

# Epidemiology of cryptonisci (Bopyridae: Isopoda) in the Gulf of Carpentaria, Australia

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**ABSTRACT:** Three Gulf-wide cruises were examined for cryptonisci and the data analysed for the effect of environmental variables on cryptoniscid abundance. Location, season and location-season interaction accounted for 80% of the variability, with each accounting for 65, 25 and 10% of the explained variability respectively. More cryptonisci were found offshore in the northern and north-western areas of the Gulf and autumn had the highest abundance. In decreasing order, depth, temperature, salinity, light and their interactions were components within season and location and they accounted for 13, 6, 6, 4 and 43% of the variability respectively. The depth effect was related to the main prawn host *Penaeus semisulcatus* which also had a preference for deeper water. Fourteen different copepod intermediate hosts were identified by direct observation of attached cryptonisci; *Canthocalanus pauper* and *Paraeuchaeta concinna* were the most important. Environment, however, was more important than intermediate hosts in explaining variation in cryptoniscid numbers.

**KEY WORDS:** Bopyrid · Plankton · Environment

## INTRODUCTION

The life cycle of bopyrid isopods involves an epicaridium larva which is released from the marsupium of the female bopyrid when the host prawn moults. The lecithotrophic larvae are positively phototactic and swim near the surface until they encounter a calanoid copepod to which they attach. The epicaridium metamorphoses via a moult into a microniscus which then develops into a cryptoniscus (Anderson & Dale 1981). The cryptoniscus then detaches and searches for a postlarval prawn as a definitive host (Beck 1980). The planktonic phase probably takes between 10 d (Anderson & Dale 1981) and 1 mo (Caroli 1928) depending on the bopyrid species and possibly the environmental factors.

To understand why adult bopyrids are distributed patchily, an understanding of the dispersal capabilities of the infective stage, the cryptoniscus, is needed. Beck (1979) suggested cryptonisci of *Probopyrus pandalicola* were capable of swimming 13 km from the habitat

of their immediate host, *Acartia tonsa*, to the nursery ground of *Palaemonetes paludosus* where infection took place. Owens & Rothlisberg (1991) described the vertical migration of bopyrid cryptonisci in the Gulf of Carpentaria, Australia, and estimated the advection distance of the larvae. That study suggested that cryptonisci were concentrated within 100 km of major foci of adult bopyrid infection. This study examined the geographic spread of cryptonisci within the Gulf of Carpentaria and the influence of environmental factors.

In the Gulf of Carpentaria, over 97% of adult bopyrid isopods found on commercial penaeid prawns were *Epipenaeon ingens* Nobili and 2% were *Parapenaeon expansus* Bourdon (in Owens & Glazebrook 1985). In this region, these 2 members of the Orbioninae usually infect only 3 prawn species: *Penaeus semisulcatus* de Haan, *P. merguensis* de Man and *P. indicus* Milne Edwards. Other members of the Bopyridae exist in the Gulf but they are not recorded often. It is assumed that the great majority of cryptonisci encountered will be *E. ingens*.

## MATERIALS AND METHODS

A maximum of 73 samples from each of 3 Gulf-wide cruises (Fig. 1) were sorted on a Bogorov tray under a dissecting microscope. The cruises were selected to give the widest possible spread of environmental parameters. June–July 1976 represented the coolest period (winter), January 1977 represented the hottest months (summer), and March 1977 (autumn) was chosen because of the low salinities associated with the rainy season. Counts of cryptoniscs from 1 stepped, oblique net (142  $\mu\text{m}$ ) tow at each site were standardised ( $\text{no. m}^{-2}$ ) for the depth and volume of water filtered. Data analysis was conducted on a DEC10 computer using the statistical packages GLIM and SPSSX. To see if data fitted certain statistical distributions (normal and Poisson), a Kolmogorov-Smirnov test was conducted on variables. Non-normally distributed variables were  $\log_{10}+1$  transformed and retested. Poisson-distributed variables were transformed by a square root transformation. Copepod species and abundance were obtained from the same samples reported in Othman (1986). A multiple regression analysis was used to correlate copepod intermediate host numbers with cryptoniscs. Initially, copepod species showing either negative or nonsignificant simple correlation coefficients with cryptoniscid numbers were eliminated as being unlikely intermediate hosts.

