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MYTILUS EDULIS CHILENSIS INFESTED WITH *COCCOMYXA PARASITICA* (CHLOROCOCCALES, COCCOMYXACEAE)

A.P. GRAY[1] , I.A.N. LUCAS, R. SEED and C.A. RICHARDSON
School of Ocean Sciences, University of Wales – Bangor, Menai Bridge, Anglesey LL59 5EY
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ABSTRACT

The association between the green alga *Coccomyxa parasitica* (Chlorococcales) and the mussel *Mytilus edulis chilensis* at Goose Green, Falkland Islands is reported. *C. parasitica* occurred within the soft tissues with an overall infestation rate of 16%. The highest levels of infestation (23%) occurred in individuals from the middle of the main mussel bed, with considerably lower levels of infestation in the upper and lower regions (<1% and 5% respectively). No consistent seasonal pattern in infestation rate was detected between September 1993 and February 1996. *C. parasitica* was most commonly observed in tissues located in the posterior territory of the host, in areas most directly exposed to light. Tissues of infested mussels were rather watery and translucent and the adductor muscle appeared weak and stringy. During the summer months when Falkland mussels are in peak reproductive condition, dry flesh weight of infested mussels was significantly lower than non-infected mussels of comparable size suggesting that infestation by *C. parasitica* may reduce reproductive output. However it is uncertain whether poor condition of the host is due to the presence of the parasitic alga or whether *C. parasitica* infests only those mussels that are already in poor condition.

INTRODUCTION

The infestation of bivalves by unicellular algae appears to be relatively common. "Green oysters" and "green-gilled clams" for example have been described by Lankaster (1886), Mitchell and Barney (1917), Medcof (1945) and Kerswill (1946). There are, however, relatively few reports of algae occurring in the soft tissue of the blue mussel, *Mytilus edulis*. Kerswill (1946) observed green coloured gills in intertidal *M. edulis* from Prince Edward Island, Canada, but did not identify the cause. Meixner (1984) observed "green spots" in the mantle and adductor tissues of *M.edulis* from the Flensburg Fjord in Denmark, and suggested that this was due to a parasitic endosymbiotic alga which he identified as a blue-green cyanobacterium, belonging to the genus *Microcystis*.

Naidu & South (1970) found green areas distributed within the mantle tissue of the giant scallop, *Placopecten magellanicus,* from the shallow waters off the west coast of Newfoundland. Naidu (1971), subsequently established that this green colour was caused by a unicellular alga and that this had a detrimental effect upon the body condition of its host, concluding that the alga was probably parasitic. This alga was identified and described by Stevenson & South (1974) as *Coccomyxa parasitica*, a new member of the Chlorococcales. However, since this alga could also be successfully cultured on an inorganic medium the parasitic relationship was considered to be facultative (Stevenson & South 1974). A facultative parasite of the genus *Chlorella* was identified by Hartman & Pratt (1976) from the siphonal tissues and surrounding mantle areas of the heart cockle, *Clinocardium nuttalli*. It was later suggested by Lauckner (1983) that Hartman & Pratt (1976) may have been unaware of the earlier descriptions of *Coccomyxa* spp. and suggested that their presumed *Chlorella* spp. may have been a member of the Coccomyxaceae. Apart from these isolated reports little is known about *C. parasitica* and its relationship with bivalves.

During an ecological study of *Mytilus edulis chilensis* (L.) from the Falkland Islands, green patches were occasionally noted in the soft body tissues of mussels from one of three sites,

[1] Present address: 3, St Marys Walk, PO Box 530, Stanley, Falkland Islands

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Goose Green, studied by Gray *et al.* (1997a & b). The organism responsible for these green areas was identified as *C. parasitica*. In this paper we report on the local abundance and distribution of *C. parasitica* within the Goose Green mussel population and briefly document its distribution within the host tissues.

MATERIALS AND METHODS

The shore at Goose Green, Falkland Islands, is gently sloping with a sheltered aspect and a substratum consisting of exposed bed-rock, coarse shingle and mud; in the lower shore there is also a dense covering of algae. As part of a general ecological study, monthly samples of ~25 mussels (25–53mm) were collected between September 1993 and February 1996 from the middle part of the main mussel zone (~ 0.67m CD). In addition a large sample of mussels was collected in November 1995 from within 5-9 random ~0.17m^{-2} quadrats from the high, mid and lower zones of the mussel bed. The shell length (maximum anterior-posterior dimension) of all mussels was measured to 0.1 mm using vernier calipers; each mussel was opened and the distribution and abundance of green patches within four distinct regions of the body tissue noted viz. 1) mantle edge; 2) (a) posterior and (b) anterior external mantle surface; 3) posterior adductor muscle and 4) (a) posterior and (b) anterior visceral mass. The degree of infestation was categorised as light ie. occasional to small patches, moderate ie. larger more abundant patches and heavy ie. the host tissue almost totally obscured. In order to determine the possible effects of this alga on the reproductive condition of the mussel the relationship between dry tissue weight and shell length of uninfested and infested mussels was determined during the summer (November) when Falklands mussels are in peak reproductive condition. The flesh of infested and non-infested mussels ranging in size from 25-53 mm, measured to the nearest 0.1mm length, was removed and oven dried for 3 days at 65 °C. The slopes and intercepts of log transformed dry tissue weights on shell length of infested and uninfested mussels were compared using the general linear model with a covariate.

