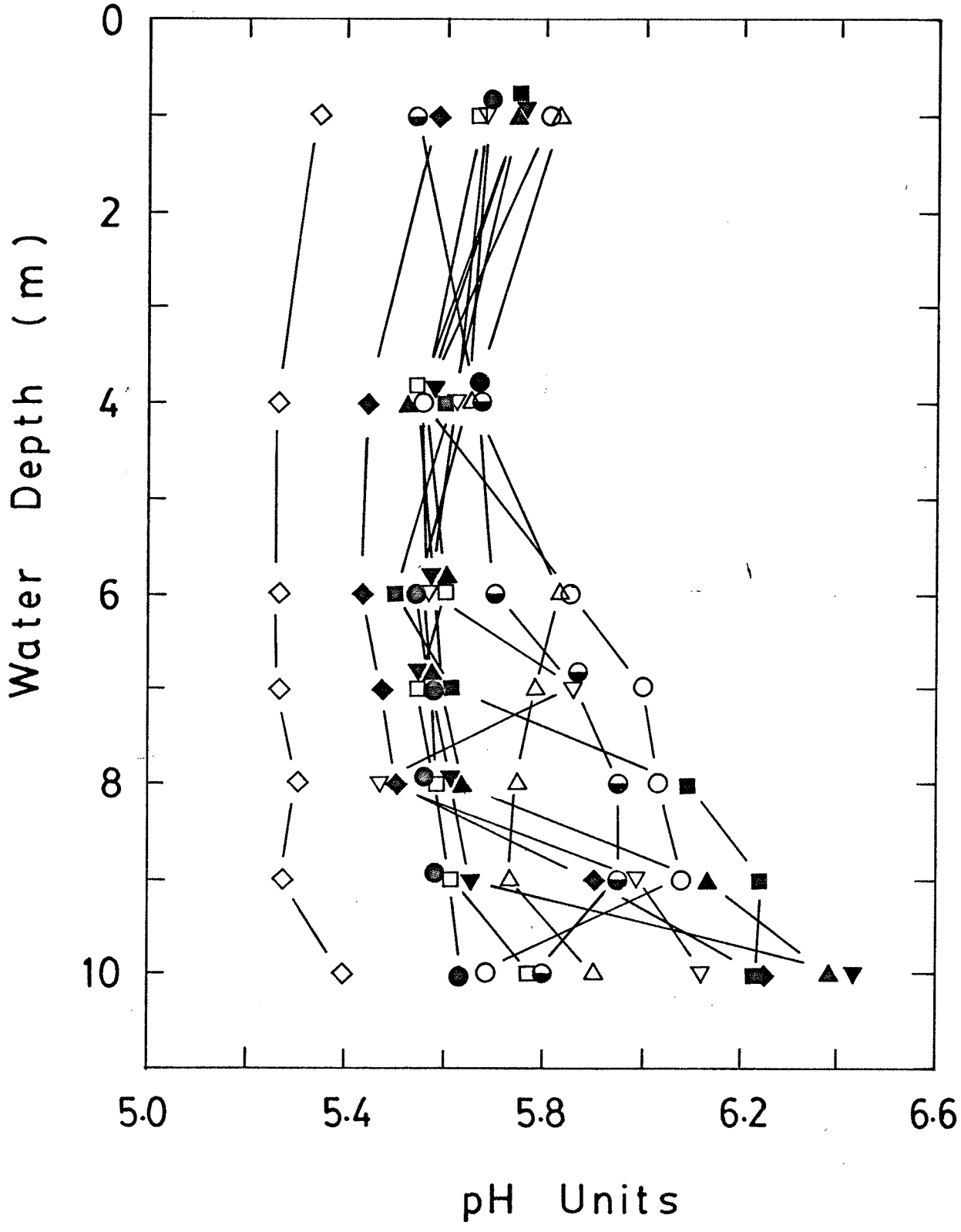

0
2
4
6
8
10
Water Depth (m)
5.0
5.4
5.8
6.2
6.6
pH Units

Fig. 11. The *in situ* dissolved oxygen concentration at various water depths of L.302S. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May and (○) June. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

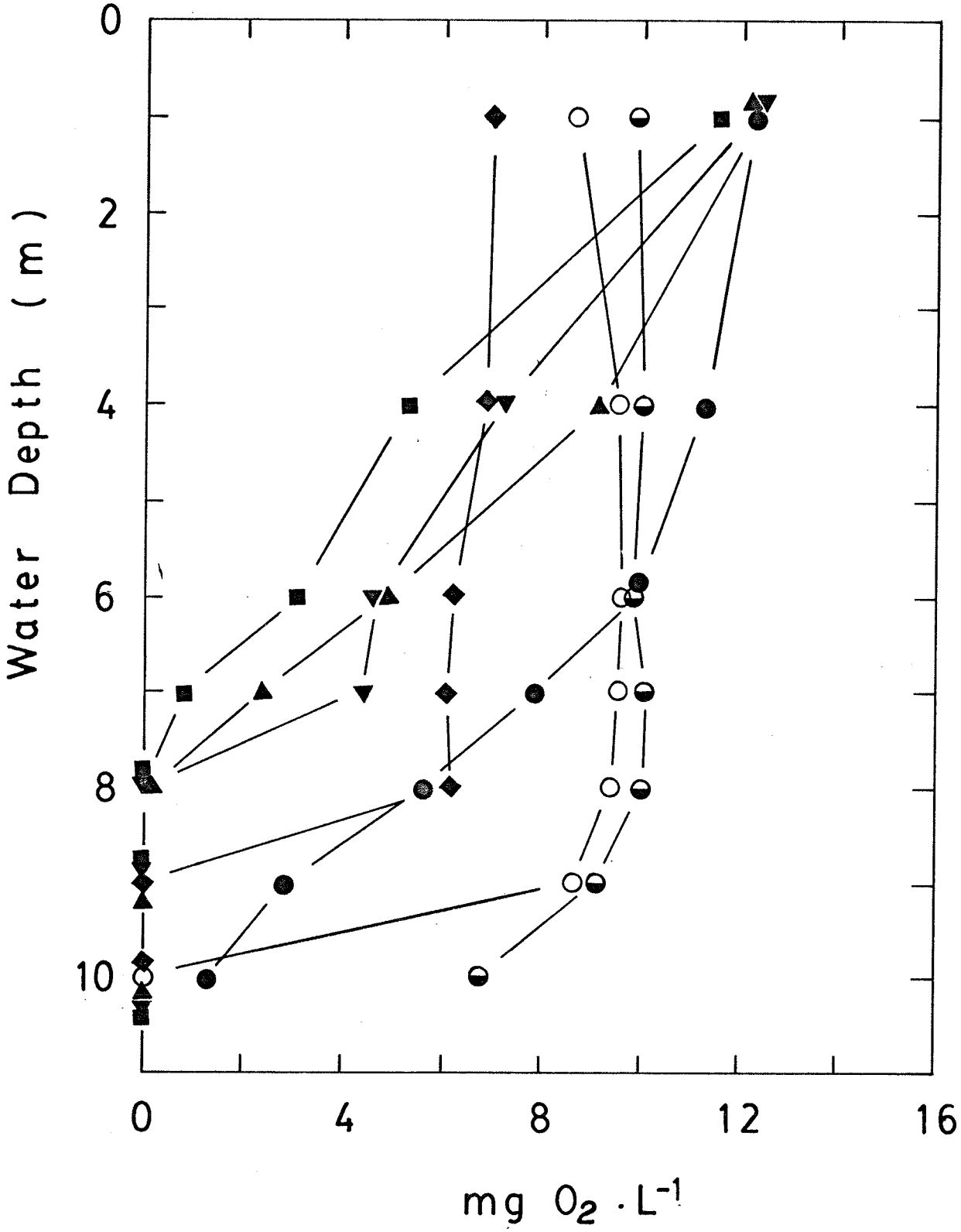

Water Depth (m)
0
2
4
6
8
10
0
4
8
12
16
mg $O_2 \cdot L^{-1}$

Fig. 12. The *in situ* dissolved organic carbon concentration at various water depths of L.302S. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (⊖) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0.10$).

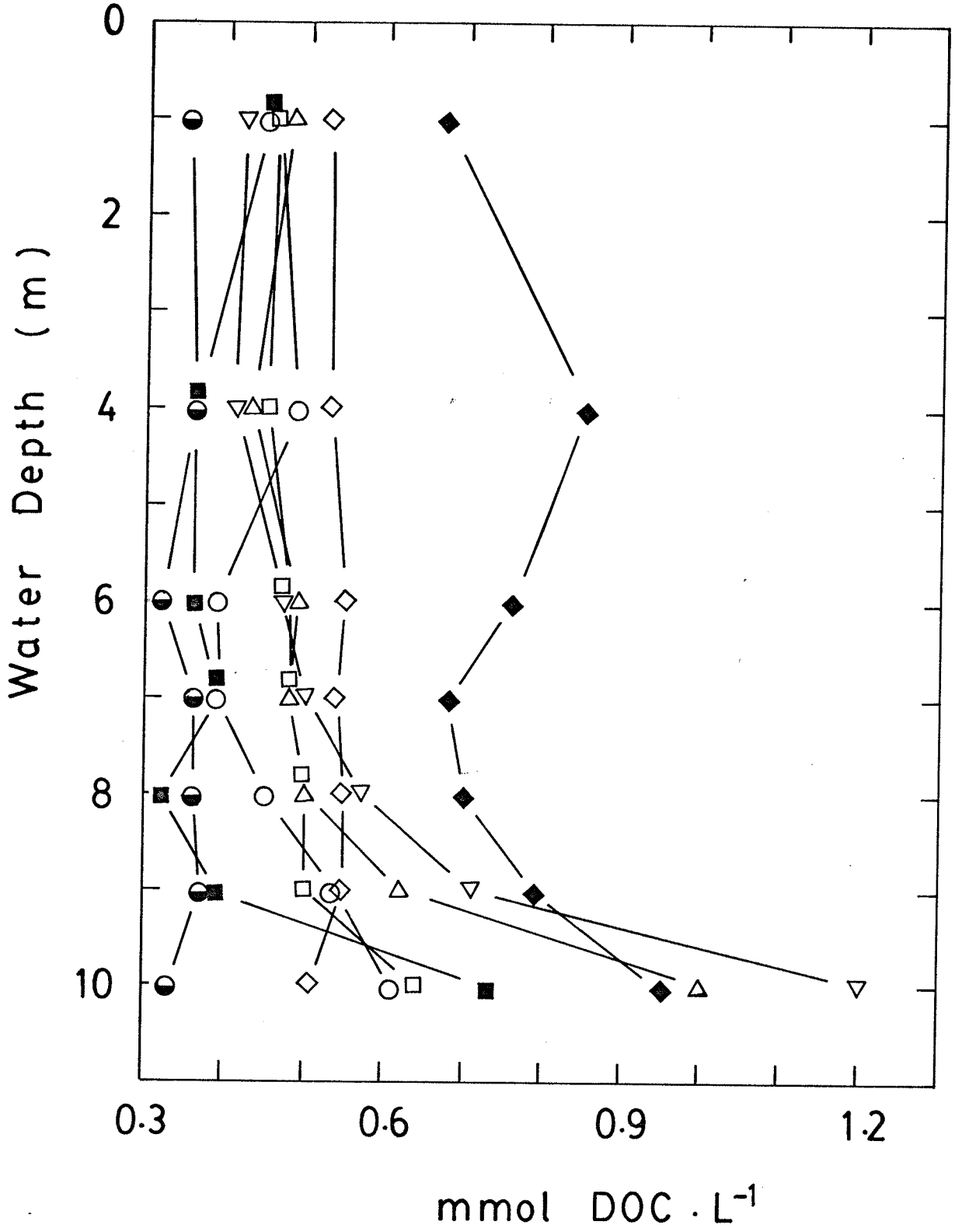

0
2
4
6
8
10
Water Depth (m)
0·3
0·6
0·9
1·2
mmol DOC · L⁻¹

Fig. 13. The in situ total dissolved nitrogen concentration at various water depths of L.302S. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (⊖) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

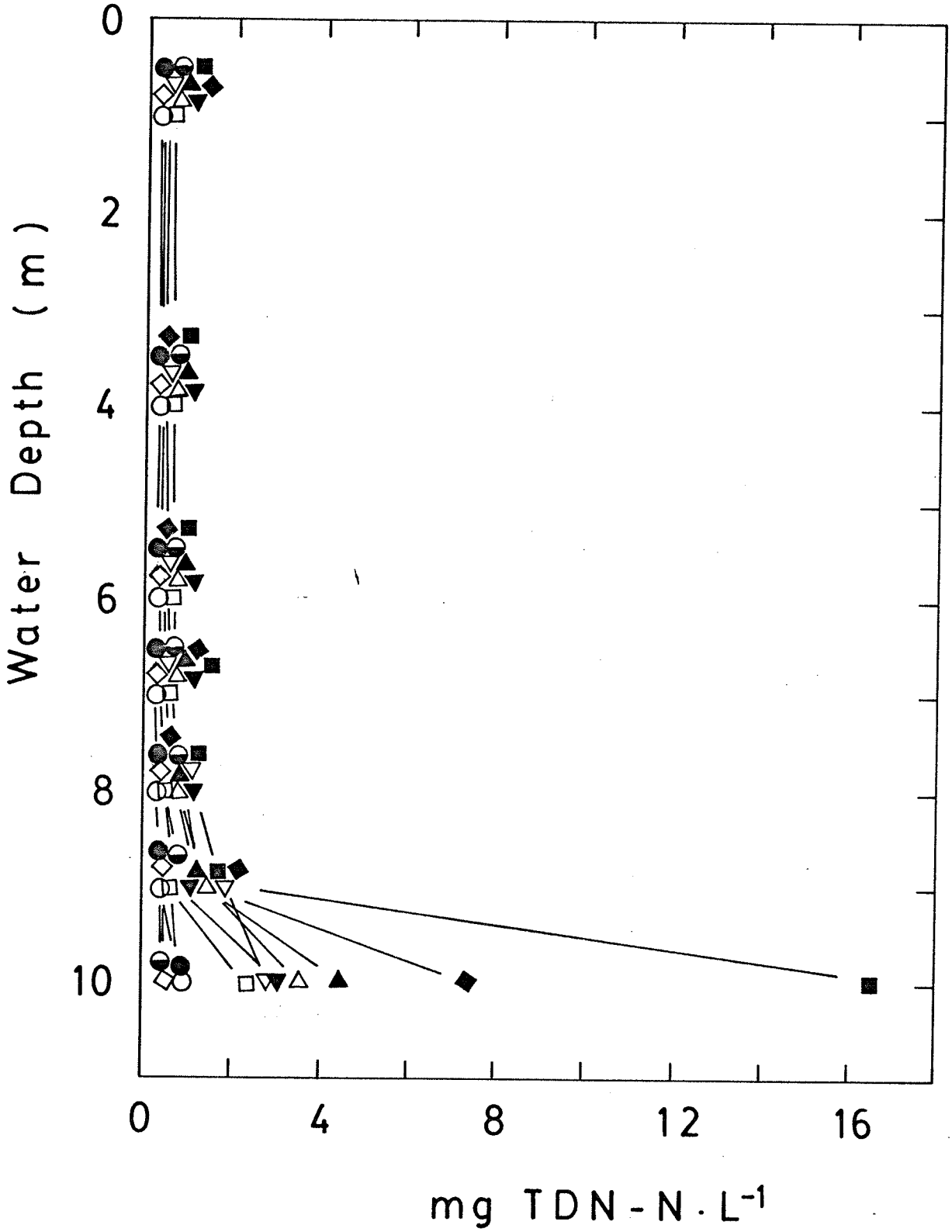

0
2
4
6
8
10
Water Depth (m)
0
4
8
12
16
mg TDN-N·L⁻¹

Fig. 14. The *in situ* ammonium-N concentration at various water depths of L.302S. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (⊖) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0.10$).

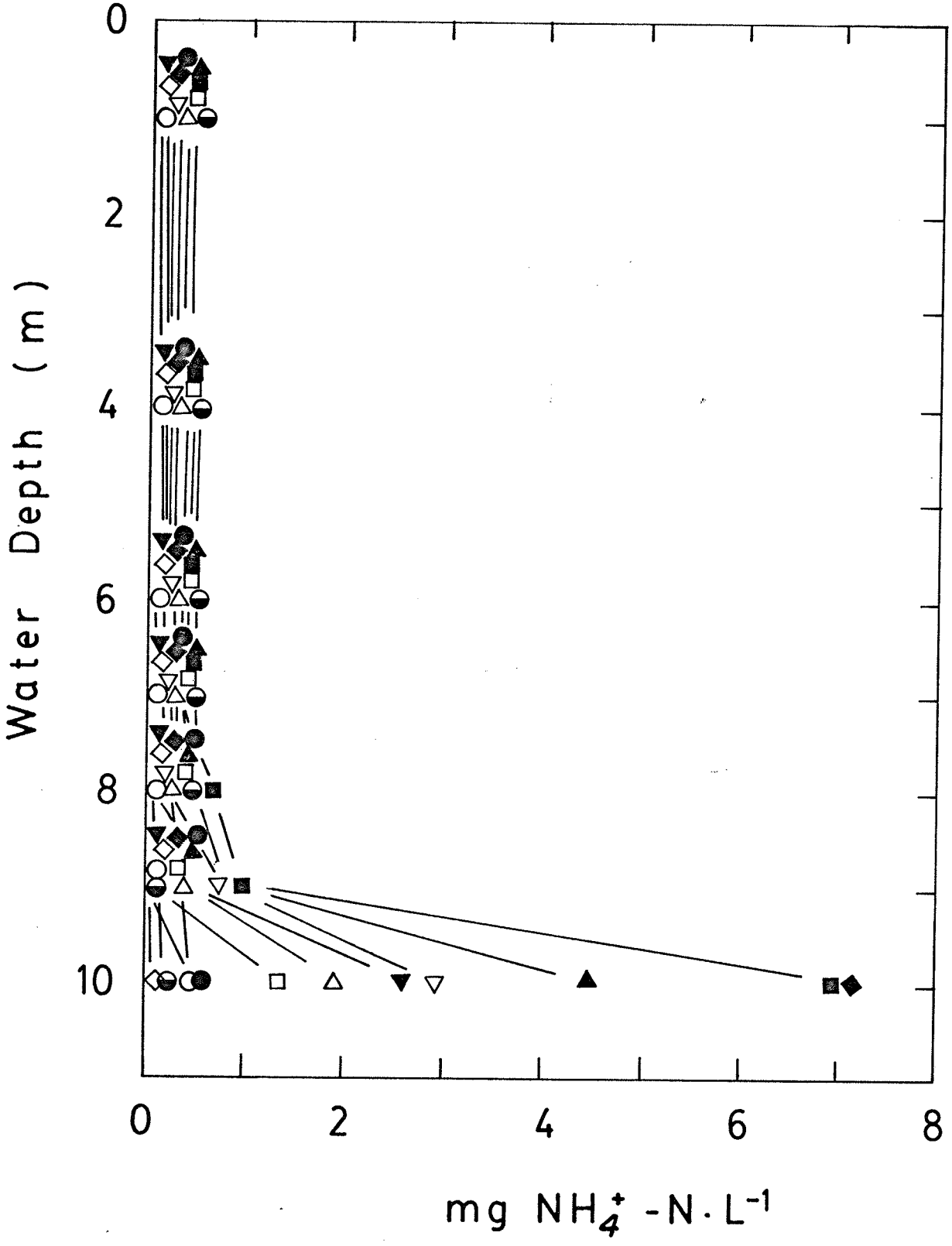

0
2
4
6
8
10
Water Depth (m)
0
2
4
6
8
mg NH_4^+ -N · L^{-1}

Fig. 15. The *in situ* nitrite-N concentration at various water depths of L.302S. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

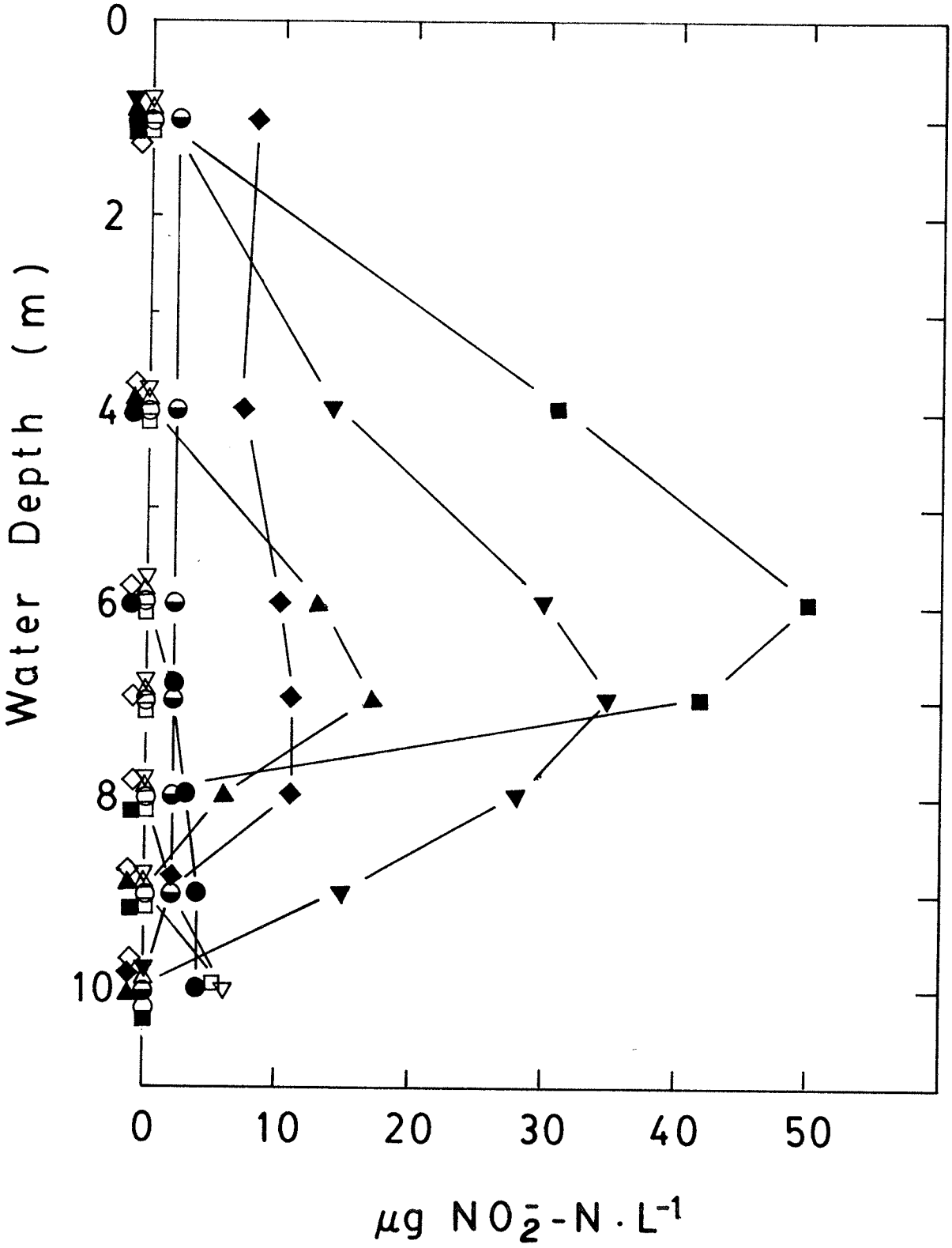

0
2
4
6
8
10
Water Depth (m)
0
10
20
30
40
50
μg NO2- - N · L-1

Fig. 16. The in situ nitrate-N concentration at various water depths of L.302S. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (⊖) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

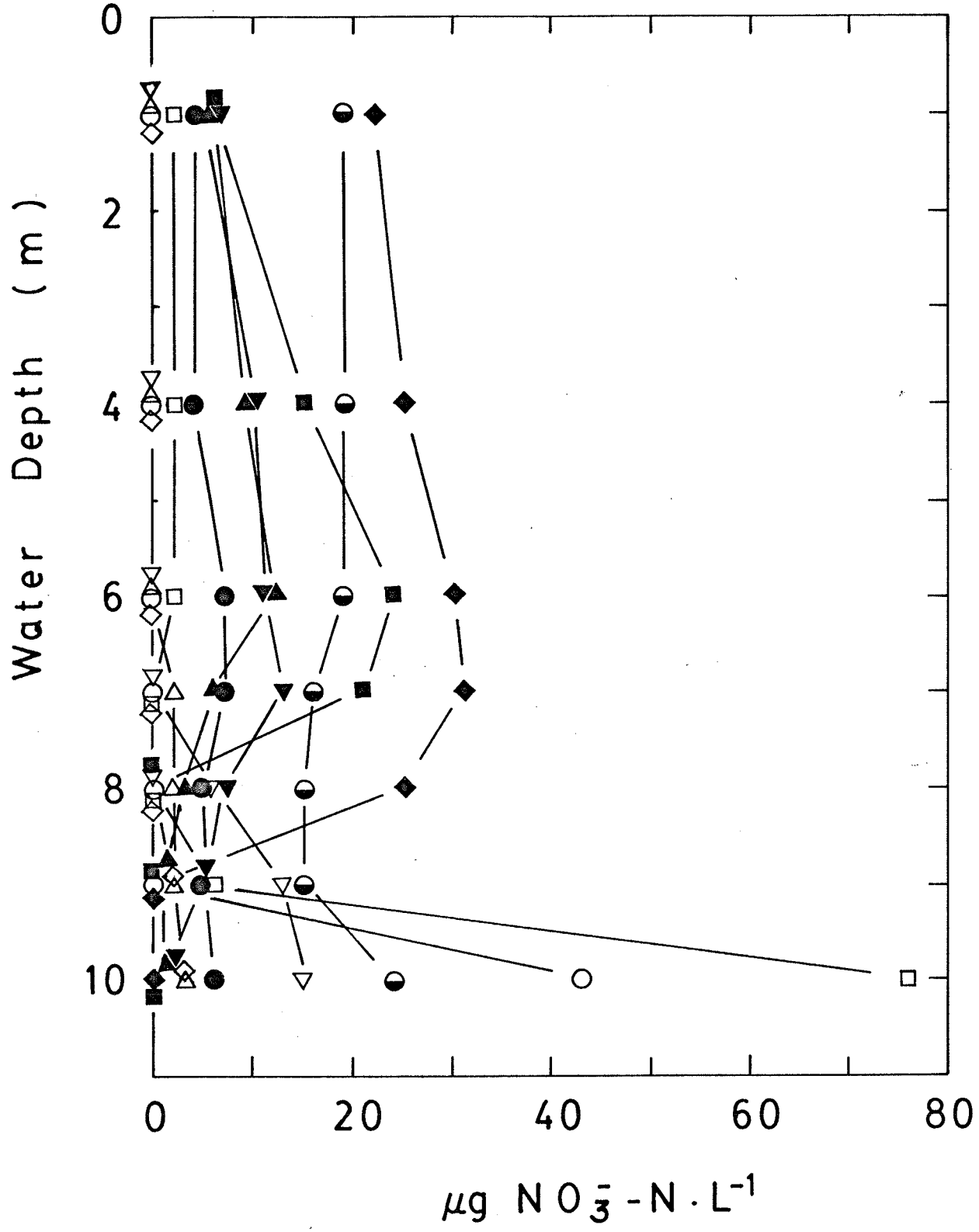

0
2
4
6
8
10
Water Depth (m)
0
20
40
60
80
μg NO3- - N · L-1

Fig. 17. The number of heterotrophic bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on nutrient agar at 15°C. Symbols are : (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

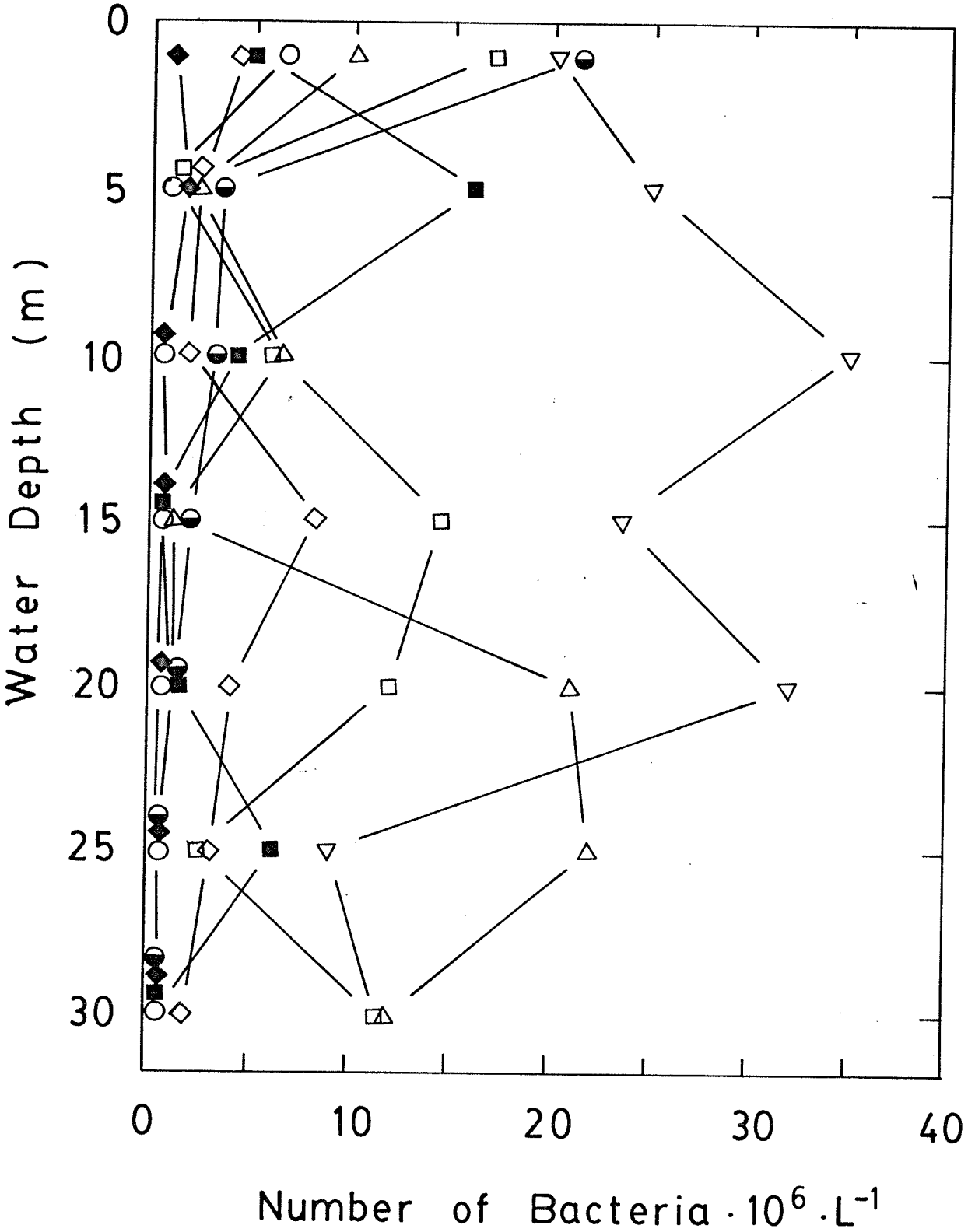

0
5
10
15
20
25
30
Water Depth (m)
0
10
20
30
40
Number of Bacteria · 10⁶ · L⁻¹

Fig. 18. The number of heterotrophic bacteria (colony-forming units) at various depths of L.239 that were capable of growth on nutrient agar at 4°C. Symbols are : (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

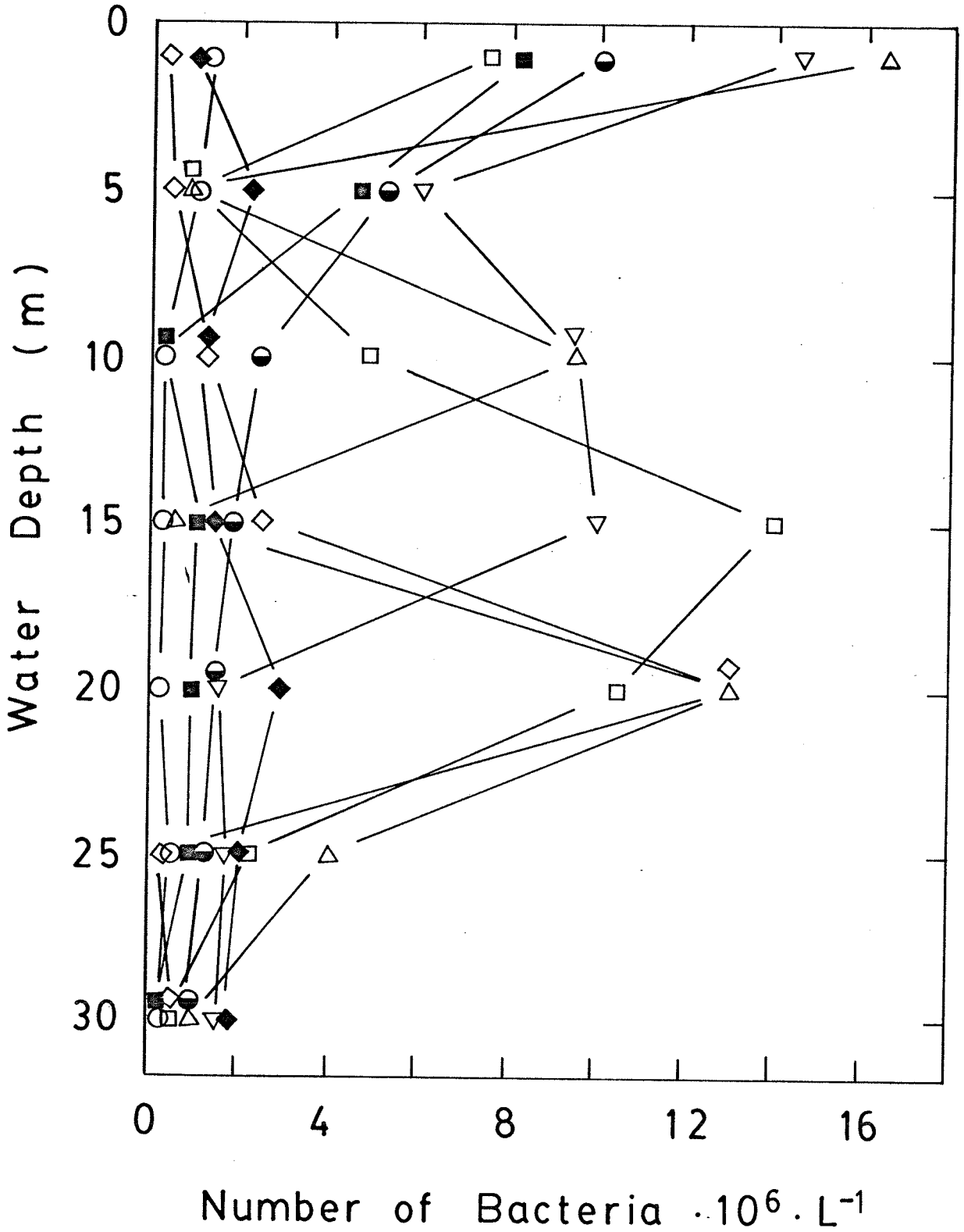

0
5
10
15
20
25
30
Water Depth (m)
0
4
8
12
16
Number of Bacteria ·10⁶·L⁻¹

Fig. 19. The number of bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on Nitrosomonas agar at 15°C and pH 5·5. Symbols are : (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

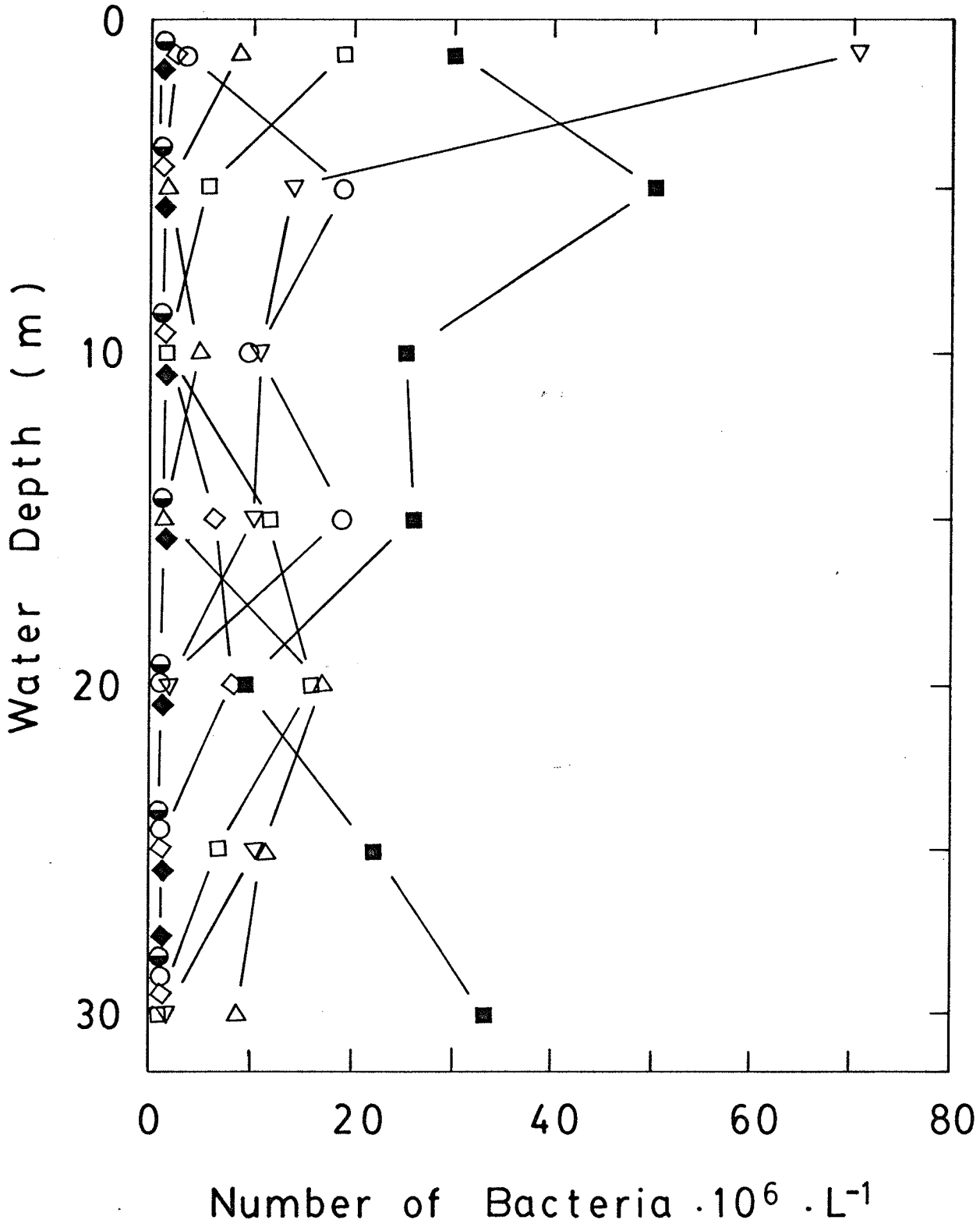

0
10
20
30
Water Depth (m)
0
20
40
60
80
Number of Bacteria ·10⁶ ·L⁻¹

Fig. 20. The number of bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on Nitrosomonas agar at 15°C and pH 7·6. Symbols are : (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

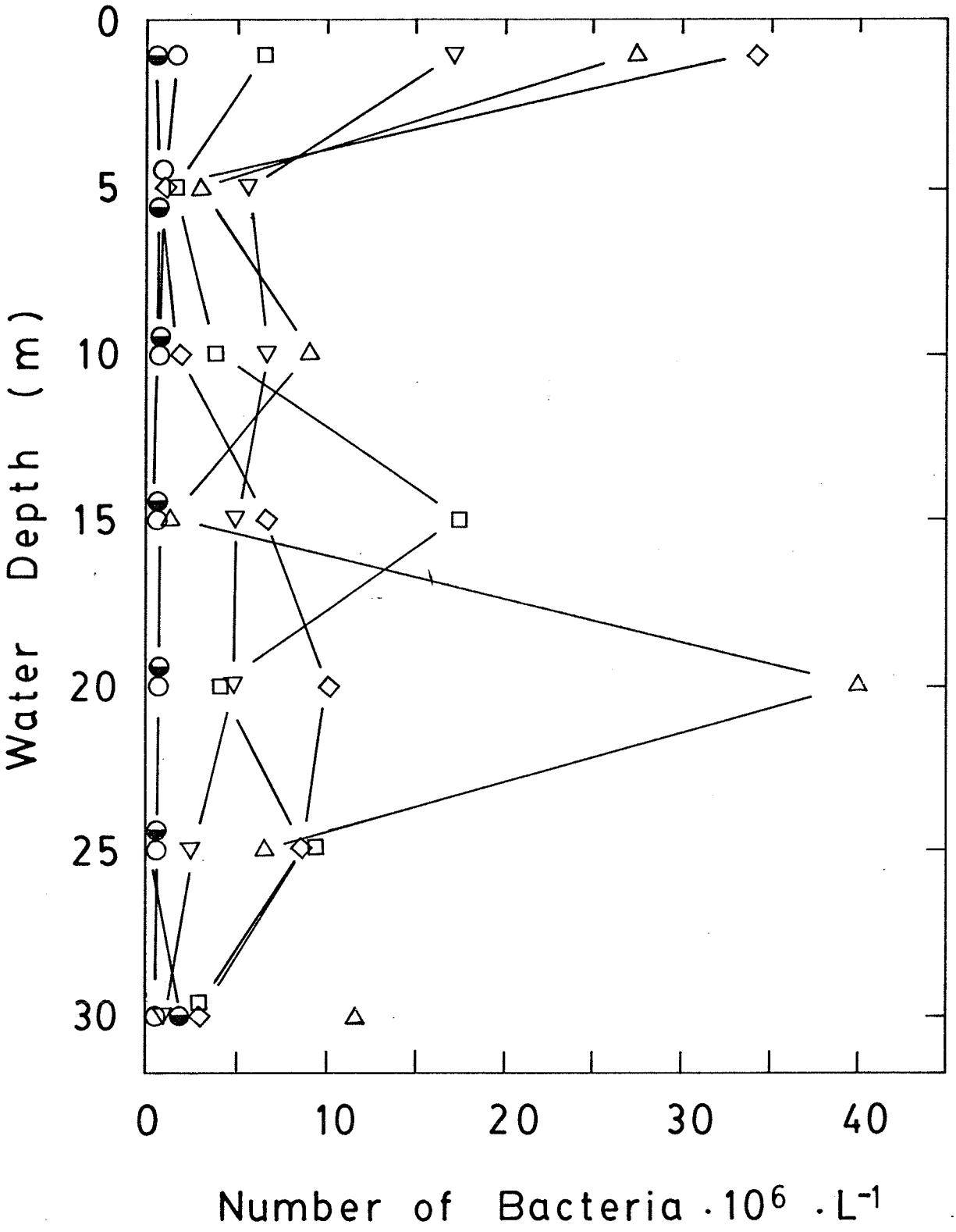

0
5
10
15
20
25
30
Water Depth (m)
0
10
20
30
40
Number of Bacteria · 10⁶ · L⁻¹

Fig. 21. The number of bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on Nitrosomonas agar at 4°C and pH 5·5. Symbols are : (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

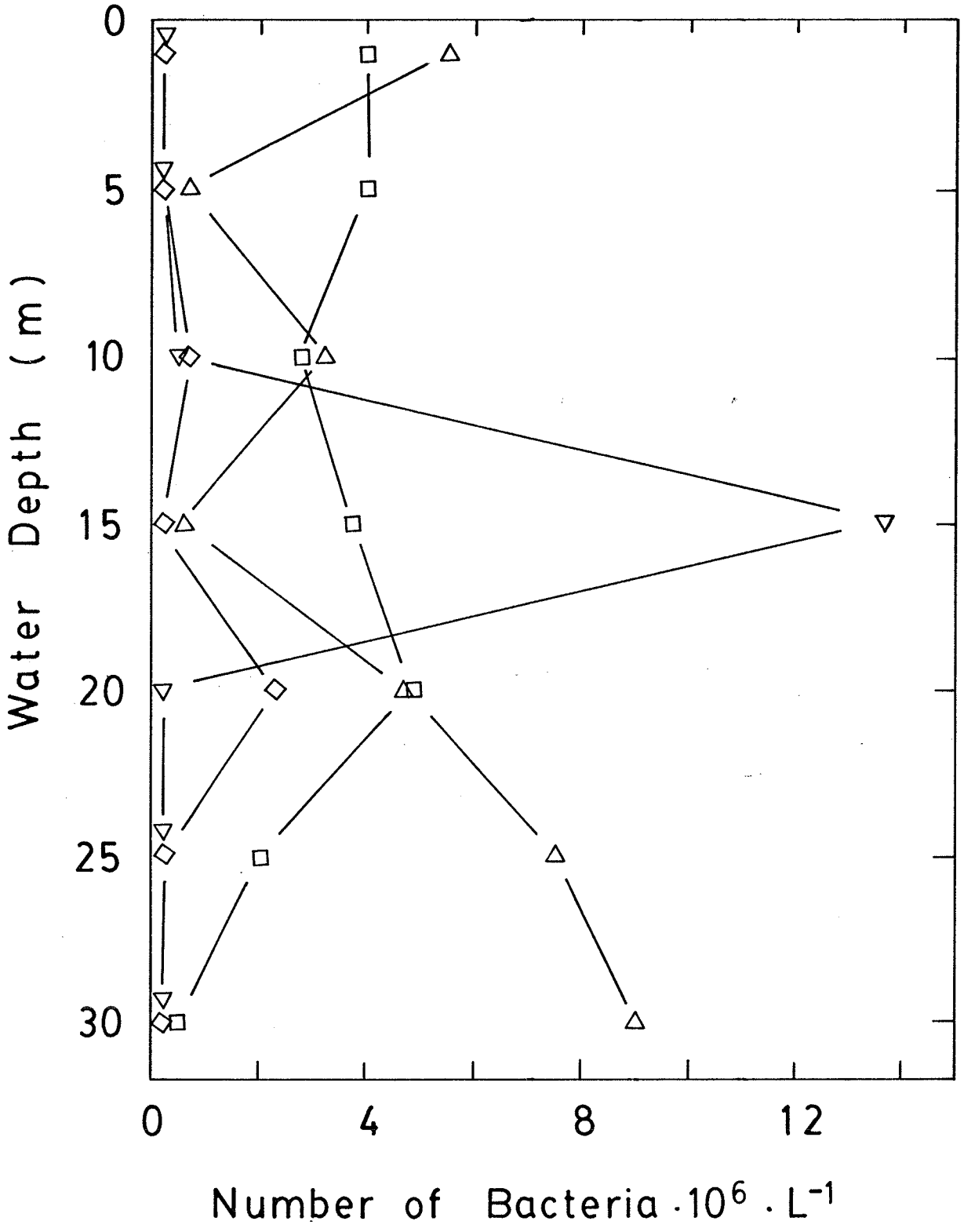

0
5
10
15
20
25
30
Water Depth (m)
0
4
8
12
Number of Bacteria · 10⁶ · L⁻¹

Fig. 22. The number of bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on Nitrosomonas agar at 4°C and pH 7·6. Symbols are : (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

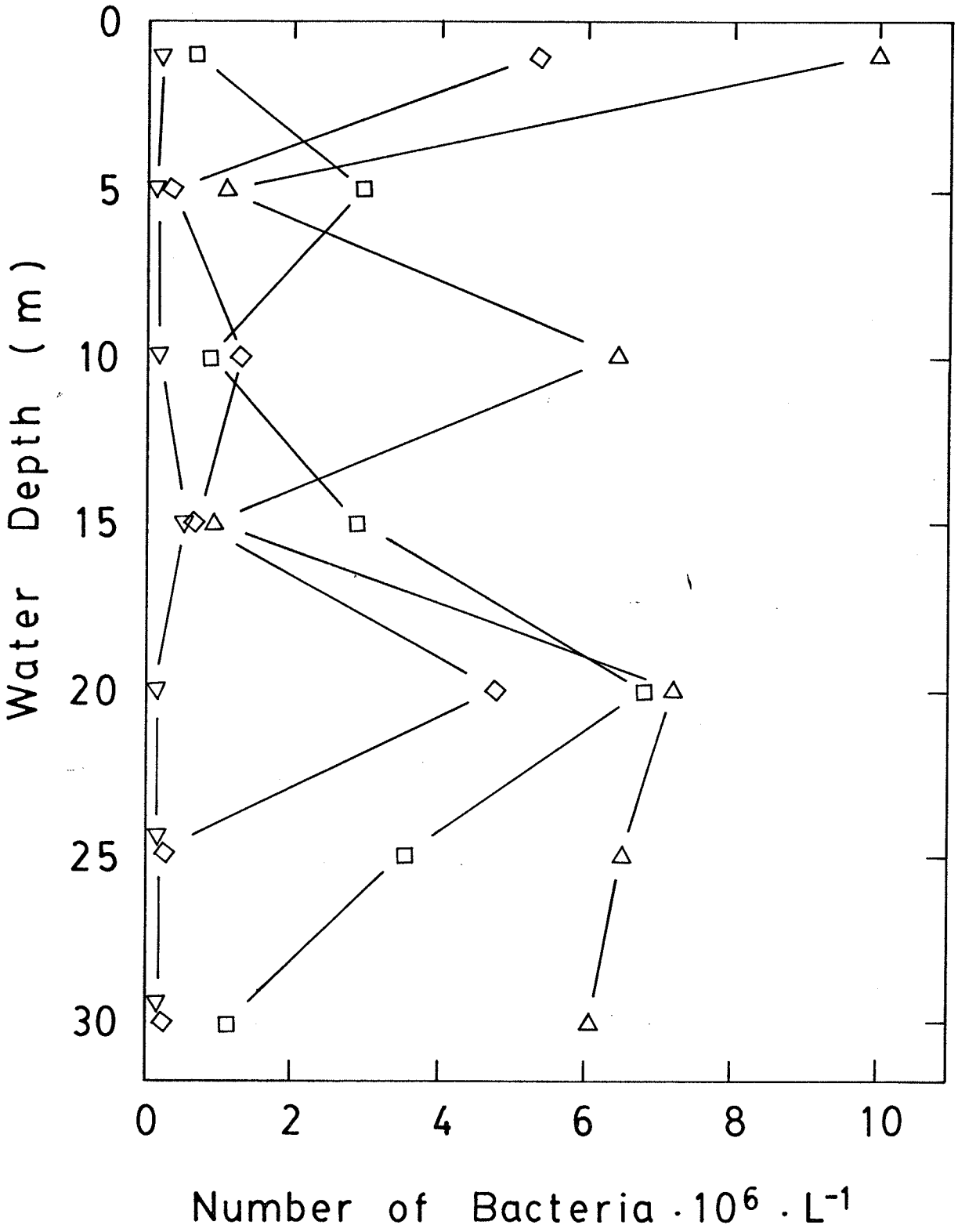

0
5
10
15
20
25
30
Water Depth (m)
0
2
4
6
8
10
Number of Bacteria · 10⁶ · L⁻¹

Fig. 23. The number of bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on Nitrobacter agar at 15°C and pH 5·5. Symbols are : (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

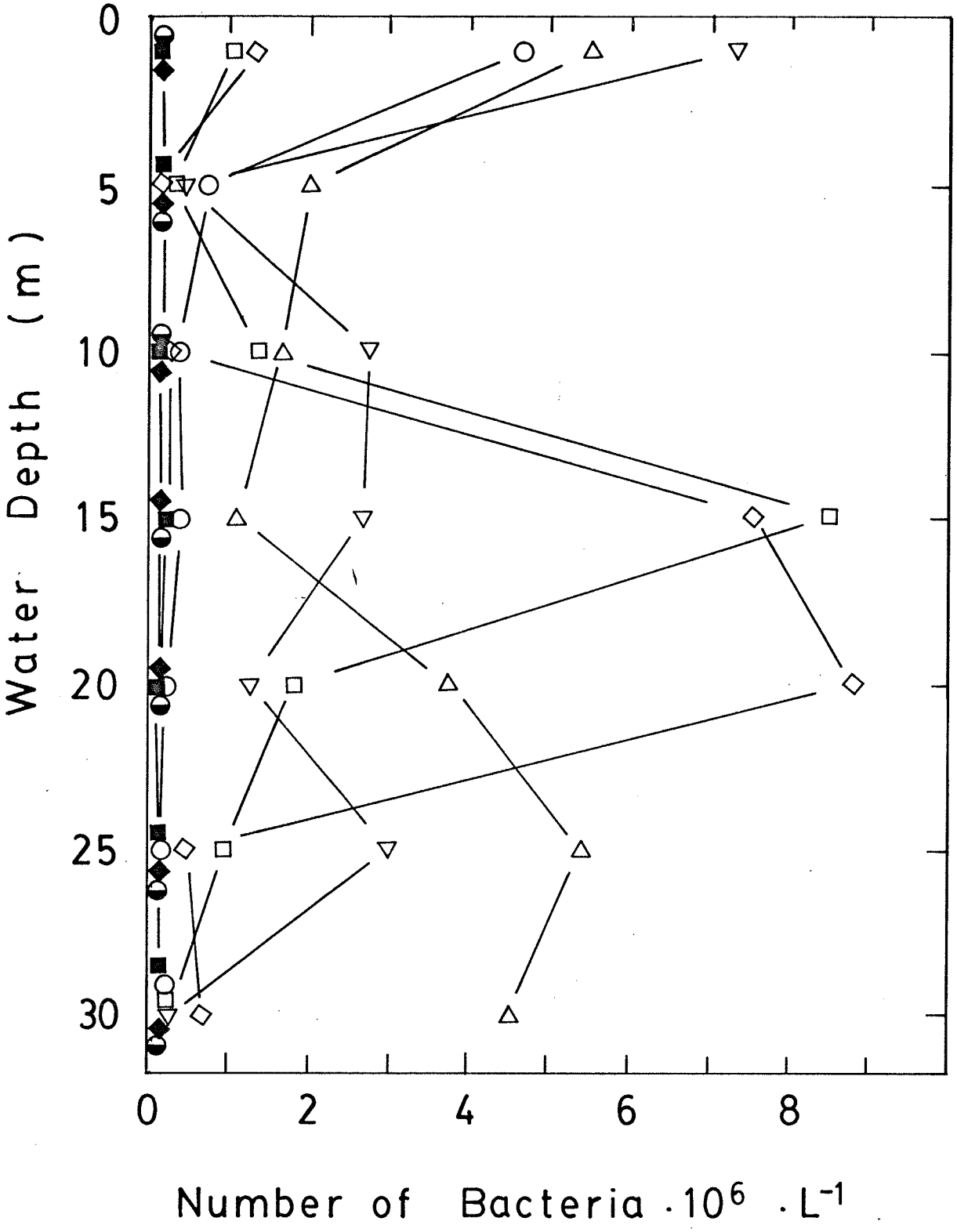

0
5
10
15
20
25
30
Water Depth (m)
0
2
4
6
8
Number of Bacteria · 10⁶ · L⁻¹

Fig. 24. The number of bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on Nitrobacter agar at 15°C and pH 7·6. Symbols are : (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

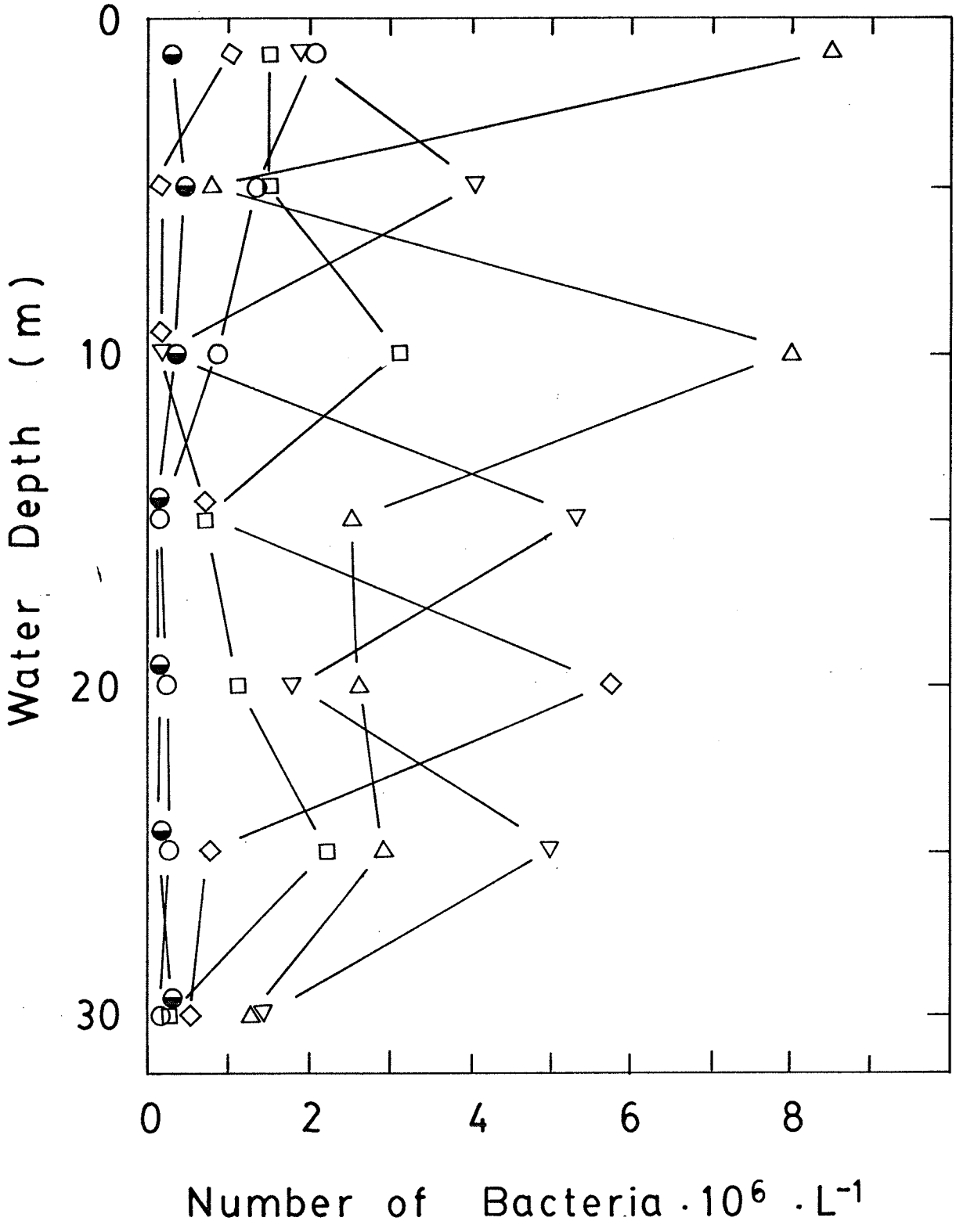

0
10
20
30
Water Depth (m)
0
2
4
6
8
Number of Bacteria · 10⁶ · L⁻¹

Fig. 25. The number of bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on Nitrobacter agar at 4°C and pH 5·5. Symbols are : (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

