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PART 4

SUPPORTING PAPERS.

Nahrungsverdauung bei *Pseudactinia flagellifera.*
B. J. KRIJGSMAN and F. H. TALBOT

Experiments on digestion in sea-anemones.
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Nahrungsverdauung bei Pseudactinia flagellifera.

Die Verdauung der Coelenteraten erschien früheren Autoren problematisch, da die Vermutung nahelag, der Inhalt der Gastralhöhle müsse jedesmal, wenn neue Nahrung aufgenommen wird, durch mit eindringendes Seewasser verdünnt werden. Sieht man von Formen wie *Rhizostoma* (Wurzelqualle) ab, die einen Pump-Filter-Mechanismus haben und insofern besondere Verhältnisse auch hinsichtlich der Größe ihre Nahrungsobjekte darbieten, so wird die gedachte Schwierigkeit, mindestens zum größten Teil, durch zwei Eigentümlichkeiten beseitigt: Zunächst schlucken die Coelenteraten nicht eigentlich, sondern sie kriechen über ihre relativ großen Beutestücke gewissermaßen hinüber und halten dabei ihre Körperwand allerseits in so engem Kontakt mit dem Nahrungskörper, daß nur wenig Seewasser mit eindringen kann. Sodann wird die Beute durch eine extrazelluläre Vorverdauung im Gastralraum so weit chemisch zerkleinert, daß die noch nicht gelösten Partikel klein genug für eine Verarbeitung durch Phagozytose und nachfolgende, von einer etwaigen Verdünnung des Magensafts unabhängige, intrazelluläre Verdauung sind. Genaueres über die bei der intrazellulären Verdauung wirksamen Enzyme ist nicht bekannt, im Magensaft verschiedener Arten wurden von BEUTLER1), HYMAN2) und YONGE3) Proteasen und eine Lipase nachgewiesen. Unsere Kenntnisse über die Verdauungsenzyme sind demnach noch recht unvollständig.

Wir haben deshalb die extrazelluläre und auch die intrazelluläre Verdauung von *Pseudactinia* untersucht. Magensaft wurde erhalten durch Verfüttern von Badeschwammstückchen, die nach der Methode von BEUTLER und HYMAN mit Fleischbrühe getränkt waren. Die Anwesenheit von Proteinase wurde untersucht mit der Gelatineverflüssigungsmethode nach FERMI und der Kaseinpräzipitationsmethode nach MICHAELIS. Es stellte sich heraus, daß eine kräftig wirkende Proteinase im Magensaft vorhanden war, mit einem Optimum im Bereich von p_H 7,3 bis 8,0. Mit der stalagmometrischen Methode wurde eine Esterase gefunden, welche Tributyrin optimal bei p_H etwas über 7,0 spaltet; Milchfett dagegen wurde kaum angegriffen. Die übliche Jodmethode ergab die Existenz einer sehr schwachen Amylase, welche nur merkliche Aktivität entfaltete bei p_H 6,3 bis 7,6. Außerdem zeigte der Magensaft eine bedeutende Labwirkung auf Milch. Disaccharasen wurden, trotz Anwendung quantitativer Methoden (HAGEDORN-JENSEN-Titration), nicht gefunden.

In Extrakten von Gastralfilamenten (hergestellt durch Verreiben mit Phosphatpuffer) wurde eine sehr starke Proteinase festgestellt, deren Optimum etwas höher lag (p_H 8,2 bis 8,5) als das Optimum der extrazellulären Proteinase. Es ist jedoch kein Grund vorhanden, die Existenz zweier verschiedener Proteinasen anzunehmen, da verschiedene Stoffe, aus dem Gewebe mitextrahiert, kleine Verschiebungen des Optimums hervorrufen könnten. Ferner wurde eine auf Tributyrin kräftig wirkende Lipase, sowie eine mäßig starke Amylase festgestellt. Auch diese Enzyme sind offenbar identisch mit der extrazellulären Lipase und Amylase. Die Labwirkung der Gewebsextrakte war sehr stark. Disaccharasen fehlten wie im Magensaft.

Unseren Resultaten, welche an anderer Stelle ausführlich publiziert werden, kann man entnehmen, daß die extrazelluläre Proteinase die Hauptrolle spielt bei der Vorverdauung der Nahrung. Dieses Enzym ist sehr wahrscheinlich adsorbiert an dem Schleim, der vom Epithel sezerniert wird als Reaktion auf Eintreten von Nahrung in die Magenhöhle. Die Möglichkeit besteht, daß dieser Schleim durch die Labwirkung des Magensaftes koaguliert wird und so eine gelatinöse Hülle um die Beute bildet. Auf diese Weise könnte Verdünnung und somit Verlust von Enzymen und Nahrungsprodukten durch eintretendes Seewasser zuvorgekommen werden. Nach der extrazellulären Vorverdauung können die aufgedeckten intrazellulären Enzyme die Verdauung vollenden.

Es ist nicht klar, warum wir weder im Magensaft noch im Gewebsextrakt Disaccharasen fanden. Während sie zur Vorverdauung im Magen nicht notwendig erscheinen, könnte man doch ihre Existenz im Extrakt erwarten, da jene Enzyme im ganzen Tierreich bei der Verdauung von Kohlenhydraten eine Rolle spielen. Auch andere Fragen warten noch auf ihre Lösung. Erstens wissen wir noch nicht, ob die Verdauungsprozesse bei allen Coelenteraten sich ähnlich sind, da bis jetzt nur wenig Arten untersucht wurden. Weiter liegen noch keine quantitativ biochemischen Untersuchungen mit gereinigten Verdauungsenzymen vor, welche deren Spezifität in Beziehung zu Verdauungsenzymen anderer Tiergruppen aufklären könnten. Es erscheint sehr wünschenswert, daß diese Probleme weitere Bearbeitung finden.

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[1]) BEUTLER: Z. vergl. Physiol. **1**, 1 (1924); **3**, 737 (1926); **6**, 473 (1927).

[2]) HYMAN: Biol. Bull. **79**, 282 (1940).

[3]) YONGE: Sci. Rep. G. Barrier Reef Exp. Brit. Mus. **1**, 59 (1930).

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EXPERIMENTS ON DIGESTION IN SEA-ANEMONES.

BY

B. J. Krijgsman and F. H. Talbot
(*University of Cape Town*)

(1 figure)

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EXPERIMENTS ON DIGESTION IN SEA-ANEMONES

BY

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(1 figure)

Introduction

Digestive processes in Coelenterates have early attracted the attention of physiologists. In most medusae as well as in sessile forms the gastric cavity is not a physiologically closed system, since its sac-like shape and wide entrance must give rise to considerable contamination of the contents with water every time the mouth opens. In some forms we even find a continuous circulation of water through the gastric cavity. Consequently special steps have to be taken by the animals to restrict dilution of digestive enzymes and loss of digested food. Coelenterates have solved this problem with the aid of phagocytosis. In those forms which take in particulate matter (i. e. plankton- and detritus feeders) phagocytosis does not introduce special difficulties. The epithelium of the gastric filaments ingests the small food particles and digestion is carried out intracellularly.

However, many Coelenterates, e. g. sea-anemones, ingest food ranging from small *Crustacea* to fishes as large as the predator itself. As phagocytosis of such large material is impossible, there must occur some preliminary disintegration in the gastric cavity. PARKER (cited by JORDAN, **18**) was the first to suggest the occurrence of preliminary digestion in the gastric cavity, by which the food might be broken down to particles small enough to be phagocytised. Subsequent intracellular breakdown could then complete the digestive processes. This suggestion directed the experimental work into two directions : 1) Investigation of enzymes in the gastric cavity which may carry out preliminary digestion, and 2) investigation of intracellular enzymes which complete digestion.

As far as the *extracellular enzymes* are concerned, early investigations exhibit many contradictions. Some authors found a digestion of animal tissue which was in contact with the gastric filaments (MULLER, **26**; GREENWOOD, **13**; BIGELOW, **6**; CHAPEAUX, **9**; SCHÄPPI, **30**; DOWNING, **11**; HADŽI, **14**). Others claimed that this contact was essential, since they did not find digestion if the food did not touch the filaments (meat in a feather shaft in the gastric cavity, LEWES cited by BIEDERMAN, **5**, KRUKENBERG, **19**). This was the reason why the term « contact digestion » was introduced. But even this contact digestion was denied by MESNIL (**23**), who did not discover any sign of digestion in the gastric cavity of sea-anemones. This, however, is in contradiction to other results, and it is easy to prove that a piece of fish or mussel is readily digested in the gastric cavity. That there is contact digestion is certain. therefore, but in addition it has been shown by WILLEM (**32**) that animals covered with a shell (*Crustacea*) were readily digested in spite of lack of contact between digestible material and gastric filaments. Furthermore, WILLEM showed that this disintegration was no autolysis, since dead *Crustacea* in seawater do not autolyse so rapidly. His opinion was supported by the results of CHAPEAUX, who found a slight digestive action of gastric juice of *Actinia* on fibrin *in vitro*. The final proof that digestive processes in Coelenterates are not confined to contact digestion was brought by JORDAN (**17**), who observed a disintegration of fibrin enclosed in a sac of filterpaper in the stomach of *Anemonia sulcata*, and by ABDERHALDEN and HEISE (**1**), who found a digestion of polypeptids. Then, for many years, scientists seem to have lost interest in this problem, for only in 1924 does BODANSKY (**7**) take up the question again. He finds very little digestive action exerted by filtered gastric juice of *Metridium*. More promising are the results of BEUTLER (**2**, **3**, **4**), who collected gastric juice from *Hydra* polyps by feeding them with small pieces of sponge. The fluid absorbed by the sponge readily digested fibrin and gelatin at a pH of 8.2. YONGE (**35**) found a proteinase in the gastric juice of the medusa *Aequora sulcata*. As far as other extracellular enzymes are concerned results are poor. CHAPEAUX, BODANSKY, BEUTLER, YONGE and HYMAN (**16**) failed to detect an amylase in the gastric cavity. BEUTLER and YONGE could not discover extracellular digestion of fat or lower esters. Only HYMAN reports a digestion of milk fat.

The picture of *intracellular enzymes* is more definite. A proteinase can be readily demonstrated in extracts of gastric filaments. According to FREDERICQ (**12**), CHAPEAUX and MESNIL, extracts of *Anemonia* and *Adamsia* break down fibrin, boiled egg-white, gelatin and casein at a pH ranging from slightly alkaline to·about 4.4. Since digestion was quickest at about pH 7, they postulated the presence of a « trypsin-like » proteinase. BODANSKY and ROSE (**8**) found, in extracts of filaments of *Stomolophus meleagris* and *Physalia arethusa*, a proteinase with two optima when acting on gelatin, but with a poor action on albumin, fibrin and casein. BODANSKY (**7**) also found a trypsin-like proteinase in filaments of Metridium. YONGE, in extracts of *Madreporia*, found a proteinase and a dipeptidase; the pH-optimum of the proteinase varied for different genera (8-8.75 or 6.9-7.9). SAVIANO (**29**) discovered a trypsin-like proteinase in the endoderm of *Anemonia sulcata*. The results concerning carbohydrases are contradictory. FREDERICQ, CHAPEAUX and MESNIL found no amylase and no sucrase, BEUTLER failed to observe intracellular digestion of starch in *Hydra*. YONGE detected an amylase in some forms; this enzyme, however, was apparently contained in zooxanthellæ, since he did not find it in zooxanthellæ-free *Madreporia*. SAVIANO did not find amylase in extracts of *Anemonia*. BODANSKY and ROSE, and BODANSKY report the existence of an amylase and a weak maltase in extracts of *Stomolophus*, *Physalia* and *Metridium*, but they did not mention the possible occurrence of zooxanthellæ. As far as lipolytic enzymes are concerned, FREDERICQ, CHAPEAUX and MESNIL report a slight action of extracts on almond milk and a reasonable action on monobutyrin; CHAPEAUX also observed intracellular digestion of olive oil. BODANSKY and ROSE found a slow action on ethyl-butyrate, amyl-acetate and milkfat; YONGE also found a lipase. SAVIANO found a strong action of extracts on tributyrin, but no breakdown of higher esters. BEUTLER did not discover any lipolytic action. A rennitic action was reported by MESNIL, BODANSKY and ROSE, and by BODANSKY. As no comparison was made with standard preparations of proteinases, it·is not certain whether this clotting action was produced by a specific rennin or merely by the action of the proteinase present.

