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定常波による砂漣生成と砂粒子の移動限界について*

阿部友三郎** 新井正一**

On the Sand Ripple Generated by Standing Wave and the Threshold Movement Sand

Tomosaburo ABE and Masakazu ARAI

Abstract: On investigating sand movement at ashore, it is fundamental and important problem to examine the threshold movement for sand. The authors examined this problem and obtained the following results. The sand ripple is generated alternately by standing wave (see Fig. 1); that is, the sand ripple is formed at the node where horizontal velocity takes the maximum value, and not formed at the loop where horizontal velocity takes the minimum. Making use of the Airy Wave Theory, they calculated the horizontal velocity at the boundary point between the place where the ripple is generated and its near by place where it is not recognized. And they discussed on the threshold movement for sand at the laminar boundary layer. Consequently, the empirical formula was obtained,

$$Sdg \tan\alpha/u_0^2 = 513\,(u_0\delta/\nu)^{-1.86}$$

where

$$S=(\rho_s-\rho) \quad \text{and} \quad \delta=\sqrt{\frac{\nu T}{\pi}}$$

d: mean grain diameter of sand, g: the acceleration of gravity, ρ_s: density of sand grain, ρ: density of fluid, T: period of the wave, ν: kinematic viscosity, u: the amplitude of horizontal velocity at the bottom, α: a static friction angle on the sand surface.

砂粒子の移動限界に関する研究は，海洋における砂粒子の移動を論ずる際に，基礎的で重要な問題である。著者らはこの問題について次の様に水槽を利用して調べた。

砂面に定常波を当てると，Fig. 1 に示す様に砂漣が交互にできる。これは底面の水平方向の流速が場所によって異なり，節の部分で最大となり，腹の部分で最小になるためであると思われる。そこで，この砂漣の生じている部分と，生じていない部分との境界における水平方向の流速を微小振幅波理論より求め，層流境界層における砂粒子の移動限界について考察し，次の様な実験式を得た。

$$Sdg \tan\alpha/u_0^2 = 513(u_0\delta/\nu)^{-1.86}$$

ただし，

$$S=(\rho_s-\rho), \quad \delta=\sqrt{\frac{\nu T}{\pi}}$$

d: 砂粒子の平均粒径，g: 重力加速度，ρ_s: 砂粒子の密度，ρ: 液体の密度，ν: 動粘性係数，T: 波周期，u_0: 底面における水平方向の流速の振幅，α: 砂粒子の安息角

1. 概 要

砂粒子の移動限界に関する問題は，漂砂を取り扱う際や，貝類などの養殖を行なう際などに関連した，基礎的で重要な問題である。この砂粒子の移動限界の問題については，理論的考察，及び模

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Table 1. The coefficient for the threshold movement.

Researcher	KURIHARA, SINOHARA, TUBAKI and YOSIOKA	ISIHARA and SAWARAGI	SATO and TANAKA	
n	1/2	1/4	1/3	
α	1.56・2.44	0.171	0.565	1.35
The type of movement	initial movement	initial movement	general movement	complete movement

型実験などかなり多数報告されている。例えば，移動限界表示としては，栗原ら[1]や石原，椹木[2]や佐藤，田中[3]，らにより次式によって示されている。

$$H_0/L_0=\alpha(d/L_0)^n \sinh 2\pi h_i/L(H_0/H) \quad (1)$$

ただし，

h_i: 移動限界水深，H_0, L_0: 沖波の波高及び波長，H, L: 水深 h_i における波高，波長である。

α, n は係数でありそれぞれ提案者によって異なり，Table 1 のようになっている。これらの各式で，係数の値が異なる理由としては，移動限界の判定の基準が人によって異なること，条件の限られた範囲における資料から導いた式を，広い範囲にまで拡張しているためと思われる。更に，この式を導くにあたり，層流境界層を仮定していること，定常流の抵抗法則を用いていることにも問題がある。以上の様な問題を加味して，種々の提案者の式を統一的に示したのが堀川，渡辺[4]である。彼らは，梶浦の振動流れにおける抵抗法則を考慮し，移動限界について述べている。ここで，著者らは砂漣と砂粒子の移動限界という点について堀川，渡辺の統一的見解を参考にし議論してみた。

一般に，砂面へ作用する波の力がある一定値以下である場合は，砂粒子は静止したままであるが，この値を過ぎると砂粒子の動きは，その作用する力の程度によって，次の様な段階が考えられる。これを MANOHAR[5] は次の様に定義した。

(1) Initial movement（初期移動）
　水底の表面に比較的突き出した粒子のいくつかが動き出す状態
(2) General movement（全面移動）
　水底の表面の第一層がほとんど動き出す状態
(3) State of producing sand ripple
　砂漣の形成される状態
(4) State of disappearing sand ripple
　砂漣の消失する状態

以上，四つに分けられるが，それぞれの判断の基準に個人差があることは考慮しなければならないことである。著者らは，この区分の(3)，砂漣の形成される程度の砂粒子の移動について議論したのであるが，従来の方法とは異なり進行波によらず，定常波を用いて調べた。

2. 波による砂粒子の移動限界の理論的考察

波の作用下における底面の摩擦応力 τ は，

$$\tau=\tau_0 e^{i\theta_1} \quad (2)$$

(2) 式を底面近くの水平方向の流速 $u=u_0e^{i\theta_2}$ を用いると，

$$\tau=\rho f u_0 u \quad (3)$$

すなわち，

$$\tau_0 e^{i\theta_1}=\rho f u_0^2 e^{i\theta_2} \quad (4)$$

ここに，τ_0: 摩擦応力の振幅，f: 抵抗係数，である。(4) 式より，

$$f=\tau_0/\rho u_0^2 \cdot e^{i(\theta_1-\theta_2)} \quad (5)$$

抵抗係数の振幅 f_0 は，

$$f_0=\tau_0/\rho u_0^2 \quad (6)$$

波による砂粒子の移動を考えるとき，鉛直力及び粒径と波高の比が小さい場合には，水平方向の加速度が無視されることが示されている。このため，その移動開始時における力の平衡関係は，(6) 式を用いて次の様に表わすことができる。

$$(\rho_s-\rho)\frac{\pi}{6}gd^3 \tan\alpha=\rho k\frac{\pi}{4}d^2 f_0 u_0^2 \quad (7)$$

ここに，k: 砂の移動の程度によって決まる定数，α: 砂粒子の安息角，である。

一方，抵抗係数 f_0 は，$u_0\delta/\nu$ との関数であると考えられるので，次の様に書ける。

$$f_0=a(u_0d/\nu)^p(u_0\delta/\nu)^q \qquad (8)$$

a, p, q: 係数

ここで，境界層が層流の場合，(8) 式において $p\ll q$ となると考えられるから，(8) 式は次の様になる。

$$f_0\fallingdotseq a(u_0\delta/\nu)^q \qquad (9)$$

(7) 式と (9) 式から，次の様な関数関係が予測される。

$$Sdg\tan\alpha/u_0^2=F(u_0\delta/\nu) \qquad (10)$$

この砂の移動限界に関する関数関係は，野田[6]らにより認められていることである。ただし，(10) 式は層流状態における関係式である。次に，この様に砂粒子の移動限界について考察するとき，その境界層が層流であるか，乱流であるかということが問題になってくる。この問題に関しては，LI and MANOHAR[7] らにより研究され，次の結果を得ている。すなわち，底面が粗面状態であるか，滑面状態であるかは，δ/d によって決まり，

$\delta/d>6.54$ ならば滑面状態

$\delta/d<4.02$ ならば粗面状態

$4.02<\delta/d<6.54$ ならば粗滑の遷移領域

である。

これらの底面の状態に対して層流，乱流の境界条件が異なり，次の値になるとしている。

滑面領域 { 乱流: $u_0\delta/\nu>566$ ／ 層流: $u_0\delta/\nu<566$

遷移領域 { 乱流: $u_0d/\nu>104$ ／ 層流: $u_0d/\nu<104$

粗面領域 { 乱流: $u_0d^{0.2}/\nu>1150$ ／ 層流: $u_0d^{0.2}/\nu<1150$

一方，KALKANIS[8] は，LI and MANOHAR の結果では遷移領域と粗面領域における層流，乱流の境界条件が異なるのは，彼らの実験の不備によるものであるとし，粗面領域でも遷移領域の式が成り立つとしている。更に，この問題に関する研究は，VINCENT[9]，CALLINS[10]，COLEBROOK and WHITE[11] らにより報告されているが，著者らは，KALKANIS の結果を使用して層流，乱流状態の決定を試みた。

3. 実　験

Fig. 1 に示すような水槽を用いて，その底に約 2～3 cm の厚さに砂を敷きつめ，その上に定常波を作る。波が作られると，底質に砂漣を生ずるが，この時生ずる砂漣は進行波によって生じた砂漣の様に，底一面に生ずるのではなく，図の様に砂漣の生じている部分と，生じていない部分が交互になっている。砂漣が生じている部分が定常波の節の付近であり，生じていない部分が腹の部分にあたる。この様な砂漣の境界に注目し，その点の水平方向の流速の振幅 u_0 を微小振幅波理論[12]により求め，砂粒子の移動限界について以下のように考察した。

1.　水平方向の流速の振幅 u_0

微小振幅波理論によると定常波による水平方向の流速 u は次式で与えられる。

$$u=\frac{Hgk}{a}\frac{\cosh k(y+h)}{\cosh kh}\sin kx\cos\sigma t \qquad (11)$$

Fig. 1 における境界付近の水平方向の流速 u は，$x=\frac{W}{2}$，$y=-h$ で与えられるから，

$$u=\frac{Hgk}{\sigma}\frac{1}{\cosh kh}\sin\frac{W}{2}k\cos\sigma t \qquad (12)$$

ここに，

$$\sigma=\frac{2\pi}{T},\quad k=\frac{2\pi}{L}$$

H: 波高，L: 波長，T: 波の周期，h: 水深，t: 時間，x: 水平方向にとった座標，y: 鉛直下向きにとった座標，W: Fig. 1 に示す距離である。

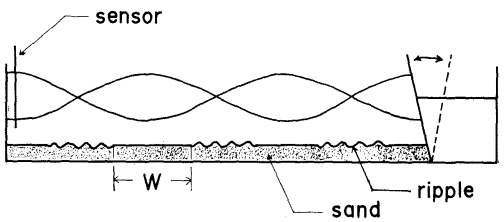
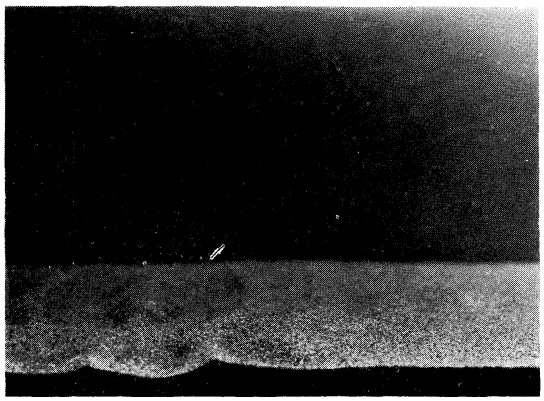
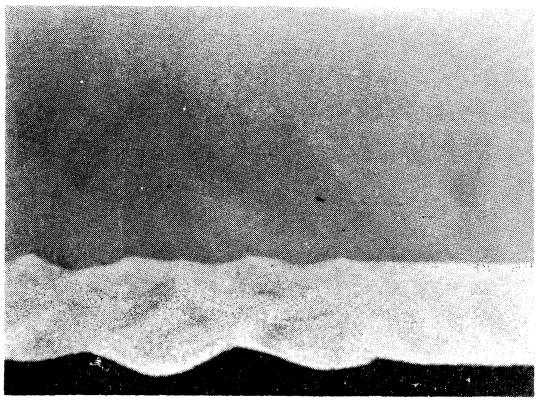


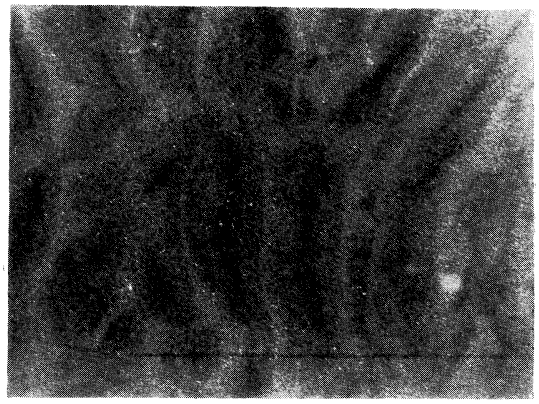
Fig. 1.　Schematic diagram of the experiment.



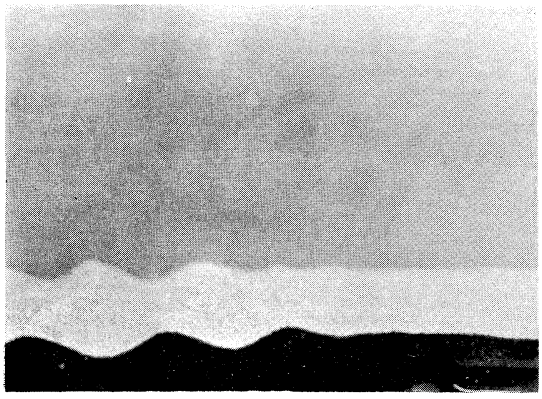
(1)
After 1 min. Photo at oblique upper position.



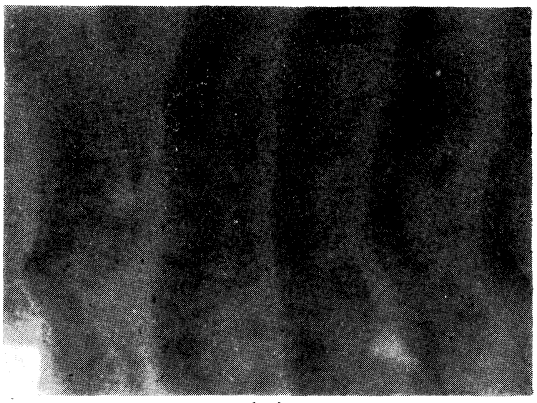
(2)
After 5 min. Photo at oblique upper position.



(3)
After 5 min. Photo at upper position.



(4)
After 10 min. Photo at oblique upper position.



(5)
After 10 min. Photo at upper position.

Fig. 2. Growing process of the sand ripple.

(12) 式より境界付近の流速の振幅 u_0 は，

$$u_0=\frac{Hgk}{\sigma}\frac{\sin\frac{W}{2}k}{\cosh kh} \qquad (13)$$

すなわち，W を測定することにより，(13) 式を用いて，境界付近の水平方向の流速の振幅 u_0 を計算することができる。

2. 砂漣と砂漣の間隔 W の測定

(13) 式より，W を測定すれば u_0 を求めることができるが，W を測定する場合に，砂漣の成長が問題になる。すなわち，砂漣の成長により砂漣が前進し，その結果 W が次第に小さくなっていく。そこで，この W のふるまいを調べるために，砂漣の成長過程を調べた。

Fig. 2 の (1) から (5) が砂漣の成長してゆく様子である。(1) は波を当ててから約 1 分後の写真であり，斜め上方から撮影したものである。この状態は，発達初期の段階で，砂粒子の移動は見受けられるが，砂漣はまだほとんど形成されていない。従って，当然その境界も明確に決定できない。(2) と (3) は約 5 分後のものであり，それぞれ，斜め上方と真上から撮影したものである。この程度になると，形は不規則ではあるが，明らかに砂漣は生じており，その境界も見分けられる。また，砂の峰の部分に注目すると，峰は複雑に交差しているのが見られる。(4) と (5) は約 10 分後の写真で前と同様に，斜め上方と真上から撮影したものである。この程度になると砂漣は十分発達しており，砂漣の波長に規則性が見られ，また，峰の部分が互いに平行になっていることもわかる。この時の砂漣の波長は約 3.0 cm，波高は約 0.5 cm であった。砂漣が発達する時間は，当てる波によってそれぞれ異なるが，その発達過程は，この様なものになると考えられる。次に，上の様な砂漣の発達に伴って砂漣と砂漣の間隔 W が，どのように変化してゆくかを示すために，W の時間変化をグラフに示した。(Fig. 3) このグラフと前述の砂漣の成長とを対応させて考察すると，砂漣が著しく前進を開始するのは，砂漣の峰の部分がほぼ平行になり始めた時点からと思われる。また，ある程度時間が経過すると，W が一定の値に近づくことから，砂漣もある程度前進すると，それ以上前進しなくなることがわかる。以上のような簡単な観察から，砂粒子の移動限界を論ずる W の値として，砂漣が著しい前進を開始する前，つまり W がほぼ一定の値になった，この値を測定値とした。と言うのは，砂漣が前進するのは，前に作られた砂漣が大きく影響し，新たに砂漣が形づくられて前進して行くものと考えられるからである。

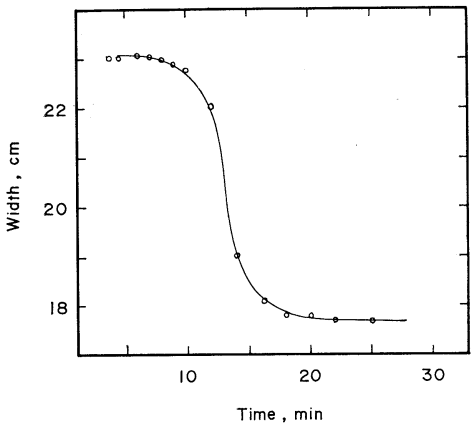


Fig. 3. Change in value of W.

