The attainment of maturity in male *Penaeus indicus*

H.F.B. Champion
Department of Zoology, University of Zululand, Private Bag X1001, Kwa Dlangezwa, 3886 Republic of South Africa

Received 15 January 1988; accepted 1 July 1988

Four criteria, namely, the formation of the petasma, the development of visible spermatophoric masses, the extrusability of the spermatophores and mating in confinement, were used to evaluate sexual maturity in male *Penaeus indicus.* It was concluded that the extrusability of the spermatophores provided a reliable indication of maturity. These results suggest the attainment of maturity at 22–30 mm carapace length with the majority being mature at 25 mm carapace length.

Vier maatstawwe, naamlik, die ontstaan van die petasma, die ontwikkeling van sigbare spermatofoor-massas, die uitdrukbaarheid van die spermatofore en paring in gevangeniskap, is gebruik om geslagsrypheid in die manlike *Penaeus indicus* te bepaal. Daar is tot die gevolgtrekking gekom dat die uitdrukbaarheid van die spermatofore 'n betroubare aanduiding is vir geslagsrypheid. Hierdie resultate dui op die bereiking van geslagsrypheid by 'n karapakslengte van 22–30 mm en dat die grootste meerderheid geslagsrypheid by 'n karapakslengte van 25 mm bereik.

Determining sexual maturity in male Penaeidae has long proved problematic because of the difficulty of identifying criteria which indicate the ability to mate (Cummings 1961; Hall 1962; Cheung 1963). Criteria which have been used include the presence of spermatozoa in the testes (King 1948) or vasa deferentia (Subrahmanyam 1965); unification of the petasmal endopodites (Kubo 1949; Young 1959; de Freitas 1980); easily extrusable spermatophores (Weymouth, Lindner & Anderson 1933), or a combination of the latter two features (Dall 1958) as well as spermatozoa in the terminal ampoule (Tuma 1967). None of these studies, however, provide convincing evidence of male mating ability, which is central to any definition of sexual maturity.

Investigations into maturation in *Penaeus indicus* males include those by Subrahmanyam (1965) and de Freitas (1980). Their conclusions on the sizes at which different stages of maturity are attained, are summarized in Table 1 (comparative values for the two size attributes used by these authors, namely, carapace length (CL) and total length (TL), are derived from the regression presented hereafter).

Table 1 The total length (TL) and carapace length (CL) values cited for the attainment of stages of maturity by *Penaeus indicus* males

Maturation stage	TL in mm	CL in mm	Author
Free spermatozoa in v.d.	120	23*	Sub.
Petasma fully formed	122–126*	23–24	deFr.
Spermatophore well developed	130	25*	Sub.
Spermatophore fully developed	140	27*	Sub.
Males mature	130–134*	25–26	deFr.

* denotes converted value to nearest mm;
deFr. = de Freitas (1980);
Sub. = Subrahmanyam (1965);
v.d. = vas deferens.

Subrahmanyam (1965) provides no quantitative evidence in support of his conclusions. He is also ambivalent on whether free spermatozoa in the vasa deferentia or the condition of the spermatophores signifies sexual maturity. De Freitas (1980) does not address this inconsistency, but cites part of Subrahmanyam's (1965) findings in support of a suggestion that maturity is attained once all males possess fully formed petasmata.

The literature thus reflects ambiguity concerning what constitutes a mature *P. indicus* male. In addition, it is not clear whether the sizes in Table 1 imply the commencement of a stage of maturity or achievement of this status by either the majority of the population or the total population.

The present study on the attainment of sexual maturity in the South African *P. indicus* male population attempts to resolve the above uncertainties. It includes assessments of the formation of the petasma, the development of the spermatophoric mass and mating in captivity.

Methods

Study area

The study area is shown in Figure 1. Estuarine samples were netted at monthly intervals from the St Lucia lake system over two annual cycles during 1966 to 1968. Offshore samples were similarly taken at monthly intervals over two annual cycles between 1966 and 1968 and intermittently, from 1972 to 1979. Offshore trawling took place 1,8 km off the mouth of the St Lucia estuary in 17 m of water and off the Amatikulu and Tugela river mouths to a depth of 50 m.