As a whole, the position is far from established and worth further attention. A factor which may have contributed to confusing results is that different authors worked with different species. Apart from that, most authors do not discuss the question of zooxanthellæ, thus is not always clear whether the reported enzymes originate from the Coelenterates themselves or from their symbionts..

Methods

After some preliminary experiments with *Bunodactis reynaudi* we used only the large sea-anemone *Pseudactinia flagellifera*, which is abundant along the coast of the Cape Peninsula. This species lives in the tidal zone, ingests a variety of animal food and does not possess zooxanthellæ. The gastric cavity often contains gastropods, Crabs, sea urchins and the like in a more or less advanced state of digestion. It is interesting to note that *Pseudactinia* never contains mussels. *Bunodactis*, on the other hand, often swallows pelecypods which it is able to crack in the gastric cavity.

In order to collect gastric juice we used the method of BEUTLER. Small pieces of sponge, soaked in diluted meat extract (Bovril) and attached to a thread, were fed to the animals and ingested readily. The use of meat extract favours ingestion and may be a stimulus for the secretion of enzymes. In the first experiments the sea-anemones were removed from the rocks, put in aerated seawater and fed in the laboratory. It was found, however, more satisfactory to feed them in their natural rock pools at low tide; this method was used in all further experiments. The sponges were left in the animals for about 90 mins., the anemones were then removed from the rocks and the sponges pulled out above water. The sponges were always found to be soaked with a mass of stiff mucus. HOLLARD (**15**) was the first author to report that the walls of the gastric cavity secrete abundant mucus. We believe that the mucus is secreted as a result of the sham feeding. In normal conditions this secretion is probably evoked by soluble organic compounds of the food, in empty animals it is absent. The sponges were squeezed out, the fluid obtained filtered to make sure that no tissue fragments were present, and diluted with boiled seawater. Control experiments showed that the meat extract did not exert any enzymatic action. In order to obtain extracts of gastric filaments, the filaments of fresh specimens were separated from the gonads with forceps, ground with chemically pure sand and diluted with boiled seawater.

Some of the experiments were carried out at room temperature. In other cases, especially in the more accurate experiments, the digestive sets were kept in a thermostat. Phosphate buffer was used to obtain the desired pH, which was checked electrometrically.

Experimental

EXTRACELLULAR ENZYMES

Acidity of gastric cavity

The pH of the gastric juice, measured electrometically, varied between 6.7 and 7.8. This is in agreement with the results of other authors, who reported 7.0-7.8 In some cases, in which we collected juice from animals while digestion was in progress, the pH of the gastric juice (7.2-7.3) was distinctly different from the pH of the water of the pools in which the animals lived (8.0).

Proteinase

Preliminary experiments with congo red fibrin as substratum, at varying pH, did not show liberation of dye within 24 hrs. Even with the highest possible concentration of gastric juice we failed to observe any digestion. Thus we continued our experiments with other substrates. Casein was used with the precipitation method of MICHAELIS (**24**), by which the amount of undigested casein is measured as a precipitate, produced by diluted acetic acid. For each experiment we used 6 ml. of gastric juice of varying concentration, mixed with 4 ml. of 0.1 % alkaline casein solution; the pH of the mixture was 8.5. At suitable intervals a sample of 1 ml. was mixed with 4 drops acetic acid and the precipitate estimated. Four gradations of the precipitate could be discriminated, labelled « strong », « moderate », « faint » and « absent ». The experiments carried out with this method gave uniform results. An example of an experiment is given in Table I (temp. 30° C). The percentage at the top of each column refers to the concentration of the gastric juice in the digestive mixture.

TABLE I

Time in hrs.	10 %	20 %	40 %	60 %	Control with boiled enzyme
0	strong	strong	strong	strong	strong
½	strong	strong	strong	strong	strong
1½	strong	strong	moderate	moderate	strong
2	strong	moderate	faint	faint	strong
3	moderate	moderate	absent	absent	strong
5	moderate	faint	absent	absent	strong
7	faint	absent	absent	absent	strong

The experiments with casein clearly prove the presence of an extracellular proteinase with considerable activity at pH 8.5.

In order to study the relation of proteolytic activity to pH we used two methods. First of all the gelatin liquefaction method of PALITZSCH and WALBUM (**27**). In this method a gelatin solution is mixed with the enzyme solution to be tested and the progress of liquefaction is observed. Many experiments were done in the pH-region 4.8-9.8. Within 3 hrs. no digestion ever occurred at a pH lower than 5.6; between pH 5.6 and about 8 digestion took place, increasing with pH, above pH 8 there was a rapid decrease and above pH about 8.5 no digestion was shown.

The gelatin liquefaction method still has a somewhat qualitative character. We therefore carried out a number of quantitative experiments in the pH-range 5-9 with the alcohol titration method of WALDSCHMIDT-LEITZ (**31**). 2 ml. of enzyme solution was mixed with 3 ml. phosphate buffer and 2 ml. of 5 % gelatin solution. The digestive sets were kept at 30° C. Titration of 3 ml. samples with 0.1n KOH in 90 % alcohol after 3 hrs. This set of experiments was repeated six times with consistent results. A typical example is given in figure 1.

From our experiments it can be concluded that the gastric juice contains a proteinase which acts on casein and gelatin, with an optimum for gelatin at a pH round 8. These results are in agreement with those of BEUTLER, who found proteolytic activity of gastric

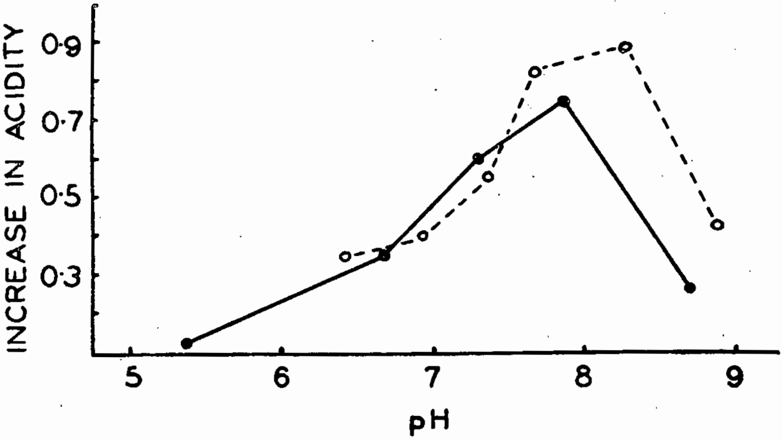

FIG. 1. — Proteolytic action of gastric juice of Pseudactinia (full line), after 3 hrs. digestion, and of extract of filaments (broken line), after 2 hrs. digestion, both on gelatin. Increase in acidity (ml. 0.1n KOH used for titration) plotted against pH.

juice at pH 8.2, and of YONGE, who observed protein digestion at pH 7.1.

Clotting action

The gastric juice of *Pseudactinia* showed a definite coagulating action on milk. 50 ml. boiled milk were mixed with 5 ml. 1 % $CaCl_2$, 2 ml. of this mixture was added to 1 ml. gastric juice and several samples of this preparation kept at 18° C. Within 3 hrs. a complete coagulation occurred, whereas controls with boiled enzyme showed no change. It is known that all enzymes which can hydrolyse casein can cause milk to clot. We had no standard preparations of proteinases available for comparison, so it is impossible to tell whether the clotting observed is due to action of a specific « rennin » or to the proteinase described above. No other authors have mentioned a rennitic action of gastric juice of Coelenterates.

Amylase

For the detection of amylase we used the well-known iodine method of MICHAELIS (**29**). 4 ml. gastric juice was mixed with 0.7 ml. 1 % starch solution, 2 ml. phosphate buffer and a few drops of toluene. At suitable intervals samples were mixed with a given small quantity of iodine solution. Even with the strongest possible concentration of gastric juice only a very weak amylolytic action was shown in 8-18 hrs., in the pH-region 6.3-7.6. This action was so weak that it was impossible to investigate the pH-optimum more closely. Former authors did not detect amylase in the gastric juice of Coelenterates.

Maltase

In order to check the presence of a maltase, experiments were made with maltose as substrate, at varied pH. Tests with FEHLING solution failed to show any increase in reducing power of digestive mixtures. Attempts to discover increase in reducing power with methylene blue as an indicator (method of LANE and ENYON, cited by COLE, **10**) also failed. More accurate experiments over a wide range of pH with the polarimetric estimation of WILLSTÄTTER et al. (**33**) and with the HAGEDORN-JENSEN titration still brought no results. Even with very concentrated extracts no maltase was found. Thus we must conclude that the gastric juice of *Pseudactinia* does not contain this enzyme in noticeable quantity.

Lipase

Preliminary experiments with milk, mixed with gastric juice and phenolphthalein as indicator, failed to show the production of fatty acids. Experiments were then carried out with olive oil as substrate according to the titration method of WILLSTATTER and WALDSCHMIDT-LEITZ (**34**). 4 ml. of enzyme solution were mixed with 2 ml. phosphate buffer and 1 ml. olive oil. The experiments were carried out at a pH range of 5.3-8.7, at 30° C. No digestion occurred within 24 hrs., the variation in KOH-consumption never exceeded the limits of error.

Since our experiments with higher esters proved to be negative, we have tried to show a breakdown of lower esters (tributyrin) by the gastric juice. These experiments were carried out with the method of RONA and MICHAELIS (**28**) with a KRIJGSMAN stalagmometer (KRIJGSMAN, **20**, **21**). A saturated, fresh solution of pure tributyrin in water was used, 9 ml. of which was added to 1 ml. gastric juice and 1 ml. phosphate buffer. Tributyrin in watery solution lowers the surface tension considerably, consequently the number of drops to a given volume is much greater than to the same volume of pure water. The breakdown of the ester causes a rise in surface tension. Consequently the breakdown can be followed quantitatively with a stalagmometer, progressive decrease of the number of drops indicating progressive breakdown. A lipolytic action was shown in all experiments. An example is given in Table II. Temperature 27° C.

TABLE II

Time in hrs.	Number of drops given by 6 ml.				
	pH 7.6	pH 7.05	pH 6.3	pH 3.5	Control with boiled enzyme
0	95	96	96	96	96
2	83	92	93	96	95
4	87	90	92	95	95
8	71	79	89	96	96

Our results clearly show the existence, in the gastric juice, of a lipolytic enzyme, which does not act on milk-fat or olive oil, but digests tributyrin fairly well at pH 7.6-7.0. No digestion occurs at pH 4. HYMAN is the only author who has found a lipolytic action by the gastric juice of Coelenterates.

INTRACELLULAR ENZYMES

Proteinase

Extracts of gastric filaments showed very little action on congo red fibrin. In other experiments, carried out with the casein precipitation method and the gelatin liquefaction method, we found a strong proteolytic action. With gelatin a number of experiments were performed at different pH, using the titration method of WILLSTÄTTER and WALDSCHMIDT-LEITZ. 2 ml. of extract were mixed with 3 ml. phosphate buffer and 3 ml. gelatin solution. Titration of 3 ml. samples after 2 hrs., temp. 30° C. Optimal digestion was found to occur round pH 8, some action still occurring at pH 6.4 and pH 8.9. A typical example of these experiments is reproduced in figure 1 (broken line). Our results are in agreement with the statements of those former authors, who found proteinase in extracts of filaments.

Clotting action

The clotting action of extracts on milk, estimated in the same way as for gastric juice, was very strong. Coagulation occurred within 10 mins. at 18° C. Extracts of other Coelenterates also show a rennitic action (BODANSKY and ROSE, BODANSKY).