3. 実験方法及び装置

装置は Fig. 1 に示したものを使用した。水槽は，波長を変化させるために次のような A, B 2 種類の水槽を使用した。

A: 幅 38 cm，長さ 300 cm，深さ 45 cm

B: 幅 15 cm，長さ 130 cm，深さ 20 cm

底質に用いた砂粒子は，平均粒径 0.16 mm，比重 2.68 と，平均粒径 0.16 mm，比重 2.94 の 2 種類を用いた。この砂の粒度分布は，平均粒径 0.16 mm 付近に約 60 % が集中している。実験は，A の水槽で波長を 180 cm と 108 cm の 2 種，B の水槽では波長 130 cm を作り，それぞれについて水深，波高を変化させ，上述のように砂漣がある程度発達するまで波を当て，W を測定した。波高の測定には，容量型波高計を Fig. 1 に示す位置にセットして測定した。周期は，この実測値を用いた。測定結果と (13) 式とより求めた u_0 の値を Table 2 に示す。

Table 2. Exprimental values.

Wave height	Wave length	Depth	Period	W	u_0	Remarks
cm	cm	cm	sec	cm	cm/sec	
5.9	180	27.7	1.26	48.0	25.3	
5.1	180	27.7	1.26	58.5	28.0	Water tank A
7.7	108	27.7	0.87	29.0	22.0	
5.7	108	24.7	0.86	33.0	22.2	$\rho_s=2.94$ g/cm^3
6.0	180	25.3	1.27	59.0	35.9	$d=0.16$ mm
4.1	180	22.2	1.29	65.0	30.4	
4.1	108	22.2	0.87	32.0	17.8	
5.1	108	22.2	0.86	28.0	18.4	
4.2	108	20.2	0.90	30.0	19.1	
4.9	130	12.0	1.22	35.0	36.7	
4.4	130	11.5	1.25	36.0	25.2	Water tank B
4.1	130	10.8	1.26	34.0	42.4	
6.4	130	10.8	1.25	21.0	28.2	$\rho_s=2.68$ g/cm^3
4.7	130	9.8	1.33	32.0	36.0	
6.4	130	9.8	1.39	24.0	34.5	$d=0.16$ mm
4.3	130	8.5	1.36	28.0	29.8	
6.3	130	10.5	1.30	17.0	23.1	
6.1	130	11.6	1.24	30.0	39.1	
5.5	130	10.0	1.29	26.0	32.2	
5.3	130	9.0	1.36	26.0	33.3	
5.4	130	11.0	1.25	23.5	26.7	

4. 結果及び考察

実験によって得られたデータを整理する時に，前述のように測面の粗滑状態に応じた層流，乱流が問題になる。著者らが得た資料について，これらの状態について考察すると，次の様になった。

底面の粗滑の状態 $\delta/d>4.05$

底面の流れの状態 $u_0d/\nu<70.6$

故に，底面の状態は粗面であり，層流状態にあると考えられる。次に，砂粒子の移動限界に関する(10)式について調べてみた。(Fig. 4) 参考のために堀川，渡辺の砂粒子の全面移動限界に関する測定値を示した。

Fig. 4 から著者らが得た結果は，ほぼ直線関係にあるように思われる。しかしながら，堀川，渡辺の資料と比較して，明らかに著者らの測定値が定数倍だけ下方向にずれている。この主な原因は前述したように，砂粒子の移動限界の程度が異なり，堀川，渡辺は，全面移動について実験を行なったのに対し，著者らは，砂漣形成という点に重点をおき砂粒子の移動限界について測定したためであろう。

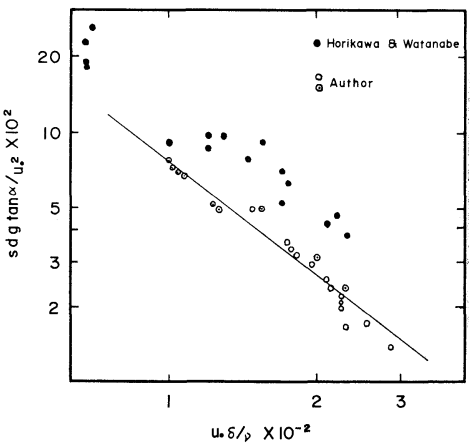


Fig. 4. The threshold state for sand.

この実験値から最小二乗法により，回帰式を求めると次のようになる。

$$\log Sdg\tan\alpha/u_0^2=-1.86\log u_0\delta/\nu+2.71$$

$$Sdg\tan\alpha/u_0^2=513\,(u_0\delta/\nu)^{-1.86} \qquad (14)$$

ただし，著者らが行なった実験においては，$\tan\alpha\fallingdotseq1$ とした。

実際にfieldにおいてどの程度の波によって砂が移動を開始するかを知るためには，(14) 式と微小振幅波理論より波高，波長，水深を求めればよい。

すなわち，微小振幅波理論によると，底面付近の水平方向の流速の振幅は次のようになる。

$$u_0=\frac{\pi H}{T\sinh 2\pi h/L} \qquad (15)$$

(14) 式と (15) 式より，

$$\frac{\pi H}{T\sinh 2\pi h/L}=\left\{\frac{Sdg\tan\alpha}{513}\left(\frac{\pi\nu}{T}\right)^{-0.93}\right\}^{7.14} \qquad (16)$$

(16) 式を満足する波が限界の波である。

しかしながら，ここで行なった実験はごく限られた条件のもとでのものであり，この式が実際にfield で適用できるかどうかは，更に検討が必要である。今後，このような定常波による砂漣についてより詳しく検討してゆきたいと考えている。

5. 結　論

定常波によって作られた砂漣の境界において，砂の移動限界に関する式

$$Sdg\tan\alpha/u_0^2\sim u_0\delta/\nu$$

の関数関係が認められた。

謝　辞

昭和48年度日仏海洋学会学術研究発表会において，佐々木忠義博士，並びに黒木敏郎博士に貴重な御教示と激励を承り，ここに深く感謝いたします。また，本研究中，当研究室の学生諸兄には種々御協力を頂き，ここに感謝申し上げます。

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A Geophysical Consideration of the Water in the Ushigomebori Moat

On the Seiche Motion of the Moat (II)*

Nobuo MORITANI** and Tomosaburo ABE**

Abstract: The Ushigomebori Moat is a basin appropriate to study of seiche motion because of its regular shape. The authors make a short quantitative discussion on observed seiches in the moat. On the assumption that the seiches are generated by the co-oscillation with the wind stress given by $\tau_0 \sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}$ and that they are subject to the uniform internal friction which is assumed to be $-2k\bar{u}$, the calculated amplitude agrees well with the observation. The drag coefficient r^2_{10} is also calculated by using the amplitude of seiches. It is 2.3×10^{-3} on the average.

1. Introduction

The present authors have carried out the observations on seiches almost every day since September 1, 1970 in the Ushigomebori Moat which is located in front of the buildings of Science University of Tokyo (Faculty of Science). The shape of the moat is roughly rectangular and the wind blows nearly parallel to the longitudinal direction throughout the year. In the moat the uni-nodal seiches are often observed. The authors have made a basic consideration on the seiches and made a phenomenal discussion about the relation between the amplitude of the uni-nodal seiches and the wind speed on the moat in an earier paper (MORITANI and ABE, 1972). In the present paper, they intend to make a short quantitative consideration on the resonated seiches generated by the wind stress under a few reasonable assumptions.

2. Theory and procedure

If we regard the Ushigomebori Moat (Fig. 1) as a rectangular basin of length L and depth h, the equation of motion is given by

$$\frac{\partial\bar{u}}{\partial t}+2k\bar{u}+g\frac{\partial\zeta}{\partial x}=\frac{\tau_s}{\rho h}\,, \qquad (1)$$

and the equation of continuity is given by

$$h\frac{\partial u}{\partial x}+\frac{\partial\zeta}{\partial t}=0\,. \qquad (2)$$

In these equations g is the acceleration due to gravity, ρ is the uniform density of the water, h is the constant depth of the moat, τ_s is the shearing stress of wind exerted on the water surface, $2k\bar{u}$ is the frictional force per unit mass, which is assumed to be proportional to the mean velocity of water ($\bar{u}$) and k indicates the frictional coefficient.

In the rectangular basin take the origin of the coordinates at one of its edges, the x-axis in the direction of the length, the z-axis and ζ the surface elevation both positive upward, as is shown in Fig. 2.

Now we seek a solution of these equations in three cases: $\tau_s=0$, $\tau_s=\tau_s(x)$ and $\tau_s=\tau_s(x,t)$.

1. Damping Seiches (in the case of $\tau_s=0$)

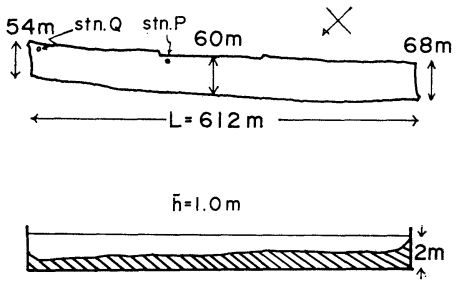

Fig. 1. Shape of the Ushigomebori Moat.

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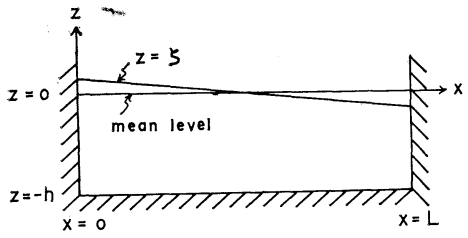

Fig. 2. A cross section of the rectangular basin.

The seiches are generated by wind stress. The amplitude of the oscillation decreases by friction immediately after the wind blows over; that is, τ_s becomes zero. In this case, for the uni-nodal oscillation,

$$\zeta=\zeta_0 e^{-kt}, \qquad (3)$$

where

$$\zeta_0=H\cos\frac{\pi x}{L}\cos\left(\frac{2\pi t}{T_1}-\theta\right)$$

$$T_1=\frac{2L}{\sqrt{gh}},\quad \theta=\tan^{-1}\frac{kT_1}{\pi}$$

and H means the amplitude of seiches.

2. *Wind set-up (in the case of $\tau_s=\tau_s(x)$)*

When the wind blows steadily and parallel to the moat (that is $\tau_s=\tau_s(x)$), seiches are not generated but wind set-up will take place. In this case, Equation (1) becomes $g\frac{\partial\zeta}{\partial x}=\frac{\tau_s(x)}{\rho h}$, that is, the generating force $\tau_s(x)$ entirely balances the hydrostatic pressure gradient. If the set-up S is defined as the difference in elevation between the two edges of the moat, S is written as

$$S=\frac{L}{\rho gh}\overline{\tau_s(x)}, \qquad (4)$$

where

$$S=\zeta_0-\zeta_L,\quad \overline{\tau_s(x)}=\frac{1}{L}\int_0^L\tau_s(x)dx$$

and ζ_0 and ζ_L means the surface elevation at $x=0$ and $x=L$, respectively. (see Fig. 2)

3. *Resonated seiches (in the case of $\tau_s=\tau_s(x,t)$)*

When the wind stress is variable as $\tau_s=\tau_0\sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}$, the resonated seiches will be occurred. And then, in the equiliblium state, the generating force will always balances the hydrostatic pressure gradient and the frictional force. In this case, for the uni-nodal oscillation,

$$\zeta=H\sin\left(\frac{2\pi t}{T_1}\pm\frac{\pi}{2}\right)\cos\frac{\pi x}{L}, \qquad (5)$$

where

$$T_1=\frac{2L}{\sqrt{gh}}\ \text{and}\ H=\frac{L\tau_0}{\rho ghT_1K}.$$

Hence,

$$\tau_s=\frac{\rho ghT_1KH}{L}\sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}. \qquad (6)$$

3. Date analysis

The wind stress τ_s is obtained from the data of the wind speed. The drag coefficient r^2 and the amplitude of seiches H are also calculated from the data of the wind speed.

Now taking a wind speed record over a proper time interval T seconds and reading it every $T/2n$ seconds as shown in Fig. 3, we carry out harmonic analysis of the time series thus obtained. Let T be equal to mT_1, where $m=1,2,3,\ldots$ and T_1 is the period of uni-nodal seiches in the moat. Since there is a bank around the moat which is about 10 m in height, it seems that the wind speed almost vanished at the edges of the moat and takes maximum value in the middle part of it. Therefore, we may assume the horizontal distribution of the wind speed is of the form $W(t)\sin\frac{\pi x}{L}$ to get the wind speed as follows:

$$W_*=\sum_{i=0}^{n-1}W_i\sin\left(\frac{2\pi it}{T}+\varepsilon_i\right)\sin\frac{\pi x}{L} \qquad (7)$$

in the case of $i=0$,

$$W_i\sin\left(\frac{2\pi it}{T}+\varepsilon_i\right)\sin\frac{\pi x}{L}=W_0\sin\frac{\pi x}{L}.$$

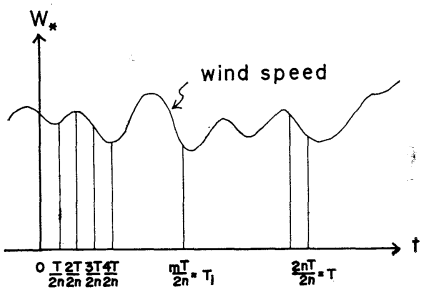

Fig. 3. Analysis of wind speed.

Now let us suppose that the wind stress exerted on water surface is proportional to the square of the wind speed, so that $\tau_*=\rho_a\gamma^2{}_{10}W_*{}^2$, where ρ_a means the density of air and W_* means the wind speed at the 10 m level. Then from Equation (7) the wind stress will be

$$\tau_*=\rho_a\gamma^2{}_{10}\left\{\sum_{i=0}^{n-1}W_i\sin\left(\frac{2\pi it}{T}+\varepsilon_i\right)\sin\frac{\pi x}{L}\right\}^2. \tag{8}$$

From Equation (6), the resonated seiches are induced by the variable wind stress $\tau\sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}$ whose wave length is $2L$ and the period is T_1. Therefore, these components of the wind stress in Equation (8) are considered to mainly generate the uni-nodal resonated seiches. Now, by Fourie's theorem Equation (8) may be represented as a sum of a number of harmonic constituents.

$$\tau_*=\sum_{i=1}^{n}\sum_{k=1}^{n'}\tau_{i,k}\sin\left(\frac{2\pi it}{T}+\theta_i\right)\sin\left(\frac{\pi kx}{L}+\theta_k\right),$$

where $\tau_{i,k}$ are constants. Only $\tau_{m,1}$ is required, for the harmonic constituent of the wave period $T_1(=mT)$ and length $2L$. To get $\tau_{m,1}$, multiply τ_* by $\sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}$ and integrate from $t=0$ to $t=T$ and from $x=0$ to $x=2L$. It follows that

$$\tau_{m,1}=\frac{8}{3\pi}\rho_a\gamma^2{}_{10}\left\{2W_0W_m\cos\varepsilon_m+\frac{8}{3\pi}W_m{}^2\cos\varepsilon_m+\frac{1}{2}W_mW_{2m}\sin(\varepsilon_m-\varepsilon_{2m})\right\}\sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}.$$

In the case of $\varepsilon_m=0$ and $W_{2m}\ll W_0+W_m$,

$$\tau_{m,1}=\frac{8}{3\pi}\rho_a\gamma^2{}_{10}W^2\sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}, \tag{9}$$

where $W^2=2W_0W_m+\frac{8}{3\pi}W_m{}^2$.

Then from Equations (6) and (9),

$$\gamma^2{}_{10}=\frac{\rho ghT_1kH}{\rho_aLW_0{}^2F(\alpha)} \tag{10}$$

or

$$H=\frac{\rho_aLW_0{}^2\gamma^2{}_{10}F(\alpha)}{\rho ghT_1k} \tag{11}$$

where $F(\alpha)=\alpha(2+\alpha)$ and $\alpha=\frac{8}{3\pi}\frac{W_m}{W_0}$.