Size measurements

TL and CL have been used to denote specimen size by the authors already cited. Prawn mass was also used in part of the present study. Comparisons of results require that the relationships between these variables be

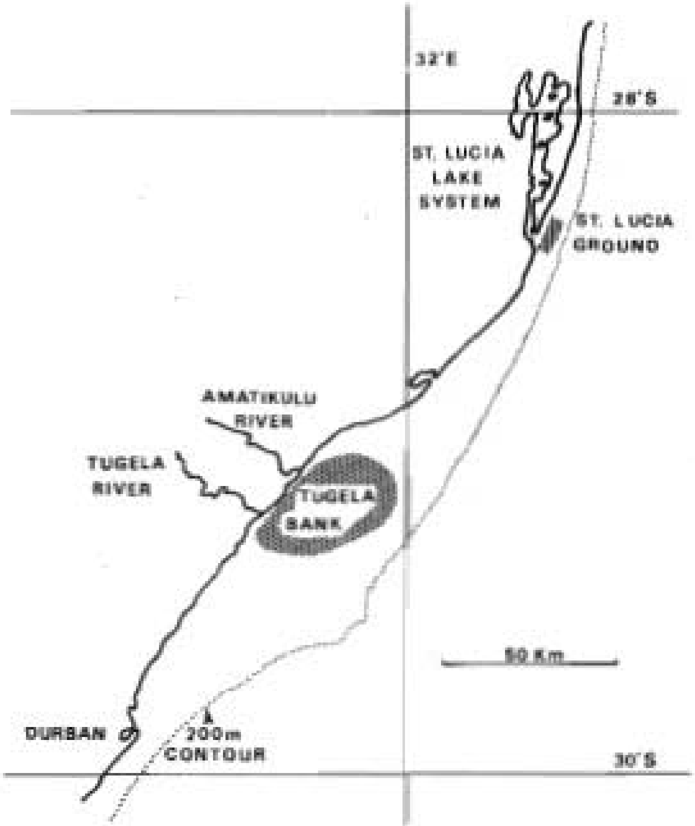

Figure 1 Map showing the estuarine (St Lucia lake system) and offshore (Tugela bank, St Lucia ground) sampling sites for *Penaeus indicus*.

determined. Freshly caught *P.indicus* males in the 9,2-41,0 mm CL size range were therefore measured for CL and TL and weighed for mass. TL and mass were then regressed against CL, which is regarded as the most reliable independent parameter indicative of size (Dall 1958; Cheung 1963). Rectilinear, allometric and polynomial regressions were tested for goodness of fit with the data (Snedecor & Cochran 1967).

CL was adopted as the standard size parameter in the remainder of this investigation and was recorded for all sampled specimens except in a confinement study where mass was used.

Reproductive development

The petasma is part of the external genitalia which is said to be concerned with the transfer of the spermatophoric mass during mating (Hudinaga 1942; Dall 1958; Cummings 1961; Tuma 1967). In early development, the pleonic endopodites making up the petasma are separate but later they become joined into a channel-like structure (Kubo 1949). The condition of the petasma of all sampled *P.indicus* males was noted, on the assumption that the joining of the petasmal endopodites signified a stage in the maturation process.

The internal reproductive organs have been described in detail by Champion (1987). They culminate in a pair of terminal ampoules, one at the base of each fifth pereiopod. A spermatophore resides within each terminal ampoule where it forms part of the spermatophoric

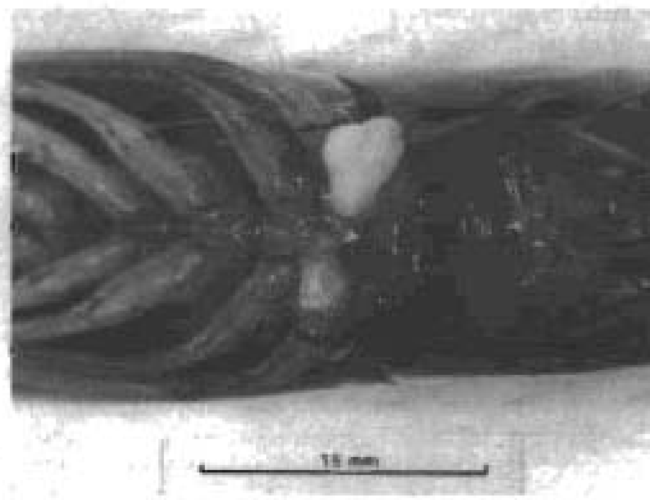

Figure 2 The ventral aspect of a *Penaeus indicus* male with extrusable spermatophores. The artificially extruded spermatophoric mass is seen erupting from the left side terminal ampoule. The right side spermatophoric mass is seen in its natural position as a white swelling at the base of the fifth pereiopod.

mass. At initial formation, these masses are discernible as medial white bodies at the bases of the fifth pereiopods. Their macroscopic development is characterized by progressive swelling which is difficult to measure. Once mature, however, mechanical extrusion is possible by gently squeezing *P.indicus* in the vicinity of the fifth pair of pereiopods: spermatophoric material readily erupts from each terminal ampoule (Figure 2). The presence or absence of spermatophoric material and the ability to extrude it when present, were thus routinely assessed in freshly netted *P.indicus* males as further signs of maturity.