Amylase

The starch-iodine method was used, as for extracellular amylase. The extracts showed an amylolytic action, which was somewhat stronger than that of gastric juice, but still so weak that it was difficult to obtain quantitative data on its action. At 28° C and after about 2½ hrs. the first indication of digestion appeared, in the pH-region 5.2-7.0, shown by a faint paling of the blue colour as compared with the control. Within 7½ hrs. the paling became quite definite in this pH-range, but no reaction was seen at a pH lower than 5.2. Attempts to strengthen the amylase action by using more concentrated extracts failed. Amylase has been found in extracts of other Coelenterates by BODANSKY and ROSE, and BODANSKY, who do not state whether their subjects contain zooxanthellæ, and also by YONGE, who attributes the presence of the enzyme to zooxanthellæ.

Maltase

Attempts to detect the presence of a maltase in extracts, with maltose as a substrate, at a wide range of pH, all faited. Increase in reducing power could not be demonstrated with the methods described for gastric juice, so we must conclude that the extracts did not contain an active maltase.

Lipase

Extracts of gastric filaments showed very little or no action on milk-fat. A very weak action on olive oil (titration method as for gastric juice) was observed. Breakdown of tributyrin, on the other hand, was very rapid in all experiments. An example (8 ml. tributyrin solution, mixed with 2 ml. buffer and 3 drops of extract, at 28° C) is given in Table III.

TABLE III

Time in mins.	Number of drops given by 6 ml.					
	pH 7.7	pH 6.7	pH 5.5	pH 5.0	pH 2.3	Control with boiled enzyme
0	95	94	94	92	93	94
30	88	88	89	89	93	94
45	79	84	84	86	94	95
60	75	79	80	82	94	94
70	72	77	79	81	93	95

From our results it is clear that a lipolytic enzyme is present in the filaments, acting on tributyrin, with a strong action at pH 7.7-6.7, and showing no action at a pH lower than about 5. Our results are in accordance with most former authors, who, however, do not give pH-ranges.

Discussion

Our results prove that the gastric juice of *Pseudactinia flagellifera* contains digestive enzymes (compare KRIJGSMAN and TALBOT, **22**). The proteinase found, although not acting noticeably on fibrin, shows considerable action on casein and on gelatin. The optimal action on gelatin occurs round pH 8. Under natural conditions the proteinase apparently acts in its optimal pH-region, since the gastric juice has a pH of 6.7-7.8. Very probably this proteinase is of the trypsin type; definite proof of its nature should be revealed by experiments with purified enzyme and activators. The proteolytic action of the gastric juice appears to be sufficient to cause disintegration of the tissues of the prey for subsequent phagocytosis.

The lipase of the gastric juice breaks down tributyrin at a noticeable speed in a neutral or slightly alkaline medium, but does not act on higher esters. The biological significance of this action on lower esters is not clear, for one cannot understand how such action could contribute to the preliminary disintegration of the food.

The amylolytic action of the gastric juice is extremely weak, this amylase apparently plays no part in extracellular digestion.

For the final intracellular digestion a strong proteinase is available, which shows an optimal activity at the same pH-range as the extracellular proteolytic action. Probably, therefore, extra- and intracellular proteinase are identical, and of the trypsin type. It is not a tissue proteinase of the kathepsin type, since kathepsins show lower pH-optima. The intracellular lipase acts strongly on tributyrin in the same pH-region as the extracellular one. Apparently both lipases are identical. Its action on milk-fat and olive oil is very poor, which is in agreement with the results of most former authors. The question arises, therefore, whether this poor action on higher esters means that Coelenterates are unable to utilise a considerable part of their food. This seems unlikely. Do they possess, then, specific lipases which act better on invertebrate than on vertebrate or vegetable fat? Although the known differences in composition between these fats do not seem to justify the assumption that Coelenterates possess specific lipases, it may be worthwhile to investigate the action of Coelenterate lipase on the fats of their normal food.

The intracellular amylase causes a slow digestion of starch. It possibly acts better on glycogen, which is the principal polysaccharide contained in the food of *Pseudactinia*. It is somewhat surprising that neither we nor former authors (except BODANSKY) have found an intracellular maltase. This enzyme is present throughout the animal kingdom as a necessary agent for the breakdown of maltose which results from the decomposition of polysaccharides. It does not seem probable, therefore, that it would be lacking in the endoderm of Coelenterates. Possibly it is bound in such a way in the cells that ordinary extraction methods fail.

It is not clear whether the strong clotting action of gastric juice and extracts has a biological significance. It may be suggested that by this action the excreted mucus is coagulated, so that it forms a solid layer coating the prey. It seems very unlikely, anyhow, that the mucus is secreted in the solid condition in which we find it in the gastric cavity. As far as we know, mucus is, throughout the animal kingdom, secreted in a fluid state.

As far as can be concluded from our experiments the digestive mechanism in *Pseudactinia* takes the following course : A prey is ingested, a process which is certainly accompanied by the swallowing of seawater. After ingestion the mouth closes, there being no continuous communication with the surrounding water. Continuous communication may occur in plankton feeders, in which a permanent stream of food particles enters the gastric cavity. In *Pseudactinia*, however, this is not the case as indicated by the difference between the pH of the gastric juice and the surrounding water. Nevertheless, *Pseudactinia* certainly does not keep its mouth closed as long as the gastric cavity contains food, frequently taking in new prey while digestion of previously swallowed food is till going on. Consequently a certain contamination with seawater must occur. Therefore mucus, secreted as a reaction to the ingested food, covers the prey and is possibly solidified by rennitic action. This mucus, which contains a fairly strong concentration of proteinase, impregnates the food, and by the protection of this stiff jelly digestion may go on without being disturbed by seawater. The particulate products of the extracellular digestion are phagocytised by the endoderm cells, and digestion is completed intracellularly. In those cases in which the prey is covered by a chitinous cuticle, the enzymes may diffuse from the surrounding jelly through the permeable regions of the cuticle. Possibly a chitinase assists the disintegration of food with a chitinous skeleton. No experimental data on chitinase are, however, available.

Summary

1) The digestive mechanism of *Pseudactinia flagellifera* was investigated.

2) The gastric juice has a pH between 6.7 and 7.8, which is not always the same as the surrounding water.

3) The gastric juice contains a proteinase (optimum for gelatin round pH 8), a lipase acting on tributyrin at about pH 6-8 and a very weak amylase. No maltase was found.

4) Extracts of filaments show a strong proteolytic action (optimum for gelatin round pH 8), a lipase strongly acting on lower esters but very poorly on olive oil and milk fat, and a weak amylase. No maltase was found.

5) Gastric juice and extracts of gastric filaments show a definite clotting action on milk.

6) The biological significance of the results obtained is discussed.

BIBLIOGRAPHY

1. ABDERHALDEN, E. und HEISE, R. — Ueber das Vorkommen proteolytischer Fermente bei Wirbellosen. *Hoppe Seyl. Z. physiol. Chemie*, 1909, LXII, 136.
2. BEUTLER, R. — Experimentelle Untersuchungen über die Verdauung bei Hydra. *Z. vergl. Physiol.*, 1924, I, 1.
3. BEUTLER, R. — Beobachtungen an gefütterten Hydroidpolypen. *Z. vergl. Physiol.*, 1926, III, 737.
4. BEUTLER, R. — Die Wasserstoffionenkonzentration im Magen der Hydra. *Z. vergl. Physiol.*, 1927, VI, 473.
5. BIEDERMANN, W. — Die Verdauung bei den Coelenteraten. *Handb. vergl. Physiol.*, *Jena*, 1911, II.
6. BIGELOW. — *Johns Hopkins Univ. Circ.*, 1891, X, 90.
7. BODANSKY, M. — Comparative studies of digestion. III. Further observations on digestion in Coelenterates. *Amer. J. Physiol.*, 1924, LXVII, 547.
8. BODANSKY, M. and ROSE, W. C. — Comparative studies of digestion. I. The digestive enzymes of Coelenterates. *Amer. J. Physiol.*, 1922, LXII, 473.
9. CHAPEAUX. — Recherches sur la digestion des Cœlentérés. *Arch. Zool. expér.*, 1893, I, 139.
10. COLE, S. W. — Practical physiological Chemistry. *Cambridge*, 1933.
11. DOWNING, A. — *Science*, D. S., 1902, XV, 523.
12. FREDERICQ, L. — La digestion des matières albuminoïdes chez quelques Invertébrés. *Arch. Zool. expér.*, 1878, VII, 213.
13. GREENWOOD. — On digestion in Hydra. *J. of Physiol.*, 1888, IX, 317.
14. HADŽI, J. — Vorversuche zur Biologie der Hydra. *Arch. Entw. Mechan.*, 1906, XXII, 38.
15. HOLLARD, M. — Monographie du genre Actinia. *Ann. Sci. Nat. Zool.*, 1851, XV, 288.
16. HYMAN, L. H. — Observations and experiments on the physiology of Medusæ. *Biol. Bull. Woods Hole*, 1940, LXXIX, 282.
17. JORDAN, H. J. — Die Verdauung bei Aktinien. *Pflüg. Arch. ges. Physiol.*, 1907, CXVI, 617.
18. JORDAN, H. J. — Vergleichende Physiologie wirbelloser Tiere, *Jena*, 1913.
19. KRUKENBERG. — *Vergleichende physiologische Studien an der Küste der Adria*, 1880, I, 38.

20. KRIJGSMAN, B. J. — Arbeitsrhythmus der Verdauungsdrüsen bei Helix pomatia. II. Sekretion, Resorption und Phagocytose. *Z. vergl. Physiol.*, 1928, VIII, 187.

21. KRIJGSMAN, B. J. — Stalagmometrische bepaling van lipase. *Natuurw. Tijdschr.*, 1928, X, 137.

22. KRIJGSMAN, B. J. und TALBOT, F. H. — Nahrungsverdauung bei Pseudactinia flagellifera. *Naturwiss.*, 1951, XXXVIII, 460.

23. MESNIL, F. — Recherches sur la digestion intracellulaire et les diastases des Actinies. *Ann. Inst. Pasteur*, 1901, XV, 352.

24. MICHAELIS, L. — *Biochem. Z.*, 1908, X, 290.

25. MICHAELIS, L. und RONA, P. — Praktikum der physiologischen Chemie, *Berlin*, 1930.

26. MÜLLER, F. — Die Magenfäden der Quallen. *Z. wiss. Zool.*, 1850, IX, 542.

27. PALITZSCH und WALBUM. — *Biochem. Z.*, 1922, XLVII, 1.

28. RONA, P. und MICHAELIS, L. — *Biochem.* Z., 1911, XXXI, 345.

29. SAVIANO, M. — Ricerche sugli enzimi digestivi dell'Anemonia sulcata. *Boll. Soc. ital. Biol. sper.*, 1936, XI, 145.

30. SCHÄPPI, Th. — *Mitt. Nat. Ges. Winterthur*, 1899.

31. WALDSCHMIDT-LEITZ, E. — Ueber Enterokinase und die tryptische Wirkung der Pankreasdrüse. *Hoppe Seyl. Z. physiol. Chem.*, 1924, CXXXII, 181.

32. WILLEM, V. — La digestion chez les Actiniens. *Bull. Soc. Méd. Gand*, 1892, 295.

33. WILLSTÄTTER, R., OPPENHEIMER, F. und STEIBELT. — Ueber Maltaselösungen aus Hefe. *Hoppe Seyl. Z. physiol. Chem.*, 1920, CX, 232.

34. WILLSTÄTTER, R. und WALDSCHMIDT- LEITZ, E. — Ueber Rizinuslipase. *Hoppe Seyl. Z. physiol. Chem.*, 1924, CXXXIV, 161.