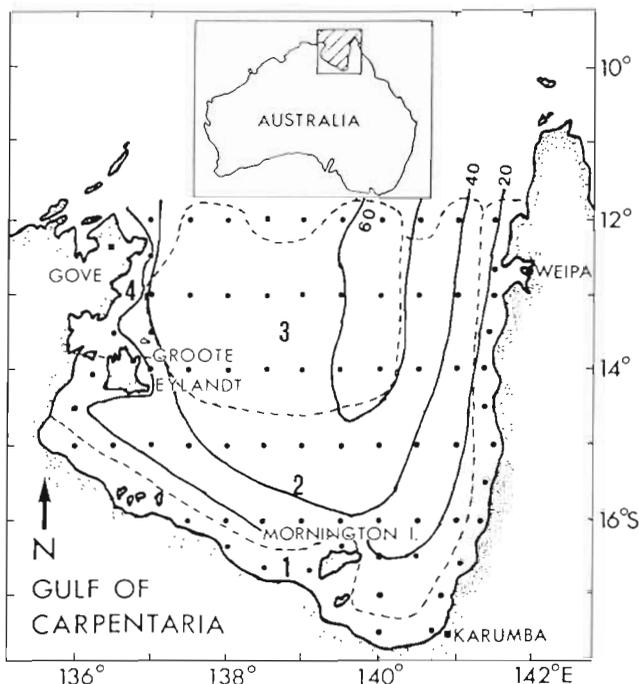


Fig. 1 Gulf of Carpentaria showing sampling sites (●), depth contours (solid lines in m) and location groupings identified by Student-Newman-Keuls ANOVA (dashed lines and numbers)

## RESULTS

The numbers of cryptoniscs in samples taken under 1  $\text{m}^2$  of surface area were distributed randomly (Poisson distribution, 1 parameter distribution) while those expressed as numbers per  $\text{m}^3$  (volume) were distributed normally (2 parameter distribution). Adult bopyrids on their penaeid host were distributed almost randomly (a 1 parameter distribution) (Owens 1988) which means the distribution that precedes the adult distribution must be a 1 parameter distribution to be capable of inducing the final adult distribution. Therefore, only cryptoniscid abundance expressed as  $\text{no. m}^{-2}$  were used in the analyses. From 177 samples sorted, an estimated 62 014 cryptoniscs were present with a mean of 350 per sample (Table 1).

Table 1. Estimates of cryptoniscs in samples from 3 Gulf of Carpentaria cruises

	Total	No. of samples	Mean no. sample <sup>-1</sup>	SD	Density ( $\text{no. m}^{-2}$ )
June/July 1976	15 144	34	445	306	30
January 1977	14 420	70	198	202	15
March 1977	32 450	73	459	334	46
Total	62 014	177	350		

### General model

A step-down linear model of all main effects and all first-order interactions was fitted to the data matrix and it accounted for 80% of the variability (equivalent to a correlation coefficient of 0.89) (Table 2). Of the fitted variables, location, season, and the location-season interaction accounted for significant ( $p < 0.001$ ) proportions of the variability, accounting for 65, 25, and 10% of the explained variability respectively. Temperature, light, day/night, depth, salinity and the other first-order interactions were not significant ( $p > 0.05$ ).

Table 2. Variability in numbers of cryptoniscs accounted for by the general maximum likelihood stepdown linear model (Baker & Nelder 1978)

	No. of cryptoniscs $\text{m}^{-2}$		
	% variability	F	p
Full model	80	17.32	0.0005
Season	25	71.7	0.0005
Location	65.2	31.1	0.0005
Season $\times$ location	9.7	2.93	0.0005
Partitioned variability	99.9		

Table 3. Student-Newman-Keuls (SNK) analysis of variance (Zar 1974) on cryptonisci numbers in geographical locations in the Gulf of Carpentaria ( $p < 0.05$ )

Location	Mean no. of cryptonisci $m^{-2}$	SD	SNK grouping
Close inshore, S and E	14.1	18.9	1
Offshore S and E	42.5	20.2	2
Central Gulf	64.8	49.5	3
Northwestern Gulf	129.4	83.9	4

The major location groups were identified by a Student-Newman-Keuls analysis of variance (Table 3). The overall trend is for cryptoniscid numbers to increase from southern inshore areas to the northern areas. The location groups progressed with increasing cryptonisci from the close inshore areas of the southern and eastern Gulf (area 1; Fig. 1) the southern-central (area 2) and northern-central Gulf (area 3) and the northwestern inshore and northern areas (area 4).

March (autumn) samples had one-and-a-half times as many cryptonisci ( $46 m^{-2}$ ) as did June–July (winter,  $30 m^{-2}$ ) samples which in turn had twice as many as January (summer,  $15 m^{-2}$ ) samples.