4. Results and discussions

1. *Wind speed*

As the moat is surrounded by a bank which is about 10 m in height, it seems that the wind blows nearly parallel to the longitudinal direction of the moat through the year (see the Wind Rose in the above cited report, 1972). The wind speed was observed by a Three-Cup Robinson Type anemometer at a level of about one meter at Station P (see Fig. 1) which is in nearly the center of the moat. The monthly average of the wind speed is shown by a thick line in Fig. 4. Also the monthly average of wind speed at the Meteorological Agency at the 52.2 m level is shown by a light line. The Meteorological Agency is located about 3 km east of the moat. Now we assume that the wind profile near the ground is indicated as (KONDO (ed.), 1964)

$$\frac{W_z}{W_{z_0}}=\left(\frac{z}{z_0}\right)^{0.24},$$

where z means the height from the ground, $z_0=10$ m, W_{z_0} and W_z are the speeds at 10 m and z m levels, respectively. Then the relationship between the wind speed at a 10 m level on the moat and that at a 52.2 m level at the Meteorological Agency was estimated by extrapolating the slope of the wind profiles from

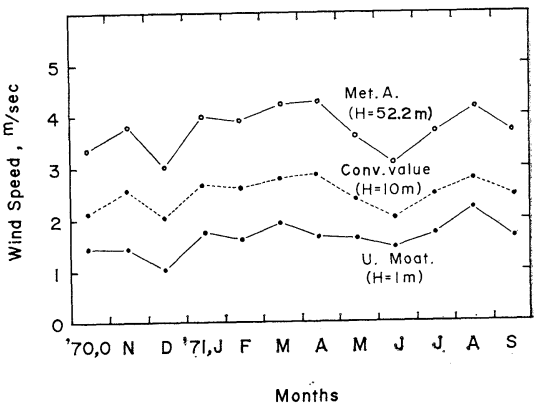

Fig. 4. Monthly average of the wind speed at the Meteorological Agency and on the Ushigomebori Moat.

Table 1. Comparison the observational period $T_{obs.}$ with the calculated period $T_{cal.}$.

Date	Interval of obs.	Mean depth (m)	$T_{obs.}$ (sec)	$T_{cal.}$ (sec)
Jan. 31, '71	09:01~14:31	1.04	406.6	39.41
Feb. 9, '71	13:21~18:12	1.01	406.0	396.0
Apr. 21, '71	08:49~11:11	1.02	405.7	394.1
May 22, '71	07:55~14:04	1.02	402.5	394.1
Jun. 4, '71	15:15~21:20	1.02	405.5	394.1
Jul. 7, '71	12:00~15:45	1.05	397.1	388.4
Mean values		1.02	403.9	393.5

10 m to 52.2 m. The extrapolated value is also shown by a broken line.

The extrapolated values of the wind speed is 1.56 times as large as that of the wind speed observed on the average. Therefore, we assume the wind speed at the 10 m level on the moat can be obtained with multipling the wind speed observed at the one meter level by 1.56.

2. Period of seiches

The observations on seiches in the moat have been made since September 1, 1970, at Station Q with float type level meter (see Fig. 1). The station is located by the edge of the moat. In the moat the predominant uni-nodal seiches are frequently observed. The observed period of the seiches becomes $T_{obs.}=403.9$ sec as shown in Table 1. The calculated period by the formula $T_{cal.}=2\sum_{i=0}^{n}\frac{\Delta x_i}{\sqrt{g\bar{h}_i}}$ (where $\Delta x_i=x_{x+1}-x_i$ and $\bar{h}_i$ is the mean depth at x_i) is 393.5 sec, which is in good agreement with the observed period. For the sake of simplicity of calculation, the period of the uni-nodal seiches in the moat is taken as $T_1=400$ sec.

3. Damped seiches

Fig. 5 shows the record of a damping seiche which was obtained during a heavy thunderstorm. The amplitude of the seiches decreases nearly exponentially because of the rapid decreasing of the wind speed. In this case, k becomes 9.3×10^{-4} from Equation (3).

Fig. 6 is a record obtained for a light wind. In this case, k is 9.2×10^{-4} sec^{-1}. Therefore, for the average value of these two cases k becomes 9.2×10^{-4} sec^{-1}. The frictional coefficient in Lake Ashinoko (about 6 km in length and 25 m in mean depth) was 1.9×10^{-5} sec^{-1}, after SUZUKI (1935). The value in the moat is remarkably large compared with that in Lake

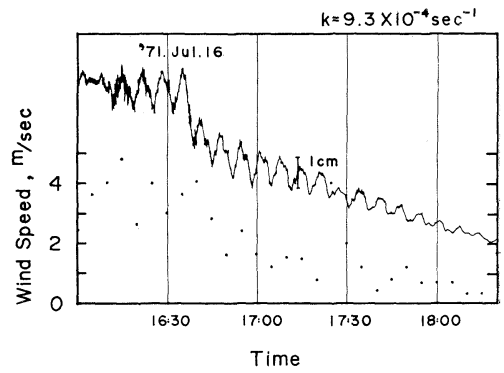

Fig. 5. Record of a damping seiche obtained during a heavy thunderstorm.

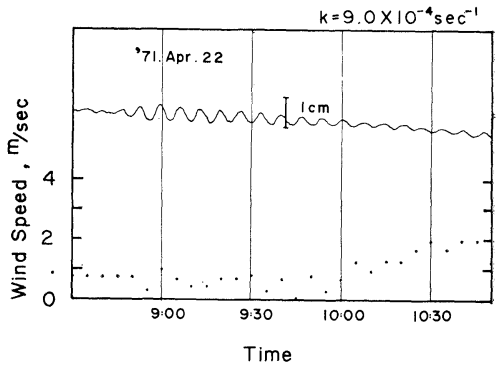

Fig. 6. Record of a damping seiche obtained for a light wind.

Ashinoko. This seems to be caused mainly by the shallowness of the Ushigomebori Moat relative to its length.

4. Wind set-up

When the wind stress exerted on the water surface has a form

$$\tau_s(x)=\rho g h\frac{T_1 k}{L}H\sin\frac{\pi x}{L}, \qquad (12)$$

Equation (4) gives the wind set-up

$$S=\frac{2HT_1k}{\pi}.$$

From Equations (5), (6) and (12), we have

$$2H=\frac{\pi}{T_1k},$$

The double-amplitude of seiches $2H$ becomes π/T_1k times a large as the set-up S. In the case of the moat, we have $T_1=400$ sec (c. f. Table 2) and $k=9.2\times10^{-4}\,\mathrm{sec}^{-1}$, so that $2H$ becomes $8.6\,S$. Hence the observation of the amplitude of seiches seems easier than that of the wind set-up in lakes or bays.

5. *Resonated seiches*

We assume that the seiches are generated by co-oscillation with the wind stress. According to Equation (11), the amplitude of seiches becomes

$$H=\frac{\rho_a L W_0^2 \gamma^2_{10} F(\alpha)}{\rho g h T_1 k},$$

where $F(\alpha)=\alpha(z+\alpha)$ and $\alpha=\frac{8}{3\pi}\frac{W_3}{W_0}$.

We put $\rho_a=1.20\times10^{-3}\,\mathrm{g/cm^3}$, $\rho=1.00\,\mathrm{g/cm^3}$, $h=1.00\times10^2$ cm, $L=6.12\times10^4$ cm, $T_1=400$ sec, $k=9.2\times10^{-4}\,\mathrm{sec}^{-1}$, $r^2_{10}=2.0\times10^{-3}$. The wind speed observed at the one meter level is used to get that at the 10 m level as mentioned before. Now we take a time length $T=1{,}200$ sec and $2n=20$. Since T_1 is 400 sec, m becomes 3 in Equation (9). We assume that the wind blows parallel to the moat and its direction is not changed in time. Since an error is inevitably introduced in the reading of the W_6 constituent (whose period is 200 sec), this constituent is not taken into account in the following calculations. That is, the wind stress which generates the resonated seiches is given by Equation (9).

6. *The amplitude of seiches versus wind speed*

Fig. 7 shows a result of the harmonic analysis of the wind speed when the uni-nodal seiche motion was prevailed as well as data of the seiches. The notation W_0 means the average wind speed, W_3 means the oscillation with a period of 400 sec and H means the amplitude of seiches obtained from Equation (11). The amplitude in the time interval 16:10 ~16:30, June 4, 1971 becomes fairly smaller than the calculated value H. This may be explained as follows; these seiches may be generated before that time and they keep relatively large amplitude in those time interval

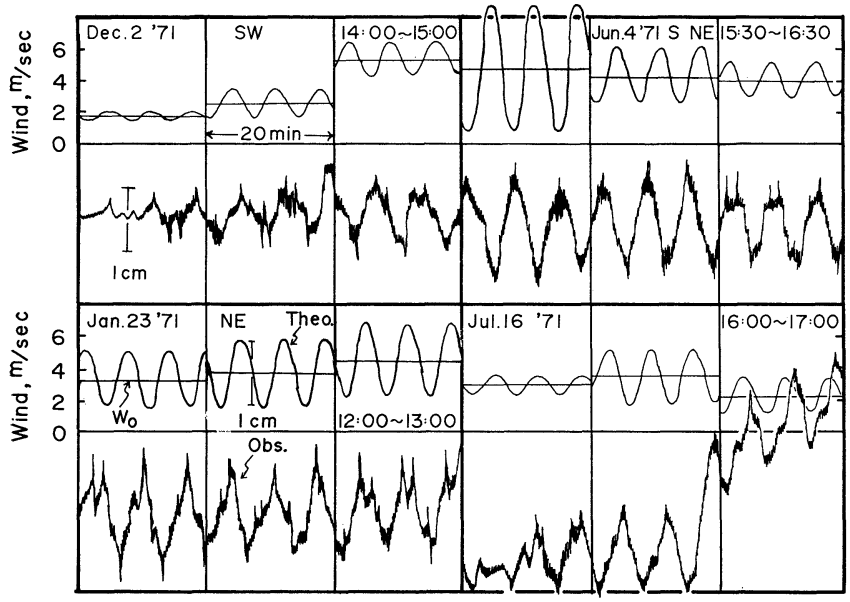

Fig. 7. Results of the harmonic analysis of the wind speed when the uni-nodal seiche motion.

without full damping effect. Since the seiches in the time interval 16:00~16:20, July 16, 1971 was generated during a heavy thunderstorm, as mentioned before, it may be expected that a front line had passed over the moat, which could give rise to the reversal of the wind direction and to the atmospheric pressure difference at the both edges of the moat in those time interval. Hence the theoretical values are considered in fair agreement with the observed results, except in the above-mentioned cases. In the moat, consequently, the seiches seems to be mainly generated by the co-oscillation with the wind stress.

The relation between the amplitude of seiches and the mean wind speed over 30 min is shown in Fig. 8. The double-amplitude $2H$ is plotted against the mean wind speed with the signs ○ and ●, respectively. The solid lines indicate the theoretical curves based Equation (11). As shown in these theoretical curves, the values of $2H$ are very small for a light wind and much larger for higher wind speeds. Now on paying attention to the intersections of the theoretical and broken lines, a characteristic of the wind over the moat may be seen as follows: The W_3 constituent, the period of which is equal to that of seiches in the moat, is nearly the same value for a light wind and the ratio W_3/W_0 becomes much smaller with higher wind speeds. The wind in general seems to fluctuate to a great extent about its mean values for a light wind, while the fluctuation seems to be smaller for higher wind speeds. When the wind blows parallel to the moat and does not change its direction, the ratio of W_3/W_0 is generally smaller than the unity. Hence in Fig. 8, the data of the amplitude of seiches, shown by sign ●, seem to be caused by disturbances such as the change of the wind direction, the pressure difference at the both edges of the moat and some others. The authors like to study on these kind of seiches in future.

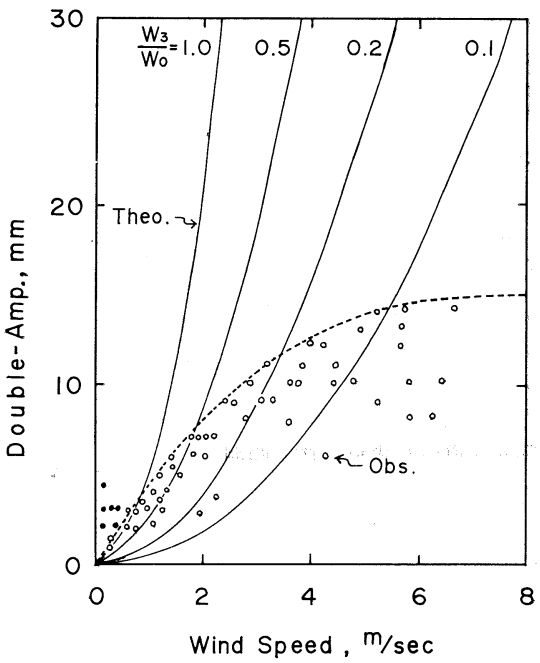

Fig. 8. The amplitude of seiches versus wind speed.

Table 2. The observational data of W_0, W_3, $2H_{obs.}$ and the calculated values r^2. In the table W_0 and W_3 means the wind speed at the level 10 m.

Date	Interval of obs.	W_0 (m/sec)	W_3 (m/sec)	$2H_{obs.}$ (cm)	r^2
Dec. 2, '70	14:20~14:40	4.18	0.89	0.45	0.0017
Jan. 22, '71	15:20~15:40	3.65	1.09	0.59	0.0019
Jan. 23, '71	12:20~12:40	5.07	0.99	0.78	0.0020
	12:40~13:00	5.68	0.73	0.90	0.0052
	13:00~13:20	6.43	1.05	0.90	0.9015
Jan. 29, '71	13:00~13:20	3.92	0.98	0.38	0.0014
Jan. 31, '71	11:30~11:50	3.00	0.97	0.55	0.0023
Feb. 6, '71	16:45~17:05	4.39	0.56	0.41	0.0028
Jun. 4, '71	15:30~15:50	7.49	1.75	1.62	0.0016
Jnl. 16, '71	16:20~16:40	5.48	1.28	1.09	0.0022
				Mean	0.0023

Table 3. The calculated values of k and ν in Ushigomebori Moat.

	Frictional coeff. k (sec^{-1})	Kinematic viscosity ν (c.g.s.)	
		In case of $\tau_b=0$	In case of $u_b=0$
Ushigomebori Moat	9.2×10^{-4}	3.5×10^{5}	4.6
Lake Ashinoko	1.9×10^{-5}	$\sim10^{5}$	1～10

7. Drag coefficient

The theoretical values γ^2_{10} is obtained from Equation (10), as

$$\gamma^2_{10}=\frac{\rho g h T_1 k H}{\rho_a L W_0^2 F(\alpha)},$$

where $F(\alpha)=\alpha(2+\alpha)$ and $\alpha=\frac{8}{3\pi}\frac{W_3}{W_0}$.

The observed data W_0, W_3, $2H_{obs.}$ and the values γ^2_{10} calculated from Equation (10) are shown in Table 2. A detailed discussion on γ^2_{10} is out of the scope, as mentioned before. However, the mean value of the $\gamma^2_{10}=2.28\times10^{-3}$ is in good agreement with the values $\gamma^2_{10}=(1.5\pm0.8)\times10^{-3}$ which is often used for lower wind speeds.

8. Kinematic viscosity

Now we assume the damping of the seiches is entirely caused by the kinematic viscosity instead of the above-mentioned friction and calculate coefficient of the kinematic viscosity ν by means of the same method as that used by SUZUKI (1936) for Lake Ashinoko. Table 3 compares the kinematic viscosity in the moat with that in Lake Ashinoko. The kinematic viscosity is calculated in the following two cases; one is for the free-slip bottom ($\tau_b=0$) and the other is for the non-slip bottom ($u_b=0$). The value becomes 3.5×10^5 c.g.s. in the former case and 4.6 c.g.s. in the latter case. The kinematic viscosity which is obtained from data on the ocean current might be between 1 c.g.s. and 10^4 c.g.s. Hence the damping in the Ushigomebori Moat may be explained more reasonably by the non-slip bottom condition.

The amplitude of the resonated seiches including the effect of the kinematic viscosity is calculated according to HIDAKA (1935) as follows; when the water does not slip at the bottom, it becomes

$$\zeta^*=\frac{L\tau_0}{\pi\rho g h}\sqrt{2}\,q\sqrt{\frac{\cosh q-\cos q}{\cosh q+\cos q}}\sin\left\{\frac{2\pi t}{T_1}-\left(2\pi\tan^{-1}\frac{\sinh q+\sin q}{\sinh q-\sin q}\right)\right\}\cos\frac{\pi x}{L},$$

where $\tau_s=\tau_0\sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}$, $q=\sqrt{\frac{2\pi h^2}{2\nu T_1}}$ and $T_1=\frac{2L}{\sqrt{gh}}$.

In our case $h=1.0\times10^2$ cm, $T_1=400$ sec and $\gamma=4.6$ c.g.s., so that q is equal to 4.1. Therefore, the maximum amplitude will be

$$\zeta^*_{Max}=\frac{L\tau_0}{\pi\rho g h}\times6.0\,.$$

On the other hand, from Equation (6), the amplitude of the variable wind stress τ_0 becomes

$$\tau_0=\rho g T_1 k h H/L.$$

Hence,

$$H=\frac{L\tau_0}{T_1\rho g h}\cdot\frac{\pi}{T_1 k}.$$

Substitution of Equations (14) and (15) into (13) gives

$$\zeta^*_{Max}=0.70\,\zeta_{Max.}$$

Consequently, the theoretical values of the amplitude of the resonated seiches with effect of the kinematic viscosity are in fair agreement with that obtained with the assumption that the friction is proportional to the velocity.