Domestic trials

Incidental observations on mating activity became possible during four domestic growth trials on *P.indicus*. Laboratory-spawned stock was used in each instance.

The prawns were grown in quadrate concrete ponds of three sizes. In the first two trials, pond dimensions were 27,5 × 1,8 × 0,45 m deep. In the third trial, they were 28,5 × 5,5 × 0,5 m deep and in the fourth, 5 × 4,5 × 0,7 m deep. Specimen size at stocking ranged from 0,78 g ± 0,1(*SE*) to 1,21 g ± 0,3 (11–12 mm CL). The stocking density was 16–17 prawns m^{-2} involving 4 650 specimens. Trials lasted 137 to 197 days. At the conclusion of each trial, specimens were sexed, weighed and the thelyca of females were inspected for spermatophoric insemination. Mass values were converted to CL measurements using the regression solution which follows.

Results

Morphometry

In both the TL/CL and mass/CL relationships, curves generated by second degree polynomial regression equations provided the best correlations and visual fits with plotted data (Figure 3).

Regression solutions were:
TL = (–5,908) + 6,693 CL + (–0,05 CL^2);
sample size (n) = 248, coefficient of correlation (r) = 0,995; standard error of estimate (SE) = 3,43;
Mass = 0,559 + (–0,421 CL) + 0,038 CL^2;
n = 297; r = 0,995; SE = 1,16.

Petasma formation

The percentage frequency of males with a united petasma per 1 mm CL, over the size range within which petasma formation takes place, is shown in Figure 4(A). The sample consisted of 2450 specimens. The number of specimens examined per size class ranged from 33 to 265.

The smallest individual with a united petasma measured 14 mm CL. More than 50% of the sample had fully formed petasmata at 20 mm CL and all specimens showed this condition from a size of 25 mm CL.

Spermatophore development

The percentage frequency per 1 mm CL of specimens in which spermatophoric material was apparent as opposed to being not readily discernible, is shown in Figure 4(B).

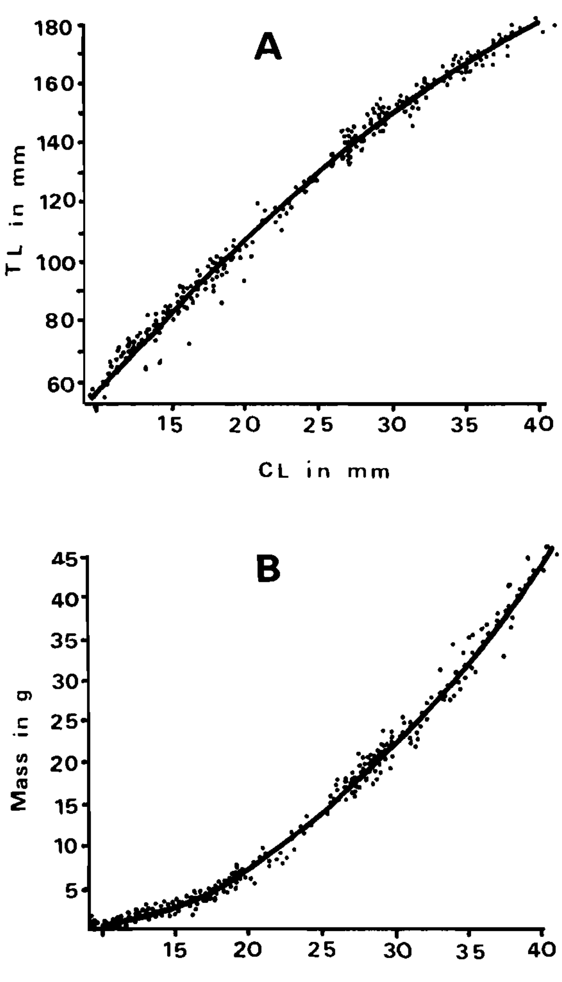

Figure 3 The relationships between total length and carapace length (A) and mass and carapace length (B) for *Penaeus indicus* males. Fitted lines are derived from second degree polynomial regression equations.