The season-location interaction showed that most southern inshore and nearshore locations in March had significantly fewer cryptonisci than estimated by season alone or location alone. During June–July, these same locations had significantly more cryptonisci. All central Gulf and northwestern sites had more cryptonisci than expected at all seasons.

#### Environmental model

Season and locality were excluded from the analysis to allow factors that characterised season and locality to emerge. In decreasing order of explained variability, temperature by depth, salinity by depth, depth, salinity by light, temperature, salinity and light accounted for 17, 14, 13, 12, 6, 6 and 4% of the variability respectively (Table 4). The overall model accounted for 71% of the variability and was far less efficient than the general model in explaining variability and it greatly reduced the degrees of freedom. Depth and its interactions were the most important and accounted for large amounts of variability with the highest significance levels.

Table 4. Variability in numbers of cryptonisci accounted for by a model of environmental factors

	No. of cryptonisci $m^{-2}$		
	% variability	F	p
Full model	71	2.97	0.0005
Temperature $\times$ depth	17.1	3.65	0.0005
Salinity $\times$ depth	13.8	2.61	0.005
Depth	12.5	6.83	0.0001
Salinity $\times$ light	12.0	2.28	0.01
Temperature	5.9	3.22	0.01
Salinity	5.8	3.19	0.01
Light	3.6	3.75	0.01
Partitioned variability	70.1		
Total explained variability	50.2		

Cryptonisci abundance maximised between 27 and  $29.5^{\circ}C$  (Fig. 2). At higher temperatures the numbers of cryptonisci dropped to one-eighth of those at the peak. The effect of salinity was very peaked compared to temperature (Fig. 2). There was a gradual increase in cryptonisci with salinity except at 33 to 33.5 ppt where a sharp peak of cryptonisci was observed. Above 33.5 ppt, the density of cryptonisci dropped precipitously.

The density of cryptonisci increased up to 55 m, almost the deepest areas in the Gulf. Here, cryptoniscid numbers were over  $80 m^{-2}$  (Fig. 2). At night (no light penetration) cryptoniscid density was moderate. At low levels of light penetration (turbid water) cryptonisci were few. Cryptonisci were most numerous where light penetrated approximately 10 to 15 m and

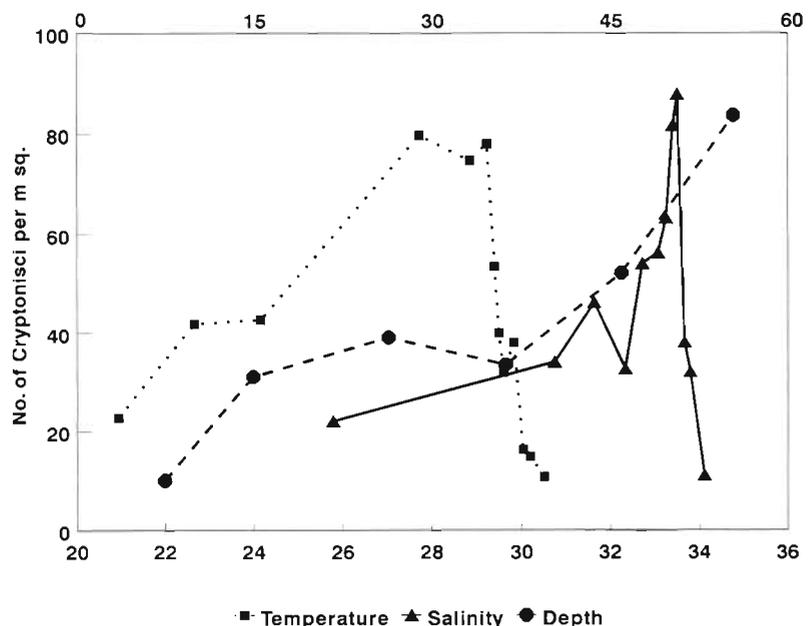


Fig. 2. Relationship between no. of cryptonisci  $m^{-2}$  and temperature, salinity and depth. The scales for temperature ( $^{\circ}C$ ) and salinity (ppt) are on the lower x-axis and the scale for depth (m) is on the upper x-axis

became less numerous where light penetrated even further.