5. Conclusion

The Ushigomebori Moat is located in front of Science University of Tokyo (Faculty of Science). The moat is considered to be a model basin suitable for making researches in seiche motion because of its geometry. Then the observations of seiches in the moat have been carried out almost every day since September

1970. The authors have made a short quantitative discussion on the seiches from the obtained data. It is as follows:

(1) If the damping of the amplitude of seiches is assumed to be mainly caused by the effect of the internal friction, proportional to the mean velocity of the water particle, the frictional coefficient k is given by $9.2\times10^{-4}\,\mathrm{sec}^{-1}$ on the average.

(2) If the seiches are generated by the co-oscillation with the wind stress in the form of $\tau_s=\tau_0 \sin\frac{2\pi t}{T_1}\sin\frac{\pi x}{L}$, and subject to the above-mentioned friction, the calculated amplitude of seiches agrees well with the observation.

(3) The drag doefficient $\gamma^2{}_{10}$ is also calculated from the amplitude of seiches. It is 2.3×10^{-3} on the average.

(4) On assuming that the damping is entirely caused by the kinematic viscosity, the coefficient of the kinematic viscosity is calculated for the non-slip and free-slip bottom. The coefficient of the kinematic viscosity becomes 3.5×10^5 c.g.s. in the former case, 4.6 c.g.s. in the latter case.

(5) The theoretical values of the amplitude of the resonated seiches with the effect of the kinematic viscosity are in fair agreement with that with the effect of the internal friction.

Consequently, the moat may be regarded as an appropriate basin for making a study of seiches from the view point of the momentum transportation from air to water. The authors will carry out more accurate observations, with emphasis on the wind profiles, to have some insight into the mechanism of the air-water interaction.

Acknowledgments

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本学前濠水の地球物理学的考察

——牛込濠の静振について（第2報）——

森谷誠生　阿部友三郎

要旨: 牛込濠はその濠が有する特質上，静振運動を考究するには格好の Model 水槽と考えられる。昭和45年10月から翌年9月まで一年間に観測された静振に関する記録をもとに若干の定量的考察を行った。

かなり理想的に減衰していると見られる静振の記録から得られた摩擦係数（k）は $9.2\times10^{-4}\ \mathrm{sec}^{-1}$，また渦動粘性係数（$\nu$）は水が底で滑るとするとき 3.5×10^{5} c.g.s.，滑らないとするとき 4.6 c.g.s. となった。当濠に発達する静振は，風との共振により生じたとすると，その実測値の振幅をかなりよく説明できた。また，この静振の振幅，風速，減衰率等の観測値から計算された抵抗係数 γ^2_{10} の値は平均で 2.3×10^{-3} となり，通常弱風時によく使用される $\gamma^2=(1.5\pm0.8)\times10^{-3}$ の値と良く一致した。

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Effects of Slag on Aquatic Life*

Yunosuke IITAKA**, Ryohei TSUDA**, Tsutomu MORINAGA**
and Hidemi KUMAI***

Abstract: In order to obtain some knowledges of slag influence on aquatic life, a series of experiments was made. First, the aquatic life adhesion to or gathering around the iron slag dropped in the sea were observed. Further, fish breeding was made in the containers either with or without slags. Finally, the growth rate of zooplankton, rotifer, and the survival rate of fish eggs and larvae were studied. The results showed minor effects on the aquatic life. The aquatic organisms adhered or gathered around slags immediately after dropped in the sea (Table 3 and Figs. 1 and 2). The growth rate of fish in containers containing slags was better than that of without (Figs. 3 and 4). The results obtained for a rotifer and fish egg or larva were not clear.

1. Introduction

Recently, slag is introduced extensively to utilize as the constructional materials for dikes and coast. It is, therefore, very important to know the effects of slag on aquatic life. It is well-known that slag is effective for fertilization of farms. Nevertheless there is no report describing the influence of slag on aquatic life. The authors thus intend to discuss this problem in the present paper.

The present study was supported by a research fund from the Japan Slag Association, Osaka, Japan.

2. Slag used

The slag used was the iron slag offered by Wakayama Slag Industry Co. Ltd., which was classified into four sized-groups as shown in Table 1.

Table 1. Various sized-groups of slag used.

Sized-group	I	II	III	X
Size (Diameter in mm)	0-5	5-25	25-40	150-300
Specific gravity	2.58	2.57	2.57	2.47

And, its chemical components are illustrated in Table 2.

Table 2. Chemical components of slag used.

SiO_2	Al_2O_3	CaO	FeO	MgO	S	MnO	TiO_2
33.5	14.5	41.5	0.4	5.1	0.9	0.9	1.6

Data: from Wakayama Slag Industry Co. Ltd.

3. Adhesion and gathering of aquatic life on slag in sea

This experiment was made in the bay of Uragami, Wakayama. Several cubic steel meshed baskets, which contained some volumes of slag of sized-group X, were dropped at the 1.0, 3.0 and 5.0 m depth layers in the bay, on June 23, 1971, respectively. For comparison, similar baskets containing some stone or brick were also set at the corresponding layers. The dimension of a basket was 35 by 50 by 40 cm length. After dropping, the appearance of

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Table 3. Species found in- and outside of baskets contained slags.

On the 48th day	On the 128th day
Japanese parrot fish, *Oplegnathus fasciatus*	Japanese parrot fish, *Oplegnathus fasciatus*
Slimy, *Leiognathus nuchalis*	Rudderfish, *Girella punctata*
Footballer, *Microcanthus strigatus*	Filefish, *Stephanolepis cirrhifer*
Scraper, *Navodon modestus*	Coralfish, *Abudefduf vaigiensis*
Boxfish, *Ostracion tuberculatus*	Footballer, *Microcanthus strigatus*
Threeline grunt, *Parapristipoma trilineatum*	Lembus rudderfish, *Kyphosus lembus*
Filefish, *Stephanolepis cirrhifer*	Small crab
Sea lettuce	Small shrimp
Acorn shell	Sea lettuce
etc.	Sea squirt
	Acorn shell
	etc.

adhesion or gathering of aquatic life on slag, stone or brick was observed and recorded by human eyes and camera using an aqualung.

According to the divers, the aquatic life adhered to and gathered around slags immediately after dropping, and rather rapidly than stones or bricks. Table 3 shows the list of fish species found inside and outside of baskets contained slags on the 48th and 128th days.

Fig. 1 is the appearance of slags dropped at 3.0 m depth layer for 128 days. Fig. 2 is the species collected in one of the baskets contained slags, left on the 306th day after dropping.

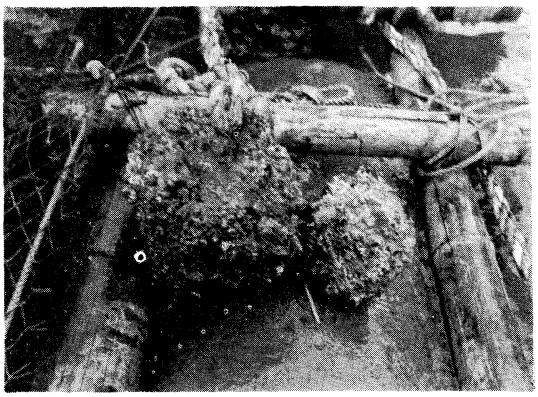

Fig. 1. Appearance of slags on the 128th day after dropping at 3.0 m depth layer.

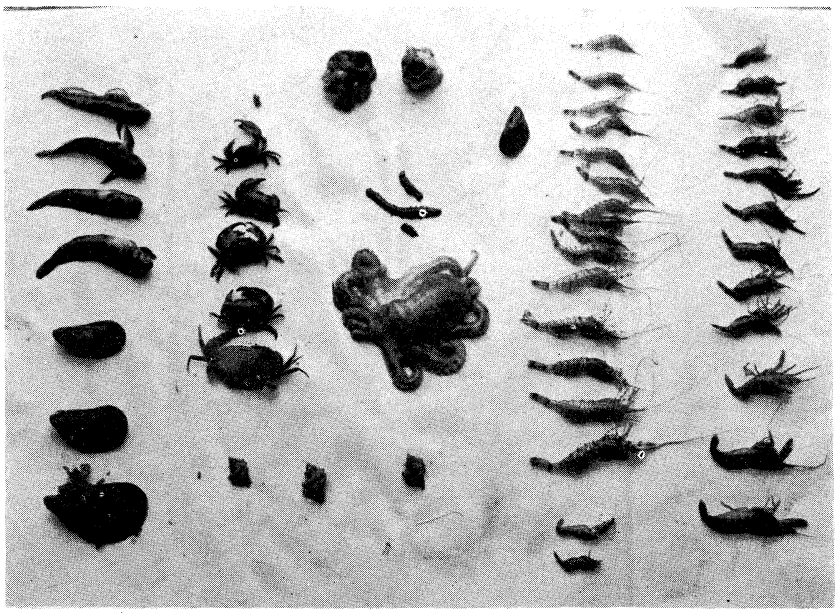

Fig. 2. Fish collected in one of the baskets with slags, on the 306th day after dropping.

4. Fish breeding in containers contained slags

This experiment was made in laboratory from August 4 to November 24, 1971. Prior to the experiment, four containers of 60 by 30 by 40 cm in length were prepared. Some slags of sized-group I, II and III, of which the weights of 5 kg each, were paved on the bottoms of the three containers, respectively. At the bottom of the last container, for comparison purpose, some pebbles of the same weight, 5 kg was placed. The fresh water of 60 *l* with ten fish of rose bitterling, *Rhodeus ocellatus ocellatus* was introduced into each vessel. The breeding conditions were the same at all containers. The water temperature was controlled as that of the room temperature, 17 to 25°C. The air of 750 m*l* per minute was circulated into each container. The pH values were not different in all containers and maintained within 8.5 to 7.2. 'Itomimizu-s', *Tubifex hattai* of 500 mg in weight per day were given into each container as food. During the experiment, the mortality of fish of each container was zero. The results are shown in Figs. 3 and 4. Fig. 3 shows the change in the mean total length of fish of each container during the experiment. Fig. 4 indicates the change in the mean body weight of fish by each vessel.

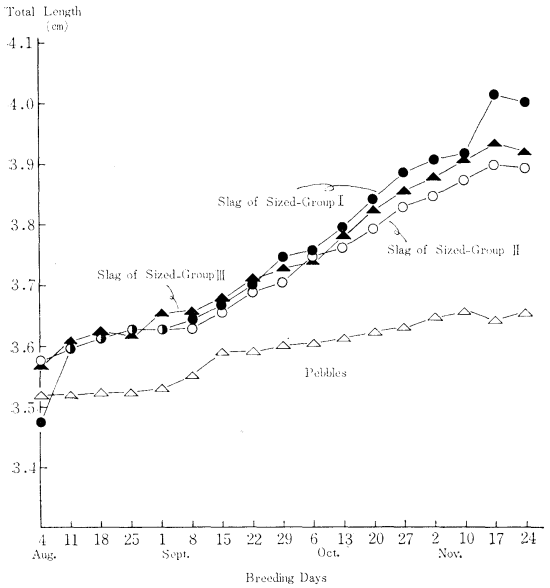

Fig. 3. Mean total lengths of fish during the experiment.

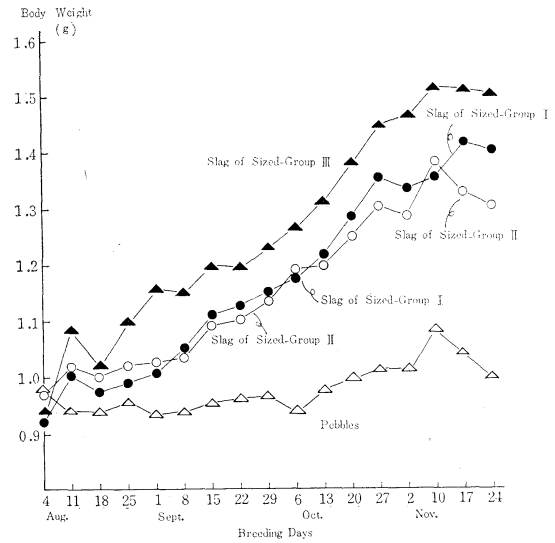

Fig. 4. Mean body weights of fish during the experiment.

From the above results, it is evident that the growth rate of fish kept in the containers contained slags was better than pebbles. There was insignificant difference in sized-groups of slag, except for the sized-group of III in body weight changing.

5. Culture of zooplankton, rotifer, in containers contained slags

The rotifer, *Brachionus plicatilis* was chosen in this experiment and its culture was conducted by using marine chlorella as food. At first, on the bottoms of five containers prepared, slags of sized-group II of 100, 300, 700 1,500 and 3,000 g weight were paved, respectively. Next, the chlorella, density of 17,000 cells per m*l* and the rotifer, density of one individual per m*l* were introduced together into each container with 30 *l* water. For comparison, one container without slag was also prepared, with the same density of chlorella and rotifer as mentioned above. The densities of chlorella and rotifer in each vessel were checked at a specific time. After stirring the culture container vigorously, ten samples were pipetted with minimal from the culture container and then the averages of chlorella cells and rotifer individuals per one m*l* were checked. During

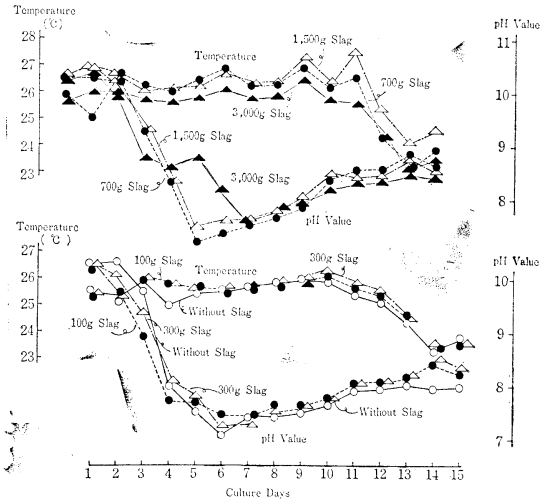

Fig. 5. Temperature and pH value of each container, during the experiment.

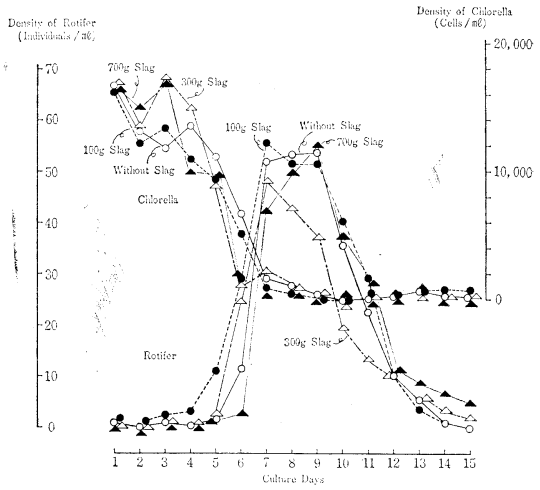

Fig. 6a. Densities of chlorella and rotifer in four experimental slag containers, from 0, 100, 300 and 700 g, during the experiment.

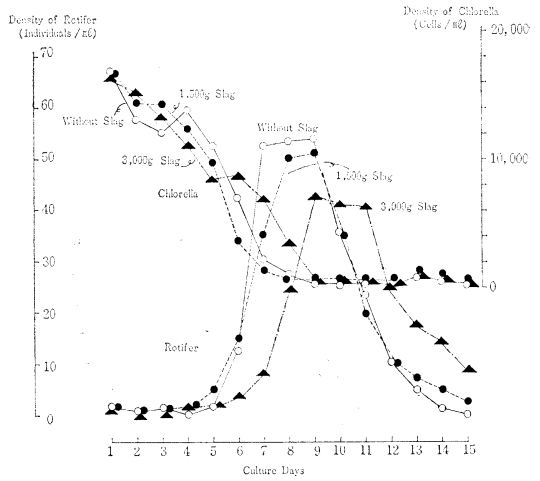

Fig. 6b. Densities of chlorella and rotifer in three experimental slag containers, from 0, 1,500 and 3,000 g, during the experiment.

the experiment, the air of 1,500 m*l* per minute was continuously circulated into each container. The temperature and pH value of water in each vessel were also observed at the same time. The results are given in Figs. 5, 6a and 6b. Fig. 5 shows the changes in temperature and pH value at a definite time of each container. Fig. 6a and Fig. 6b indicate the changes in the densities of chlorella and rotifer in each container.

Fig. 5 shows that the temperature and pH value lied within 27 to 23°C and 10.5 to 7.5 in all vessels. There was no statistical significant. From Figs. 6a and 6b the number of rotifer density increased during the first 8 days and gradually decreased due to the insufficient supply of chlorella. As far as the effect of slag concerned, it can be said that the growth rate of rotifer is depending upon the amount of slag. The great amount of slag retards the growth rate and in *vice versa*.

6. Survival of fish eggs and larvae in containers contained slags

In this experiment, the normal fertilized eggs of Japanese parrot fish, *Oplegnathus fasciatus* were used. Two 500 *l* containers, one containing slag of 2 kg, were prepared. In this experiment, slag was suspended in water. The normal fertilized eggs of 10,000 were introduced into containers obviously, without feeding. The aeration of 510 m*l* per minute was continuously pumped into each container. The water temperature and pH value in each vessel were between 21 to 25°C and 8.6 to 8.2 throughout the experiment. The eggs introduced were hatched out from 30 to 40 hours. The number of terminated eggs and larvae, thus was counted

Table 4. Numbers of terminated eggs and larvae, and measurements of total length (TL) and body depth (BD) of alive larvae.