35. YONGE, C. M. — *Sc. Rep. G. Barrier Reef Exped. Brit. Mus.*, 1930, I, 59.

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NOTES ON THE BIOLOGY OF THE WHITE STUMPNOSE, *RHABDOSARGUS GLOBICEPS* (CUVIER), AND ON THE FISH FAUNA OF THE KLEIN RIVER ESTUARY

By F. H. Talbot
East African Marine Fisheries Research Organization, Zanzibar

(With three text-figures)

(Read November 17, 1954)

The fish fauna of the Klein River estuary is discussed in relation to food, shelter, and hydrographic conditions.

The biology of the white stumpnose is described on the basis of an examination of 1,905 specimens from estuarine and sea samples.

The growth rate of the white stumpnose as determined by length-frequency data and by otolith examination is suggested to be about 60 mm. per year.

Only juvenile white stumpnose are present in estuaries, entering annually, and remaining until they reach a length of about 140 mm., then leaving for the open sea and not returning.

Females are considered to mature at between 220 mm. and 240 mm. in length. Males were first found with well-developed gonads at 275 mm. Males and females were first found breeding in their third year.

Introduction

The white stumpnose, *Rhabdosargus globiceps* (Cuvier), is a common South African inshore fish. It ranges from Walvis Bay to Durban, and is of angling and commercial importance on the Cape south and west coasts. Adult fishes, often in large shoals, are found along the coastline and down to forty fathoms (Barnard, 1927), being caught by shore seiners, line boats, and trawlers. Juveniles, up to about 15 cm. in length, frequent estuaries, where they may be netted and sun-dried to form 'Bokkems', a term covering other small estuarine fishes such as juvenile white steenbras (*Lithognathus lithognathus*) and mullet or haarders (*Liza ramada*).

Little is known about the general biology of the species, or its movements, as is unfortunately the case with most South African fishes. This work was, therefore, undertaken as a preliminary study of factors such as rate of growth, size at sexual maturity, migrations and others.

The *R. globiceps* population of the Klein River estuary near Hermanus and the Milnerton estuary near Cape Town were studied, and samples of marine fish were bought from Kalk Bay line boats. The fish populations of the two estuaries were found to be nearly identical and it was therefore considered necessary to describe only the Klein River estuary fully and merely to refer to the Milnerton estuary where it showed differences or provided additional information. To give a clear picture of the biotope of *R. globiceps* the fish fauna of the Klein River estuary is fully described. Scott, Harrison, and Macnae (1952) made a qualitative survey of the bottom fauna of this estuary.

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METHOD

Samples were obtained by regular nettings made over a period of eighteen months at Milnerton estuary and the Klein River estuary. Samples of *R. globiceps* were also bought from Kalk Bay line boats. This sampling was irregular because catches at times contained no *R. globiceps*, and because boats leave this harbour during the period that the snoek, *Thyrsites atun* Euphrasen, is abundant around the Peninsula. Two collecting expeditions by the ecological survey team of the Zoology Department, University of Cape Town, to the Breede River estuary, in which I was able to participate, yielded a summer and a winter sample of *R. globiceps*. Odd records were also obtained from trawlers and shore anglers. (Table I.)

TABLE I

Place and dates	*Gear*	*Number of fish measured*	*Number of fish opened*	*Size range*
Klein River estuary .. May '50–July '51	Large seine	1,166	371	52–154 mm.
Klein River estuary .. Jan., Feb., March., April 1951	Drag net	12	12	27–105 mm.
Milnerton estuary .. April '50–June '51	Small seine	631	238	28–98 mm.
Breede River estuary.. July 1951	All nets	44	44	35–124 mm.
Kalk Bay .. .. Jan.–July '51	Hand lines	41	41	128–300 mm.
Cape Infanta .. .. July '50, July '51	Trawl at 30 fathoms	5	5	128–260 mm.
Miller's Point .. May 1951	Angling	6	6	200–300 mm.
Buffels River mouth .. July 1951	Angling	–	6	300–350 mm. (approx.)
	TOTALS:	1,905	723	27–350 mm.

Nets of three types were used for samplings:

(i) *The Large Seine.* This is a net of English design (the Saltash Tuck Seine), weighted to sink when pulled so that it rides the bottom, and does not float as do the seine nets of commercial fishermen used for shore and estuary fishing in South Africa. It is 152 ft. in length, with a purse of 19 ft., is 5 ft. deep in the wings and

7 ft. deep at the purse. The mesh of the wings is 1½ in. bar, and that of the purse ¾ in. bar. The net has 250 ft. ropes at each end, and is shot from a boat.

Fish below 70–80 mm. escape through the meshes of the large seine, so that length-frequency curves are skewed from this size and below. With capture by net only, there is a possibility that the larger and faster fish may more easily escape this gear, resulting in skewing of the length-frequency curves of the larger sizes. Estuarine *R. globiceps* do not reach a large size, however, and it is unlikely that this type of error is affecting the large seine captures.

(ii) *The Small Seine.* This is a much smaller net than the above, being 40 ft. in length, 4 ft. deep in the wings, and 6½ ft. deep at the bunt. Unlike the large seine it has no long purse, but has a slight belly of about 6 ft. The mesh is ½ in. bar. The small seine is pulled by two persons without ropes, and is therefore limited to water that is shallow enough for wading (about 4½ ft.). The small seine was used at Milnerton estuary, as the large seine dug into the soft muddy bottom. This net has considerable netting error. Small fish of about 30 mm. and below are able to escape through the meshes, and larger fish of about 120 mm. approx. have often been seen to swim out of the net due to the slow pulling.

(iii) *The Drag Net.* This is a small conical net pulled by two people. Ropes 10 ft. long are attached to each end of the mouth which is 6 ft. 9 in. wide. The depth of the net is 15 in., its length 10 ft., and mesh ½ in. bar. The net has retaining flaps half-way down its length, helping to retain fish already caught. The head rope of the net is corked, with the foot rope attached to a length of chain. The net is basically a trawl (originally intended to be kept open with a beam) that has been put to a different use. It was found to be ideal for dragging through weed beds of *Zostera capensis* and *Ruppia maritima* as the chain prevented the foot of the net rolling up. Because this net has a narrow mouth, and because the operators dragging it pass near the region to be netted, the drag net can be considered to give a representative sample of fish from 30 to 60 mm. only. It is of little use for length-frequency data but was useful for showing the presence or absence of smaller fish than those caught with the large seine in areas where the small seine was useless because of weed beds.

Due to the selectivity of all the above nets, all length-frequency distribution below 80 mm. is inaccurate (fig. 2).

Preservation of the fish was done immediately after netting. An incision was made in the abdominal wall and the fish put into 20 per cent formaldehyde. This stopped digestion immediately, and gut and gonads were preserved in good condition.

Gut contents of the whole alimentary canal were washed out and the different types of food organisms and the numbers of each type present were noted.

Maturity. Gonads were weighed. In males the presence or absence of milt was noted, and in females the colour of the ovary, the transparency of the ova, and maximum ovum diameter were recorded.

Age determination was made by counting otolith growth rings. The concave dorso-lateral surfaces of the otoliths were flattened by grinding down on a ground-glass screen using powdered glass as an abrasive. Growth rings showed clearly when the otoliths were examined by reflected light.

Sample errors. The samples from which the length-frequency curves were built are considered adequate, as successive monthly samples show very similar curves.

The monthly food comparisons were made on samples of thirty fish. Some idea of the error due to sampling alone was afforded by the occurrence of an Acanthocephalan parasite in successive monthly samples of fish. The following are the percentages of fish that contained these parasites in monthly samples:

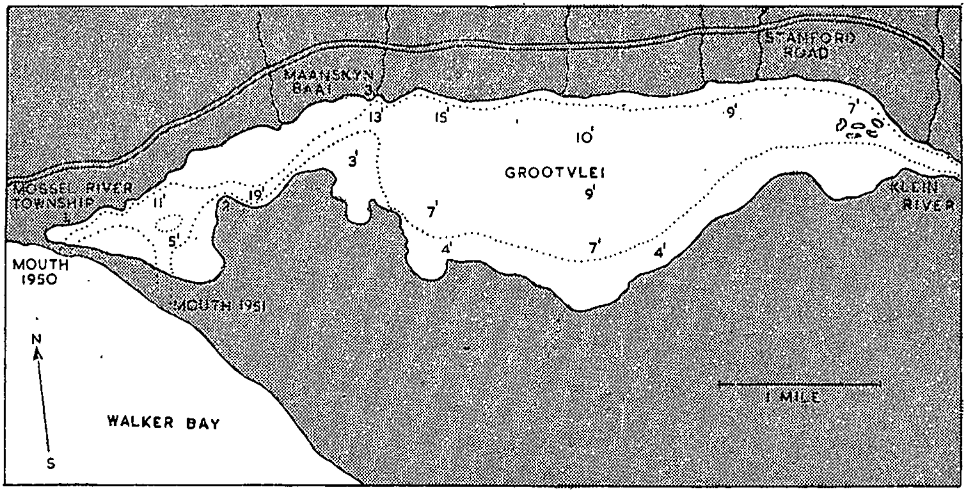

FIG. 1. The Klein River Estuary. Dotted line indicates extent of lagoon at lowest water level.

1950		1951				
November	*December*	*January*	*February*	*March*	*April*	*May*
20%	16%	45%	16%	40%	34%	14%

The parasite was firmly attached to the inside of the rectum, usually about one to four being present in infected fish. The adult parasite is non-motile. It can be assumed that the percentage of infected fish in the lagoon, at least from February to May 1951 when the lagoon was closed to the sea, was fairly constant. Infected fish were in excellent condition, so the number of parasitized fish was not being reduced through deaths. An increase or decrease in percentage infection would be expected to be fairly gradual, yet the percentages given above are extremely erratic. If the assumption is correct that the total percentage of parasitized fish in the lagoon is roughly constant, these fluctuations must be due to numerical sampling error. This can be utilized in evaluating the monthly occurrence of food items. If a food item is present in samples over a period of months in 20 per cent of the total population, percentages in monthly samples of thirty fish would be expected to show fluctuations similar to those of the parasites given above. These variations would reflect no change in the feeding habits of the population. Only very marked changes in the monthly percentages of food items can therefore be considered as indicative of food changes in the fish population.

Identification and terminology of the fishes is based on Smith (1949).

Lengths of fishes are standard lengths (i.e. excluding the caudal fin).

The Klein River Estuary

Description

As a detailed description of this estuary and its bottom fauna has been made by Scott, Harrison, and Macnae (1952), only a short account will be given here.

The Klein River and a number of smaller streams flow into a wide lagoon, separated from the sea along its eastern edge by a range of low sand-dunes. At the southern edge of the lagoon these dunes flatten out to form a low, sandy strip where the mouth of the estuary is situated.

The lagoon is six miles long and at most half a mile wide, with an average depth of 8 ft. in summer and a few feet deeper in winter when the level is raised by flood water. Depths of up to 19 feet have been recorded in the channel (fig. 1).

In the summer little water enters the lagoon, a sand-bank is built up at the mouth by wave action and the lagoon becomes land-locked. Evaporation causes a slight drop in level during the summer months and then the winter rains swell the lagoon until the increase in area of the lake interferes with farming at its margin. When this happens local farmers dig a channel through the sand-bank. By the time the dammed-up water has poured out, a wide mouth is formed and the lagoon becomes tidal for most of its length. The mouth then closes gradually, usually taking about five months.

There are thus fairly regular periods every year during which the mouth is alternately open and closed, which has profound effects on the lagoon fauna. During 1950 and 1951 when this study was in progress the state of the mouth was as follows:

1950	Mouth closed	.. February-September	Open	.. Oct.-Jan. (1951)
1951	Mouth closed	.. February-June	Open	.. July onwards

This more or less regular opening and closing of an estuary is a common occurrence along both the Cape and Natal coasts.