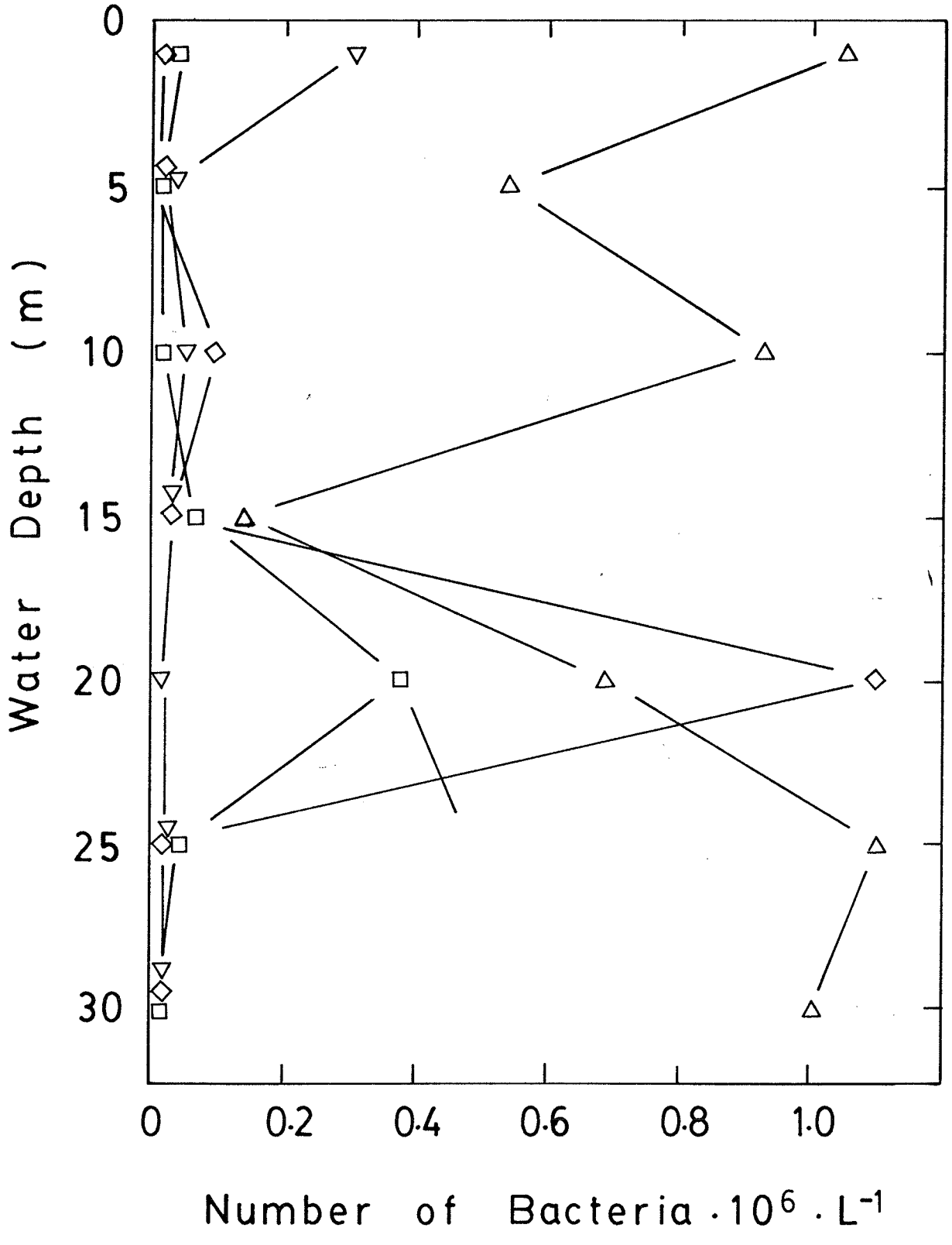

0
5
10
15
20
25
30
Water Depth (m)
0
0·2
0·4
0·6
0·8
1·0
Number of Bacteria · 10⁶ · L⁻¹

Fig. 26. The number of bacteria (colony-forming units) at various water depths of L.239 that were capable of growth on Nitrobacter agar at 4°C and pH 7·6. Symbols are : (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

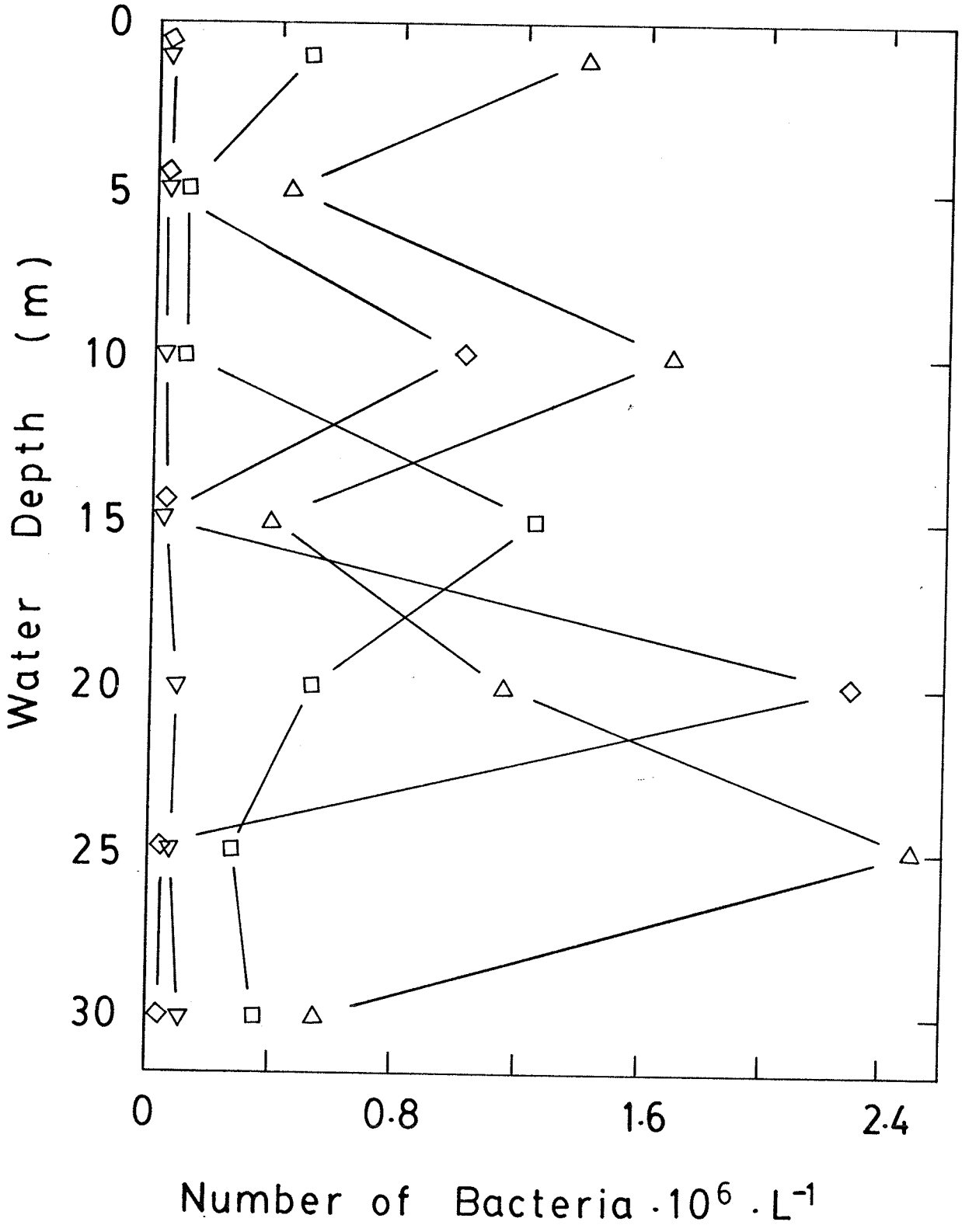

0
5
10
15
20
25
30
Water Depth (m)
0
0.8
1.6
2.4
Number of Bacteria $\cdot 10^6 \cdot L^{-1}$

Fig. 27. The number of heterotrophic bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on nutrient agar at 15°C. Symbols are : (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

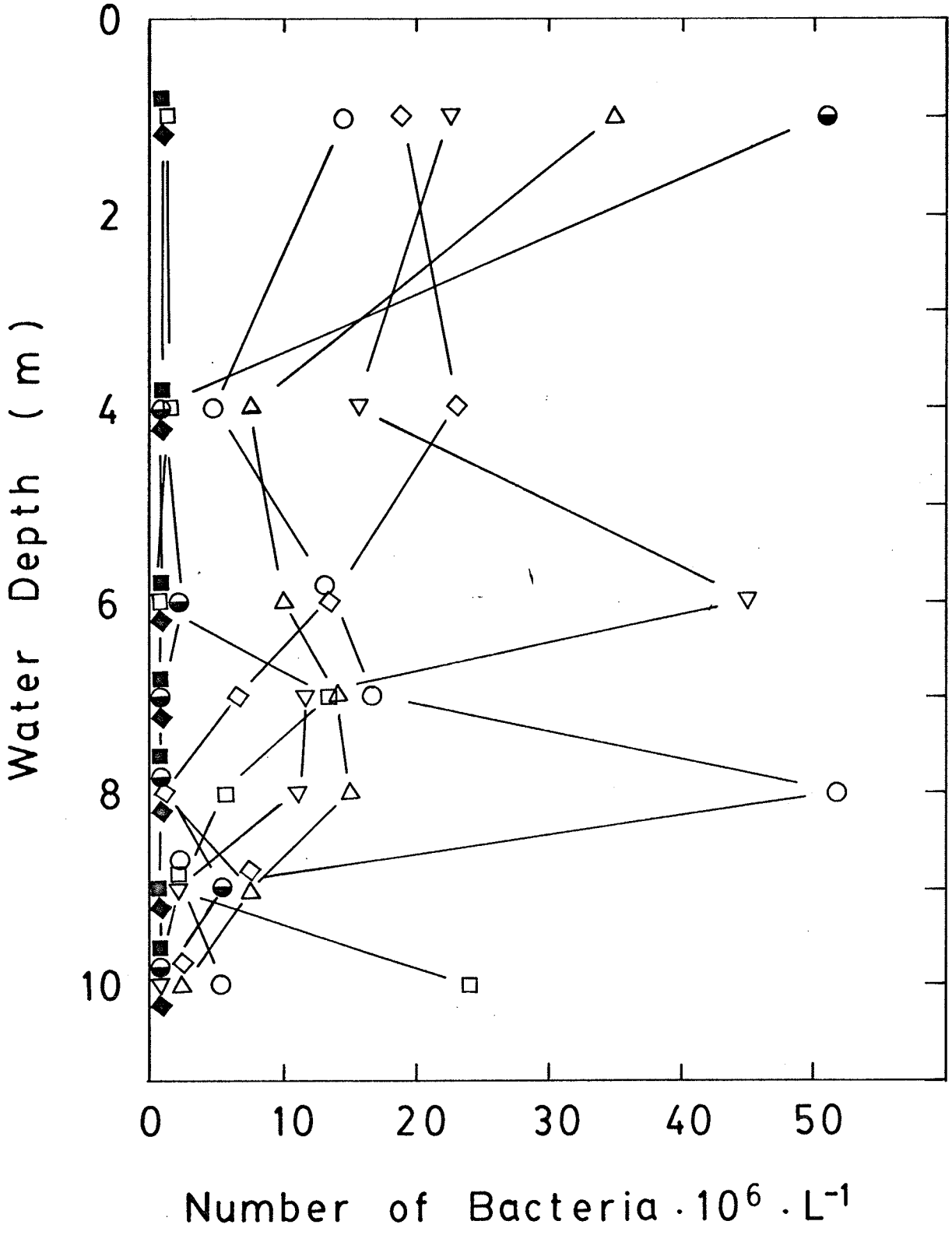

0
2
4
6
8
10
Water Depth (m)
0
10
20
30
40
50
Number of Bacteria · 10⁶ · L⁻¹

Fig. 28. The number of heterotrophic bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on nutrient agar at 4°C. Symbols are the : (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

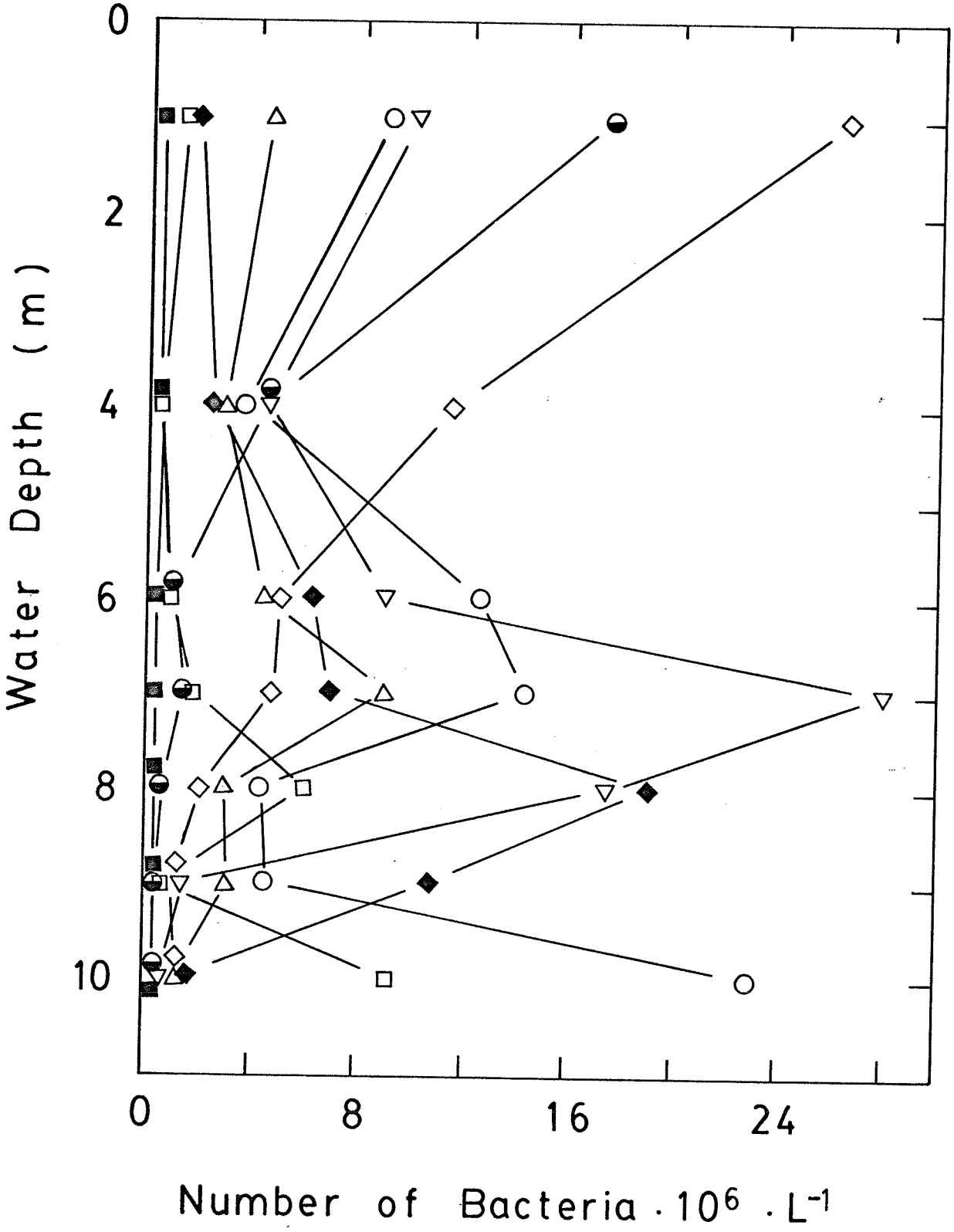

0
2
4
6
8
10
Water Depth (m)
0
8
16
24
Number of Bacteria · 10⁶ · L⁻¹

Fig. 29. The number of bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on Nitrosomonas agar at 15°C and pH 7·6. Symbols are : (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

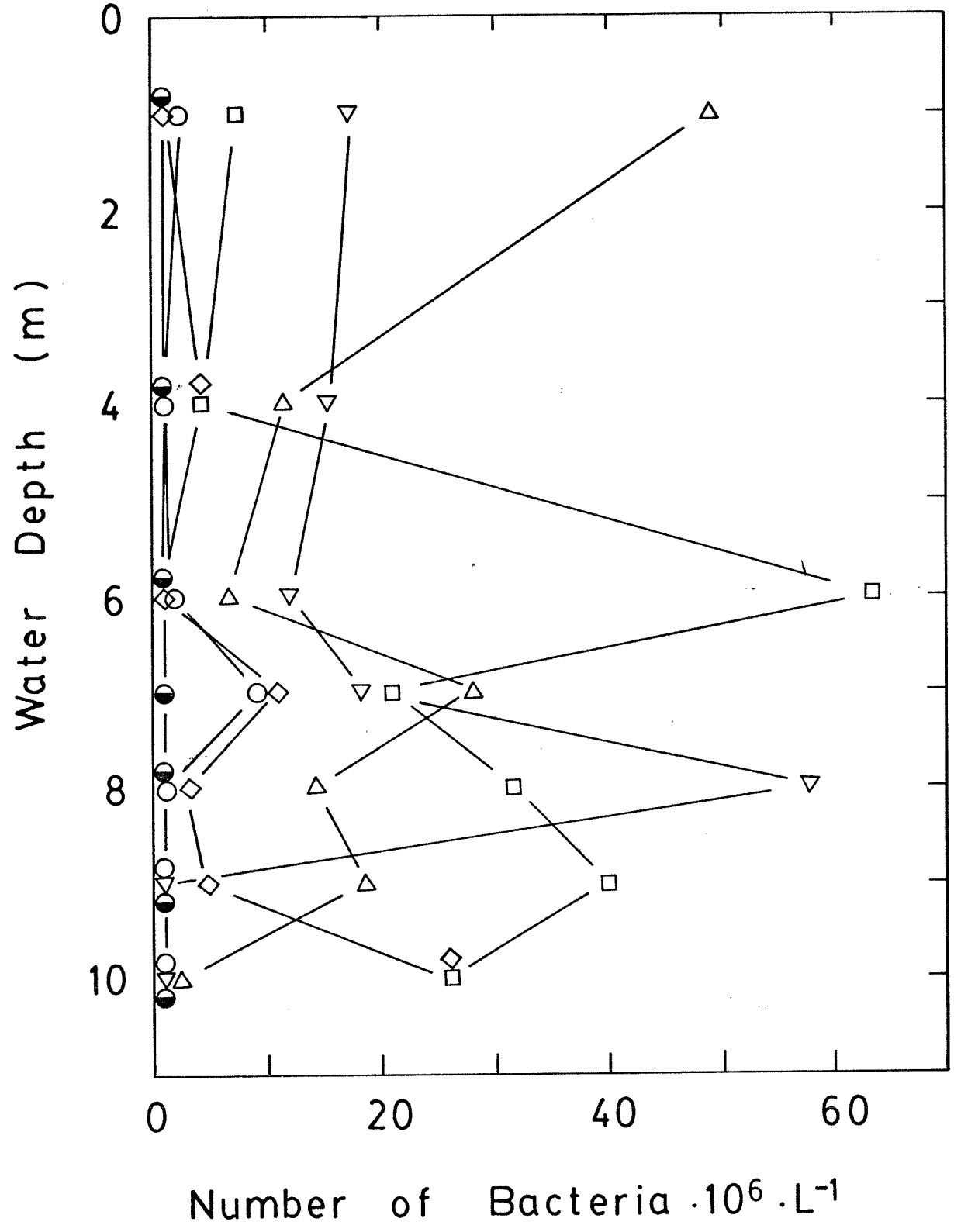

0
2
4
6
8
10
Water Depth (m)
0
20
40
60
Number of Bacteria ·10⁶ ·L⁻¹

Fig. 30. The number of bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on Nitrosomonas agar at 4°C and pH 7·6. Symbols are : (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

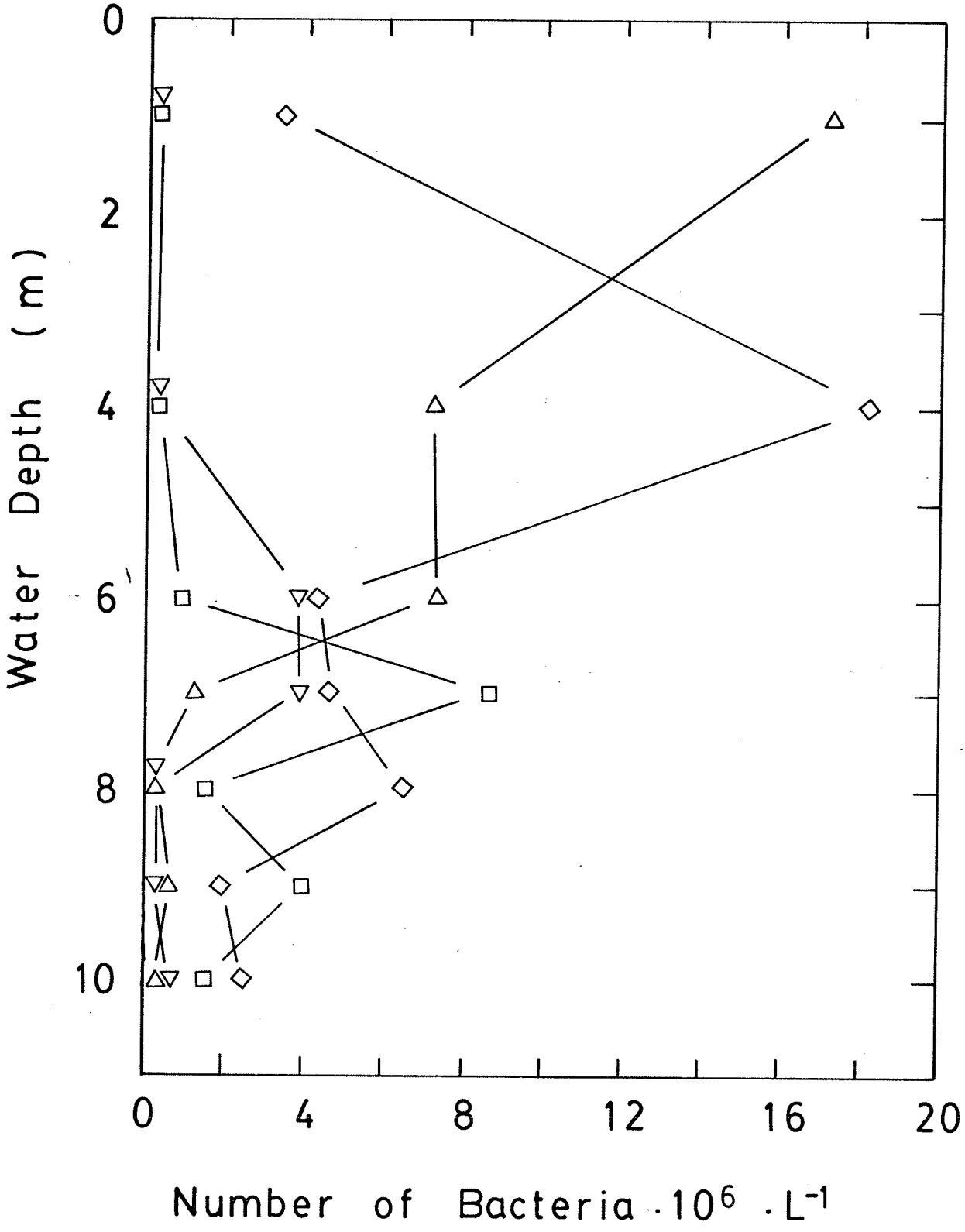

0
2
4
6
8
10
Water Depth (m)
0
4
8
12
16
20
Number of Bacteria · 10^6 · L^{-1}

Fig. 31. The number of bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on Nitrosomonas agar at 15°C and pH 5·5. Symbols are : (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

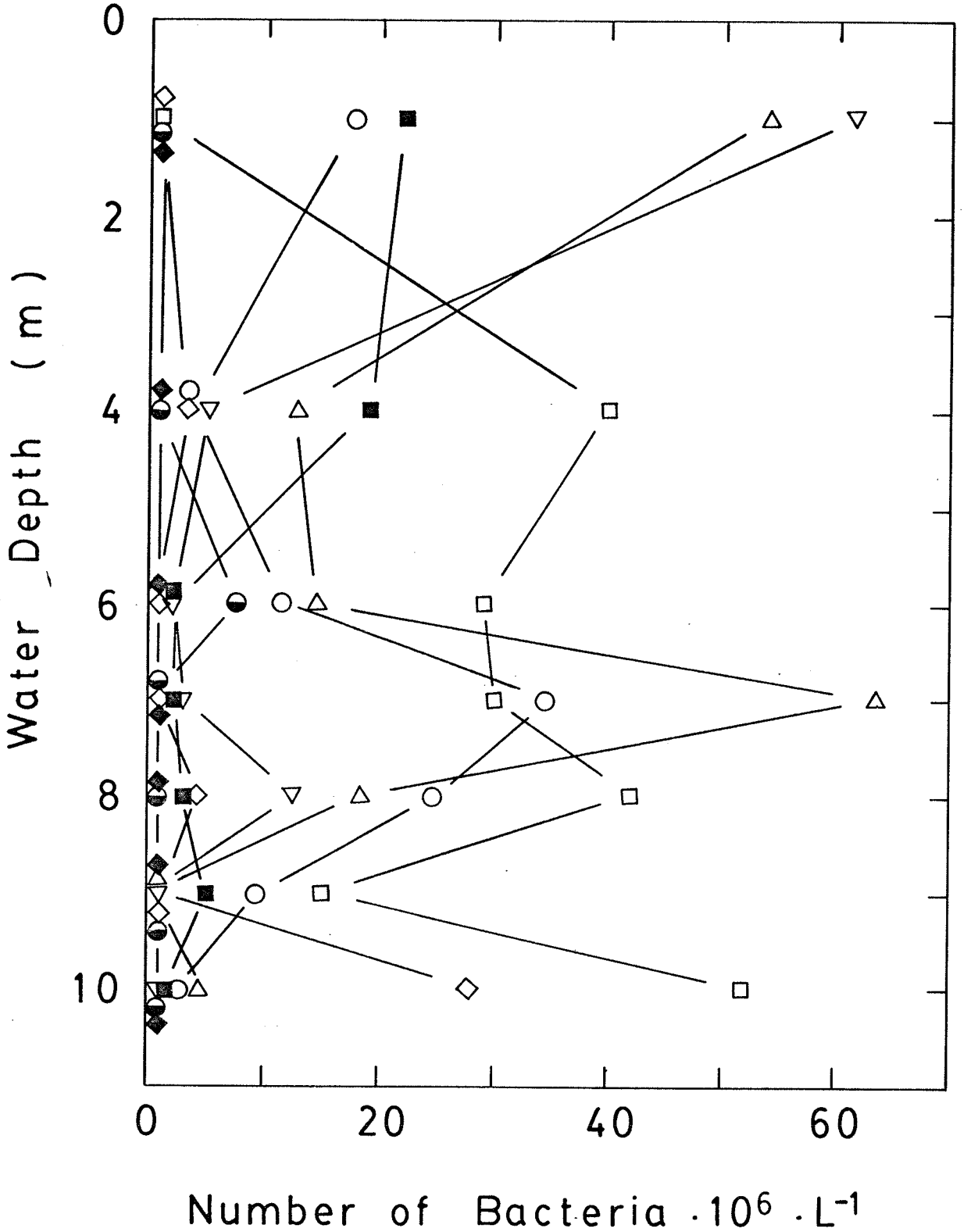

0
2
4
6
8
10
Water Depth (m)
0
20
40
60
Number of Bacteria ·10⁶ ·L⁻¹

Fig. 32. The number of bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on Nitrosomonas agar at 4°C and pH 5·5. Symbols are : (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

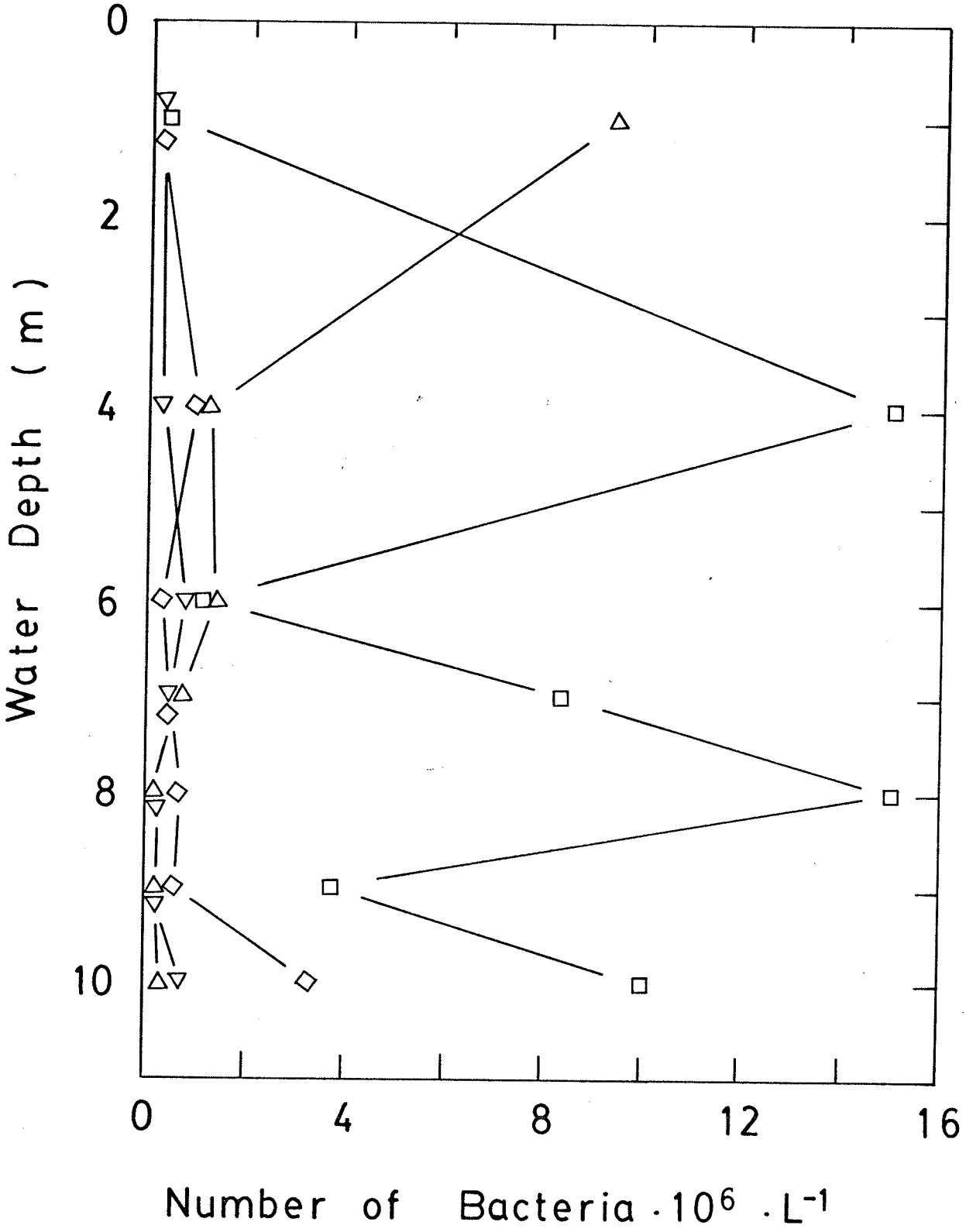

0
2
4
6
8
10
Water Depth (m)
0
4
8
12
16
Number of Bacteria · 10⁶ · L⁻¹

Fig. 33. The number of bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on Nitrobacter agar at 15°C and pH 7·6. Symbol are : (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

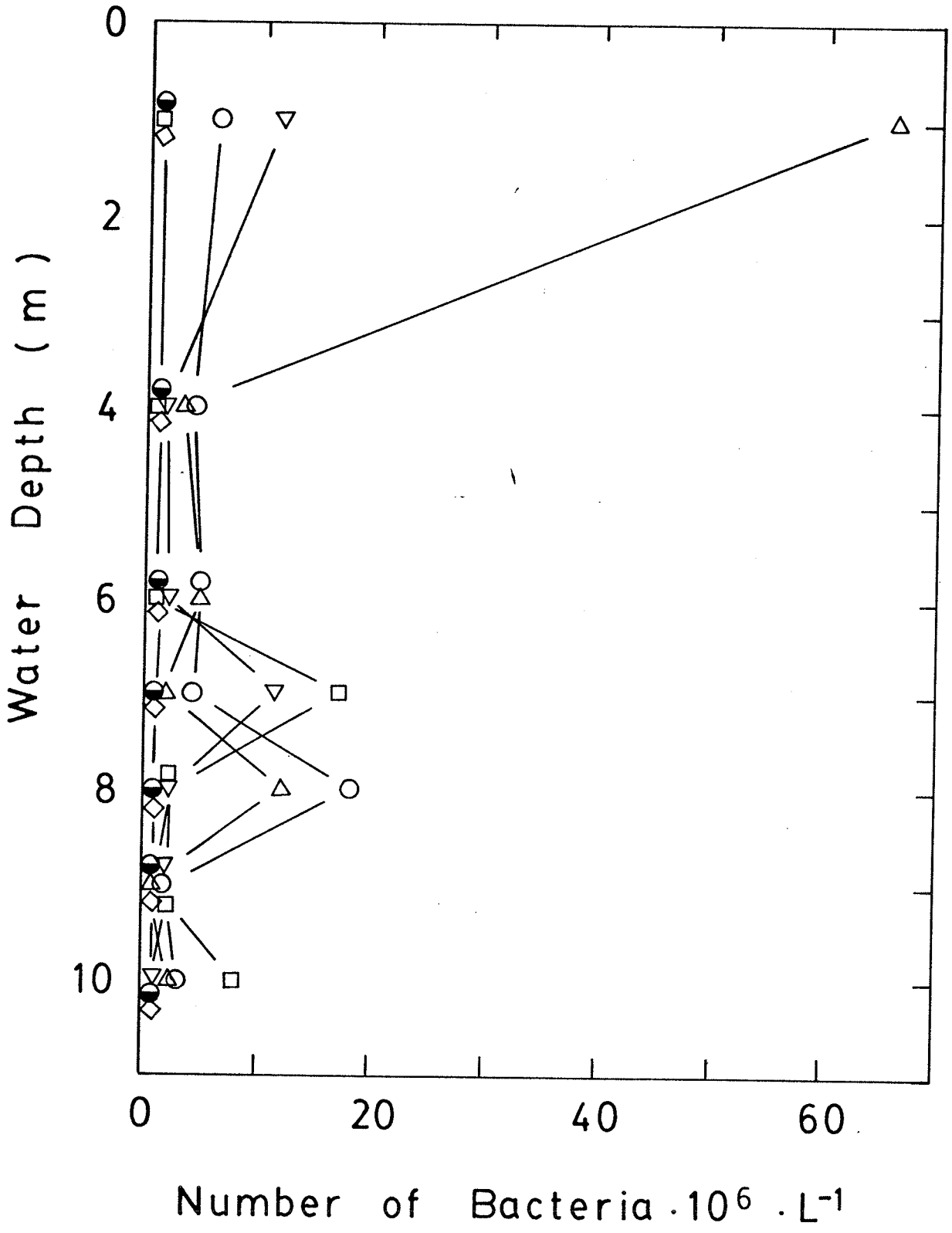

0
2
4
6
8
10
Water Depth (m)
0
20
40
60
Number of Bacteria · 10⁶ · L⁻¹

Fig. 34. The number of bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on Nitrobacter agar at 4°C and pH 7·6. Symbols are : (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

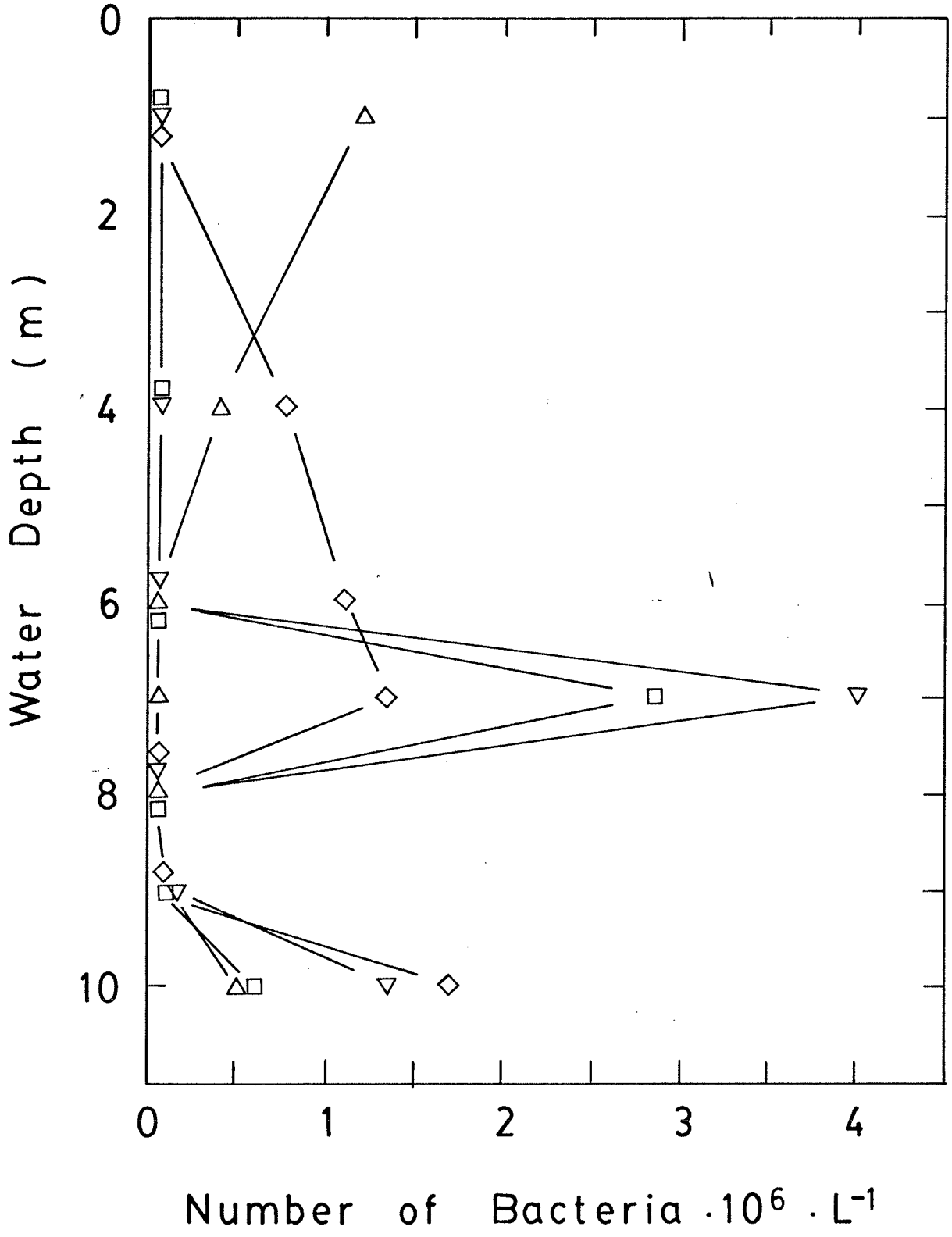

0
2
4
6
8
10
Water Depth (m)
0
1
2
3
4
Number of Bacteria ·10⁶ · L⁻¹

Fig. 35. The number of bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on Nitrobacter agar at 15°C and pH 5·5. Symbols are : (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

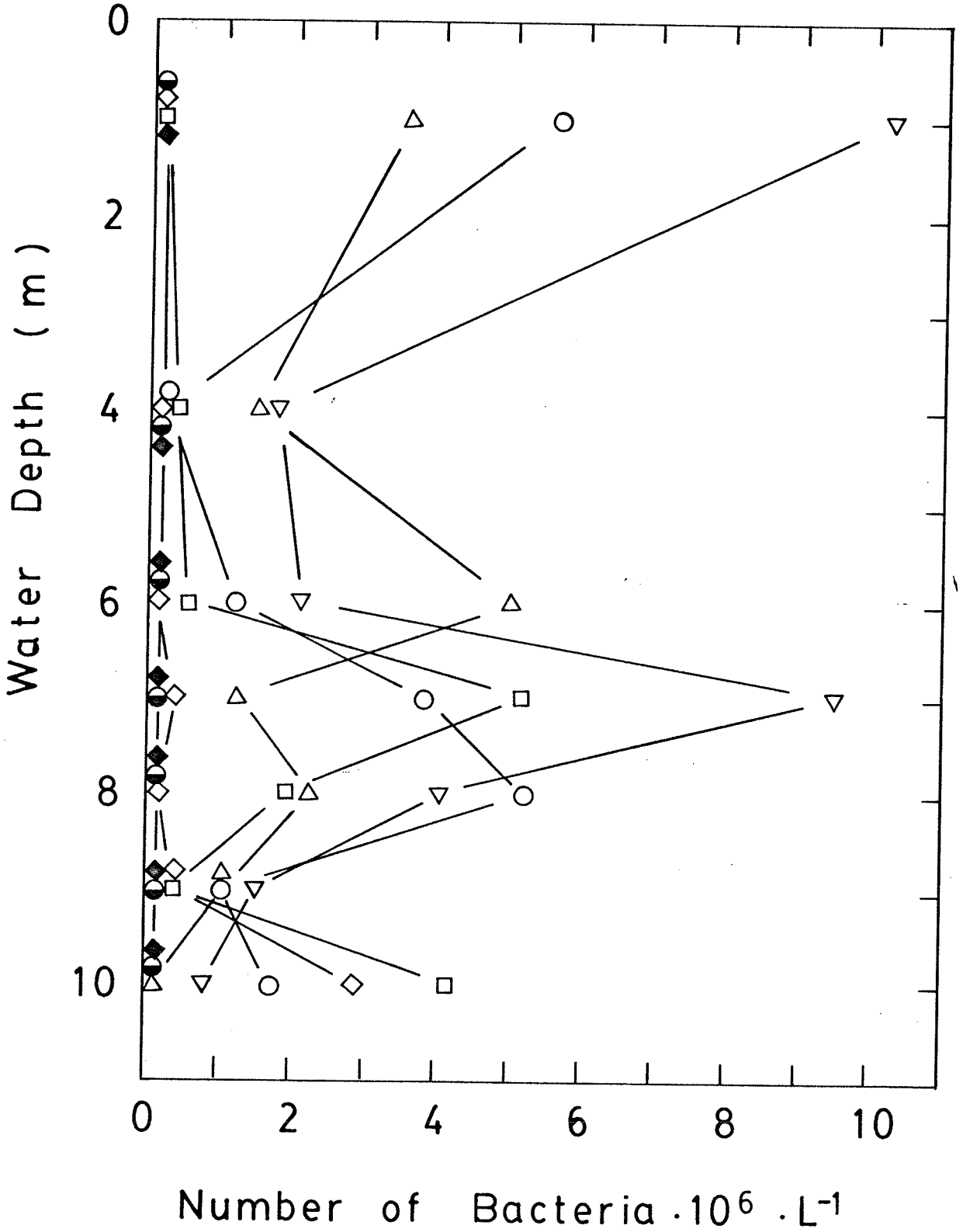

0
2
4
6
8
10
Water Depth (m)
0
2
4
6
8
10
Number of Bacteria · 10^6 · L^{-1}

Fig. 36. The number of bacteria (colony-forming units) at various water depths of L.302S that were capable of growth on Nitrobacter agar at 4°C and pH 5·5. Symbols are : (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

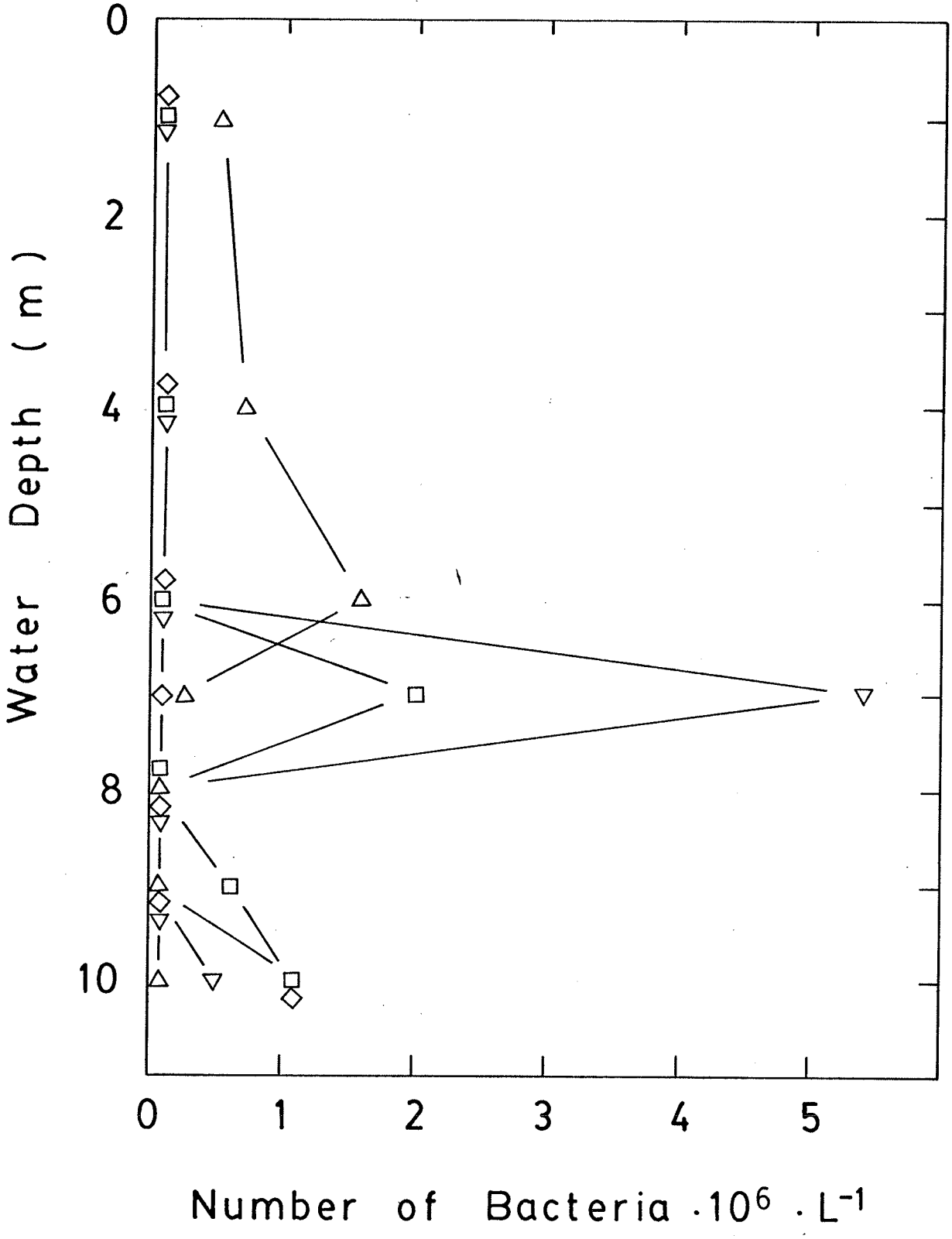

0
2
4
6
8
10
Water Depth (m)
0
1
2
3
4
5
Number of Bacteria ·10⁶ ·L⁻¹

Fig. 37. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in December, 1983 and incubated at 15°C without any supplements. Symbols are: (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

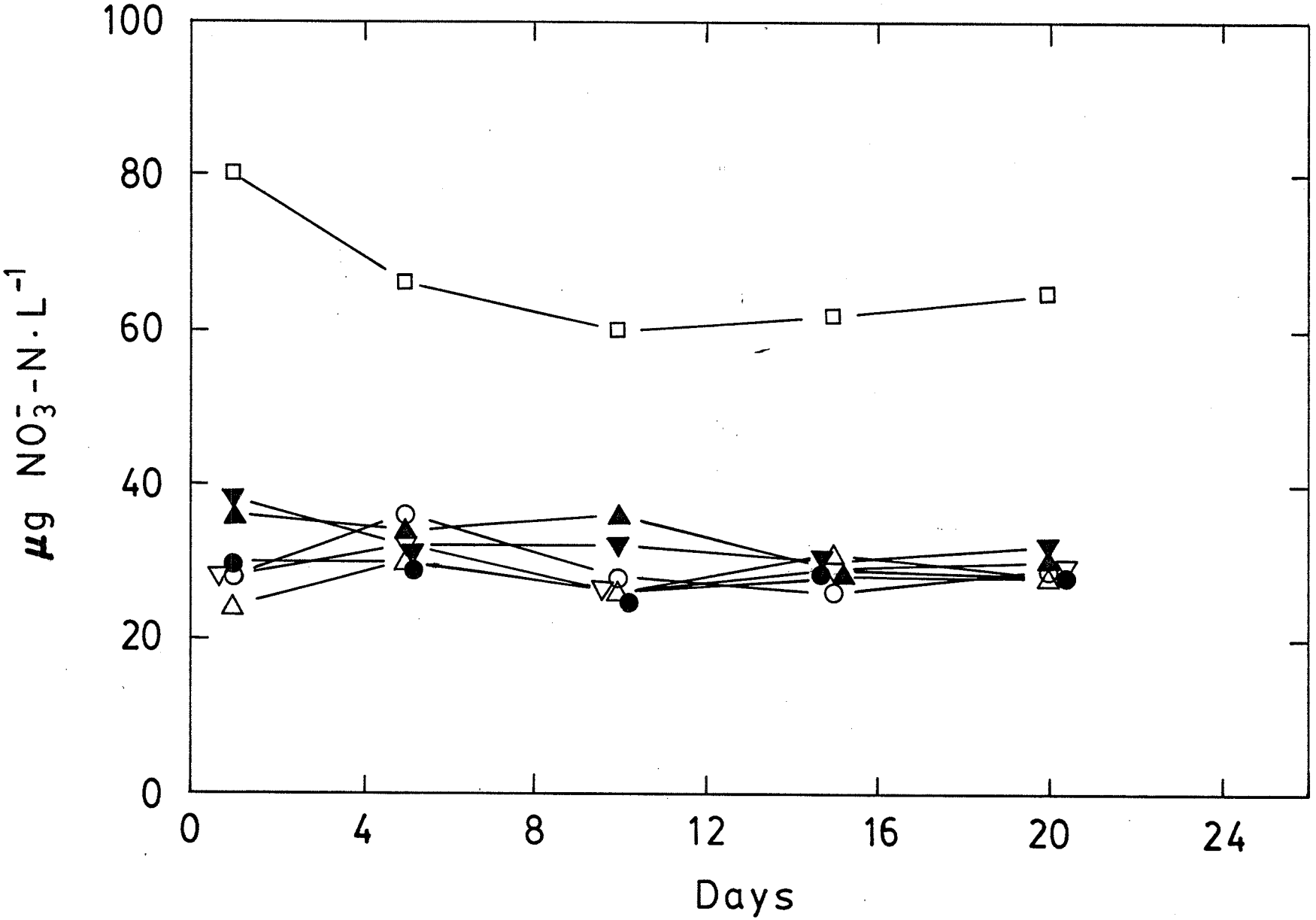

100
80
60
40
20
0
0
4
8
12
16
20
24
Days
µg NO3-N·L-1

Fig. 38. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in January, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

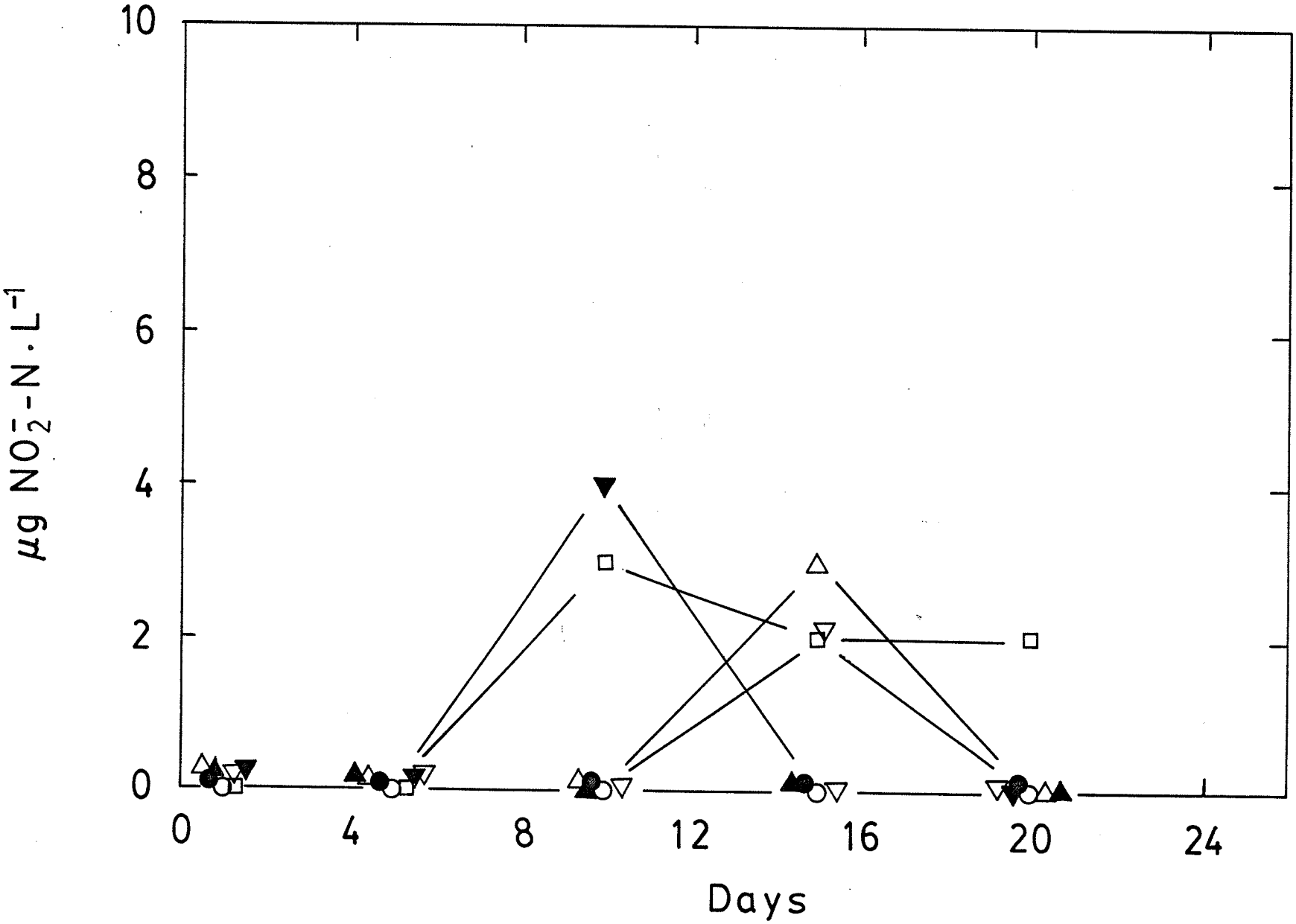

10
8
6
4
2
0
0
4
8
12
16
20
24
Days
µg NO2-N·L-1

Fig. 39. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in January, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

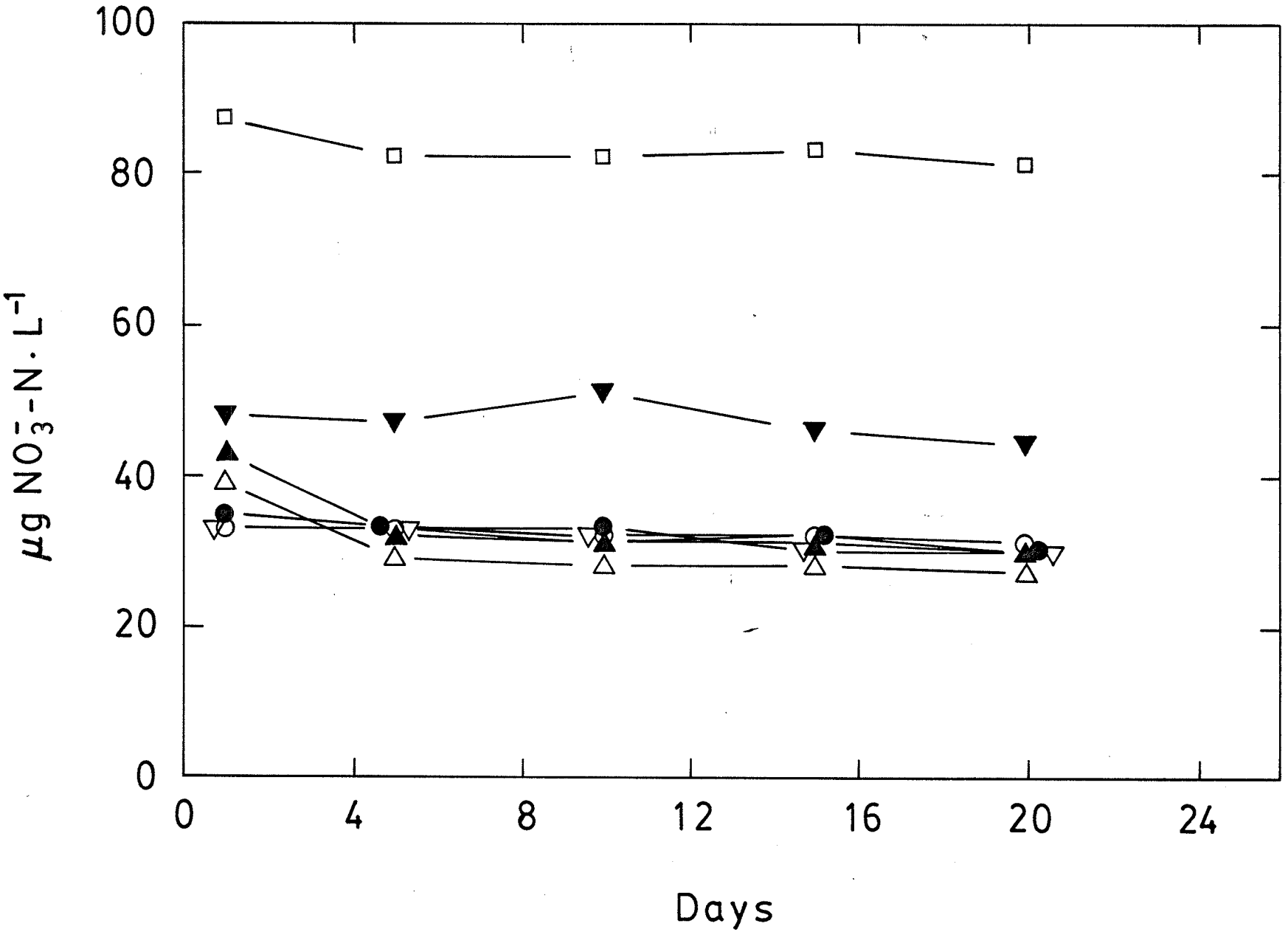

100
80
60
40
20
0
0
4
8
12
16
20
24
Days
µg NO3-N . L-1

Fig. 40. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in February, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