Pieces of infested mussel tissue ~1 mm^2 were excised, fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 12 hours at 5°C and fixed for a further 1 hour in 2% osmium tetroxide. Tissues were then washed thoroughly with phosphate buffer, dehydrated to absolute alcohol and then embedded in 'SPURR' resin (Spurr, 1969). Thick sections (1 μm) were stained with toluidine blue (1%) and examined under a light microscope. Thin sections (50 nm) were collected onto 'celloidin' coated grids, stained with uranyl acetate and lead citrate and examined in a Philips EM 301 transmission electron microscope (TEM).

RESULTS

Microscopical observations showed that the host tissues had been invaded by a spherical unicellular green alga containing a number of chloroplasts and similar in shape and size to the alga *Coccomyxa parasitica* described by Naidu & South (1970) and Stevenson & South (1974). Within the host connective tissue, algal cells were concentrated into large aggregations ~ 400 μm in diameter, (Fig. 1A). At higher resolutions in the TEM these aggregations comprised host cells, probably leukocytes, each typically containing several *Coccomyxa* cells within individual vacuoles (Fig. 1B). The presence of the vacuole wall surrounding each algal cell ensures that the *C. parasitica* is effectively isolated from the leukocyte cytoplasm. The rounded algal cells ranged from 1-4 μm in diameter (Fig. 1C). Within the algal cell cytoplasm one or two chloroplasts each containing stacks of between two and seven thylakoids and starch grains could be detected but there were no obvious pyrenoids, no flagella nor flagella bases (Fig. 1C). Each algal cell also typically contained profiles of mitochondria (Fig. 1C), distinct ribosomes, rough endoplasmic reticulum, electron dense vesicles (possible storage products) together with a central nucleus and obvious nucleolus (Fig. 1B,C). Each algal cell was enclosed by two membranes (20–40nm thick), an outer membrane which appeared to be of host origin and an inner one of algal origin. Reproduction appeared to be achieved by cell division into either two or four daughter cells or autospores (1.7–2.5 μm) before the parental membrane ruptures (Fig. 1D); these autospores are a characteristic feature of chlorococcalean algae. It is not known whether this cell division occured prior to or during infestation of the host tissue. The presence of multilamellar bodies within some of the vacuoles of the leucocytes suggested that the algal cells were being digested by the host (Fig. 1B).

Infestation rates were highest (23%) in mussels from the mid mussel zone (Table 1) and lowest in the upper and lower parts of the zone (<1% and 5%, respectively). Of the infested mussels 71% were lightly infected, 23% moderately infected and 6% heavily infected. Despite the occurrence of relatively high levels of infestation by *C. parasitica* at Goose Green, extensive sampling of mussel populations at two other Falkland Island sites provided no evidence of algal infestation (Gray *et al.*, 1997a).

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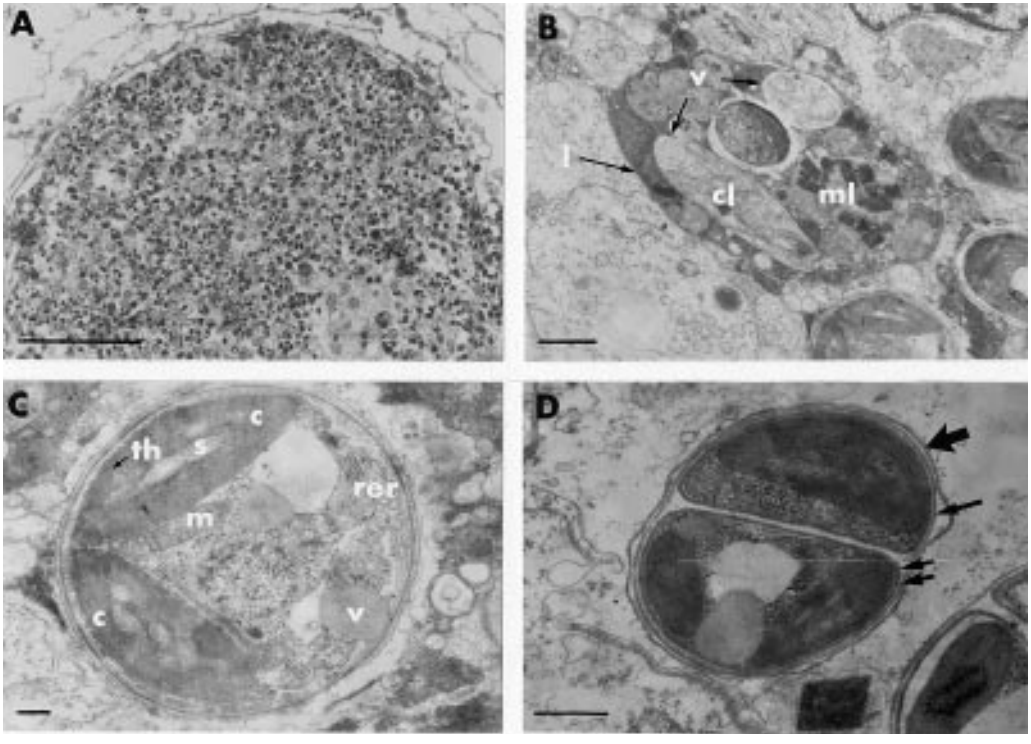