Both the flora and the fauna of the lagoon are rich. Beds of *Zostera capensis*, *Ruppia maritima*, and filamentous algae shelter and feed abundant life. Animals such as the small gasteropod, *Assiminea* sp., the bivalve, *Modiola capensis*, the amphipods, *Corophium triaenonyx* and *Melita zeylanica* and Chironomid insect larvae abound in the weed beds. Mudprawn *Callianassa kraussi* is found in muddy sand in the shallows near the mouth and along the margins of the lagoon; in the firmer sand of the mouth region the razor shell *Solen capensis* is abundant.

The lagoon has therefore the main elements for the support of a very rich fish fauna, providing cover from predators, and plant and animal foods in plenty.

Two stations were chosen for regular nettings of the lagoon: the region of the mouth (between 1 and 2) and Maanskynbaai (3) (fig. 1). The first lay in the channel; the second at the edge of the open lagoon.

Fish Fauna of the Lagoon

Table II is a list of fish species netted in the estuary during 1950 and 1951. Division into three groups gives an idea of relative abundance: A—abundant, C—Common, R—rare. At least twenty of the twenty-five species found were marine fishes tolerant of estuarine conditions.

Of the other five species *Gilchristella aestuarius*, *Atherina breviceps* and *Psammogobius knysnaensis* are chiefly estuarine. In the western Cape the remaining two, *Rhabdosargus tricuspidens* and *Syngnathus acus*, are also known mainly from estuaries. An unexpected omission from the list is *Mugil cephalus*, a mullet common in estuaries both east and west of the Klein River. It is possible that the fish was present in the lagoon and not netted, for it is known for its habit of swimming upwards when disturbed, at times leaping clear of the water over the top of a floating net. The large seine, sweeping the bottom of the lagoon, would probably have allowed them to escape.

Table II

Species according to families	*Standard length range*	*A.*	*C.*	*R.*
RHINOBATIDAE				
Rhinobatos annulatus (M. &. H.)	740 mm.			x
STOLEPHORIDAE				
Gilchristella aestuarius (G. & T.)	38–50 mm.		x	
TACHYSURIDAE				
Tachysurus feliceps (Valenciennes)	60–250 mm.		x	
SOLEIDAE				
Heteromycteris capensis Kaup.	20–70 mm.		x	
Solea bleekeri Boulenger	90–110 mm.		x	
SYNGNATHIDAE				
Syngnathus acus Linn.	61–155 mm.		x	
CARANGIDAE				
Hypacanthus amia (Linn.)	225–530 mm.		x	
POMATOMIDAE				
Pomatomus saltator (Linn.)	205–300 mm.		x	
SCIAENIDAE				
Atractoscion aequidens (Cuv.)	200 mm.			x
POMADASYIDAE				
Pomadasys olivaceum Day	95–156 mm.			x
SPARIDAE				
Rhabdosargus globiceps (Cuv.)	27–158 mm.	x		
Rhabdosargus tricuspidens Smith	112–260 mm.		x	
Diplodus sargus Linn.	70–105 mm.			x
Diplodus trifasciatus (Rafinesque)	70–100 mm.			x
Lithognathus lithognathus (Cuvier)	90–330 mm.	x		
Lithognathus mormyrus (Linn.)	75–170 mm.			x
Sarpa salpa (Linnaeus)	105–120 mm.		x	
Spondyliosoma emarginatum (Cuv.)	85 mm.			x

TABLE II (*contd.*)

Species according to families	*Standard length range*	*A.*	*C.*	*R.*
MUGILIDAE				
Liza ramada (Risso)	50–260 mm.	x		
ATHERINIDAE				
Atherina breviceps Cuv.	15–60 mm.		x	
GOBIIDAE				
Psammogobius knysnaensis Smith	27–50 mm.		x	
Gobius nudiceps Cuv.	50–115 mm.		x	
CLINIDAE				
Clinus superciliosus (Linn.)	62–110 mm.		x	
TRIGLIDAE				
Trigla capensis Cuv.	140–200 mm.			x
LAGOCEPHALIDAE				
Amblyrhynchos honkenii (Bloch)	120–145 mm.		x	

Fish netted in the Klein River estuary during 1950 and 1951

(i) *Migration*

Many coastal fishes are known to migrate and it seems probable on *a priori* grounds that migration to and from Cape estuaries should take place, as there are considerable changes in temperature, salinity and turbidity between summer and winter in most estuaries.

At the Klein River estuary, however, whatever migrations might take place are prevented in most years by the closure of the mouth during the whole of the winter, and fish that have entered during the summer months are forced to remain in an environment of low salinity, low temperature, and high turbidity.

In 1951 the mouth of the estuary was cut earlier than usual, and was open during the latter half of the winter. Due to exceptionally heavy rains the lagoon was very muddy, and cold water poured out through the mouth. Scott, Harrison, and Macnae (op. cit.) state that under conditions of this type, temperatures in the estuary may go down to 12° C. and salinities down to 20 parts per thousand. During this period white stumpnose, *R. globiceps*, elf, *Pomatomus saltator*, and leerfish, *Hypacanthus amia*, were absent from nettings.

It seems therefore that fish trapped in the lagoon are able to withstand the average winter conditions, but that a number of species would migrate away from the lagoon if this were possible.

(ii) *General Note on Feeding*

Few fishes feed directly on the vegetable matter in the lagoon. Perhaps the only complete herbivore is the mullet, *Liza ramada*, whose diet consists chiefly of diatoms and unicellular algae, the cell walls of which are crushed by its exceptionally muscular stomach filled with sand grains.

Rhabdosargus globiceps, *R. tricuspidens*, *Sarpa salpa*, *Diplodus sargus* and *D. trifasciatus* were found to be omnivorous, cropping plants such as *Zostera capensis*, *Ruppia maritima*, and filamentous algae (mainly *Enteromorpha* sp.), and also feeding on the small crustaceans, polychaetes, and molluscs of the weed beds and bottom.

Lithognathus lithognathus, the white steenbras, although taking vegetable matter occasionally, is mainly carnivorous, feeding on the animals sheltering in the weeds, and using its elongated snout to suck or blow *Callianassa kraussi* and *Solen capensis* out of the sand.

The small sole, *Heteromycteris capensis*, and the small goby, *Psammogobius knysnaensis*, are common on the sandy shallows near the mouth of the lagoon. *P. knysnaensis* had fed on ostracods, amphipods and sphaeromid isopods, and the few *Heteromycteris capensis* examined had eaten ostracods and foraminifera. *Solea bleekeri*, the larger sole present in the lagoon and not restricted to the mouth region, had a more varied diet including isopods, amphipods, *Assiminea* sp., and occasional small crabs and small fish.

The whitebaits, *Atherina breviceps* and *Gilchristella aestuarius*, swim in shoals, feeding on planktonic copepods and amphipods.

Two large fish predators were present in the lagoon, the leerfish or garrick, *Hypacanthus amia*, and the elf or shad, *Pomatomus saltator*. These two species were only occasionally netted, but being exceptionally speedy fish, and probably swimming near the surface, they may be present in greater numbers than shown by the large seine nettings.

Three species of cormorant were present in the lagoon, all fish predators. These were *Phalacrocorax neglectus* (the white-breasted cormorant), *P. capensis* (the Cape cormorant or trek-duiker), and *P. africanus* (the reed cormorant). *Anhinga rufa* (the darter), another fish-eater, was also occasionally seen. Predation of the fish population due to these birds cannot be heavy, for an average daily count of all species was about twelve to fifteen birds.

Biology

Age Determination and Rate of Growth

Many workers have found that bands or rings are present on the calcareous otoliths, and also on the scales and other bony parts of many fish, which may be annual in nature and are formed by varying rates of growth causing material of different transparencies to be laid down. The scales, otoliths, and supra-occipital crests of *Rhabdosargus globiceps* were examined to see whether any such markings could be found.

Although banding was occasionally present on scales and supra-occipital crests, otoliths gave much more consistent results, and eventually only this method of age determination was used.

Otoliths of all fish had opaque centres. Clear banding was normal in estuarine *R. globiceps*, which were one or two years old. Fish from the sea samples were older, and rings toward the periphery of the otoliths were crowded together, and sometimes unreadable.

In previous work done on age-reading using otoliths, some investigators have simply assumed the annual nature of the rings (Roux, 1947, on the Cape Stockfish). In all cases where the validity of this has been tested (Menon, 1950, on the Poor

Cod; Hickling, 1931, on the Hake; Jones and Hynes, 1950, on the Stickleback, among others) it has been found to hold. The commonest method of testing is to find out from periodic samples the seasons during which each type of band is laid down at the periphery.

Hickling (1931) found that in the hake the light and dark bands were due to alternating layers of thick and thin organic lamellae embedded between radially arranged inorganic crystalline material. The cause of the change in the thickness of these lamellae is not known. Most workers have found that the transparent band is formed in winter, and the opaque in summer. This is not an invariable rule and may alter with the species of fish studied.

(i) *Time of Formation of Opaque and Transparent Bands in the Otoliths of R. globiceps*

In small specimens of *R. globiceps* it is easy to see whether an opaque or transparent band of otolith material is being laid down at the periphery. In larger fishes the bands become very narrow at the periphery and it is more difficult to see which type of band is forming. The thinning of the otolith at its edge interferes by making an opaque zone look lighter. Thus the larger size groups taken by line fishing gave no clue to the time of year the bands were produced.

Sea fishes entering the Klein River estuary in October 1951 had thin opaque bands at the periphery, suggesting that opaque material would continue to be deposited during the summer. Fish, caught at the same time, that had been in the estuary during the winter showed wide transparent bands, with no trace of opaque material. Estuarine conditions must therefore be able to effect a change in the normal banding. The new estuarine population, as soon as the mouth closed in January, also started forming transparent material.

It is probable therefore that in marine *R. globiceps* an opaque band is formed in summer and a transparent band in winter. *R. globiceps* trapped in the lagoon, however, lay down transparent material during the greater part, or possibly the whole, of the year.

In the following discussion it is assumed that the ringing is annual, and that the transparent bands are always counted. In spite of the interference of the normal banding by estuarine conditions, fish trapped and laying down transparent material from February to October would still have the total number of bands unaltered.

(ii) *Age Groups*

If the modes in length-frequency distribution (fig. 2) represent year groups, it would be expected that the fish in one mode would have the same number of otolith bands. This was found to be so. Fish of mode A taken in May, August, and September all had two transparent otolith rings. Fish of the smaller mode B entering the lagoon in October had one transparent otolith band.

TABLE III

Length in mm.	1 *ring*	2 *rings*	3 *rings*	4 *rings*
60	x			
70				
80	x			
90	x	x		
100	x	x		
110	x	x		
120		x		
130		x		
140		x		
150		x		
160				
170				
180		X		
190		X	X	
200		X	X	
210			X	X
220			X	
230				
240			X	
250			X	X
260				X
270				
280				X
290			X	X
300			X	
310				
320				X
Number of fish examined	17	21	10	7

The number of transparent otolith rings in *R. globiceps* of different sizes from the Klein River estuary (x) and from Kalk Bay line boats (X).

A second transparent band started to form on the otoliths of these fishes in January. Mode C fishes had one transparent band. Otoliths of mode X fish were not examined.

Mode B in October 1950 and mode C in March 1951 consist of one-year fish. Mode A in May, August, and September 1950 and mode B in April 1951 are composed of two-year fish. Mode x is considered as a small element in the lagoon fauna of fish under one year.

(iii) *Rate of Growth*

This can be assessed in a number of different ways from the data available. Modes A and B are different year groups of *R. globiceps*, mode A consisting of two-year fish and mode B in October 1950 consisting of one-year fish. The difference between these two modes is about 60 mm., representing one year's growth.

From the movement of a single mode in successive months increase in average size of fish is between 5 and 10 mm. per month, i.e. between 60 and 120 mm. per year.