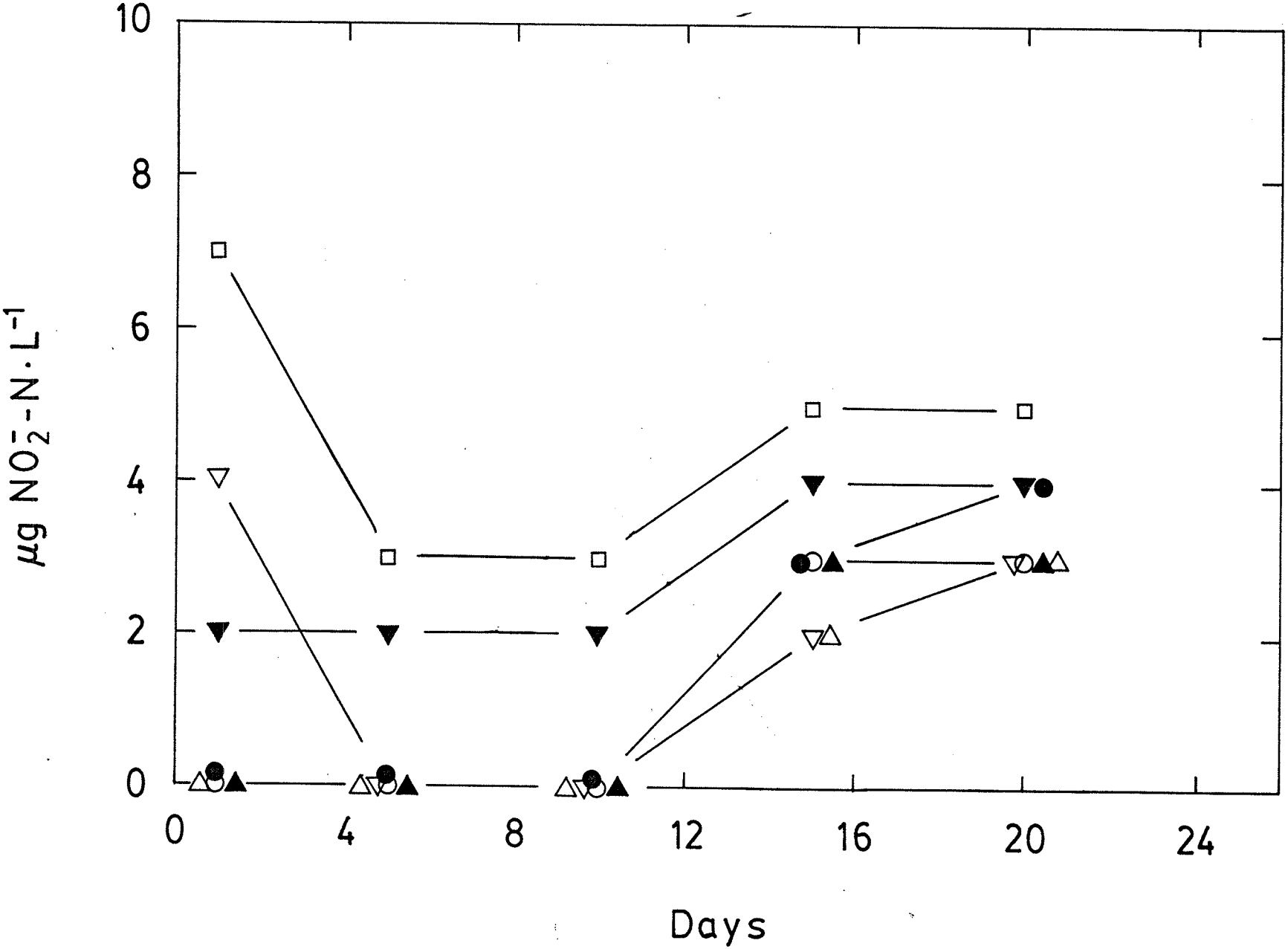

10
8
6
4
2
0
0
4
8
12
16
20
24
µg NO2-N·L-1
Days

Fig. 41. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in February, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

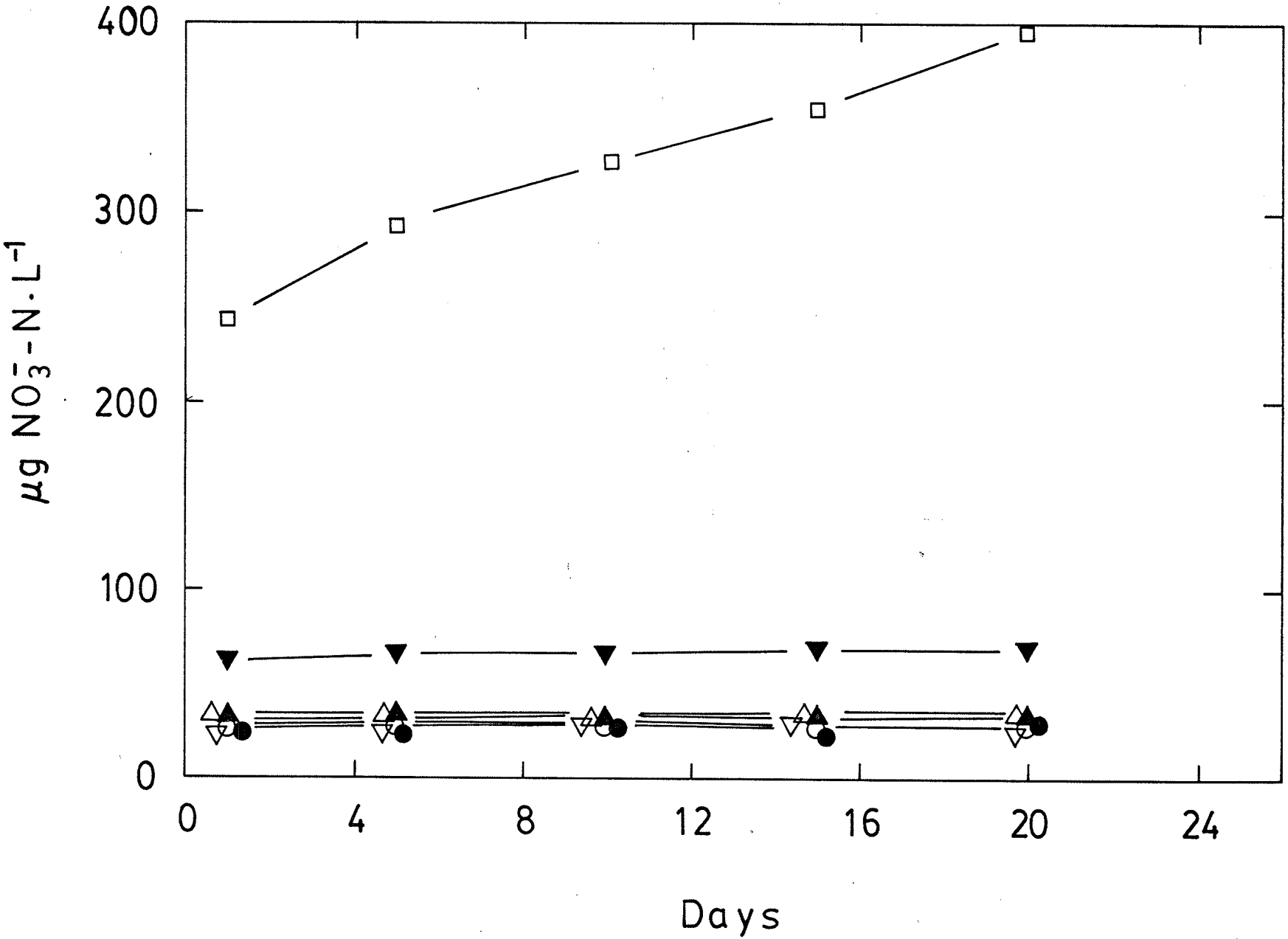

400
300
200
100
0
µg NO_3^--N·L^{-1}
0
4
8
12
16
20
24
Days

Fig. 42. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in March, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

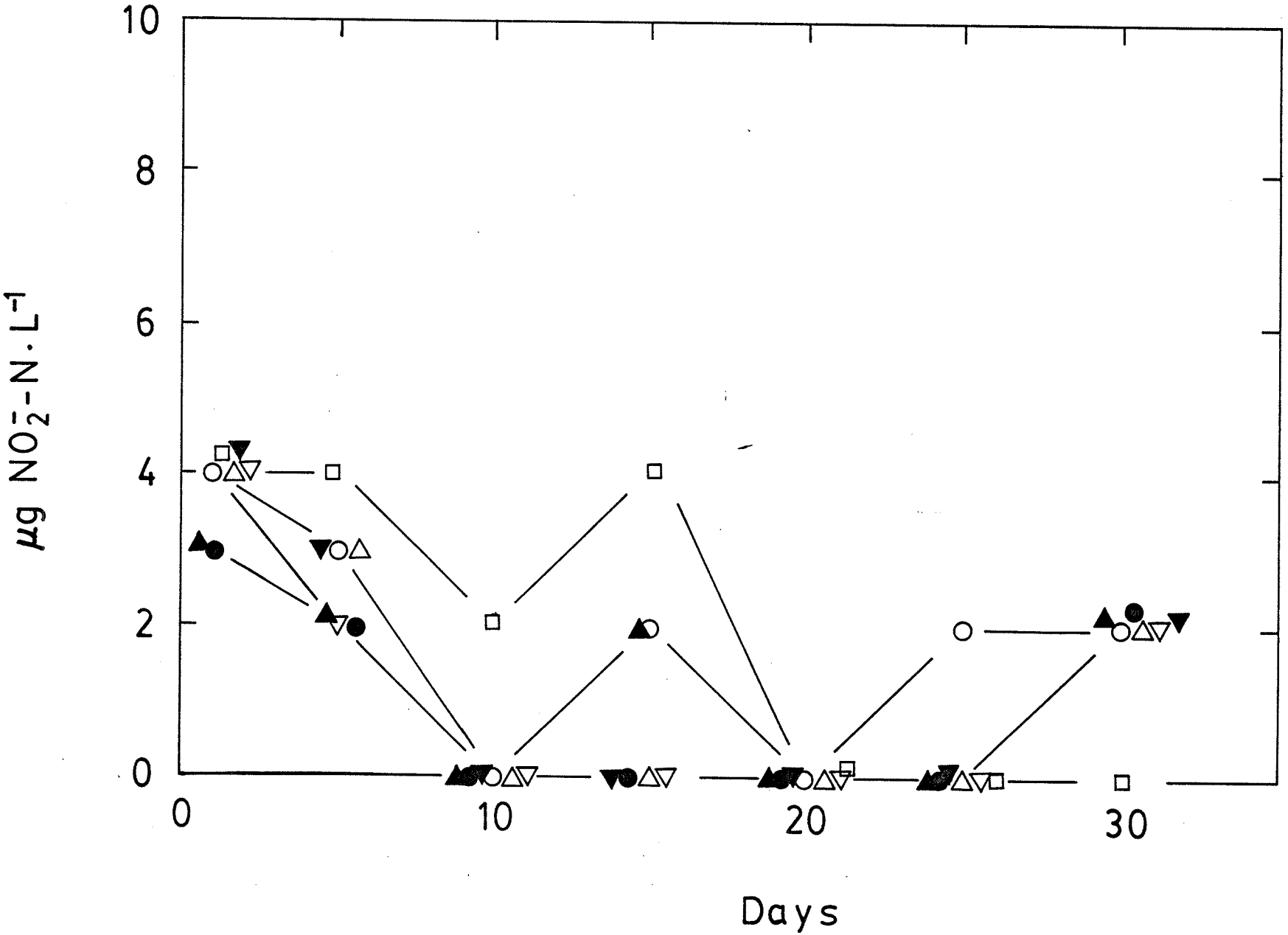

10
8
6
4
2
0
0
10
20
30
µg NO_2^--N.L^{-1}
Days

Fig. 43. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in March, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

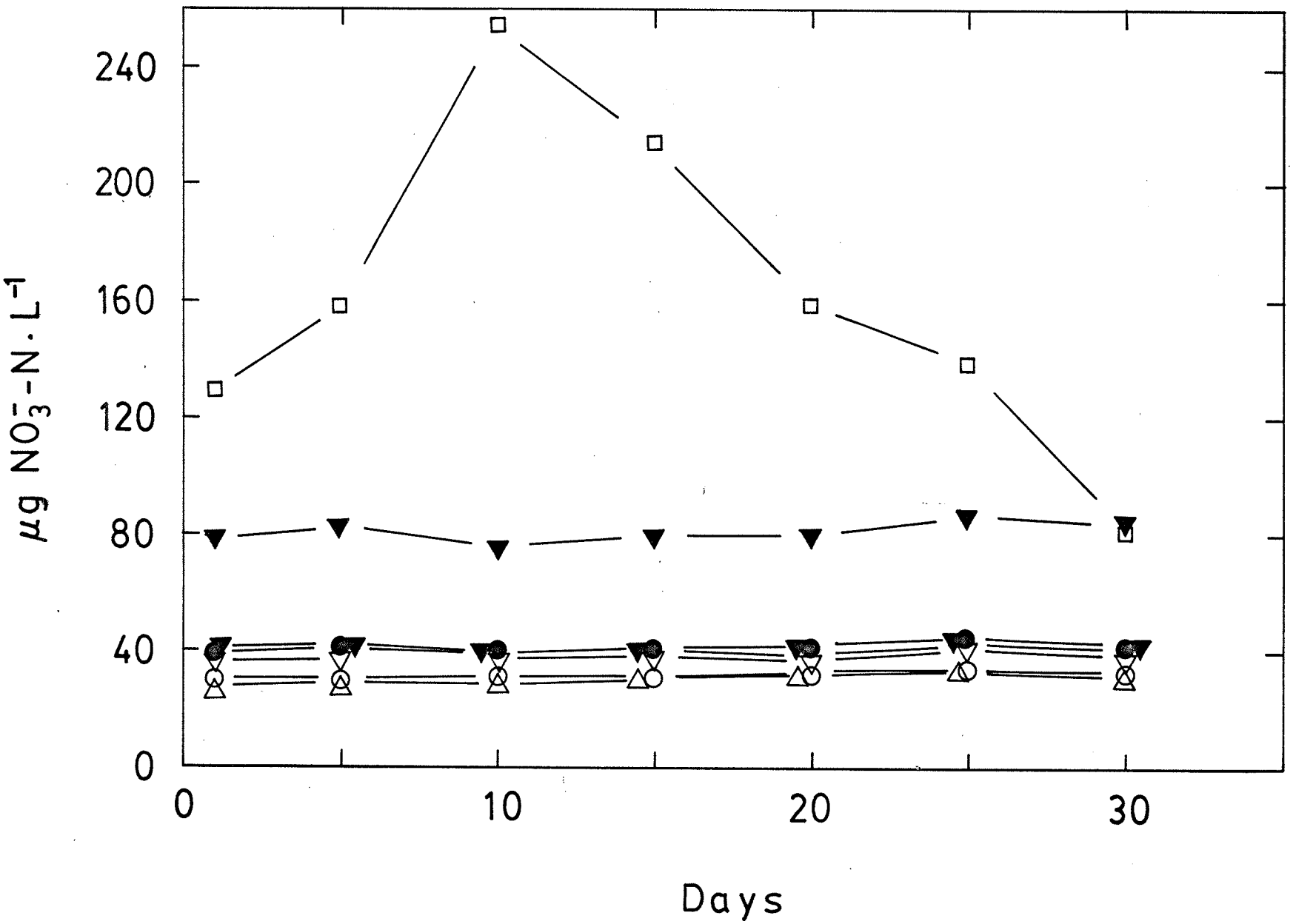

240
200
160
120
80
40
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 44. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in April, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

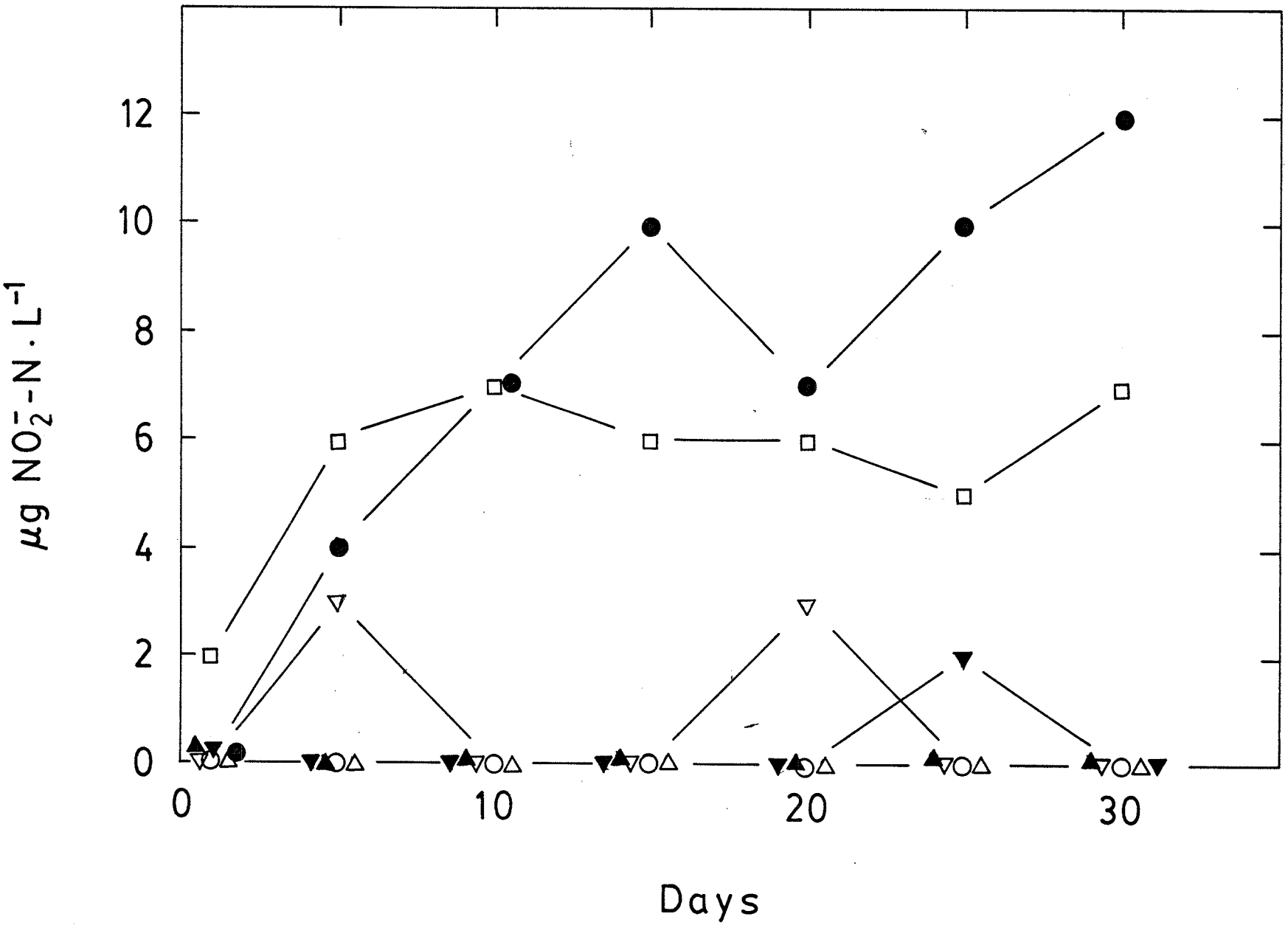

12
10
8
6
4
2
0
0
10
20
30
Days
µg NO2-N . L-1

Fig. 45. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in April, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

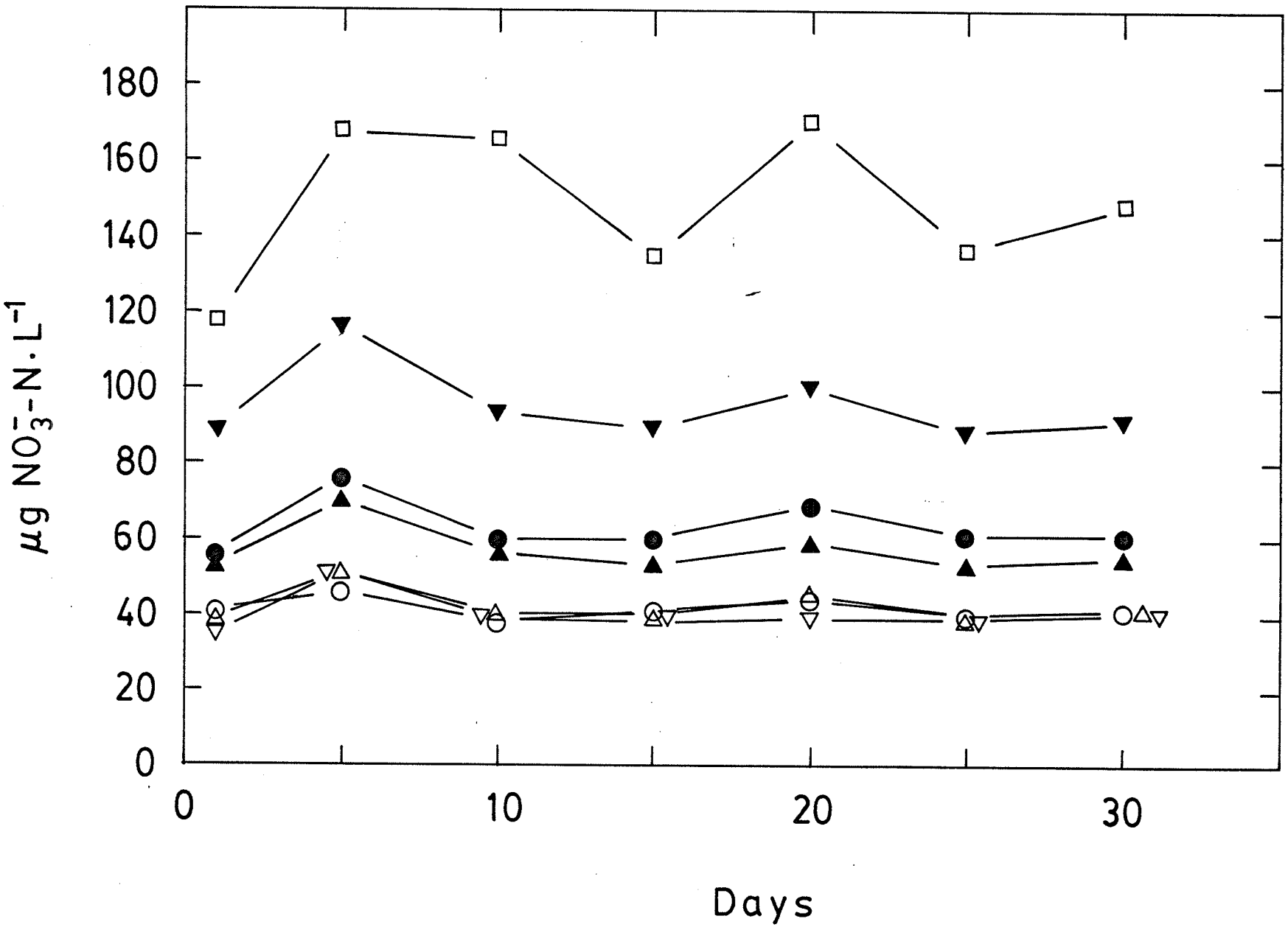

180
160
140
120
100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N.L-1

Fig. 46. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in May, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

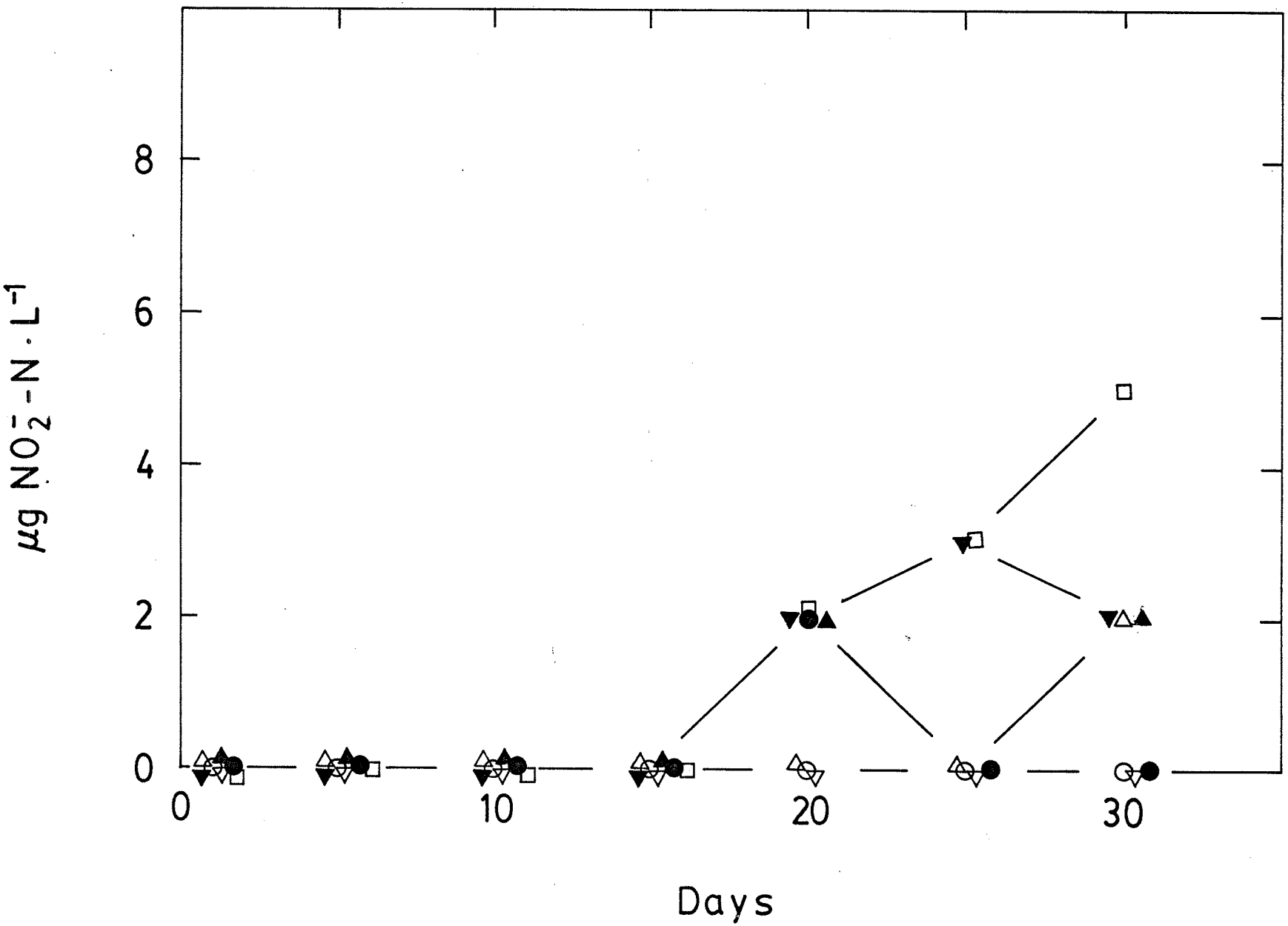

8
6
4
2
0
0
10
20
30
Days
µg NO2-N · L-1

Fig. 47. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in May, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

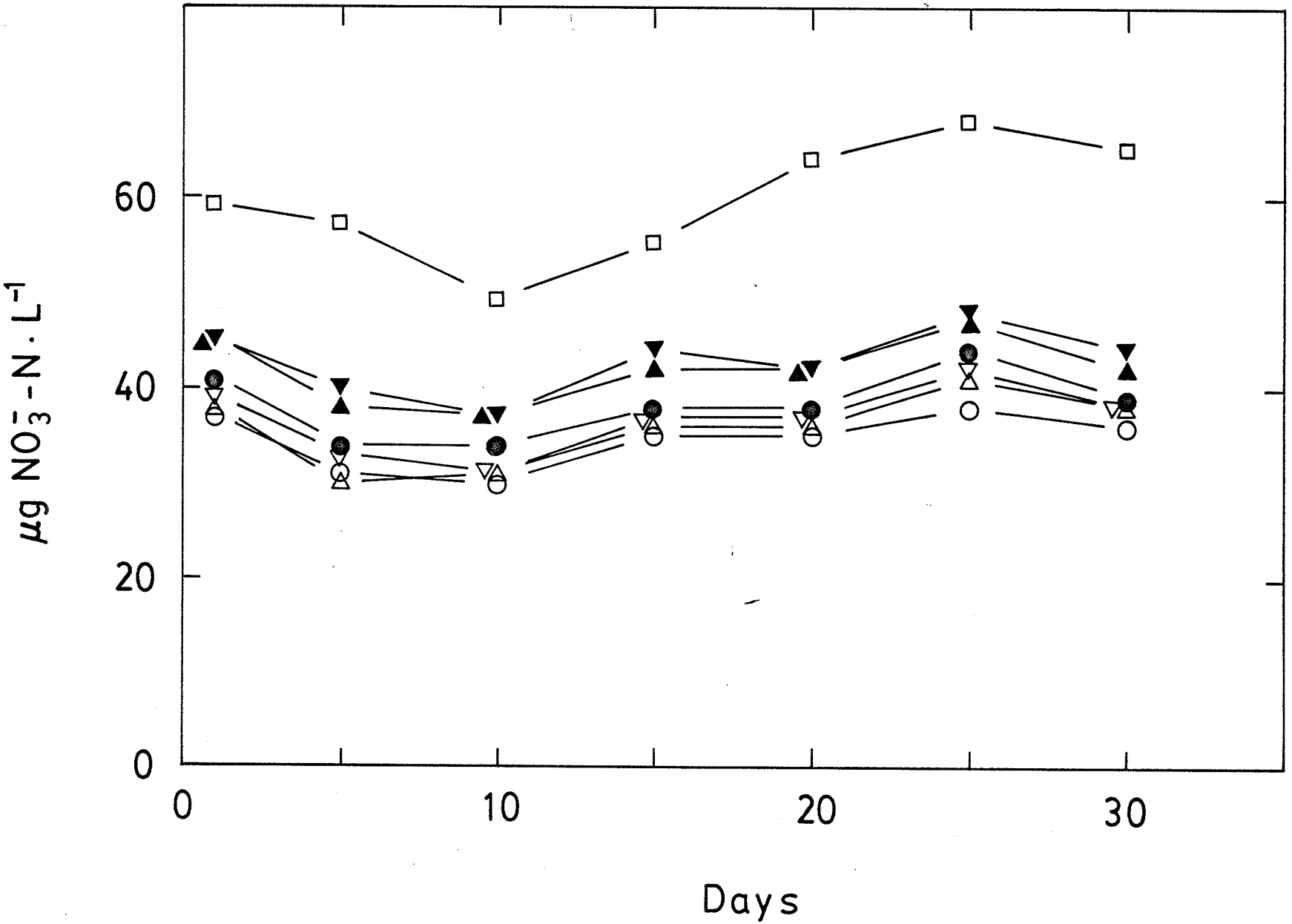

60
40
20
0
0
10
20
30
Days
µg NO3-N . L-1

Fig. 48. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in June, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

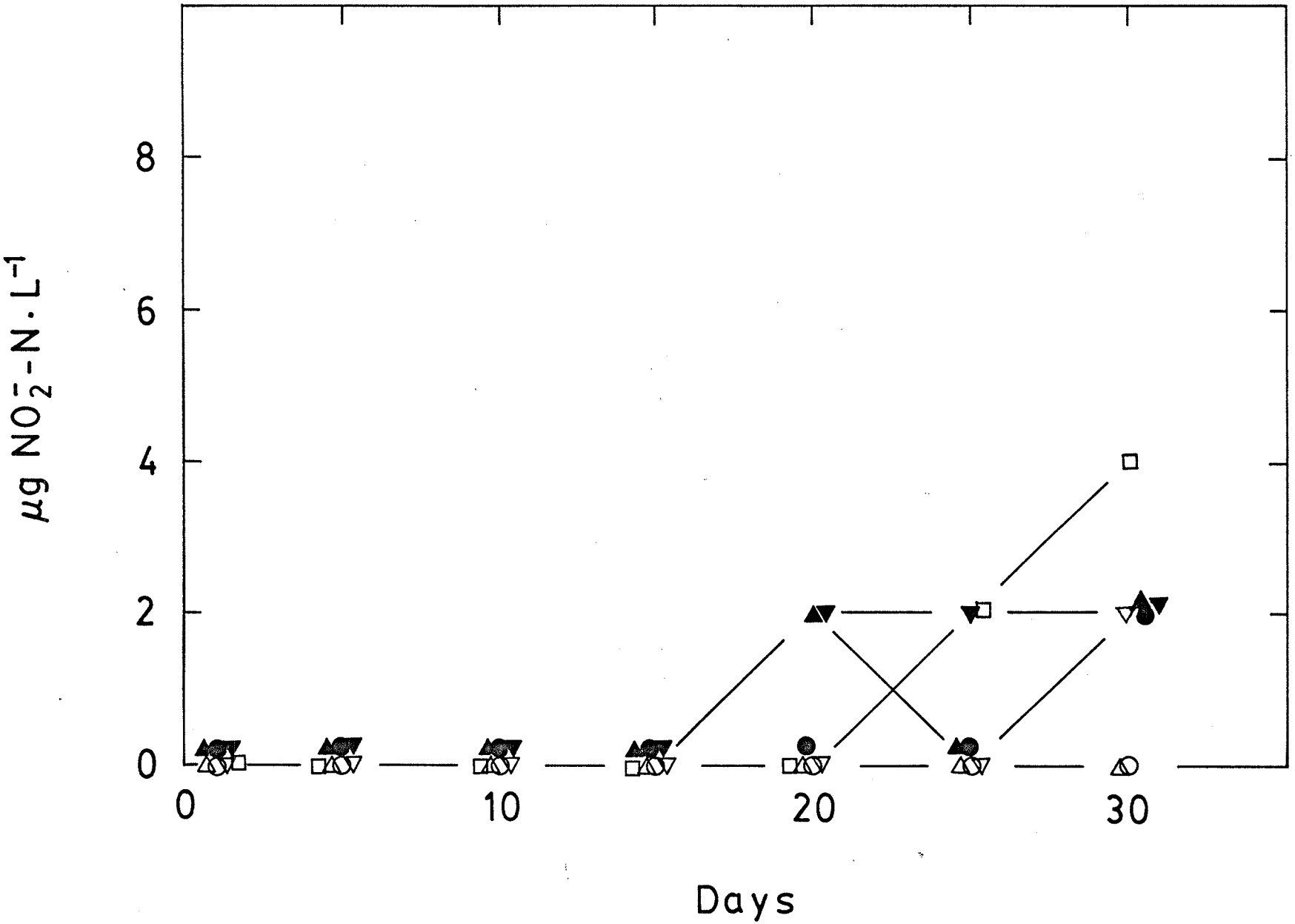

8
6
4
2
0
0
10
20
30
Days
µg NO2-N · L-1

Fig. 49. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in June, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

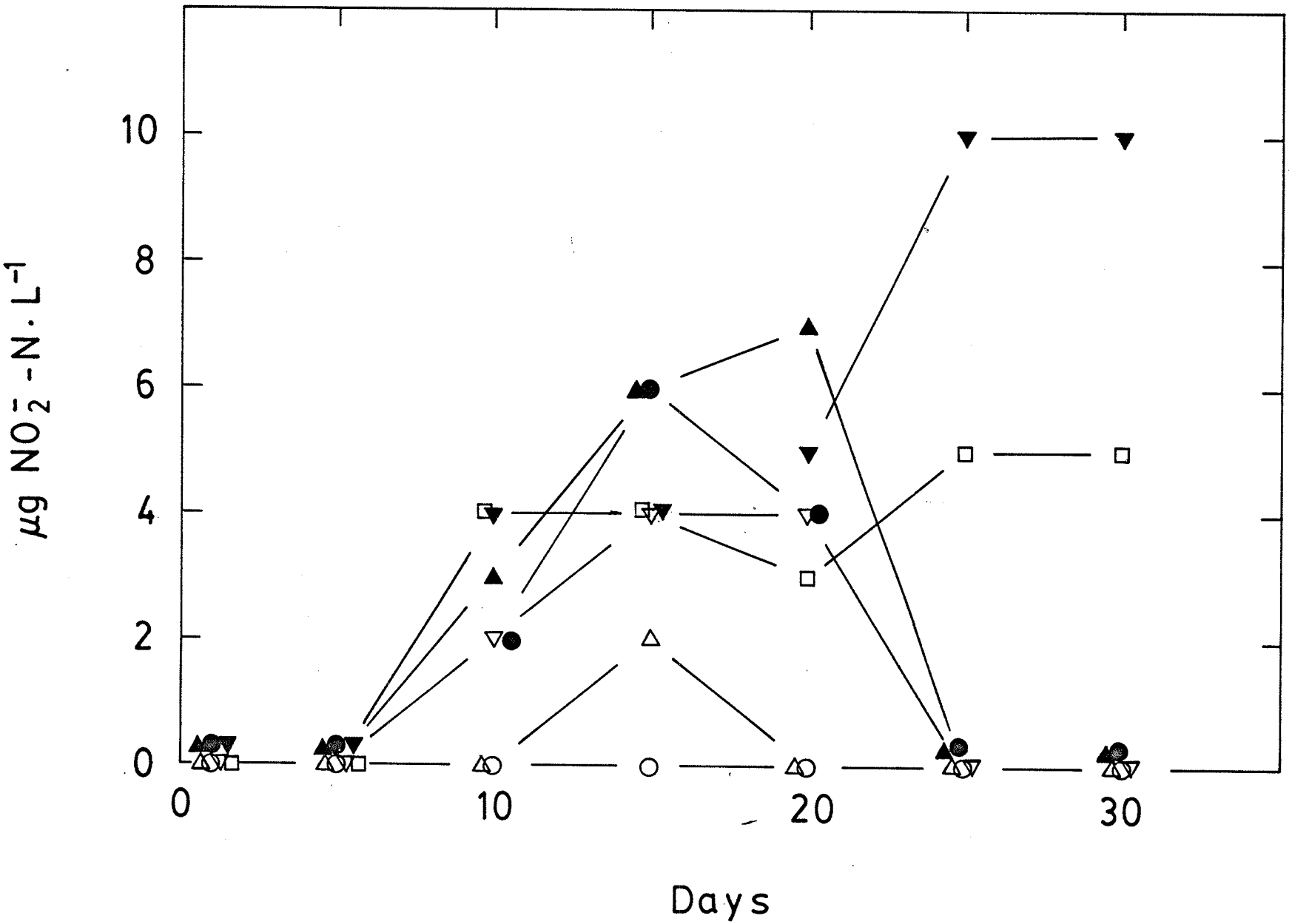

10
8
6
4
2
0
0
10
20
30
Days
µg NO2- -N . L-1

Fig. 50. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in June, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

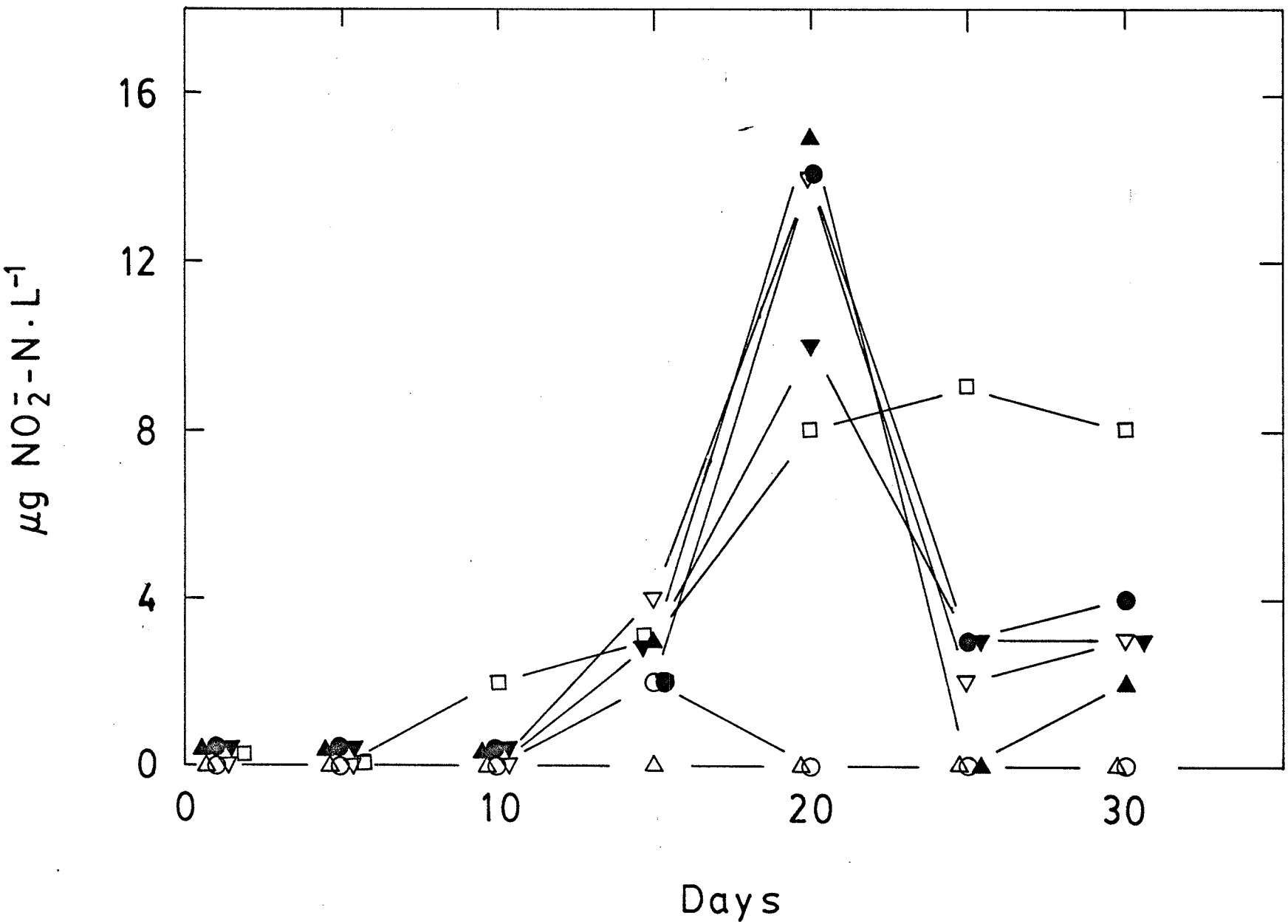

16
12
8
4
0
0
10
20
30
µg NO2-N · L-1
Days

Fig. 51. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in June, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

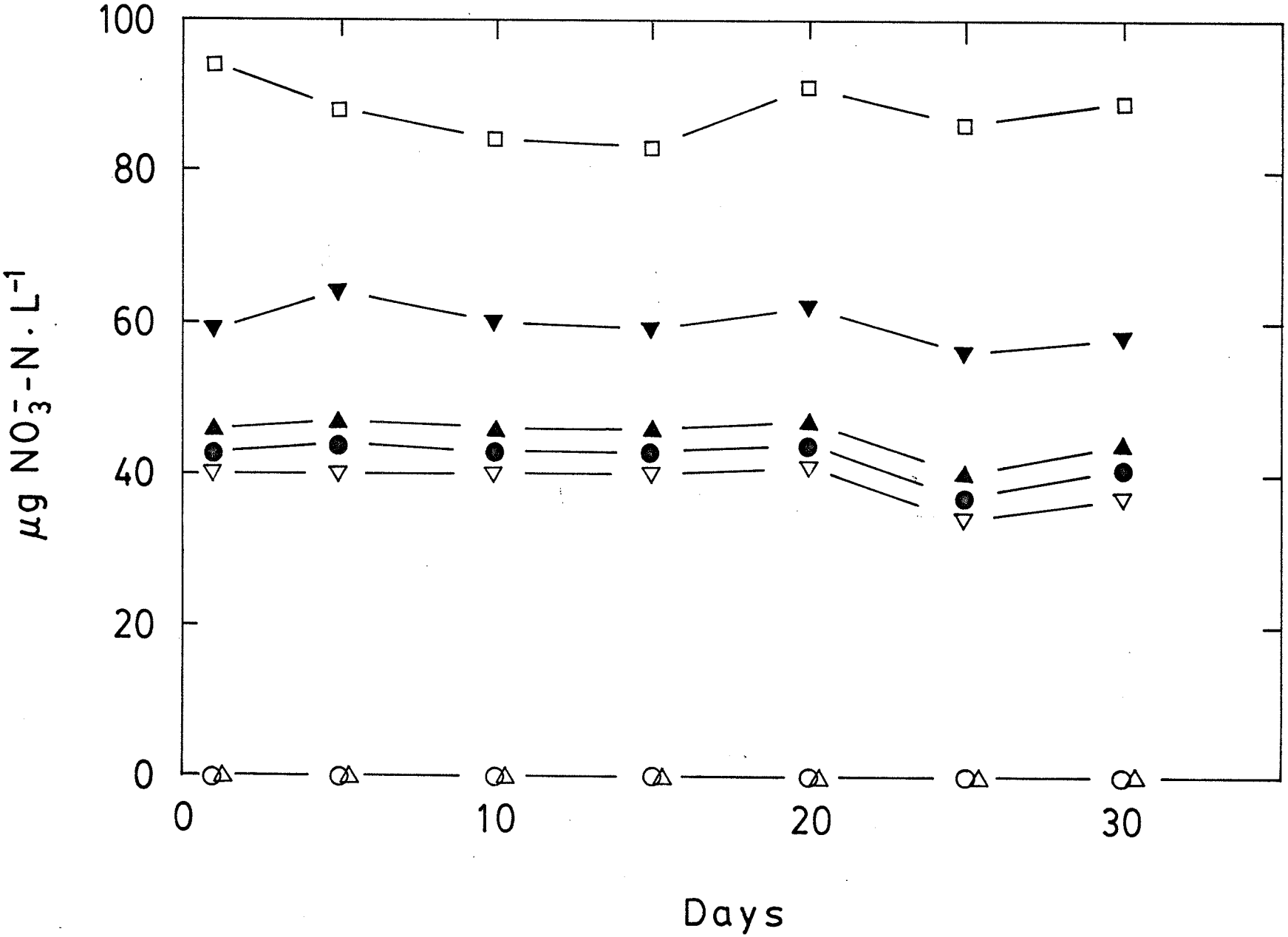

100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N . L-1

Fig. 52. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in June, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

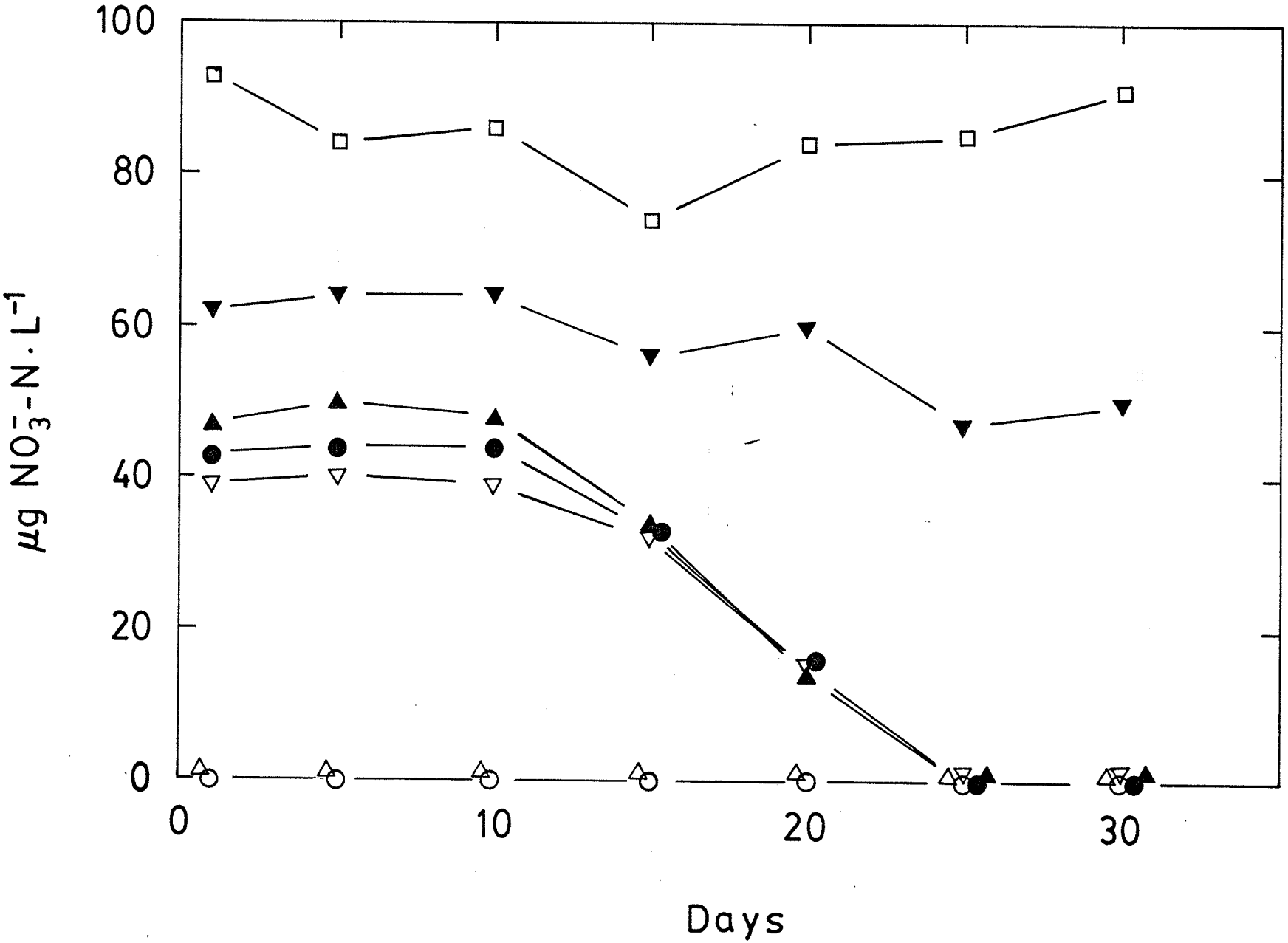

100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 53. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in June, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

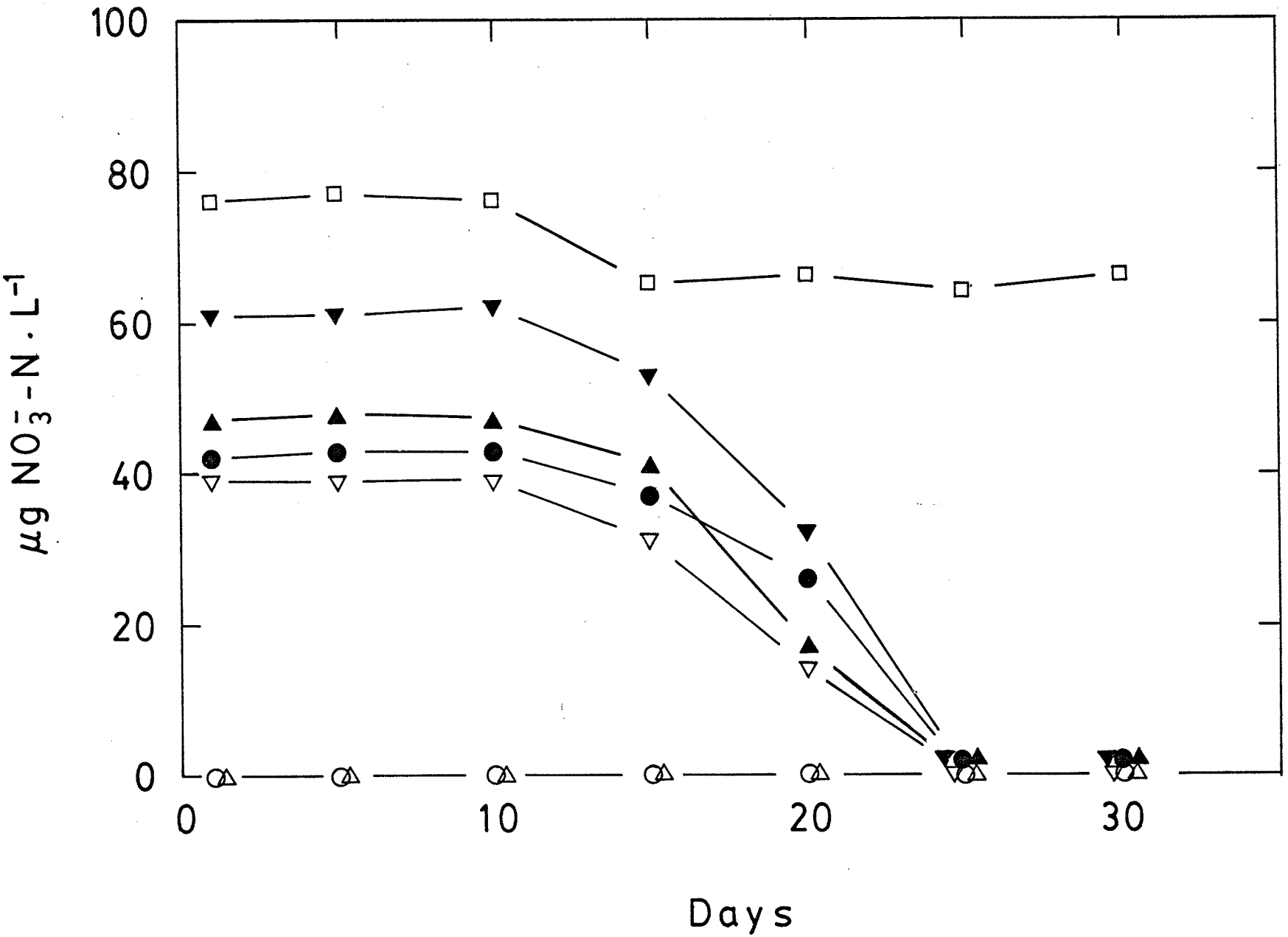

100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 54. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in July, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

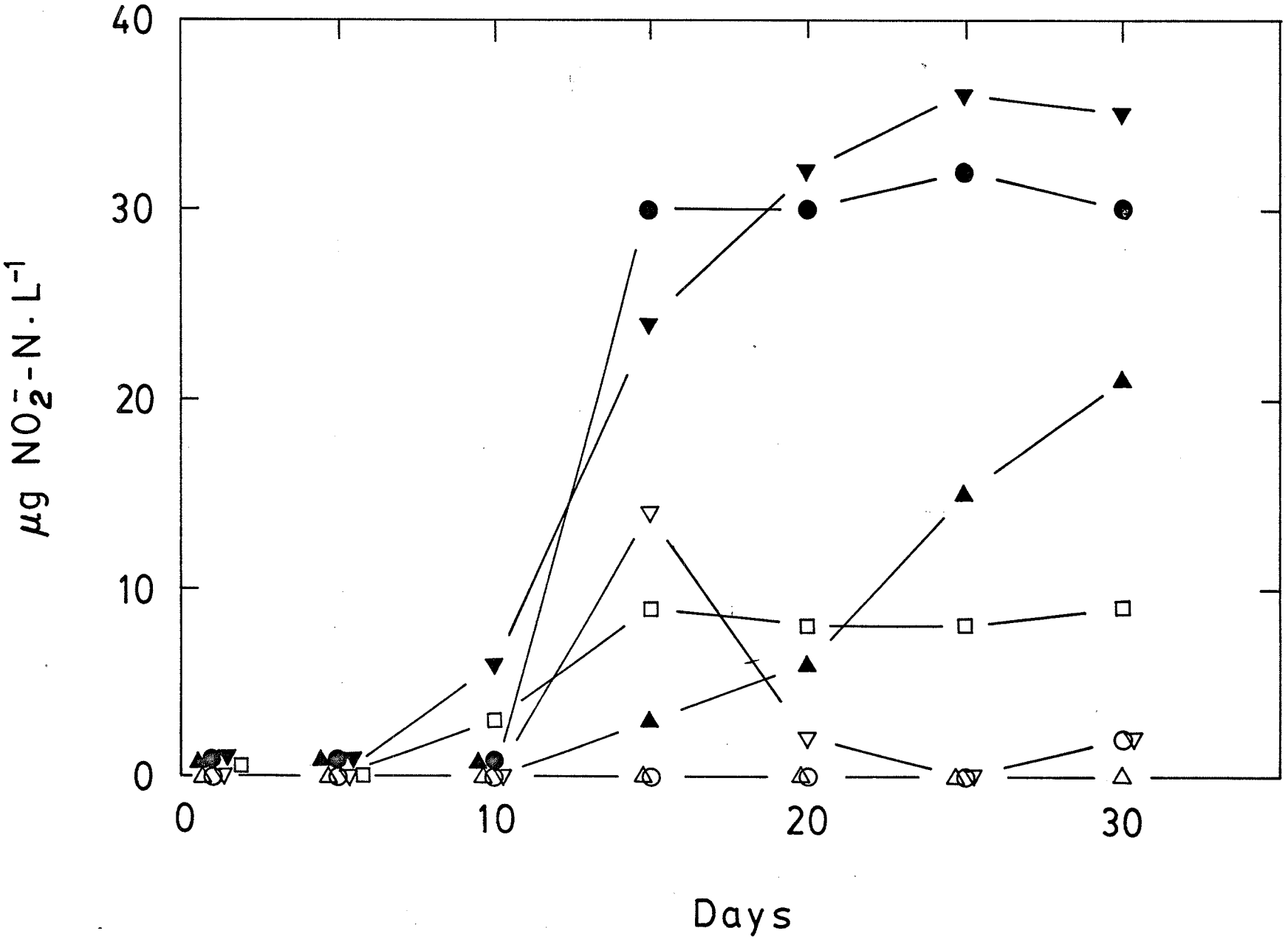

40
30
20
10
0
0
10
20
30
μg NO2-N · L-1
Days

Fig. 55. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in July, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

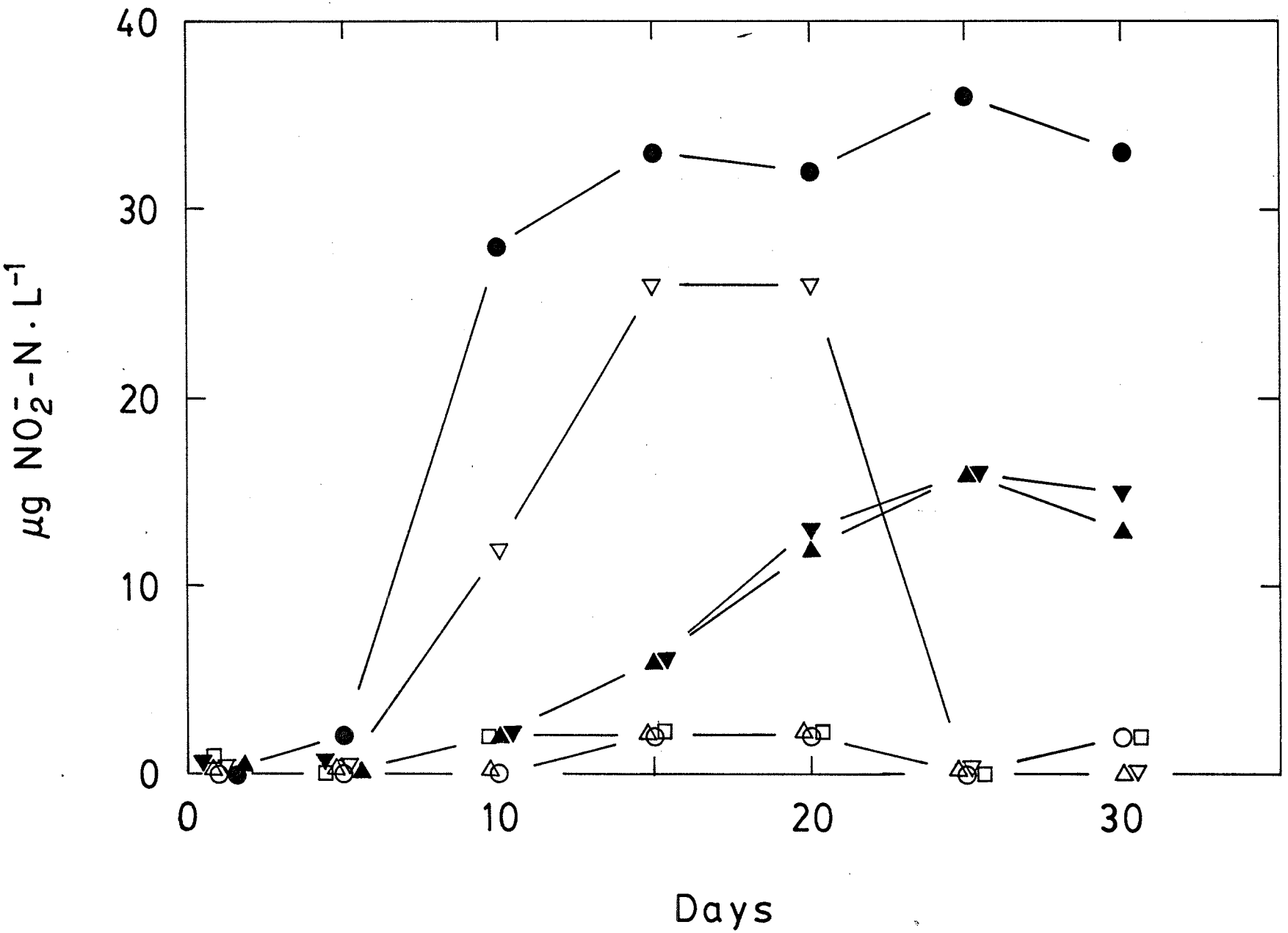

40
30
20
10
0
0
10
20
30
µg NO2-N . L-1
Days

Fig. 56. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in July, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

