J. Appl. Ichthyol. 18 (2002), 87–94

ISSN 0175–8659

Received: November 27, 2000
Accepted: July 30, 2001

Sublethal effects of aquatic pollution in Lake Maryût on the African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822)

By K. G. Adham

Zoology Department, Faculty of Science, University of Alexandria, Moharram Bey, Alexandria, Egypt

Summary

Some aspects of the physiology and biochemistry of the African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), were studied along with an array of physicochemical characteristics of the water in Lake Maryût, Egypt. Data were compared to those of a reference fish hatchery. At least 11 of the conventional water pollutants (Cd, Cu, Fe, Pb, Mn, Hg, Ni, Zn, turbidity, chemical oxygen demand and ammonia nitrogen) were elevated in the most polluted main basin of the lake. In turn, serologic analysis of the indigenous catfish *C. gariepinus* pointed at functional impairment of the liver, heart and kidney as reflected by the elevated activities of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, cholinesterase, glucose and creatinine. Reduced nucleic acid measurements [the ratio RNA/DNA and the relative RNA content (r)] indicated diminished protein synthesis and impaired growth in polluted fish. In some instances, glucose and nucleic acid measures were elevated in favour of fish from moderately polluted basins of the lake rather than in reference fish. Fish seemed to profit from the typical geographical habitat in the lake, regardless of the virtual contamination there, versus reference fish that seemed to suffer congestion stress and food competition in the confined and overcrowded commercial pond.

Introduction

Inland waters, including seas, rivers and lakes, receive massive flux loaded with industrial and anthropogenic wastes that can exert a huge impact on aquatic life. In Egypt, lakes of the Nile Delta that used to be of special economic and social importance are now under potential risk due to unremitting runoff of several types of polluted discharge. Lake Maryût, the smallest and most polluted of these, is situated to the south of the city of Alexandria (5 million inhabitants) along the Mediterranean Sea at latitude 31°10′ N and longitude 29°55′ E (Fig. 1). As a result of pollution, this lake that used to be one of the most productive nationwide, became highly eutrophic with greatly diminished fish production. The lake environment became a substantial threat to native fish, higher-rank predators, fishermen and human consumers. This was concurrent with a significant reduction in its size due to reclamation projects, road construction and other urbanization schemes. Lake Maryût thus became artificially disjoined into five principal basins (Fig. 1): the main basin (MB) and four smaller basins (East, E; North-west, NW; South-east, SE; South-west, SW).

Physicochemical changes in aquatic environments could drastically influence fish physiology. Blood glucose was reported as a reliable and sensitive indicator for environmental stress in fish (Van Vuren et al. 1994), whereas elevated serum creatinine is routinely used to detect kidney dysfunctions in humans (Kaplan et al. 1988). Because it is excreted through the glomeruli of the kidney, retention thereof could be an index of glomerular insufficiency (Kaplan et al. 1988). Circulating creatinine is derived from metabolism of tissue creatine, and plasma levels would be expected to increase with increased tissue breakdown (Kaplan et al. 1988).

There is mounting evidence that serum enzymes [alanine aminotransferase (ALAT, formerly SGPT); aspartate aminotransferase (ASAT, formerly SGOT); lactate dehydrogenase (LDH); creatine kinase (CK)] possess special diagnostic magnitudes to several pollutant categories (Krajnoviæ-Ozretiæ and Ozretiæ 1987; Nemcsók et al. 1987; Adham et al. 1997). Additionally, the relative RNA and DNA content is used as an integrative indicator of contaminant stress and as a sensitive and rapid indicator for assessing the effects of contaminants on fish growth (Haines 1973; Barron and Adelman 1984; Rodreguez-Ariza et al. 1999). Earlier literature on nucleic acids mainly targeted the liver as a principal metabolic organ in vertebrates. Based on its significance to fish physiology, the gill tissue was used in this study instead. In contrast to land animals, which eliminate nitrogen waste products through the kidney, fish rely heavily on their gills for this function (Swann 2000).

Cholinesterases (ChEs) are a class of serine hydrolases that are ubiquitous in the animal kingdom. Based on substrate specificity and sensitivity toward specific inhibitors, there are two isoenzymes of cholinesterase: cholinesterase (ChE, also known as pseudocholinesterase or buterylcholinesterase) and acetylcholinesterase (AChE, true cholinesterase) (Smith 1983). ChE is secreted by liver cells into the plasma and this is the one that is routinely measured (Moss et al. 1986). AChE is a tissue enzyme found principally in the nervous tissue (gray matter), red cells and muscle. Inaccurately, the term 'cholinesterase' was also used in some of the older literature to refer to the red cell/muscle enzyme. This use is discouraged to avoid confusion. Inhibition of the enzyme AChE by several organophosphate, carbamate and chlorinated hydrocarbon pesticides is well documented in the literature (Becker and Rahmann 1995). However, very few reports dealt with the inhibitory action of pesticidal and non-pesticidal pollutants on the enzyme ChE (Van Vuren et al. 1994).

In field studies, setting apart different types of pollutant is quite complex. As an example, in Alexandria and surroundings, many of the polluting activities are mixed together. These activities include several industries such as textiles, paper and pulp, petrochemicals, oil and soap, fertilizers, tanneries, distilleries, rubber and plastics. Domestic sewage, agronomic effluents and wastes from 'slaughter and cattle-raising houses'