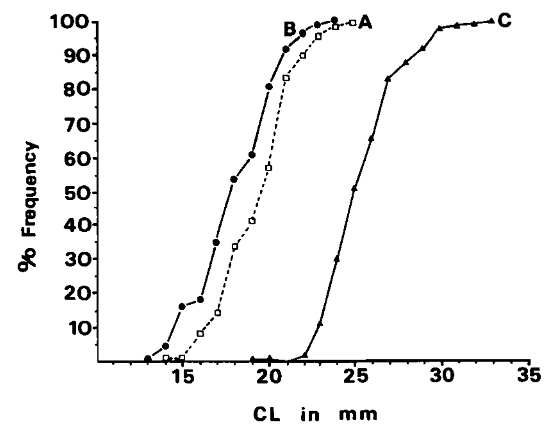

Figure 4 The percentage frequencies per 1 mm carapace length of *Penaeus indicus* males with united petasmal endopodites (A), visible spermatophoric masses (B) and extrusable spermatophores (C).

The similar size-frequency curve of specimens in which spermatophores were not only visible but could also be easily extruded, is shown in Figure 4(C). The sample comprised 8 873 *P.indicus* males in the size range 12–34 mm CL. The number of specimens examined per size class ranged from 210 to 699.

Initial formation of the spermatophoric mass became apparent in the 13–24 mm CL interval with more than 50% of the sample bearing visible spermatophores at 18 mm CL. All males inspected possessed visible spermatophoric material at 25 mm CL (Figure 4 B).

The smallest male with extrusable spermatophores measured 19 mm CL and the largest male with unextrusable spermatophores measured 32 mm CL. Spermatophores could be extruded from more than 50% of the sample at 25 mm CL (Figure 4C).

Mating

The results of the four confinement trials in which evidence of mating activity was monitored, are summarized in Table 2. Combined survival was 79,9%

Table 2 The mean size, size range and number of surviving males versus the incidence of inseminated females and the total percentage survival at the conclusion of each of four growth trials on *Penaeus indicus*

	Males			Females		
Trial	Mean size	Size range	n	Number mated	Mated %	Survival %
1	24	17,7–26,5	355	84	25	81.6
2	25,7	20,2–31,2	288	102	27,8	79
3	26,6	19,5–31,4	1 067	323	31,9	80
4	26,4	24 –29,3	147	102	72	72
Combined	26	17,7–31,4	1 857	611	32,9	79,9

Size = CL in mm; n = number of specimens comprising 1 857 males and 1 859 females. Of the survivors, 611 females (32,9%) were found to have been inseminated. The size interval containing males responsible for these inseminations was 17,7–31,4 mm CL. No significant mortalities of larger-sized males took place prior to harvest.

Whereas the results do not indicate the size at which male mating activity commences, the 84 inseminations recorded in Trial 1 were made by males smaller than 26,5 mm CL (Table 2). A more detailed analysis of the size composition of these males at harvest, is presented in Table 3. This shows that four males were in the 26,0–26,5 mm CL size class, 56 were in the 25,0–25,9 mm CL size class and 127 were in the 24,0–24,9 mm CL size class.

Discussion

Figure 4 shows the progressive development of three successive events in the maturation of *P.indicus* males. The cumulative profile for the initial appearance of the spermatophore (Figure 4B), precedes by 1–2 mm CL the similar profile for the formation of the petasma (Figure 4A), which in turn, is followed at a further interval of 5–7 mm CL by maturation of the spermatophore to the extrusable state (Figure 4C). Details of the curve representing petasma formation (Figure 4A) are in general agreement with the previous findings of Hall (1962) (other than his over-estimate of the size at which formation is initiated), George & Rao (1968) and de Freitas (1980). Comparable data is not available on the development of the spermatophore (Figure 4B, C) although Subrahmanyam's (1965) proposals (Table 1) fall within the 19–33 mm CL 'extrusable spermatophore' interval.

Since formation of the petasma has been shown to take place some time before the spermatophores are fully developed and since direct evidence of mating is limited, this assessment of the attainment of sexual maturity must rely mainly on the significance of spermatophoric extrusability.