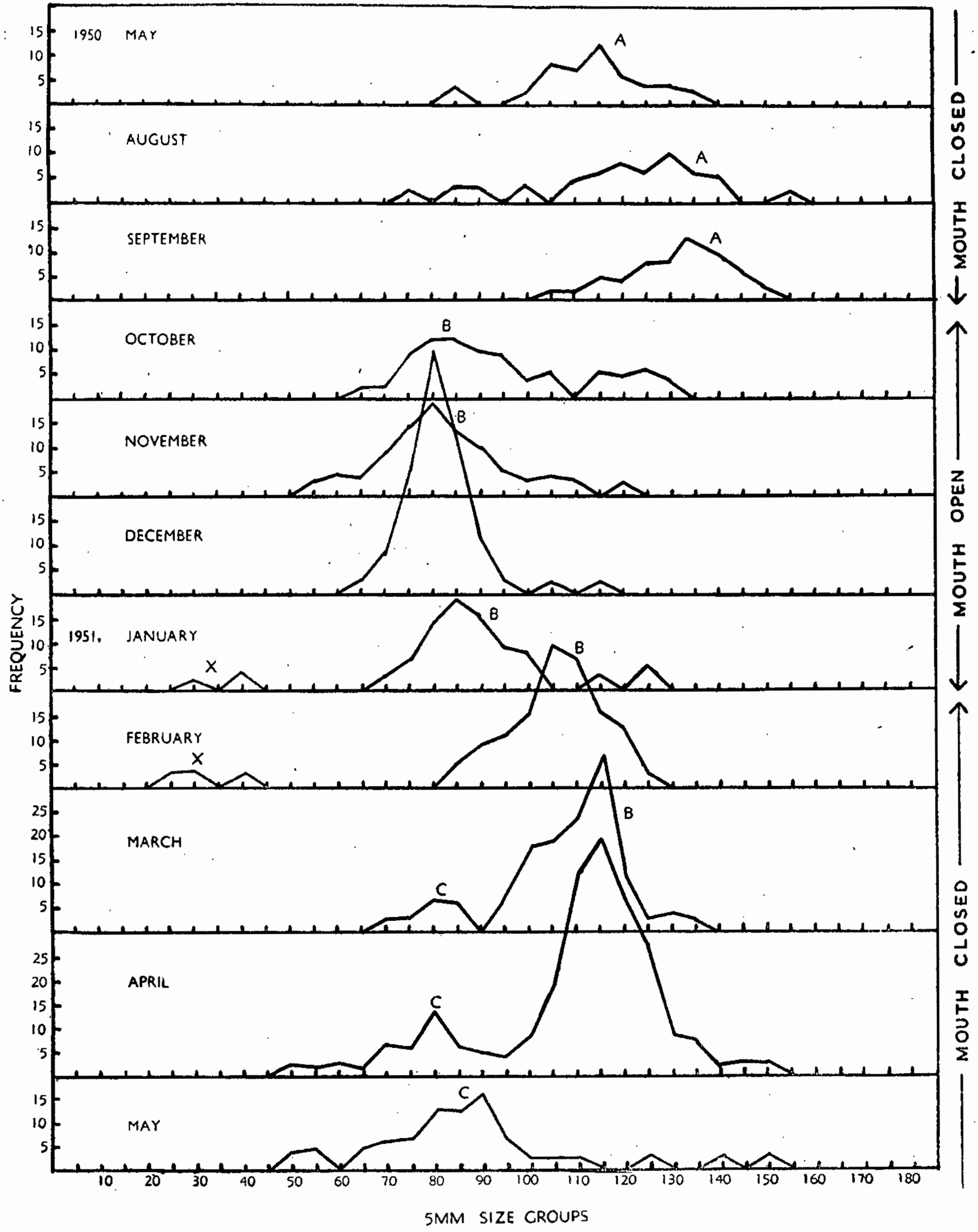

FIG. 2. Length-frequency distribution of *Rhabdosargus globiceps* in the Klein River estuary.

Table III also gives an estimate of growth. From the table it can be seen that sea fish have reached a length of about 260 mm. in their fourth year, averaging a growth of about 60 mm. per year.

The three calculations are therefore in fair agreement. The only growth rate that has been calculated for Cape fish to my knowledge is that of the stockfish (*Merluccius capensis*), a member of the Gadidae. Roux (1947) found it to be from 90 to 110 mm. per year.

The Population in the Klein River Estuary

As has been shown, juvenile *R. globiceps* are among the commonest fishes in the Klein River estuary. The population of *R. globiceps* during the time of study consisted of immature fish up to 15 cm. in length. If normal growth is taking place larger fish than this must be migrating out of the lagoon, with smaller fish entering and continually replacing the stock.

From the beginning of sampling in May 1950 until September of the same year the population consisted mainly of a single year group of two-year-old fishes (mode A, fig. 2). On the opening of the lagoon in October this mode immediately began to diminish in size, the fish migrating out to sea, until by December this group was hardly represented.

Simultaneously a new mode appeared in the length-frequency data (mode B, fig. 2), consisting of one-year-old fish which replaced the older population during these three months.

The younger fish are therefore attracted to the rich estuary, but at a slightly larger size they migrate out to sea. This change in choice of environment is probably related to the physiological changes in the fish connected with development to maturity. Spawning takes place at about 240 mm., but marked increase in gonad weight is already seen at 200 mm.

The drag nettings occasionally caught much smaller fish of about 30 mm., well below the netting range of the large seines (mode X, fig. 2). Mode C, first seen in the large seine nettings of March 1951, is considered to be formed of the larger members of this O-year group, just beginning to fall within the large seine nettings. The fact that mode C does not show growth movement to the right in successive months shows that the majority of the fish are still below the mesh size.

In May 1951 mode B was not represented. At this time the lagoon was filled with fresh water from heavy rains. Turbidity in the lagoon was high. Mode B fish were out of range of the nettings, so must have been in the deeper, more inaccessible parts of the lagoon. It is suggested that the inflowing fresh water overlay the denser more saline water, which would be least diluted in the deepest portions of the lagoon. That the larger fish of mode B and not the smaller fish of mode C should seek these deep areas is consistent with the difference in choice between larger and smaller fish on the opening of the lagoon in October 1950.

This preference of two-year fish for the open sea, ensuring that an estuarine population consists always of immature fish, seems to be a general rule for *R. globiceps*, and also for a number of estuarine fishes. This has also been found to hold by Hartley (1940) in England and by Irvine (1947) on the Gold Coast.

Feeding of R. Globiceps

(i) *Estuarine Fish*

Table IV lists all the food organisms found in *Rhabdosargus globiceps* netted in the Klein River estuary. A wide variety of both hard and soft foods was found. As well as small animals, vegetable matter in the form of algae, blades of *Zostera capensis* and *Ruppia maritima* and seeds are eaten.

Table IV

PROTOZOA
Foraminifera.

POLYCHAETA
Spionid. ?*Prionospia* larva.
Lumbriconereis sp.
Glycera sp.
Pectinaria sp.

MOLLUSCA
Haminea alfredensis Bartsch.
Modiola capensis Krauss.
Solen capensis Fischer.
Assiminea sp.

INSECTA
Chironomid larvae.
Syrphid larvae.
Syrphid pupae.

CRUSTACEA
Ostracods.
Copepods.
Melita zeylanica Stebb.
Parorchestia rectipalma Brnd.
Corophium triaenonyx Stebb.
Exosphaeroma hylecoetes Brnd.
Tanais philetaerus Stebb.
Callianassa kraussi Stebb.
Hymenosoma orbiculare Desm.

PISCES
Psammogobius knysnaensis Smith.
Fish scales.

ALGAE
Enteromorpha sp.
Ulva sp.

ANGIOSPERMAE
Grass seeds.
Potamogetonaceae seeds.
Ruppia maritima Linnaeus.
Zostera capensis.

Food organisms found in *R. globiceps* from the Klein River estuary.

The commonest food item occurring in estuarine *R. globiceps* is filamentous algae (*Enteromorpha* sp.) (fig. 3). It is possible, however, that fish do not derive as much food value from vegetable as from animal matter, and it has also been suggested that they may often eat algae for the animals that shelter in them. The latter is not the case with *R. globiceps*, for many fish of a sample may have the stomach and intestine packed with algae, although no animal remains are present. In some months, noticeably October and November 1950, filamentous algae formed by far the bulk of the food.

The remainder of the common food organisms are animals that shelter in the weed beds, such as the amphipods *Melita zeylanica* and *Corophium triaenonyx*, the isopod *Exosphaeroma hylecoetes* and the small gastropod *Assiminea* sp., and bottom animals, such as the bivalve *Modiola capensis*. Cropped pieces of *Ruppia* were often found, sometimes packing the alimentary tract. Grass seeds and a potamogetonaceous seed were occasionally present, the latter sometimes having begun to germinate. No whole specimens of *Solen capensis* were ever found, although this bivalve is abundant on the estuary bottom in some areas. Unlike *Lithognathus lithognathus*, *R. globiceps* seems unable to dig the whole animal out of the sand. However, the siphons of *Solen capensis* were often present, suggesting that the fish had apparently bitten off the siphons without destroying the animal in the sand below.

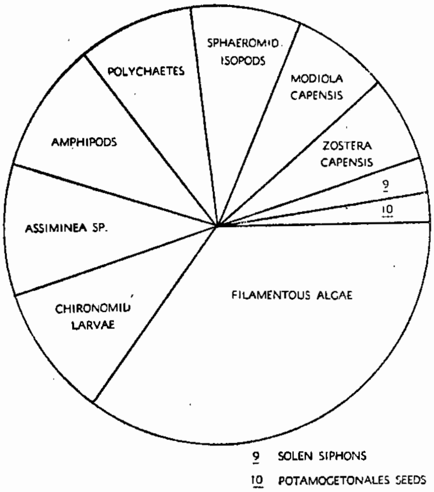

FIG. 3. Relative proportions of the ten main food organisms of *R. globiceps* in the Klein River estuary.

Steven (1930) and other authors have found that most species of fish will take the commonest foods available to them in their particular habitats. This general rule also holds for *R. globiceps*, because its diet is composed of organisms which Scott, Harrison, and Macnae (1952) found to be commonest in the estuary.

Although the rule applies to the fish sample as a whole, there is evidence of choice of particular types of food by individuals. In a sample of fish one individual may be found to have fed only on seeds, another only on chironomid larvae, another only on *Assiminea* sp., and a fourth may have only algae in its gut. This occurs where a whole sample has been netted over one weed bed, and therefore cannot be due to fish feeding on different organisms in different areas. Lebour (1919) carried out experiments on the feeding habits of small fish and found that they would often eliminate one species of plankton food before feeding on another. Dannevig (from Lebour, 1918) found that young plaice will only eat one food at a time, but that different individuals will be selecting different species of food at the same time. This is what occurs in *R. globiceps*. It seems possible that after snapping up the first likely food it finds, a hungry fish may ignore other types of food present, and continue to take this first type because it is satisfying the hunger. This has not, however, been checked by aquarium experiments.

Some change in choice of food organisms takes place with increasing size of fish. This is shown in Table V.

It is known that the larval fish feed on plankton (Gilchrist, 1904), and later become bottom feeders. The smallest fishes found in the Klein River estuary are just at this transition period. At a length of just over 20 mm. they have a diet consisting chiefly of planktonic copepods and amphipods, and also take sphaeromids, ostracods, and algae.

As the fish increase in size other animals become available to them as food, and the larger fishes have a more varied diet. Increase of mouth size, strength of jaw, size of teeth, and speed of movement must effect this. Large polychaetes such as *Lumbriconereis* sp. and chironomid larvae are eaten by fish of 40 mm. and larger. The first hard foods (the gastropod *Assiminea* sp. and the bivalve *Modiola capensis*) are taken by fish over 70 mm. Above this size larger animals such as *Haminea alfredensis* and *Hymenosoma orbiculare* are included in the diet, and the first pieces of *Solen* siphon are taken. Hard molluscs become increasingly important as foods of the larger fish over 100 mm., and *Callianassa kraussi* is also eaten.

