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Thermal Stress for Hexadecane Decomposition in Seawater of a Natural Environment*

Humitake SEKI**

Abstract: The thermal alteration of microbial activity was studied on hexadecane decomposition by microorganisms in salt water of a natural pool of Shimogamo Hot Spring. There was a significant linear relationship between the logarithm of the *in situ* potentiality of hexadecane decomposition, $m/\mu\text{g hexadecane/liter seawater/hr}$, and temperature, *i.e.*, $F_0=7.81$ and $F(1, 10; 0.05)=4.96$. The regression line was determined statistically to be $y = -0.0152x + 1.82$ with unbiased variance $\sqrt{V_{yx}}=0.106$, where $y = \log_{10}$ of the *in situ* potentiality of hexadecane decomposition and $x = \text{temperature } (^{\circ}\text{C})$, within the temperature range of 42.8 to 63.9 $^{\circ}\text{C}$ in the pool.

1. Introduction

Serious regional problems are induced by thermal alteration of aquatic ecosystems as the result of thermal pollution due to the effect of heated effluents or cooling towers on man's environment, sometimes with temperature ranging from normal to well above 50 $^{\circ}\text{C}$ (*e.g.*, GIBBONS and SHARITZ, 1974). One of the problems is thermal effect on the mechanism of self-purification in aquatic environments: This might be most important for degradation of hydrocarbon pollutants, because they spread over as surface film on water where the thermal effluents discharged might be restrictive with their influence being greatest.

The effect of temperature on microbial degradation of hydrocarbons have been examined extensively over a wide range of temperature in the laboratory as reviewed in ZOBELL (1969) or CROW *et al.* (1974), but no scientific works have been reported on the effect of high temperature in natural aquatic environments.

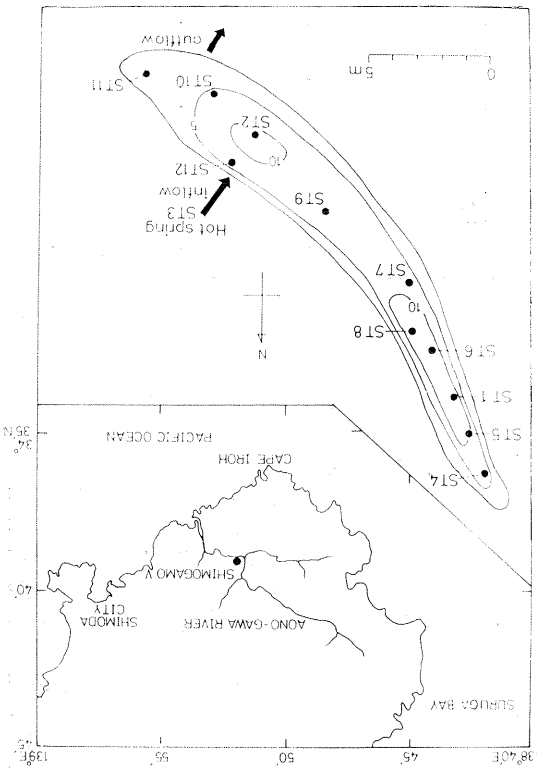
The water of hot springs in Shimogamo Village, Shizuoka Prefecture of Japan, is saline as influenced greatly by seawater of the Pacific Ocean (Office Hygiene of Shizuoka Prefecture, 1957), although the springs distributed along the Aono-gawa River in the village where is

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Fig. 1. Location of Shimogamo Hot Spring and stations in a natural pool at a spring effluent. Numbers in the pool represent depth in cm.



about 4 km from the seashore (Fig. 1). A natural pool (Fig. 1) is formed on the river beach at a spring effluent, where the water is

number of bacteria was directly counted under a phase contrast microscope. The heterotrophic bacteria (SEKI *et al.*, 1974) were enumerated by a plate count method with Medium 2216 (salt medium for seawater bacteria) and Medium 2216 with distilled water instead of 75% seawater (freshwater medium for freshwater bacteria). The details of environmental conditions in the pool have been already reported in SEKI *et al.* (1975a).