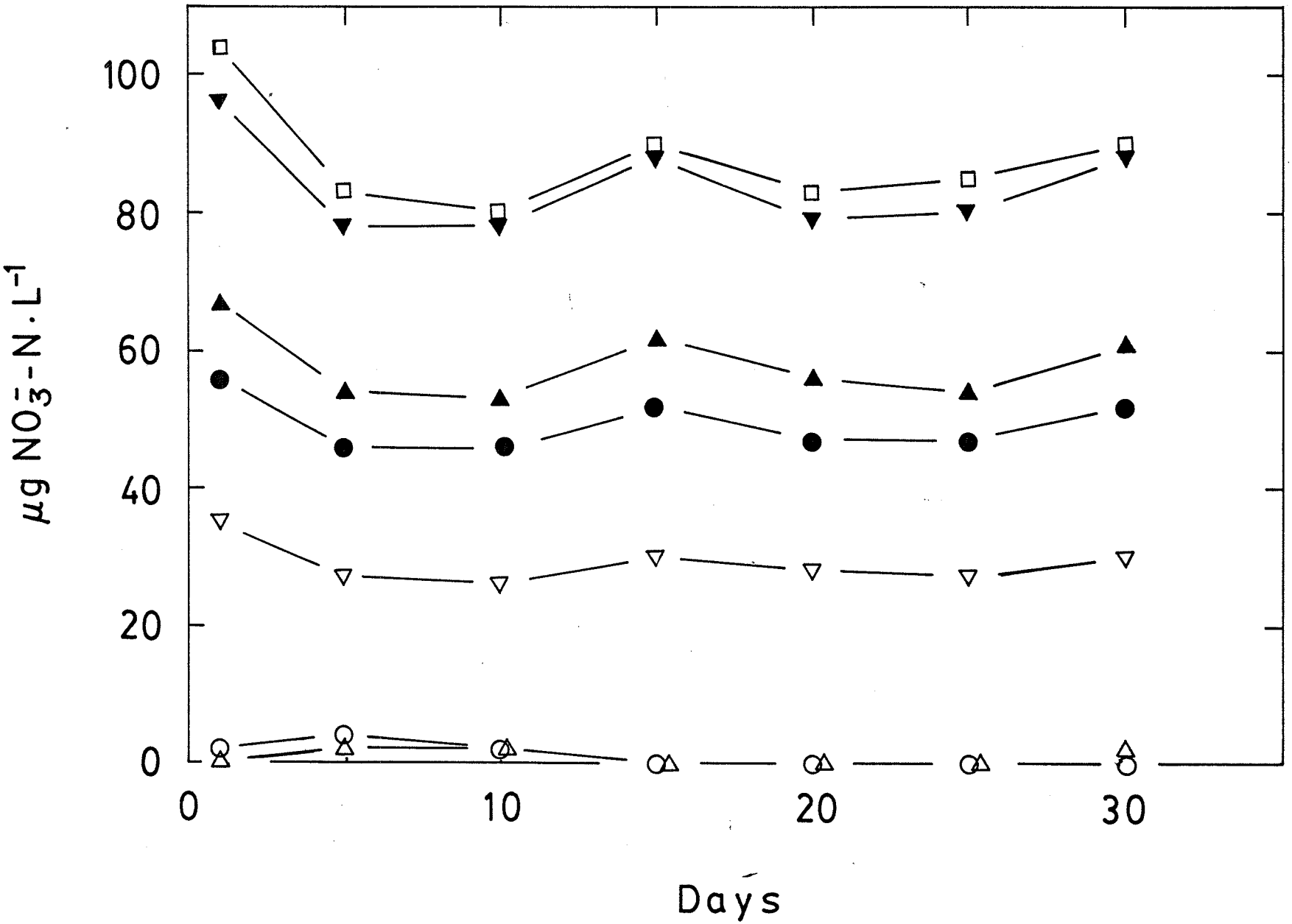

100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N.L-1

Fig. 57. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in July, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

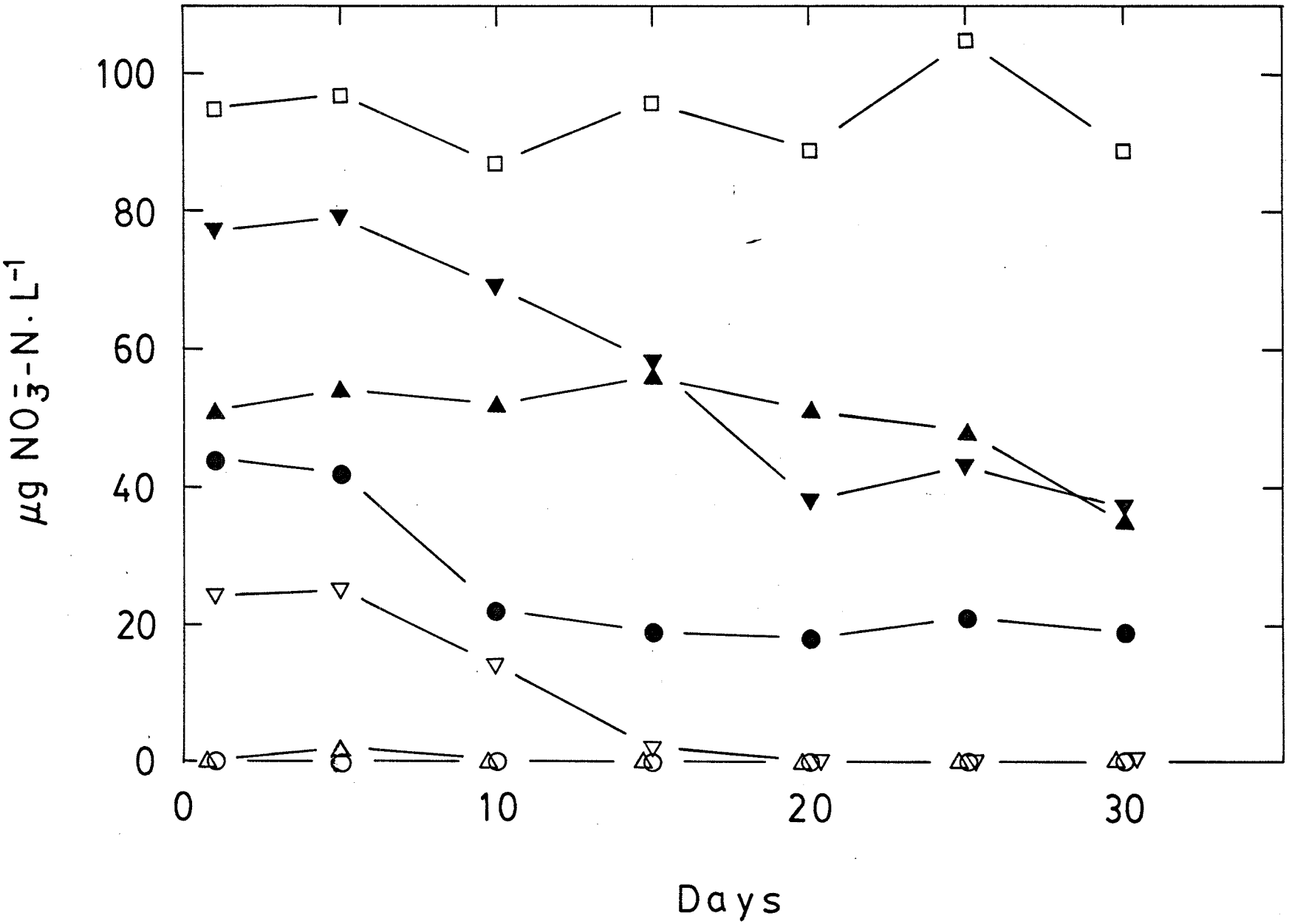

100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 58. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in July, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

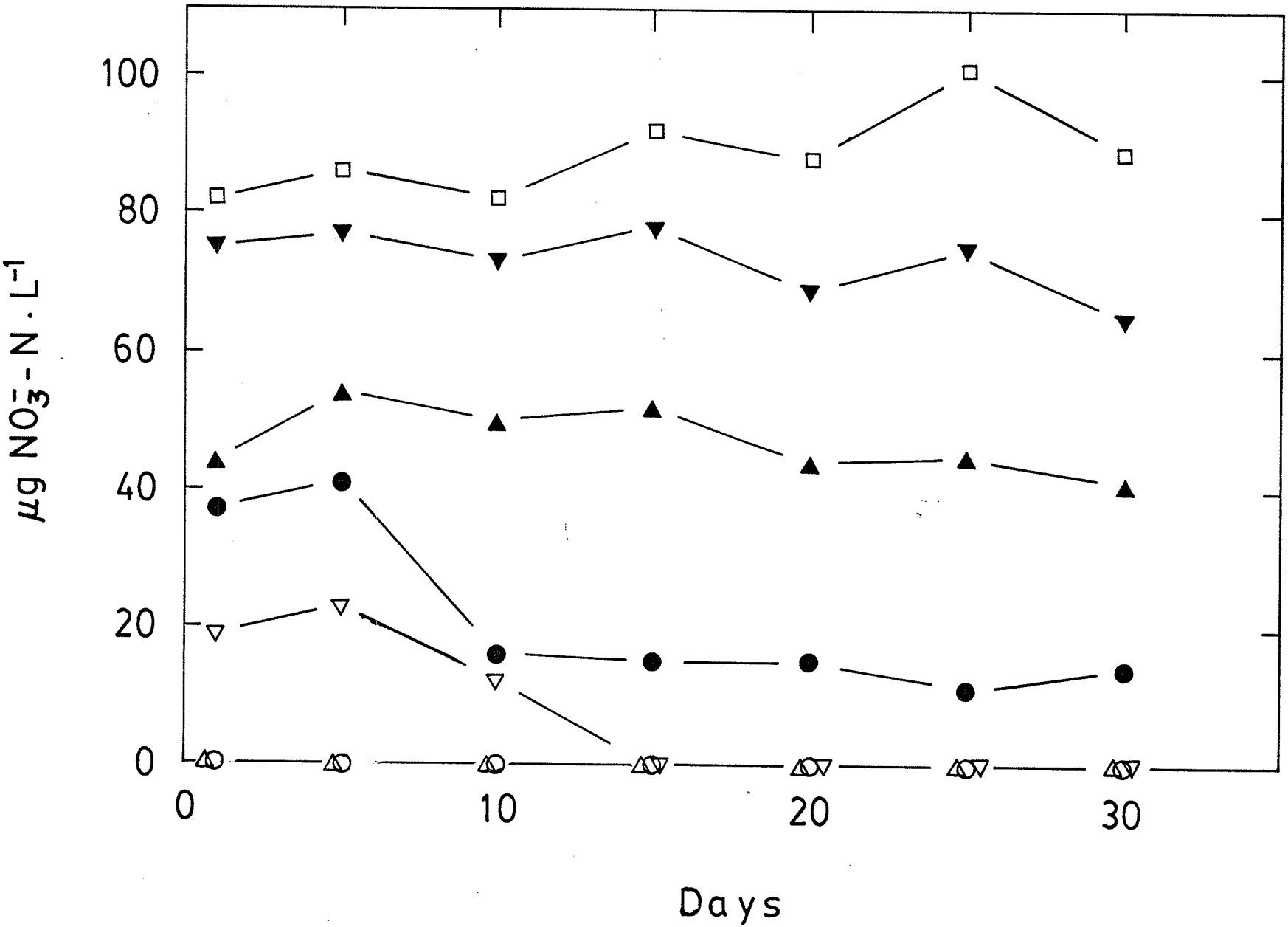

100
80
60
40
20
0
0
10
20
30
Days
µg NO3- - N . L-1

Fig. 59. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtaind in August, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

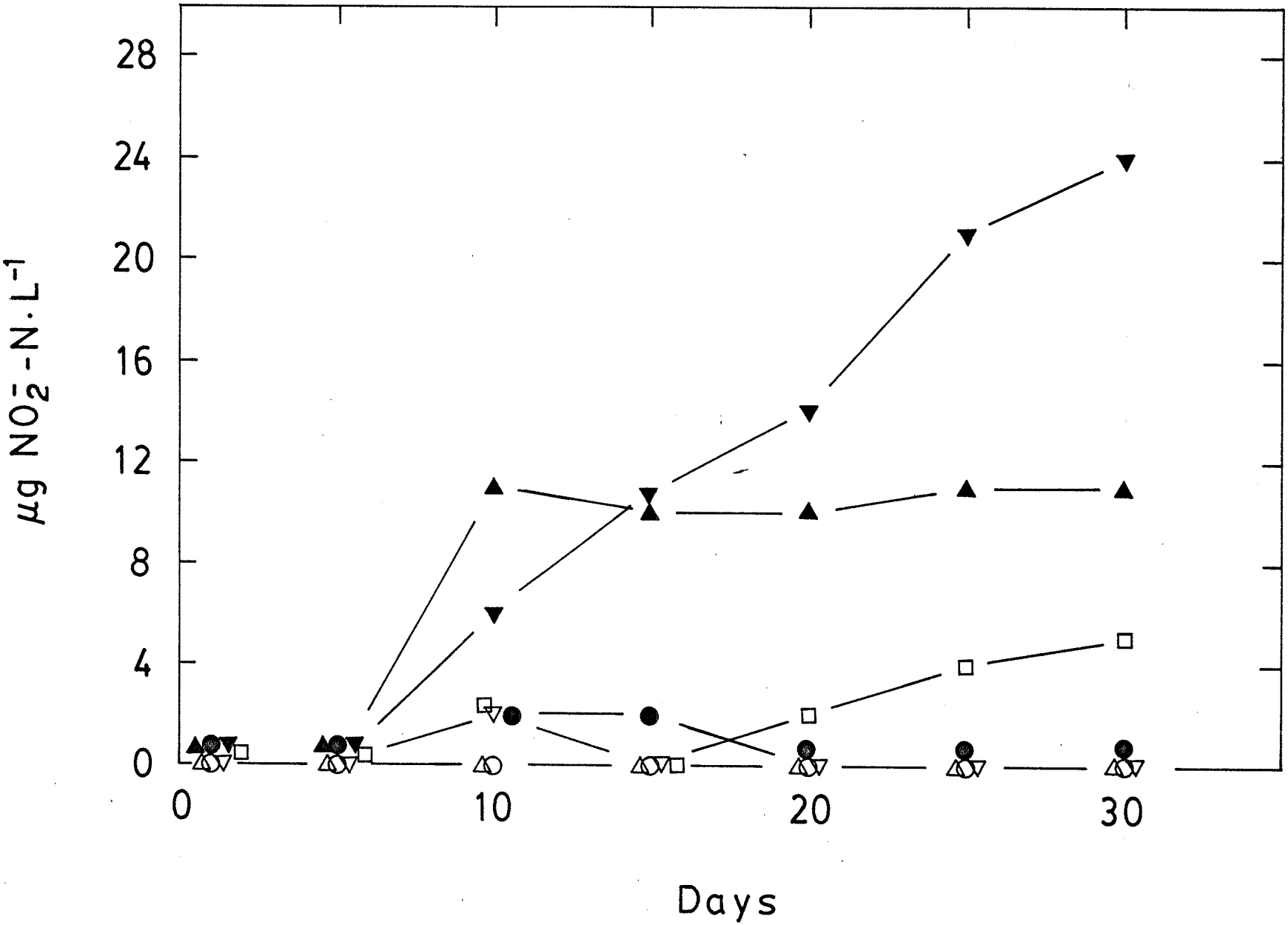

28
24
20
16
12
8
4
0
0
10
20
30
µg NO2- -N.L-1
Days

Fig. 60. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in August, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

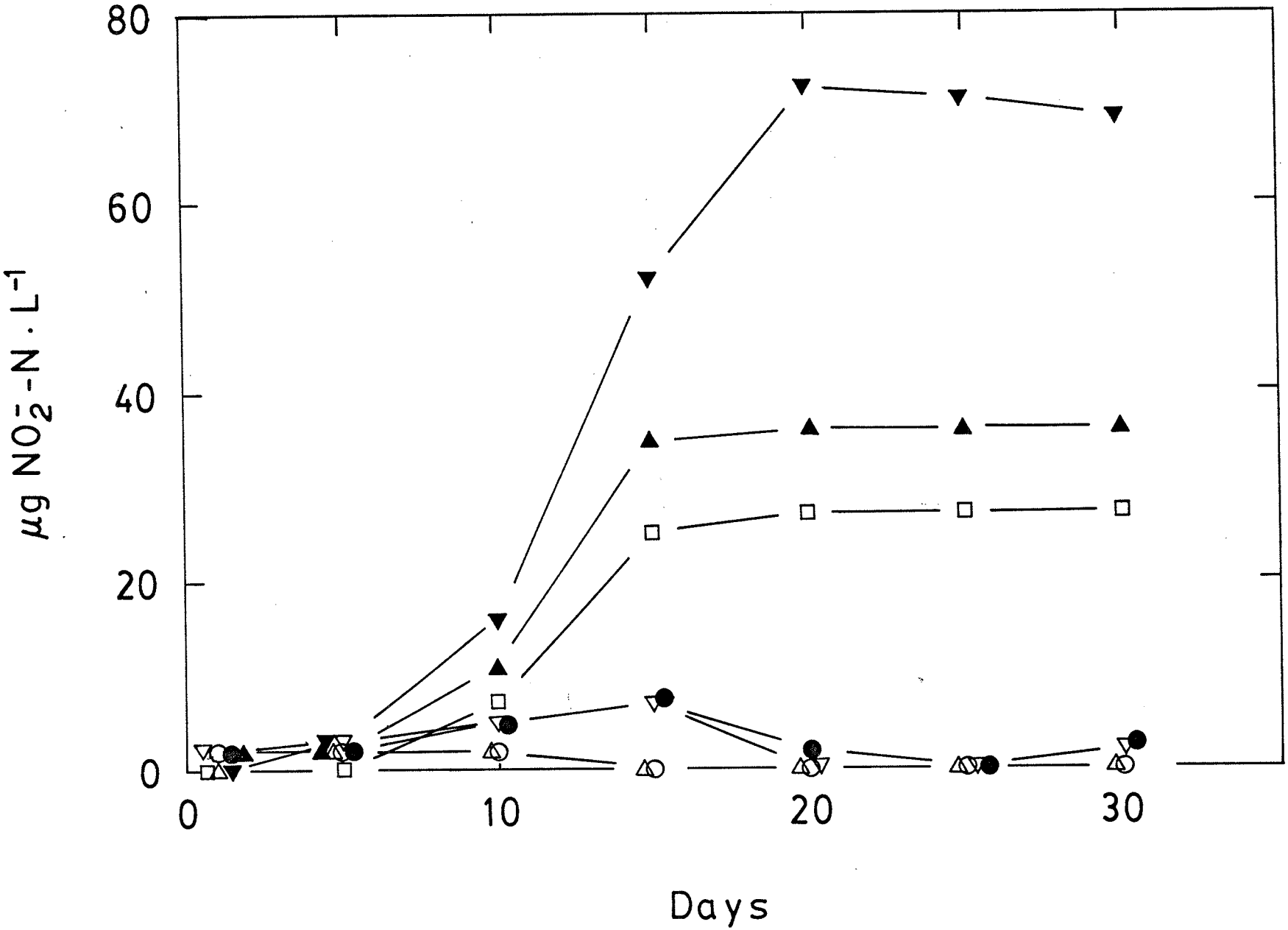

80
60
40
20
0
0
10
20
30
µg NO2-N . L-1
Days

Fig. 61. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in August, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

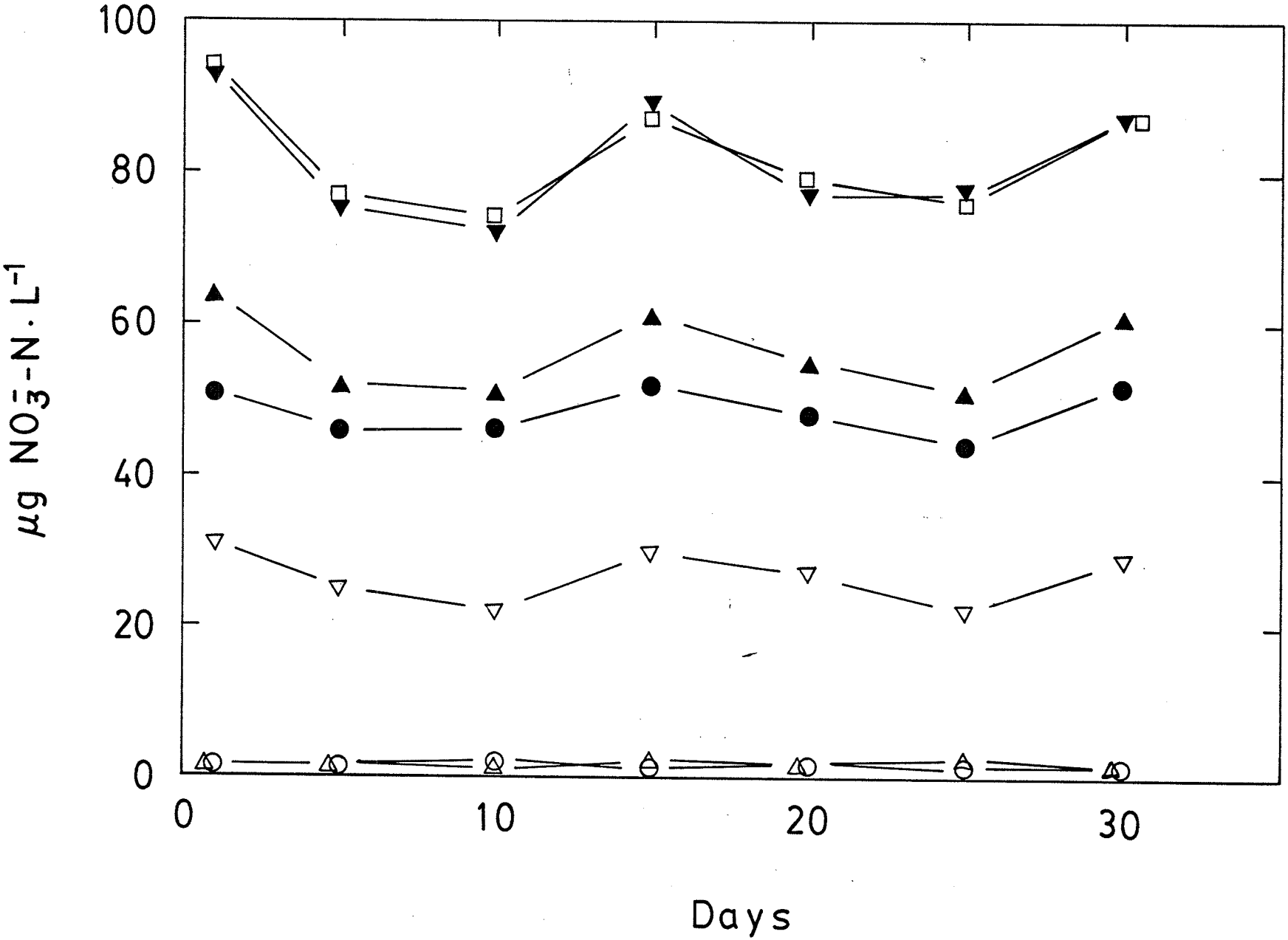

100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 62. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in August, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

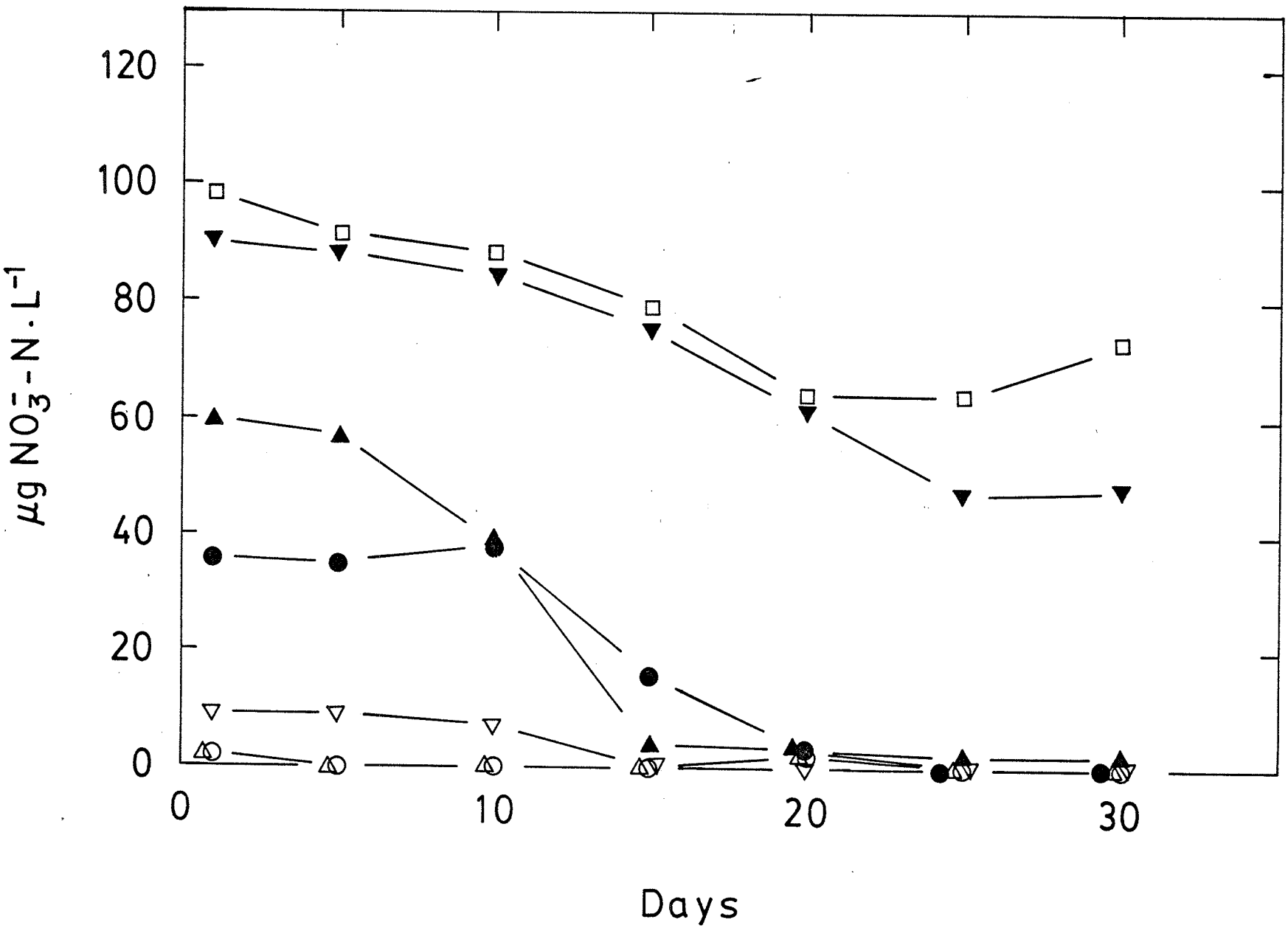

120
100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N.L-1

Fig. 63. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in August, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

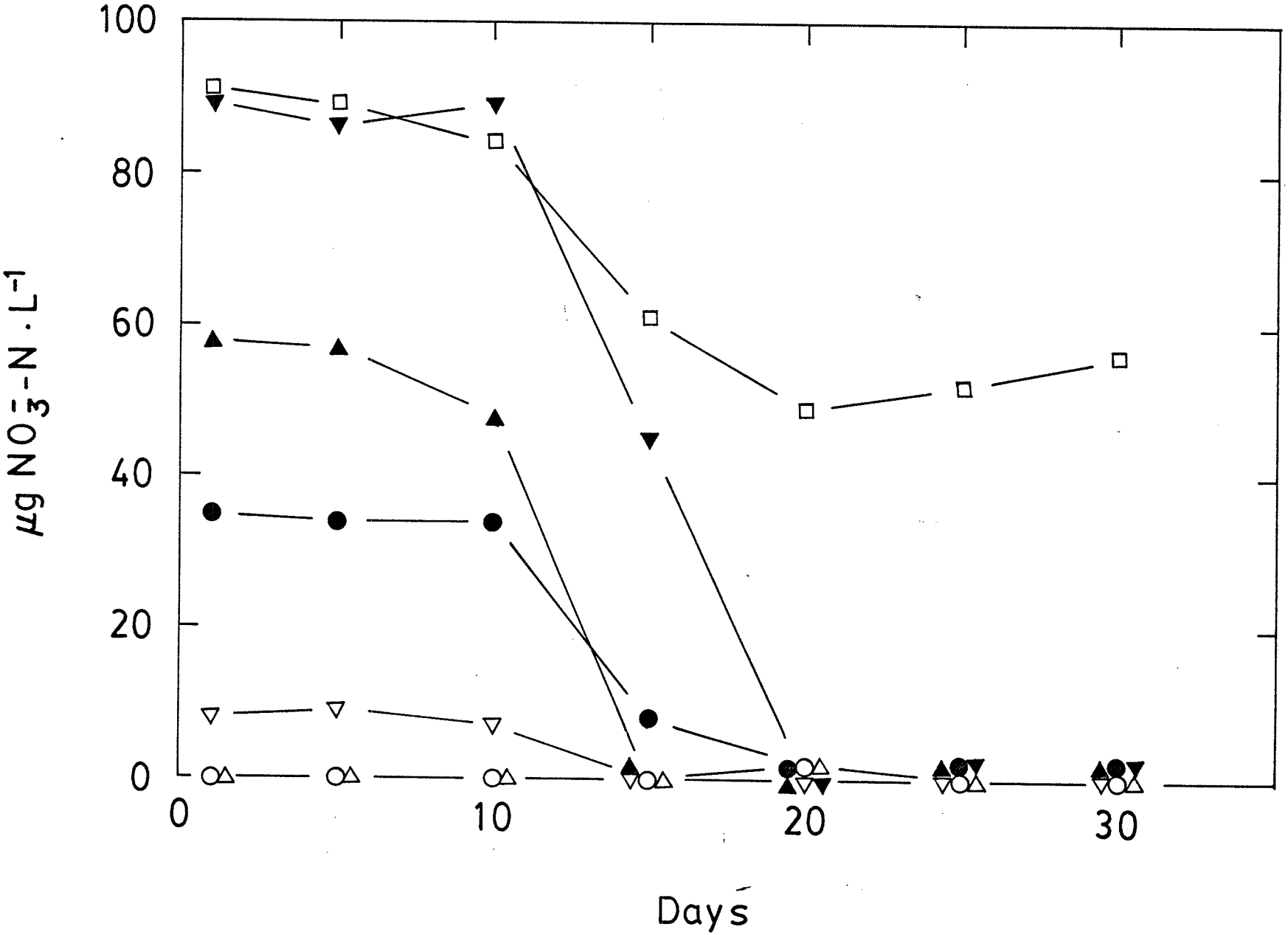

100
80
60
40
20
0
0
10
20
30
µg NO3-N · L-1
Days

Fig. 64. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in September, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

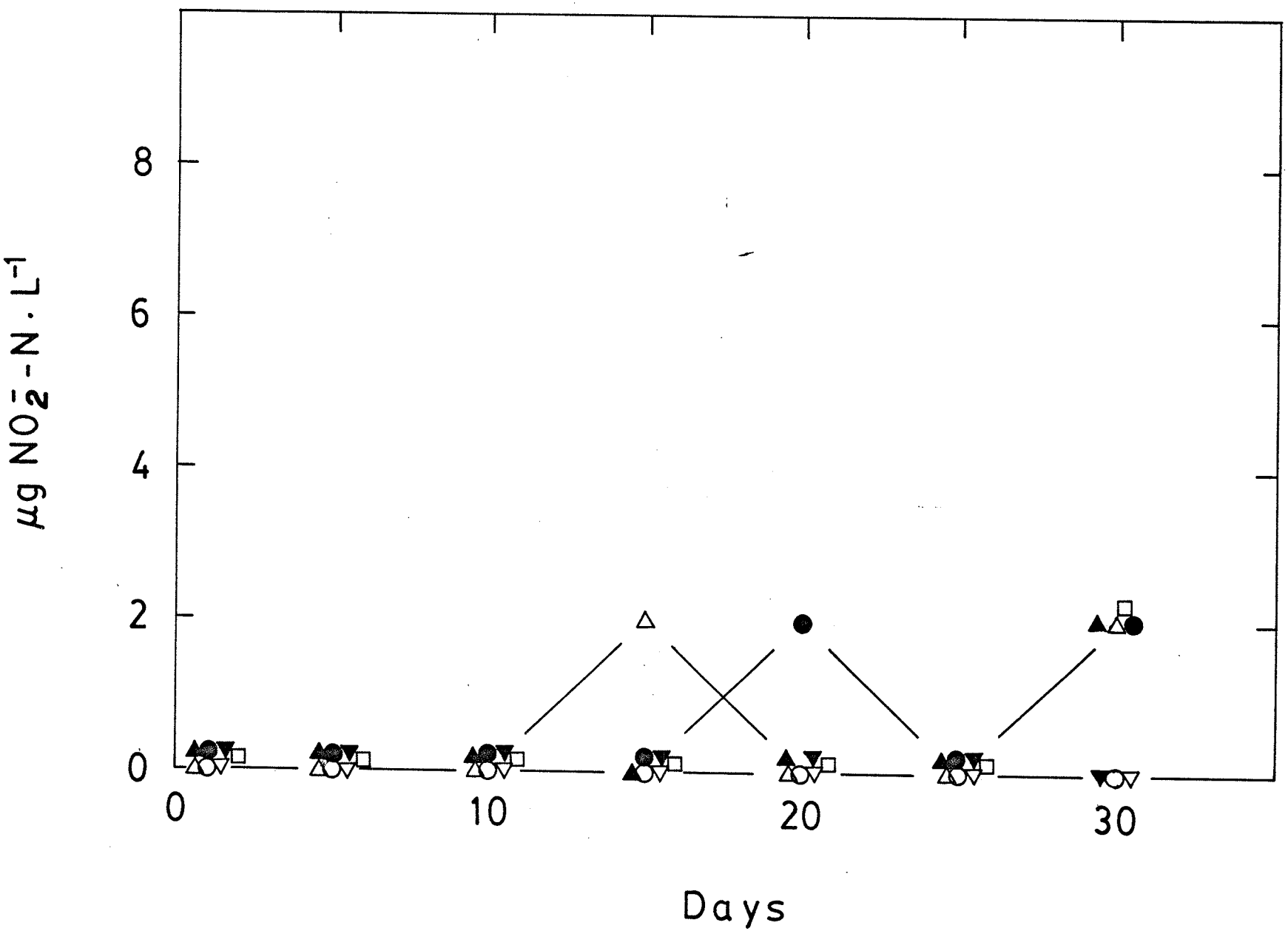

8
6
4
2
0
0
10
20
30
Days
µg NO2-N · L-1

Fig. 65. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in September, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0 \cdot 10$).

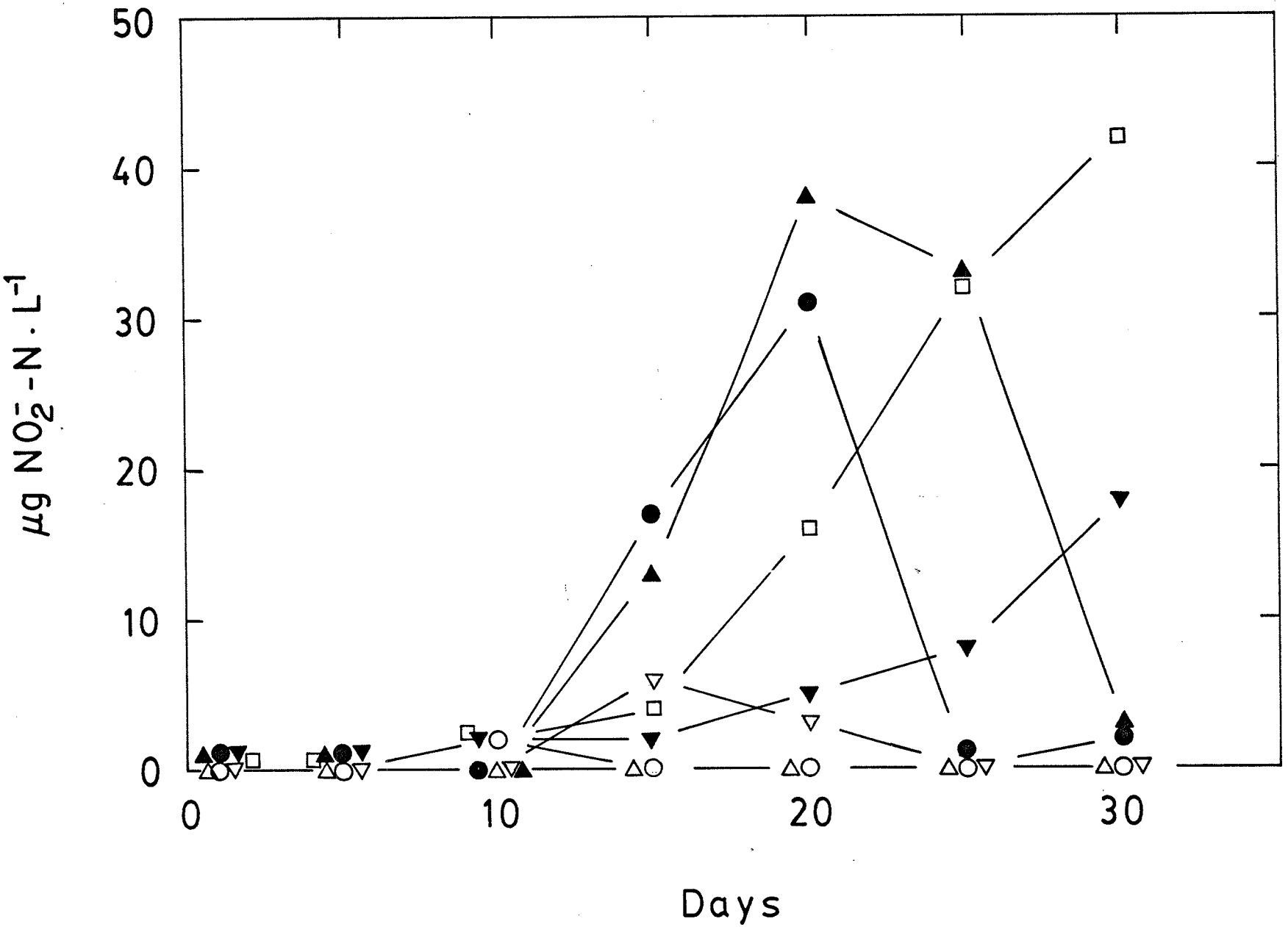

50
40
30
20
10
0
0
10
20
30
µg NO2-N · L-1
Days

Fig. 66. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in September, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

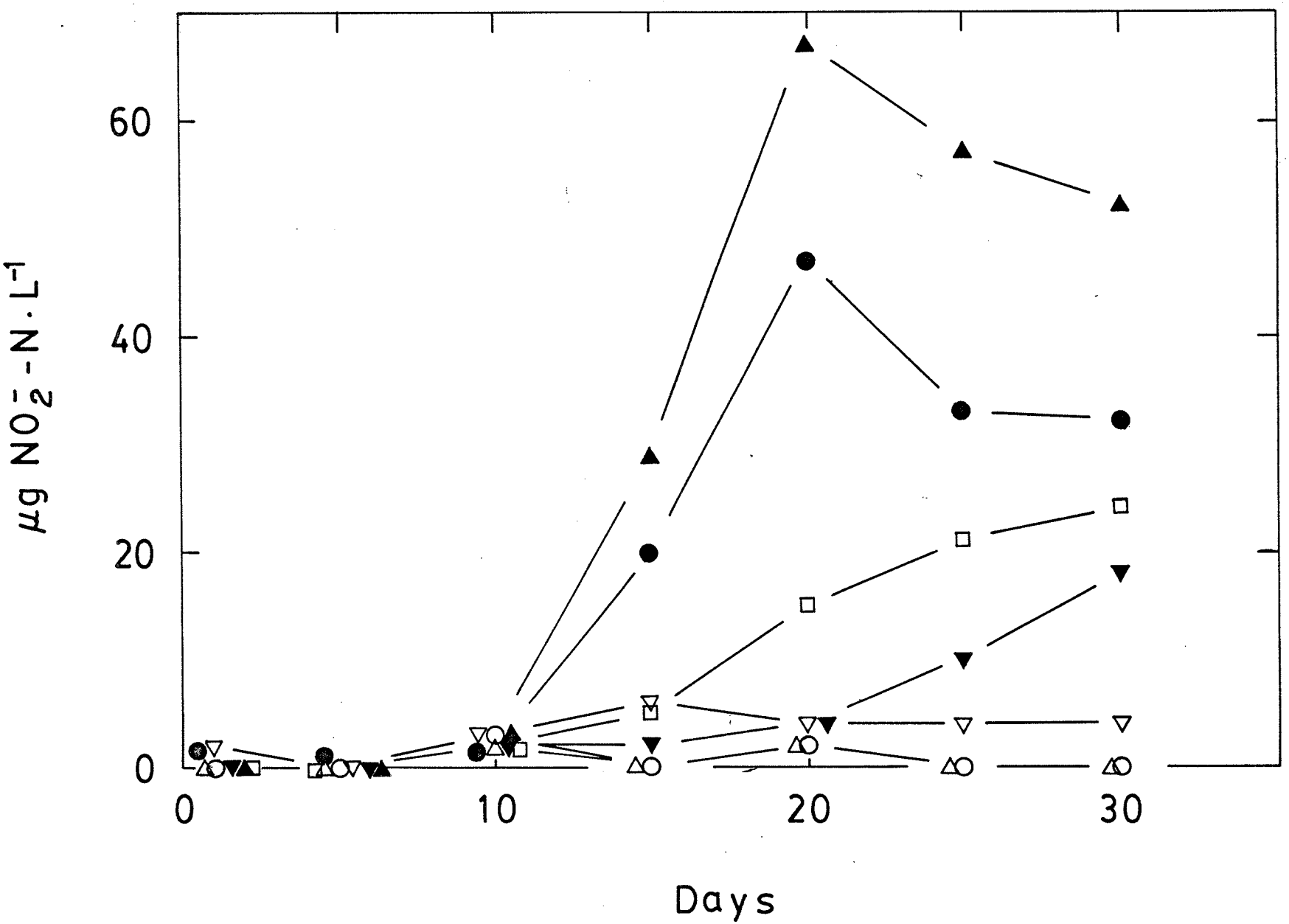

60
40
20
0
0
10
20
30
µg NO2-N.L-1
Days

Fig. 67. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in September, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

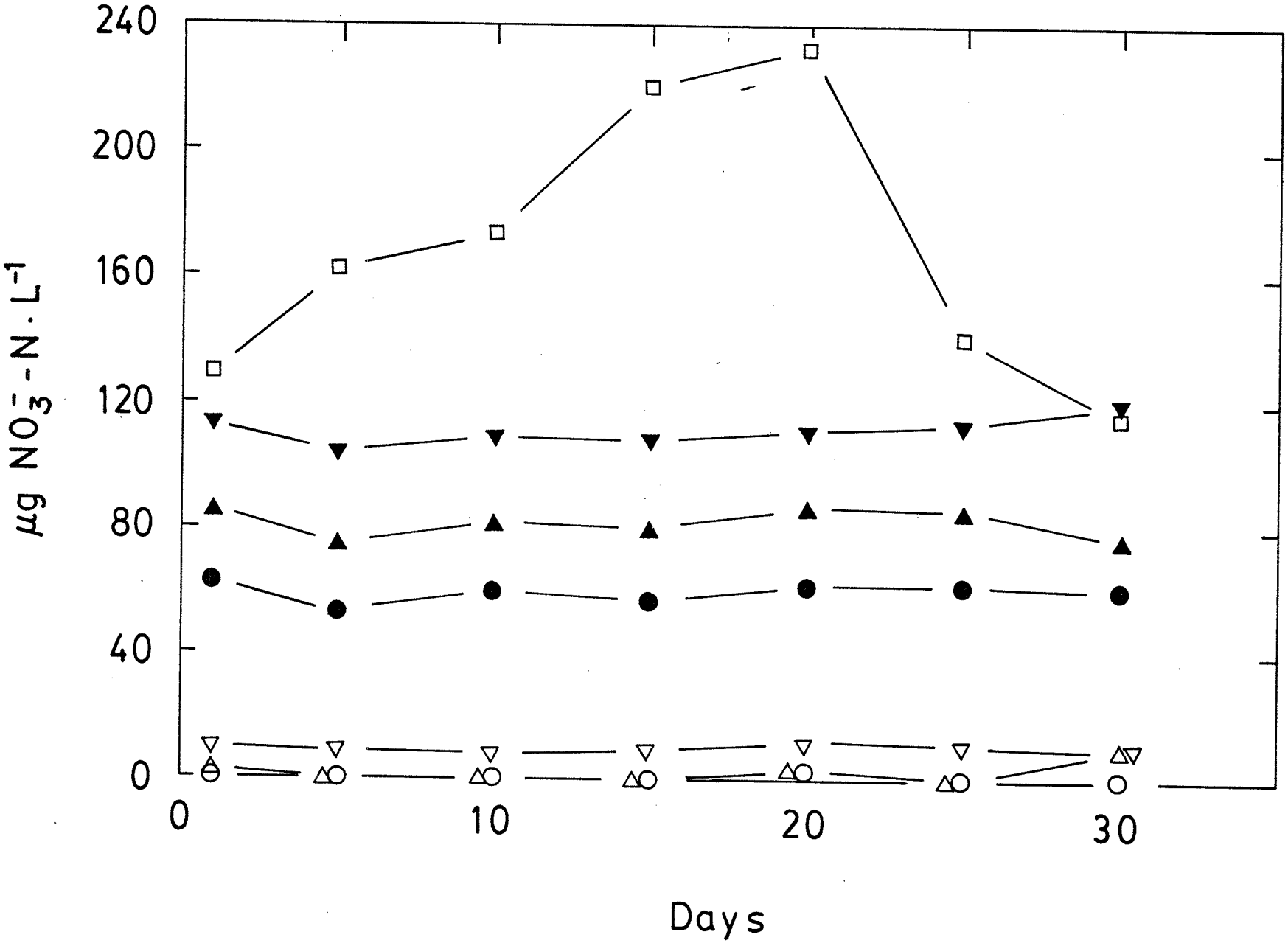

240
200
160
120
80
40
0
0
10
20
30
Days
µg NO3- -N . L-1

Fig. 68. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in September, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

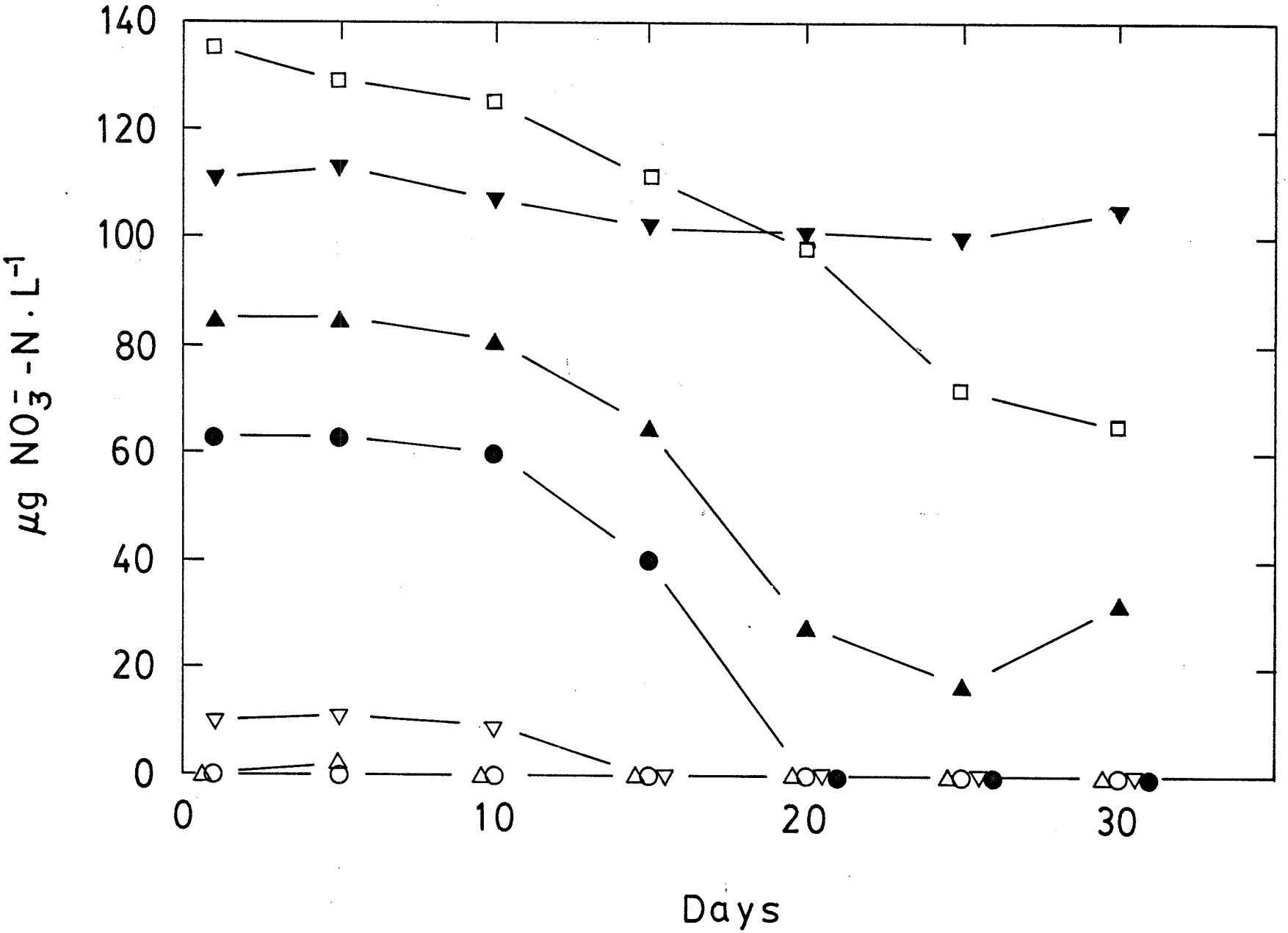

140
120
100
80
60
40
20
0
0
10
20
30
Days
µg NO3- -N . L-1

Fig. 69. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in September, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

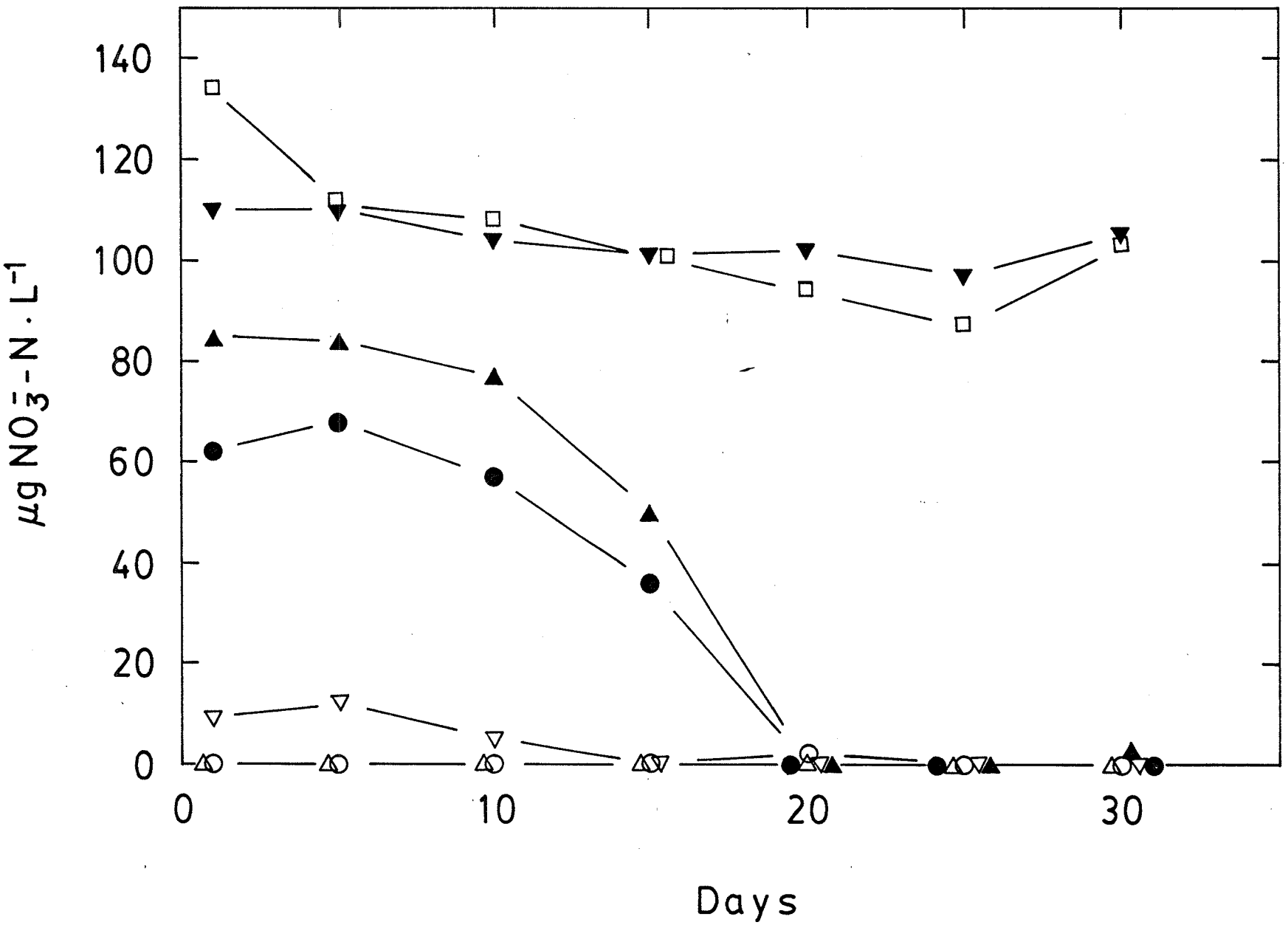

140
120
100
80
60
40
20
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 70. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in October, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

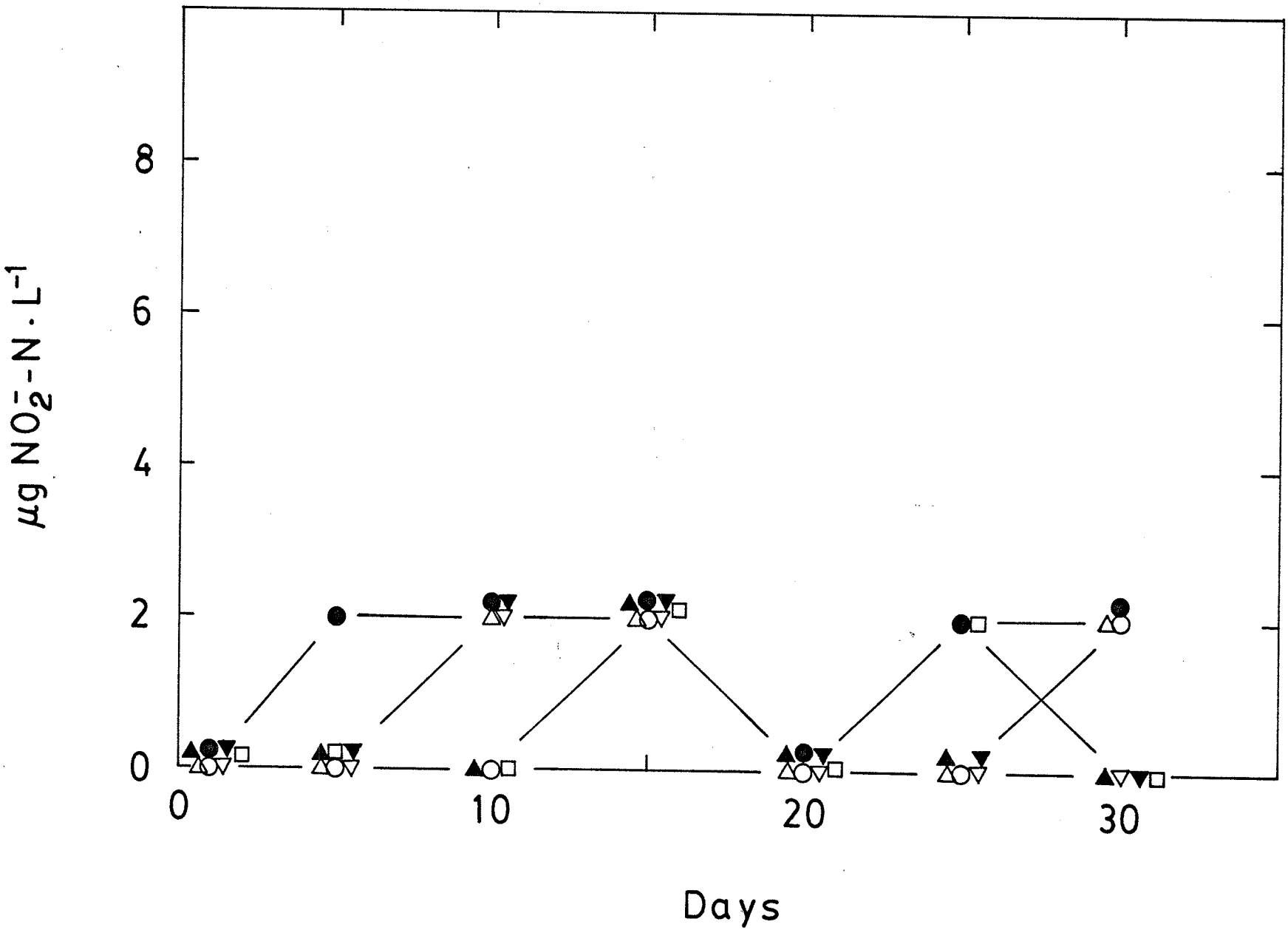

8
6
4
2
0
0
10
20
30
Days
µg NO2-N · L-1

Fig. 71. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in October, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

