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Investigation on the Cause of a Fish-Kill (*Epinephelus*) in the Kisamos Gulf, Crete

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Alteration in the chemical composition of the natural marine environment usually affects a change in the behaviour and health of fish. This alteration may be caused by the presence of chemical wastes in the aquatic environment, such as heavy metals, pesticides, crude oil, phenolic and chlorine derivatives, arsenicals, etc. The degree of toxicity produced by these poisonous substances is dependent upon environmental conditions, such as temperature, pH value, oxygen content and presence of certain other substances (VAN DUIJN 1966)

Spontaneous lesions in fish, displaying degeneration of the anterior lateral line and alteration in the olfactory sensory structure, have occured in many marine areas, for example, Gaspee Point in Narragansett Bay and Millston Point in Connecticut, as well as in the coastal waters of New Jersey and New York (SINDERMANN 1970; GARDNER 1975). These disorders were observed in adult menhaden and resemble those resulting from toxicity in the aquatic environment (GARDNER & LA ROCHE 1973). It has been demonstrated, moreover, that the sensory organs of some teleosts are sensitive to heavy metals such as copper, mercury and silver. This sensory disability and difficulty in orientation may cause to be more susceptible to enemies and predation, diseases or other hazards, due to an inability to relate to a viable environment.

This study deals with events which occurred in the Kisamos Gulf, Crete, where more than 6,000 kg of fish sickened and died within a three month period.

DESCRIPTION OF THE AREA

The Gulf of Kisamos is located in northwestern Crete and occupies an area 72 square nautical miles (Fig. 1a). Its deepest point exceeds 110 m in depth, and the seafloor exhibits alternatively sandy and rocky areas. The land of the western peninsula of the gulf consists exclusively of limestones. The remaining terrestrial area is formed by alluvial deposits-older fluvial terrace-, marls with gypsum occurences, alternating beds of scales and argillaceous sandstones and crystalline schists with iron ore occurences (I.G.M.E. 1963).

Vegetation along the perimeter of the gulf is minimal, and systematic cultivation is centered chiefly in the area surrounding the small town of Kisamos. There is no industry in this area, and, therefore, the waters of the gulf are free from pollution by indu-

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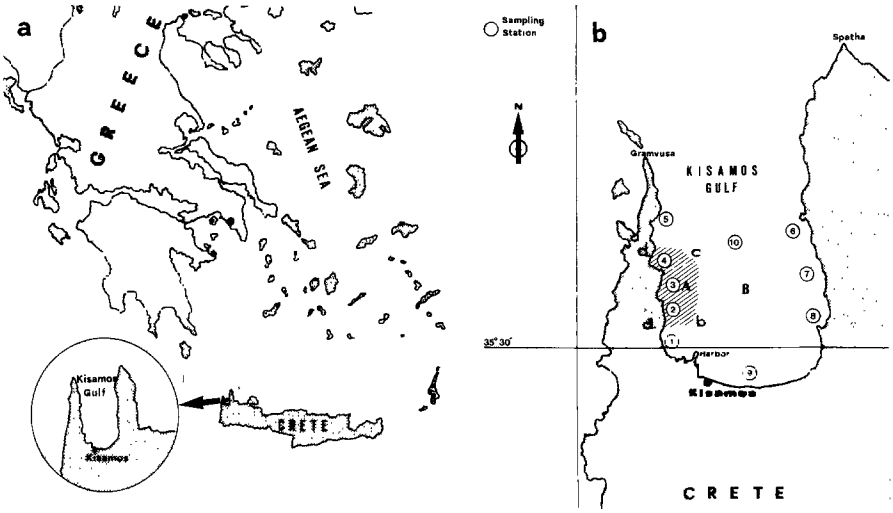

Fig. 1.(a). Gulf of Kisamos, Crete.
(b). Areas designated for sampling and on-the-spot analysis of marine life and water of the gulf of Kisamos.

strial wastes. Commercial freight and ferry boat traffic is quite limited.

DESCRIPTION OF THE EVENT

Toward the end of July 1979, various species of fish, chiefly *Epinephelus quaza*, *E.gigas* and *E.alexandrinus* weighing between 3 to 15 kg each, began to be washed ashore in the gulf, in a half-dead state. These fish were observed swimming upright in circular patterns with their heads above the surface, and their mouths open.