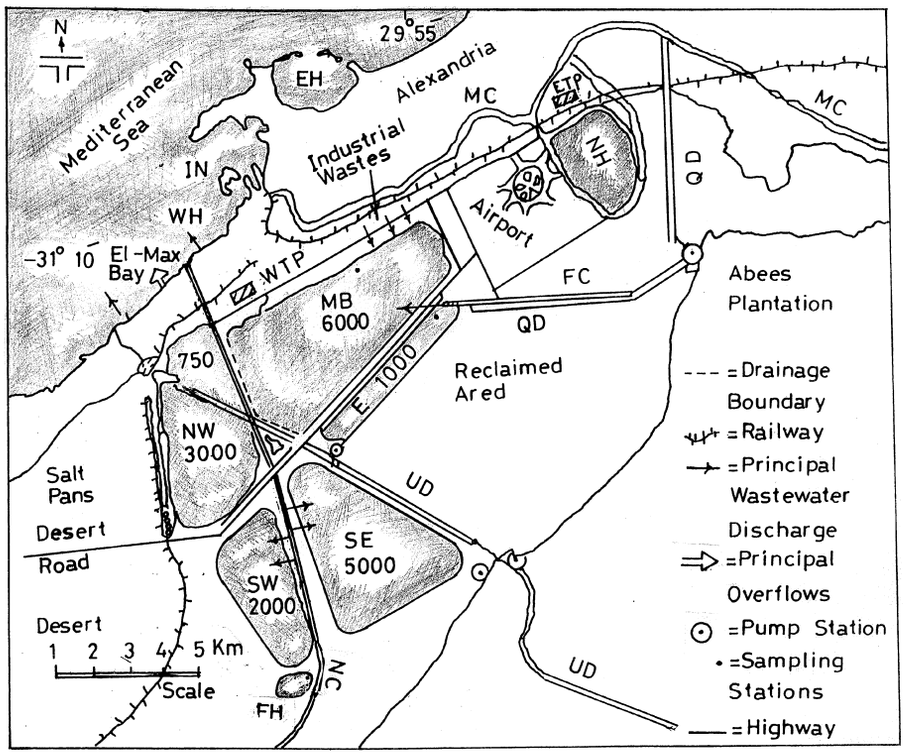


Fig. 1. General surface structure of Lake Maryût and its position in relation to the Mediterranean Sea as well as the supporting canal system; MB, main basin; E, east basin; NW, north-west basin; SE, south-east basin; SW, south-west basin; FH, fish hatchery; NH, Nozha Hydrodrome; EH, east harbor; IN, inner harbor; WH, west harbor; MC, Mahmoudiya Canal; NC, Nubarriya Canal; QD, Qualaa Drain; UD, Umoum Drain; FC, freshwater canal; ETP, east treatment plant; WTP, west treatment plant; sampling stations are shown in the MB, E and FH. For more information about positions, see the Introduction and Materials and Methods

are mixed together with industrial pollutants and all customarily flow into Lake Maryût (El-Rayis and El-Sabrouti 1998). The environment of the lake is formed from the total sum and possible interactions of this mixture. This study aimed to assess the collective pollution effects on some health aspects of aquatic biota using *Clarias gariepinus* as an experimental model.

The Nile sharptooth catfish, *C. gariepinus*, which is a senior synonym of *C. lazera*, is the most common species in rivers, lagoons and estuaries. This fish species grows to 60 cm or more and is of considerable importance as a food fish, particularly in inland areas (Adamek and Sukop 1995). In common with most other clariids, this species is highly resistant to muddy waters and can survive extremes of aquatic deoxygenation and even desiccation (Bok and Jongbloed 1984). It is omnivorous and a general scavenger, with a marked tendency to feed on benthic organisms and detritus (Bok and Jongbloed 1984). *C. gariepinus* is probably the most widely distributed fish in Africa and is found throughout woodland-savanna zones of the Afrotropical region from the Nile to as far south as the Orange system and the East Coast and as far north as the Mediterranean Sea.

To relate fish physiology to environmental impact and to assess how the lake water had deteriorated, a wide array of trace metals (cadmium, Cd; chromium, Cr; cobalt, Co; copper, Cu; iron, Fe; lead, Pb; magnesium, Mg; manganese, Mn; mercury, Hg; nickel, Ni; zinc, Zn) and some other features of water quality [pH, turbidity; dissolved oxygen, DO; chemical oxygen demand, COD; biochemical oxygen demand, BOD; hardness; alkalinity; Cl^-, chlorides and nutrient salts (orthophosphate-phosphorus PO_4^{3-}-P; ammonia-nitrogen, NH_4^+-N; nitrate-nitrogen, NO_3^--N; nitrite-nitrogen, NO_2^--N)] were compared to data of an authorized fish hatchery. Thus, an attempt was made in this study to construct a national record for some serologic and tissue indices in *C. gariepinus* from clean and polluted waters that proved significant in this respect.

Materials and methods

Study area and sampling locations

Main features of the study area and sampling stations along the MB, E basin and FH are shown in Fig. 1. The first station (Forn El-Geraya), where fish and water were sampled, lies at the north-western edge of the MB (6000 acres). The second station (Abu-Azzaam) lies north-west inside the E basin (1000 acres). A fish production pond lying south of the SE basin (5000 acres) of the lake served as the third and reference station ($\sim$ 200 acres); this commercial pond is run by private sector under the auspices of the 'Fish Development Bureau'.

Sampling strategy

Biweekly surveys covering two sampling periods supplied the present data. The first sampling period (I) extended from the beginning of November 1999 until late January 2000, representing the cooler months of the year (autumn/winter). Period II stretched out between early May and mid-July 2000 as the warmer months (spring/summer). Prior to performing the required assays, pilot tests were run on a brief scale for all experimental indices. In these pilot tests, equal numbers ($n = 5$) of either sex of *C. gariepinus* were sampled and analysed for each parameter to test the reliability of considering the sex factor in test groups. No significant differences were found for the same index between the two sexes; data for males and females were thus combined. Metcalfe-Smith et al. (1996) reported among a number of biological factors, including species, size and age, that sex was the least important factor influencing metal concentrations in unionids, amounting to 3% of the overall variability in the data. Similar conclusions were reported for the arctic char and the African catfish (Köck et al. 1995; Adham et al. 1999).

Sampling of fish

A total approximate number of 230 individuals of *C. gariepinus* were caught at random from the selected locations. Fish were trapped in closed meshed nets, maintained alive and quickly transported to the laboratory in large vessels filled with aerated lake water. As much as was possible, all fish used were of uniform size (25.0 ± 5.0 cm) and weight (250.0 ± 28.0 g). In the laboratory, fish were placed in two-thirds-filled 73.5-L glass aquaria (60 × 35 × 35 cm). Fish sampling procedures were undertaken only a few hours after catch; until then fish were allocated into groups (eight individuals/aquarium) according to the specific sampling location. In the aquaria, fish were kept in closed freshwater systems equipped with physical and biological filters and aeration was monitored continuously. In such conditions, fish recovered quickly after capture.

Sampling of lake water

Together with the fish catch, 2 L of water were collected from the particular catch area. Because the depth of Lake Maryût averaged 120 cm (El-Rayis and El-Sabrouti 1998), only two water samples (one surface and one bottom) covering the whole depth were taken each time. Surface water samples were collected about 20 cm below the water surface to avoid floating matter. In all cases, the reported data were averages for both clear measurements that proved to be well mixed. Water samples were filtered in the field using a polypropylene syringe fitted with a 0.45-μm millipore cellulose acetate filter, collected in acid-washed polyethylene bottles and acidified for preservation.