3. Results

1. Hexadecane-decomposers in the microbial flora

Hexadecane-decomposers in the microbial flora at each water temperature of the pool are shown in Table I. In salt water of the hot spring, the densities of hexadecane-decomposers and kerosene-decomposers were almost the same, as has been observed in seawater of the days of Shimoda and Tokyo (SEKI, 1974). The density of hexadecane-decomposers in each water sample of the pool was 0.00001% to 0.00001% of the total bacteria or 0.01 to 0.1% of the heterotrophic bacteria: Approximately one order magnitude lower in bacterial flora (as indicated by total bacteria) or one order magnitude higher in the heterotrophic bacteria, compared to that in each water sample of the days of Shimoda and Tokyo (SEKI, 1974). Slightly halophilic bacteria were predominant

characterized as temperature of 63.9°C, chlorinity of 10.47‰, pH of 8.82 and Eh of 537 mV. Environmental gradient can be observed even in such a small pool as influenced primarily by temperature gradient within the range of 41.5 and 43.9°C (for details see SEKI *et al.*, 1975a). Thermal effect of microbial degradation of a hydrocarbon, hexadecane, was studied in the natural pool of Shimogamo Village, where the slightly halophilic microorganisms are considered to be acclimatized to the thermal gradient in a high temperature environment (SEKI *et al.*, 1975a).

2. Materials and methods

An investigation was made at a natural pool (Fig. 1) of hot spring on river beach of the Aono-gawa River in Shimogamo Village, Shizuoka Prefecture, on September 22, 1974. Water samples were collected at a few cm depth from the surface with sterilized 50 ml plastic syringes. The petroleumytic microorganisms were enumerated by the method of SEKI (1973), as rate of hexadecane decomposition in seawater was determined by a simulated *in situ* method of SEKI (1975), except the incubation being made *in situ*. The rate of hexadecane oxidation measured using ¹⁴C hexadecane-1-¹⁴C by this method shows the maximum attainable rate of hexadecane decomposition *in situ*. The total

Table I. Density (bacteria/l water) of total bacteria, heterotrophic bacteria both saline and fresh water, kerosene-decomposers, hexadecane-decomposers in a natural pool of the hot spring.

Station	Temperature (°C)	Total bacteria	Heterotrophic bacteria	Freshwater	Kerosene-decomposers	Hexadecane-decomposers
1	46.0	6.7×10 ⁹	9.6×10 ⁴	1.8×10 ⁸	6×10	8×10
2	53.5	8.6×10 ⁹	2.4×10 ⁴	1.2×10 ⁸	2×10	4×10
3	63.9	2.5×10 ⁹	7.3×10 ³	4×10 ⁷	1.0×10 ⁷	1.2×10 ⁷
4	42.8	8.0×10 ⁹	7.5×10 ⁴	1×10 ⁷	4×10	4×10
5	43.0	1.3×10 ¹⁰	7.1×10 ⁴	2.0×10 ⁸	1.2×10 ⁷	1.4×10 ⁷
6	47.0	1.5×10 ¹⁰	1.9×10 ⁵	2.0×10 ⁸	8×10	8×10
7	48.4	6.7×10 ⁹	2.2×10 ⁵	3.0×10 ⁸	2×10	4×10
8	51.4	3.3×10 ⁹	1.1×10 ⁵	1.0×10 ⁸	2.6×10 ⁷	1.0×10 ⁷
9	51.8	2.9×10 ⁹	1.7×10 ⁵	4.6×10 ⁸	1.0×10 ⁷	4×10
10	56.0	2.5×10 ⁹	1.2×10 ⁵	3.2×10 ⁸	1.0×10 ⁷	4×10
11	50.9	6.7×10 ⁹	3.3×10 ⁴	2.3×10 ⁸	4×10	1.2×10 ⁷
12	53.5	2.5×10 ⁹	9.6×10 ⁴	3.1×10 ⁸	1.0×10 ⁷	4×10