Most of these fish were alive, but exhibited minimal reaction to external stimuli and reduced capacity for orientation. Extreme irritability was evident with slight mechanical stimuli. However, following stimulation, the fish submerged to swin away or accelerate in a rather normal fashion.

Macroscopic Changes: Affected fish collected from the above mentioned area, exhibited, macroscopically, morphological changes (haemorrhage and ecchymoses) in the buccal cavity, the gills and at the base of the fins (Fig. 2). The eyes of many fish appeared bulged (exophthalmic) and hemorrhagic (Fig. 2a). Corneal turbidity was also noted.

The distal parts of the fins lost colour and appeared abnormally white or transparent, whereas the ends of the got freyed (Fig. 2a,b). Excretion of slime on the skin was observed as well as on the epithelium of the gill sheets, which was caused to swell and get loose of its substractum (Fig. 2c). Ulceration was noted the abdominal wall of the fish, and upon dissection, their stomachs were found to be empty, with noticeable thickening of the stomach wall (Fig. 2d). The liver, spleen and heart demonstrated foci of degene-

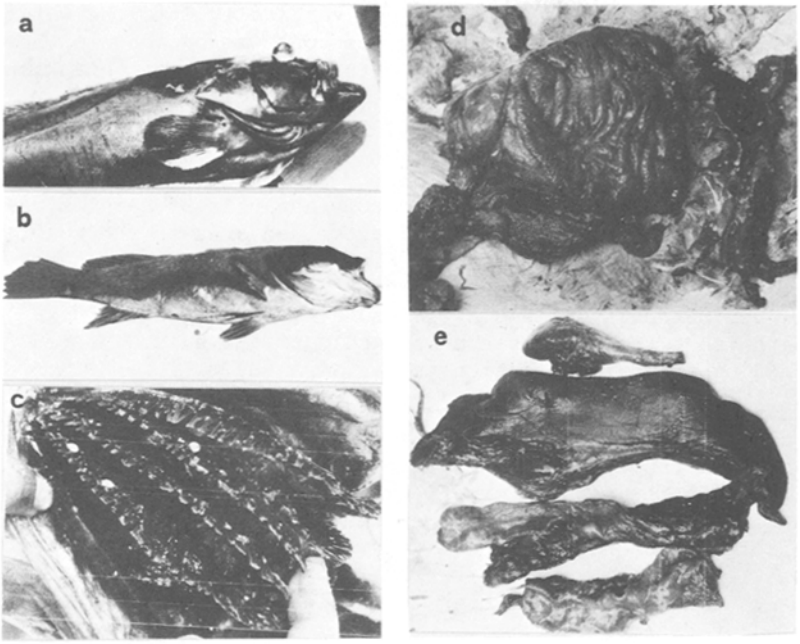

Fig. 2. Macroscopic changes in the external and internal organs of the fish.

ration (Fig. 2e).

METHODS OF STUDY

To determine the causative factors responsible for the event mentioned above, 10 sampling points in the gulf area were chosen (Fig. 1b), where on-the-spot analyses were conducted, and, in addition, water samples and speciments of the affected fish were also obtained for routine laboratory and toxicologic analysis.

Methodology and Instrumentation: The following were determined by on-the-spot analysis: sea water temperature, pH value, conductivity, oxygen content, ammonia and cyanide content. Ammonia and cyanide determinations were conducted according to the SLAWYK & MacISAAK (1972) and LANCY & ZABBAN (1962) methods, respectively.

Heavy metal analysis was accomplished by means of atomic absorption spectrophotometry. Finally the identification and determination of organochlorine compounds, polynuclear aromatic hydrocarbons and paraffins were accomplished using the gas chromatographic methods by JOHNSON (1965), ACHESON et al. (1976) and VALFRE et al. (1976), respectively. The remaining determinations were conducted in accordance with the spectrophotometric methods of STANDARD METHODS (1975), while the turbidity of the water was measured by the HACH, DR-EL/2 apparatus.

Plankton samples were obtained at 10 m depths from No 2,6,8 and 9 sampling stations by vertical tows in the following manner: a cone with a plankton net 15 cm in diameter and made of nylon cloth

with a mesh opening of 28 μm, was lowered vertically into the water of the gulf, and samples were taken as the cone was brought to the surface again, samples were preserved with lugol solution. Plankton individuals were counted under an inverted light microscope. The results tabulated as individuals X 10^3 per cubic meter of sea water.