Sampling of blood for biochemical analysis

Immediately after fish recovery, fish were sampled for biochemical and histological analysis. To avoid handling stress reactions, fish were slightly anaesthetized. Blood was rapidly drawn from the caudal vessel using untreated sterile plastic syringes fitted with 21-gauge needles (Hrubec et al. 1997). For serum preparation, blood was allowed to clot on ice for 1 h. Serum was separated from whole blood by centrifugation (model: VM 310) at 14 000 *g* for 5 min. Blood samples from three fish were pooled to give one composite specimen. Eight composite specimens for each type of measurements were analysed for statistical evaluation. Packed cells were discarded and the supernatant serum samples were separated into plain serum tubes and kept at – 4 °C until later use for physiological analysis within a few hours.

Cytological preparation for RNA and DNA determinations

After blood sampling, fish were sacrificed; gill arches were excised and immediately prepared for DNA and RNA determinations as described by Traganos et al. (1977). For making gill blots on glass slides, gills were cut into small portions and then immersed in 40% formaldehyde for 24 h. Gill portions were then removed from formaldehyde and kept in 70% ethyl alcohol until used. Gill portions (comparatively coarse) were then transferred to glass slides and cut again into fine portions with the addition of a few drops of 40% acetic acid, pressing carefully with a spatula until tissue granules became fine and homogenous. Bigger particles were lightly removed with another glass slide. Gill samples were allowed to dry in air and thus became ready for staining within 1 h. From each individual gill tissue, two samples were prepared on separate slides, one for the determination of DNA and the other for RNA.

Analysis of lake water

Trace element determinations in the lake water were conducted at three random intervals in each sampling period. However, other physicochemical characteristics of water were measured only once because of the high cost of analysis. Concentrations of heavy metals were measured in filtered lake water according to Riley and Taylor (1968). Except for Hg, metals were measured by graphite furnace atomic absorption spectroscopy (Perkin-Elmer model 2380) under the recommended conditions and the detection limits (DL) in the manual for each metal. Hg was measured by cold vapour atomic absorption spectroscopy (Perkin-Elmer model 3100) at DL = 0.03. Other physicochemical qualities of water [pH, turbidity, DO, COD, BOD, hardness, alkalinity, chlorides, and nutrient salts (PO_4-P, NH_3-N, NO_3-N, NO_2-N)] were measured by standard methods for the analysis of natural and treated wastewater as described by APHA (1992).

Colorimetric determinations of blood serum

For spectrophotometric determinations, Ultraspec III, Pharmacia LKB Biochrom Ltd, was used as an experimental device. Serum samples were incubated in color reaction at 25 °C; incubation period varied, according to the manufacturer's recommendations for each set of assays. Absorbance was detected at an appropriate wavelength ranging from 330 to 550 nm according to the parameter tested. ALAT and ASAT were measured according to IFCC (1986) using commercial kits (Ecoline 25, Merck KGaA, 64271, Darmstadt, Germany). LDH was measured according to Bergmeyer and Bernt (1974) using kits from the Human Gesellschaft für Biochemica und Diagnostica mbH, Germany. CK was measured according to Szasz et al. (1976) by clinical kits from the Human Gesellschaft für Biochemica and Diagnostica mbH, Germany. ChE was determined according to Weber (1966) using standard kits (Test-combination Boehringer Mannheim GmbH Diagnostica). All enzyme activities were expressed as UL^{-1}. Glucose was measured according to Trinder (1966) by kits of Reactivos Spinreact, S.A. San Ant°.M[a] Claret, Spain. Creatinine was determined according to Thomas (1992) using kits of Boehringer Mannheim GmbH Diagnostica. Concentrations of glucose and creatinine are listed in mg/dl.

Nuclear image measurement and cell classification

Air-dried films of gill tissue were immersed in fixative (15 parts 100% methyl alcohol, five parts 5% acetic acid, one part 37% formaldehyde and five parts distilled water) for 10 min and then washed in tap water for 2 min. They were then immersed in 6 N HCl at room temperature for 10 min and at 60 °C for 8–10 min. Slides were then rinsed in 6 N HCL at room temperature for 2 min and stained with basic Fuchsin for 2 h. At that point, they were rinsed in SO_2 water for 1–2 min and washed with tap water for 10–15 min before being dehydrated in increasing concentrations of ethyl alcohol (5 min in 70%, 10 min in 80% and 10 min in 100%). Slides were then cleared in xylene and mounted with Canada balsam. Films were then stained in Feulgen Reaction for DNA and gallocyanine-

chromalum for RNA. The computerized cell image processor (Leica Quantiment 520) was then used to estimate and analyse the gray values of the cells as an indicator of the relative DNA and RNA content of these cells (Brady 1973; Darzynkiewicz et al. 1984; Crissman et al. 1985; McNally et al. 1997).

Feulgen reaction is perhaps the most specific for DNA among histochemical reactions. It owes its specificity to the presence of an aldehyde in deoxyribose and the fact that there are no naturally occurring substances other than DNA that would yield an aldehyde group under the conditions of gentle hydrolysis employed in this method (Brady 1973). The reaction is based on the acid hydrolysis of its components into purine and pyrimidine bases and its pentose sugar (deoxyribose). The latter releases aldehyde groups that form a stable compound with the Schiff's reagent and localizes DNA. Nuclear chromatin stains pink depending on the concentration of DNA. The nuclear chromatin of immature cells stains pale pink, indicating a low DNA concentration, whereas nuclear chromatin of mature cells stains deep magenta, indicating a high DNA concentration. Nucleoli are defined and counted because they do not stain but are often surrounded by rings of heavy chromatin. Gallocyanine-chromalum was used as a stain for RNA[20, 21] (Romeis et al. 1989). Gallocyanine is a basic dye that stains, in violet-purple, cell components rich in RNA. The more basophilic the cytoplasm, the more RNA it contains. As cells mature, the amount of RNA in the cytoplasm and nucleus diminishes. A fall in the intensity of nuclear and cytoplasmic gallocyanine-chromalum indicates a diminution in RNA and relapse to mature cells. Incorporation of radioactive ^{14}C-uridine[22] into RNA brought to an end any stain inaccuracy. One of the arithmetic figures implicated in assessing growth adequacy using data of tissue RNA and DNA is relative RNA content referred to as the factor r [α or $r = \text{RNA}/(\text{RNA} + \text{DNA})$] (Traganos et al. 1982); assessment procedures also included the ratio RNA/DNA.