formed primarily by a strong current passes from the inflow to the outflow through short axis at the southern part of the pool. Hexadecane decomposition at each station (42.8-63.9°C) is shown in Fig. 2. As is shown in Table 1, hexadecane-decomposers at each station were almost the same in their population density, the difference in the rate of decomposition has to be attributed chiefly to thermal stress. The activity was lower at higher temperature and there was a significant linear relationship between the logarithm of the *in situ* rate of hexadecane decomposition and temperature (Table 2). The rate is measured as the maximum attainable rate of decomposition *in situ* or the heterotrophic potentiality *in situ*. The regression line was determined statistically to be $y = -0.0152x + 1.82$ with unbiased variance $\sqrt{V_{y^2}} = 0.106$, where $y = \log_{10}$ of the *in situ* potentiality of hexadecane decomposition (mg hexadecane/l/hr) and temperature (°C). The relationship indicates that the microorganisms responsible for the hexadecane decomposition were facultative psychrophiles which had been acclimatized to the thermal environment as eurythermal organisms: This leads to the deduction that the microorganisms in the salt water of the spring must be chiefly marine microorganisms as influenced little by microorganisms from other origin. As a matter of fact, the relationship between logarithm of the *in situ* potentiality of hexadecane decomposition and temperature in the bottom layer of Shimoda Bay, as indicated in the cross mark in Fig. 2, fits also on the regression line obtained at the hot spring. The population density of hexadecane-decomposers in the bottom layer of the sea was almost the same with that in the hot spring.

in the bacterial flora of the salt water of the spring, where chlorinity of the water was 10.47%. As has been observed in a natural estuarine environment (SEKI *et al.*, 1969), bacteria which grow best in the freshwater medium inhabit water in an estuary with a salinity of less than 1.9‰ that is equivalent to a chlorinity of 10.65‰. These differences show that high temperature might be favourable for the activity and survival of halophilic bacteria in more freshwater environment. Population density of total bacteria is very high, many of which must be dead cells because of bacteria by the viable count being not so numerous.

2. Hexadecane decomposition at different water temperatures

The water temperature ranged from 41.5 to 63.9°C in the pool. The temperature gradient seems to be chronic in the pool as formed chiefly by the existence of two gyres, that are

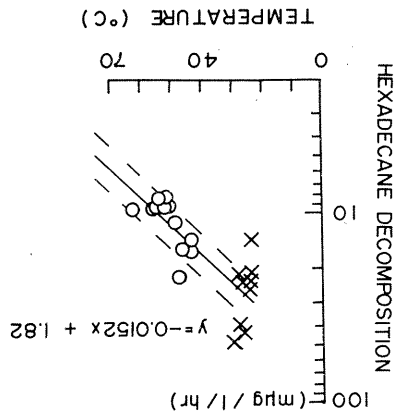


Fig. 2. Relationship between the heterotrophic potentiality of hexadecane decomposition by microorganisms and temperature.
 ○ : in the pool of Shimogamo Hot Spring
 × : in the bottom layer of Shimoda Bay

Table 2. Regression analysis of logarithm of the *in situ* potentiality of hexadecane decomposition (mg hexadecane/l/hr) and temperature (°C).

Source of variation	Sum of square	Degree of freedom	Mean square	F_0
Linear regression	0.0885	1	0.0885	7.81*
Residual	0.1133	10	0.01133	
Total	0.2018	11		

* Probability level $F(1, 10 : 0.05) = 4.96$
 $F(1, 10 : 0.01) = 10.10$

4. Discussion

The existence of petroleumlytic activity by microorganisms was confirmed in a natural pool of Shimogamo Hot Spring. The rate of hexadecane decomposition in salt water of the pool was approximately 10 mg/hexadecane/l per hr, which is almost equivalent to that in some mesotrophic watermass in the sea as little affected by petroleum pollution (SEKI, 1974). This has been already confirmed in the former studies from the point of eutrophication (SEKI *et al.*, 1975a and b). The activity rate had the close relationship with water temperature, and a regression relationship can be determined statistically among the data not only from the pool of the hot spring but also from the water of the bottom layer of Shimoda Bay, where is believed to be the origin of salt water in Shimogamo Hot Spring (Office Hygiene of Shizuoka Prefecture, 1957). Thus the relationship shown in Fig. 2 must be one of the typical example of the thermal stress for hexadecane decomposition by microorganisms in seawater of a natural environment.