RESULTS

From on-the-spot testing and laboratory analysis of samples taken at the sampling stations mentioned above, an area of the gulf, designed as area (A), was identified (Fig. 1b). This area measures approximately 2.5 nautical miles in length and 1.0 nautical mile in width. Its perimeter is defined by points a,b,c and d, and it extends lengthwise along the western peninsula of the gulf. The remaining area of the gulf is designated as area (B).

The physical and chemical parameters determined in area (A) differed significantly from corresponding parameters of area (B). The results obtained from the determinations of these parameters are given in Table 1. The results of the examination of fish samples, as well as, those obtained from the examination of plankton, are given in Tables 2, 3 and 4, respectively.

Table 1. Results Obtained Refer to Samples Taken from Water of Kisamos Gulf.

P A R A M E T E R S	AREA (A)	AREA (B)
Temperature	24-25°C	22-25°C
pH	8.2-8.6	7.1-8.1
Conductivity (mmhos)	350-550	450-500
Dissolved oxygen (mg/ℓ)	7.1-7.6	6.2-8.3
Turbidity (FTU)	<20	<20
Salinity (°/oo)	33.0	34.4
Sulfate (SO_4) (mg/ℓ)	2.2-3.5	1.5-2.1
Nitrate (NO_3) (mg/ℓ)	1.9-2.2	0.6-0.7
Nitrite (NO_2) (μg/ℓ)	3-4	0.1
Phosphate (PO_4) (μg/ℓ)	2.0-2.9	2.2-2.7
Silica (SiO_2) (μg/ℓ)	70-82	72-90
Cyanide (CN) (μg/ℓ)	30-40	2-3
Ammonia (NH^+) (μg/ℓ)	48-64	6-8
Bromine (Br) (mg/ℓ)	48-56	50-57
Fluoride (F) (mg/ℓ)	0.8-1.4	0.8-1.1
Arsenic (As) (μg/ℓ)	<1	<1
Chromium Hexavalent (Cr) (μg/ℓ)	6-8	10-18
Copper (Cu) (μg/ℓ)	160-220	39-56
Mercury (Hg) (μg/ℓ)	0.4-0.6	0.5-0.6
Lead (Pb) (μg/ℓ)	<0.1	<0.1
Detergents (ABS) (μg/ℓ)	<10	<10
Phenolic substances, as phenol(μg/ℓ)	<0.1	<0.1
Paraffins (μg/ℓ)	<0.1	<0.1
Organochlorines (ppt)		
-DDT	<5	<5
-BHC	<5	<5
-PCBs	10	10
Polynuclear Arom. Hydroc. (ppb)	<0.1	<0.1

Table 2. Results Obtained from the Analysis of Fish of Kisamos Gulf.

CHEMICALS	CONCENTRATION (ppb wet weight)
Copper (Cu)	3,040-4,400(*)
Mercury (Hg)	87-98
Lead (Pb)	350-420
Paraffins	<0.1
Polynuclear Aromatic Hydrocarbons	<0.1
Organochlorines:	
-DDT	69
-BHC	18
-PCBs	63
(*) Copper concentration in liver	8,500-14,900

Table 3. Phytoplankton and Zooplankton of the Area (A) and (B) of Kisamos Gulf.

GROUP	Concentration (individuals X $10^3/m^3$)	
	Area (A)	Area (B)
Phytoplankton		
Diatomeae	230.0	130.0-170.0
Dinoflagellatae	0.4	0.2 0.4
Zooplankton		
Cladocera	-	- 0.1
Copepoda adults	0.2	0.1 - 0.2
Copepoda nauplii	0.2	0.2 - 0.4
Heteropoda	-	- 0.06
Polychaeta larvae	0.06	0.06- 0.11
Radiolaria	0.1	- 0.06
Rotatoria	190.0	13.00-23.0
Tintinnidae	0.2	0.1 - 0.4

Table 4. Species of Phyto- and Zooplankton at the Areas (A) and (B) of Kisamos Gulf.

SPECIES	Concentration (individuals X $10^3/m^3$)	
	Area (A)	Area (B)
Rotatoria TOTAL	190	13- 23
Keratella tropica (Apstein)	190	13- 23
Diatomeae TOTAL	230	130-120
Coscinodiscus excentricus (Ehrb)	89	14- 27
Rhizosolemia styliformis (bright)	110	110-130

DISCUSSION

It should be noted that the determinations of the above mentioned parameters were carried out at various depths of the sea water of the gulf. The samples, for investigation of plankton, were collected from 2,6,8 and 9 sampling stations.