Data analysis

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) software for IBM-compatible PC was used. Trace metal concentrations in water were measured in parts per million (p.p.m., mg/L) except for Hg, which was measured in parts per billion (p.p.b., μg/L). The normal probability, frequency distribution, arithmetic mean, standard deviation, standard error and percentage coefficient of variation (%CV) were determined according to Turner (1970). Analysis of variance (one-way ANOVA) was calculated according to Snedecor and Cochran (1969). Duncan's multiple-range test was used to determine the specific differences between groups. In all cases, $P < 0.05$ was the accepted significance level. For quality assurance, replicate samples were submitted on a regular basis and data were compared to results of an authorized fish hatchery as reference. Calibration verification was used to validate analytical methods. In all cases, concentrations and activities in procedural blanks were below detection limits.

Results

Physical and chemical water characteristics

Figure 2 shows trace metal concentrations in the water of Lake Maryût at the selected sites in autumn/winter 1999/2000 (Fig. 2a) and spring 2000 (Fig. 2b). In both periods, Cd, Cu, Fe, Pb, Mn and Hg were significantly higher in the water of MB than in other sites (E and FH). Shared with other

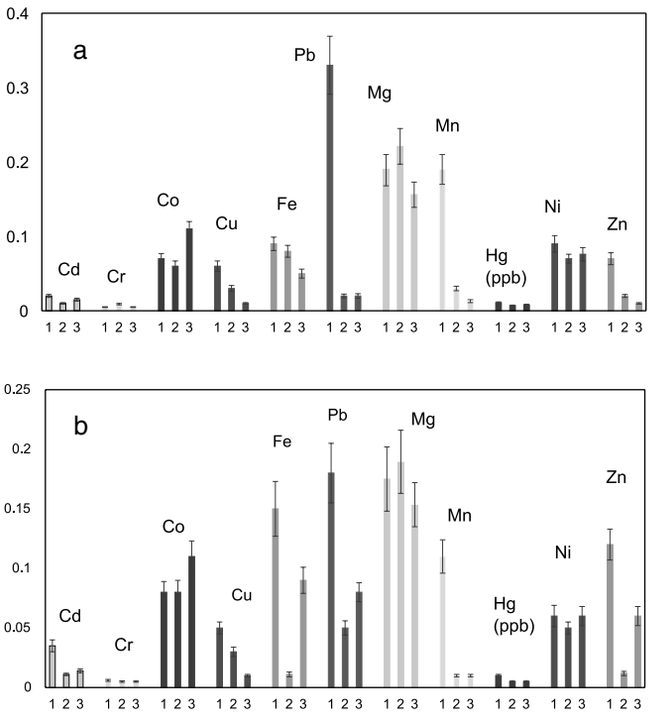


Fig. 2. Mean ± SE metal concentrations ($n = 8$) of lake water collected during: (a) the cooler months (autumn/winter 1999/2000) and (b) the warmer months (spring/summer 2000); all concentrations are expressed as mg/L (p.p.m.) except Hg that is referred to as μg/L (p.p.b.); each presented Mg concentration is only a factor of 1000 of the actual concentration; 1, 2 and 3 refer to sampling locations (MB, E and FH, respectively)

locations, Ni and Zn were generally elevated in the MB. Co was characteristically elevated (0.11 p.p.m.) in FH in period I. In both periods, Cr in E basin hit highest levels (0.009 p.p.m.). According to international standards and thresholds for metal concentrations in lakes and lagoons, Cd, Pb, Mn and Hg generally exceeded corresponding maximum contaminant levels (MCL; 0.005, 0.015, 0.17 and 0.002 mg/L, respectively) (USEPA 1987). A group of physicochemical qualities of lake water, other than metals, are shown in Fig. 3 (a,b). pH ranged 7.0–8.2 in all sites and was generally on the alkaline side. Water in the MB was significantly higher in turbidity (45.4 NTU), BOD (59.0 p.p.m.), COD (141.6 p.p.m.), NH_3-N (0.2 p.p.m.) and NO_3-N (0.9 p.p.m.) than in other sites, whereas DO in all sites ranged from 7.5 to 7.8 p.p.m.

Serologic features

Activities and concentrations of serum enzymes and metabolites, in periods I and II, are given in Tables 1 and 2, respectively. No substantial differences were detected for serologic parameters of fish analysed in the cooler or warmer months. Concentrations of serologic enzymes were fairly high in *C. gariepinus* when compared to many other vertebrates. ALAT, ASAT, LDH and CK were considerably higher in MB fish (61.3, 3135.1, 9628.5 and 32488.7 UL^{-1}, respectively) than other fish groups. LDH was generally elevated in fish analysed during the cooler months. Activities of ChE were commonly reduced in serum of MB fish; inhibition was maximal during sampling period II (120.0 ± 13.0 UL^{-1}). Mean values of glucose were extremely high in MB fish (244.6 and 204.6 mg/dl in periods I and II, respectively). In E fish, mean values were as low as 61.8 and 81.3 mg/dl in periods I and II, respectively).

Table 1
Mean ± SE activities and concentrations of serum enzymes and noncellular components of *Clarias gariepinus* collected from Lake Maryût at the selected sites (MB, E and FH) during autumn/winter 1999/2000

	MB	E	FH
ALAT	46.0 ± 6.1[a]	21.3 ± 2.75[b]	13.0 ± 1.7[b]
ASAT	3135.1 ± 390.8[a]	1040.0 ± 136.8[b]	1247.0 ± 159.5[b]
LDH	9628.5 ± 1090.3[a]	4912.3 ± 696.8[b]	771.0 ± 105.8[c]
CK	32488.7 ± 4632.5[a]	1057.5 ± 148.2[b]	9060.5 ± 1190.4[b]
ChE	130.0 ± 14.4[a]	150.0 ± 16.5[b]	170.0 ± 20.0[c]
Glucose	244.6 ± 28.9[a]	61.8 ± 7.3[b]	142.0 ± 17.4[c]
Creatinine	0.70 ± 0.08[a]	0.30 ± 0.04[b]	0.55 ± 0.06[a]

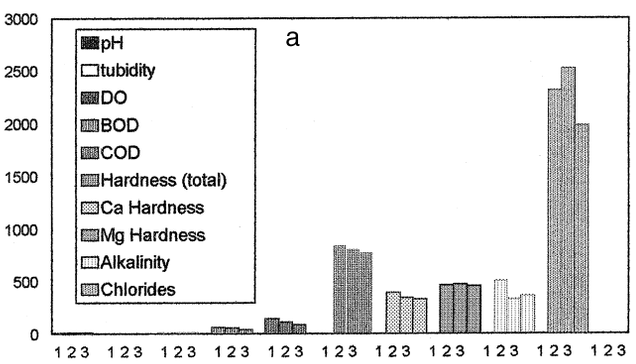
Activities of ALAT, ASAT, LDH, CK and ChE are expressed as UL^{-1}; concentrations of glucose and creatinine are expressed as mg/dl. Different superscripts differ significantly ($\alpha < 0.05$).