The interaction terms were difficult to interpret. At mid-range temperatures of 26 to 29°C, significantly more cryptonisci were found at 10 to 15 m; at temperatures above 29°C, fewer cryptonisci were found at 10 to 15 m than expected by depth alone. Although other temperature-depth interactions showed considerable variation, none were significant. There was some variation in salinity by depth; at shallow depths (8 m) and at salinities between 33 to 33.5 ppt there were significantly fewer cryptonisci than indicated by depth alone or salinity alone. Where light penetrated to 10 m, there were significantly more cryptonisci at 33 ppt than expected by salinity alone or light alone.

### Intermediate hosts

A total of 31 copepods representing 14 different species were collected with cryptonisci or micronisci attached (Table 5). The most common intermediate hosts were *Canthocalanus pauper* and *Paraeuchaeta concinna*. Most copepod species only occurred once or twice with cryptonisci attached. All were calanoid copepods except for *Oncaea clevei* from the suborder Cyclopoida. No harpacticoid copepods found had cryptonisci attached.

In an analysis of 83 species of copepods from a subset of 54 sites, Othman (1986) showed that only 4 species of copepod accounted for 58% of the variation in cryptonisci abundance. Those species were the 3 calanoid copepods *Canthocalanus pauper*, *Paraeu-*

*chaeta concinna* and *Metacalanus aurivilli*, and the cyclopoid *Corycaeus asiaticus*. Although analysis of individual months showed some variation, *C. pauper* and *P. concinna* were present in 2 of the 3 cruises and prominent in both regression and direct observation of attached cryptonisci.

Numbers and species of copepods that were examined by Othman (1986) were combined with the general model factors (i.e. season, location, season-location) and then with the environmental factors (i.e. temperature, salinity, depth, light) and reanalysed by ANOVA with covariates (Zar 1974). Only 23 of the 70 Gulf-wide sampling sites examined by Othman (1986) had copepods, environmental information, and cryptonisci. However, in both analyses, the environmental variables were significant and the copepod species were nonsignificant, indicating cryptoniscid abundance was more strongly linked to the environmental factors than copepod numbers.

## DISCUSSION

### Environment

Seasonality of reproduction of bopyrids is determined by 3 main factors: the prevalence of infection, the size of the bopyrid, and the reproductive activity of the bopyrid. The abundance of copepod intermediate hosts in the Gulf shows very little temporal change (Othman 1986) and can be discounted as a limiting factor in reproduction, especially since the main hosts (*Canthocalanus pauper* and *Corycaeus asiaticus*) were ubiquitous. The

Table 5. List of copepod species with no. of cryptonisci found attached and multiple regression analysis. I: Inshore species; O: offshore species, U: ubiquitous (Othman 1986)

Copepod species	Number	Change in R on addition of copepod species	Partial correlation coefficient	Copepod group
<i>Calanopia elliptica</i>	1			I
<i>Canthocalanus pauper</i>	10	0.59	0.32	U
<i>Centropages furcatus</i>	1			I
<i>Clausocalanus minor?</i>	1			O
<i>Corycaeus asiaticus</i>	0	0.09	0.20	U
<i>Eucalanus subcrassus</i>	2			U
<i>Eucalanus</i> sp.	2			
<i>Eucalanus monachus</i>	2			
<i>Metacalanus aurivilli</i>	0	0.05	0.34	I
<i>Oncaea clevei</i>	1			U
<i>Paracalanus aculeatus</i>	1			U
<i>Paracalanus elegans</i>	1			U
Paracalanidae 'C'	3			
<i>Paraeuchaeta concinna</i>	4	0.03	0.45	O
<i>Scolecithricella orientalis</i>	1			O
<i>Undinula vulgaris</i>	1			O
Total	31			

period of peak prevalence of *Epipenaeon ingens* on *Penaeus semisulcatus* was April to June (Owens & Glazebrook 1985). Size increased over the calendar year with reproduction starting in January–February and individuals produced more larvae as their size increased. However, cooler water temperatures during winter depress the spawning activity of the bopyrid (Owens & Glazebrook 1985). January had the lowest number of cryptonisci because most bopyrids were just coming into reproductive activity. By March, prevalence was rising, bopyrids were larger and reproductive activity was high. In June–July, prevalence was higher than in March but falling rapidly, size was still increasing but due to cooler water temperatures reproductive activity was low (Owens & Glazebrook 1985). This is reflected by a lower number of cryptonisci than in March, suggesting reproductive activity to be the most important component to reproductive output. Similarly, Greenwood (1980) found peak abundance of cryptonisci in Moreton Bay, Australia, in autumn (early May) and again in spring (November) with the November peak being twice as large as the May peak.