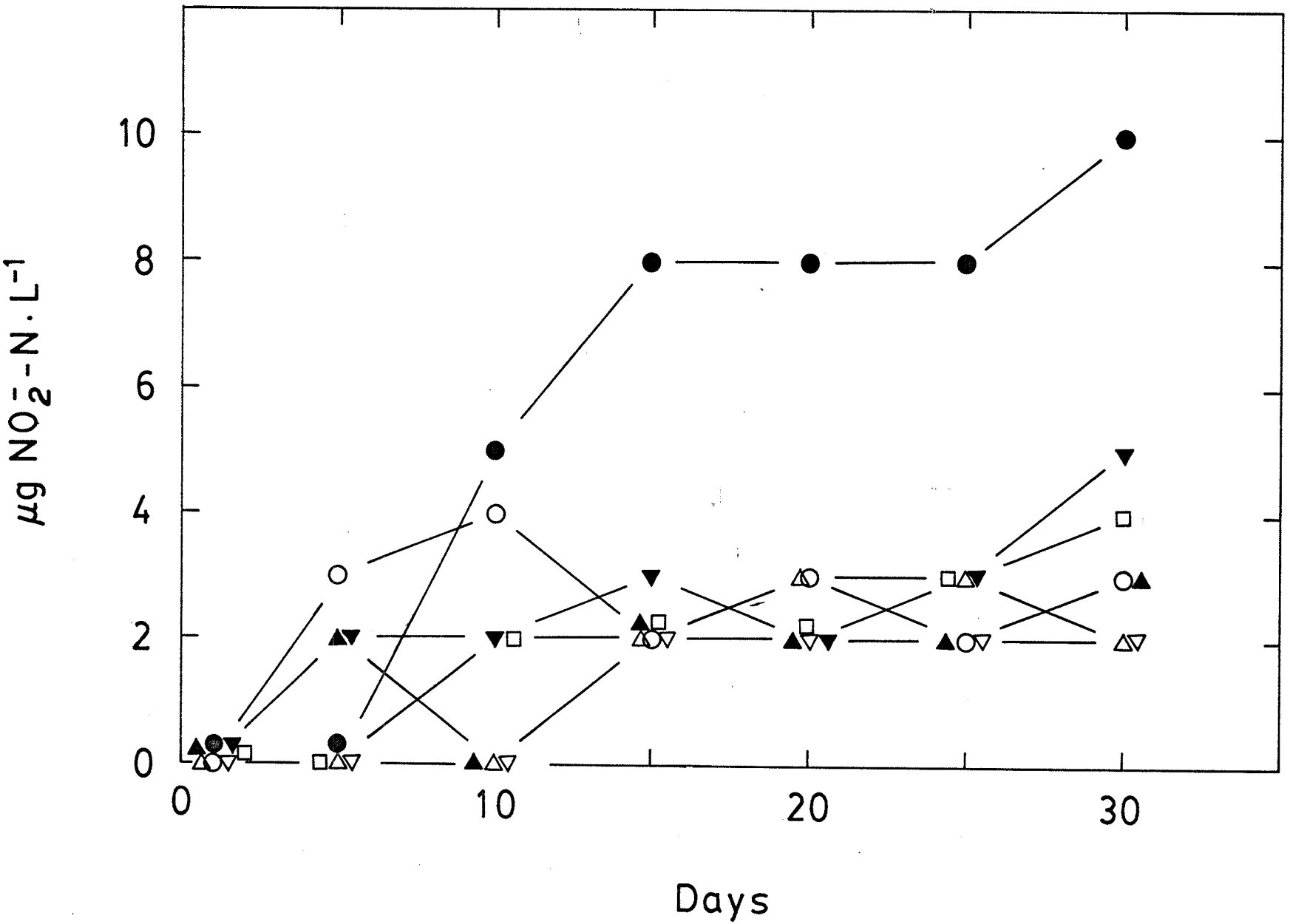

10
8
6
4
2
0
0
10
20
30
µg NO2-N.L-1
Days

Fig. 72. The production and disappearance of NO_2^- at various water depths of L.239. Samples were obtained in October, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

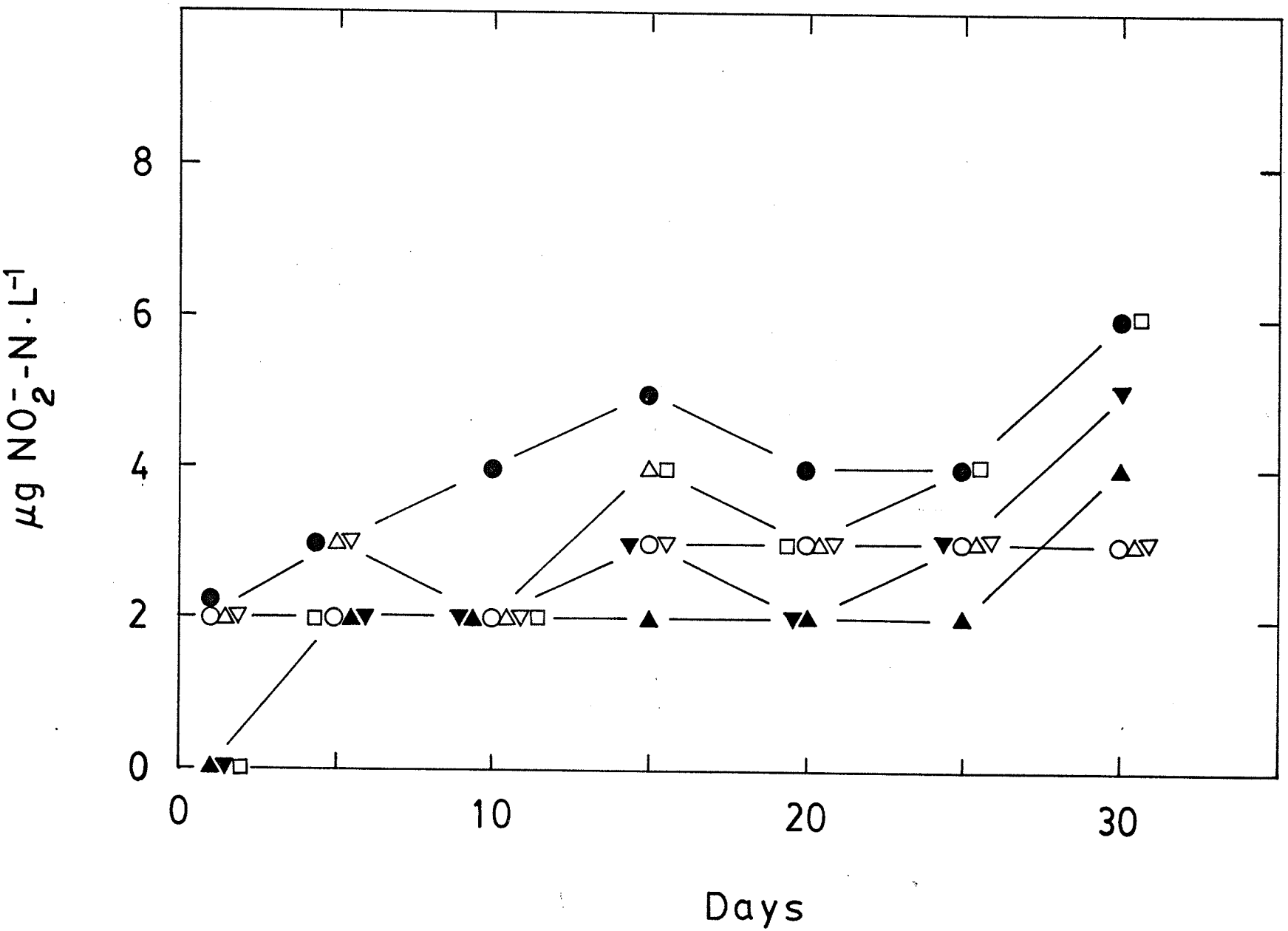

8
6
4
2
0
0
10
20
30
Days
µg NO2-N.L-1

Fig. 73. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in October, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

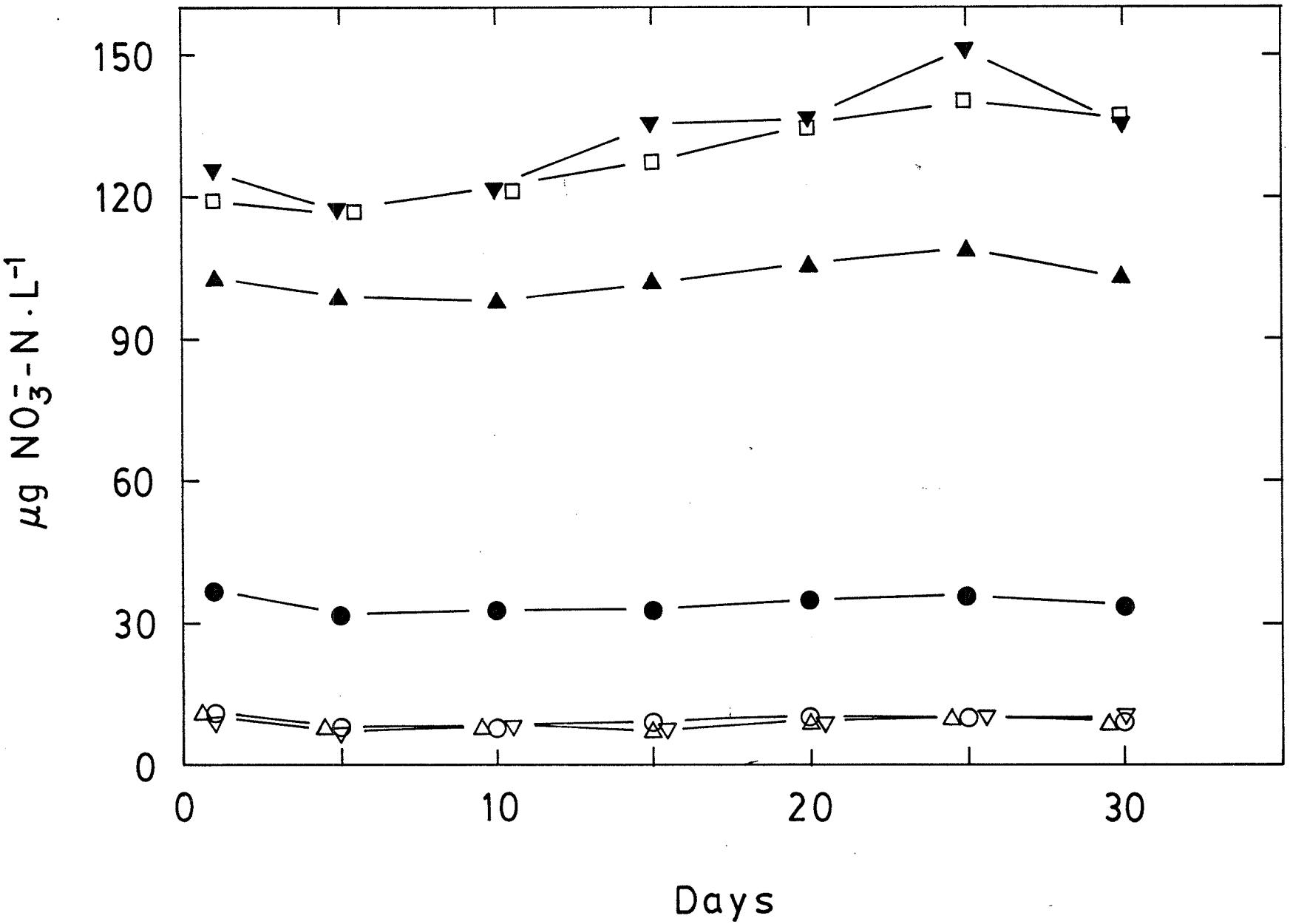

150
120
90
60
30
0
0
10
20
30
Days
µg NO3-N . L-1

Fig. 74. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in October, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0 \cdot 10$).

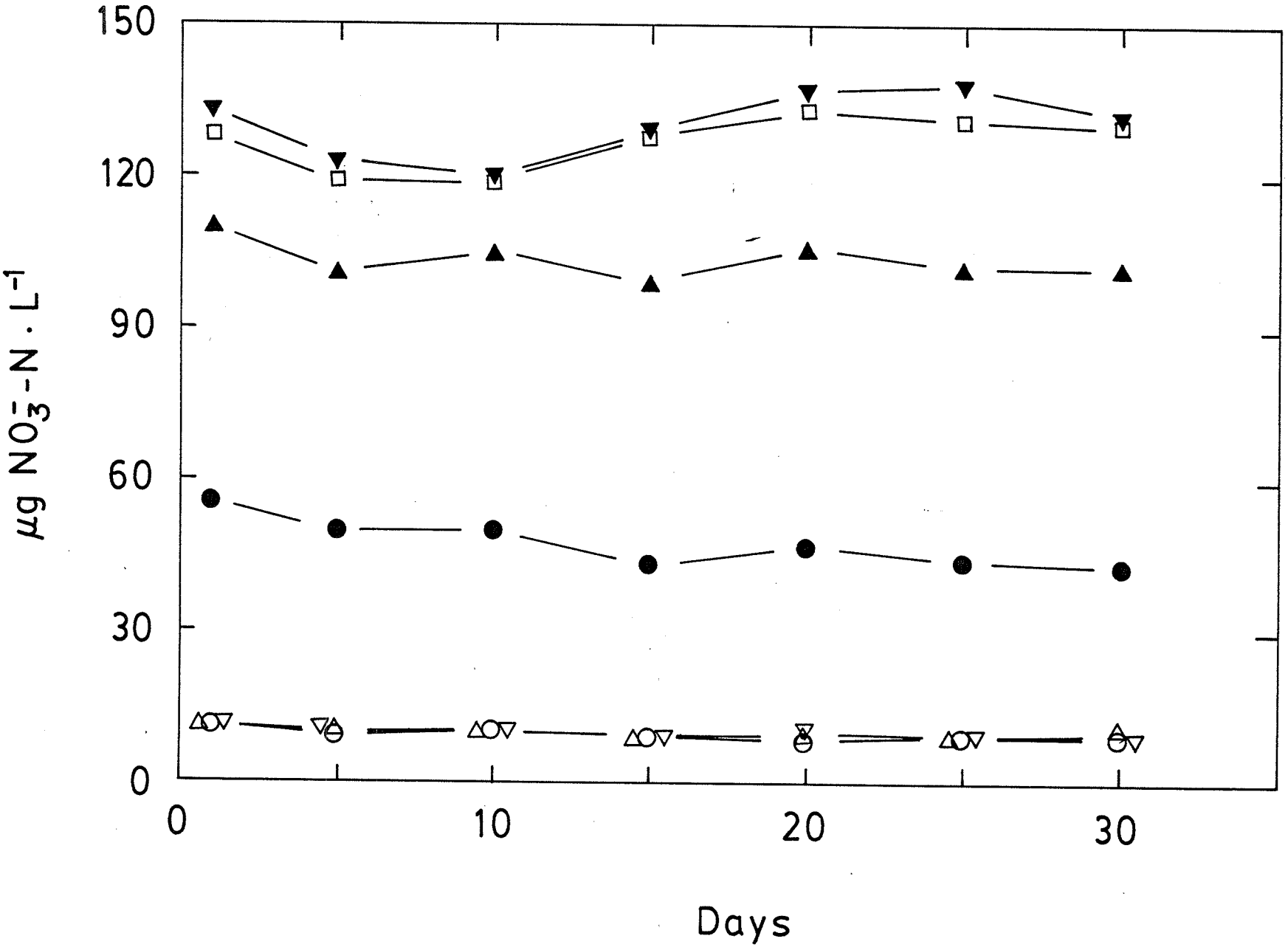

150
120
90
60
30
0
0
10
20
30
Days
µg NO3-N . L-1

Fig. 75. The production and disappearance of NO_3^- at various water depths of L.239. Samples were obtained in October, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 5m, (▽) 10m, (●) 15m, (▲) 20m, (▼) 25m and (□) 30m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

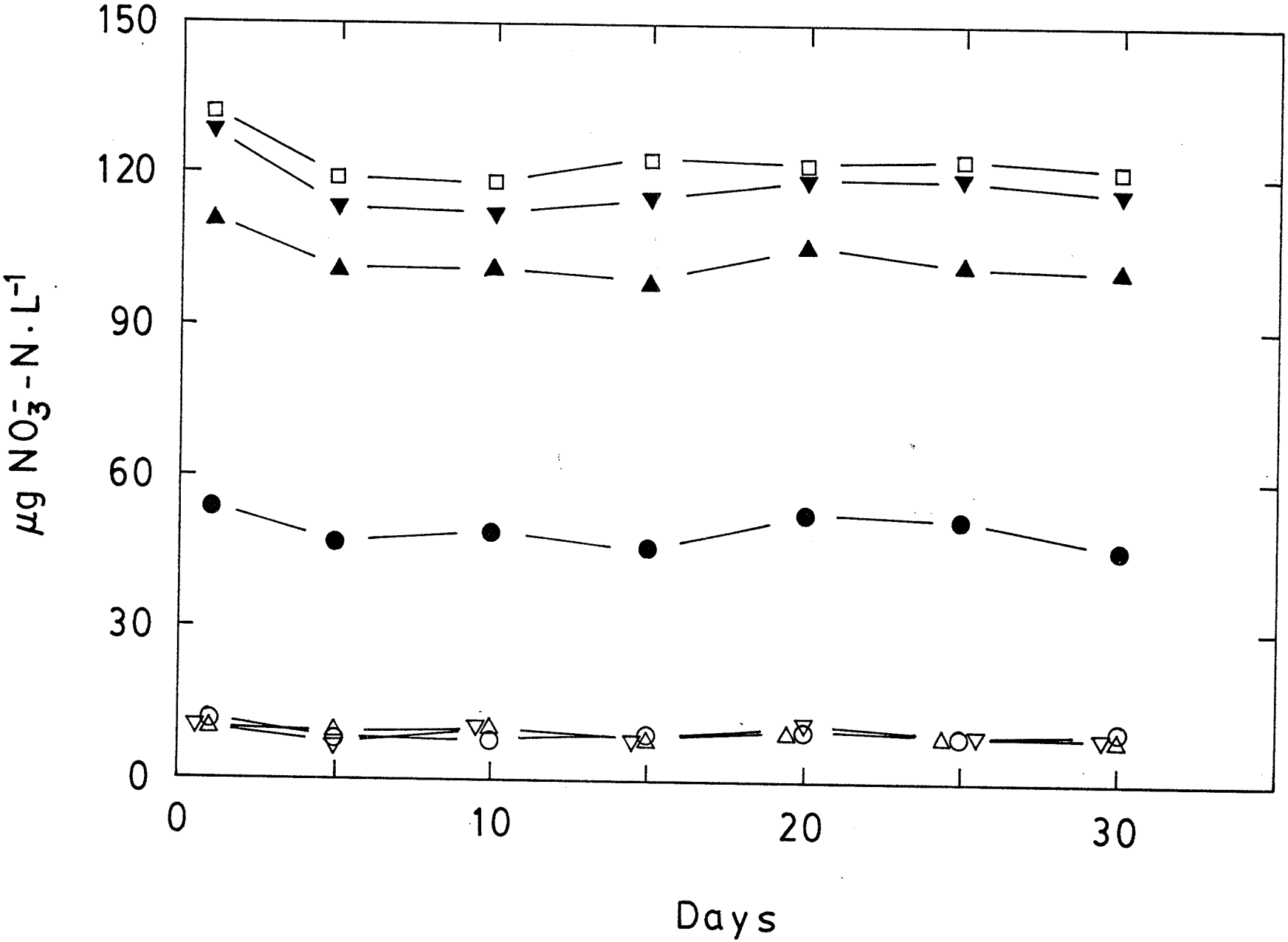

150
120
90
60
30
0
0
10
20
30
Days
µg NO3- - N . L-1

Fig. 76. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in December, 1983 and incubated at 15°C without any supplements. Symbols are: (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

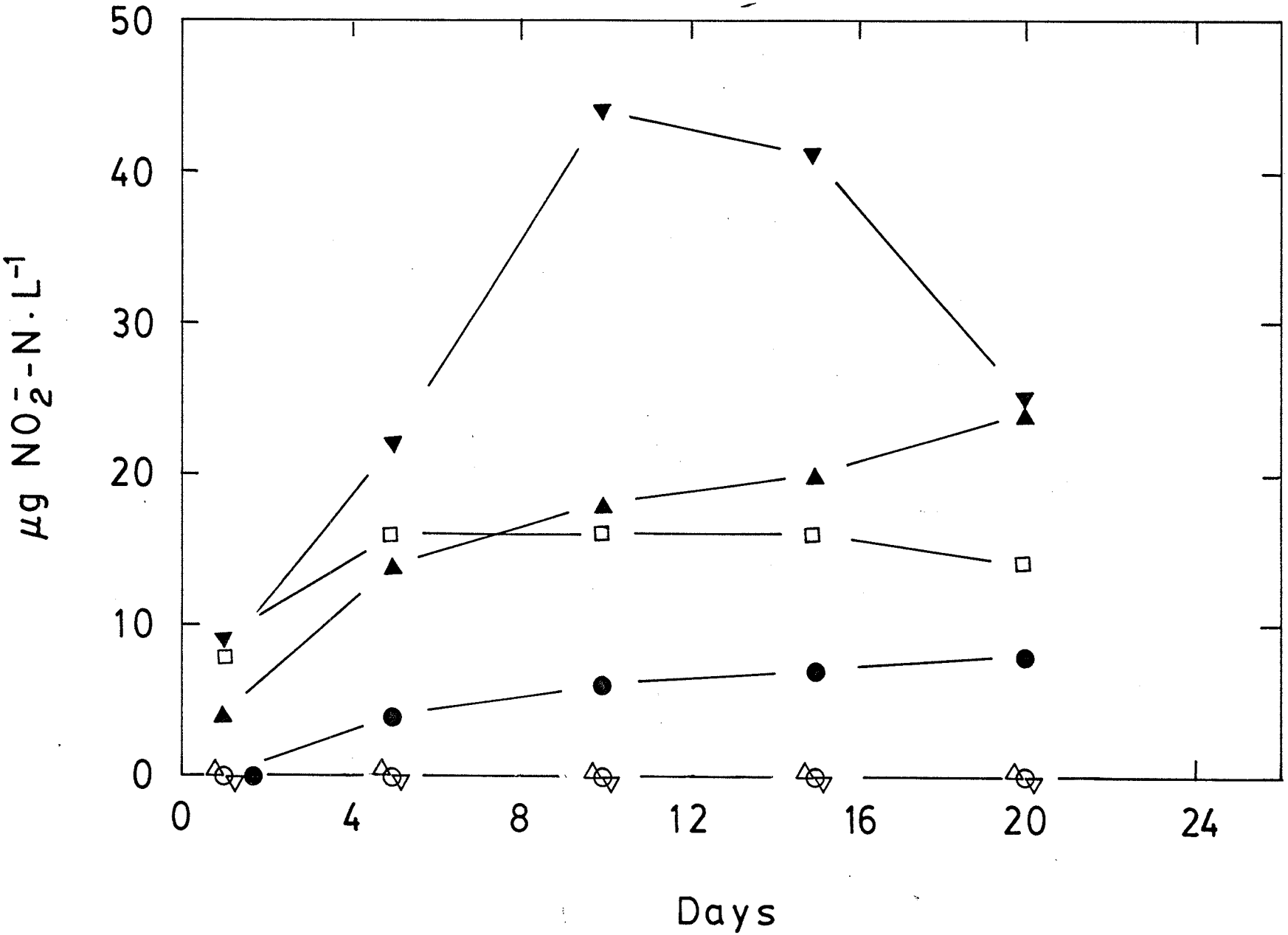

50
40
30
20
10
0
0
4
8
12
16
20
24
µg NO2-N.L-1
Days

Fig. 77. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in December, 1983 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

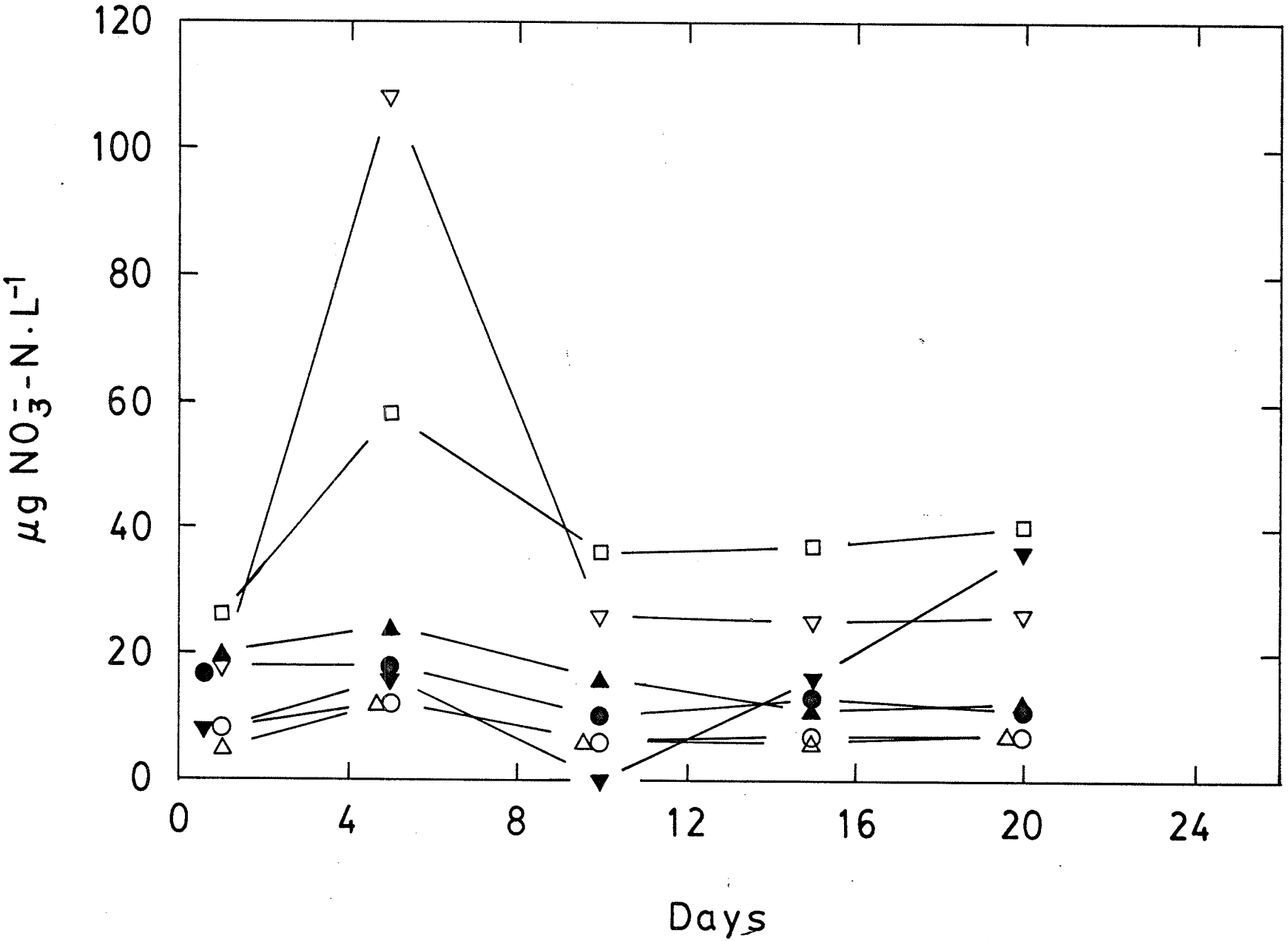

120
100
80
60
40
20
0
µg NO3-N.L-1
0
4
8
12
16
20
24
Days

Fig. 78. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in January, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

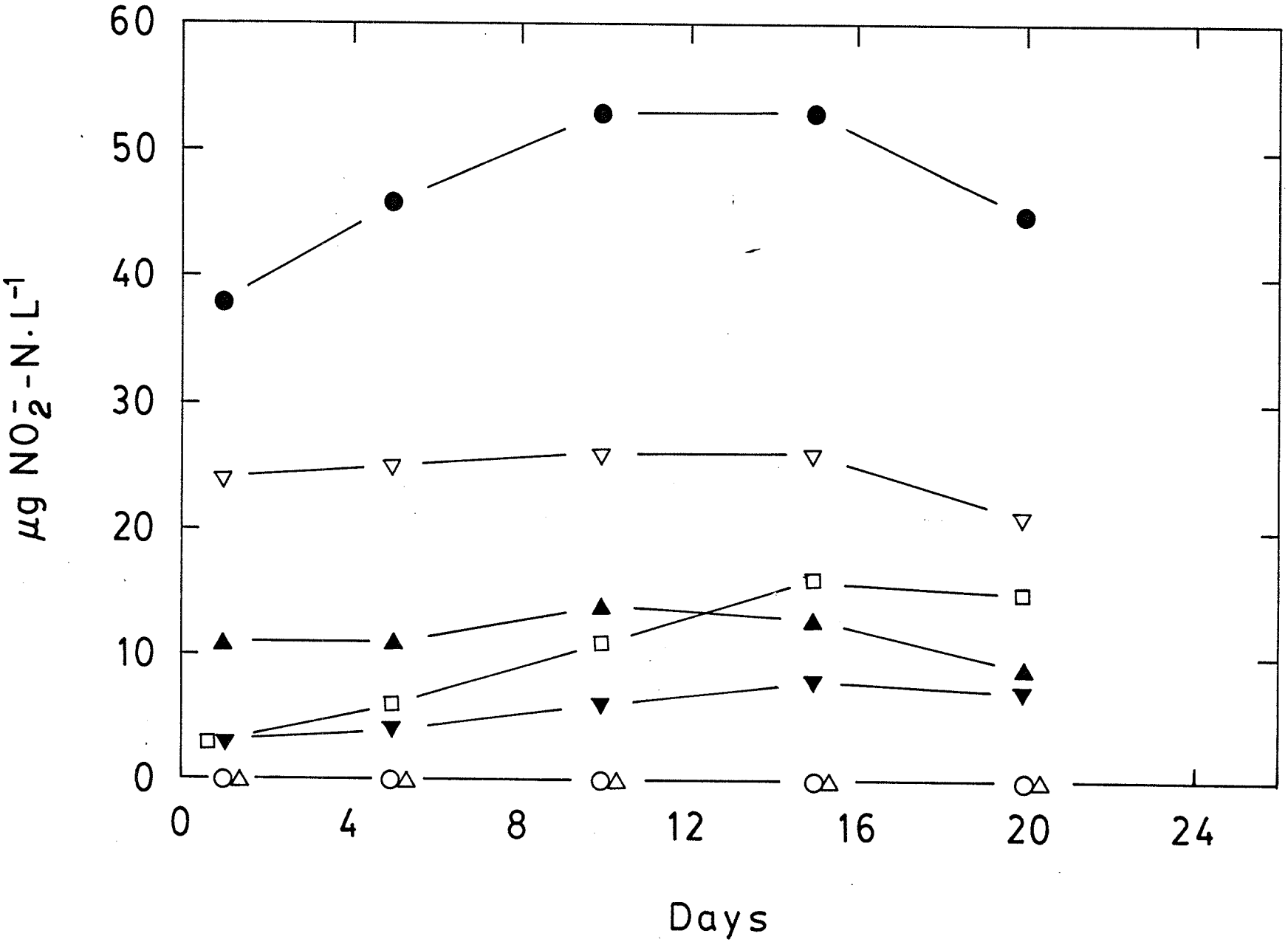

60
50
40
30
20
10
0
0
4
8
12
16
20
24
µg NO2-N.L-1
Days

Fig. 79. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in January, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

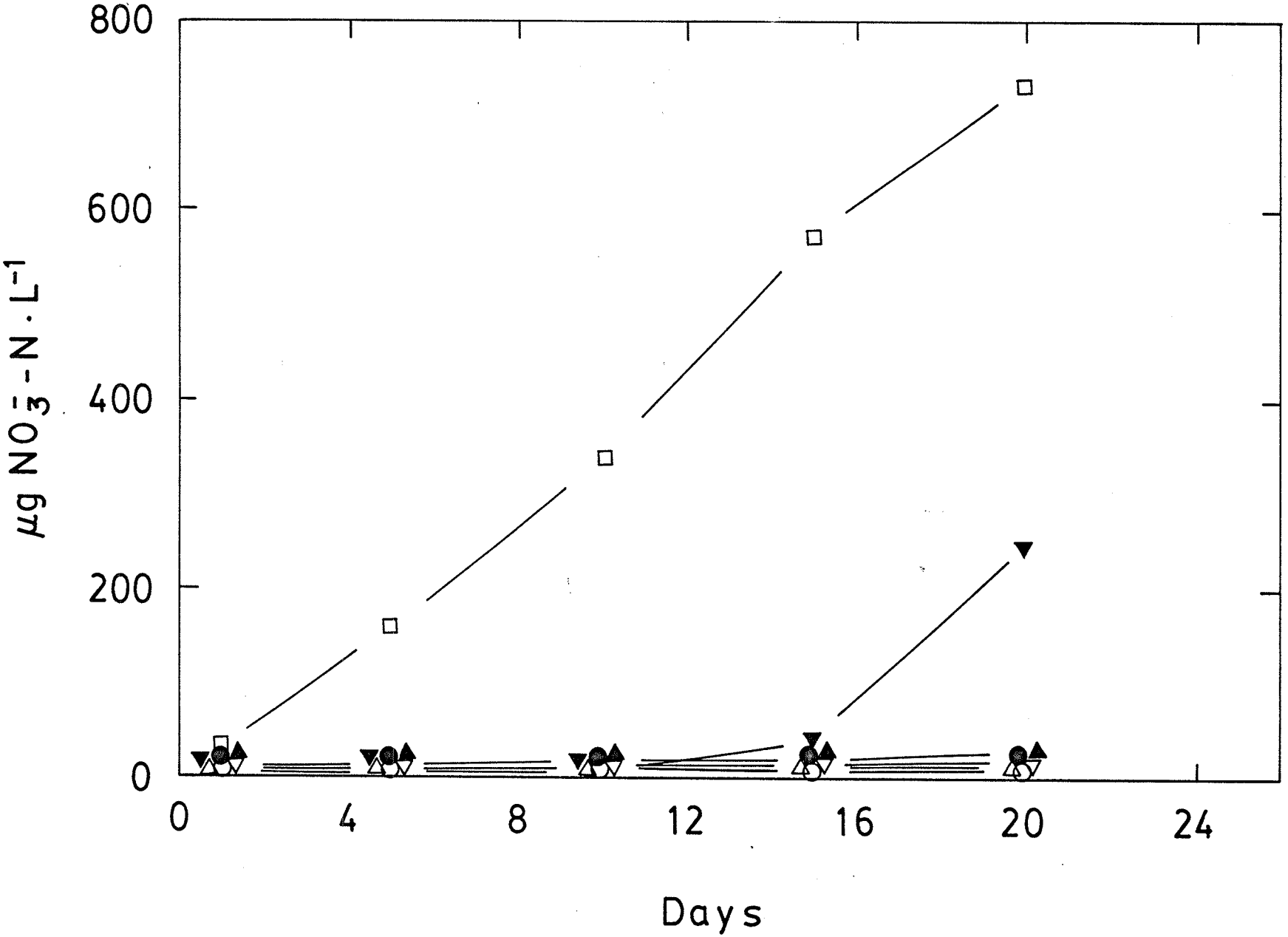

800
600
400
200
0
0
4
8
12
16
20
24
Days
µg NO3- - N . L-1

Fig. 80. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in February, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

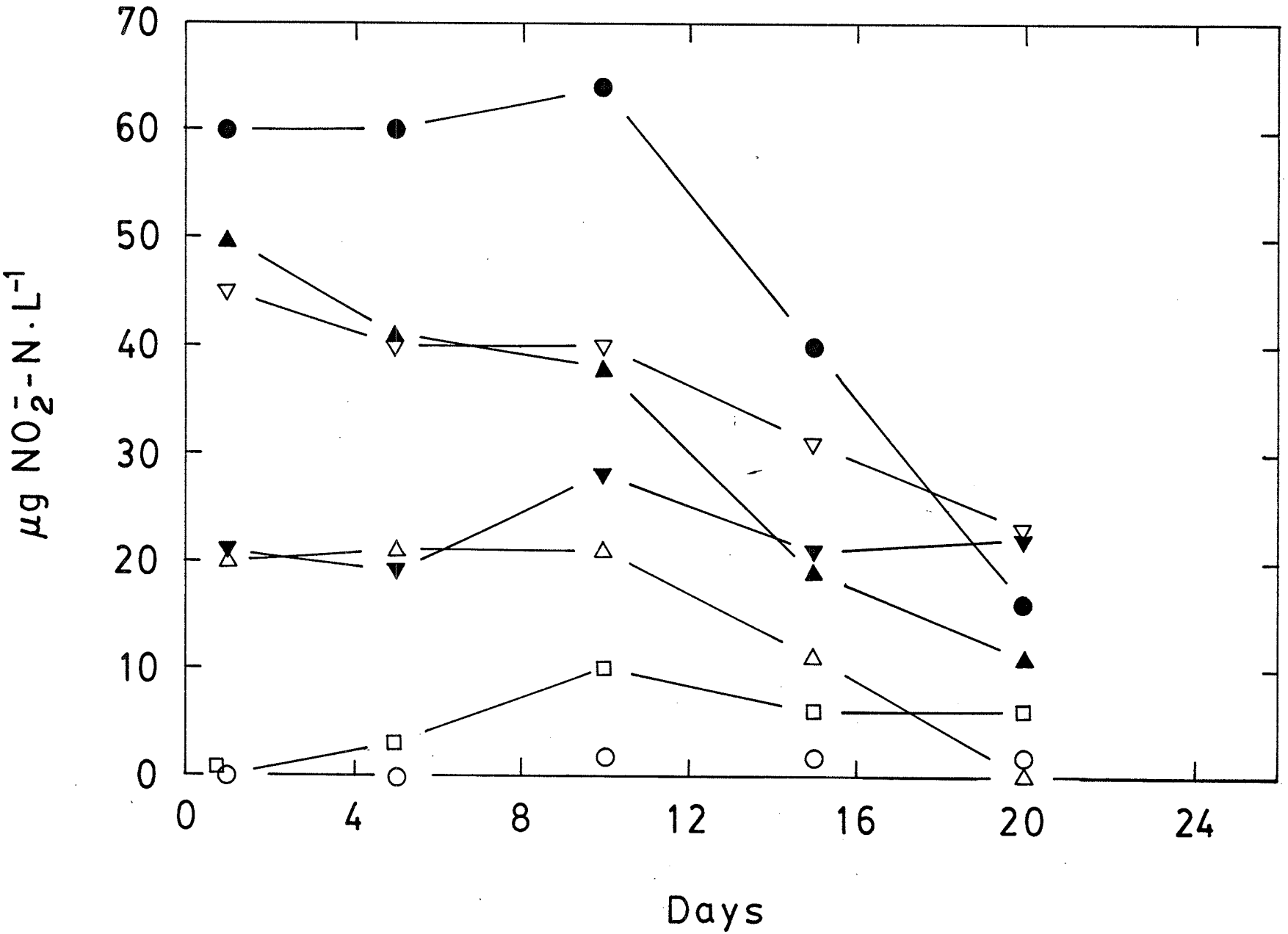

70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
µg NO2-N.L-1
Days

Fig. 81. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in February, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

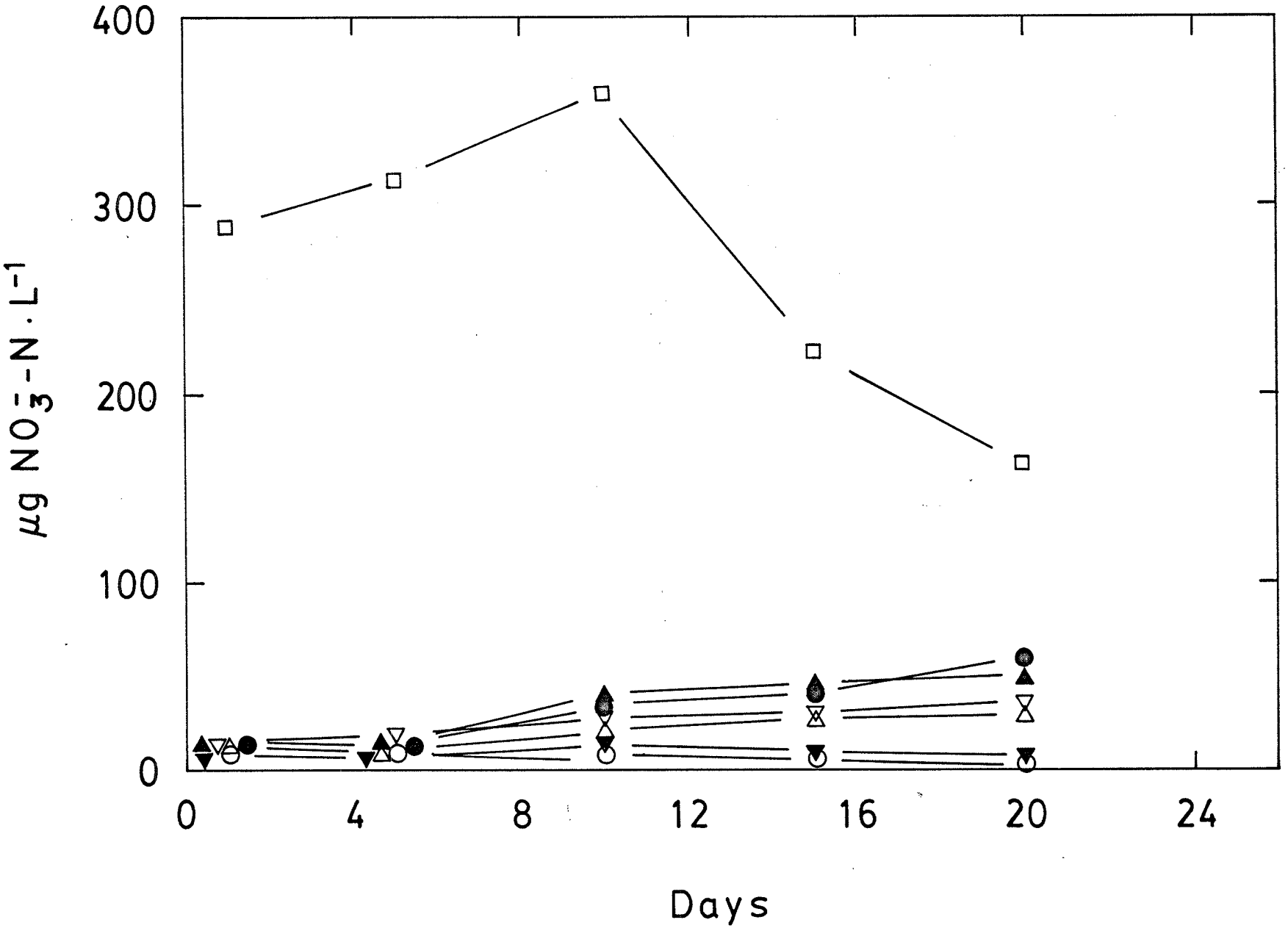

400
300
200
100
0
µg NO3-N . L-1
0
4
8
12
16
20
24
Days

Fig. 82. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in March, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

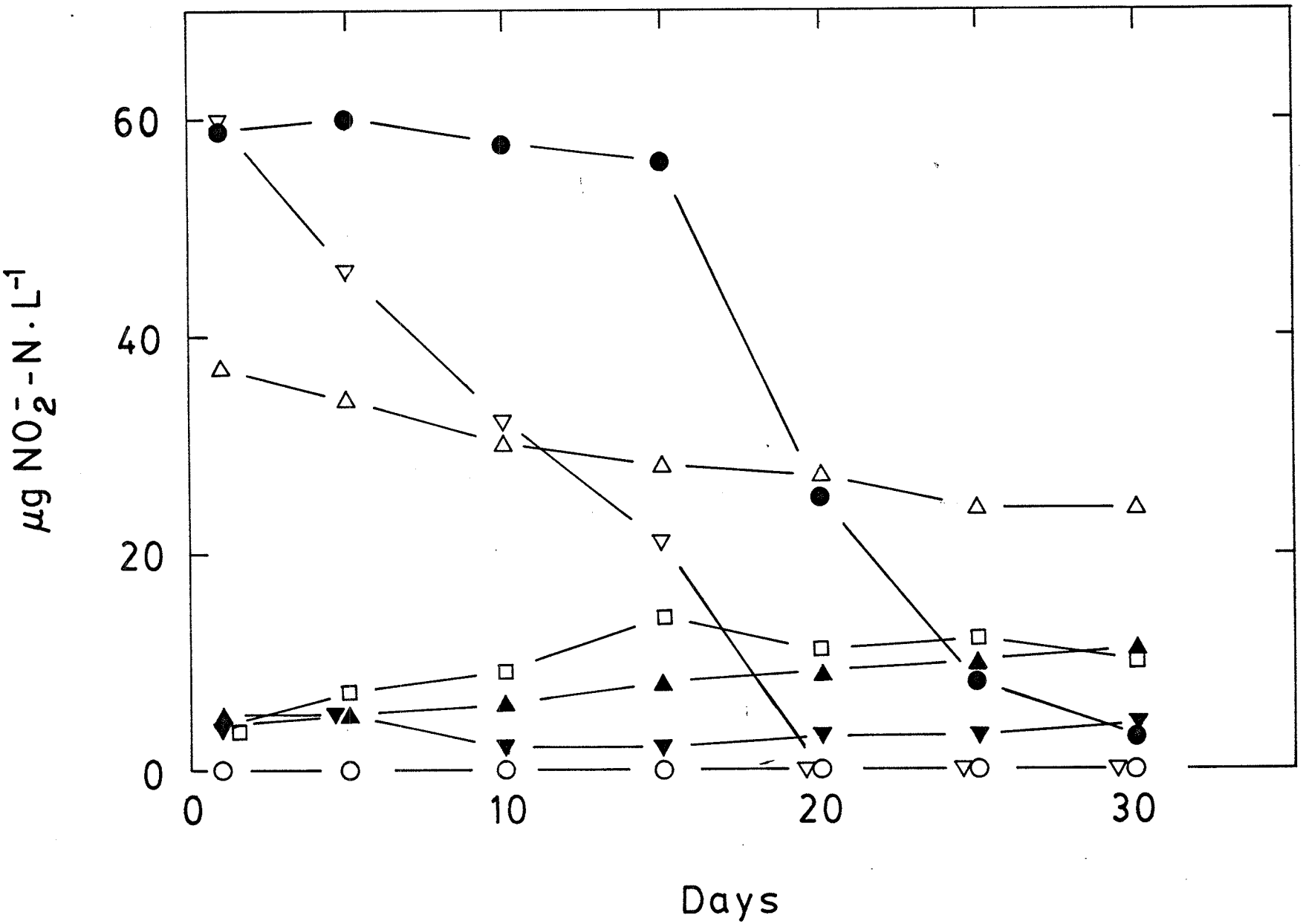

60
40
20
0
0
10
20
30
µg NO2-N.L-1
Days

Fig. 83. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in March, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

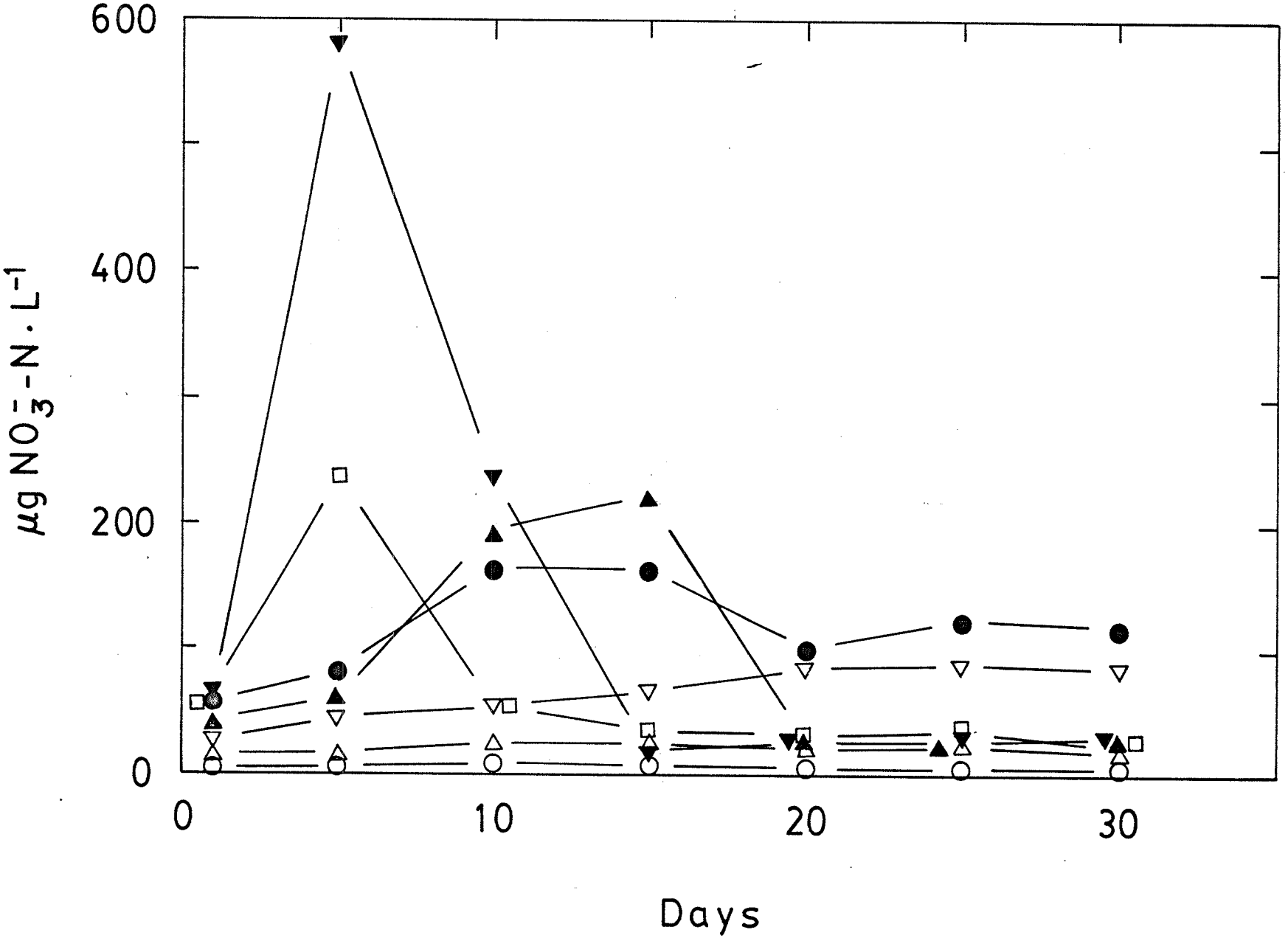

600
400
200
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 84. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in April, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

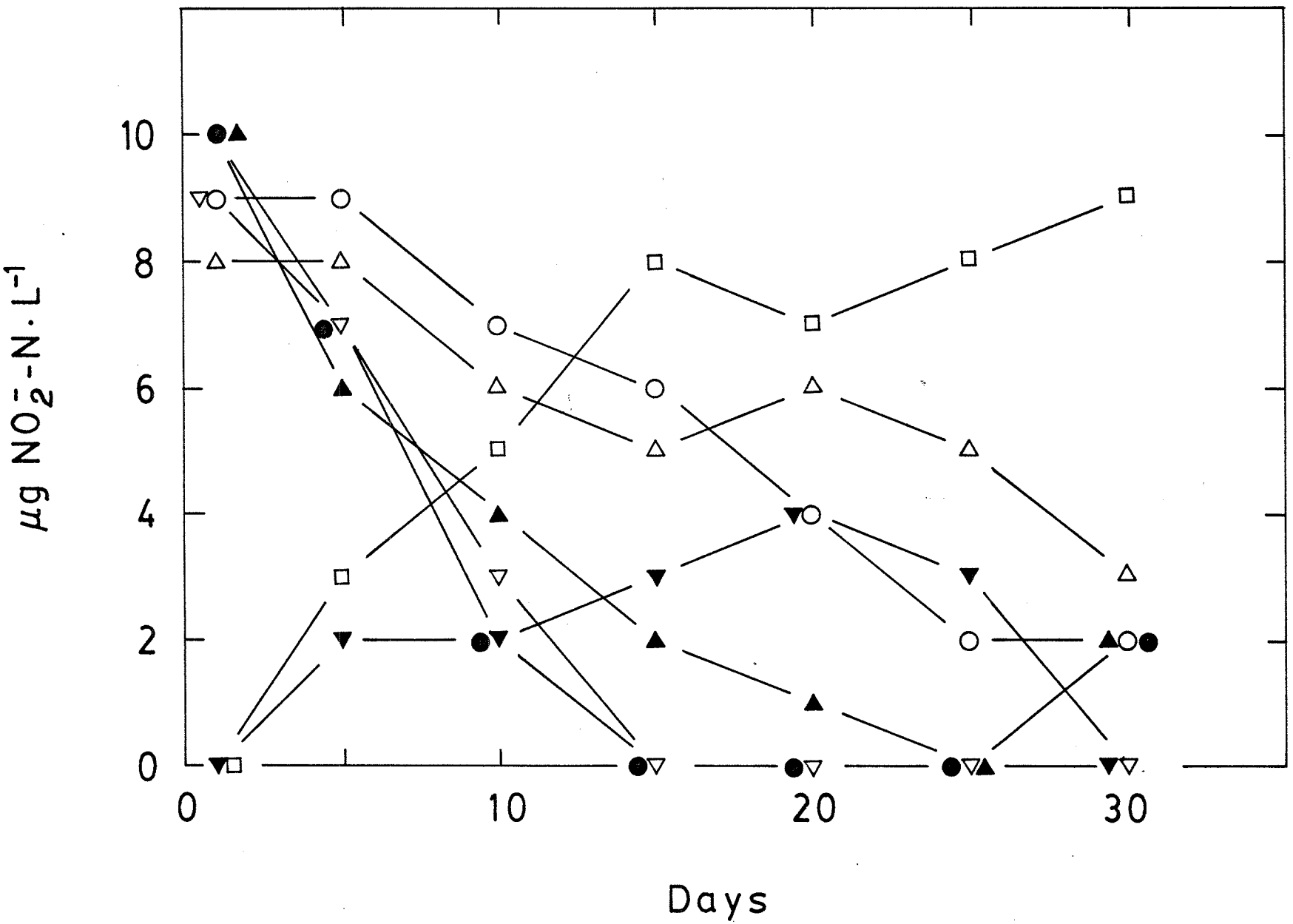

10
8
6
4
2
0
0
10
20
30
Days
µg NO2-N.L-1

Fig. 85. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in April, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (○) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

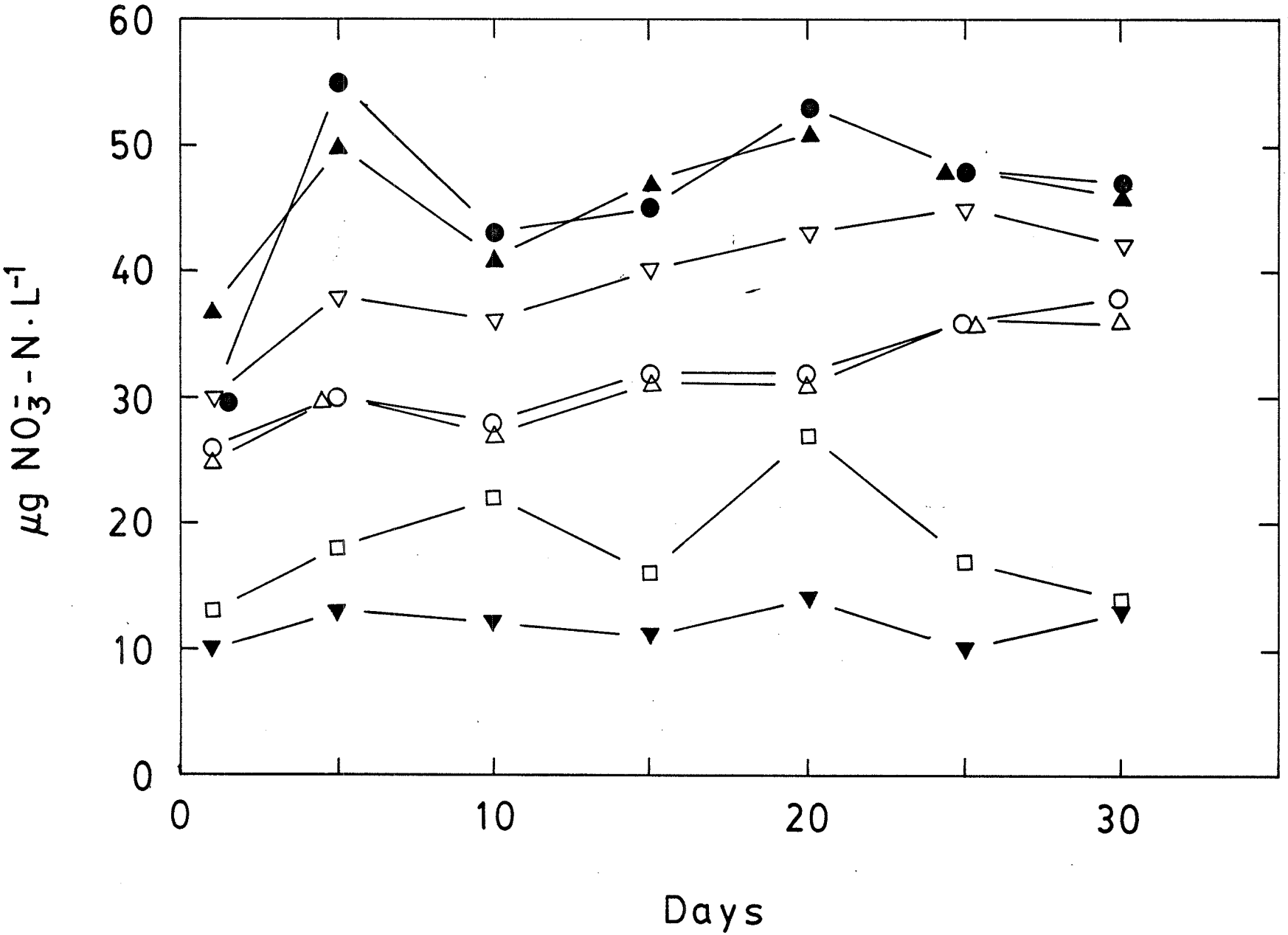

60
50
40
30
20
10
0
0
10
20
30
Days
µg NO3-N.L-1

Fig. 86. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in May, 1984 and incubated at 15°C without any supplements. Symbols are: (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

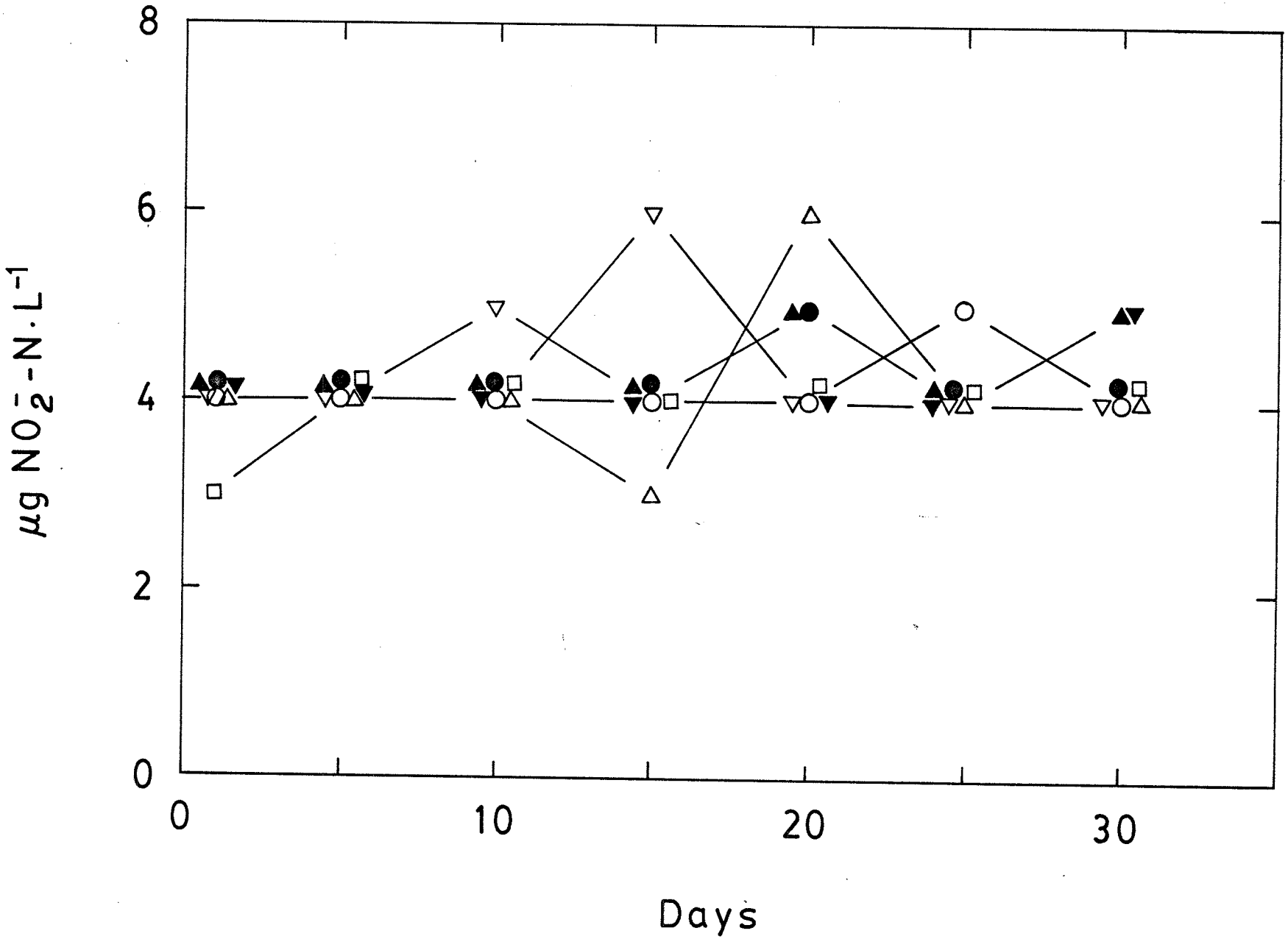

8
6
4
2
0
0
10
20
30
Days
μg NO2-N·L-1

Fig. 87. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in May, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

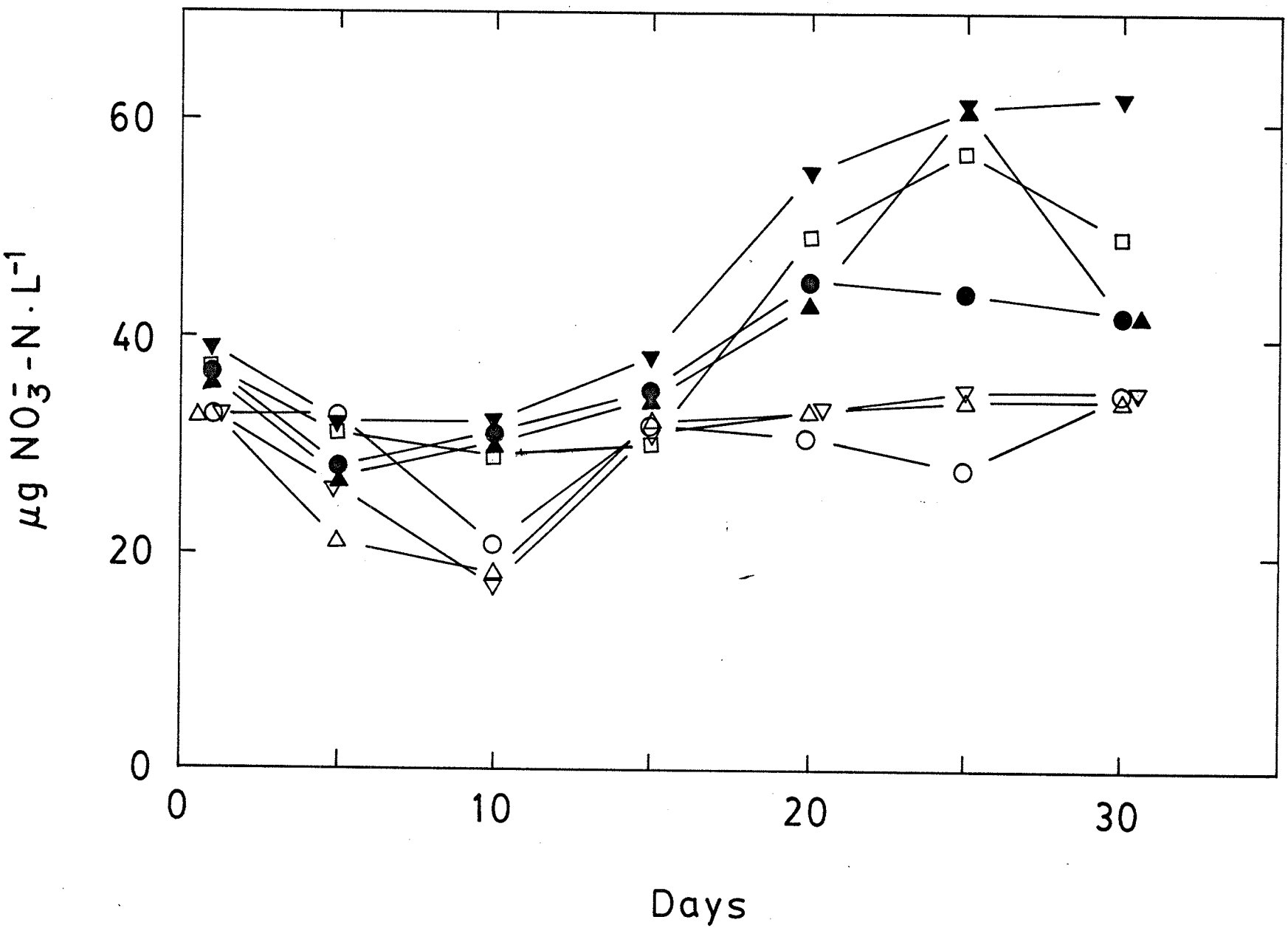

60
40
20
0
0
10
20
30
Days
µg NO3-N.L-1

Fig. 88. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in June, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0 \cdot 10$).