Table 2
Mean ± SE activities and concentrations of serum enzymes and noncellular components of *C. gariepinus* collected from Lake Maryût at the selected sites (MB, E and FH) during spring/summer 2000

	MB	E	FH
ALAT	61.3 ± 8.2[a]	25.9 ± 2.9[b]	8.3 ± 0.9[c]
ASAT	739.8 ± 95.5[a]	719.3 ± 91.2[a]	134.3 ± 19.0[b]
LDH	3992.1 ± 450.6[a]	1526.7 ± 212.2[b]	1045.0 ± 150.6[b]
CK	27501.6 ± 3885.3[a]	12922.6 ± 212.5[b]	1625.4 ± 182.9[c]
ChE	120.0 ± 13.0[a]	350.0 ± 45.0[b]	320.0 ± 50.0[b]
Glucose	204.6 ± 26.0[a]	81.3 ± 11.7[b]	153.0 ± 19.7[c]
Creatinine	0.63 ± 0.07[a]	0.26 ± 0.03[b]	0.48 ± 0.06[c]

Activities of ALAT, ASAT, LDH, CK and ChE are expressed as UL^{-1}; concentrations of glucose and creatinine are expressed as mg/dl. Different superscripts differ significantly ($\alpha < 0.05$).

Therefore, the normal glucose level in this species seems to fall within this range. Concentrations of creatinine were minimum in E fish in period I (0.30 mg/dl) and II (0.26 mg/dl), whereas MB fish possessed the highest (0.70 and 0.63 mg/dl in periods I and II, respectively).



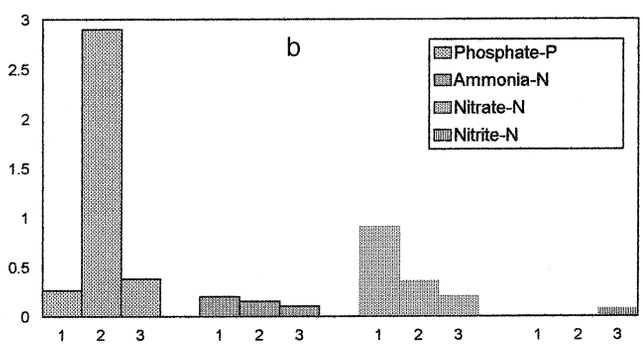


Fig. 3. Some physical and chemical characteristics ($n = 8$) of lake water at locations MB, E and FH (1, 2 and 3, respectively); (a) DO, dissolved oxygen; BOD, biochemical oxygen demand; COD, chemical oxygen demand; nutrient salts (b) include orthophosphate-phosphorus, ammonia-nitrogen, nitrate-nitrogen and nitrite-nitrogen; unless otherwise stated, all measurements are expressed as mg/L (p.p.m.); no unit is defined for pH; turbidity is measured in NTU (nephelometric turbidity units)

Cellular RNA and DNA in gills

Cellular determinations of nucleic acids (Fig. 4) in the gill tissue revealed enhanced RNA/DNA and relative RNA content (r) in E and FH fish. In some instances, as in the 2C stage, E fish exceeded reference FH fish. Simply, this means that protein synthesis and cellular growth were in favour of E fish. On the contrary, MB fish seemed to suffer unbalanced growth and inadequate protein synthesis. RNA/DNA and the relative RNA content (r) proved to be reliable tools in assessing growth and protein adequacy in *C. gariepinus* impacted by water pollution.

Discussion

Data of several priority inorganic pollutants proved to be highest in the water of the MB, lower in the reference site and least in E basin. No admissible levels have been put to action, so far, for pH, DO, turbidity, BOD, COD and ammonia. Being on the alkaline side, pH values in all sites were quite

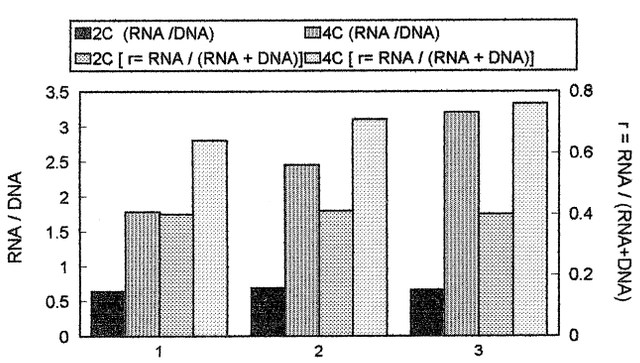


Fig. 4. Average values ($n = 4$) of the formula RNA/DNA and the factor (r) based on the frequency (arbitrary units, AU) of these nuclear components in the cells of the gill arches of *Clarias gariepinus*; 1–3 refer to sampling locations MB, E and FH, respectively; data are the results of nuclear image measurements (Image Acquisition and Processing); Feulgen reaction and gallocyanine- chromalum were used for staining DNA and RNA, respectively

safe, yet the elevated turbidity levels at MB could restrict light penetration and limit photosynthesis (US Fish & Wildlife Service 1984). The oxygen requirement of fish is temperature-dependent; for example, in countries with higher atmospheric temperatures (e.g. Egypt), the oxygen requirement certainly increases as the metabolic rate doubles for each 18 °F increase in temperature (Swann 2000). DO in all studied locations seems unfavourable and far less than the recommended (55.0 p.p.m.) for healthy aquaculture (Dunn et al. 1993). The current DO levels (7.5–7.8 p.p.m.) are, however, not lethal to fish as levels of less than 2 or possibly 3 p.p.m. will result in death (Swann 2000). Toxicity levels for un-ionized ammonia depend on the individual species. However, levels recorded in all locations (0.1–0.2 p.p.m.) are hazardous to fish based on the statement that levels above 0.02 p.p.m. are considered unsafe (Swann 2000).

BOD is defined as the laboratory measurement of the amount of oxygen consumed by microorganisms while decomposing organic matter in a product. BOD levels are indicative of the effect of waste on fish or other aquatic life, which require oxygen to live, and though not a specific compound, it is defined as a conventional pollutant under the Federal Clean Water Act (US Fish and Wildlife Service 1984). COD is defined as the laboratory measurement of the amount of oxygen used in chemical reactions that occur in water as a result of the addition of wastes. A major objective of 'Conventional Wastewater Treatment' under the agenda of the US Environmental Protection Agency is to reduce COD and BOD. Levels of BOD and COD were significantly elevated in the MB versus other sites. This is mostly due to the direct discharges of industrial wastes plus untreated sewage from Alexandrian districts into the MB. This site is known to receive sewage effluent from Gheit El-Enab, El-Metrass and Quabbary in addition to industrial effluent from various manufacturing complexes.