Day	With slag						Without slag					
	Temp. C°	pH	Numbers of term. eggs and larvae		Larval size, mm		Temp. C°	pH	Numbers of term. eggs and larvae		Larval size, mm	
				Suc. t.	TL	BD				Suc. t.	TL	BD
1	21.4	8.55	913				21.6	8.55	1227			
2	22.1	8.40	97	1010	2.08	0.74	22.0	8.40	136	1363	2.12	0.73
3	22.3		18	1028			22.3		1	1364		
4	22.4		12	1040			22.4		13	1377		
5	22.0	8.41	11	1051			22.1	8.41	9	1386		
6	21.8		44	1095	3.11		21.8		26	1412	3.12	
7	21.5	8.41	51	1146			21.5	8.41	57	1469		
8	21.9		914	2060			21.9		1307	2776		
9	22.2		823	2883			22.2		1722	4498		
10	22.1	8.39	720	3603			22.1	8.39	1263	5761		
11	21.9		661	4264	3.56	0.79	22.0		590	6359	3.35	0.63
12	22.4		449	4713			22.4		243	6594		
13	21.7	8.30	97	4810	4.33	0.82	21.8	8.32	105	6699	3.94	0.73
14	20.9		87	4897			20.9		86	6785		
15	20.8	8.40	122	5919	4.24	0.90	20.8	8.39	144	6929	3.98	0.77
16	21.4		105	5124			21.5		164	7093		
17	21.7	8.40	20	5144	4.11	0.80	21.6	8.30	207	7300	3.73	0.75
18	21.7		5	5149			22.1		166	7466		
19	22.1	8.49	14	5163	4.59	0.95	22.2	8.42	237	7703	4.50	0.89
20	22.3		15	5178			22.6		74	7777		
21	23.0	8.37	18	5196			24.5	8.45	144	7921		
22	24.5		6	5202			24.8		184	8105		
23	25.1		0	5202	5.99	1.33	24.9		0	8105	4.34	0.83
24			0	5202			24.8		0	8105		
25		8.25	12	5214				8.23	128	8233		
Within 25 days,												
Total term. eggs and larvae				5,214						8,233		
Alive				258						0		
Unknown				4,528						1,767		
Eggs introduced				10,000						10,000		

Temp.: temperature, term.: terminated, TL: total length, BD: body depth, Suc. t.: successional total

at a certain time every day. When the experiment was completed, eggs in the container without slag were all terminated. During the experiment, the total length (TL) and body depth (BD) of alive larvae were measured and compared. The results are tabulated in Table 4.

It is evident from Table 4 that the survival rate of eggs and larvae in container with slags was slightly better than container without slags. The total length and body depth of alive larvae were also better in the vessel contained slags.

7. Discussion and conclusion

From the results of the experiment, the following conclusions are derived;

(1) the aquatic life adheres to gathers around the iron slag immediately after dropping in the sea (Table 3 and Figs. 1 and 2),

(2) the growth rate of fish reared in the containers contained slags is slightly better than that of without slags (Figs. 3 and 4),

(3) the survival rate of eggs and larvae, and the growth rate of alive larvae are slightly

better in the vessel contained slags than the container without slags (Table 4), and

(4) the increasing or decreasing of growth rate of the zooplankton, rotifer, is considered depending upon the amount of slags supplied in the containers (Figs. 6a and 6b).

These may, generally, lead to the conclusion that the effect of slag on the aquatic life is fairly good. The authors, however, suggest that the experiments mentioned above might have been carried out with sufficient aeration. Conducting the experiment with insufficient aeration, may yield in poor results, due to some undesirable substances gushed from slags absorb oxygen quantity in water. This is resulted in Figs. 6a and 6b.

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鉱滓の水族に対する影響

飯高勇之助　津田良平
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要旨: 鉱滓の水族に対する影響を知る目的で二，三の実験を行った。先ず，鉱滓，自然栗石，レンガを直接海水中に吊してそれらに付着，集合する水族を観察した。その結果は，鉱滓の方に水族が幾分早く付着，集合するようであった (Table 3, Figs. 1, 2)。鉱滓を敷きつめた水槽でタイリクバラタナゴの飼育を行い，小石を敷いた水槽での飼育と比較してみると，鉱滓水槽での魚の成長がよかった (Figs. 3, 4)。鉱滓の量を変化させた水槽でワムシの増殖実験を，また，鉱滓を吊した水槽でイシダイの卵を発生させ，その生残率と成長率を調べた。この場合，鉱滓の効果は幾分見られるようではあったが，明確には結論できなかった (Figs. 6a, 6b, Table 4)。

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寄　　稿

速さの変化に対するサボニアス・ロータの反応

——予　備　試　験——*

高　野　健　三**　松　山　佐　和***

Réponse d'un moulinet de Savonius à l'écoulement variable en temps

Kenzo TAKANO et Sawa MATSUYAMA

ロータで速度の大きさを測り，流向板で速度の向きを測る方式は古くから使われてきた。速度がひんぱんに変わるとき，この方式の弱味は，(1)ロータや流向板の時定数が大きく，流れの変化に追いつけないこと，(2)一般に，ロータと流向板の時定数は等しくないから，測定の誤差が大きくなること，である。

流速測定があまり行なわれていなかった時代には，流速計の性能に対する関心はうすく，感知部や流速計全体の検定も重視されていなかったようである。

最近はようやく，かなり精度の高い測定ができるようになってきたので，流速計の性能をよく調べておくことが必要となったが，定常な流れに対する性能だけを調べるのがふつうであって，定常ではない流れでの検定はまれであった。

そこで，流れの大きさが変わる場合，ロータがどのようにまわるか，ということだけをいちおう調べてみる。

ロータとしてはサボニアス・ロータ[1]を使う。現在のサボニアス・ロータの形は，サボニアスの原型とは違うが，そのまわりやすさ，作りやすさ，使いやすさなどを考えると，この先なお当分の間は，速度の大きさの感知部としてしばしば使われるだろう。

この試験には，EG & G 流速計（102型，写真式）を利用した。ロータは，高さ 8.5 cm あまり，直径 16 cm あまりのものを上下に2段重ねにしてある。Woods Hole 海洋研究所での検定によると，回転しているこのロータを静水中におくと，回転速度が e^{-1}（=0.368）になるまで約10秒，停止するまで約4分かかる。

水槽の上を走る台車からこの流速計を吊下げ，台車の速さをいろいろに変える。台車の速さの記録と，流速計の記録とを比べれば，台車の速さ（流れの速さ）とロータの回転との関係がわかる。

この種の試験には，EG & G の流速計は適していない。この流速計では，ロータの1回転ごとに一つのパルスしか出てこない。その上，フィルム送りが遅い（3 mm /min）ため，パルス発生の時刻を正確に読むことがむずかしい。フィルム送りを早め，1/4 回転，または 1/8 回転ごとに一つのパルスが出れば，回転の細部までわかるはずである。EG & G の流速計をそのように変えることはむずかしくないが，今回は，サボニアス・ロータの試験だけではなく，このロータを使った EG & G 流速計の試験という意味もかねて，パルスの数，フィルムの送りの早さについては手を加えず，そのまま使用した。

結果を第 1～3 図に示す。横軸が時間(分)，縦軸が速さで，実線はロータの回転から計算した速さ，点線は台車の速さである。実線が段階状になるのは，あるパルスと次のパルスの時刻を読み，その時間差から計算した速さを，その時間内での平均速さとしているからである。回転と速さの関係式としては，メーカの取扱説明書どおり，82 RPM/knot を使った。

台車の速さが変わるとき，ごく短い間だけ急に遅くなるのは，変速ギヤの入れかえのためである。

第1図は台車の速さを一定に保って2分間走らせた後，停止させたものである。台車の速さは，10 cm/sec

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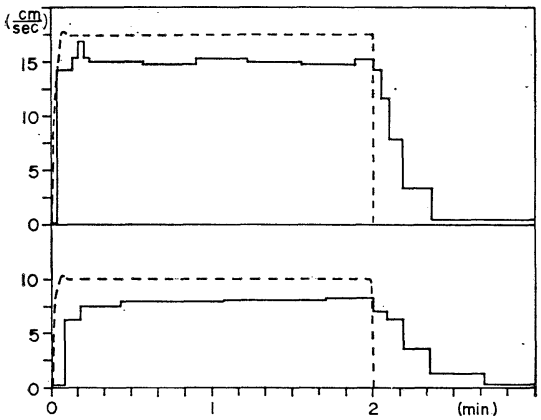
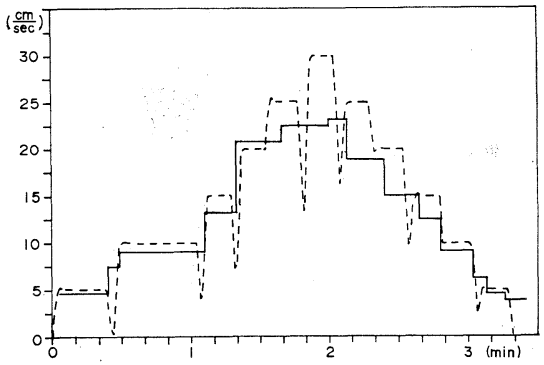
と 17.5 cm/sec の 2 種類である。どちらの場合も，ロータの立ちあがりは早いが，停止する方は時間がかかる。82 RPM/knot という換算率は，このロータに対しては適切ではない。ちなみに，この型のロータによる測定誤差は ±2.5 cm/sec とされている。台車が停止してからロータの回転が非常に遅くなるまでの時間は，もとの速さが 10 cm/sec でも，17.5 cm/sec でもほぼ同じである。第 2 図は台車の速さを 30 秒ごとに，第 3 図は 15 秒ごとに変えたものである。第 2, 3 図から，ロータは，30 秒ごとの流速変化には十分についてゆくといえるが，15 秒ごとの変化には追いつけない。一般に加速に対する反応は早いが，減速に対する反応は遅い。

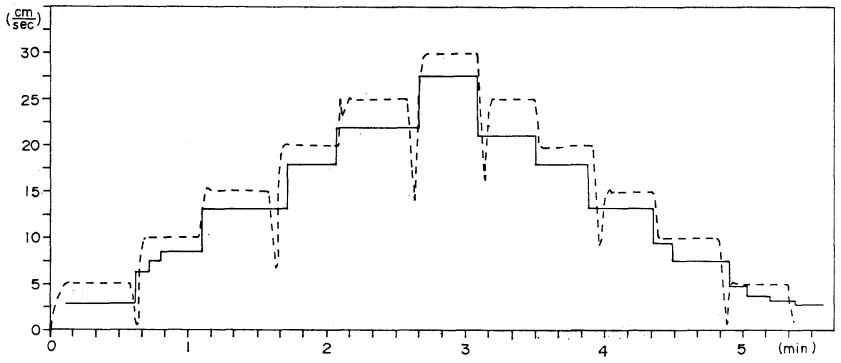
上で述べた理由によって，この装置ではロータの反応を細かく調べることはできないが，1 分から数分程度よりも長い時間規模の現象を研究対象とする時には，このロータで十分である。



第 1 図　ロータの回転の加速と減速の 2 例。
実線はロータの回転から計算した速さ，
点線は台車の速さ。



第 3 図　台車の速さの変化とロータの回転の変化。
実線はロータの回転から計算した速さ，
点線は台車の速さ。



第 2 図　台車の速さの変化とロータの回転の変化。
実線はロータの回転から計算した速さ，点線は台車の速さ。

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Compte rendu

The Biology of Aquatic Chitinoclastic Bacteria and their Chitinolytic Activities*

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1. Distribution of chitinoclastic bacteria

Pelagic region. Chitinoclastic microorganisms have been isolated from numerous environments: fertilized garden soils, lake waters, plankton, exoskeletons of insects and crustaceans, intestines of both vertebrates and invertebrates, muds, and sands (as reviewed by VELDKAMP, 1955; and BENTON, 1935). The marine environment, which is rich in chitinous material, is an excellent source of chitinoclastic bacteria.

The initial, most notable study conducted in the pelagic zone or deep marine waters, was that of ZOBELL and RITTENBERG (1938). A very uneven distribution of chitinoclasts was found in the sediments off the California coast. This lack of uniformity was attributed to random distribution of substrate particles and substrate affinity or colonization of the bacteria. The most abundant biomass (10^3 cells/g) was in the topmost sediment layers; concentrations of the bacteria decreased with core depth. No relationship was noted between bacterial biomass and depth of the overlying waters or distance from the mainland. Coarse sedimentary material, such as sands, supported the largest number of chitinoclastic microorganisms, due to a "concentration of the particles by the sorting action of sedimentation forces." Within these waters and muds only 0.1 to 1.0 % of the total bacterial biomass were chitinoclastic.

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VELDKAMP (1955) also noted that soil types (terrestial) influenced the quantity of chitin decomposers. Highest concentrations of chitinoclasts were detected in acid sandy soils. In these soils, actinomycetes formed the largest part of the total chitinoclastic populations. HOCK (1940) reported similar results in the Woods Hole area. Mud core samples from a depth of 878 m showed a concentration of 1.3×10^2 cells/g at the water-sediment interface. At a depth of 5 cm below the surface of the sediment, the number of chitinoclastic bacteria decreased to 5 cells/g. However, one mile offshore in five fathom waters, 1.5×10^2 cells/ml were found, further indication of the somewhat inconsistent pattern of distribution of chitin utilizers in pelagic waters. Similarly, LEAR (1963) demonstrated the scarcity of chitin decomposers in waters deeper than 1,000 m off the coast of California but noted a correlation between the abundance of the bacteria and depth. All samples showed less than 5.0×10^2 cells/ml; as depth of the water column decreased, the quantity of cells decreased proportionally. BIANCHI (1971) also found a scarcity of chitinoclasts in the deep sediments of the Mediterranean Sea. Of the 90 samples examined, only 10 revealed chitin decomposers: one of the samples, however, contained 2.4×10^4 cells/g.

One of the most comprehensive studies dealing with chitin utilizers is contained in a series of papers by SEKI and associates. SEKI and TAGA (1965c) conducted microbiological investigations in Sagami Bay, whose surface waters (100 m deep) originate from the neritic or coastal zone and the Kuroshio current. The intermediate waters (below 100 m and

above 1,000 m) are derived from the Oyashio undercurrent, while the deep bottom waters (below 1,000 m) represent waters from the Pacific Ocean. Each water mass had a characteristic quantitative and qualitative distributional pattern of chitinoclastic bacteria. The surface waters contained an average of 1.0–2.0 × 10^3 cells/m*l* with the concentration of the bacteria decreasing to a depth of 200 m. From the intermediate waters, a maximal bacterial biomass was noted at 600 m, but chitin decomposers could not be detected in the bottom layers. The percentage of chitinoclasts within the total micro-biota decreased with depth, *i.e.*, the upper layers contained the highest percentage of chitinoclasts while the lower masses contained less. The abundance of the chitin decomposers also varied with temperature. A ten-fold increase was noted from February to May when water temperatures were an average of several degrees higher than during the winter months. In contrast, a two-fold increase was observed in the intermediate waters where temperatures showed considerably less variation.

Along with a distinct biomass of chitinoclasts within each water mass, characteristic species were identified. These included *Beneckea lipophaga*, *B. hyperoptica*, *B. idolthetica* and *B. chitinovora*. *B. lipophaga* appeared to dominate in the surface waters during the summer, while *B. hyperoptica* was prevalent during the other months. *B. hyperoptica* and *B. indolthetica* appeared in the upper intermediate waters, while *B. hyperoptica* constituted the sole *Beneckea* species of the deeper intermediate waters. SEKI and TAGA (1965c) also identified the plankton of the waters and noted distinct species within each layer. The data suggested that certain bacterial types were associated with specific plankton.

Neritic region. Within the shallow waters of the coastal zone the abundance of chitinoclastic bacteria is noticably greater than in the open ocean. CHAN (1970) disclosed high concentrations of chitin utilizers, comprising slightly less than 10 % of the total biota, in seawater and sediment from the Puget Sound estuary. Sediments from deep subtidal, intertidal, and freshwater sites contained 2.0×10^4 cells/g, 6.8×10^4 cells/g, and 3.7×10^3 cells/g, respectively. Similar to data from the pelagic region, the greatest concentrations of chitin decomposers were at the surface of the sediments with chitinoclastic bacteria decreasing with sediment depth. Surface waters averaged 2.5×10^2 cells/m*l* while bottom waters exhibited fewer cells, 2.0×10^2/m*l*. Seasonal variations were not apparent at deep sampling stations but in shallow areas, microbial biomass correlated with temperature.