One of the most important environmental variables associated with cryptoniscid abundance was depth and its interactions. The location boundaries roughly follow the depth contour lines with greater numbers of cryptonisci being found in deeper water, especially >40 m. Paradoxically, while cryptonisci are in deep water, prawn postlarvae become infected in shallow water nursery areas (sea grass beds in the case of *Penaeus semisulcatus*; Owens & Glazebrook 1985). The area north of Groote Eylandt where deep water is relatively close inshore, thus increasing the probability of cryptonisci successfully finding postlarval prawns close to the neighbouring nursery grounds, is a site of high prevalence of bopyrids on *P. semisulcatus*.

*Penaeus semisulcatus* is the main host for *Epipenaeon ingens* in the Gulf of Carpentaria. Adult *P. semisulcatus* have a preference for deeper water with 97% of the population captured in waters >20 m in depth and almost one-third at depths >40 m (Somers et al. 1987). This may in part explain the strong correlations between depth and cryptoniscid number and probably explains why the southern and eastern Gulf coastline areas, which are less than 20 m in depth, have few cryptonisci. Furthermore, in November, the southeastern corner has salinity >36.5 ppt and temperature >30°C (Anon 1977), both of which were associated with low numbers of cryptonisci. In fact, the whole eastern area of the Gulf is characterised at this time of the year by temperatures >30°C which appear unfavourable to cryptonisci and hence contribute to low numbers.

Low numbers of bopyrid cryptonisci in the southern inshore Gulf are associated with seasonal advection.

Directions of advection for vertically migrating larvae are different at different times of the year. In March, the advection direction is northwards, whilst in October, it is southwards (Rothlisberg et al. 1983). Cryptonisci would follow this scheme (Owens & Rothlisberg 1991), and this may account for the location-season interaction, with fewer numbers of cryptonisci inshore during March. It also suggests that by July the advection direction may be inshore as shown by greater numbers of cryptonisci in that area at that time.

### Intermediate hosts

Othman (1986) showed that the copepod fauna of the Gulf of Carpentaria is divided into offshore, intermediate and inshore faunas. We found these faunal groups had highly significant differences in depth across all 3 cruises analysed. A temperature effect was very highly significant ( $p < 0.001$ ) across fauna groups in January and highly significant ( $p < 0.01$ ) in June–July and March whilst a salinity effect was highly significant ( $p < 0.01$ ) in June–July and March but not significant in January ( $p > 0.05$ ). Both cryptonisci and copepods seem to respond to the same environmental variables with the same order of importance and this explains why environmental factors exceed the availability of copepod intermediate hosts as a determinant of cryptoniscid abundance.

There was good agreement between the copepods found with cryptonisci attached and those possible intermediate hosts indicated by multiple regression analysis, at least in the most important species, *Canthocalanus pauper* and *Paraeuchaeta concinna*. These differed markedly from those copepod species implicated as intermediate hosts during a study of vertical migration of cryptonisci (Owens & Rothlisberg 1991) with only 1 species being shared, *Calanopia elliptica*. In the vertical migration study, *C. elliptica* had the largest simple regression coefficient with cryptoniscid numbers, but it was displaced by depth in a multiple regression analysis. The Mornington Island, Australia, site for the vertical migration study was a relatively high density area for cryptonisci characterised by inshore species of copepods (Othman 1986). In Owens & Rothlisberg (1991), 5 of the 8 copepods recorded were inshore species, and 10 of 13 classifiable copepods listed in this study (Table 5) were ubiquitous or offshore species (Othman 1986). In the present study, most cryptonisci were found in offshore habitats. Therefore, the inshore species of copepods could have been masked by the greater abundance of the offshore species.

A further consequence of the use of ubiquitous offshore species of copepods as intermediate hosts was

the spread of cryptonisci throughout the whole Gulf. Spawning *Penaeus semisulcatus*, as indicated by protozoal larvae, were found in depths greater than 40 m (Rothlisberg et al. 1987) and possibly some parasitised *P. semisulcatus* might also be present there, although Somers & Kirkwood (1991) found that most of the parasitised population (median depth 27 m) does not migrate as far into the deep waters as the unparasitised population (median depth 40 m). Released epicaridium larvae of the bopyrids would have no trouble finding ubiquitous copepod intermediate hosts to develop to cryptonisci. Whether these cryptonisci contribute to the next bopyrid generation or not is undetermined but the consequent dispersive powers of the bopyrid are considerably larger than first expected (Owens & Rothlisberg 1991).

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