Although seasonal fluctuations are routinely considered in environmental studies, no major differences were observed between data of the selected indices in cooler or warmer periods. Data of serum ALAT, ASAT, LDH and CKP correlated positively to different water qualities in Lake Maryût. One of the possible roles disease might play in relation to enzymes is that the damaged tissue can 'leak' enzymes into the surrounding body fluids (Kaplan et al. 1988). It is therefore concluded that the increased activities of ALAT, ASAT, LDH and CK in serum from more polluted MB fish are a sign of some functional damage in such tissues as the heart and liver, which in turn lead to the leakage of these cellular enzymes into the blood. Healthy cells would most likely not import any of the 'leaky' enzymes because their cell membranes would be functioning normally (Pappas 1989).

In the literature, linking disease to changes in serum ALAT and ASAT is well documented (Raccicot et al. 1975; Adham et al. 1997). ALAT is present in high concentrations in the liver and to a lesser extent in skeletal muscles, kidney and heart (Pappas 1989) and is considered a good indicator of liver pathology (Neff 1985). It is also known to be very symptomatic of hepatic cytotoxic injury (Ellsaesser and Clem 1987; Van Vuren et al. 1994) and elevated activities are also thought to be associated with disease multiplicity (Wroblewski 1959). ASAT is mainly located in the liver (i.e. liver guiding enzyme) and any change in its activity is suggested as reflecting the functional state of the liver (Pfeifer et al. 1977; Van Vuren et al. 1994). Elevated activities of serum ALAT and ASAT in fish caught from the highly polluted MB were typical to liver dysfunction.

Among other enzymes, CK displayed exceptionally elevated rates in *C. gariepinus*. In cooler months, CK averaged as high as 32 488 UL^{-1} in MB fish compared to 1057.5 and 9060 UL^{-1} for E and SE fish, respectively. Although statistical methods were followed in data analysis, it is sometimes useful to retrieve raw data for positive assessment. For instance in MB fish, CK in one sample reached as high as 47 388 UL^{-1}. The significantly elevated CK and LDH activities in blood serum of MB fish could be consequent to damaged liver and/or to myocardial infarction (necrosis of the heart tissue). Increased blood activities of LDH were suggested to indicate liver damage in humans, while increased levels of CK and LDH were shown to occur after myocardial infarction (Pappas 1989).

Water quality in the MB showed inhibitory aptitude towards serum ChE in *C. gariepinus*. Since water in this site was highest in turbidity, NH_3, COD and some toxic metals (Cd, Pb Mn, Hg Ni), it is possible that these nonpesticidal pollutants possessed anti-ChE activity. ChE is synthesized by liver cells, which might be chronically injured in *C. gariepinus* due to water pollution in the MB. It is then expected that the synthesis of this enzyme would be blocked, inhibited or reduced in polluted fish. In addition to anticholinesterases (as organophosphates), hepatic parenchymal disease as hepatitis and cirrhosis could inhibit this enzyme (Pappas 1989). Pesticide analysis in water was not targeted in this study. However, Van Vuren et al. (1994) showed that many nonpesticidal pollutants could display powerful anti-ChE activity.

As a general rule, *C. gariepinus* from MB seemed to be subjected to several factors that multiplied stress upon them resulting in significantly elevated levels of serum glucose (244.6 mg/dl in autumn/winter). Individual cases of MB fish recorded glucose levels as high as 433 and 338 mg/dl. In contrast, glucose significantly decreased in E fish referring to minor stress impact. Glucose measures of individual E fish ranged between 30 and 45 mg/dl, and this could be within the normal range for this species. Exposure to water pollutants including organophosphorus pesticides and heavy metals similarly resulted in elevated serum glucose levels in *Cyprinus carpio* L., *Clarias gariepinus* and acutely stressed channel catfish (Ellsaesser and Clem 1987; Nemcsók et al. 1987; Van Vuren et al. 1994). Reference fish, unpredictably, displayed higher glucose levels than did E fish, despite the virtually similar qualities of their water habitats. The possible throng in the cramped commercial pond seemed to keep fish jam-packed and more food-competitive, resulting in this stress response.

Furthermore, water can hold large amounts of heat with a relatively small change in temperature. This heat capacity has far reaching implications. It permits a body of water to act as a buffer against wide fluctuations in temperature. The larger the water body, the slower the rate of temperature change. Most aquatic organisms take on the temperature of their environment and cannot tolerate rapid changes in temperature (Swann 2000). This easily explains the reason why fish from E basin (1000 acres) were less susceptible to environmental stress than were fish in the commercial pond ($\sim$ 200 acres).

There is evidence for a relationship between pollutant exposure and kidney disease in humans. Parallel studies in several animal groups have reinforced these findings in humans. However, complete data for fish are still missing. One of the major indices for assessing kidney function is serum creatinine. According to Shell (1961), serum creatinine is the least variable nitrogenous constituent of fish blood and is normally excreted in constant amounts. However, the present data show inconsistent values (ranging from 0.1 to 1.0 g/dl) of

serum creatinine in *C. gariepinus*. Perhaps this is associated with acute fish disease and/or physiological imbalance that characterize fish populations dwelling in polluted habitats. At any rate, serum creatinine was insignificantly enhanced in *C. gariepinus* from MB denoting renal, muscular or kidney ailment. Higher creatinine levels in polluted *C. gariepinus* were typical of data given previously for cases of renal failure (Emmerson 1973) and increased muscular tissue catabolism (Pappas 1989).

In mammals, there are some conflicting data about creatinine. Although no effect of Pb application was found on creatinine levels in male rats (Mahaffey et al. 1981), Bishop et al. (1992) attributed the significantly increased serum creatinine in Pb-treated rabbits to impaired renal function. In one of the few reports on teleosts, Ellsaesser and Clem (1987) indicated statistically significant differences in the serum levels of creatinine in acutely stressed channel catfish. Similarly, in *Tilapia zilli*, Adham et al. (1997) reported elevated creatinine levels due to water pollution in Lake Maryût. Serum creatinine was unexpectedly lower in E fish than in reference fish. Again, proper dimension of the facility and fish multitude would appear as potential indices for healthy aquaculture.