The semi-logarithmic depression of the microbial activity of hexadecane decomposition by high temperature from 24.0°C at the bottom layer of Shimoda Bay up to 63.9°C in the hot spring was observed in a water of the same water-type. The depression is shown to be very small perhaps because of acclimatization of facultative psychrophiles to the high temperature environment, as the activity of typical marine microorganisms could not be detected with thermal shock at 50°C in the case of no acclimatization. Moreover, the density of hexadecane-decomposers were almost the same in the thermal range of 24.0 to 63.9°C in the water.

In conclusion, thermal stress must be greatest at the thermal shock for microflora in the marine environment, as far as self-purification of petroleum pollution is concerned. However, once the thermal pollution becomes chronic in a certain region of the sea, the thermal stress for the self-purification might not be great with only slight depression as the eurythermal characteristics of facultative psychrophiles.

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自然環境において高温が海洋微生物の キサデカノ分解作用に及ぼす影響

関 文 威

要旨：下賀茂温泉の塩水中における，温度とキサデカノ分解速度との関係を調査研究した。本温泉の泉質や地質構造から，泉水は海水の影響を強く受けていることが推定されている。微生物作用によるキサデカノ分解速度と水温との間には，次の関係があることが統計学的に明らかたされた。

$$y = -0.0152x + 1.82$$

但し， y はキサデカノ分解速度 (mg hexadecane/l/hr) の常用対数。 x は水温 ($^{\circ}\text{C}$)。

× パチに関する水産海洋学的研究—II*

東部熱帯太平洋におけるマクロバエなわ漁場と

水温躍層および溶存酸素量との関係

花 本 栄 一**

Fishery Oceanography of Bigeye Tuna—II

Thermocline and Dissolved Oxygen Content in Relation to Tuna Longline Fishing Grounds in the Eastern Tropical Pacific Ocean

Eiji HANAMOTO

Abstract: In this report, the author examined the fishing grounds of bigeye tuna (*Thunnus obesus*) in the eastern tropical Pacific Ocean in relation to the thermocline depth and dissolved oxygen content, and found the following:

1) The areas with high catch rate (catch per 100 hooks) of bigeye tuna are located along 7°N and 12°N, west of 135°W; and along 7°N, east of 135°W, and extend to the equatorial area between Galapagos Is. and off Ecuador. There is also a high catch rate area along the equator extending from the western part of Galapagos Is. to 115°W, in the northern hemisphere. In the southern hemisphere, high catch rate areas are located between 1°S and 7°S, west of 140°W; and 1°S and 10°S, between 105°W and 140°W. In the area between 93°W and 105°W, the high catch rate area is separated into two areas: between 1°S and 4°S, and between 7°S and 11°S.

In areas other than those listed above, the catch rates are generally low. Moreover, in the coastal area of Peru and in the area along latitude 10°N east of 135°W, no bigeye tuna are taken at all.

2) The depth of the top of the thermocline is generally shallower than 100 m in the eastern tropical Pacific. Ridges in the thermocline lie along the equator, off Ecuador, and in the area along latitude 10°N extending from east to west off Costa Rica.

3) The depth of the thermocline generally coincides with that of the oxycline. The dissolved oxygen content decreases rapidly within the thermocline. There are two types in the tendency of decreasing oxygen: 1. areas where the dissolved oxygen content decreases to less than 1 ml/l below the thermocline, and 2. the areas where the dissolved oxygen content is greater than 1 ml/l although it does decrease below the thermocline.