TABLE V

	20–39	40–69	70–99	100–129	130–159 mm.
	%	%	%	%	%
Copepods	83		2	1	
Amphipods	83	38	23	25	34
Ostracods	17	8	3	3	5
Fil. Algae	17	46	70	74	67
Sphaeromids	17	23	15	16	20
Chironomids		38	22	16	26
Lumbriconereis		31	8	4	2
Zostera, Ruppia		8	13	16	18
Glycera sp.			1	1	
Haminea			1	4	
Solen siphons			6	6	
Modiola			5	16	48
Assiminea			7	29	56
Grass seeds			1	5	13
Potamogetonales seeds			1	7	10
Hymenosoma			1	6	5
Callianassa				1	2
NO. OF FISH	6	13	140	140	61

Change of food organisms with size of *R. globiceps* from the Klein River estuary. The number of times an organism is found in each size group is represented as a percentage of the total number of fish in that group. Fish in 20 mm. groups.

Three fairly distinct stages are seen. The very small fish (20-40 mm.) feed mainly on planktonic organisms, although a small proportion of bottom forms are are also taken. Medium-sized lagoon fish, up to 100 mm., feed on a wide range of small animals, but only a very small proportion of these have hard shells. Above 100 mm. hard molluscs become one of the most important food organisms in the diet. Throughout all sizes filamentous algae and amphipods are important foods.

Little change of food with season was found. When the mouth of the lagoon opened in October some difference was seen, but here smaller one-year fishes were replacing the two-year fish, and size may have affected the comparison. The young fish entering the lagoon feed mainly on filamentous algae, *Zostera* and *Ruppia*, *Modiola capensis* and *Corophium triaenonyx*, and the quantities of *Assiminea* sp., the isopod *Exosphaeroma hylecoetes*, polychaetes and chironomid larvae show a sudden reduction from the September sample. Scott, Harrison, and Macnae (1952) found almost no change in the bottom fauna when the mouth opened, and suggest that there is little difference in the abundance of the common animals. The change in food is then not due to a change in the food organisms present in the lagoon.

It is unlikely that the small size of the group entering the lagoon accounts for the change in diet, because most of the food organisms showing a reduction are animals which are normally taken by very small fish, as can be seen from Table V.

The average size of fish remains the same in the samples of the next three months, but the four food species which showed a reduction are again taken by the fish in increasing numbers during these three months.

It seems, therefore, that although a number of food species are available to the one-year fish, they select certain foods on entering the lagoon, and only gradually are other foods taken. This 'lag' in the use of available food may be due to the feeding habits before the fishes enter the lagoon. Accustomed to feeding on other types of food outside the estuary, the fish would have to learn new feeding habits before some of the new food species could be taken. Lebour (1918, 1919a, 1919b, 1920), Scott (1922 from Hartley, 1940), and others have shown that some fish do learn definite feeding habits, and it is reasonable to suppose that it must take some time to change these.

Feeding of *R. globiceps* in the Milnerton and Breede River estuaries showed patterns similar to those in the Klein River estuary.

(ii) *Sea Fish*

Fifty-eight specimens of *R. globiceps* were obtained from the sea. Unlike estuarine fish, where the percentage of fish with no food in the alimentary canal was negligible, nineteen out of fifty-eight fish had both stomach and intestine empty, or contained only bait. The only record of the feeding of marine *R. globiceps* is that of Biden (1930), who mentions that black mussels (*Mytilus perna*, *Mytilus meridionalis*) and polychaete worms are important foods.

Table VI lists the food organisms found in *R. globiceps* from sea samples. Amphipods were by far the commonest food, being found in abundance in Kalk Bay and west coast fish. Bivalves were also important. Six fish caught by Mr. Len Reed when angling at Miller's Point were packed with *Mytilus perna*, and many fish caught by other anglers at the same time also contained mussels.

TABLE VI

Place and date	*Size*	*Foods*	*No. of fish*
Kalk Bay Line Boats Summer and autumn 1951	128–300 mm.	Amphipods, Barnacle shells Bivalve remains Sand Ostracods Crab remains *Chorisochismus dentex*	41
Cape Infanta Trawled 36 fathoms. July 1950	260 mm.	Crab remains Bivalves Polychaete worms	3
Cape Infanta Trawled 35 fathoms. July 1951	128–203 mm.	Crushed echinoid Amphipods Crab leg	2

TABLE VI (*contd.*)

Place and date	*Size*	*Foods*	*No. of fish*
Miller's Point, Cape Peninsula Angling by Mr. Reed. May 1951		*Mytilus perna*	6
Buffels River mouth, West Coast Angling by Mr. Guy Currie, July 1951	300–350 mm.	Amphipods, including *Hyale saldanha* Chilton *Paridotea* sp. *Ascidians* *Ulva* sp. Sponge	6
			58

Food organisms found in *R. globiceps* from the shore to 36 fathoms.

Small white bivalve fragments (sp. indet.) were also found in Kalk Bay fish, and a small brown bivalve (sp. indet.) was present in a sample of three fish from Cape Infanta. Crab remains were found in a few stomachs, and the other organisms listed in Table VI were present in one or two fish.

No change of food with size or with season could be ascertained from the data.

In conclusion it can be stated that: (*a*) amphipods and bivalves are important foods; (*b*) ostracods, isopods, crabs, echinoids, barnacles and fish are also taken; and (*c*) vegetable matter is seldom eaten. The fish are solely bottom feeders, as all the organisms found, including the amphipods and fish, were bottom forms. The powerful incisors and molars enable adult fish to remove and crush barnacles and bivalves from rock surfaces.

SPAWNING AND SEXUAL MATURITY

R. globiceps lays eggs which float and on hatching give rise to pelagic larvae (Gilchrist, 1904). Spawning probably takes place close inshore, for fertilized eggs have been pumped into the old St. James aquarium through the water inlet (Biden, 1930). Little is known about the spawning season but Gilchrist states that an abundance of ripe males and females can be obtained from trawlers in November and December, and he performed successful fertilization experiments with the eggs and sperm of these fish. Biden mentions that very enlarged yellow ovaries can be obtained throughout the year from fish caught by the Kalk Bay line boats.

In December 1950 it was decided not to restrict the records of *R. globiceps* to estuarine fish, and from this date until August 1951 the gonads of fish bought from Kalk Bay line boats were examined. Although gonads that were enlarged and yellow were found in December, January and February, the only fish that had fully ripe eggs (large, round, perfectly transparent, and with a central oil globule) were taken in August. From the few facts that are available, it would seem that there is an extended breeding season from August to about February. It is possible that the fish may breed throughout the year, but Biden's observations are not definite enough to establish this.

TABLE VII

Size groups	*Average egg diameters*
60–79	0·04
80–99	0·04
100–119	0·06
120–139	0·08
140–159	0·09
160–179	—
180–199	0·08
200–219	0·07
220–239	0·60
240–259	0·68
260–279	0·60
280–299	0·10
300–319	—
320–339	0·50

Average diameter of ova of *R. globiceps* from the Klein River estuary and Kalk Bay, for different size groups. All measurements in millimetres.

The eggs seem to go through three stages during their formation. They are at first transparent, closely packed, and of different shapes dependent on how they have been pressed by their neighbours. Later they become granular, yellow, and more rounded. Finally the egg ready for shedding has again become transparent and is rounded and soft, with one distinct oil globule in the centre.

Gilchrist measured the eggs of one female, and found the variation of fifty eggs measured to be from 0·85 mm. to 0·89 mm. Ovaries from two females taken in August 1950 contained eggs that corresponded to Gilchrist's descriptions, but the average diameter was 1·05 mm.

Table VII gives the egg diameters of different size groups of *R. globiceps* taken from marine and estuarine fish.

Females under 60 mm. had ovaries that were fine threads, and the egg diameter averaged about 0·04 mm. At about 220 mm. the egg diameter suddenly increases about ninefold.

Gonad weights showed a sudden increase in weight at almost the same fish length. Fish of up to 180 mm. had a maximum gonad weight of 1·7 gms. At 200 mm. ovary weights were up to 8·0 gms., or about five times the weight. At 240 mm. some fish had tremendously enlarged ovaries, up to 41 gms. Females can therefore be considered to mature at 220-40 mm. in length.

The condition of maturity of the male is more difficult to determine, but the first males to be described as 'faintly milky' were 275 mm.

The ratio of males to females is difficult to determine among juvenile fish in the estuaries, for there is little visible difference between females with extremely undeveloped ovaries and immature males. However there are definitely more females. The ratio among the sea fish from Kalk Bay samples was 2:1 females to males. Biden (op. cit.) mentions that ratios of 10:1 and 20:1 females to males are common in large shoals of *R. globiceps*.

In conclusion it can be said that *R. globiceps* mature at the beginning of their third year, at a length of just over 200 mm., with males maturing at a slightly larger size than females.

PARASITISM IN R. GLOBICEPS

A trematode, some small nematode parasites, and an acanthocephalan were found in the alimentary tracts of estuarine *R. globiceps*.

The trematode (unidentified) was a small parasite about 5 mm. long present in the intestine of *R. globiceps* that entered the Klein River estuary and Milnerton estuary in the spring of 1950. Percentage infection was at first high in fish from both estuaries, but rapidly decreased over a period of three months, after which it was rarely found. No parasitized fish seemed in poor condition.

The acanthocephalan parasite, with a body about 5 mm. long and a proboscis of about 2 mm., was attached to the rectum just inside the anus. Usually two or three per fish were present, but as many as twelve were found. About 20 per cent of Klein River estuary fish were infected, and none found were in poor condition.

No parasites were found in sea fish.

CONCLUSION

It is well known that estuarine faunas are derived mainly from marine forms, but include a few purely estuarine species, and a few fresh-water forms in the upper reaches of the estuary (Day, 1951). This has been shown specifically for fish faunas by Hardenberg (1931), Hartley (1940), and Irvine (1947), and was found to be the case in this survey. Day has divided estuarine faunas into five components; (*a*) the fresh-water component, restricted to waters of low salinity; (*b*) the stenohaline marine component at the mouth of the estuary; (*c*) the euryhaline marine component, being marine forms able to tolerate the estuarine conditions; (*d*) the estuarine component, comprising the few species restricted to estuaries, and (*e*) the migratory component whose members spend only part of their lives in estuaries.

It is interesting to note that at least 80 per cent of the fish fauna of the Klein River estuary must be considered as falling under component (*e*), migratory animals not spending their whole lives in the estuary. The fish of this component must be further divided into two categories, those fishes present in the estuary only as juveniles, such as *Rhabdosargus globiceps*, *Lithognathus lithognathus* and *Pomatomus saltator*, and those fishes present both as juveniles and adults, but which leave the estuary for the purposes of spawning.

Clinus superciliosus, *Gobius nudiceps* and *Syngnathus acus* seem to represent the euryhaline marine component, being marine fishes extending into the estuary and able both to live and to breed there.

The truly estuarine component (*d*) is represented by *Psammogobius knysnaensis* and *Gilchristella aestuarius*, although the latter is also known from fresh water (Barnard, 1947).

No fresh-water component was found in this survey, but Scott, Harrison, and Macnae (personal communication) found a small silurid in the upper reaches of the estuary.

No stenohaline marine forms were found at the entrance of the estuary, or moving in and out with the tide, although nettings were made a hundred yards from the mouth.

It has been suggested that the Klein River estuary might be suitable for fish farming. It is quite possible that fertilization of the lagoon might produce more abundant growth of both animals and plants, and the growth rate of the fish fauna might be increased. Annual replenishment of fish stocks by young *R. globiceps* and possibly other species would obviate restocking by more laborious methods.

It has been stressed by MacGinitie and MacGinitie (1949) that the basic foodstuff supporting estuarine faunas is detritus; both that derived from the green matter in the estuary and that brought down by the river system. In the Klein River estuary, *R. globiceps* and mullet *Liza ramada* form a considerable proportion of the fish fauna, and both these fishes are supported in the main by the green matter of the estuary, before it has been broken down to detritus. The latter feeds almost exclusively on attached diatoms, and the former has filamentous algae as one of its main foods, and the two food organisms next in importance (*Assiminea* sp. and chironomid larvae), are eaters of green matter, not detritus. The bulk of the estuarine fauna is undoubtedly directly or indirectly supported by detritus or the bacteria living on detritus, but in a weedy estuary of this type much of the fish population is more directly dependent on living plant matter.