One of the arithmetic figures implicated in assessing growth adequacy using data of tissue RNA and DNA is the factor r [α, $r = \mathrm{RNA}/(\mathrm{RNA} + \mathrm{DNA})$]. According to Traganos et al. (1982); the factor (r) refers to the relative RNA content as a function of total cellular nucleic acid content. Amongst other formulae tested, the factor (r) provided the most comprehensive data for *C. gariepinus* in relation to water quality. This was particularly obvious during the 4C stage. Reference fish and E fish seemed to surpass those on the MB in growth adequacy and protein balance since they possessed higher rates of the factor (r) and the ratio RNA/DNA. In a similar report, Rodreguez-Ariza et al. (1999) noticed significantly elevated hepatic DNA in fish exposed to moderate levels of urban and industrial pollution. According to Kearns and Atchison (1979), the ratio RNA/DNA is an integrative indicator of contaminant stress and effects on fish growth. In the literature, lowered energy reserves of fish in contaminated streams were shown to be responsible for reduced growth (lowered RNA/DNA; Lee et al. 1983), while increased DNA was attributed to exposure to hepato-carcinogens (Ramadan and Samy 1976). In brief, the deteriorated water habitat of the MB of Lake Maryût caused adverse effects and exerted high stress impact upon fish resulting in retarded growth and impaired functions of the liver, kidney and heart. A broader survey with the analysis of numerous fish and monitored indices should follow this limited-scale survey. This is crucial for a more comprehensive assessment of the physiological consequences of polluted semiclosed lakes or lagoons such as Lake Maryût.

References

Adamek, Z.; Sukop, I., 1995: Summer outdoor culture of African catfish (*Clarias gariepinus*) and tilapias (*Oreochromis niloticus* and *O. aureus*). Aquat. Living Res. **8,** 445–448.

Adham, K. G.; Hassan, I. F.; Taha, N.; Amin, T. H., 1999: Impact of Hazardous exposure to metals in the Nile and Delta lakes on the catfish, *Clarias lazera*. Environ. Monitor. Assess. **54,** 107–124.

Adham, K. G.; Khairalla, A.; Abu-Shabana, M.; Abdel-Maguid, N.; Abd El-Moneim, A., 1997: Environmental stress in Lake Maryût and physiological response of *Tilapia zilli* Gerv. J. Environ. Sci. Health **A32,** 2585–2598.

APHA (American Public Health Association) 1992: Standard Methods of Water and Wastewater, 18th edn. American Public Health Association, American Water Works Association, Water Environment Federation publication. APHA, Washington DC.

Barron, M. G.; Adelman, I. R., 1984: Nucleic acid, protein content, and growth of larval fish sublethally exposed to various toxicants. Can. J. Fish. Aquat. Sci. **41,** 141–150.

Becker, K.; Rahmann, H., 1995: Influence of ambient temperature on content and composition of brain ganglioside in vertebrates. Comp. Biochem. Physiol. **111B,** 299–310.

Bergmeyer, H. U.; Bernt, E., 1974: Lactate dehydrogenase, UV-assay, with pyruvate and NADH. In: Methods of Enzymatic Analysis, Vol. 2 (Ed. by H. U. Bergmeyer), Academic Press, New York, pp. 574–579.

Bishop, M. L.; Duban-Engelkirk, J. L.; Fody, E. P., 1992: Clinical Chemistry Principle Procedures Correlations, 2nd edn. JB Lippincott Co, Philadelphia.

Bok, A. H.; Jongbloed, H., 1984: Growth and production of sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae), in organically fertilized ponds in the Cape Province, South Africa. Aquaculture **36,** 141–155.

Brady, T., 1973: Feulgen cytophotometric determination of the DNA content of the embryo proper and suspensor cells of *Phaseolus coccineus*. Cell Differ. **2,** 65–75.

Crissman, H. A.; Darzynkiewicz, Z.; Tobey, R. A.; Steinkamp, J. A., 1985: Correlated measurements of DNA, RNA and protein in individual cells by flow cytometry. Science **228,** 1321–1324.

Darzynkiewicz, Z.; Crissman, H. A.; Traganos, F.; Steinkamp, J., 1984: Cell heterogeneity during the cell cycle. J. Cell Physiol. **113,** 465–474.

Dunn, C.; Brown, S.; Young, K.; Stein, S.; Mistichelli, M., 1993: Current water quality best management practices design guidance. Transportation Res. Record **1483,** 80–88.

Ellsaesser, C. F.; Clem, L. W., 1987: Blood serum chemistry measurements of normal and acutely stressed channel catfish. Comp. Biochem. Physiol. A. **88,** 589–594.

El-Rayis, O. A.; El-Sabrouti, M. A., 1998: Pollution problems and proposals for restoration. J. Arab Acad. Sci. Technol **23,** 16–28.

Emmerson, B. T., 1973: Chronic lead nephropathy. Kidney Int. **4,** 1–5.

Haines, T. A., 1973: An evaluation of RNA-DNA ratio as a measure of long-term growth in fish populations. J. Fish. Res. Board Can. **30,** 195–199.

Hrubec, T. C.; Robertson, J. L.; Smith, S. A., 1997: Effects of temperature on hematologic and Serum biochemical profiles of hybrid striped bass (*Morone chrysops* x *Morone Saxatilis*). Am. J. Vet. Res. **58,** 126–130.

IFCC (International Federation of Clinical Chemistry) 1986: Methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotranferase, EC 2.6.1.1). J. Clin. Chem. Clin. Biochem. **24,** 497–510.

Kaplan, A.; Ozabo, L. L.; Ophem, K. E., 1988: Clinical Chemistry. Interpretation and Techniques, 3rd edn. Lea & Febiger, Philadelphia.

Kearns, P. K.; Atchison, G. J., 1979: Effects of trace metals on growth of yellow Perch (*Perca flavescens*) as measured by RNA-DNA ratios. Environ. Biol. Fish. **4,** 383–387.

Köck, R.; Hofer, R.; Wögrath, S., 1995: Accumulation of trace metals (Cd, Pb, Zn) in arctic char (*Salvelinus alpinus*) from oligotrophic Alpine lakes: relation to alkalinity. Can. J. Fish. Aquat. Sci. **52,** 2367–2376.

Krajnoviæ-Ozretiæ, M.; Ozretiæ, B., 1987: Estimation of the enzymes LDH, GOT and GPT in the plasma of gray mullet *Mugil auratus* and their significance in liver intoxication. Dis. Aquat. Org. **3,** 187–193.

Lee, R. M.; Gerking, S. D.; Jezierska, B., 1983: Electrolyte balance and energy mobilization in acid stressed rainbow trout, *Salmo gairdneri*, and their relation to reproductive stress. Environ. Biol. Fish. **8,** 115–123.