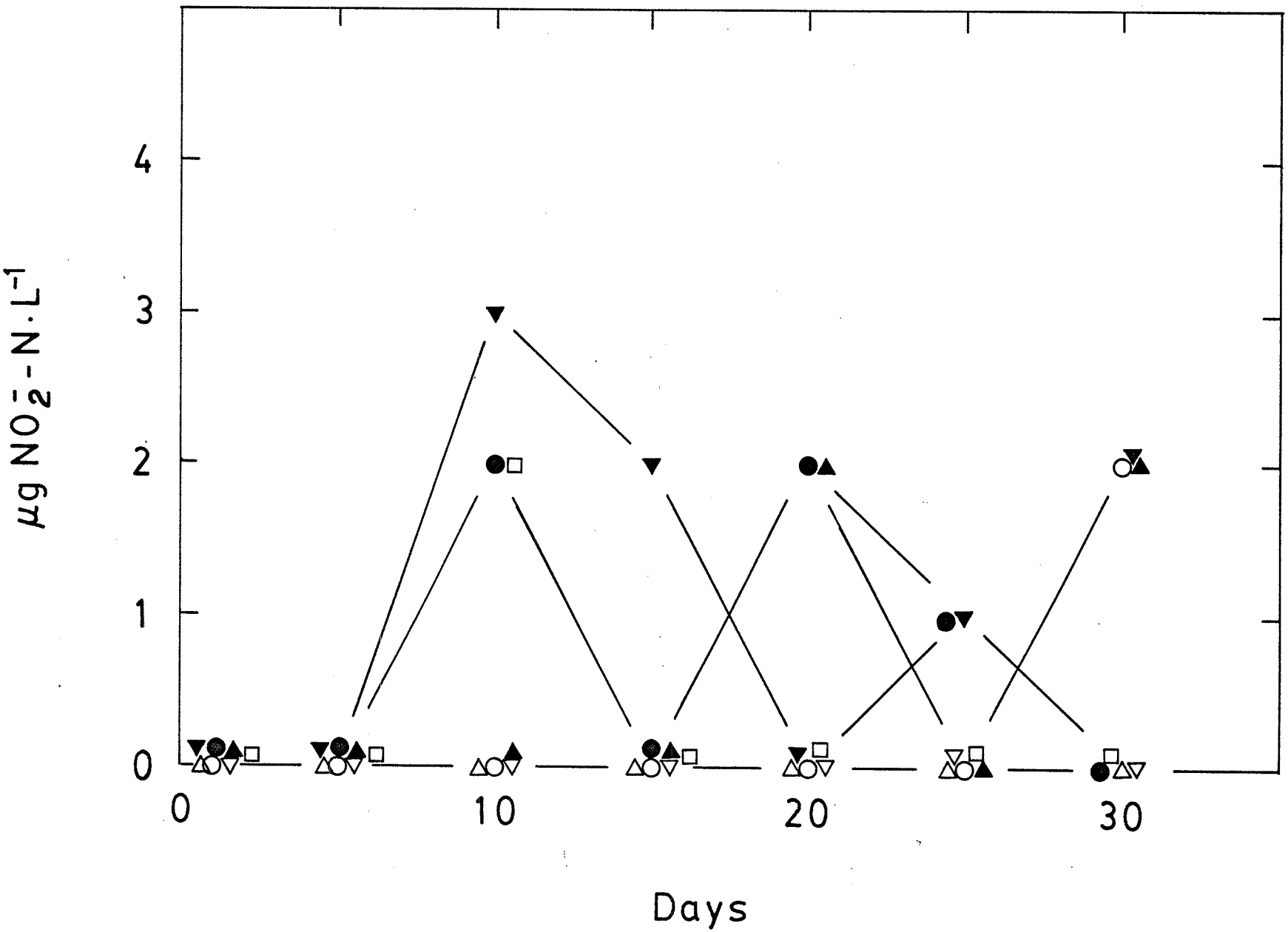

μg NO2- - N · L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 89. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in June, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

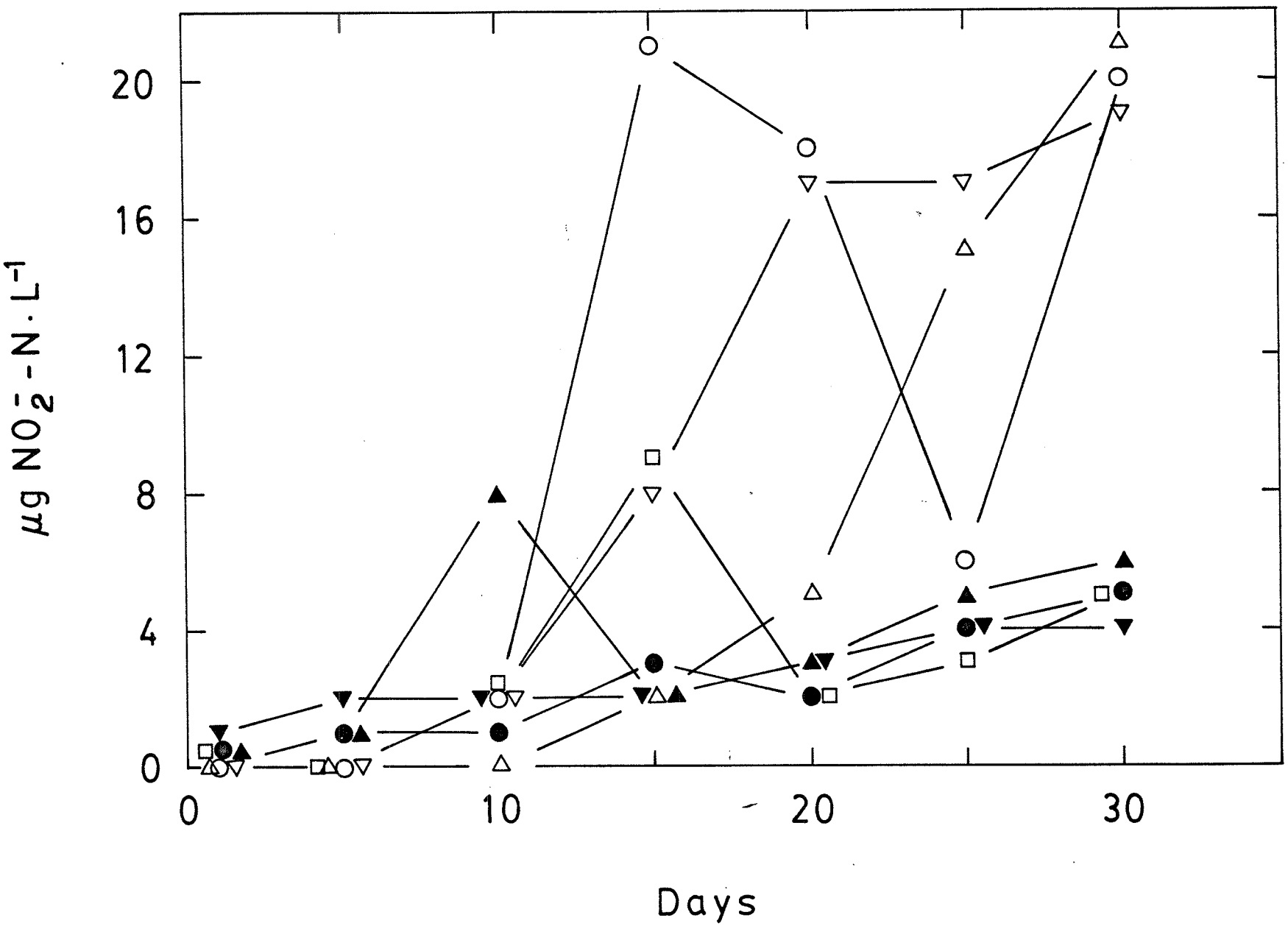

20
16
12
8
4
0
0
10
20
30
µg NO2- -N . L-1
Days

Fig. 90. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in June, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

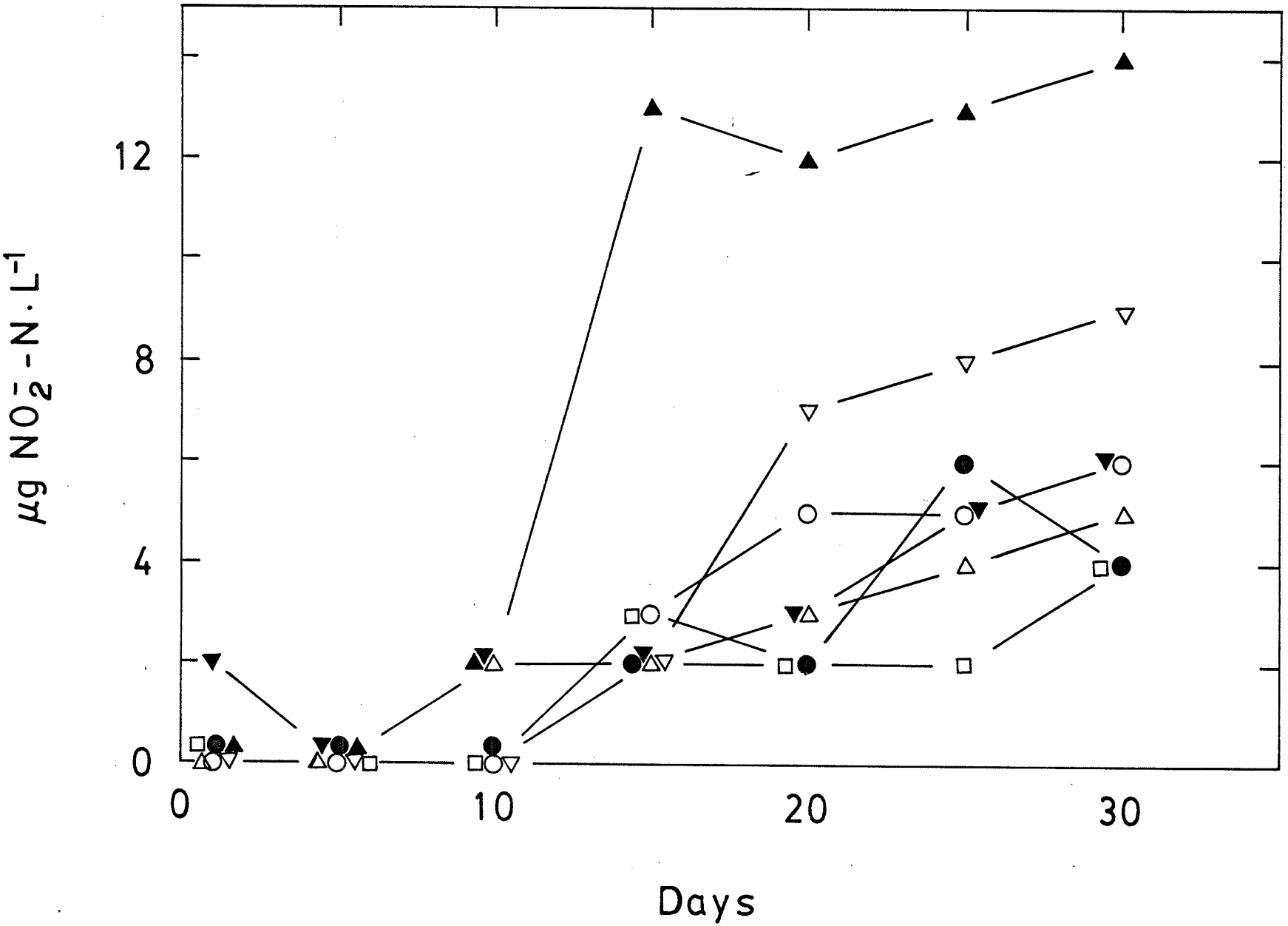

µg NO2-N.L-1
12
8
4
0
0
10
20
30
Days

Fig. 91. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in June, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

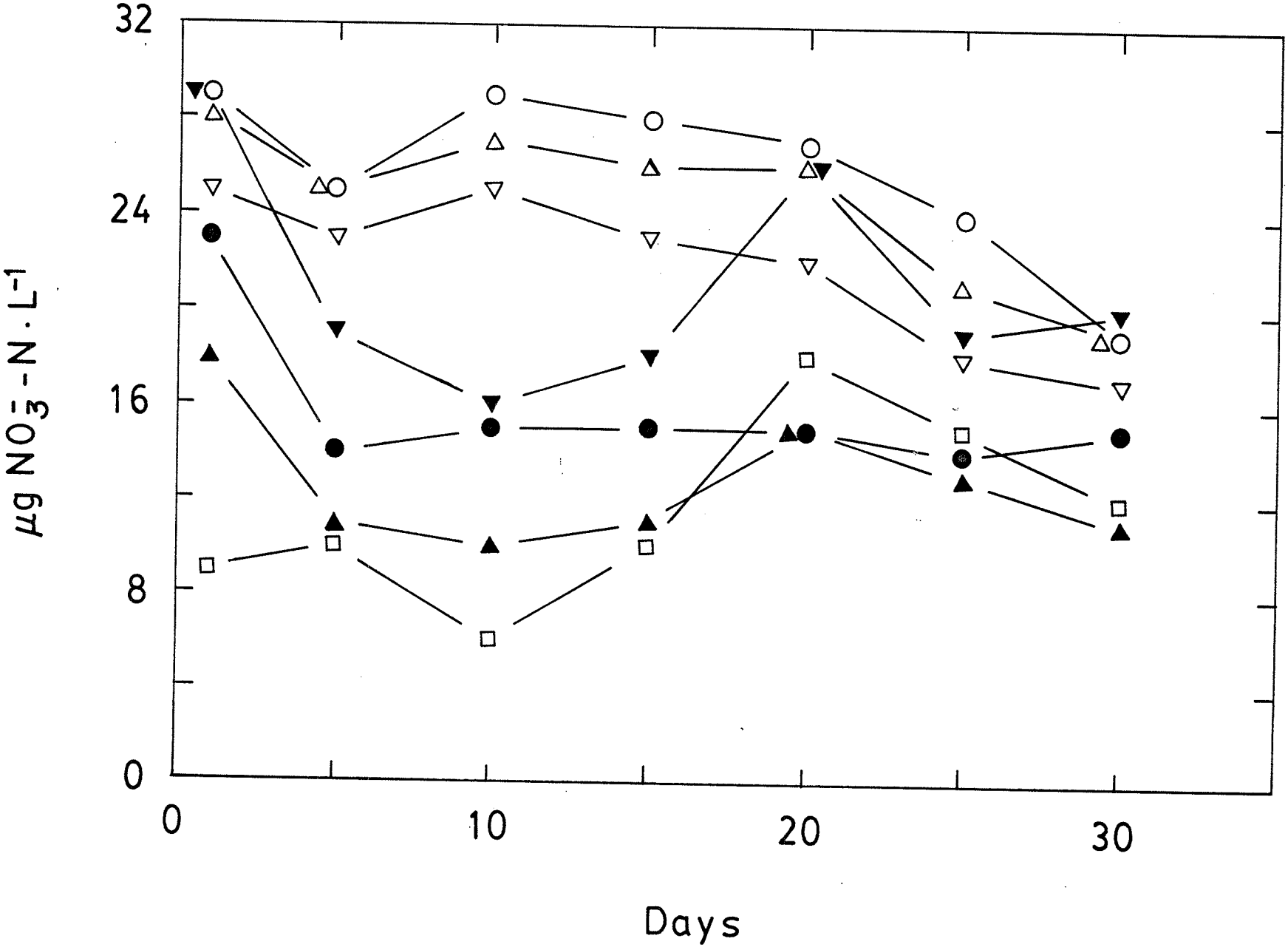

32
24
16
8
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 92. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in June, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0 \cdot 10$).

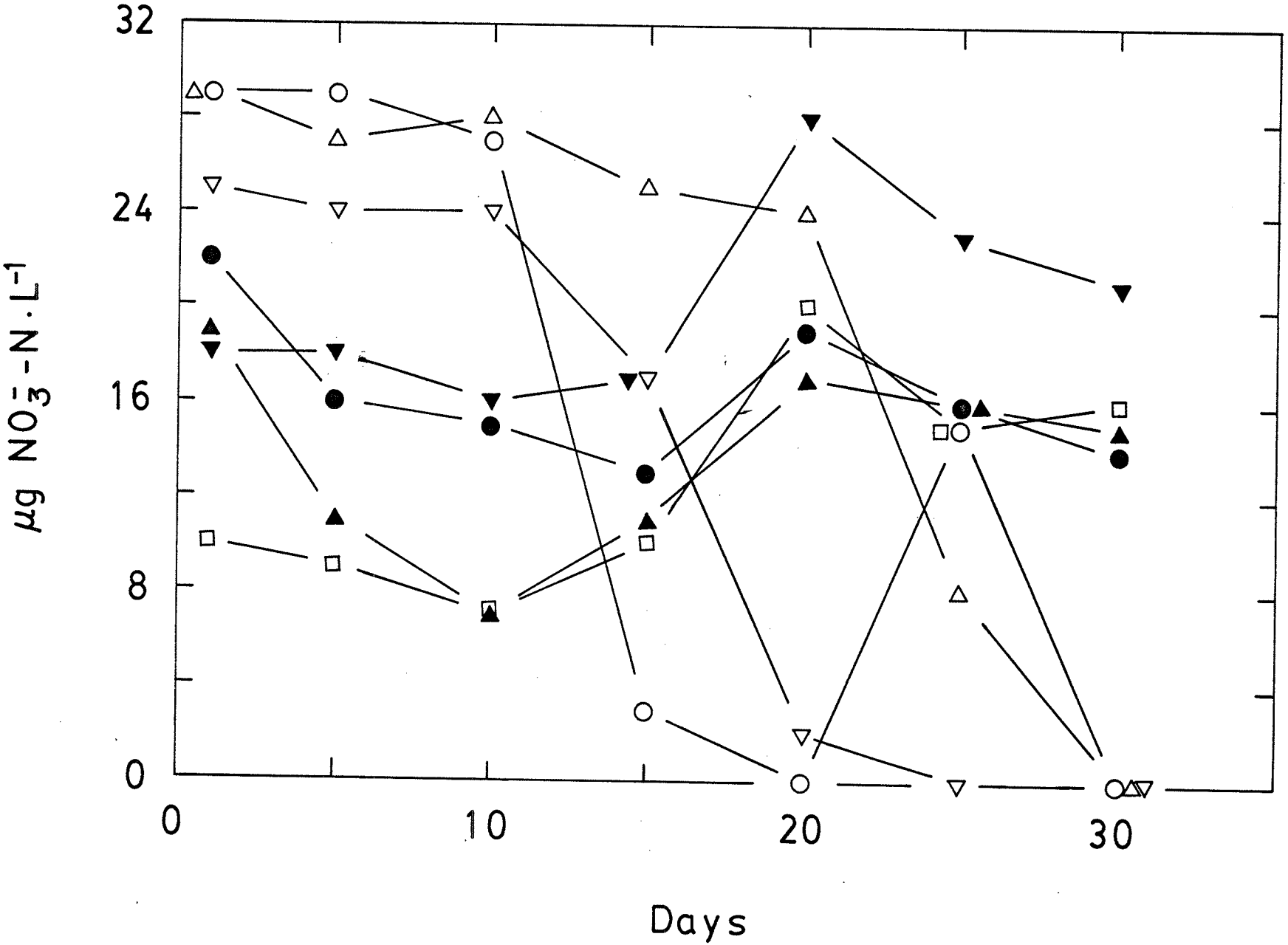

32
24
16
8
0
0
10
20
30
Days
µg NO3-N.L-1

Fig. 93. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in June, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

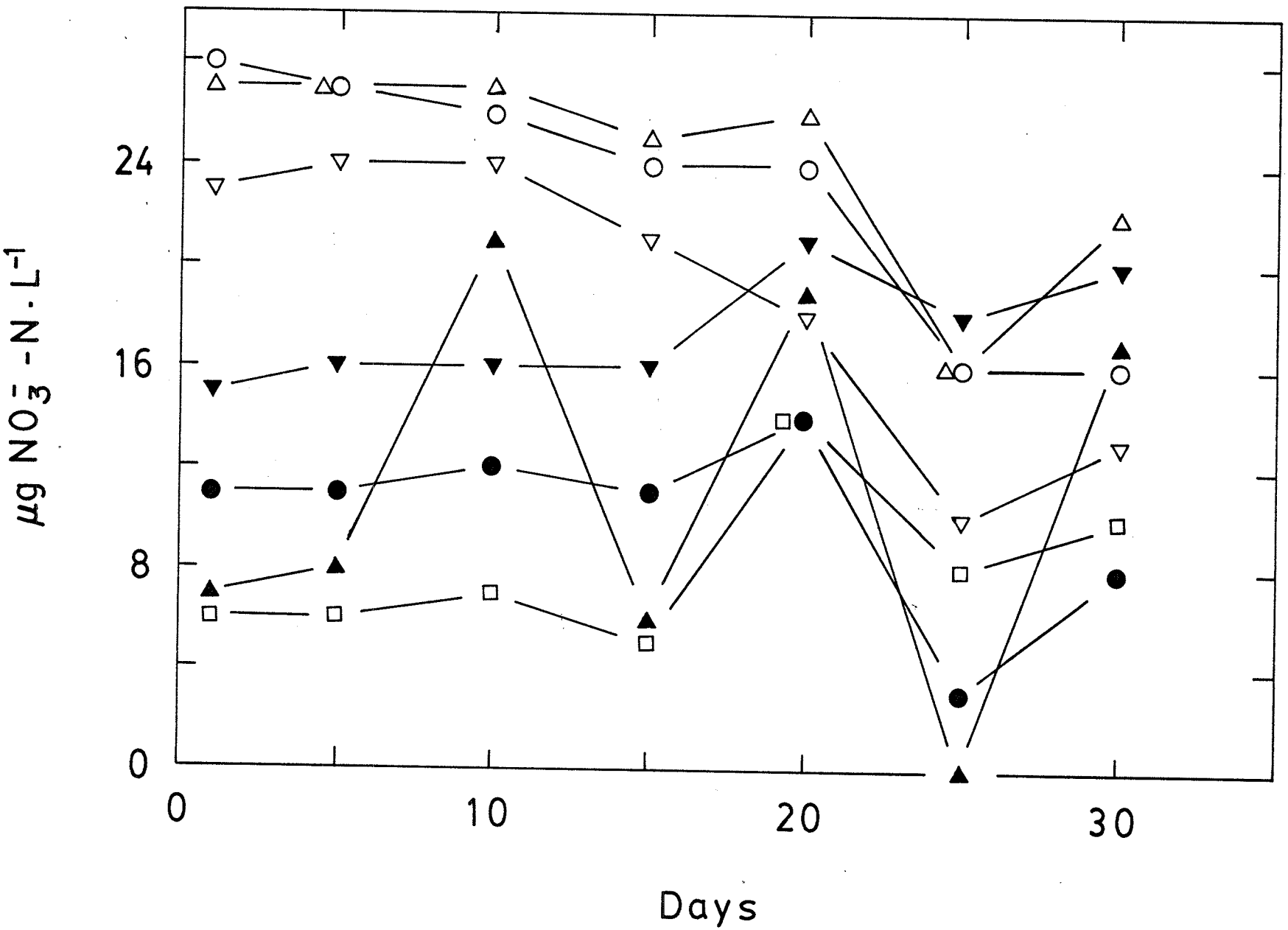

µg NO_3^--N·L^{-1}
0
8
16
24
0
10
20
30
Days

Fig. 94. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in July, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0 \cdot 10$).

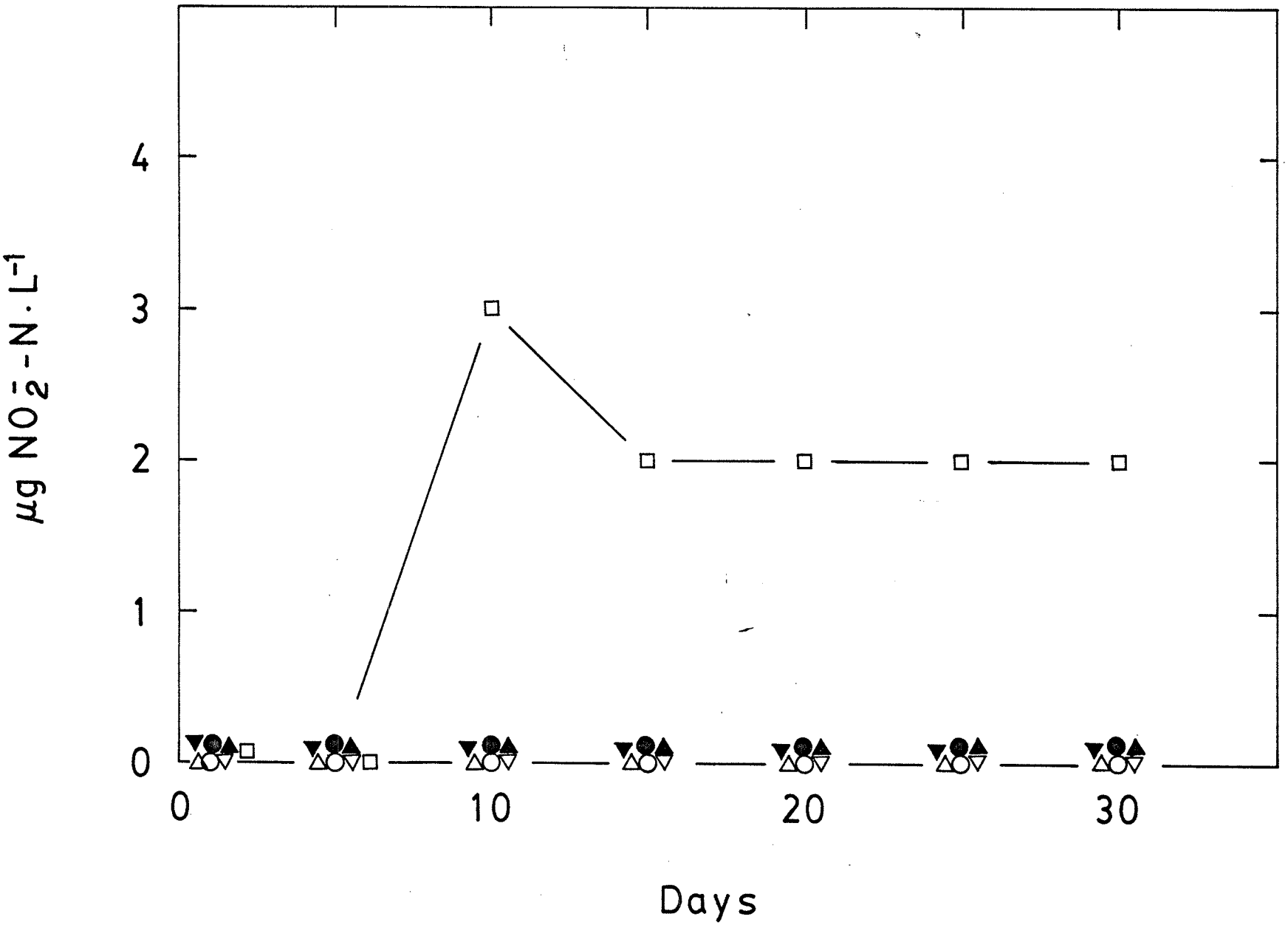

μg NO2-N.L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 95. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in July, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

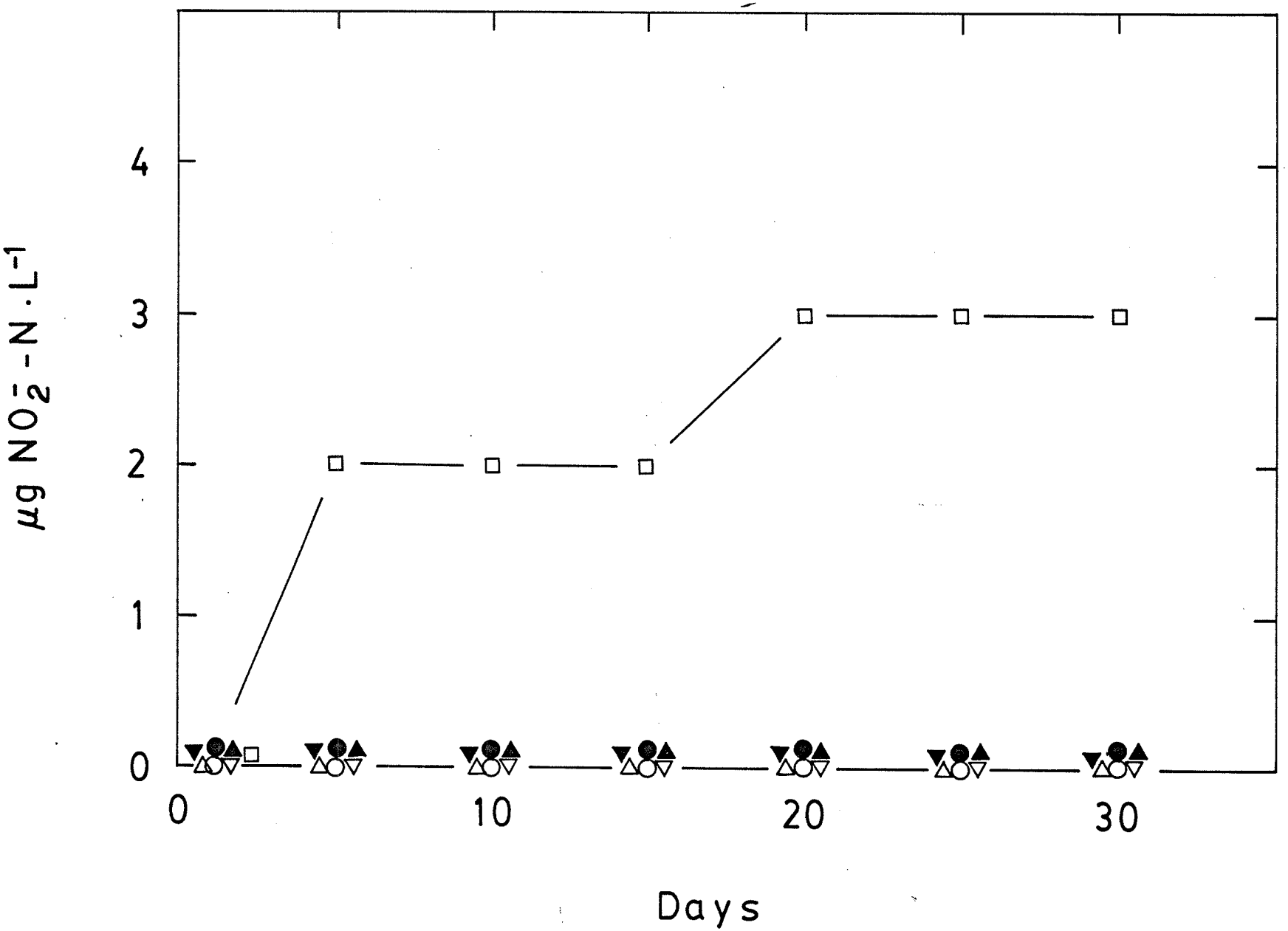

μg NO2- N . L-1
0
1
2
3
4
0
10
20
30
Days

Fig. 96. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in July, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

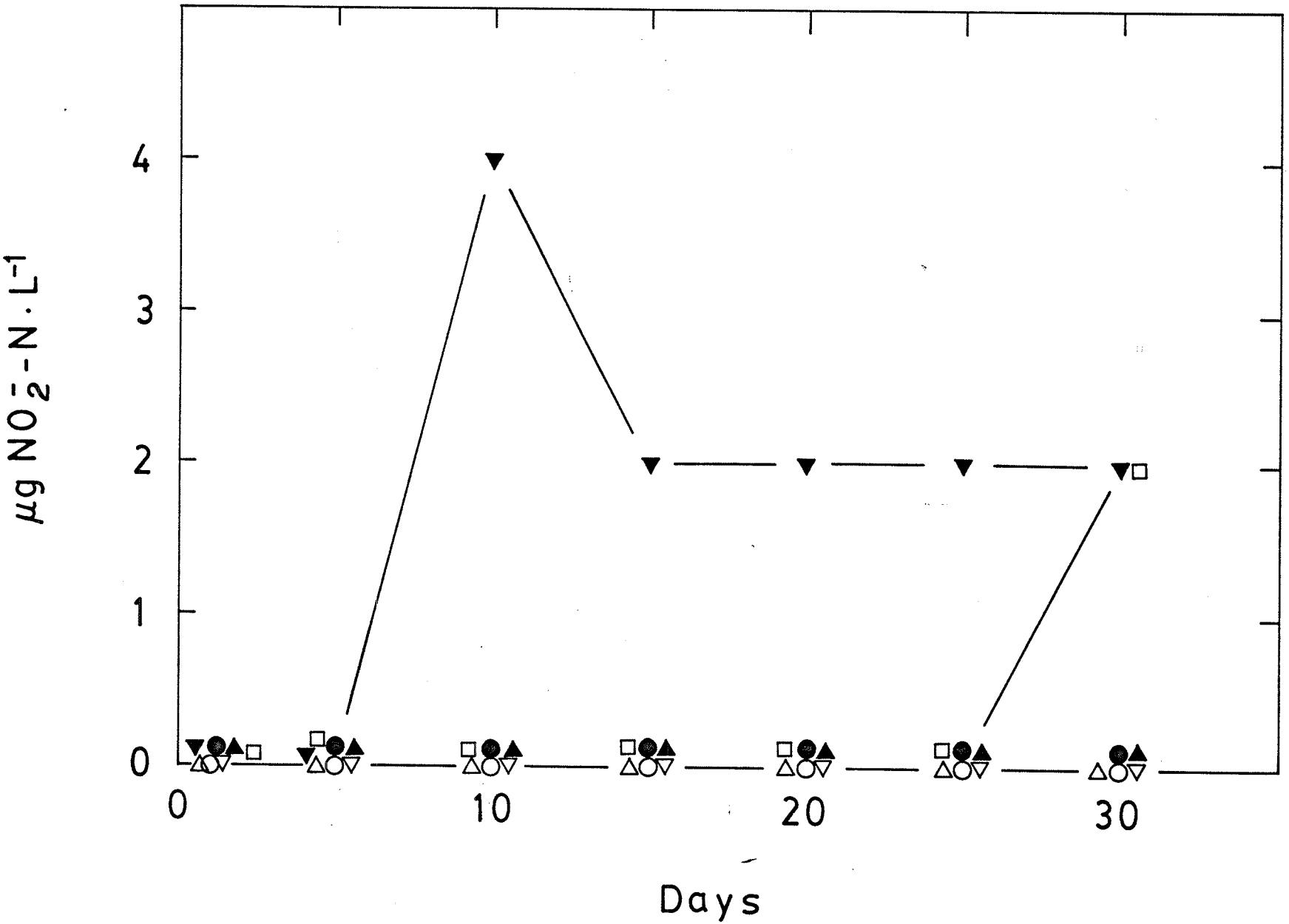

μg NO2-N . L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 97. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in July, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

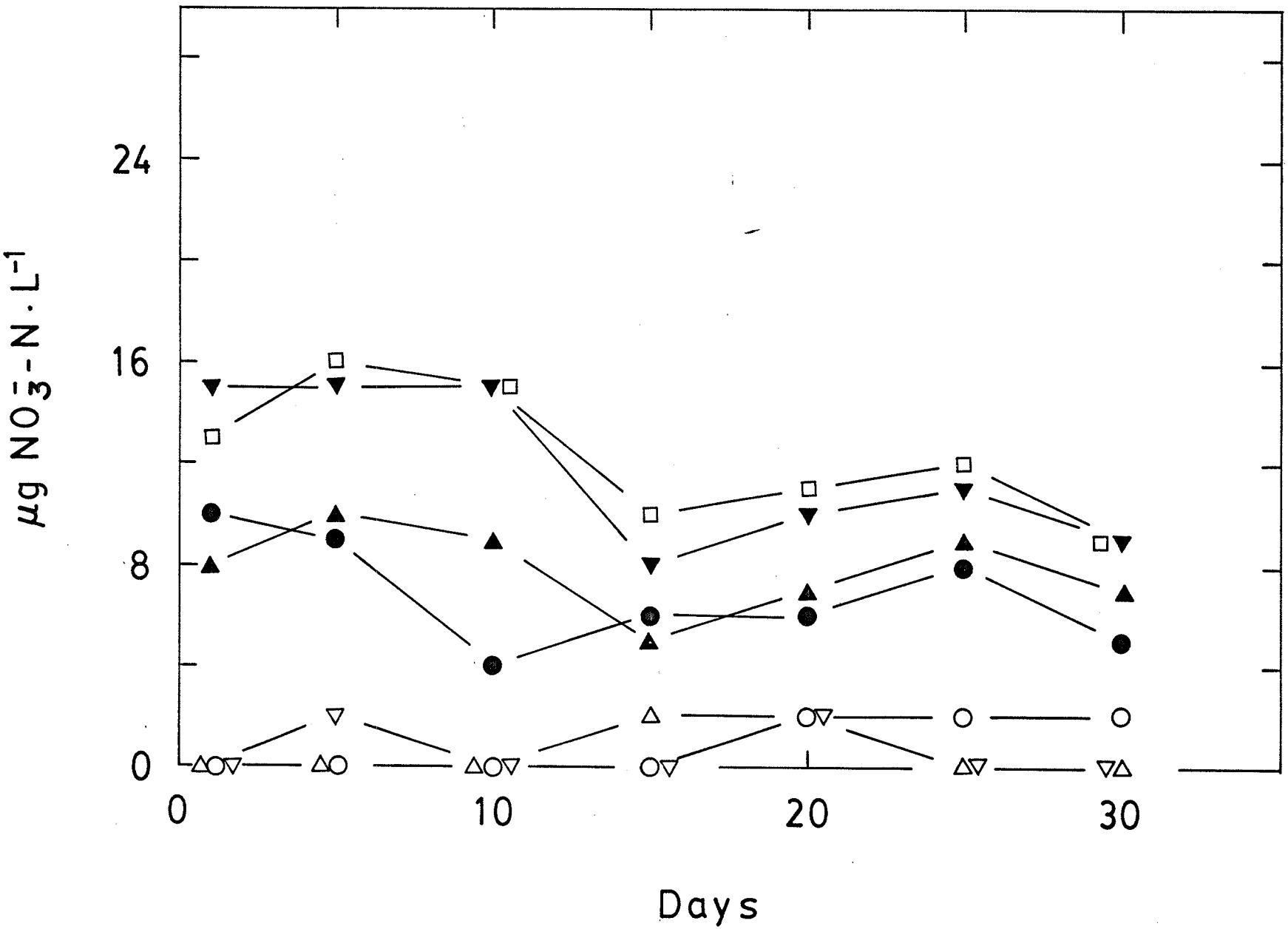

24
16
8
0
0
10
20
30
µg NO3-N.L-1
Days

Fig. 98. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in July, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0 \cdot 10$).

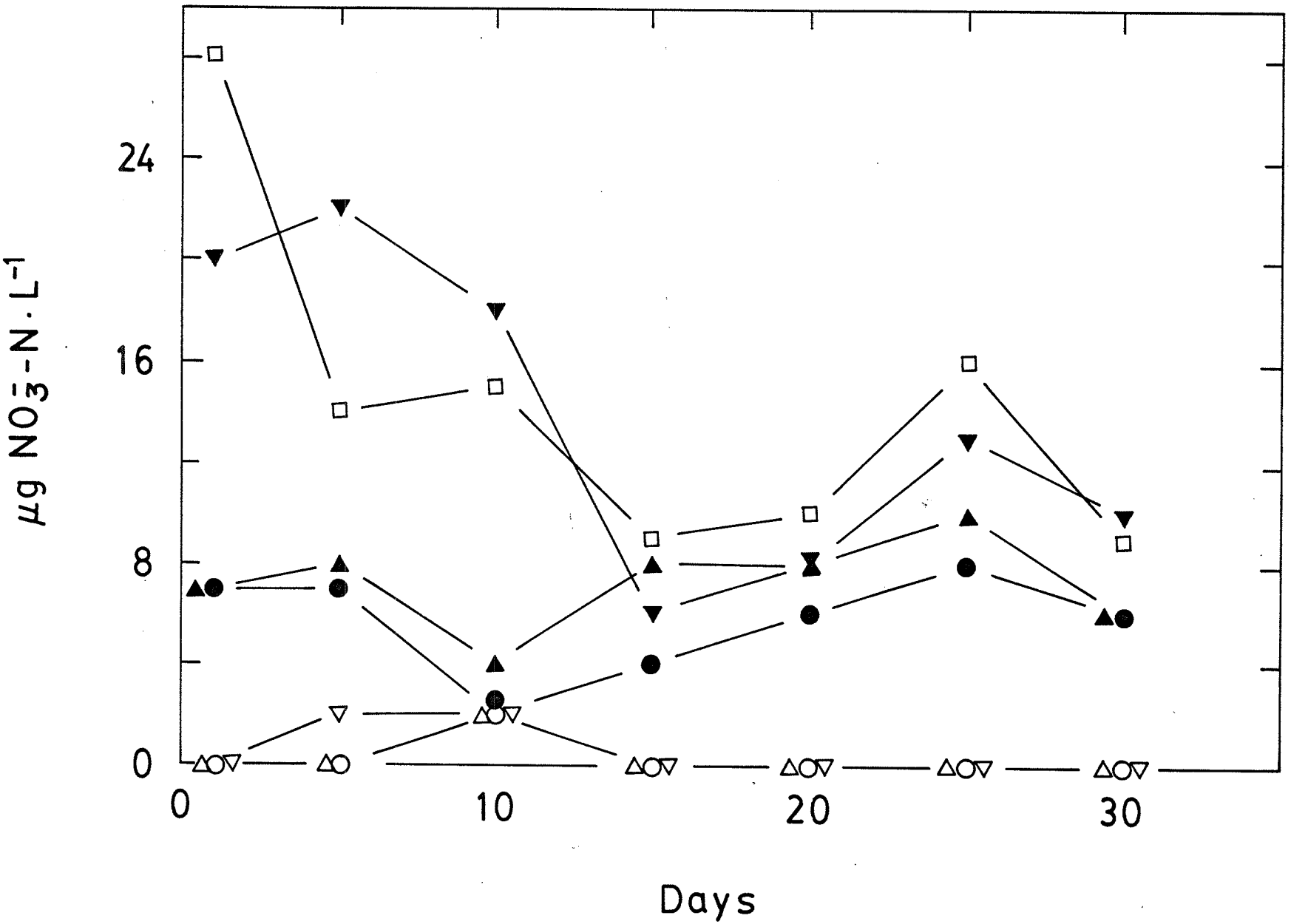

24
16
8
0
0
10
20
30
Days
μg NO3-N.L-1

Fig. 99. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in July, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

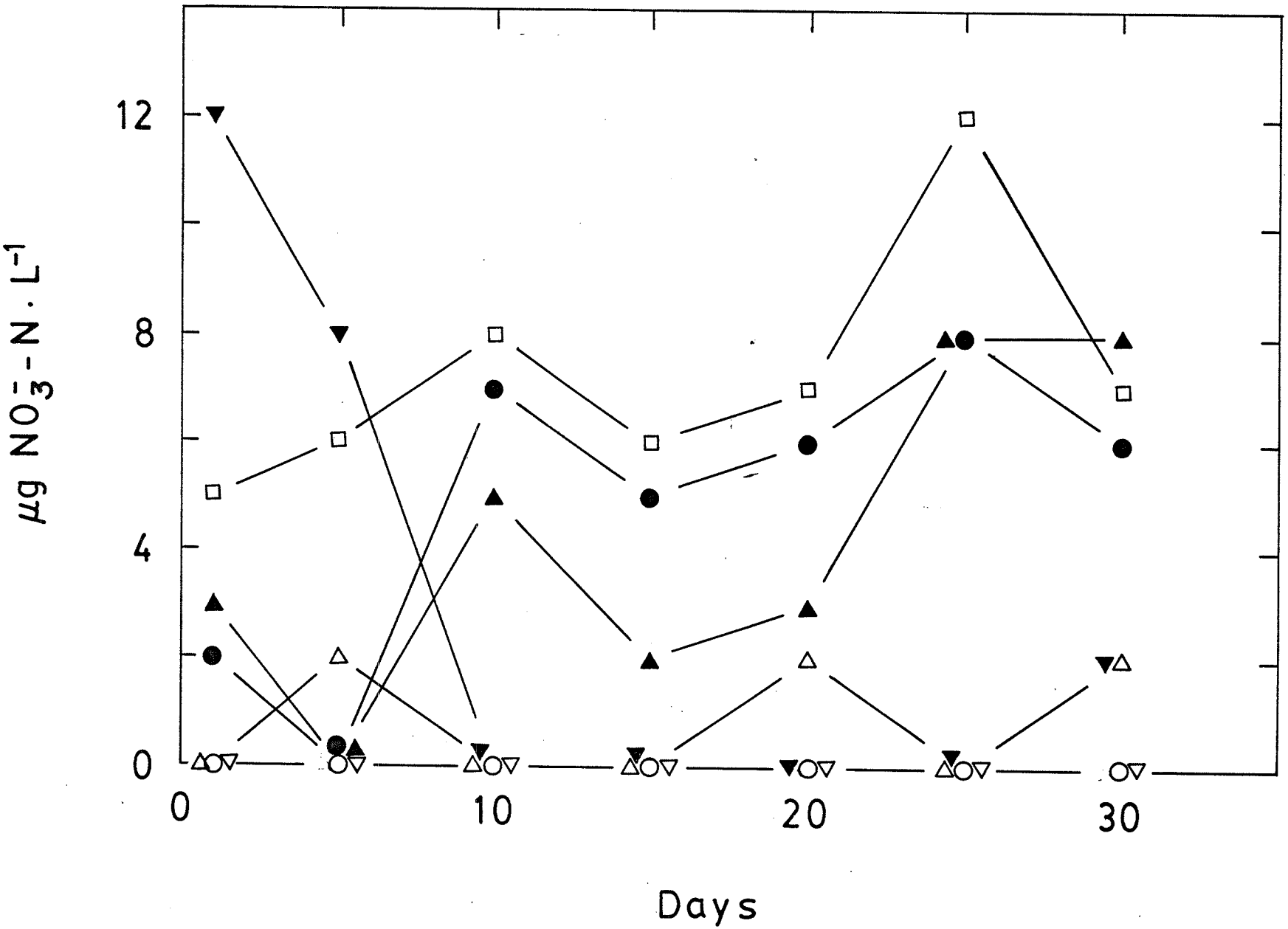

12
8
4
0
0
10
20
30
Days
μg NO3- - N · L-1

Fig. 100. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in August, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

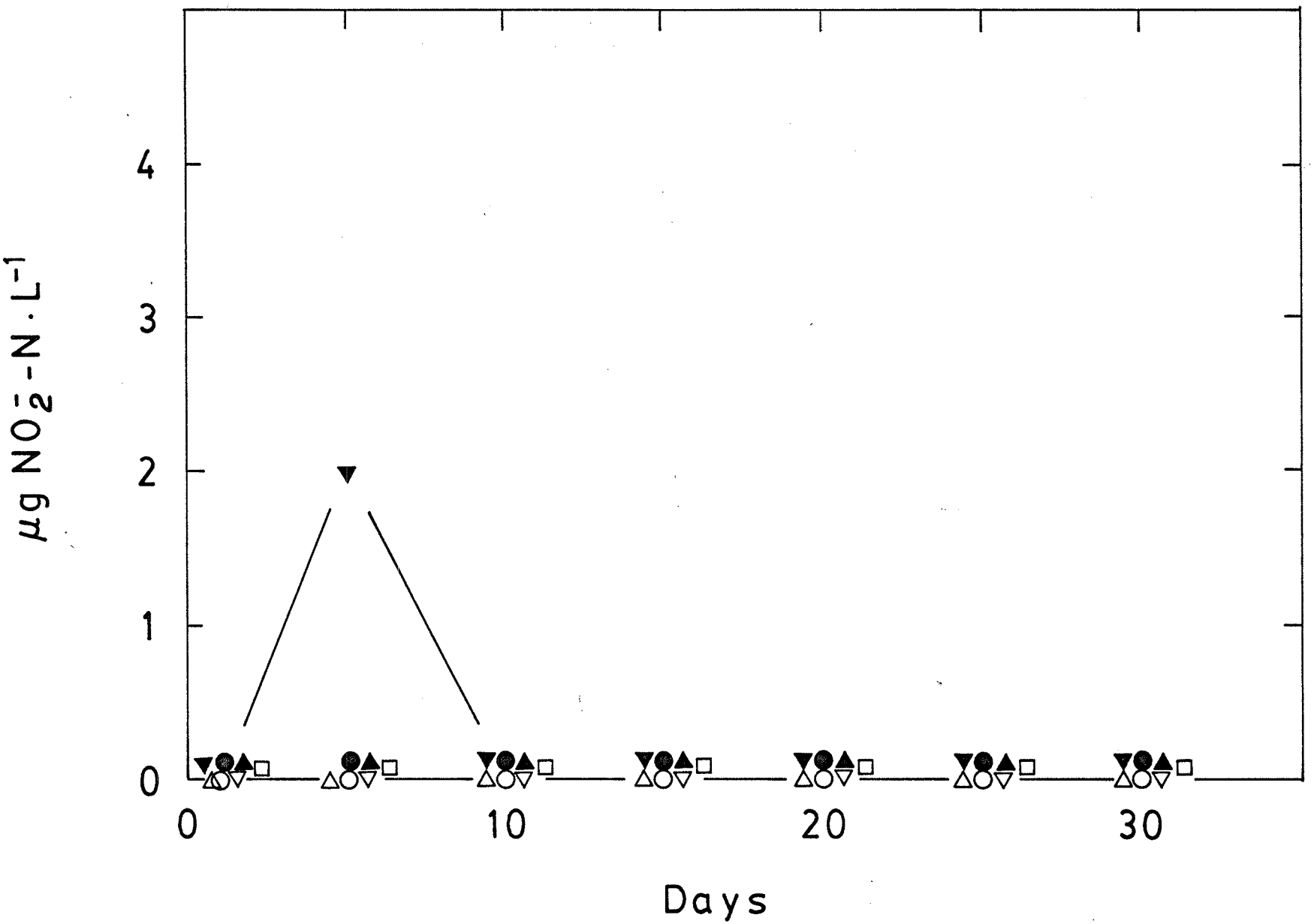

μg NO2-N · L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 101. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in August, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

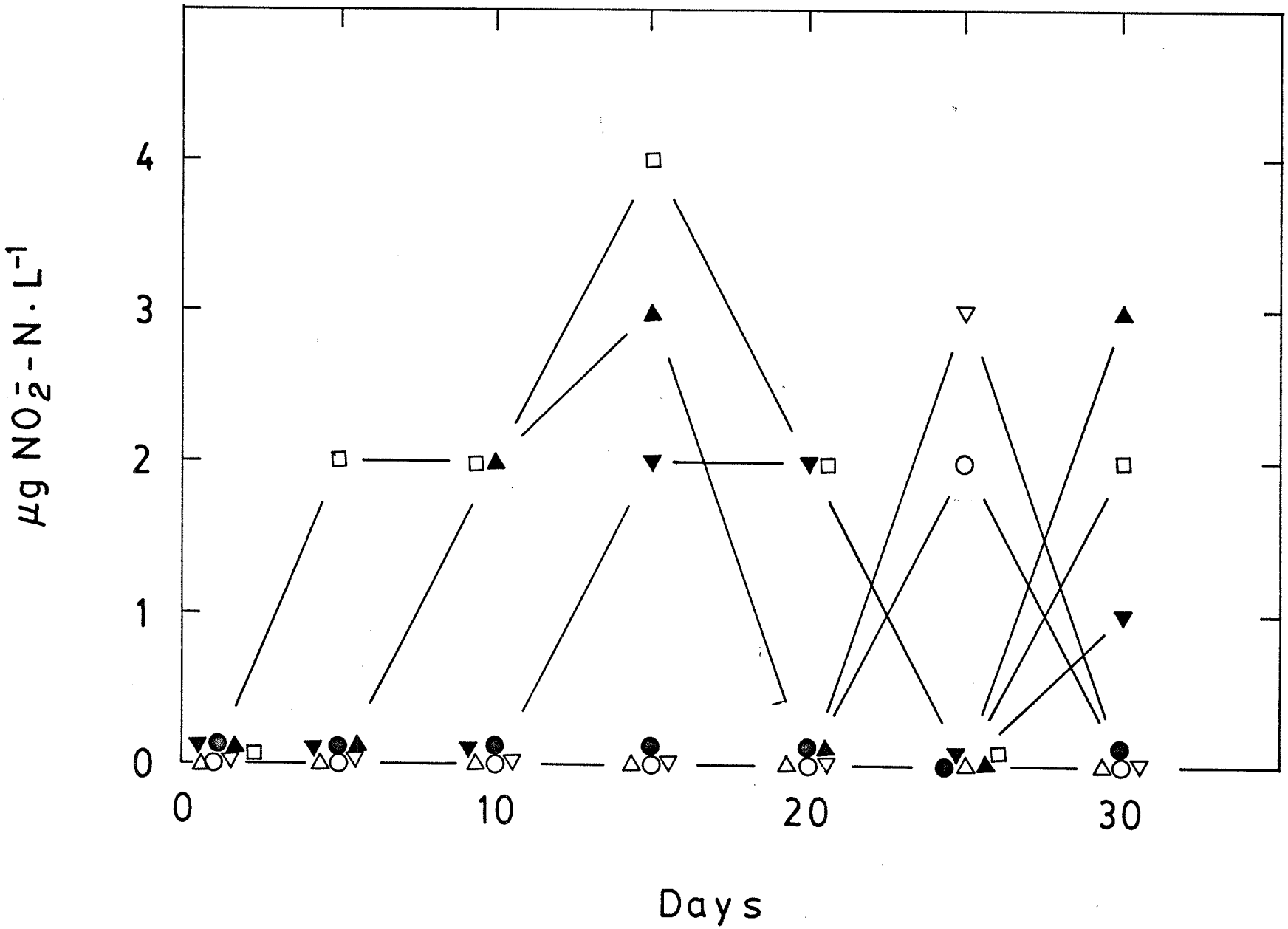

μg NO2-N · L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 102. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in August, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

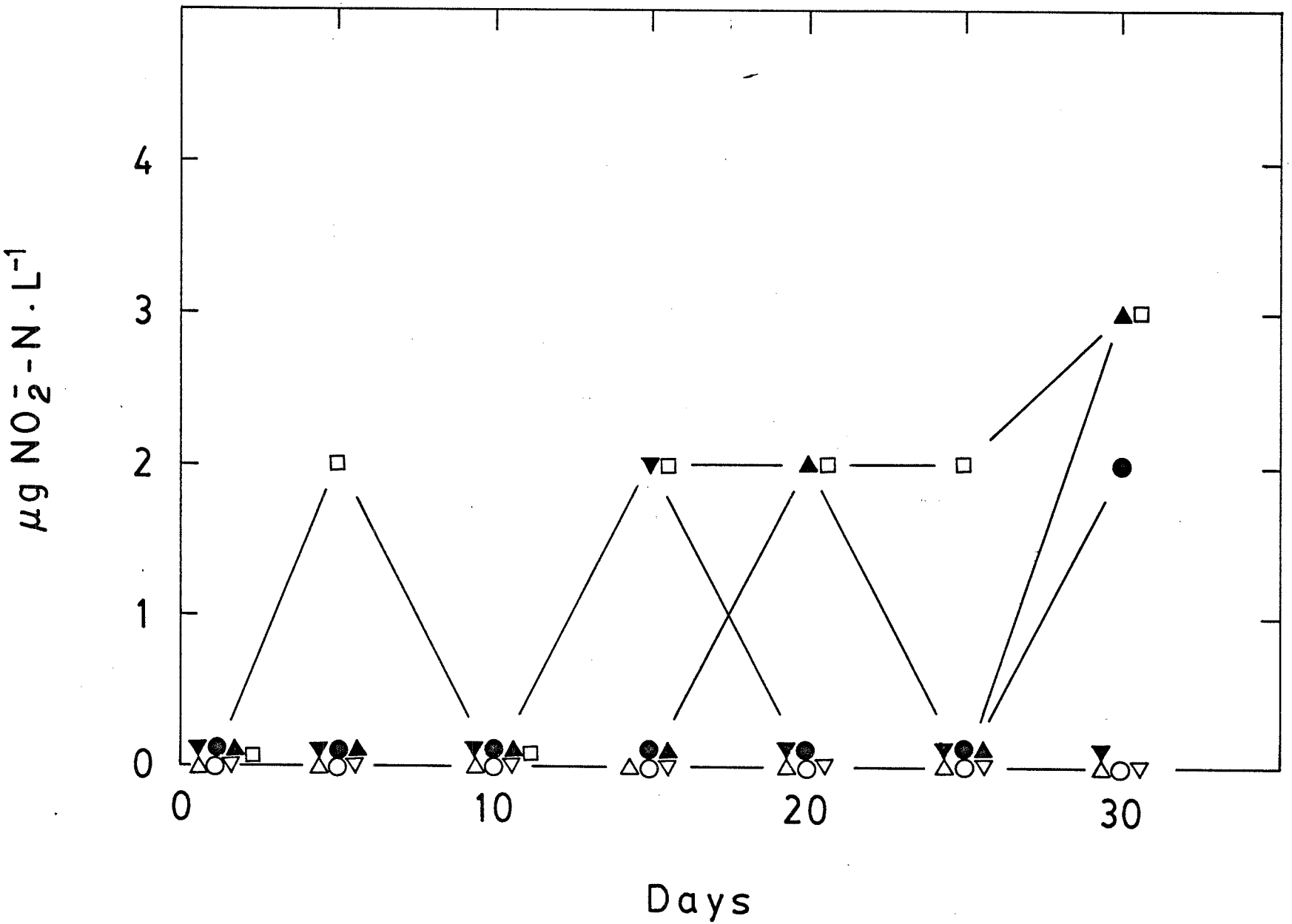

μg NO2-N · L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 103. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in August, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

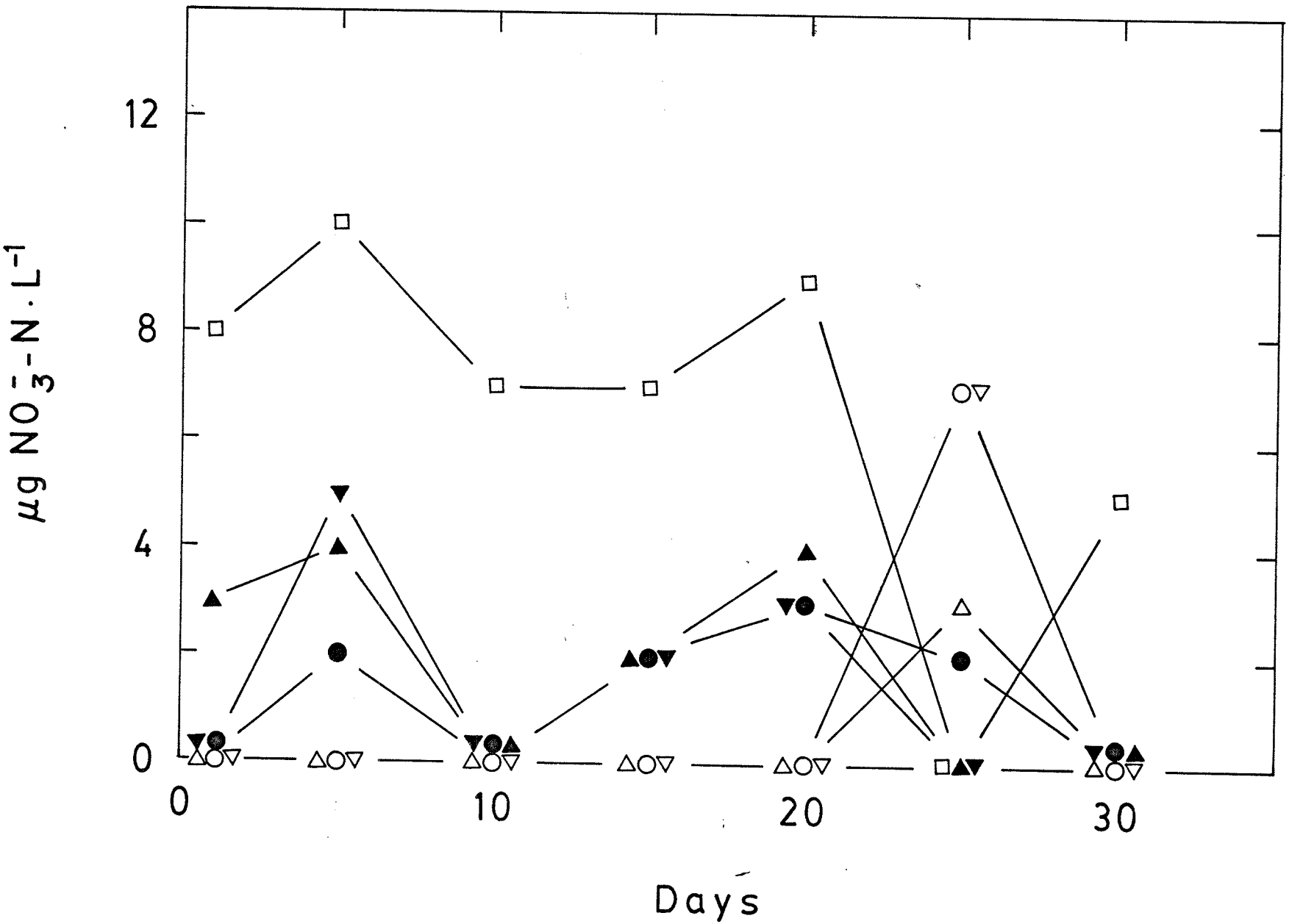

12
8
4
0
0
10
20
30
Days
µg NO3-N.L-1

Fig. 104. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in August, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0 \cdot 10$).