In spite of the prevalent opinion of South African anglers and commercial fishermen, few fish seem to breed in estuaries. Protection of estuarine fish stocks is possibly useful, not to protect breeding-grounds, but to protect nurseries of young marine fish. What proportion of young *R. globiceps* enter estuaries out of the whole young population of the species cannot be assessed from the present meagre knowledge of marine *R. globiceps*.

REFERENCES

Barnard, K. H. 1927. 'A Monograph of the Marine Fishes of South Africa.' *Ann. S. Afr. Mus.*, XXI (I) and (II), 1-419.

—— 1947. *A Pictorial Guide to South African Fishes.* Maskew Miller, Cape Town, xvii and 226.

Biden, C. L. 1930. *Sea-angling Fishes of the Cape.* Oxford University Press.

Dannevig. 1897, from Lebour 1918.

Day, J. H. 1951. 'The Ecology of South African Estuaries. Part I. A Review of Estuarine Conditions in General.' *Trans. Roy. Soc. S. Afr.*, xxxiii (I), 53-91.

Gilchrist, J. D. F. 1904. 'Development of South African Fishes. Part I.' *Marine Invest. S. Afr.*, No. 2.

—— 1905. 'Development of South African Fishes. Part II.' ibid., No. 3.

Hardenberg, J. D. F. 1931. 'The Fish fauna of the Rokan Mouth.' *Treubia*, xiii (I).

Hartley, P. H. T. 1940. 'The Saltash Tuck-net Fishery and the Ecology of some Estuarine Fishes.' *Journ. Mar. Biol. Assoc.*, xxiv (I), 1-68.

Hickling, C. F. 1931. 'The Structure of the Otolith of the Hake.' *Quart. Journ. Micr. Sci.* (N.S.), lxxiv (4), 547-61.

Irvine, F. R. 1947. *The Fishes and Fisheries of the Gold Coast.* University Press, Cambridge, xv and 352.

Jones, J. W., and Hynes, H. B. N. 1950. 'The Age and Growth of *Gasterosteus aculeatus*, *Pygostius pungitius* and *Spinachia vulgaris* as shown by their Otoliths.' *Journ. Anim. Ecol.*, xix (I), 59-73.

Lebour, M. V. 1918. 'The Food of Post-Larval Fish.' *Journ. Mar. Biol. Assoc.*, xi (4), 433-69.

—— 1919*a*. 'Feeding Habits of some Young Fish.' ibid., xii (I), 9-21.

—— 1919*b*. 'Food of Post-larval Fish. Part II.' ibid., xii (I), 22-47.

—— 1920. 'Food of Young Fish. Part III.' ibid., xii (2), 261-324.

MacGinitie, G. E., and MacGinitie, N. 1949. *Natural History of Marine Animals.* McGraw-Hill, New York, xii and 473.

Menon, M. D. 1950. 'Bionomics of the Poor Cod (*Gadus minutus*) of the Plymouth Area.' *Journ. Mar. Biol. Assoc.*, xxix (1), 185-239.

Roux, E. R. 1947. 'Growth Rate of the Cape Hake or Stockfish.' *S. Afr. Journ. Sci.*, i (2).

Scott, K. M. F., Harrison, A.D., and Macnae, W. 1952. 'The Ecology of South African Estuaries. Part II. The Klein River Estuary, Hermanus, Cape.' *Trans. Roy. Soc. S. Afr.*, xxxiii (3), 283-331.

Smith, J. L. B. 1949. *The Sea Fishes of Southern Africa.* Central News Agency, Cape Town, xvi and 550.

Steven, G. A. 1930. 'Bottom Fauna and the Food of Fishes.' *Journ. Mar. Biol. Assoc.*, xvi (3), 667-705.

Tinbergen, N. 1951. *The Study of Instinct.* Clarendon Press, Oxford, xii and 228.

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A PRELIMINARY NOTE ON THE BREEDING AND GROWTH OF TILAPIA IN MARINE FISH PONDS ON ZANZIBAR ISLAND

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The following notes describe an initial experiment in the stocking of marine fish ponds operated by the Organization with a species of wild *Tilapia* found in the estuarine reaches of the Mzinga Mzinga stream, at the north-western end of Zanzibar Island. This species had been collected twice previously from Zanzibar. It is closely allied to *Tilapia mossambica* (Peters) and to *Tilapia pangani* Lowe, being fairly similar to the latter but differing from it in the lateral line scale count. It is possibly a distinct species, and Dr. Trewavas of the British Museum is working on its systematic position.

The species is hardy, as is shown by its tolerance of widely varying environments. For instance, *Tilapia* fry were netted in the Mzinga Mzinga stream where the water was almost completely fresh, and where in places the oxygen content was very low—only about one quarter saturation. When transferred to the fish ponds, they successfully adapted themselves to water slightly saltier that the outside sea, and supersaturated with oxygen. The temperature difference between the two environments was about 12° C. (21° F.), and, whilst the fish pond water (like the open sea) is always alkaline, the stream water was slightly acid.

Two series of water samples were collected from the Mzinga Mzinga stream: the first series just before low water springs in February, 1956, and the second at about half-tide a month later a few hundred yards downstream from the earlier location. In each case the flow was seaward. Sampling at the pond was carried out in March, one day before the periodic "topping-up" that is effected twice a month at each high water spring tide. Salinity and temperature are here affected by rain, drainage from the surrounding land, evaporation and the prevailing meteorological conditions. At the time of sampling there had been no rain for two weeks and the sky was clear. As the atmospheric temperature in March is at its maximum for the year, the conditions of sampling probably represent the highest extremes of temperature and salinity to which *Tilapia* have so far been subjected.

The analyses of the stream and pond samples are given in the Appendix, Tables I, II, III and IV. Where a range of values is given (e.g. for oxygen in the Mzinga Mzinga stream) these are the limits of all samples, the bottles having been filled at random in various parts of the stream. So far no *Tilapia* have been caught at high tide; but this is more likely to be due to the physical difficulties of netting in deeper water than to the absence of the fishes.

THE EXPERIMENTAL POND

The fish ponds at Chukwani, some four miles from Zanzibar Town, are designed on the simple mud-walled system of the East, but with cement gateways, grooved to fit heavy wooden doors. Every spring tide the ponds are topped-up by placing a wire-netting sieve over the cement entrance on the flooding tide, and opening the doors until high tide.

The pond used for *Tilapia* is half an acre in extent, and normally 30 to 20 inches deep over most of the area at average filling, but with a deeper pool just inside the sill, and a deep ditch 18ft. by 60 ft., these providing cooler water and shade when the water level is low. The pool varies from 10 inches when all doors are removed to 40 inches in depth at average filling, and the ditch from 25 inches when all doors are removed to 55 inches at average filling. The water in the pond will be subject to considerable variation in properties, since, during the period of each two weeks prior to topping-up, its salinity, temperature and oxygen content will vary with meteorological conditions, especially over the course of the year as the seasons change. These changes can be expected to affect the flora and micro-fauna of the pond, which will themselves in turn affect such important factors as oxygen and nutrient salt content and pH. Furthermore, at each topping-up the pond suffers an influx of sea-water (itself varying in properties with the seasons) which has passed over a mangrove tidal flat. These conditions severely limit the number of species able to live and grow in the ponds. Of those species stirring is necessary to disperse it. After stirring, the emulsion descends and the fish can be seen to react to this descending "barrier", immediately diving to the bottom as they encounter it. It is the normal behaviour of *Tilapia* to dive to the bottom of the pond when disturbed, and in this way many of them may escape the poison. Further tests will be carried out in the laboratory when supplies of fish are avaialble from the pond, to establish the minimal concentration of rotenone necessary for lethal effect on various species of fish. It would seem that it may be possible to "clean" the pond of all species of fish other than *Tilapia* by judicious use of the rotenone poison.

The rotenone used was in the form of "Staff Allens" Rotenex resin, containing 25 per cent rotenone. This is dissolved in warm xylene and one volume of this solution is added to an equal volume of emulsifying agent. The latter consists of a 50:50 mixture of solutions of "Manoxol O.T." (Hardman & Holden Ltd., Manchester) and "Ethylan C.P." (Lankro Chemicals Ltd., Manchester). These materials were obtained through the courtesy of the Zanzibar Government Entomologist, Dr. F. L. Vanderplank.

For help with identifications our thanks are due to Dr. Ethelwyn Trewavas (*Tilapia*), Dr. Isabella Gordon (Crustacea), Miss D. Ballantine (Algæ), Mr. F. Williams (Carangidæ and Sphyrænidæ) and Mr. J. F. C. Morgans (Serranidae).

REFERENCES

Hartley, P. H. T. (1940). *J. Mar. biol. Ass. 24*, 1–68.

Hickling, C. F. (1951). *Proc. Rhod. sci. Ass. 43*, 1–6.

Irvine, F. R. (1947). The Fishes and Fisheries of the Gold Coast. Cambridge.

Schuster, W. H. (1952). Indo-Pacific Fish. Council, Special Publications 1.

Talbot, F. H. (1954). *Trans roy. Soc. S. Afr. 34*, 387–407.

APPENDIX

TABLE I

Water samples collected from the Mzinga Mzinga stream at 10.30 hrs. on 13th February, 1956 (low water springs 11.11 hrs.). A slow but steady flow seawards was observed. Sandy bed with no black mud or deposit visible. Greatest depth of water about 1½ ft. High banks densely vegetated, hindering wind access. Water cloudy:—

Temperature—26·2° C.
pH—6·7/6·8.
Oxygen { 1·6 to 2·65 mils/litre.
27% to 45% saturation at 26·2° C.
Alkalinity { Bound CO_2 1·12 millimols/litre.
Total CO_2 2·15 millimols/litre.
Total solids { Organic matter 0·237 grams/litre.
Mineral residue 0·867 grams/litre.
Silver nitrate titre 0·8 to 1·0 p.p. mille.
Calcium { Total—60 milligrams/litre.
Dissolved—40 milligrams/litre.
Phosphate—Negligible (less than 2 micro-gram atoms P/litre).
Ammonia—Negligible (less than ·04 p.p.m.).

TABLE II

Water samples collected from the Mzinga Mzinga stream at 09.00 hrs. on 16th March, 1956 (high water springs 06.10 hrs.). The site visited was a ford a few hundred yards downstream from that used in February. A strong current seawards was observed, and shoals of small mullet and a few large prawns were seen. Greatest depth of water, 3 ft.

Temperature—28·1° C.
Oxygen { 2·6 to 2·8 mils/litre.
49% to 53% saturation at 28·1° C. and chlorinity 8·5 p.p.mille.
pH—7·5/7·9.
Salinity—15·5 p.p.mille (Silver nitrate titre).

TABLE III

Water samples were collected from Chukwani main pond on 12th March, 1956, the bottles being filled from the sump adjacent to the gates one day before the periodic topping-up.

Temperature { Sump—37° C.
Periphery 38° C.
pH { Surface—8·4 (*in situ*; all corrections applied
Bottom—8·4 for salinity and temperature.)
Oxygen { Surface 4·7 mils/litre (117·5% saturation).
Bottom 3·3 mils/litre (82·5% saturation).
Salinity—35·10 p.p.mille (Silver nitrate titre).
Phosphate—0·25 micro-gram atoms P/litre.

TABLE IV

Figures for surface water in the open sea off Zanzibar at this time of the year are as follows:—

Temperature—29·35° C.
pH—8·49.
Oxygen—4·4 mils/litre (97% saturation).
Salinity—34·8 p.p.mille.
Phosphate—0·5 micro-gram atoms P/litre.

Methods of analysis will be described in a subsequent publication, together with results of a histological examination of *Tilapia* kidneys and further biometric data on this fish.