Mahaffey, K. R.; Capar, S. G.; Gladen, B. C.; Fowler, B. A., 1981: Concurrent exposure to lead, cadmium, and arsenic. Effects on toxicity and tissue metal concentrations in the rat. J. Lab. Clin. Med. **98,** 463–481.

McNally, J. G.; Cogswell, C.; Fekete, P. W.; Conchello, J. A., 1997: Comparison of 3D microscopy methods by imaging a well characterized test object. In: Three-Dimensional Microscopy: Image Acquisition and Processing, Vol. IV, C. J. Cogswell, J.-A. Conchello, and T. Wilson, Chairs/Editors, Proceedings of the 1997 SPIE Biomedical Optics Symposium (BiOS-9), 2984: 52–63,

February 1997: Conference Paper; Inst. for Biomed. Comput., Washington Univ., St. Louis, MO, USA.

Metcalfe-Smith, J. L.; Green, R. H.; Grapentine, L. C., 1996: Influence of biological factors on concentrations of metals in the tissues of freshwater mussels (*Elliptio complanata* and *Lampsilis radiata radiata*) from the St Lawrence River. Can. J. Fish. Aquat. Sci. **53,** 205–219.

Moss, D. W.; Henderson, A. R.; Kachmar, J. F., 1986: Enzymes. In: Textbook of Clinical Chemistry, 9th edn (Ed. by N. W. Tietz). WB Saunders Co., Philadelphia.

Neff, J. M., 1985: Use of biochemical measurements to detect pollutant-mediated damage to fish. In: Aquatic Toxicology and Hazard Assessment: 7th Symposium, ASTM STP 854 (Ed. by Cardwell, R. D.; Purdy, R.; Bahner, R. C.). American Society for Testing and Materials, Philadelphia, pp. 155–183.

Nemcsók, J.; Asztalos, R.; Víg, E.; Orbán, L., 1987: The effect of an organophosphorus pesticide on the enzymes of carp (*Cyprinus carpio* L.). Acta Biol. Hungarica **38,** 77–85.

Pappas, N. J. Jr, 1989: Diagnostic Enzymalogy (Ed. by Pappas, N. J. Jr). Clin. Lab. Med. **9,** 595–826.

Pfeifer, K. F.; Weber, L. J.; Larson, R. E., 1977: Alanine aminotransferase (GPT) in rainbow trout: plasma enzyme levels as an index of liver damage. Proc. West. Pharmacol. Soc. **20,** 431–437.

Raccicot, J. G.; Gaudet, M.; Leray, C., 1975: Blood and liver enzymes in rainbow trout (*Salmo gairdneri* Rich.) with emphasis on their diagnostic use: study of CCl_4 toxicity and a case of *Aeromonas* infection. J. Fish. Biol. **7,** 825–835.

Ramadan, A. A.; Samy, N., 1976: Cytochemical studies of rat liver nucleoproteins under the effect of hepatocarcinagens. Gg. Morph. Jahrb. Leipzig **122,** 771–786.

Riley, J. P.; Taylor, D., 1968: Chelating resin for concentration of the trace elements from sea water and their analysis in conjunction with atomic absorption spectrometry. Anal. Chim. Acta. **40,** 479–485.

Rodreguez-Ariza, A.; Alhamam, J.; Deaz-Mendez, F. M.; Lopez-Barea, J., 1999: Content of 8-Oxod G in chromosomal DNA of *Sparus aurata* fish as biomarker of oxidative stress and environmental pollution. Mutat. Res. **438,** 297–107.

Romeis, B.; Denk, H.; Künzle, H.; Plenk, H.; Rüschoff, J.; Sellner, W., 1989: Mikroskopische Technik, 17th edn. Urban and Schwarzenberg, München.

Shell, E. W., 1961: Chemical composition of blood of small mouth bases. US Bureau Sport Fish Wild Res. **59,** 36.

Smith, W. G., 1983: Cholinesterase. Chemicals Pesticide Program. Cornell Cooperative Extension Information. New York State College of Agriculture and Life Sciences. Cornell University, Ithaca, NY. Electronic version: http://ace.ace.orst.edu/info/extoxnet/tibs/cholines.htm.

Snedecor, G. W.; Cochran, W. G., 1969: Statistical Methods, 6th edn. The Iowa State University Press, Ames, Iowa.

Swann, La. D., 2000: A Fish Farmer's Guide to Understanding Water Quality. Electronic version: http://ag.ansc.purdue.edu/aquanic/publicat/state/il-in/as-503.htm IIlinois-Indiana Sea Grant Program, Purdue University, West Lafayette, IN.

Szasz, G.; Gruber, W.; Bernt, E., 1976: Creatine kinase in serum: 1. Determination of optimum reaction conditions. Clin. Chem. **22,** 650–656.

Thomas, L., 1992: Labor und Diagnose, 4th edn. Medizinische-Verlagsgesellschaft, Marburg, Germany.

Traganos, F.; Darzynkiewicz, Z.; Melamed, M. R., 1982: The ratio of RNA to total nucleic acid content as a quantitative measure of unbalanced cell growth. Cytometry **2,** 212–218.

Traganos, F.; Darzynkiewicz, Z.; Sharpless, T.; Melamed, M. R., 1977: Simultaneous staining of ribonucleic and deoxyribonucleic acids in unfixed cells using acridine orange in a flow cytofluorometric system. J. Histochem. Cytochem. **25,** 46–56.

Trinder, P., 1966: Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. **6,** 24.

Turner, L. C., 1970: The Normal Probability Distribution Modern Applied Mathematics. The English Universities Press, London, pp. 202–207.

USEPA (US Environmental Protection Agency), 1987: Quality Criteria for Water: 1986. Report no. USEPA 440/5–86–001. US Environmental Protection Agency, Washington, DC.

US Fish and Wildlife Service, 1984: Third Report to Fish Farmers. (Ed. by Dupree, H. K.; Huner, J. V.). US Fish and Wildlife Service, Washington, DC.

Van Vuren, J. H.; Van der Merwe, M.; du Preez, H. H., 1994: The effect of copper on the blood chemistry of *Clarias gariepinus* (Clariidae). Ecotoxicol. Environ. Safety **29,** 187–199.

Weber, H., 1966: Quick and simple ultra-micro-method for the determination of serum cholinesterase. Dtsch. Med. Wschr. **91,** 1927–1932.

Wroblewski, F., 1959: Serum transaminase activities. Am. J. Med. **27,** 911.

Author's address: Dr Khadiga G. Adham, Zoology Department, Faculty of Science, University of Alexandria, Moharram Bey, Alexandria 21511, Egypt.
E-mail: kadham@link.net kadham_100@yahoo.com