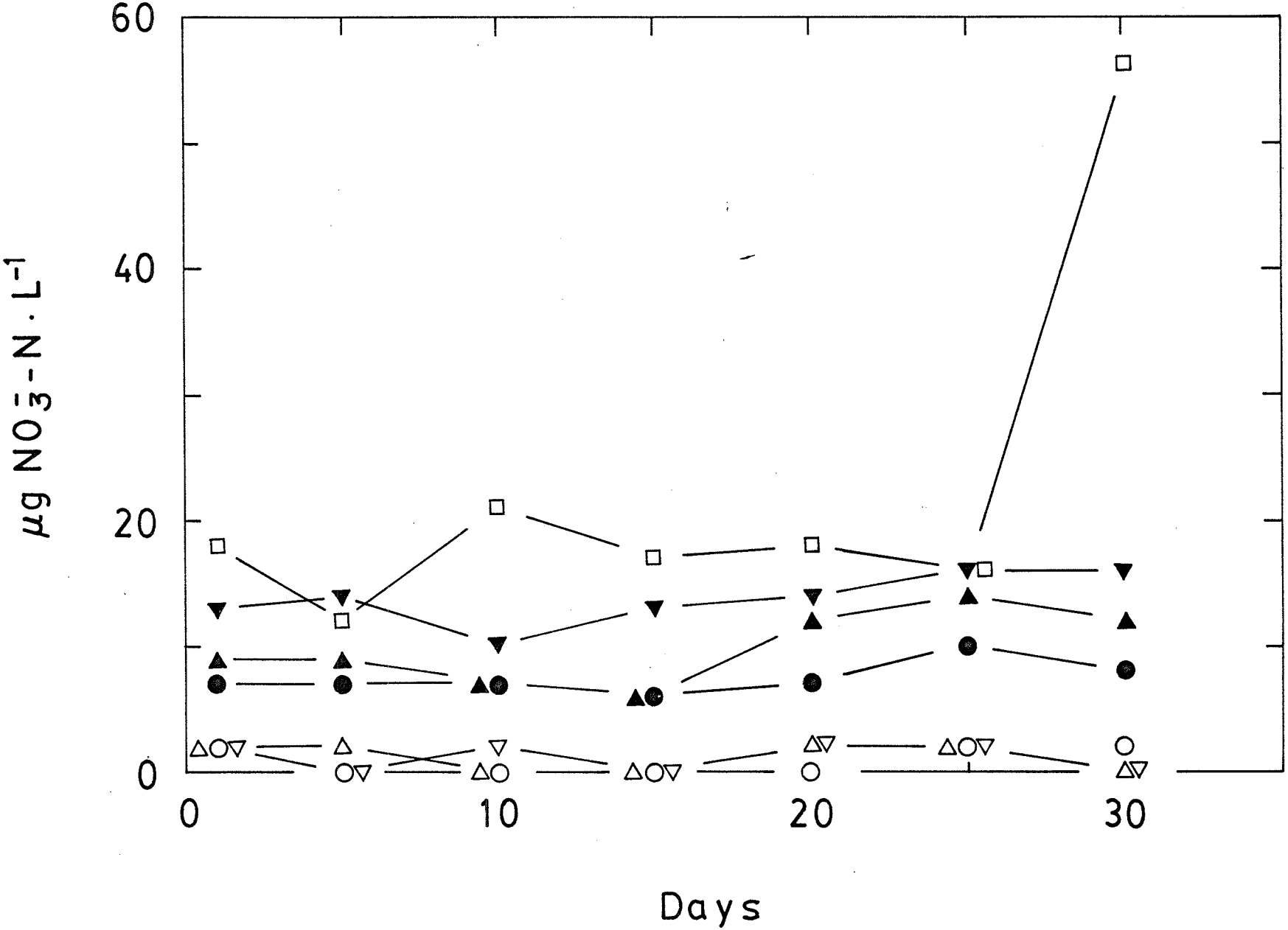

60
40
20
0
0
10
20
30
μg NO3-N . L-1
Days

Fig. 105. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in August, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

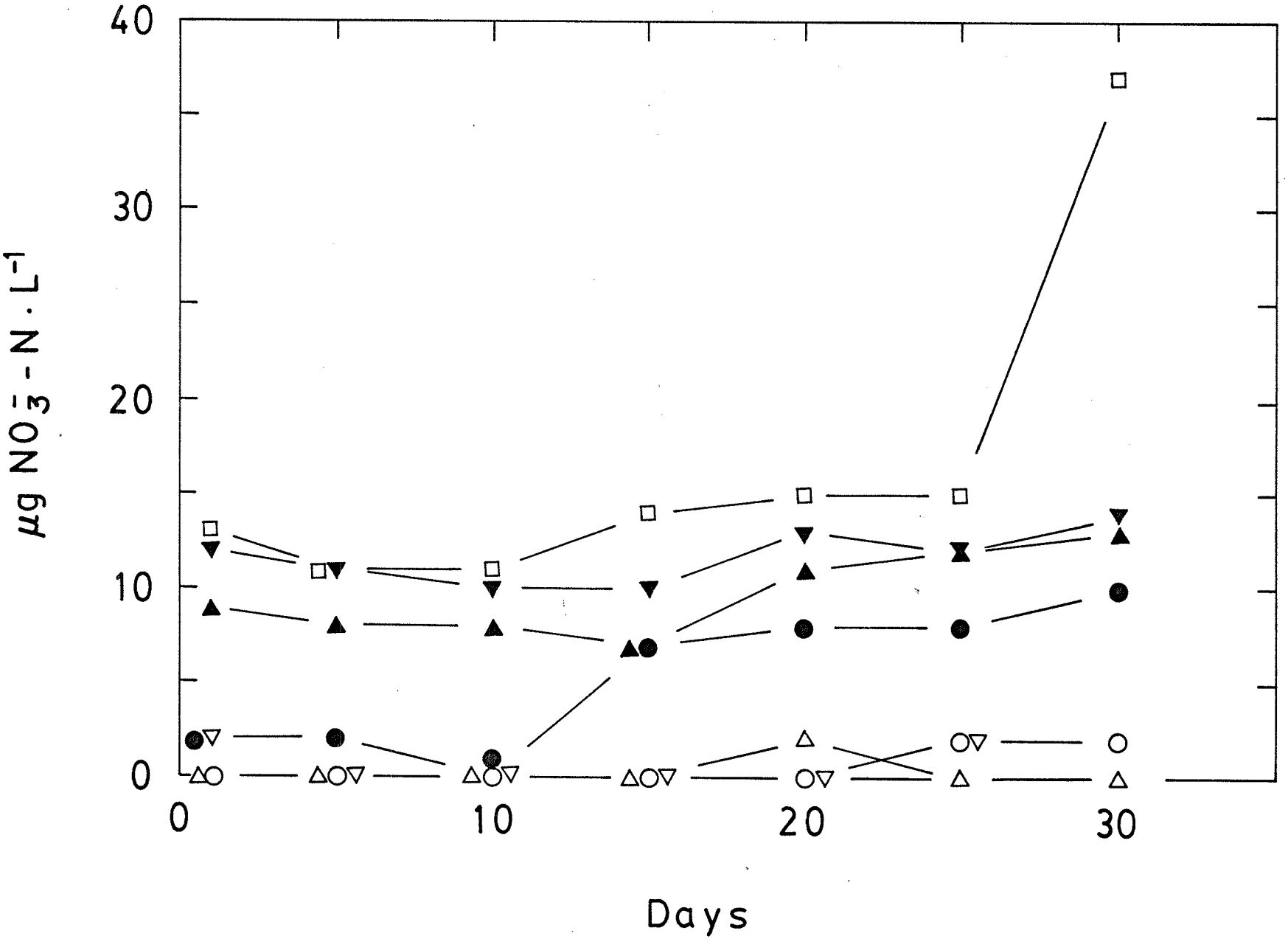

40
30
20
10
0
0
10
20
30
μg NO3- - N · L-1
Days

Fig. 106. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in September, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

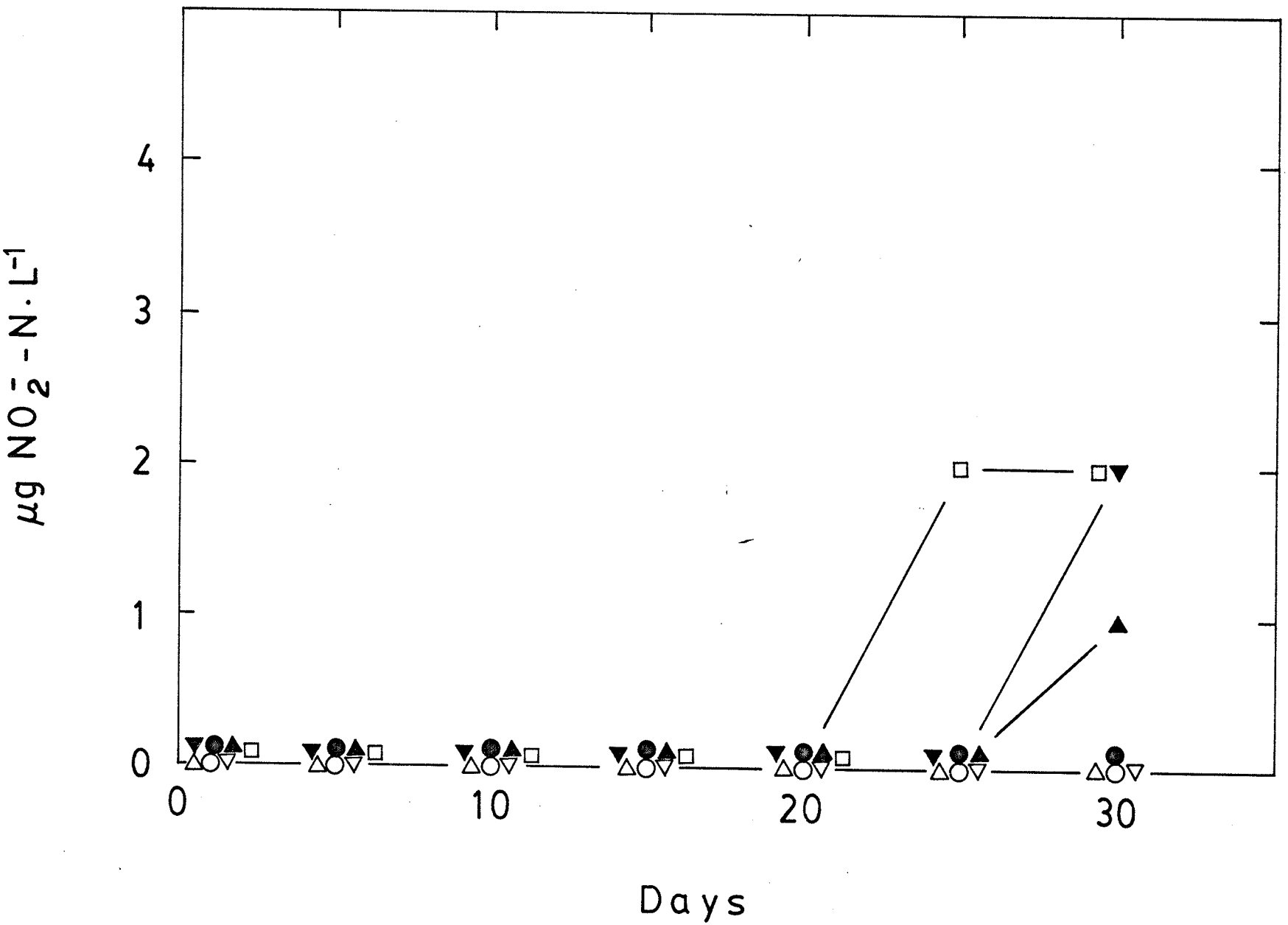

μg NO2- - N · L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 107. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in September, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

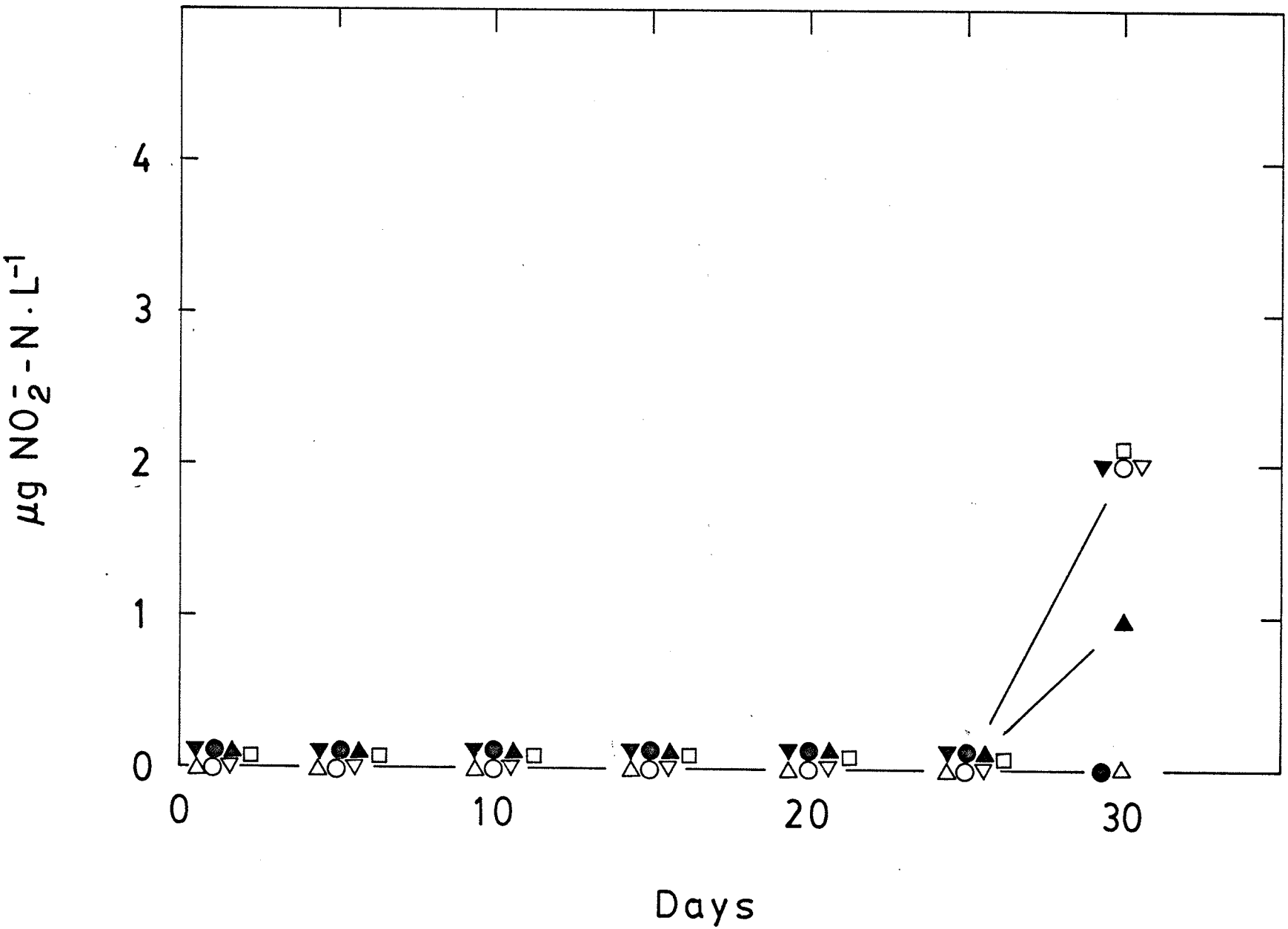

μg NO2- - N . L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 108. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in September, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

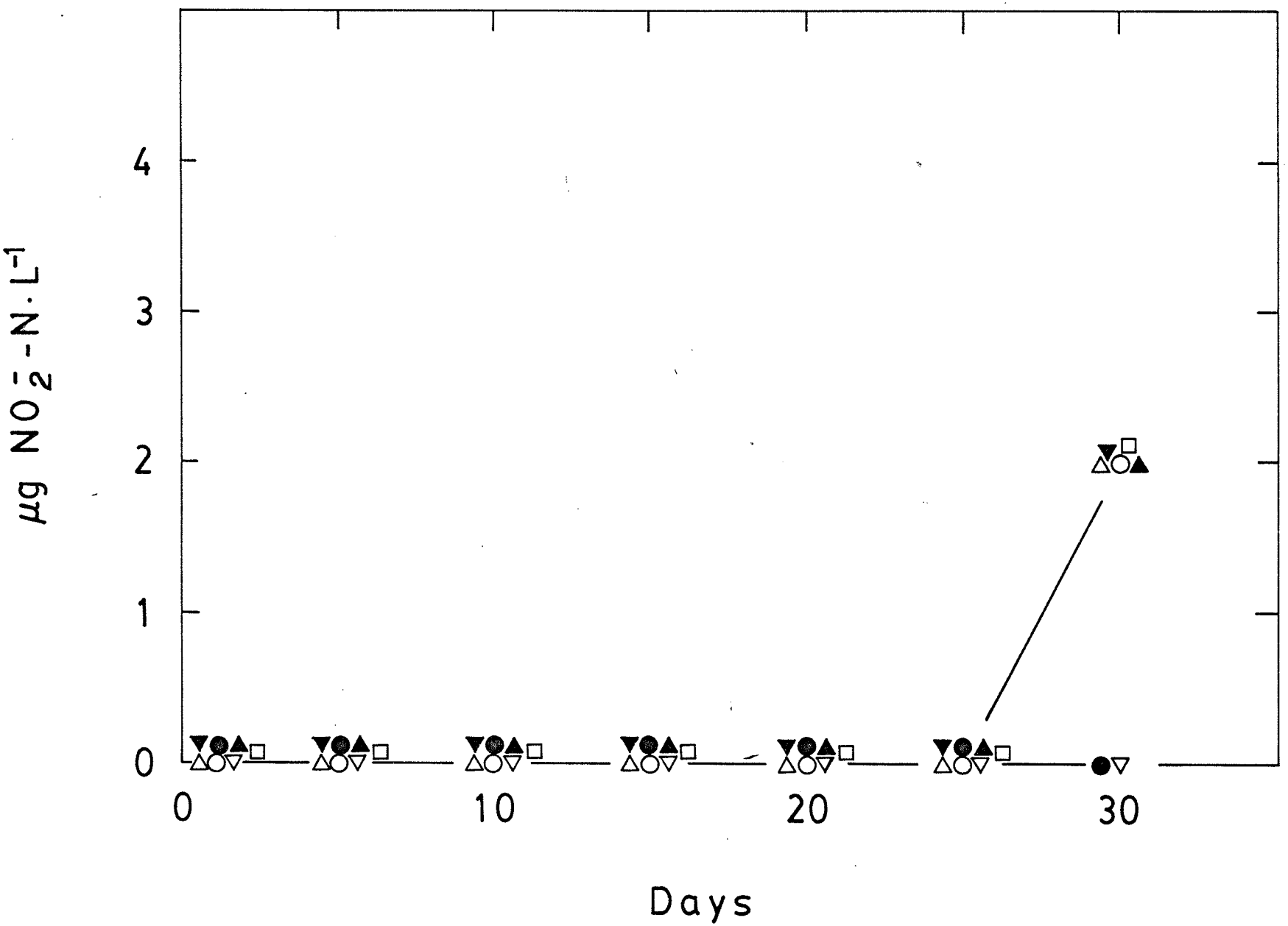

μg NO2-N·L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 109. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in September, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

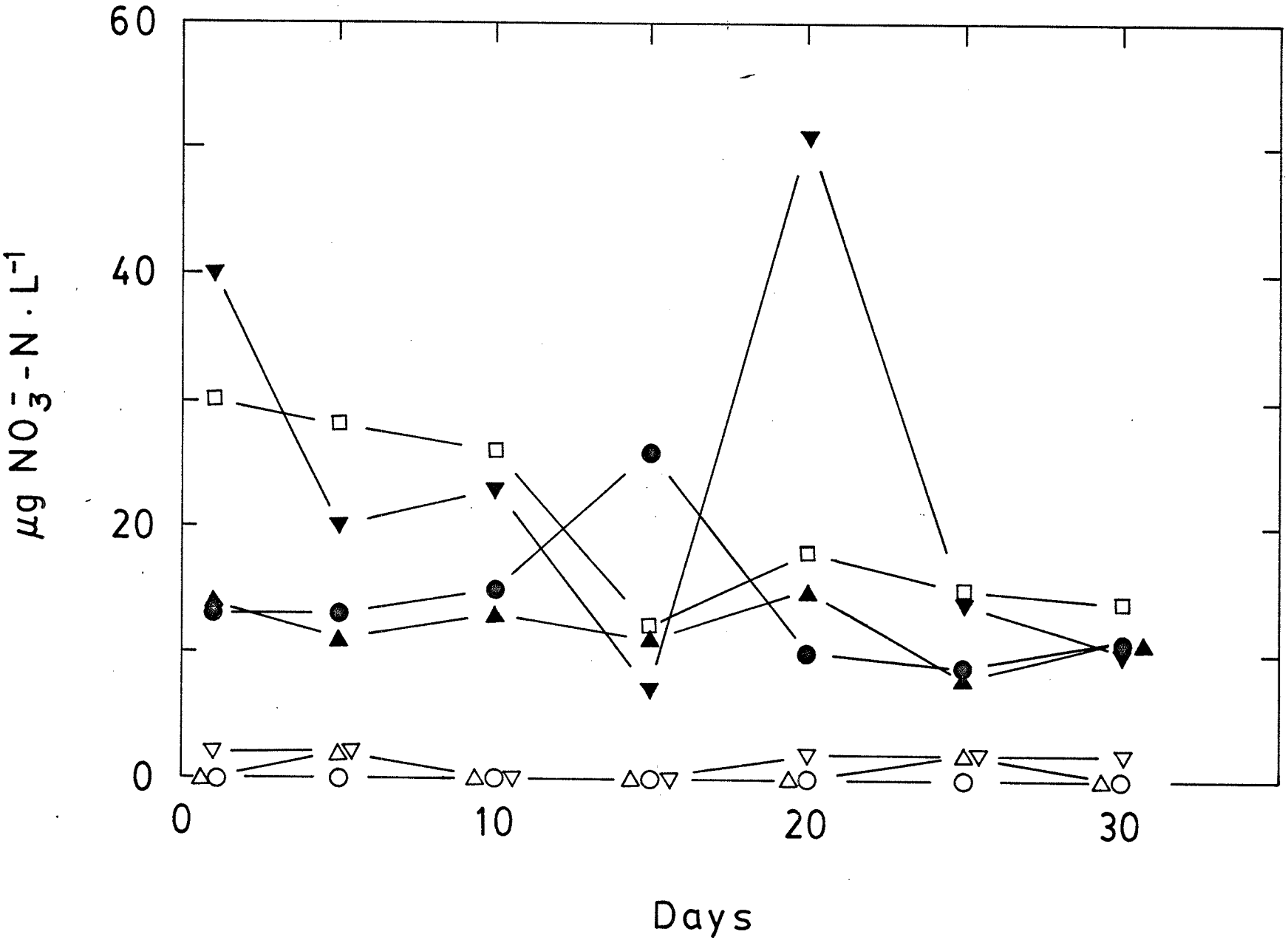

60
40
20
0
0
10
20
30
Days
µg NO3-N · L-1

Fig. 110. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in September, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (○) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

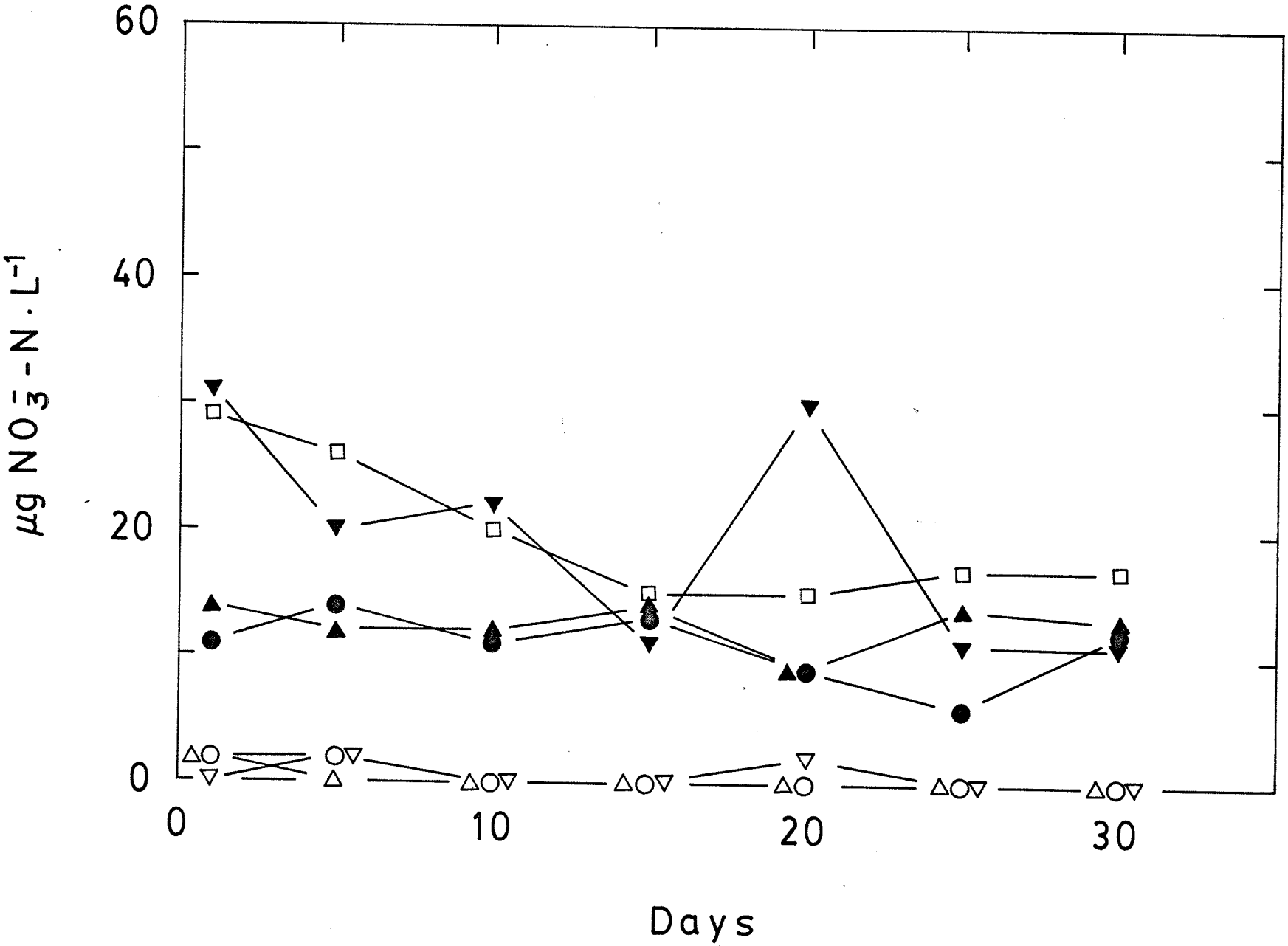

60
40
20
0
0
10
20
30
Days
µg NO3-N . L-1

Fig. 111. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in September, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

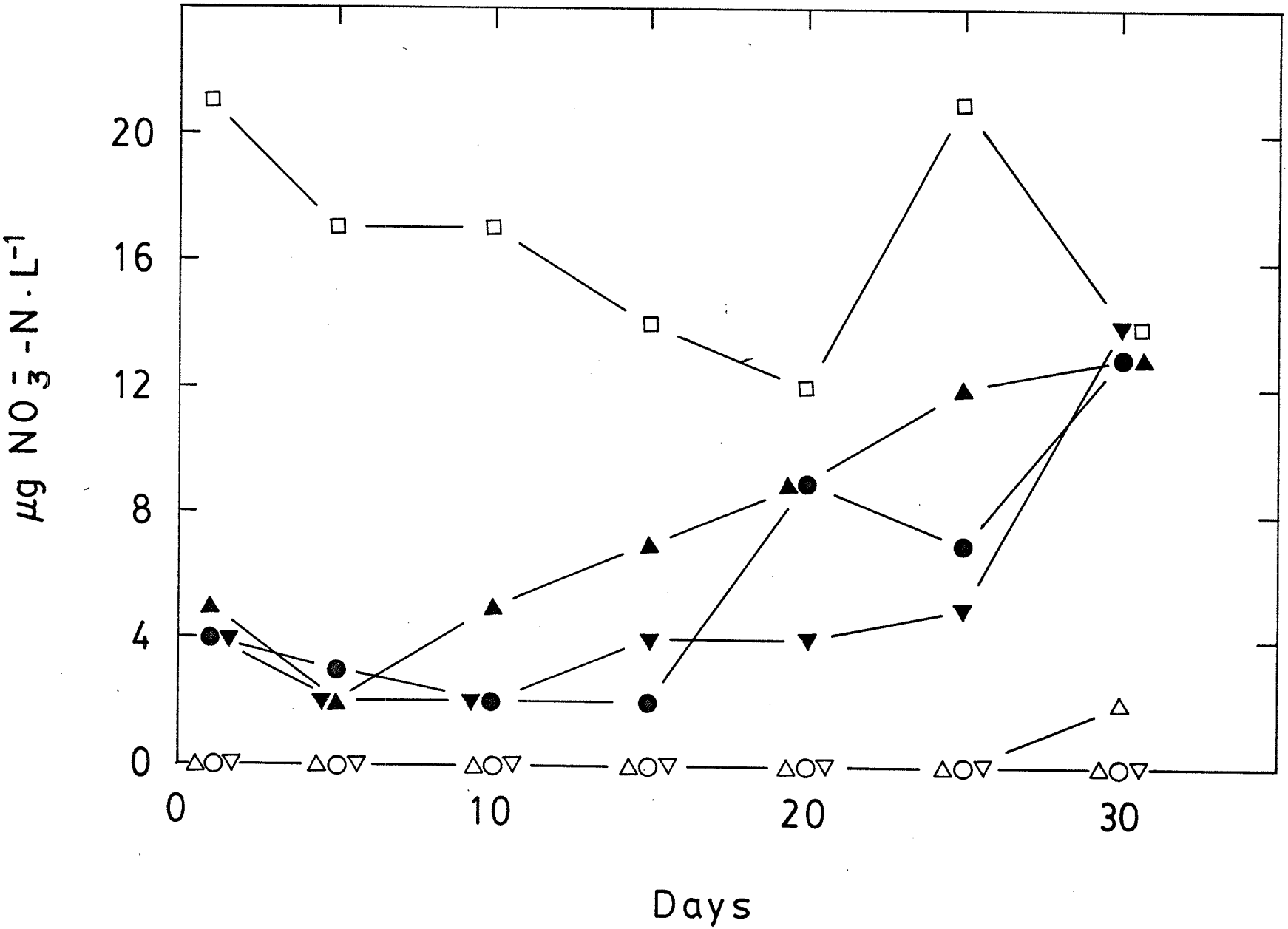

μg NO3-N · L-1
20
16
12
8
4
0
0
10
20
30
Days

Fig. 112. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in October, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0 \cdot 10$).

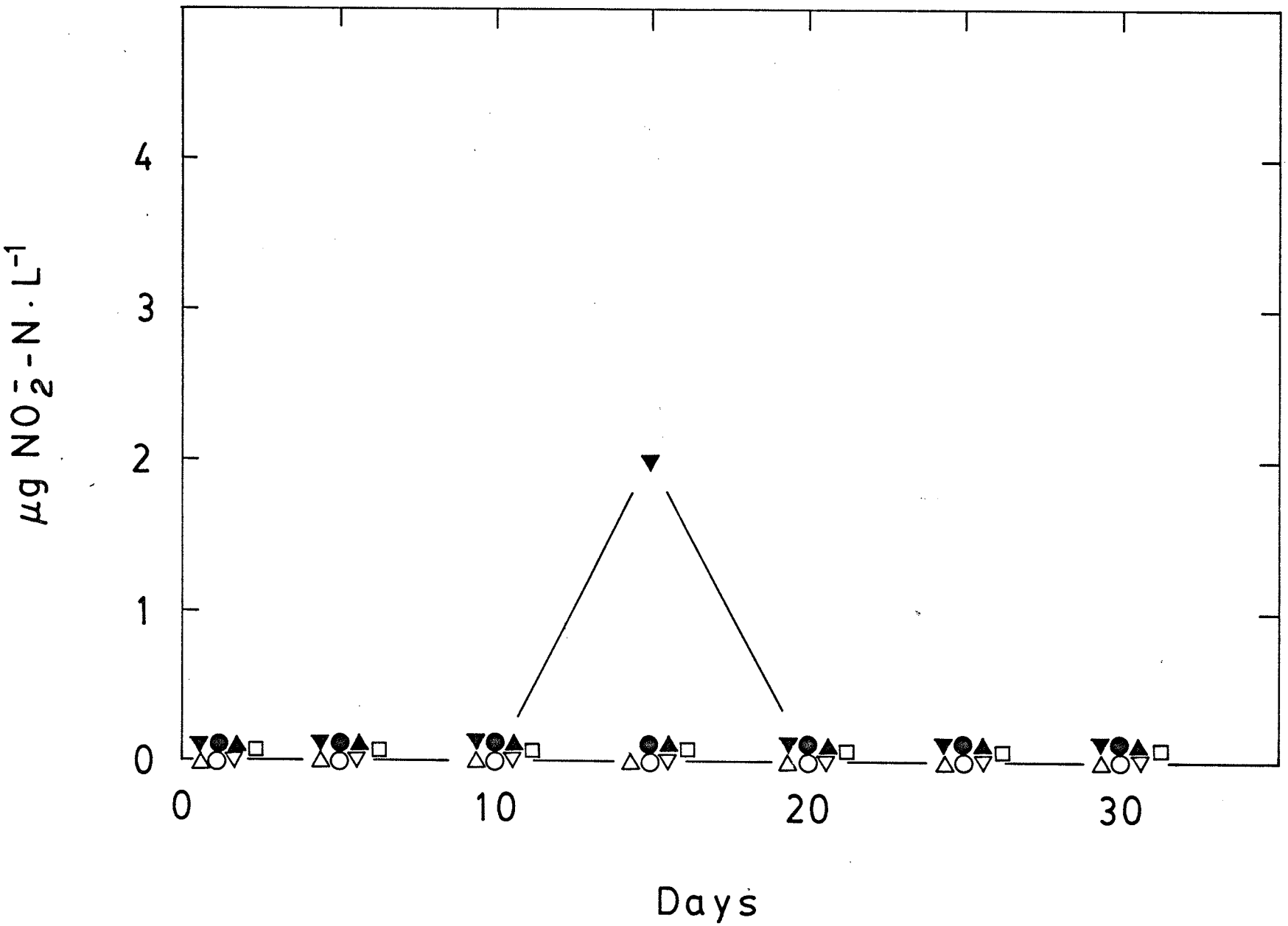

μg NO2-N . L-1
4
3
2
1
0
0
10
20
30
Days

Fig. 113. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in October, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

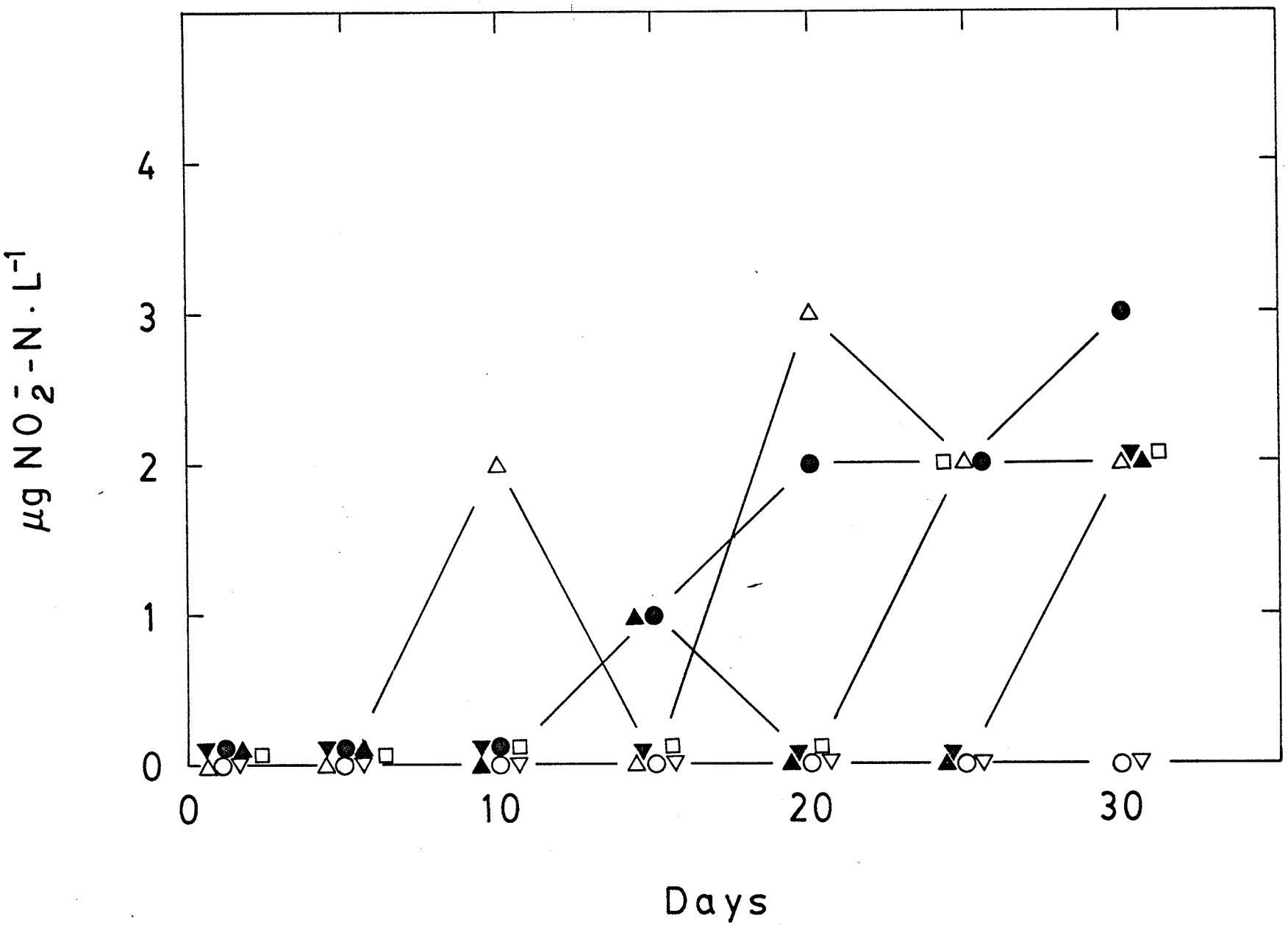

μg NO2-N · L-1
0
1
2
3
4
0
10
20
30
Days

Fig. 114. The production and disappearance of NO_2^- at various water depths of L.302S. Samples were obtained in October, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

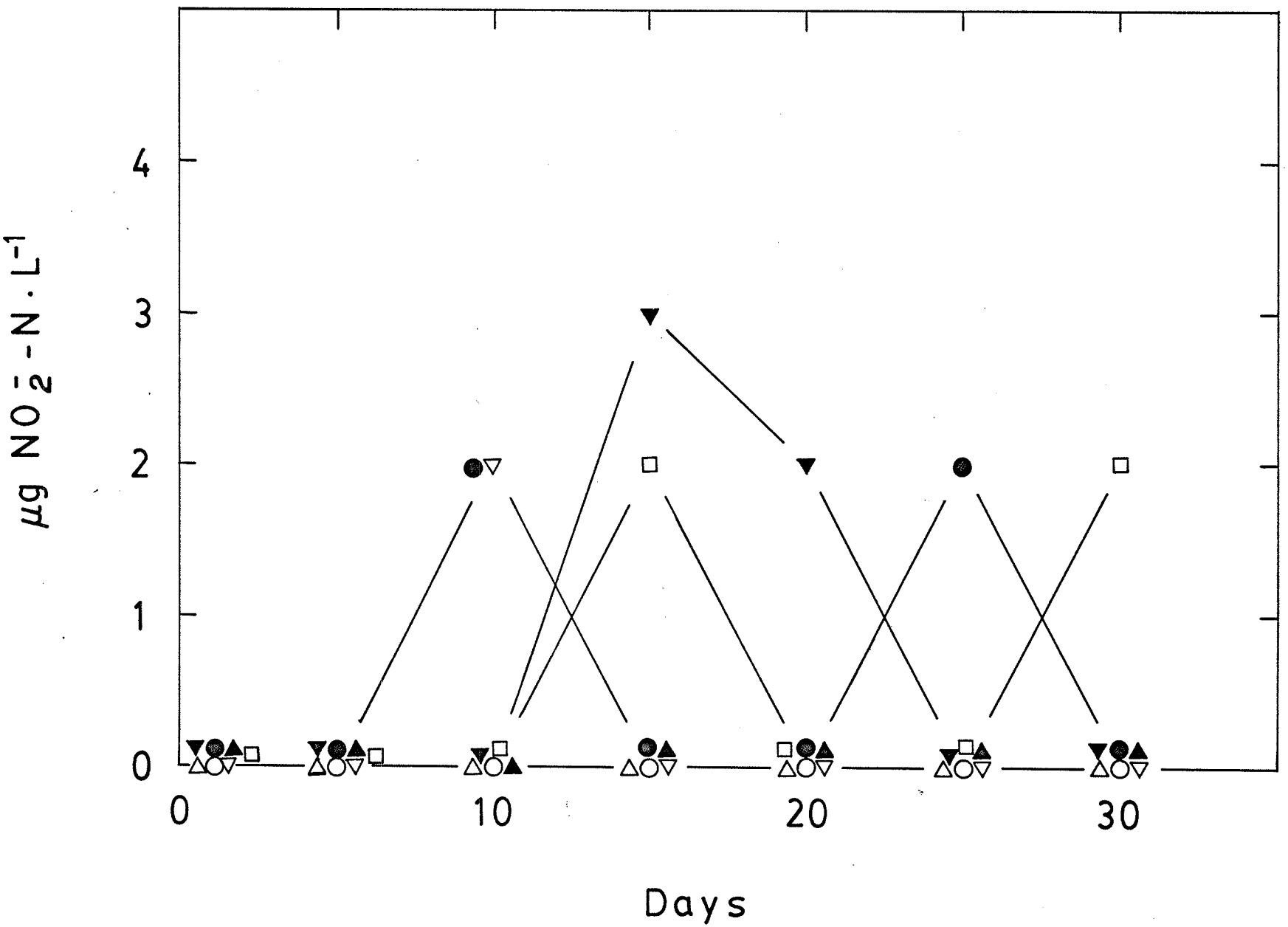

μg NO2-N · L-1
0
1
2
3
4
0
10
20
30
Days

Fig. 115. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in October, 1984 and incubated at 15°C without any supplements. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

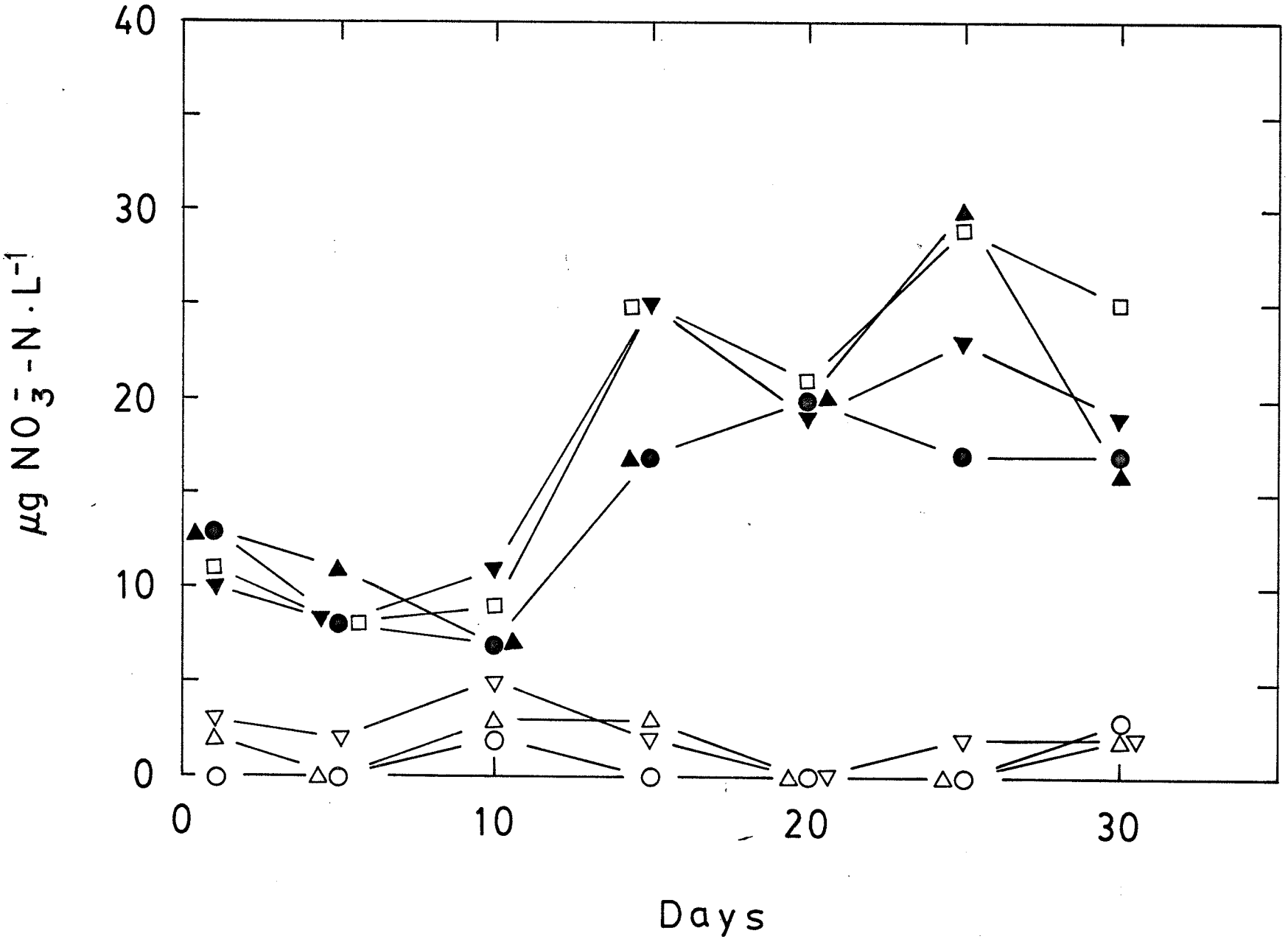

40
30
20
10
0
0
10
20
30
Days
µg NO3- -N . L-1

Fig. 116. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in October, 1984 and incubated at 15°C with NaAc added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0{\cdot}10$).

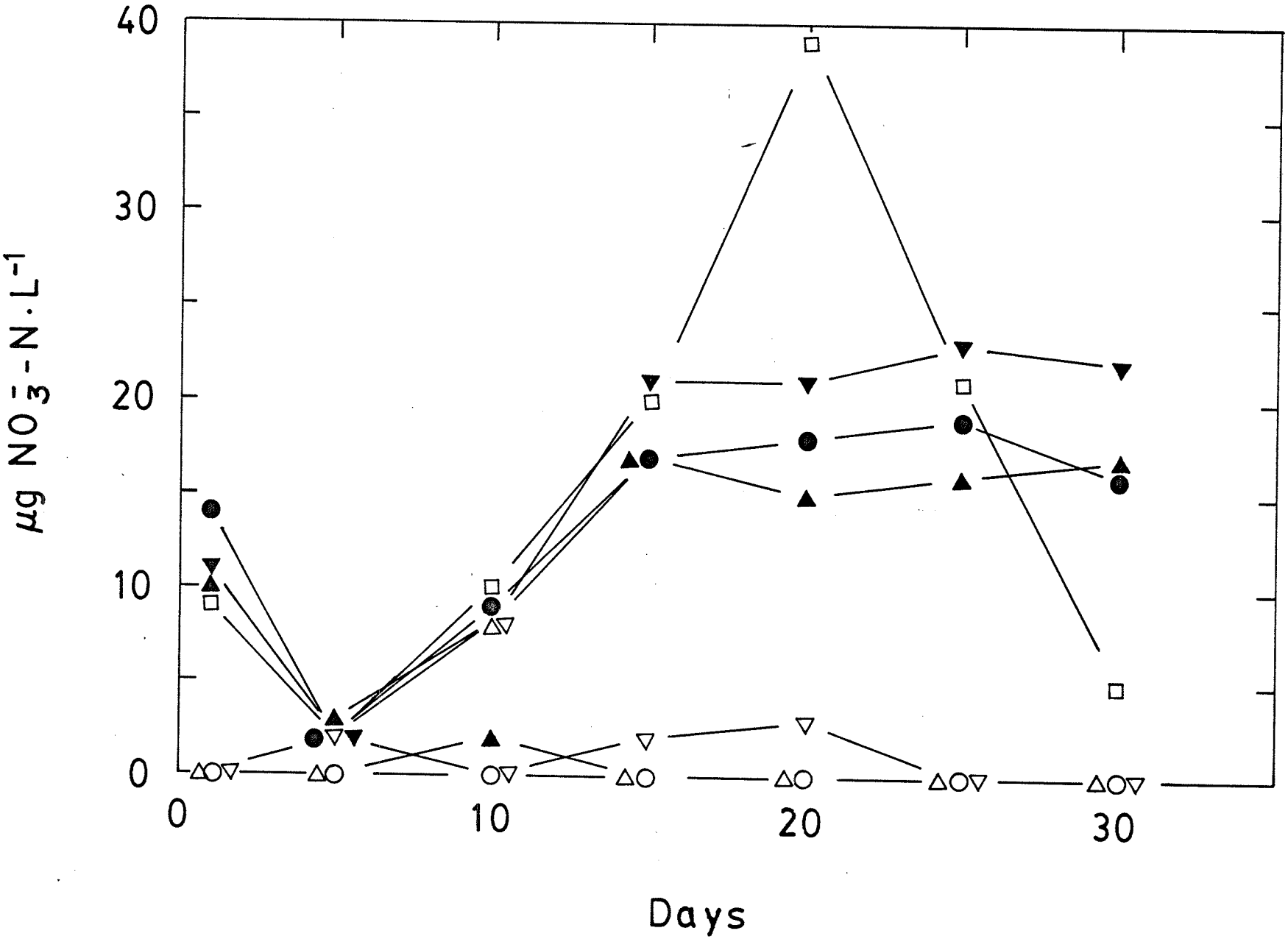

40
30
20
10
0
0
10
20
30
µg NO3-N·L-1
Days

Fig. 117. The production and disappearance of NO_3^- at various water depths of L.302S. Samples were obtained in October, 1984 and incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) 1m, (△) 4m, (▽) 6m, (●) 7m, (▲) 8m, (▼) 9m and (□) 10m. All data points that are overlapping or placed horizontally on the same line for the same day of sampling are not significantly different ($p = 0.10$).

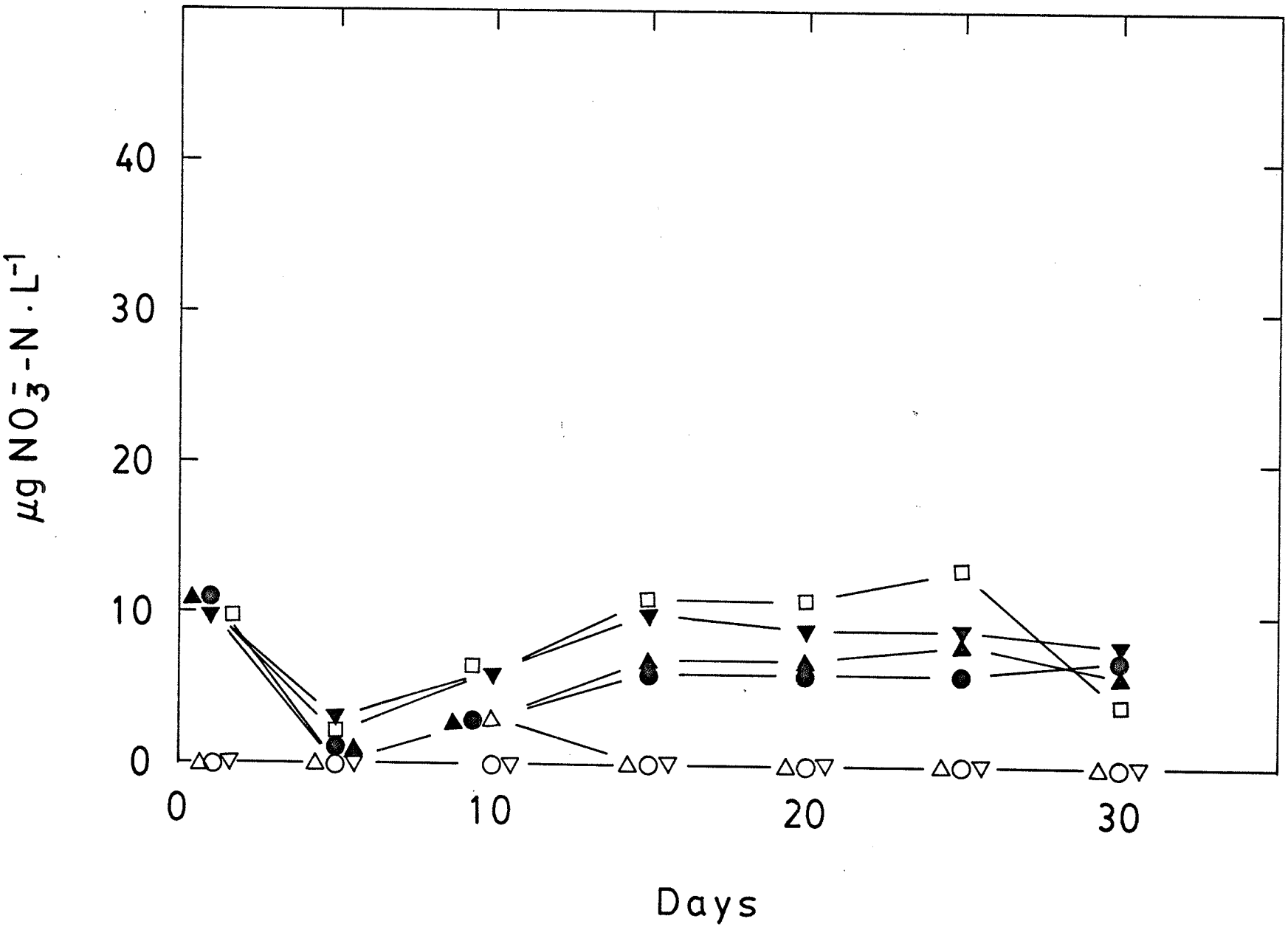

40
30
20
10
0
0
10
20
30
Days
μg NO3-N · L-1

Fig. 118. The increments of pH during heterotrophic nitrification at various water depths of L.239. Samples were incubated at 15°C without any supplements. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (◒) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0.10$).