In the neritic waters of Aburatsubo Inlet, SEKI and TAGA (1963a) found that only 0.4 % of the total heterotrophic bacteria were chitinoclastic. No attempt was made to explain this unusually low concentration of chitinoclastic microorganisms. It was observed, however, that a considerable number of the bacteria were attached to living copepods, and a correlation was postulated between the percentage of chitin decomposers and planktonic crustaceans within the water column. Although the populations of bacteria on plankton and suspended matter showed little variation, a slight increase was noted during the summer and autumn months, probably related to a rise in temperature. An inverse relationship was also observed between the chemical oxygen demand (COD) and the percentage of chitinoclasts present. Five species of *Beneckea* comprised the biota. *Beneckea lipophaga* predominated in summer and *B. hyperoptica* appeared in winter and early spring. *B. indolthetica* and *B. chitinovora* were always found in association with plankton or suspended matter throughout the year, but dominated in late spring and autumn. *B. labra* was the least abundant of all the species.

In order to elucidate the ecological factors affecting the distribution of chitinoclastic bacteria, SEKI and TAGA (1965b), conducted a series of investigations defining those conditions for bacterial survival. The optimum temperature for all strains, excluding *B. chitinovora*, was 30 °C with cessation of growth at 40 °C. However, the isolates demonstrated strong heat resistance compared to other marine bacteria. A temperature of 50 °C for 30 minutes was

needed to achieve bactericidal effects. All species exhibited a wide range of pH tolerance, with an optimum between 7.0 and 9.0. Growth was retarded below pH 7.0, while a pH of 4.0 stopped growth completely (SEKI and TAGA, 1963b). The bacteria proved to be strongly resistant to ultraviolet light, *i.e.*, almost four times the amount of energy was required compared with that necessary to kill the same quantity of cells of *Escherichia coli*. All of the aforementioned *Beneckea* species grew well in a media containing 0.5 to 5.0 % NaCl; however, salinities of 12 % stopped cell division, and 25 % NaCl caused death of all cells after 24 hours. A pressure of 200 atmospheres inhibited growth but pressures of even 600 atmospheres did not destroy the cells (SEKI and TAGA, 1965b).

Distribution of chitinoclastic bacteria in the Barataria Bay salt marsh environment (Hood, 1973) appeared to be related to factors of 1) organic matter, 2) chitin deposition, and 3) to a lesser extent, temperature. Highest concentrations of chitinoclasts (10^6 cells/g sediment) were observed in areas of high organic content, *i.e.*, the emerged marshland soil. The water column contained the least number of chitinoclasts (10^2–10^3 cells/m*l*). Peak bacterial biomass occurred simultaneously with optimum numbers of chitin-producing animals in the water column. Species of the genus *Beneckea* comprised the predominant bacterial biota, although within the marshland soil a much greater diversity of species was found. Large concentrations of chitinoclasts were associated with the intact exoskeleton and the digestive tract of penaeid shrimp. The function of the bacteria in the shrimp digestive tract is possibly that of enzyme production (chitinase) and elaboration of growth factors. Bacteria ingested by the animal may also provide a direct food source.

Association of chitinoclastic bacteria with aquatic animals. It is becoming increasingly evident that chitinoclastic bacteria are closely associated with certain marine invertebrates. Various reports suggest a commensal or symbiotic relationship between these microorganisms and plankters. SEKI and TAGA (1963a) found considerable quantities of chitinoclasts attached to living copepods within the water colum. LEAR (1961) reaffirmed this phenomenon, suggesting that the external region of plankters served as the locus for microbial attachment. JONES (1958), using a radiolarian species, demonstrated the annexation propensities of marine bacteria in general. The surface of the radiolarian, *Castanidin longispinum*, contained a one thousandfold increase in chitin decomposers compared to the biomass of chitinoclasts in the seawater. BUCK and BARBAREE (1971) detected higher concentrations of chitin utilizers within large copepod species, than in the surrounding waters from which the copepods were collected. These *Beneckea* species were demonstrated to be indigenous to the copepod and were able to reproduce within the crustacean under certain conditions.

Several noteworthy disease-producing bacteria are found within the chitinoclastic group. These more widely studied organisms have been detected in conjunction with zooplankton and crustaceans. HESS (1937) reported a disease of the exoskeleton of living lobsters attributed to chitin decomposers. Chitinoclastic bacteria have been suggested as the causative agent of "brown spot" in shrimp (SINDERMANN, 1971). A significant interaction has been documented between *Vibrio parahaemolyticus*, the causative agent of more than 70 % of the food poisoning in Japan, and fish and shellfish. This pathogen was identified in blue crabs in Chesapeake Bay (KRANTZ *et al.*, 1969), commercial species of shrimp in the Gulf of Mexico (VANDERZANT and NICKELSON, 1970), and oysters (BARTLEY and SLANETZ, 1971). Of the several confirmed incidents of food poisoning in the United States, *V. parahaemolyticus* was traced to steamed crabs in Maryland (USPHS, 1971) and boiled shrimp in Louisiana (USPHS, 1972). KANEKO and COLWELL (1973) found that concentrations of *V. parahaemolyticus* and related species were correlated with population dynamics of zooplankton in Chesapeake Bay. The internal bacterial biomass in the zooplankton remained constant throughout the seasons whereas the external bacterial populations varied with

temperature, rising sharply during the summer months.

It appears that both the external and internal regions of marine animals offer an excellent microenvironment for the proliferation and survival of chitinoclasts. The concept of a truly indigenous biota within the digestive tract of fish and other aquatic animals is rather controversial. LISTON (1957) noted a distinct relationship between bacterial species and the species of fish. In contrast, POTTER and BAKER (1961) failed to find evidence of a consistent biota in fish or any relationship between species of fish and bacterial types. The ease with which the bacteria were removed indicated that the fish were only passive carriers of the microorganisms. MARGOLIS (1953) regarded the bacteria of fish to be a function of the ingested food, finding that intestinal tracts of non-feeding fish were virtually sterile. The focal point of this problem seems to be centered around the term "indigenous". An indigenous biota does not necessarily imply that only one or two species of bacteria will be found consistently in a specified environment. It does, however, suggest a restriction in bacterial types for, obviously, certain bacteria are more suited to a specific microenvironment than to others.

The microbiota of the digestive tract of marine fish as well as plankton were shown to have a distinct generic composition, *i.e.*, *Vibrio* and *Aeromonas* (AISO *et al.*, 1968); and had complex nutritional requirements, many hydrolyzing chitin. A microbiota consisting of a large percentage of chitinoclastic bacteria, sometimes exclusively chitinoclasts, has been reported for both marine invertebrates and vertebrates. For example, CHAN (1970) noted 1.0×10^7 chitinoclastic cells/g in the intestines of numerous species of fish from the Puget Sound estuary. Similarly, in the digestive tracts of the Japanese sea bass, OKUTANI (1966) recorded 1.7×10^4/g chitin degraders within the stomach contents, 5.4 to 1.6×10^7 cells/g within the intestines, and 2.3×10^7 cells/g within the pyloric caeca. In the stomach and intestines of yellow tail, chitinolytic bacteria were at concentrations of 2.0×10^4 and 1.4×10^5 cells/g, respectively (OKUTANI *et al.*, 1967a). The intestines of octopus, squid and swell fish contained 1.5×10^5 cells/g, 4.3×10^5 cells/g, and 4.4×10^3 cells/g, respectively (SEKI and TAGA, 1963d). In addition whales were shown to contain chitinoclasts in their digestive tracts (SEKI and TAGA, 1965a).

Since the microenvironment of the digestive tract of marine animals is characterized by a restriction in bacterial species and a high microbial biomass, the question arises as to the function of these bacteria. Are these organisms coexisting in an active "partnership" or does the relationship involve a passive incorporation, wherein the endosymbionts are merely ingested? Unfortunately, little conculsive information is available on the role of such microorganisms, although several functions have been suggested: 1) the bacteria provide a direct food source, and 2) the organisms provide growth factor, *i.e.*, vitamins, secreted by-products from cellular respiration, and products resulting from breakdown of substrates by bacterial enzymes (ALEXANDER, 1971).

It has been well established that bacteria serve as food for protozoans (LUCK *et al.*, 1931). ZOBELL and FELTHAM (1938) demonstrated an analogous situation in higher invertebrates as well. Mussels, *Mytilus californianus*, when submerged into a suspension of bacteria, removed large quantities of cells, rejecting some as pseudo-feces but ingesting most of the cells. Likewise, the ingestion of bacterial cells was observed using oysters. Bacteria in different stages of lysis were noted in the stomach content of the mussels and oysters. Using 31 strains of bacteria as the exclusive component of the diet, the mussels survived and gained weight. The waters in which the mussels lived, however, had to be changed often due to the accumulation of toxic bacterial metabolites. Similar results were obtained when bacteria were fed to the sand crab, *Emerita analoga*, although the sand crabs showed a more sensitive reaction to high quantities of bacteria than did the mussels. Sipunculid Gephyrean worms, *Dendrostroma zootericola*, failed to grow on a bacterial diet but main-

tained their initial weight. Concomitant with these studies, extracts of the digestive enzymes from the aforementioned animals were able to lyze a number of species of bacterial cells. In summation, ZoBELL and FELTHAM (1938) concluded that the abundance of bacteria in marine sediments provided an important secondary food source or even an exclusive food source for bottom feeders.

Other workers reported similar data. MACGINITE (1932) supplying a *Pseudomonas* species to the bottom feeder, *Urechis caupo*, observed excellent growth. BURKE (1933) found development of larval stages of the frog, *Rana pretiosa*, on a bacterial diet comparable to that on a controlled diet. Recently, CONDREY *et al.* (1972) demonstrated that diets of diatoms plus bacteria gave greater feeding efficiencies in penaeid shrimp than diets without bacteria, supporting the theory that bacteria act as an important supplementary food.

Unfortunately, it is much more difficult to establish any specific relationship between a bacterial "probiotic", or growth factor, and a higher animal. Such relationships as those of rumen bacteria (HUNGATE, 1960) and insect microorganisms (STEINHAUS, 1960) have been well studied, but very little information is available concerning the interactions of microorganisms and marine animals. Certain amino acids (VELDKAMP *et al.*, 1963), vitamins and coenzymes (WILSON and PARDEE, 1962), polysaccharides such as dextrans and levans (WILKINSON, 1958) and other probiotics (LILLY and STILLWELL, 1965) "are produced by heterotrophic microorganisms in excess of their needs and released as extracellular products to the environment." May investigations, however, failed to isolate the probiotic produced by the microorganism or to show the effect of the growth factor on the animal with which the bacteria is associated.

In an effort to define the role of the chitinoclastic bacteria within the digestive tracts of marine invertebrates, SEKI and TAGA (1963e) calculated the maximum theoretical value of bacterial chitinase activity in the octopus, *Polypus vulgaris*, the squid, *Loligo edulis*, and the marine fish, *Canthigaster rivulatus*. Having determined the number of chitin decomposers present in the digestive tract, the amount of chitin the microbial population could degrade under ideal conditions, the amount of chitin in the diet of the invertebrates, and the resident time of the substrate in the tract, the value obtained was a mere 3×10^{-4} mg of chitin decomposed/cubic centimeter of digestive tract. This negligible quantity, 0.003 to 0.0008 %, suggested that the bacteria do not produce enough enzyme to significantly degrade chitin.

2. Taxonomy of chitinoclastic bacteria

BENECKE (1905) was one of the first to describe a bacterium, *Bacillus chitinovorous*, which utilized chitin. There are numerous investigations concerning non-marine chitinoclastic microorganisms representing a variety of genera such as *Flavobacterium*, *Chromobacterium* (VELDKAMP, 1955), *Bacillus* (BAXBY and GRAY, 1968) as well as actinomycetes of the genera *Micromonospora*, *Streptomyces*, *Nocardia* (VELDKAMP, 1955) and fungal species (GRAY and BELL, 1963; LEOPOLD and SEICHEVTOVA, 1967; OTAKARA, 1964). The following discussion, however, will concern only marine bacteria (MACLEOD, 1965).

ZoBELL and RITTENBERG (1938) isolated thirty-one chitin utilizers from marine sources but did not attempt to classify them, although two species were tentatively assigned *Vibrio* species. HOCK (1941) described the species, *Bacterium chitinophilum* and *Bacterium chitinochroma*, but failed to mention flagella type, an important criterion for later reclassification. Gram-negative chitin decomposers, fermenting glucose with the production of acid but no gas, were isolated by CAMPBELL and WILLIAMS (1951). The polarly flagellated strains were placed in the genus *Pseudomonas*, while those with peritrichous flagella were included in the genus *Achromobacter*. Those assigned to *Pseudomonas*, however, were placed in an unsatisfactory group since this genus is restricted to bacteria having an oxidative metabolism rather than a fermentative one. The new species described and assigned to *Achromobacter* were subsequently placed into a newly created genus *Beneckea* (BREED *et al.*, 1957).

OKUTANI (1966) described and proposed the name of six chitinoclasts from the digestive tract of marine fish as follows: *Vibrio gerris*, *V. orphus*, *V. labrakos*, *Aeromonas skiaina*, *A. chitinophthora*, and *Alginomonas channe*. Other chitin utilizers isolated were similar to those previously named: *V. piscium* (BREED *et al.*, 1957), *V. anguillarum* (SAKAZAKI *et al.*, 1970), *V. parahaemolyticus* (SAKAZAKI *et al.*, 1963), *Aeromonas liquefaciens*, *A. punctata*, *A. hydrophila* (BREED *et al.*, 1957), *Bacterium chitinophilum* (HOCK, 1941) and *B. lepidorthosae* (CAMPBELL and WILLIAMS, 1951).

Six isolates from Japanese waters were classified as *Agarbacterium*, *Beneckea* and *Pseudomonas* (KIHARA and MOROOKA, 1962). SEKI and TAGA (1963a) described thirty-nine strains of chitinoclastic bacteria using Bergey's scheme (BREED *et al.*, 1957). They also isolated species similar to V. *alopsis*, first described by ZOBELL and UPHAM (1944), and *Pseudomonas cryothasia* (CAMPBELL and WILLIAMS, 1951). CHAN (1970) separated the chitinoclasts from Puget Sound estuary into the following genera: *Vibrio*, *Pseudomonas*, *Cytophaga*, *Aeromonas*, *Photobacterium*, and *Streptomyces*. He reported the abundance of *Vibrio* species, similar to *V. marinus* (redefined by COLWELL and MORITA, 1964) and *V. gerris*. *V. alginolyticus*, also isolated, was first named *Oceanomonas alginolyticus* by MIYAMOTO *et al.* (1961) but SAKAZAKI (1968) designated it as a second biotype of *V. parahaemolyticus* and gave the organism its present name.

Upon examination of the criteria for separation of marine *Vibrio*, *Pseudomonas*, *Beneckea*, and *Aeromonas* it becomes evident that little difference exists using classical techniques. *Vibrio* is defined in Bergey's Manual (BREED *et al.*, 1957) on the basis of cell curvature and flagellation. The genus is restricted to polarly flagellated oxidase positive, curved rods which are faculatively anaerobic and ferment glucose with acid production but no gas. DAVIS and PARK (1962) noted the subjectivity of using cell curvature as a criterion and expanded the genus to include straight rods. *Aeromonas* consists of oxidase positive, facultatively anaerobic polarly flagellated or non-motile, straight rods fermenting glucose with or without the production of gas (EDDY, 1960). To distinguish the two genera, molecular techniques are required. *Aeromonas* contains 50 to 60 moles ratio GC (DNA base composition) (HILL, 1966), while *Vibrio* has 40 to 50 moles ratio GC (COLWELL, 1970). *Beneckea* is composed of peritrichously flagellated, facultatively anaerobic straight rods of marine origin which decompose chitin and ferment glucose with the production of acid but no gas (BREED *et al.*, 1957). ALLEN and BAUMANN (1971) have shown that flagellation within *Beneckea* species may vary with cultural conditions, exhibiting peritrichous flagella when grown on solid medium but having a single, polar flagella when grown in liquid medium.

In taxonomic studies of marine bacteria, BAUMANN *et al.* (1971) re-evaluated the classification of the gram-negative rods of marine origin capable of fermenting glucose without production of gas. Using classical biochemical and morphological tests, DNA base composition, and numerical analysis, 145 strains representing *Beneckea*, *Vibrio*, *Aeromonas*, *Pseudomonas*, and *Photobacterium* were reassigned to *Beneckea*, sensu BAUMANN. Although the BAUMANN scheme has received criticism, it represents an excellent attempt to group a number of similar species of marine origin.

3. The rate of chitin degradation

It is difficult to discuss degradation of a substrate in "non-enzymatic" terms, particularly if an enzyme system is the primary mechanisms by which the specified substrate is decomposed. However, enzymes are limited not only by their indigenous properties but also by those factors which limit the growth and development of the organisms which elaborate the enzymes. For example, if an organism produces an extracellular enzyme whose cell-free characteristics include inactivation at pH 6-7, but the organism is neither found in nature nor can survive at pH's other than 6 and 7, the enzyme, for all practical purposes, is non-functional within that biological system. It is with these considerations in mind that the process of chitin degradation

is discussed.