4) The areas with high bigeye tuna catch rate are found in shallow thermocline areas such as off Ecuador and along the equator. However, the area east of 135°W and centered along latitude 10°N where no bigeye tuna are taken is also seen to have a shallow thermocline. The catch rates are low in the areas where the depth of the top of the thermocline is deeper than 100 m.

5) The depths of capture of the bigeye tuna occur principally within or below the thermocline.

* 1975年3月5日受理

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6) The water temperatures at the depths of capture of bigeye tuna range widely from 12°C to 27°C. This study has shown that bigeye tuna are caught in waters of lower temperature than hitherto believed.

7) The bigeye tuna are caught in waters where the dissolved oxygen content is greater than 1 ml/l, but not in waters with oxygen content below 1 ml/l. It is thus considered that 1 ml/l is a minimum requirement of dissolved oxygen for bigeye tuna.

8) No bigeye tuna are caught in waters of the Costa Rica Dome in spite of the shallow thermocline and high biological productivity in the area. This phenomenon is likely due to low dissolved oxygen content (less than 1 ml/l) at the hook depths of the tuna longline gear.

1. はじめに

東部熱帯太平洋海域は日本のえなわ漁業にとりて重要な漁場となっている。この海域におけるマクロ類、海洋環境およびこれらに関する研究は種々あるが(例えば、BRANDHORST, 1958¹⁾; CROMWELL, 1958²⁾; WOOSTER *et al.*, 1958³⁾; 久米, 1969a⁴⁾), マバチと海洋環境に関する研究は須田ら(1969⁵⁾)の研究以外ほとんどなされてはいない。

東部熱帯太平洋では水温躍層が浅く、このような海域では動物マクロトノ現存量が高く(BRANDHORST, 1958¹⁾), マクロ類は比較的動物マクロトノ現存量の高い海域に集るといふ現象がみられている(例えば、CROMWELL, 1953⁶⁾; KING *et al.*, 1957⁷⁾; BRANDHORST, 1958¹⁾)。一方、水温躍層と酸素躍層の深さはほぼ一致し、水温躍層における溶存酸素量は急激に減少している。そこで、本報ではマバチ漁場の特性を解明すべく、餌料の多寡の一指標となり得る水温躍層、および水温躍層下で激減し、魚の生活に直接影響を及ぼす溶存酸素量を海洋環境として取り上げ、これらとマバチ漁場との関連を検討したので報告する。

本研究を進めるにあたって FAO, ローマ水産局林繁一博士, 海洋水産資源開発センター奈須敬二博士には始終懇切なる御指導, 御助言を頂いた。また、遠洋水産研究所の山中一郎博士, 山中一室長は原稿を校閲して下さい、同所の久田幸一技官には有益なる討議を、同所の上柳昭治博士, 須田明博士, 久米漸技官には助言をして頂いた。

National Marine Fisheries Service, Hawaii Area Fishery Center の Tamio ORSU 室長から

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は英文要約を吟味して頂いた。以上の方々の御援助と御協力に対し心から感謝の意を表する。

2. 資料および方法

1. 釣獲率分布

遠洋水産研究所に集められている 1966~1970年の5年間によるはえなわの漁獲資料から緯度、経度それぞれ1度区画ごとのマバチの年平均釣獲率分布図を作成した(Fig. 1)。ここで1度区画ごとの年平均釣獲率(\bar{r})とは月別に求めた平均値を更に年間にわたって平均したものである。すなわち、

$$\bar{r} = \frac{1}{m} \sum_{i=1}^m r_{ij}$$

ただし、

r_{ij} : i 年 j 月における1度区画の釣獲率

m_j : その1度区画で j 月に資料の得られた年の数

m_i : その1度区画で資料の得られた月の数

Fig. 1の階級は1度区画別平均釣獲率の頻度分布(Fig. 2)に基づいて決めた。この頻度分布に

は0.7, 0.8%の階級にそれぞれ出現頻度11%の約13%を占める副次モードが存在している。ここで、主モードの上端である0.9%以上の高釣獲率域、副次モードである0.2%以下の0釣獲率域を考え、更に両者の中間である0.6%を境にそれ以上の中釣獲率域、それ未満の低釣獲率域に分けて四つの階級を設定した。