The extrusion method, as employed by Weymouth *et. al.* (1933), Dall (1958) and Tuma (1967), assumes that specimens from which spermatophores are easily extruded, are capable of mating. The validity of such an assumption depends on three conditions being met. Firstly, the method is arbitrary to the extent that assessment of the ease of extrusion relies on personal interpretation. Also, it does not recognize the specimen whose spermatophoric material is depleted owing to recent mating. There is thus the need to demonstrate that the method yields reliable results. Secondly, since male maturity implies an ability to fertilize ova, it must be shown that extrusable spermatophores contain spermatozoa. Thirdly, evidence is required that males with extrusable spermatophores are capable of mating.

The reliability of the method may be gauged by comparing the extrusability curve (Figure 4C) with those obtained for the initial appearance of spermatophoric material (Figure 4B) and the unification of the petasmal endopodites (Figure 4A), respectively. Although the tails of the extrusability curve seem excessively long, the similar shapes of the three curves and the consistency of the intervals between them give credibility to the method.

With respect to the second condition, microscope inspections in the present study, revealed the presence of spermatozoa in extruded spermatophores without exception, but these inspections were not conducted systematically. Subrahmanyan (1965) found that spermatozoa were not yet formed at 110 mm TL (20,5 mm CL), were present in the vasa deferentia at 120 mm TL (23 mm CL) and in the terminal ampoules at 130 mm TL (25 mm CL). Champion (1987) confirmed the presence of spermatozoa in the spermatophores of males larger than 28 mm CL. A comparison between Subrahmanyam's (1965) results and the curve obtained for spermatophore extrusability (Figure 4C) shows that males with a CL of 20,5 mm lie in the lower tail of the curve. Only one of 370 males in this size class possessed extrusable spermatophores. There is thus agreement that *P.indicus* males up to this size are virtually all immature. The 23 mm CL size at which spermatozoa were found in the vasa deferentia, but not the terminal ampoules, corresponds to a position on the extrusability curve where 11% of the males possessed extrusable spermatophores. The majority of males at this size would therefore be regarded as immature and could conceivably lack spermatozoa in their terminal ampoules. The 25 mm CL size at which spermatozoa were found in the terminal ampoules conforms to a class in the present study where the majority (51%) of males possessed extrusable spermatophores which were considered to be mature. Previous findings do not therefore contradict the evidence gathered in this investigation that extrusable spermatophores contain spermatozoa.

Finally, the third condition which refers to mating ability, is at least partly met by the evidence that males smaller than 26,5 mm CL were responsible for 84 inseminations in a confinement trial (Table 2). Since attainment of maturity is a function of size, it seems reasonable to assume that the males responsible came from the large end of the size spectrum, as shown in Table 3. It is highly improbable though that the four males larger than 26 mm CL were responsible for all 84 inseminations and it is thus concluded, that a proportion

Table 3 The size composition of *Penaeus indicus* males at the conclusion of a growth trial which yielded 84 inseminated females (Trial 1)

CL in mm	Mass in g	Specimens
17,7–22,9	5,0–10.8	35
23,0–23,9	10,9–12,2	133
24,0–24,9	12,3–13,6	127
25,0–25,9	13,7–15,1	56
26,0–26,5	15,2–16,1	4

of the 56 males in the 25,0–25,9 mm CL size class were also mature (Table 3). No inferences on mating ability are possible on the males of the progressively smaller size classes. These results disagree with Subrahmanyam's (1965) finding that the spermatophore is only fully developed at 140 mm TL (27 mm CL), since specimens smaller than this have been shown to possess extrusable spermatophores and be capable of mating.

It is concluded from the above evidence, that spermatophoric extrusability indicates the attainment of sexual maturity in *P. indicus* males. Ignoring the extended tails of the extrusability curve (Figure 4C), it is proposed that maturity is attained by *P. indicus* males in South Africa within the size range 22 mm CL (2% extrusability) to 30 mm CL (98% extrusability) with more than half the population becoming mature at 25 mm CL, when all males possess fully formed petasmata and visible spermatophores as shown in Figure 4.

To place these results in perspective, an analysis was carried out to establish what proportions of *P. indicus* males sampled in the St Lucia system and offshore respectively, were mature according to the extrusion method. It is well established that adults breed at sea (Mohamed 1969). It is therefore of interest to determine whether maturity is attained exclusively at sea or in the estuarine environment as well. Furthermore, breeding at sea implies that a large proportion of the offshore male population is mature. Consequently, any assessment of maturity could be regarded as suspect if it failed to confirm this.