The qualitative and quantitative analyses of plankton indicated that the *Keratella tropica* (Apstein) was the main species of zooplankton. The concentration of this species in area (A) was approximately, ten times more that the area (B). The *Keratella tropica* is a tropicopolitan perennial species and demonstrated that, this species development under anaerobic conditions. In addition, other conditions, such as temperature, pH, turbidity etc, influence with its development and distribution (BENZINS 1955).

Although morphological changes were not detected in the cells of the peripheral blood by light microscopy, degenerations and hemorrhages were observed, as herein reported, in the gills, eyes, liver, spleen and epithelium of the stomach of fish.

From the evaluation of the data obtained from the analyses of sea water, plankton and fish of the gulf of Kisamos, and with the aid of documented research, the following conclusions can be drawn:

a) There is generally no basis for suspecting that the Gulf of Kisamos is subject to pollution by domestic, agricultural or industrial wastes. This, moreover, accords with the physical features of the region; given that extensive vegetation does not exist in this area, the use of agricultural chemicals and fertilizers must be limited. Besides, the area is not heavily populated and, consequently, lacks heavy industry.

b) Area (A) of the Gulf of Kisamos differs from the remaining area (B) in its physical, chemical and biological constituents. The results obtained from measurements in both areas of specific parameters such as pH, nitrates, nitrites, copper, ammonia, cyanides,plankton etc. display marked differences.

c) The concentrations of certain chemical substances found in area (A) are sufficiently elevated to produce a toxic effect on the aquatic life.

The copper concentrations of the water of area (A) (160-220 μg/ℓ) are approximately ten times greater than those in marine area (B) of the gulf. Comparing these copper concentrations of area (A) with those of the other marine areas in the Mediterranean, which range from 1-80 μg/ℓ (HUYNH et al. 1979, SERBANESCU et al. 1970, CURI 1979) confirmed that those of area (A) are elevated to such a level as to be toxic to fish. BOND & STRAUB (1973) assent that copper concentrations in water of 120-130 μg/ℓ are sufficient to cause death in fish within a span of 160-192 h. According to the views of REICHENBACH-KLINKE (1966), copper concentrations ranging from 140-330 μg/ℓ may produce a chronic toxic condition in fish, characterized by thickening of the epithelium and degeneration of the parenchymal organ systems. The affected fish found in the Gulf of Kisamos exhibited exactly these symptoms.

The influence of copper in sea water of fish is evident, considering the concentrations of this element determined in the tissues of the fish. The copper concentrations in the tissues of the fish of the gulf are considerably more elevated than those given in various studies of copper concentrations in fish from other Mediterranean areas (BALKAS et al. 1979).

The ammonia concentrations determined in area (A) are about nine to ten times those calculated in area (B). These ammonia concentrations found in area (A) are again higher than those found in other areas of the Eastern Mediterranean (FRILIGOS 1979). SCHRENKENBACH & SPANGENBERG (1978) state that ammonia concentrations of 1-69 µg/ℓ produce chronic toxemia in fish. In accordance with this theory, then, the fish found in the Kisamos Gulf might well have been affected by the ammonia concentrations of the water. This chronic effect is characterized by degeneration of liver, as well as discoloration and hemorrhage of the entire body surface of the fish,especially in the region of the gills and the fins (VAN DUIJN 1966, RIBELIN 1975). Again, these symptoms were demonstrated in the affected fish of the gulf.

According to VAN DUIJN (1966), toxic cyanide levels range from 50-230 µg/ℓ. Thus, the cyanide concentrations determined in area (A) should not, by themselves, have a toxic effect on fish, but where cyanide is found in conjunction with ammonia, it may be potantially dangerous to fish (BRODERIUS & SMITH 1979).

One can concluded from the above discussion that the toxic substances responsible for the death of fish in the Gulf of Kisamos are primarily copper and ammonia, and, secondarily, cyanides, which acting synergistically, increase the toxicity of ammonia. Since there is no apparent reason to believe that the toxicity present stems from either environmental or agricultural pollution, the possibility of stealthy human interference or an accident of nature seem to be more plausible causative factors.

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