2. 水温、溶存酸素量

水温躍層上限の深さ、水温、溶存酸素量鉛直断面分布および100 m層の水温、溶存酸素量水平分布は日、米、ソの調査船12隻、48航海の資料によ

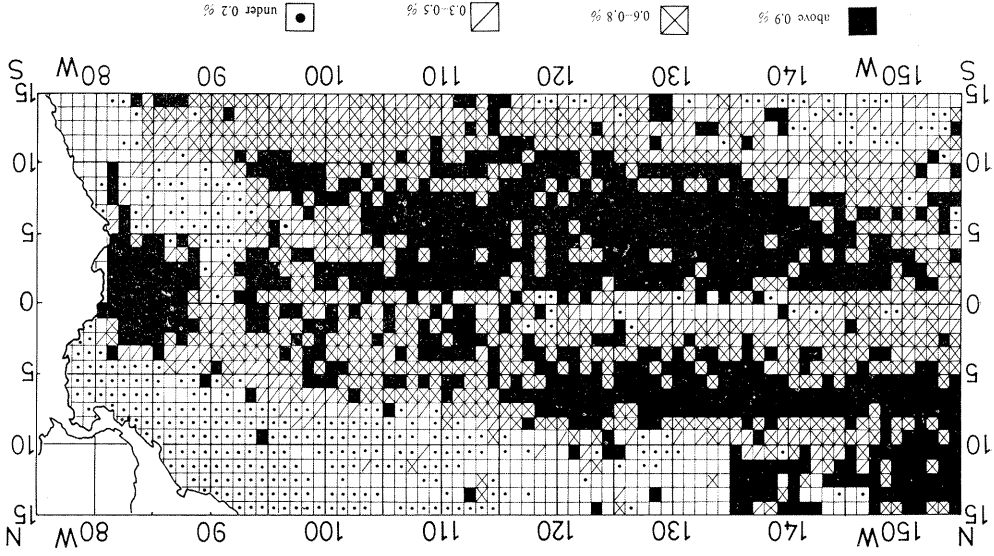
Table 1. Tabulation of available oceanographic observation.

Vessel	Cruise	Period of survey	T.	O.	Source of data		
Sagami maru	3	Apr.-July, 1958	○	○	K N S, 1968		
	4	Sep.-Nov, 1958	○	○			
	6	Sep.-Oct, 1959	○	○			
	8	Apr.-July, 1960	○	○			
	11	July-Sep, 1961	○	○			
	12	Jan.-Feb, 1962	○	○			
	18	Feb, 1964	○	○			
	Taisei maru	13	July-Sep, 1961	○		○	M E S, 1963
		15	June-Aug, 1962	○		○	
		17	June-Sep, 1963	○		○	
18		Dec, 1963-Mar, 1964	○	○			
19		June-Nov, 1964	○	○			
20		Feb.-May, 1965	○	○			
21		Sep, 1965-Jan, 1966	○	○			
22		Apr.-Sep, 1966	○	○			
23		Dec, 1966-May, 1967	○	○			
24		Sep, 1967-Jan, 1968	○	○	H G S, 1969		
25		May-Sep, 1968	○	○			
26		Jan.-June, 1969	○	○			
Shoyo maru		27	Oct, 1969-Mar, 1970	○	○	S M H, 1963	
	12	Dec, 1962-Mar, 1963	○	○			
	13	Dec, 1963-Feb, 1964	○	○			
	14	Dec, 1964-Feb, 1965	○	○			
	Kaiyo maru	5	Sep.-Oct, 1950	○	○		S S R, 131, 1954
		8	Jan.-Feb, 1951	○	○		
		11	Sep.-Oct, 1951	○	○		
		31	Sep.-Oct, 1955	○	○		
		33	Mar, 1956	○	○		
		35	Aug.-Oct, 1956	○	○		
		38	Jan.-Mar, 1957	○	○		
		45	Apr.-June, 1958	○	○		
		Koyo maru	Eastern Pacific Ocean	Dec, 1964	○		
Horizon			Step 1	Sep.-Dec, 1960	○	○	
	Dorado		July-Aug, 1965	○	○		
	Dolphin		Apr.-May, 1958	○	○		
	Downwind		Oct.-Nov, 1957, Feb, 1958	○	○		
	Eastropic		Oct.-Dec, 1955	○	○		
	Tethys		June-July, 1960	○	○		
	Costa Rica Dome		Nov.-Dec, 1959	○	○		
	TO-58-2		Nov, 1958	○	○		
	Scot		May-June, 1958	○	○		
	Eastropic	Oct.-Dec, 1955	○	○			
S. F. Baird	TO-59-1	Jan.-Feb, 1959	○	○	O O P, 1965		
	Doldrums	Aug, 1958	○	○			
	Scope	Nov.-Dec, 1956	○	○			
	Unsel III	May, 1949	○	○			
	U.S.S. Serrano	Swan Song Exp.	Sep.-Nov, 1961	○		○	D R, S I O Ref. 66-1, 1965
		Argo	May, 1949	○		○	