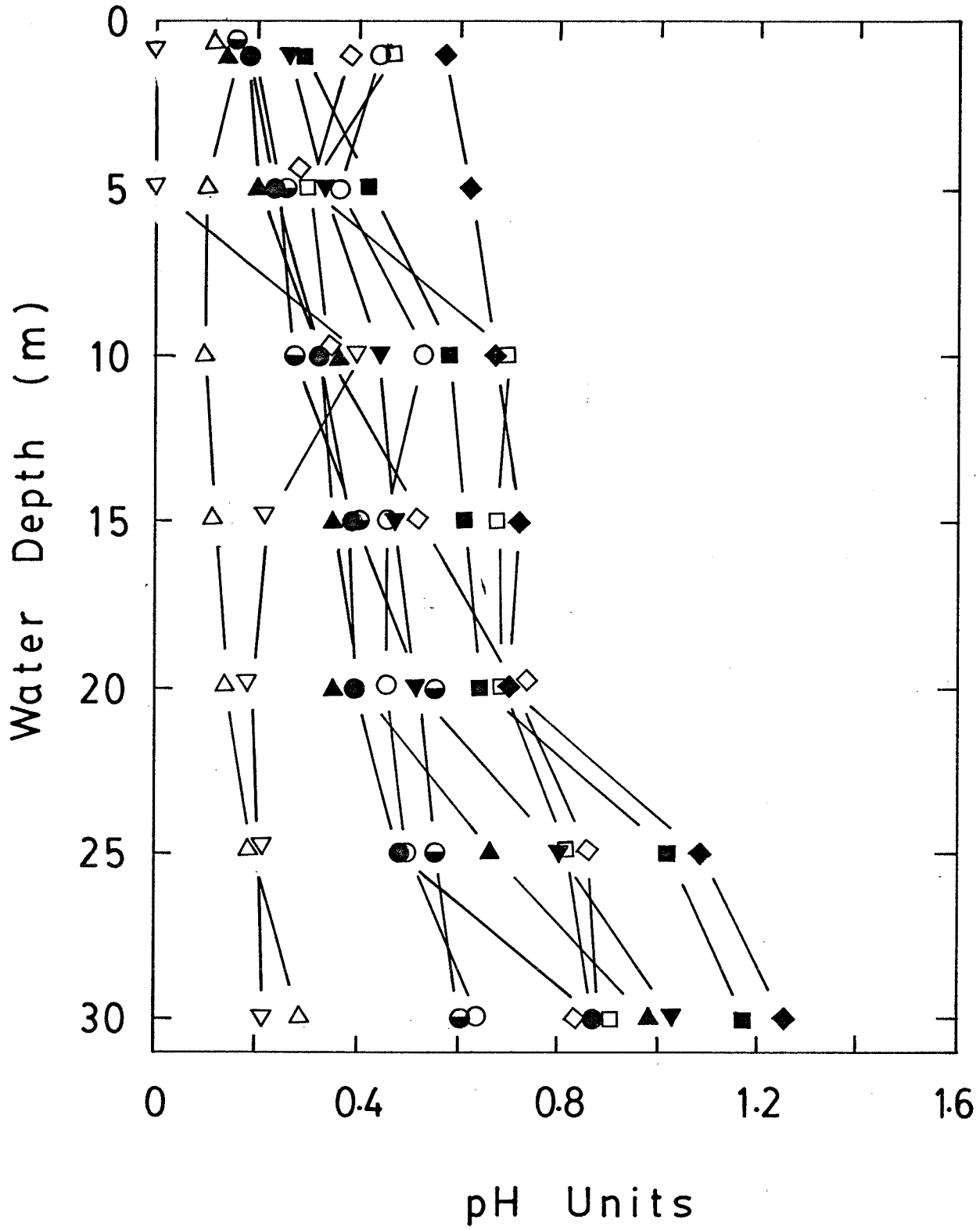

0
5
10
15
20
25
30
Water Depth (m)
0
0·4
0·8
1·2
1·6
pH Units

Fig. 119. The increments of pH during heterotrophic nitrification at various water depths of L.239. Samples were incubated at 15°C with NaAc added. Symbols are : (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

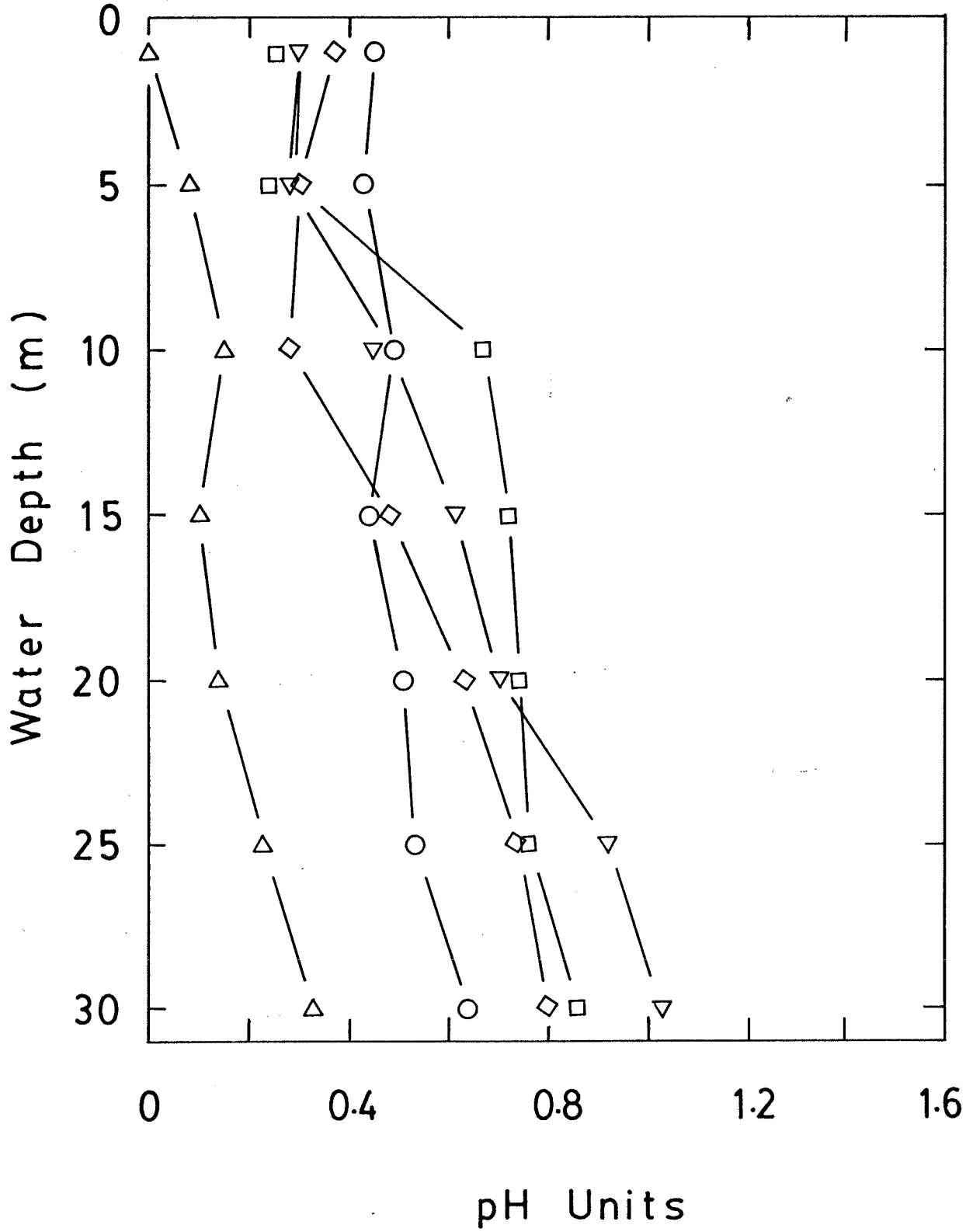

0
5
10
15
20
25
30
Water Depth (m)
0
0·4
0·8
1·2
1·6
pH Units

Fig. 120. The increments of pH during heterotrophic nitrification at various water depths of L.239. Samples were incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

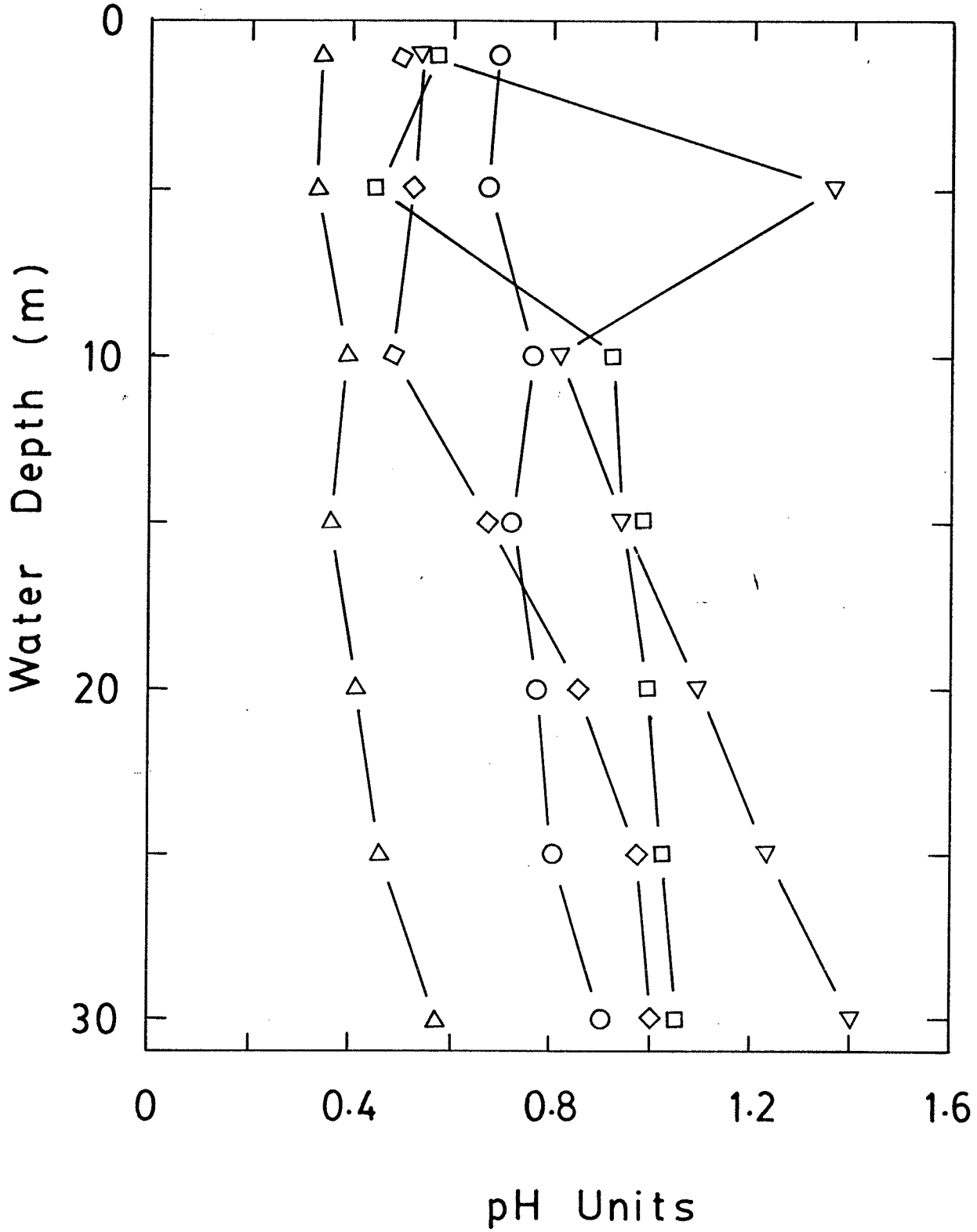

0
10
20
30
Water Depth (m)
0
0·4
0·8
1·2
1·6
pH Units

Fig. 121. The increments of pH during heterotrophic nitrification at various water depths of L.302S. Samples were incubated at 15°C without any supplements. Symbols are : (●) December, (▲) January, (▼) February, (■) March, (◆) April, (○) May, (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

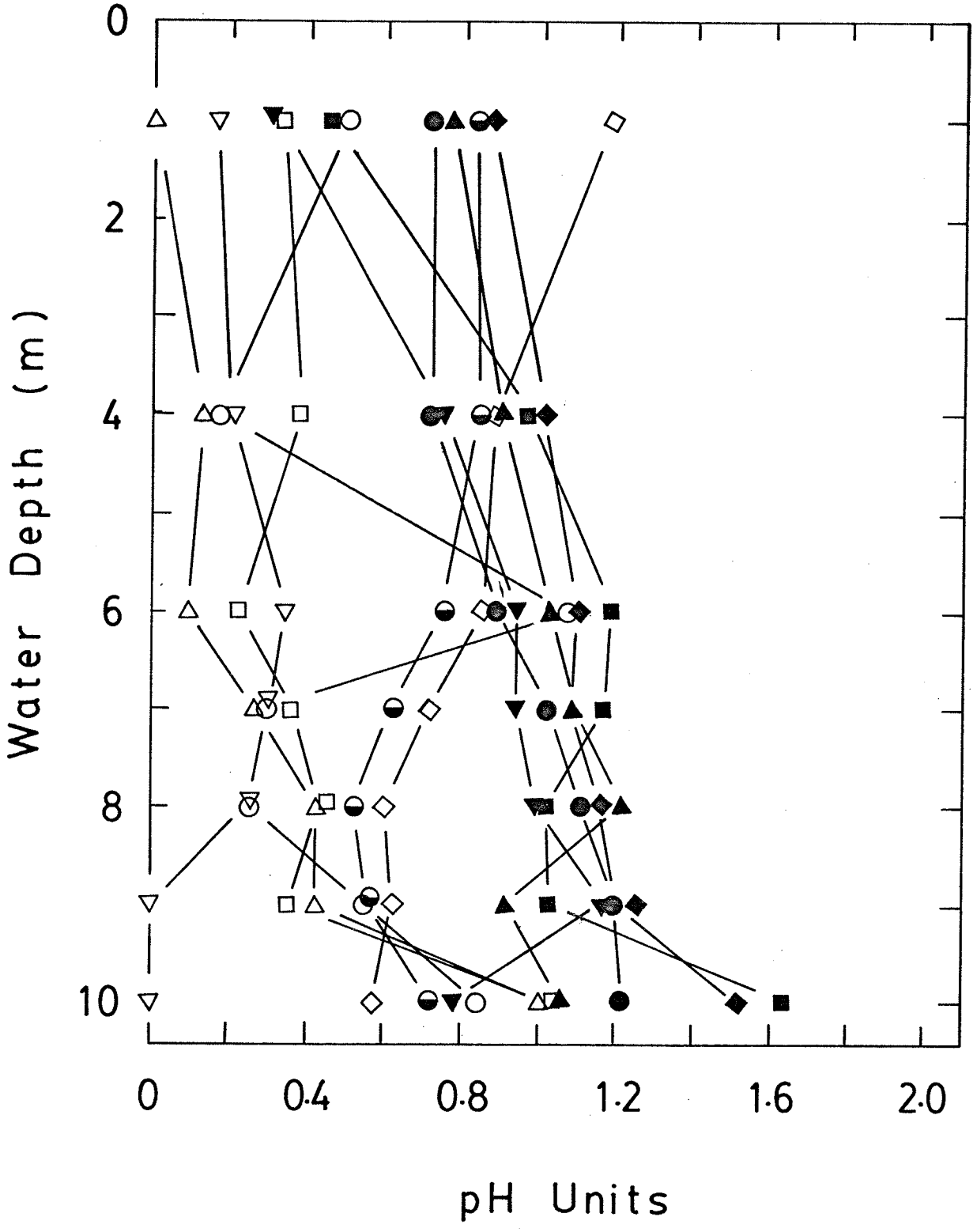

0
2
4
6
8
10
Water Depth (m)
0
0·4
0·8
1·2
1·6
2·0
pH Units

Fig. 122. The increments of pH during heterotrophic nitrification at various water depths of L.302S. Samples were incubated at 15°C with NaAc added. Symbols are : (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different (p = 0·10).

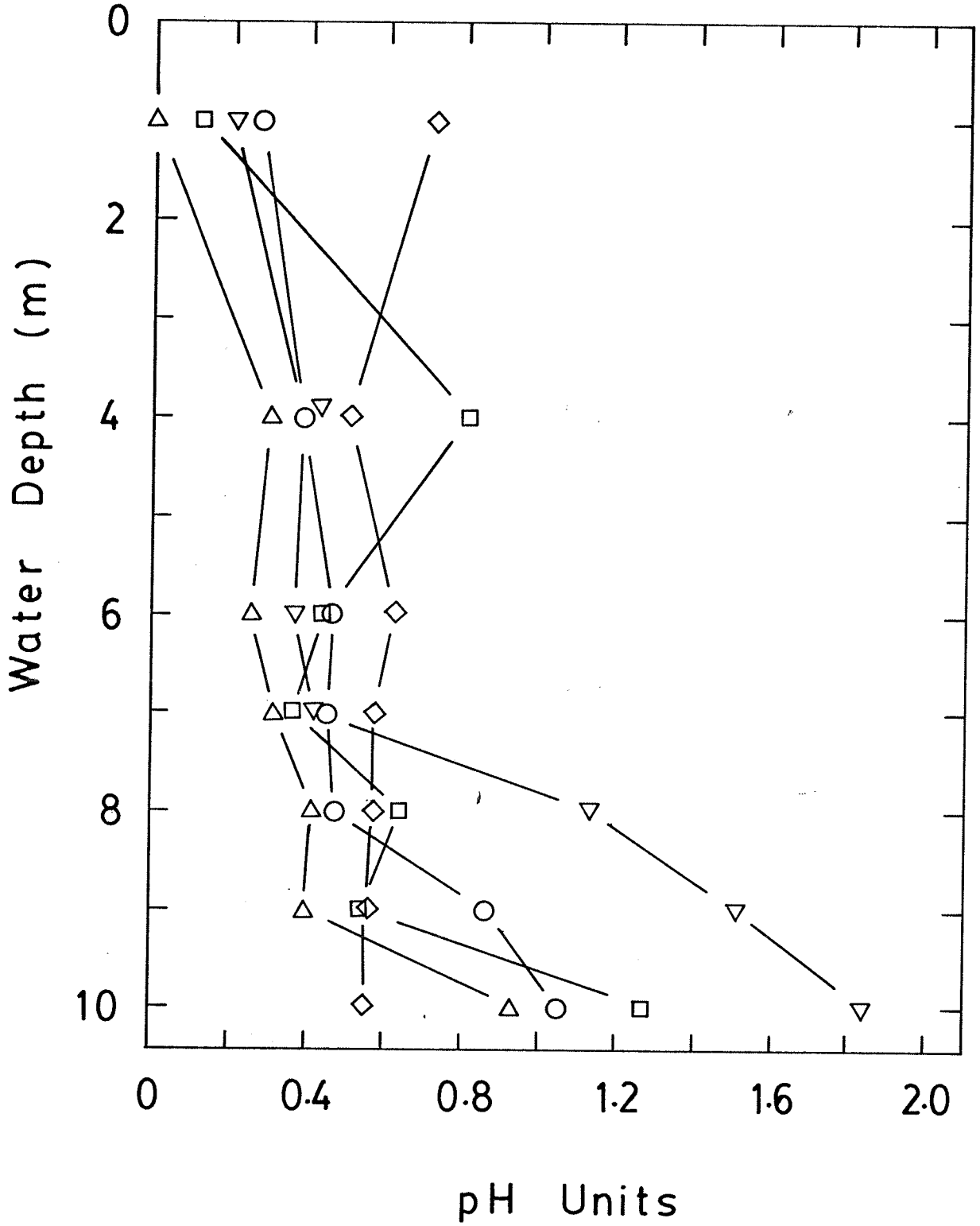

0
2
4
6
8
10
Water Depth (m)
0
0.4
0.8
1.2
1.6
2.0
pH Units

Fig. 123. The increments of pH during heterotrophic nitrification at various water depths of L.302S. Samples were incubated at 15°C with NaAc and $(NH_4)_2SO_4$ added. Symbols are : (○) June, (△) July, (▽) August, (□) September and (◇) October. All overlapping data points are not significantly different ($p = 0{\cdot}10$).

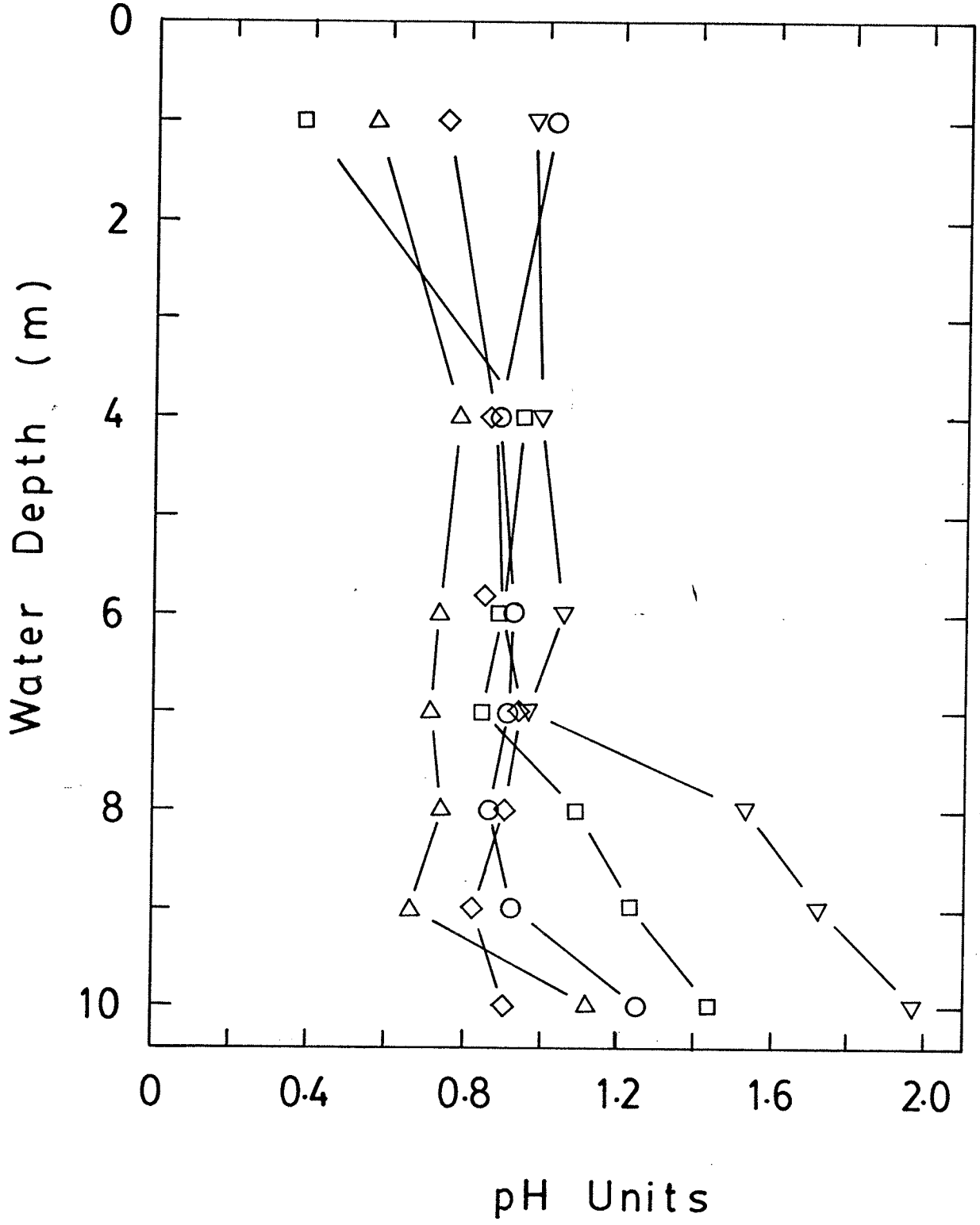

0
2
4
6
8
10
Water Depth (m)
0
0·4
0·8
1·2
1·6
2·0
pH Units

Fig. 124. The production and disappearance of NO_3^-, NO_2^- and NH_4^+ (A) and the effects of NH_4^+ (B) and allylthiourea (C) on NO_3^- production at 30 m of L.239. Samples were obtained in March, 1984 and incubated at 15°C. Symbols in (A) are : (○) NO_3^-, (▽) NO_2^- and (△) NH_4^+. Symbols in (B) are : (○) Control and (□) NH_4^+ added. Symbols in (C) are : (○) Control and (□) allylthiourea added. Bars represent standard deviation of duplicate samples.

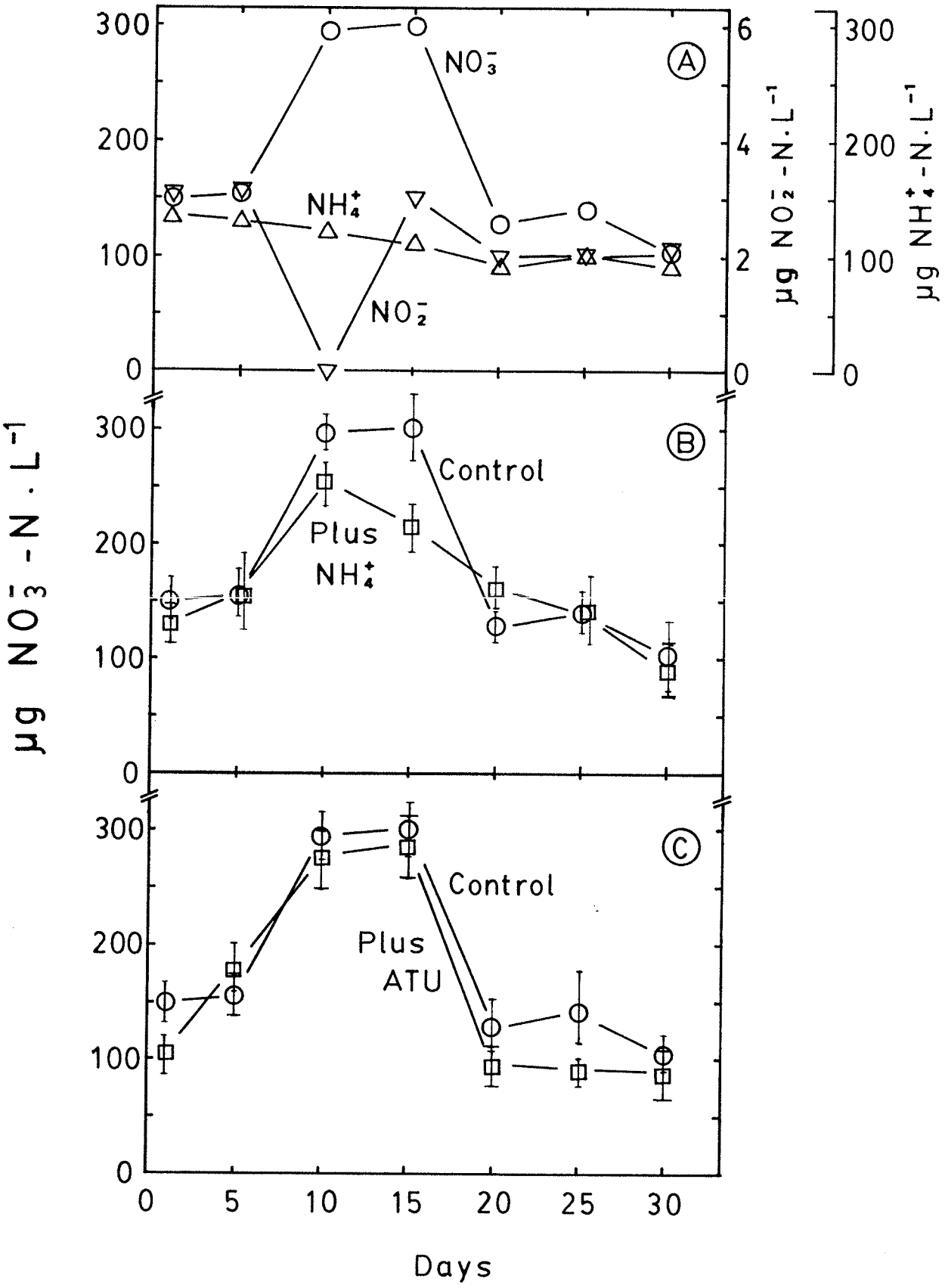

μg NO3- -N · L-1
300
200
100
0
NO3-
NH4+
NO2-
A
6
4
2
0
μg NO2- -N · L-1
300
200
100
0
μg NH4+ -N · L-1
B
Control
Plus NH4+
C
Control
Plus ATU
0
5
10
15
20
25
30
Days

Fig. 125. The production and disappearance of NO_3^-, NO_2^- and NH_4^+ (A) and the effects of NH_4^+ (B) and allylthiourea (C) on NO_3^- production at 9 m of L.302S. Samples were obtained in March, 1984 and incubated at 15°C. Symbols in (A) are : (○) NO_3^-, (▽) NO_2^- and (△) NH_4^+. Symbols in (B) are : (○) Control and (□) NH_4^+ added. Symbols in (C) are : (○) Control and (□) allylthiourea added. Bars represent standard deviation of duplicate samples.

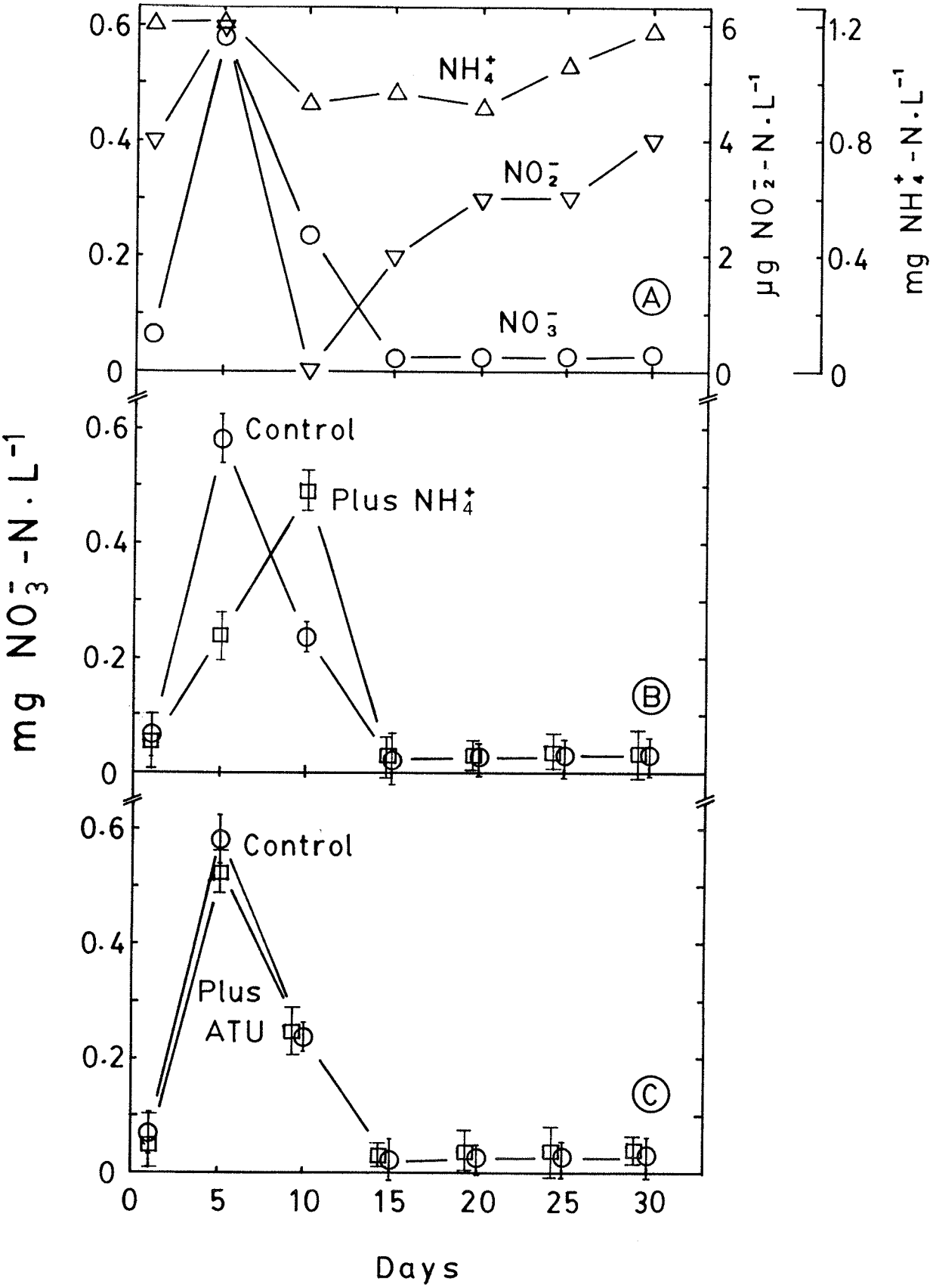

0.6
0.4
0.2
0
6
4
2
0
1.2
0.8
0.4
0
NH_4^+
NO_2^-
NO_3^-
A
$\mu g\ NO_2^- \text{-} N \cdot L^{-1}$
$mg\ NH_4^+ \text{-} N \cdot L^{-1}$
Control
Plus NH_4^+
B
Control
Plus
ATU
C
$mg\ NO_3^- \text{-} N \cdot L^{-1}$
0
5
10
15
20
25
30
Days

Fig. 126. The *in situ* concentrations of dissolved N_2O (○) and the *in vitro* rates of N_2O production (●) at various water depths of L.239 in March, 1984.

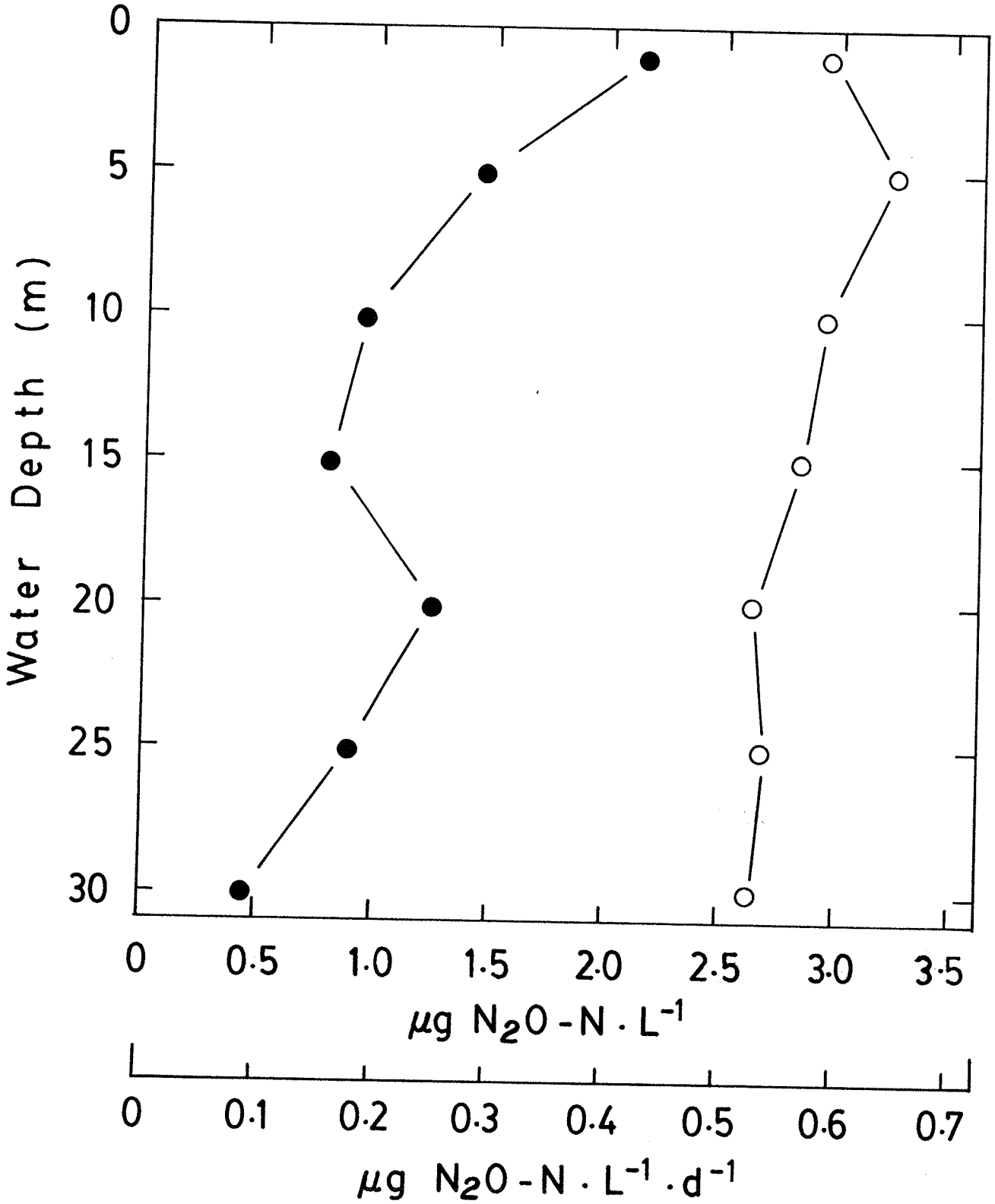

Water Depth (m)
0
5
10
15
20
25
30
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
μg N2O-N · L-1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
μg N2O-N · L-1 · d-1

Fig. 127. The *in situ* concentrations of dissolved N_2O (○) and the *in vitro* rates of N_2O production (●) at various water depths of L.302S in March, 1984.

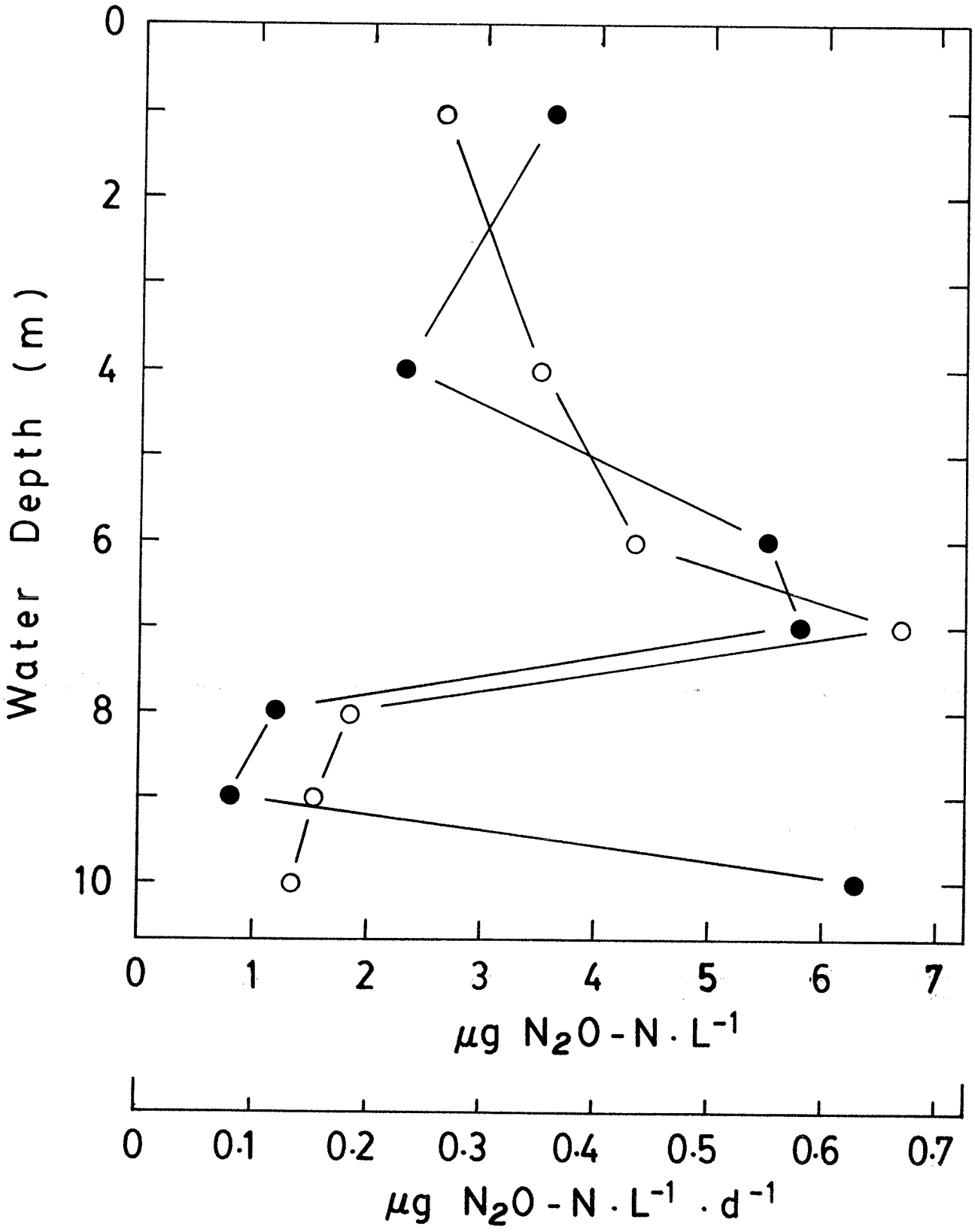
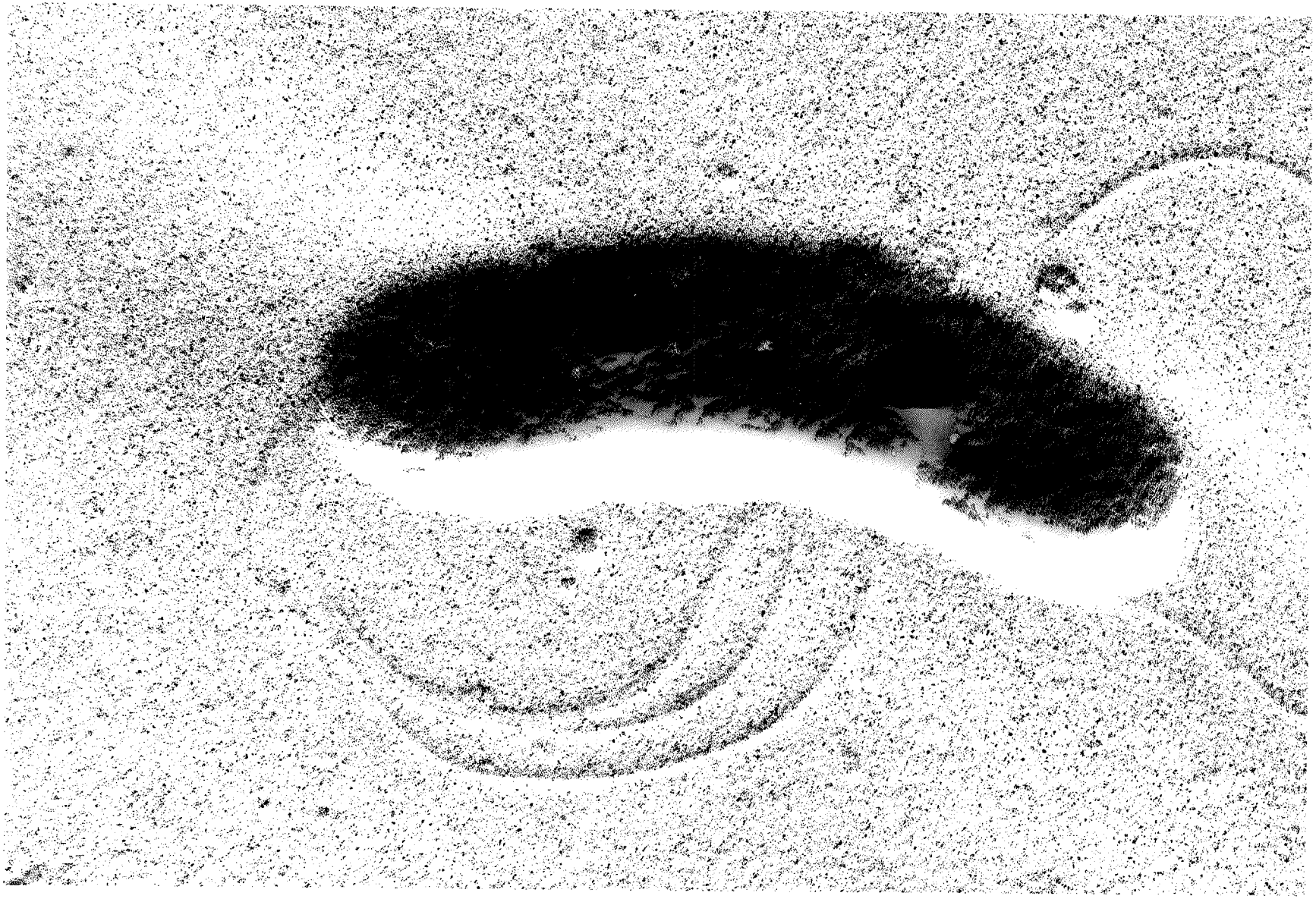

Water Depth (m)
0
2
4
6
8
10
0 1 2 3 4 5 6 7
μg N2O - N · L-1
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
μg N2O - N · L-1 · d-1

Fig. 128. Electron micrograph of the heterotrophic nitrifying isolate, Pseudomonas fluorescens. (59,568X magnification).



D I S C U S S I O N

D I S C U S S I O N

The qualitative and quantitative ecological significance of heterotrophic nitrification in freshwater lacustrine ecosystems was demonstrated by the present study. The ecological importance of heterotrophic nitrification has not previously been recognized, reported or validated. Focht and Verstraete (1977) suggested that this mode of microbial activity may possibly be environmentally significant in only two types of extreme ecological niches, namely acid soils and highly alkaline, nitrogen-rich aquatic environments such as sewage sludge. The two lakes used for the present study did not fall into these categories, with one being oligotrophic and at near neutral pH and the other one being meso-oligotrophic and at acidic pH. Moreover, no such in situ heterotrophic nitrification in highly alkaline, nitrogen-rich aquatic ecosystems has been reported in literature. Verstraete and Alexander (1973) reported that some water samples from rivers, lakes and sewage produced NH_2OH, 1-nitrosoethanol, NO_2^- and NO_3^- only when the samples were amended with acetate and $(NH_4)_2SO_4$, incubated on a rotary shaker and adjusted to neutral or alkaline pH. They attributed the production of NO_2^-, NO_3^-, etc., in the water samples to heterotrophic nitrification. They also noticed the increase in pH during the nitrification process and in the case of a sewage sample, the pH rose from 7·4 to 9·0.

On the other hand, the present study used several direct and indirect tests to confirm the occurrence of heterotrophic nitrification in both L.239 and L.302S. Indirect evidence comprised (a) the lack of stimula-

tion of NO_2^- and NO_3^- production following the addition of $(NH_4)_2SO_4$. In fact the production was sometimes depressed by the presence of added NH_4^+. These findings are in agreement with the observations of Schimel et al. (1984); (b) the addition of NaAc to give a final C:N ratio of 3:1 stimulated the production of NO_3^-; similar phenomena have been observed with pure cultures of heterotrophic nitrifiers and in soil and water samples (Verstraete, 1975; Verstraete and Alexander, 1972a; 1973); (c) the production of alkalinity during NO_2^- and NO_3^- production; similar results have been reported by Castignetti and Gunner (1981) and Verstraete and Alexander (1973); (d) the disappearance of the produced NO_2^- without the concomitant increase in NO_3^- concentrations under aerobic conditions. Castignetti and Gunner (1981) also noticed the rapid assimilation of the produced NO_2^- by Alcaligenes sp. under acidic conditions but were unable to explain the observations; and (e) the disappearance of NO_3^- without the concomitant increase in NH_4^+ concentrations under aerobic and NH_4^+-nonlimiting conditions. Direct evidence comprised (a) the lack of inhibition of NO_2^- and NO_3^- production in the presence of autotrophic nitrification inhibitors, allylthiourea. Similar observations have previously been reported (Focht and Verstraete, 1977; Hynes and Knowles, 1983; Schimel et al., 1984; Verstraete, 1975); (b) the absence of culturable autotrophic nitrifying bacteria in the lake water samples. Many workers studying heterotrophic nitrification in acid soils had similar observations (Ayanaba and Omayuili, 1975; Focht and Verstraete, 1977; Ishaque and Cornfield, 1974; 1976; Lemee, 1976; Overrein 1971); (c) the presence of a large number of heterotrophic nitrifying bacteria in the samples that were able to grow on autotrophic nitrifying

media and also at low pH and low temperatures, and (d) the ^{15}N tracer techniques used in the present study indicated that the N atoms in NO_2^- and NO_3^- produced in the lake water samples were not derived from NH_4^+-N. Schimel et al. (1984) reported similar findings for heterotrophic nitrification in acid forest soils.

Although the additions of NaAc or NaAc plus NH_4^+ stimulated NO_2^- production in the present study, NO_2^- and NO_3^- were also produced in some samples without any amendments. The experimental designs would critically influence the subsequent interpretations and extrapolations of the results obtained. Verstraete and Alexander (1973) added abnormally high concentrations of C and N source to their samples and yet only some of their samples did produce NO_2^- and NO_3^-. Subsequently, they concluded that heterotrophic nitrification would only be of ecological significance in ecosystems having such high levels of C and N loadings. In addition, none of their experiments included any autotrophic nitrification inhibitor as a control. The present study, however, appears to be more representative of natural lake conditions. Every treatment used in the present study included a control with an autotrophic nitrification inhibitor added so that any portion of NO_2^- and NO_2^- produced by autotrophic nitrifying bacteria can be estimated. In addition, the incubation temperatures used and the amounts of NH_4^+ or NaAc added in the present work were within the range of the natural environmental conditions of the two lakes studied. This was possible because of the availability of the physical, chemical and biological records before, during and after the perturbations of the two lakes. The results presented here could possibly be applied to other precambrian Shield lakes in Canada.

The accumulation of NO_2^- in lakes and oceans is quite unusual (Painter, 1970). Few studies reported the accumulation of NO_2^- at particular depths of some waters (Karl et al., 1984; McCarthy et al., 1984; White et al., 1977). This phenomenon is usually attributed to: (a) higher rates of NH_3 oxidation to NO_2^- than the rates of NO_2^- oxidation to NO_3^-; (b) the lack of autotrophic NO_2^- oxidizers; and (c) reduced oxygen tensions allowing NH_3 oxidizers but not NO_2^- oxidizers to nitrify. However, the accumulations of NO_2^- in the two lakes studied during the ice-cover periods cannot be explained with the facts mentioned above. Since the nitrification process involved was heterotrophic in nature and yet the pathways and the mechanisms involved are not known, only tentative explanations are permissible on the basis of in vitro and in situ results obtained from the present study. It appeared that the in situ acid pH and low temperature did not inhibit the production of NO_2^-. Previous studies on pure cultures of heterotrophic nitrifiers and heterotrophic nitrification in acid soils showed that NO_2^- and other nitro compounds were the main products of heterotrophic nitrification (Castignetti and Hollocher, 1982; Gode and Overbeck, 1972; Gowda et al., 1977; Hirsch et al., 1961; Jensen, 1951; Malashenko et al., 1980; Odu and Adeoye, 1970; Tate, 1977).

The rapid disappearance of NO_2^- and NO_3^- under aerobic and NH_4^+-non-limiting conditions suggested that in some samples the rates of NO_2^- and NO_3^- disappearance were probably much greater than their rates of production resulting in small amount or no NO_2^- and NO_3^- accumulated. Consequently, the occurrence of heterotrophic nitrification cannot be estimated solely by the amount of NO_2^- and NO_3^- produced. The fact that the pH

of these samples did increase suggested that heterotrophic nitrification had occurred. Castignetti and Gunner (1981) reported the production of NO_2^- by Alcaligenes sp. under both alkaline and acidic conditions and that the produced NO_2^- was rapidly assimilated under acidic conditions. However, they did not offer any explanations. The mechanisms involved are still not understood. Axler et al. (1982) used ^{15}N and ^{13}N tracer techniques to study nitrogen assimilation in a subalpine lake and concluded that NH_4^+ was the preferred N-substrate for assimilation even when NH_4^+ concentrations were far lower than the concentrations of NO_2^- and NO_3^- present.

The present study indicated that heterotrophic nitrification was responsible for the production of NO_2^- and NO_3^- in the two lakes studied and that it occurred throughout the water columns of both lakes during the ice-cover periods. Focht and Verstraete (1977) compared the rates of heterotrophic and autotrophic nitrification by pure cultures that were available in literature and concluded that heterotrophic nitrification would be insignificant because their rates were 10^3 to 10^4 times lower than those of autotrophic nitrifiers. In L.239, highest rates of NO_2^- accumulation were noted in August at 25-m depth, whereas in L.302S it occurred in January at the 7-m depth. The highest rates of NO_3^- accumulations of both lakes occurred in March, 1984 being 19·2 and 128·75 ug NO_3^--N·L^{-1}·d^{-1} at the 30-m depth of L.239 and 9-m depth of L.302S, respectively. These rates were much higher than the autotrophic nitrification rates reported in the literature. Vincent and Downes (1981) found that the oligotrophic Lake Taupo in New Zealand had autotrophic nitrification rates of 0·50-4·03 ug NO_3^--N·L^{-1}·d^{-1}. In Lake Vanda, Ant-

arctica, these rates were 0·08-1·95 ug NO_3^--N·L^{-1}·d^{-1} (Vincent et al., 1981). The rates in the mesotrophic Lake Grasmere, England, were in the range of 5·49-6·86 ug NO_3^--N·L^{-1}·d^{-1} (Hall and Jeffries, 1984). Lean and Knowles (1982) reported that the autotrophic nitrification rate under the ice-cover periods in the eutrophic Lake St. George, Ontario, was 10 ug NO_3^--N·L^{-1}·d^{-1}. They also noted that nitrification occurred throughout the water column of the lake during the ice-cover periods. However, the autotrophic nitrification rate reported for Lake St. George sometimes did not fully account for the total NO_3^- production (R. Knowles, personal communication). Methylotrophic bacteria are also considered as heterotrophic nitrifiers because of their ability to nitrify NH_3 (Hutton and ZoBell, 1953; O'Neill and Wilkinson, 1977; Malashenko et al., 1980). However, the involvement of these bacteria in the heterotrophic nitrification process of the two lakes studied are unlikely since methylotrophic bacteria nitrify NH_3 but not organic nitrogen and their nitrifying activity is inhibited by autotrophic nitrification inhibitors such as N-Serve (Salvas and Taylor, 1984).

The growth of heterotrophs on the autotrophic nitrifying agar media suggested, rather than their capability to fix CO_2, the presence of some unknown organic carbon compounds in the media. This was probable since a trace amount of arganic carbon could be present as contaminants in the water, chemicals or the agar used (Harrison, 1984). On the other hand, the number of heterotrophic nitrifiers estimated for each selected water depth did not correlate to their nitrifying activities since the amounts of NO_2^- and NO_3^- produced were not proportional to the population size. Indeed, Verstraete (1975) pointed out that the heterotrophic nitrifica-

tion activity was most often not associated with cell growth nor proportional to the overall cellular biomass.

The production of N_2O during heterotrophic nitrification was confirmed by the present study. The rates of N_2O production appeared to be correlated with the heterotrophic nitrification activities. The production of NO (Verstraete, 1981) and N_2O (Topp and Knowles, 1982) during heterotrophic nitrification by methylotrophs has previously been reported. The accumulation of high concentrations of N_2O in the surface water of the two lakes was probably the result of two factors: (a) the waters were under the ice cover thus preventing any dissolved N_2O in the surface water from escaping to the atmosphere, and (b) high N_2O production rates in the surface waters were probably in part due to the presence of algae (DeBruyn et al., 1984) actively producing N_2O. It has been demonstrated that some green algae were capable of nitrification (Kessler and Oesterheld, 1970; Spiller et al., 1976) and that the produced NO_2^- was converted to N_2O by most of the green algae commonly found in aquatic ecosystems (Weathers, 1984).

The production of alkalinity via heterotrophic nitrification in both neutral and acid lake waters was clearly demonstrated by the present study. The findings are in good agreement with observations by other workers (Castignetti and Gunner, 1981; Verstraete and Alexander, 1973) and have important implications regarding the lake acidification process. The addition of a metabolizable carbon source, which is also essential for denitrification and sulfate reduction to occur, stimulated heterotrophic nitrification. While denitrification and sulfate reduc-

tion, which only occur in anoxic sediments, are considered as the major bacterial processes to provide alkalinity against lake acidification, nitrification has been speculated to increase the acidity of lakes in ELA (Kelly et al., 1982). In contrast, the present study clearly indicated that heterotrophic nitrification was the sole source of NO_2^- and NO_3^- production in the two lakes at ELA and that the process would possibly provide alkalinity against lake acidification. The advantage of having heterotrophic nitrification would be two fold. Firstly, it is an aerobic process that provides alkalinity in oxygenated waters and sediments, i.e. not being restricted to the anoxic sediments. Secondly, most of the heterotrophic nitrifying bacteria have also been found to be active denitrifying bacteria under anaerobic conditions (Castignetti and Hollocher, 1981; 1984). Conversely, the NO_2^- and NO_3^- produced by heterotrophic nitrifying bacteria under aerobic conditions could be denitrified by the same microorganisms and other denitrifying bacteria under anaerobic conditions. The latter process, denitrification, is known to produce alkalinity. Consequently, the possibility of monitoring the heterotrophic nitrification process in nature to provide alkalinity against lake acidification should not be overlooked.

The disadvantages of having such microorganisms actively oxidizing nitrogen-containing compounds are due to the many end products other than NO_3^- such as NO_2^-, NH_2OH, nitro compounds and nitroso compounds produced during heterotrophic nitrification. These compounds are toxic and some of them are mutagens or carcinogens (Hayes, 1964; Venulet and Van-Ettan, 1970). According to Verstraete (1975), due to their stability in natural waters, the presence of these compounds in the natural environ-

ments, even at a concentration of 1 uM, could be hazardous to human and animals. However, it appears that the environmental conditions dictate the types and the quantities of end products to accumulate in nature. Clearly, more studies on the mechanisms of heterotrophic nitrification are needed.

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