Several papers, notably those of SEKI (1965a, b) and SEKI and TAGA (1963c), have attempted to determine the rates of chitin mineralization in the ocean environment. Initially, pure cultures of the five predominant chitinoclastic bacteria, all *Beneckea* species, were grown in a chitin medium under conditions approximating those in the sea. Total bacterial growth and chitin removal were simultaneously monitored. The rate of decomposition obtained was 30 mg chitin/24 hours/10^{10} bacterial cells. Little variation in the rate of degradation was noted with the bacterial type, the initial concentration of bacterial inoculum, or the initial concentration of chitin. The surface area of the particle affected the rate of decomposition inversely, *i.e.*, the smaller the particle, the more rapid the material was broken down. Hydrostatic pressure also affected the rate; a pressure of 200 atmospheres decreased the rate by 40 %. Assuming that the average chitin particle in the ocean is less than 0.033 cm, the rate of decomposition in the environment was calculated as 27 mg/24 hours/g of chitin at 25 °C. The complete mineralization of deposited chitin was then postulated to occur within 38 to 67 days.

CHAN (1970) demonstrated that *Vibrio* species from the Puget Sound estuary decomposed chitin at a rate of 80–130 mg/hour/10^{10} bacterial cells at 22°C or 19.2–31.2 mg/24 hours/10^{10} cells. Using a simulated model seabed system, LISTON *et al.* (1965) demonstrated a rapid loss of $CaCO_3$ initially from a chitin substrate followed by a slower loss of protein and chitin. The rate of chitin degradation with Puget Sound sediment was 18.8 mg chitin/day, but with mixed coastal sediments it was 4.5 mg chitin/day (LISTON *et al.*, 1966). The carbon conversion rate, however, was higher for offshore sediments, *i.e.*, 48.4 % compared with 10.7 % for Puget Sound sediments. The difference was postulated to reflect dissimilarities between the microbiota of the two environments.

In *in vitro* studies, SEKI (1965b) revealed areas of higher chitin decomposition, *i.e.*, 75 mg chitin degraded/30 days, just below the water-sediment interface, than in the water column or in the deeper sediment zones. Collected cores of sediment from 2 to 10 cm deep and seawater, sterile seawater, sterile chitin strips were incubated in containers at 20°C and 10°C for 30 days. The pH and Eh were maintained but the bacterial cell crop was not enumerated. While the closed systems do not duplicate conditions in the natural environment due to lack of exchange, nutrient inflow and waste outflow, the data identify microenvironments capable of supporting greater chitin mineralization.

In situ rates of chitin degradation "native chitin" in the salt marsh environment (HOOD, 1973) were extremely high, *i.e.*, 87 mg/day/g chitin, while pure culture and *in vitro* rates were similar to those reported in the literature.

From the results of the aforementioned workers (SEKI, 1965a. b; SEKI and TAGA, 1963c; CHAN, 1970), it is apparent that chitin degradation is primary a function of temperature and available organic matter. Factors which do not fluctuate greatly in the marine environment, *i.e.*, pH and salinity, have little effect on the growth of chitinoclastic bacteria or on the rate of chitin decomposition. The indigenous microbiota may also determine the rate at which the substrate is mineralized.

4. The chitinase enzyme system

The structure of chitin. The term " chitin " has been applied in this review to the chemically pure polymer of N-acetylglucosamine as defined earlier. Since the term chitin may have one meaning to the biologist and another to the chemist, it is necessary to differentiate the terms " native chitin," " decalcified chitin ", and " chitin ".

Pure chitin is rarely found in the natural environment but occurs in combination with proteins and inorganic salts, primarily calcium salts. When in such conjunction, it is considered native chitin. The composition of native chitin in the cuticle of animals varies with species. Hard exoskeleton, such as found in crabs, contains more salts and tightly bound protein than soft cuticle typical of shrimp. The pure chitin in the exoskeleton is highly organized

into linear or fiberous micelles, the orientation of the molecular chains resulting in several X-ray diffraction patterns. On the basis of the patterns, chitin is separated into α, β, and γ types, each composed of chains of the N-acetylglucosamine unit linked in the 1-4 β glucosidic manner but arranged in a different physical structure (RICHARDS, 1951).

The backbone structure of α chitin from insects and crustaceans contains two helical polysaccharide chains oriented in opposite directions with screw axes along the chain directions. There are four asymmetrical N-acetylglucosamine residues per cell unit. The spatial configuration of side groups are given in Fig. 1. The two chains are linked by hydrogen bonds, the first between the NH (amine) group on one chain and the CO group in the neighboring amino-acetyl group of the other chain. A second bond is between the oxygen and the hydroxyl group of the hydroxyl methyl side group. The bonds give the chains a bent or buckled configuration. For a more detailed explanation of the bonding see DEWELTZ (1960), CARLSTRÖM (1962), and RAMAKRISHNAN and PRASAD (1972).

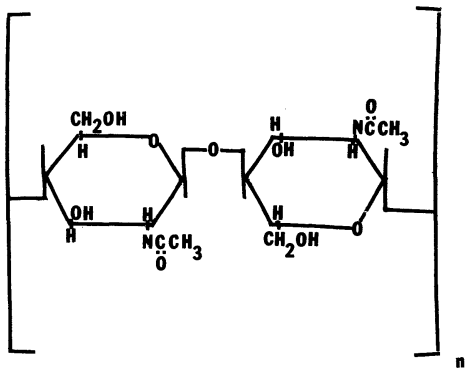

Fig. 1.

The γ and β forms of chitin are found associated with an elastin counterpart (RUDALL, 1967) but their exact configuration at this time is unknown. The β type is produced by Coelenterata, Annelida, Mollusea, and Brachiopoda and is associated with collagen cuticle (HACKMAN, 1964). The γ type is found in cuttlefish shell and shows a more disoriented X-ray diffraction pattern than the other chitins (HACKMAN, 1960).

The exact nature of the association between protein and chitin is unknown. However, HACKMAN (1960) suggested that the protein is linked by stable covalent bonds, resulting in a glycoprotein complex. The protein appears to be linked to the chitin chains through the aspartyl and histidyl residues (HACKMAN, 1964). In soft cuticle a large portion of the protein can be removed by hot water, suggesting a loosely-bound water soluble protein, while a small amount can be removed by 5 % KOH extraction. Contrariwise, in hard cuticle less protein is removed by water and more is removed by KOH extraction (RICHARDS, 1951). DeMets and Jeuniaux, as reported by RICHARDS, (1951), extracted a *Streptomyces* chitinase, which could not attack chitin until the protein was removed by alkaline digestion. BULL (1970) demonstrated a similar phenomenon with a chitinase from *Aspergillus nidulans*. Melanin-bound chitin proved extremely resistant to this chitinase system, but activity was restored when the melanin was removed.

Calcium carbonate exists in the chitinous exoskeleton of crustaceans in the primary form of calcite. The percent composition varies with each species ranging from 16 to 60 % of the dry weight of the cuticle. Other inorganic salts include $MgCO_3$, $Ca_3(PO_4)_2$, SiO_2, $(Al, Fe)_2O_3$. MgO, CaO, P_2O_5 and $CaSO_4$, but the salts are found only in trace quantities. Calcite may occur in either micro or macro crystals in granular, platelike, circular symmetrical, or disc-like spherical patterns. Commonly, the crystals result in a mosaic pattern but there is no fusion of the crystallites and each crystal is a self-differentiating unit (RICHARDS, 1951).

Enzymatic mechanisms involved in the degradation of chitin. Several enzymes are responsible for the breakdown of chitin by an exocellular system to products which can be taken up by the cell and then utilized for intracellular metabolic processes. The following schematic is a composite of previously reported steps involved in chitin degradation.

$$\text{Chitin} \xrightarrow[\text{factor } (CH_1)]{\text{prehydrolytic}} \text{Chitin (Susceptible)}$$

$$\text{chitinase} \longrightarrow \underset{\text{short chain soluble chitin residues}}{\text{(cellular uptake) Chitodextrins}} \xrightarrow{\text{chitobiase or chitinase}}$$

$$\underset{\text{Chitobiose dimer}}{\text{(cellular uptake)}} \xrightarrow{\text{chitobiase}}$$

$$\underset{\text{N-acetylglucosamine monomer}}{\text{(cellular uptake)}} \xrightarrow{\text{deacetylase}}$$

$$\underset{\text{Glucosamine}}{\text{(cellular uptake)}} \xrightarrow{\text{deaminase}} \underset{\text{Glucose}}{\text{(cellular uptake)}}$$

REYNOLDS (1954) found that the exocellular chitinase produced by a *Streptomyces* species attacked chitin with the formation of two end products, the monomer, N-acetylglucosamine, and the dimer, N, N-diacetylglucosamine. Like many other polysaccharides, one or more enzymes degrade the substrate to a biose stage which is then split by another enzyme to the monomer stage. No chitodextrin, glucosamine, or glucose were detected as products of the enzyme system. BERGER and REYNOLDS (1958) resolved a chitinase system of *Streptomyces griseus* into two chitinases with similar activities, and a chitobiase. Chitin was hydrolyzed by the chitinases to N-acetylglucosamine and N, N-diacetylchitobiose without the formation of detectable concentrations of higher saccharides. Chitodextrin was cleaved randomly, forming a number of intermediate saccharides by the chitinase and then further hydrolyzed to the monomer and the dimer. The trimer, N,N,N-triacetylchitotriose, and the tetramer, N,N,N,N-tetra acetylchitotetraose were broken down to the monomer and dimer stages also. The chitobiase hydrolyzed the β phenylglycosides of N-acetylglucosamine, the di- and trisaccharides of N-acetylglucosamine but none of the higher saccharides. KIMURA *et al.* (1967a) demonstrated the presence of three products from chitin hydrolysis by a chitinase extracted from the snail. N-acetylglucosamine and two oligosaccharides of the N-acetylglucosamine were detected on chromatographic analysis.

OKUTANI (1966) showed that the end products of a chitinase system from an animal (a marine fish, *Lateolabrax japonicus*) and a bacterial source (*Vibrio gerris* and *Aeromonas chitinophthora*) were N-acetylglucosamine, and its oligosaccharide as well as glucosamine. It was suggested, however, that the glucosamine present was due to deacetylation of chitin during preparation. The composition of the oligosaccharide was not identified but it was probably the dimer, N,N-diacetylchitobiose. Other workers (HACKMAN, 1964; VELDKAMP, 1955) reported glucosamine as an end product of chitin degradation, but in each case it was suggested that this was due to deacetylation in the preparation of chitin. When chitin is treated with acid (either dilute HCl in the decalcification procedure or with concentrated HCl in the process of swelling chitin, *i.e.*, "molecular chitin"), some of the N-acetylglucosamine units can be deacetylated. VELDKAMP (1955), however, detected the accumulation of acetic acid in the culture fluids of *Pseudomonas chitinovorans* in the presence of chitin under conditions in which bacterial metabolism was inhibited. It was concluded that the only mechanism by which the acetic acid could accumulate was by direct deacetylation of N-acetylglucosamine to glucosamine and acetic acid by the action of a deacetylase.

A prehydrolytic factor similar to that reported for cellulolytic systems was suggested by MONREAL and REESE (1969). Little correlation was found between activity of the chitinase on crystalline and swollen chitin. If only one component were involved there would have been a strong correlation between the activities on both substrates. The chitinase of *Serratia marcescens*, however, showed increased activity on swollen chitin with incubation, but decreased activity on crystalline chitin. The data indicated that a special enzyme (CH_1) was required to modify the crystalline chitin to a form susceptible to the glycanase. A synergistic effect was not evident on combining the chitinolytic factors; however, JEUNIAUX (1955, 1959a) observed such effects when several chitinase fractions were combined.

The chitinase of *Serratia marcescens* was also demonstrated to be a highly specific

enzyme acting only on the β 1,4 polymer of N-acetylglucosamine (MONREAL and REESE, 1969). The mechanisms of the enzyme system were characterized as similar to those for the enzyme system of *Streptomyces* (BERGER and REYNOLDS, 1958): 1) chitinase, which included a random endoglycanase producing soluble intermediates upon chitin hydrolyzation, and 2) a glycosidase which hydrolyzed these intermediates to the monomer stage.

The terminal steps in the breakdown of chitin have not been established at this time. To date, no evidence has been found to indicate the presence of an exocellular deacetylase or a deaminase elaborated by an organism which also produces a chitinase or a chitobiase. ZOBELL and RITTENBERG (1938) first suggested that chitin degradation might occur from the splitting-off of the amino-acetyl group. These workers detected ammonia and acetic acid in the culture media of marine chitinolytic bacteria grown in the presence of chitin. Data compiled by OKUTANI and KITADA (1968a, b) showed large amounts of acetic acid and lactic acid, as well as other organic acids, which accumulated in the media when marine chitinoclasts were grown on chitin as the sole carbon source. Acetic acid also accumulated but to a lesser extent in media devoid of chitin. These experiments do not definitely demonstrate that an exocellular deacetylase occurs, but rather suggest that the organic acids detected are metabolic intermediates produced by complex intracellular reactions. However, FAULKNER and QUASTEL (1956) noted a specific deacetylase in *E. coli*. The enzyme was exocellular and capable of attacking the acetyl group on the N-acetylglucosamine unit (DOBROGOXZ, 1968). WU and WU (1971) demonstrated that the deacetylated glucosamine was then transported into the cell by a specific enzyme system. For a review of the pathway see OKUTANI and KITADA (1970).

Little information is available concerning deamination of glucosamine. Although the assimilation of amine compounds by marine microorganisms has been shown, the enzymatic mechanisms for the utilization of the amines have not been clearly demonstrated (MEYERS and NICHOLSON, 1970). Utilization of methyl amine by marine bacteria occurs by a demethylation process rather than a deamination or amine oxidase mechanism (BUDD and SPENCER, 1968). Glucose as well as N-acetylglucosamine was detected in the culture fluid of a *Chytrimyces* species grown with chitin (REISERT, 1972). It was explained, however, that the glucose might have been a product indigenous to the spore, transferred to the fluid and released upon germination rather than a breakdown product.

Distribution of chitinase systems. Chitinases are found in a variety of organisms including bacteria, fungi, invertebrates and vertebrates (even some mammals). Of these, microorganisms probably provide the most convenient source of chitinase and for this reason these systems are more generally studied. Whether the systems found in the various animals examined are similar to those of microbial origin remains to be demonstrated. JEUNIAUX (1971) considered all chitinase systems examples of regressive evolution or "enzymapheresis." Chitinases and chitobiases in the primitive unicellular organism are widespread and well-developed systems. In the more advanced phyla, however, chitinase systems are less widespread and less developed, in many cases characterized by the loss of one of the chitinolytic enzymes. Only in those organisms recognized as chitin consumers are the enzymes retained. Animals which have adapted to diets devoid of chitin have subsequently lost the ability to produce chitinases.

Microbial chitinases: cultural conditions. The first consideration in the study of microbial chitinases is the disclosure of conditions which promote production of the enzyme. Such cultural conditions were reviewed by MONREAL and REESE (1969). The type and concentration of chitin on which the organisms were grown proved to be important. Little enzyme was elaborated on mushroom chitin or on beetle (*Tribolium*) chitin, while shrimp chitin permitted higher enzyme production. Maximal yields of chitinase were obtained on highly purified commercial chitin. BAXBY and GRAY (1968) also noted increased growth of

bacteria on shrimp chitin than on lobster chitin. In the investigation, the degree of purity of the substrate may have contributed to the results. The shrimp was highly purified while the lobster chitin was only partially purified. Other factors influencing chitinase production were particle size and initial concentration of chitin. With the reduction of substrate particle size an increase in chitinase activity was noted. Maximal yields of enzyme occurred on 1.5 % to 2.0 % chitin. For the fungus, *Chytriomyces*, only 0.2 % substrate was required for highest enzyme production (REISERT and FULLER, 1962). Greatest activity was detected on the substrate chitin, with the soluble dimer, N,N-diacetylchitobiose, giving approximately one-third the yield. The monomer, N-acetylglucosamine, gave less than 7 % of the activity while chitosan, glucosamine, cellulose, cellobiose, glucose, and lactose gave little activity. Therefore, the probable inducers of chitinase are soluble oligomers derived from chitin (MONREAL and REESE, 1969). However the chitinase of the fungus, *Beauveria bassiana*, produced chitinase without the addition of chitin to the medium, indicating that this system is a constitutive one (LEOPOLD and SAMSINKOVA, 1970).

OKUTANI and KITADA (1968a,b) observed inhibition of chitinase production when several chitinoclastic marine bacteria were grown on acetate and lactate, but litte inhibition was noted when the bacteria were grown on succinate. CLARKE and TRACEY (1956) found that glucose in the culture medium depressed chitinase production by a factor of 3 to 5.

Highest chitinase yields were obtained in 4 to 5 days for *Serratia marcescens* and several other species (MONREAL and REESE, 1969), 4 to 5 days for the fungus, *Beauveria bassiana* (LEOPOLD and SAMSINKOVA, 1970), and 6 days for the *Streptomyces* species (REYNOLDS, 1954). Optimum temperature for several bacteria and an *Aspergillus* species was 30 °C. The initial pH for highest enzyme production varied with species type but correlated with the microbial growth pattern, *i.e.*, bacteria showed optimum chitinase production at neutral pH 7.0-7.5, fungi at pH 4.5 (MONREAL and REESE, 1969).