Emmerson (1980) has shown that the completion of one generation in the life history of *P. indicus* (development from ovum to adult breeding) can be achieved in 255 days in confinement. In order to cover all biological development phases as well as minimize temporal effects brought about by migratory movements, monthly sample data from two annual cycles were combined for each sampling area. Figure 5 thus shows the size distribution of males netted in the St Lucia lake system, in 1966 and 1968 (n = 13 348).

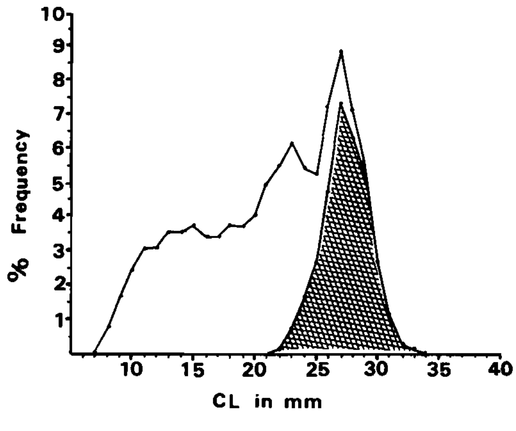

Figure 5 The size composition (percentage frequencies per 1 mm carapace length) of *Penaeus indicus* males netted in the St Lucia lake system. The sample comprises the combined monthly catches for 1966 and 1968. The hatched area represents the percentage of mature males in each size class.

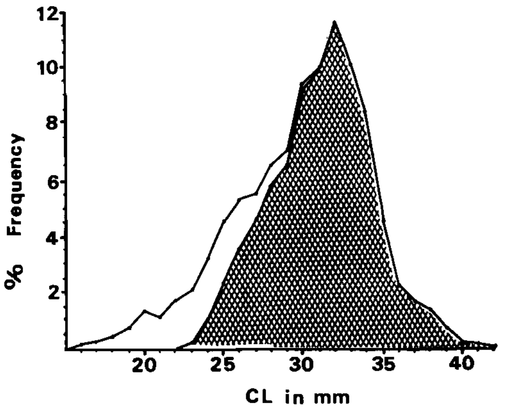

Figure 6 The size composition (percentage frequencies per 1 mm carapace length) of *Penaeus indicus* males netted offshore. The sample comprises the combined monthly catches from July 1966 to June 1968. The hatched area represents the percentage of mature males in each size class.

Similarly, Figure 6 shows the size distribution of males sampled offshore from July, 1966 to June, 1968 (n = 3 493). Mature males (with extrusable spermatophores) are expressed as a proportion of the sample in each 1 mm CL size class and are shown by the hatched areas in the two figures.

The skewed shape of the distribution curve representing the St Lucia population indicates an imbalance in sample returns. Notwithstanding this, Figure 5 does suggest that the estuarine environment *per se* poses no impediment to male maturation. A significant number of mature *P. indicus* males (n = 4 363) were sampled in the lake system. Males which attain the appropriate size thus develop to maturity prior to departing the estuarine habitat for offshore breeding grounds.

Analysis of the offshore data yielded a normal size–frequency curve with a modal size class of 32 mm CL (Figure 6). Males classed as mature comprised 83% (n = 2 905) of the sample. A comparison between the data from each of the annual cycles making up the sample showed that both the size distribution and proportion of males with extrusable spermatophores remained consistent. Figure 6 is therefore regarded as a fairly typical portrayal of the offshore *P. indicus* male population. The finding that the overwhelming majority of offshore males possess extrusable spermatophores provides further support for the conclusion that these males are sexually mature.

Acknowledgements

Sampling prior to 1969 took place under the auspices of the Oceanographic Research Institute. Funding by SANCOR and the generous co-operation of the Natal Parks Board in facilitating work at St Lucia is gratefully acknowledged. Subsequent sampling and experimental work was funded by the Fisheries Development Corporation. The late Mr W. Taylor and Mr C. Taylor of Sterling Fisheries are thanked for making trawlers available for offshore trawling. The data was analysed and written up at the University of Zululand. Dr W.T. Barham and Prof. H.J. Schoonbee are thanked for constructive comments on the text. Prof. J.G.J. Visser is thanked for assistance with the translation of the summary into Afrikaans. Finally, the two referees are thanked for their helpful comments on restructuring the presentation.

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