た。なお、2 回以上の観測がなされた 1 度区画に
 対してはその平均値を求めた。
 3. メバチの主漁獲層
 著者は前報(花本, 1974⁹⁾で枝縄別漁獲割合お
 よび漁研型深さ計による釣鈎の深さから 東部熱帯
 太平洋においてメバチがはなわにより主として
 漁獲される深さ(主漁獲層)を推定した。それに
 よると、主漁獲層の深さは 3°N~9°N の海域

り求めたものである (Table 1)。
 ある観測点における水温躍層とは、川合 (1969⁹⁾
 の説に従い水温深度曲線上で水温の鉛直傾度が
 5°C/100 m を越える層とし、その頂上部の深度を
 水温躍層上限の深さと定義した。この定義に従っ
 て、水温深度曲線上で読み取った深さを、また水
 温、溶存酸素量水平分布を求めるにあたっては各
 観測点の資料を緯度、経度 1 度区画ごとに整理し

Fig. 1. Geographical distribution of yearly average catch rate (catch per 100 hooks) of bigeye tuna by one degree square in the eastern tropical Pacific Ocean, 1966-1970.



- T. : Temperature
- O. : Dissolved oxygen
- KNS : Kanagawa ken Suisanshikenjo Chosa Hokoku, Kanasushi Shiryo No. 113, Kanagawa Prefectural Fisheries Experimental Station
- MES : Mie Ken Suisanshikenjo Jiygo Hokoku, Mie Prefectural Fisheries Experimental Station
- HGS : Mie Ken Hamajima Suisanshikenjo Jiygo Hokoku, Mie Prefectural Fisheries Experimental Station
- SMH : Chosasen Shoyo Maru Hokokusho, Research Division, Fisheries Agency of Japan
- KMH : Kaiyo Maru Chosa Hokokusho, Fisheries Agency of Japan
- DOO : Data of Oceanographic Observations and Exploratory Fishings No. 3, Shimomoseki University of Fisheries
- SSR : Special Scientific Report—Fisheries, United States Department of the Interior Fish and Wildlife Service
- OOP : Oceanic Observations of the Pacific, Scripps Institution of Oceanography of the University of California
- DR, SIO : Data Report, Scripps Institution of Oceanography
- IGY, OR : IGY Oceanography Report No. 3, Oceanographic observations in the Intertropical Region of the World Ocean during IGY and IGC, Part II B: Pacific Ocean, Department of Oceanography and Meteorology, Agricultural and Mechanical College of Texas, College Station, Texas, U.S.A.