Microbial chitinases: properties of the cell-free extract. It has been documented that extraneous protein in an enzyme extract causes marked changes in the rate of substrate breakdown and in the effect of pH on the activity of the enzyme. Thus, a necessary prerequisite for characterization of an enzyme is purification (TRACEY, 1955). Since such purification is an arduous and time consuming task, many of the reports concerning the properties of chitinase have involved crude extracts. With these observations in mind, some of the properties of chitinase are reviewed below.

Chitinases of several species of *Streptomyces* have been purified and obtained in large enough quantities to determine many of the physical and chemical properties (SKUJINS *et al.*, 1970; JEUNIAUX, 1951, 1956, 1959b). The pH for optimal activity was pH 5.0 for the chitinase of *Streptomyces antibioticus* (SKUJINS *et al.*, 1970) while for another *Streptomyces* species it was pH 6.2 (BERGER and REYNOLDS, 1958; REYNOLDS, 1954). The enzyme of *S. antibioticus* was shown to be relatively stable with respect to drying and heating, with inactivation at 65 °C within 3 hours. The sequence of ion inhibition was as follows: $Mg^{++}<Co^{++}<Zn^{++}$ (with the Na^{+} ion inhibiting the enzyme more effectively than the Ca^{++} ion). The Ca^{++} ion stabilized the enzyme in small quantities (SKUJINS *et al.*, 1970; JEUNIAUX, 1959b). In an *Actinomyces* species, Cu^{++} had a greatly reducing effect on the activity of the chitinase, while Mg^{++} caused some activation of the enzyme (WIGERT, 1962).

Sedimentation tests revealed a molecular weight of the *Streptomyces* chitinase of 29,000 (SKUJINS *et al.*, 1970; JEUNIAUX, 1959b). Because the chitinase reaction takes place after adsorption of the enzyme to the surface of the substrate and, the Michaelis-Menten equation is not applicable to enzymatic reactions at surfaces, the K_m of the chitinase is not a valid characteristic (SKUJINS *et al.*, 1970).

A study of the chitinase of the fungus, *Chyrtriomyces hyalinus*, disclosed greatest activity at a pH similar to those in *Streptomyces*, *i.e.*, pH 5.5 (REISERT, 1972). Activity

was negligible below 10 °C, optimal at 25 °C, and completely lost at 45 °C. Neither N-acetylglucosamine nor glucose inhibited the activity, but Cu^{++} and Cd^{++} caused total inhibition, while Co^{++}, Li^{++}, Mg^{++}, and Na^{+} decreased activity in the respective order. Similar results were reported for the chitinase system of the fungus, *Aphanomyces astaci* (UNESTAM, 1968).

The chitinases of several marine chitinolytic bacteria, *Aeromonas chitinophthora* and *Vibrio gerris*, were partially purified and characterized as follows: pH optimum 5.5-6.0 and 7.0, respectively; stability maintained within a range of pH 5.0-9.0; and temperature optimum 40 °C (OKUTANI, 1966). The crude extract of chitinase from *Serratia marcescens* exhibited maximum activity at pH 6.4 and 50 °C with 50 % loss of activity at 50 °C for 1 hour at pH above 7.2 and below pH 4.8 (MONREAL and REESE, 1969).

Chitinase of marine invertebrates. Numerous reports document the presence of chitinase systems in animals. However, since it is beyond the scope of this review to discuss distribution in general, this discussion will be limited to the properties of chitinases from marine invertebrates. For a more complete review of the subject, see ELYAKOVA (1972), JEUNIAUX (1961), TRACEY (1955) and FRANKIGNOUL and JEUNIAUX (1965).

A series of investigations by OKUTANI (1966), OKUTANI *et al.* (1967a, b) and SERA and OKUTANI (1968) described the properties and mechanisms of the chitinase systems of the Japanese sea bass, *Lateolabrax japonicus*, the yellow tail fish, *Seriola quinqueradiata*, the rainbow trout, *Salmo irideus*, and the sea bream, *Acanthopagrus schlegedi*. Highest activity was noted in the stomach of the vertebrates with some activity in the pyloric caeca and the intestines. The chitinase from the sea bass gave optimum activity under the following conditions: pH 4.0, 50 °C, stability range of 3.0-8.0 at 60 °C for 30 minutes. Similar results were observed with the chitinase of the yellow tail. The trout chitinase was slightly less stable than the other enzymes with an optimum pH and temperature of 4.5 and 30 °C, respectively. The optimum pH and temperature of the bream chitinase was 3.4-4.0 and 60 °C, respectively.

The enzymatic products of chitin hydrolysis were identified as N-acetylglucosamine and higher oligosaccharides of N-acetylglucosamine (OKUTANI, 1966). It was suggested that a chitinase randomly attacked the linkages of chitin forming the dimer, trimer, and higher chain units, after which a chitobiase hydrolyzized the dimer and trimer to the monomer, N-acetylglucosamine. It thus appears that this system resembles that found in micro-organisms.

A chitinase from a snail, *Helix* species, was partially purified by KIMURA *et al.* (1967a, b). The state of purity of the substrate was shown to be very important for maximum activity. After decalcification of chitin an increase of 80 % in activity of the enzyme was found, while after deproteinization a 20 to 30 % increase in activity was noted. The former increase was suggested as a result of removal of enzyme inhibitors, Ca^{++} ion primarily. Certain other ions, Fe^{++}, Mn^{++}, and Zn^{++}, were demonstrated to increase activity. The chitinase was specific and did not react with any other polysaccharides or mucopolysaccharides other than chitin. Upon digestion of the substrate, identification on chromatographic paper revealed N-acetylglucosamine.

A chitinase system was demonstrated in the gastic juices of the American lobster, *Homarus americanus*, by BROCKERHOFF *et al.* (1970). A highly active chitobiase was detected but very little chitinase activity was noted. The assay used for chitinase activity, chitin azure, may have been a poor one, with low activity possibly attributed to the substrate or other conditions. The optimum pH and temperature for the chitobiase was pH 5.0 and 30 °C and for the chitinase, pH 3.0-8.0 and 37 °C, respectively. Of all the enzymes detected in the gastic juice, only the chitiobiase demonstrated an optimum pH similar to that of the normal pH of the gastic juice within the animal. KOOIMAN (1964) reported chitinase activity at optimum pH of 3.0-4.0 for related species, *Astacus fluviatilis* and *Homarus vulgaris*.

The problem proposed by KOOIMAN (1964)

was as follows: enzymes found in the digestive tract of crustacea may not be indigenous, but rather produced by the endosymbionts within the animals. To date, no crustacean producing chitinase has been found with chitinoclastic bacteria absent. KOOIMAN (1964) demonstrated the indigenous nature of enzymes by showing that chitinase was secreted by the digestive gland, the hepatopancreas, and that chitinolytic microorganisms were not present in this gland in sufficient concentrations to produce a bacterial chitinase. However, the method used by KOOIMAN to detect bacterial chitinase was designed to detect *only* a constitutive chitinase rather than an adaptive one. Thus, the question of the presence of an indigenous enzyme has still to be demonstrated.

Enzymatic studies revealed high chitinase activity by the chitinoclasts associated with shrimp as well as a moderately active indigenous chitinase produced by the hepatopancreas of the shrimp in the salt marsh environment (HOOD, 1973). Optimum activity of both enzymes was at 40 °C and pH 7. The Na^{+} ion inhibited the bacterial enzyme while the Co^{++} ion had the greatest inhibitory effect on the penaeid enzyme. The bacterial enzyme was shown to be induced while the shrimp enzyme was constitutive in nature. Both bacteria and hepatopancreas produced 1) a chitinase, 2) a chitobiase, 3) no chitosanase, and 4) a deacetylase. The end products of chitin degradation are oligosaccharides of N-acetylglucosamine, N-acetylglucosamine, and glucosamine.

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学　会　記　事

1. 昭和48年11月8日，東京水産大学において編集委員会が開かれ第11巻第4号の編集が行われた。

2. 昭和48年11月19日，東京水産大学において第1回日仏海洋学会賞受賞候補者推薦委員会が開かれ互選の結果，森田良美氏が委員長となり，評議員全員に往復葉書によって12月15日までに受賞候補1件ずつを推薦してもらうよう依頼することを決定した。

3. 死　亡

評議員上野福三氏は，昭和48年10月31日逝去された。謹んで御冥福を祈る。

4. 下記の諸氏が入会された。

正会員

氏　名	所　属	紹介者
寒川　強	公害資源研究所	佐々木忠義
中島　真固	東海大学海洋学部	〃
辻　勇雄	もがみ士官室	川原田　裕
菅野　尚	東北区水産研究所	宇野　寛
Mary A. Hood	Louisiana State University, U.S.A.	関　文威

賛助会員

株式会社大林組	佐々木忠義
東亜港湾株式会社	清水　博
東レ株式会社	佐々木忠義
芙蓉海洋開発株式会社	〃

5. 退　会

東京急行電鉄株式会社，舶用電球株式会社

6. 交換及び寄贈図書

1) 神戸海洋気象台彙報，No. 190.
2) 海洋産業研究資料，**4**(8～10).
3) 研究実用化報告，**22**(8, 9).
4) 海洋機器開発，**5**(10).
5) 鯨研通信，266，267.
6) 国立科学博物館研究報告，**16**(3)，1973.
7) 海洋開発の動向（鋼材倶楽部）.
8) なつしま，No. 4，1973.
9) 港湾技術研究所報告，**12**(3)，1973.
10) 港湾技研資料，No. 164～170，1973.
11) 港湾技術研究所年報，昭和48年版.
12) 神奈川県立博物館研究報告，No. 6，1973.
13) Ocean Age, **5**(11, 12).
14) Science et Pêche, N° 226, 1973.
15) Bulletin de l'Association de Géographes Français, N° 406～409, 1973.
16) The Scientific Reports of the Whales Research Institute, No. 25, 1973.

日仏海洋学会役員

顧　問　ユベール・ブロッシェ　ジャン・デルサルト　ジャック・ロベール　アレクシス・ドランデール

名誉会長　ベルナール・フランク

会　長　佐々木忠義

常任幹事　永田　正，大柴五八郎

幹　事　阿部友三郎，石野　誠，井上　実，今村　豊，岩下光男，宇野　寛，川原田　裕，神田献二，菊地真一，鬼頭正隆，草下孝也，斎藤泰一，佐々木幸康，杉浦吉雄，高木和徳，高野健三，辻田時美，冨永政英，奈須敬二，西村　実，根本敬久，半沢正男，松生　洽，松尾邦之助，丸茂隆三，森田良美，山中鷹之助　（五十音順）

監　事　久保田　穣，岩崎秀人

評議員　赤松英雄，秋山　勉，阿部宗明，阿部友三郎，新崎盛敏，有賀祐勝，石野　誠，市村俊英，井上直一，井上　実，今村　豊，入江春彦，岩崎秀人，岩下光男，岩田憲幸，宇田道隆，宇野　寛，大内正夫，大柴五八郎，大村秀雄，岡部史郎，梶浦欣二郎，金谷太郎，川合英夫，川上太左英，川口守一，川村輝良，川村文三郎，川原田　裕，神田献二，菊地真一，鬼頭正隆，木村喜之助，草下孝也，楠　宏，国司秀明，久保田　穣，黒木敏郎，小林　博，小牧勇蔵，近藤　仁，西条八束，斎藤泰一，斎藤行正，坂本市太郎，佐々木忠義，佐々木幸康，猿橋勝子，椎野秀雄，柴田恵司，下村敏正，庄司大太郎，末広恭雄，杉浦吉雄，多賀信夫，高木和徳，高野健三，高橋淳雄，田畑忠司，田村　保，千葉卓夫，土屋靖彦，辻田時美，寺本俊彦，冨永政英，鳥居鉄也，中井甚二郎，中野猿人，永田　正，永田　豊，奈須敬二，奈須紀幸，西村　実，新田忠雄，根本敬久，野村　正，花岡　資，半沢正男　半谷高久，菱田耕造，日比谷　京，桧山義夫，平野敏行，深沢文雄，福島久雄，淵　秀隆，星野通平，増沢譲太郎，松井　魁，松生　洽，松尾邦之助，松崎卓一，松平康男，丸茂隆三，溝口哲夫，三宅泰雄，宮崎千博，宮崎正衛，元田　茂，森田良美，森安茂雄，安井　正，矢部　博，山路　勇，山中鷹之助，山中　一，依田啓二，渡辺貫太郎，渡辺精一　（五十音順）
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うみ(日仏海洋学会誌)

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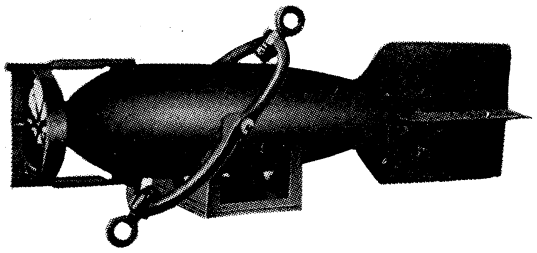
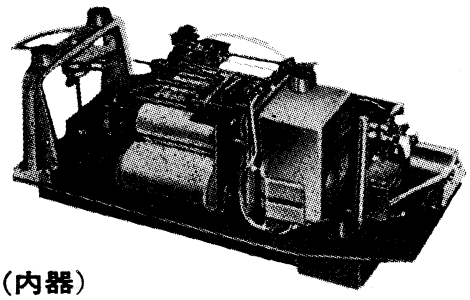
長期捲自記流速計

(NC-Ⅱ)

本流速計は海中に設置し、内蔵した記録器に流速流向を同時に記録するプロペラ型の流速計で約20日間の記録を取る事が出来ます。但し流速は20分毎に3分間の平均流速を又流向は20分毎に一回、共に棒グラフ状に記録しますから読取が非常に簡単なのが特徴となって居ります。



(外器)



(内器)

プロペラはA，B，C三枚一組になって居り

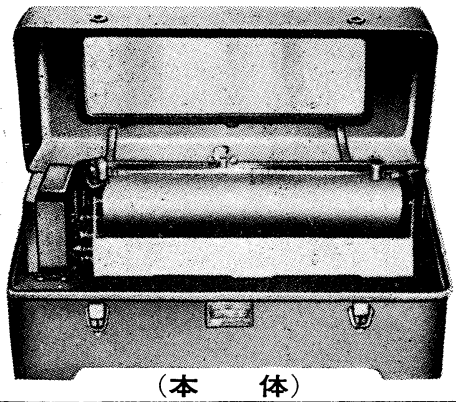
A(弱流用)……1m/sec
B(中流用)……2m/sec
C(強流用)……3m/sec

} 迄で一枚毎に検定してあります。

弱流ペラーに依る最低速度は約4cm/secです。

フース型長期捲自記検潮器

(LFT-Ⅲ)



(本　体)

営業品目

階段抵抗式波高計
ケーブル式波高計
フース型検潮器
小野式自記流速計
自記水位計
港施型土圧計
理研式水中カメラ
その他海洋観測諸計器

協和商工株式会社

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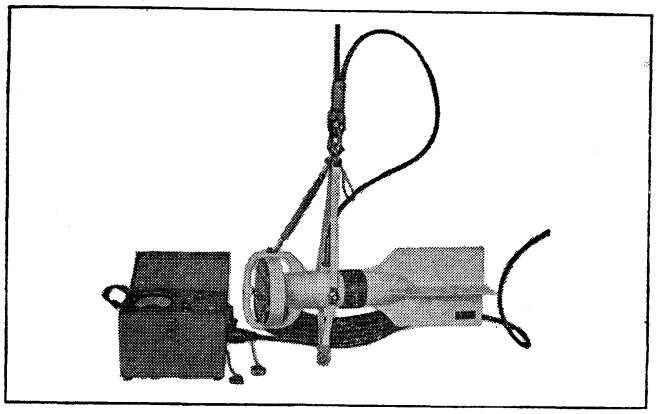

Direct-Reading Current & Direction Meter

Model

CM-2

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Products

KM-2 : Direct Reading Knot-Meter for Trawl-Boats to Control Adequate Speed

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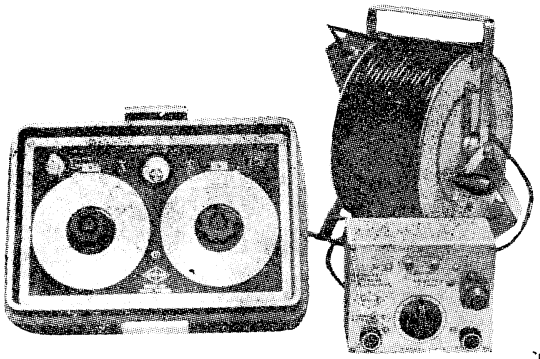
Model 602



オート・ラブ誘導起電式精密塩分計に引続いて，開発された温度と塩分の現場測定用の可搬型海洋測器です。温度，塩分ともダイアルで直読出来，簡便で堅牢しかも高精度なソリッドステートのユニット結合構造の最新鋭計器です。

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塩　分 : Scale 1. 0～32 ‰S 確度 ±0.1 ‰S
Scale 2. 32～42 ‰S 確度 ±0.03 ‰S

電　源 : 電池 9 V，200 時間使用可能

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