Figure 1 A. Photomicrograph of a section of *Mytilus edulis chilensis* mantle tissue showing the presence of large aggregations of *Coccomyxa parasitica* within the host conective tissue. **B**. Low power TEM of the aggregations to show they comprise host cells, probably leukocytes (l), each typically containing several *C. parasitica* cells (cl) within individual vacuoles (v); multi-lamellar bodies (ml). **C**. TEM of a rounded *C. parasitica* cell containing chloroplasts (c), mitochondrial profiles (m), rough endoplasmic reticulum (rer), thylakoids (th), starch grains (s) and electron dense vesicles (v). **D**. TEM of a C. *parasitica* cell dividing into 2 daughter cells. The animal cell membrane (large arrow), the algal cell parental membrane (small arrow) and algal daughter cell membrane (small double arrows) can be seen. Scale bar =100nm (A) and 500nm (B-D).

Table 1. The infestation of *Coccomyxa parasitica* within *Mytilus edulis chilensis* from Goose Green in the Falkland Islands during November 1995

Zone	Number examined	Number infested	Infestation		
			Light	Moderate	Heavy
High	106	1	100	0	0
Mid	572	134	69	25	6
Low	245	12	92	8	0
Total	923	147	71	23	6

Coccomyxa parasitica was most commonly distributed as densely packed colonies in the posterior mantle edge tissues and occurred with progressively lower abundances through the following sequence; posterior visceral mass, outer posterior mantle surface and posterior adductor muscle (Table 2). Very low infestations were observed in tissues located within the anterior territory of the host (Table 2).

The size distribution and infestation of *M. e. chilensis* in the three zones within the Goose Green mussel population during November 1995 are shown in Fig. 2. Both the mean and maximum size of mussels increased progres-

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Table 2. The degree of infestation of *Coccomyxa parasitica* in different body tissues of *Mytilus edulis chilensis* collected from Goose Green in the Falkland Islands during November 1995

Tissue	Level of Infestation – number (%)			
	None	Light	Moderate	Heavy
a. mantle edge	21 (14)	48 (33)	48 (33)	30 (20)
b. anterior of outer mantle edge	137 (94)	8 (6)	1 (<1)	0 (0)
c. posterior of outer mantle edge	68 (47)	55 (38)	21 (14)	2 (1)
d. posterior adductor muscle	75 (51)	62 (42)	8 (6)	1 (<1)
e. anterior area of visceral mass	141 (97)	5 (3)	0 (0)	0 (0)
f. posterior area of visceral mass	64 (44)	65 (45)	16 (11)	1 (<1)

sively with decreasing tidal elevations. Whilst infestation by *C. parasitica* occurred predominantly among the larger size classes; smaller mussels were only rarely infested. The shells of mussels infested by *C. parasitica* were often severely damaged by erosion of the outer shell surface or else showed irregular shell growth at the posterior margin; non infested shells were rarely damaged in this way. There was no evidence for a seasonal pattern in infestation although considerable variation over the study period was evident (Fig. 2 inset).

Dry flesh weights of infested mussels collected during the summer when Falklands mussels are in peak reproductive condition were significantly lower than those of uninfected mussels of comparable size (for slopes $F= 2.75$, $\rho > 0.05$; for intercepts $F= 347.74$, $\rho < 0.01$), strongly suggesting that *C. parasitica* has an adverse affect on reproductive condition.

DISCUSSION

The alga described in this study was identified as *Coccomyxa parasitica*, a member of the Chlorococcales. It is similar in shape (spherical, ovoid or sausage-shaped), size (3-11 μm), number of chloroplasts (1 or 2) and reproduction (formation of 4, 8 or 16 daughter cells), to the species described in association with *Placopecten magellanicus* from Newfoundland, Canada by (Naidu & South, 1970; Naidu, 1971; Stevenson & South, 1974; 1975). *Mytilus edulis chilensis* from the mid zone of the bed at Goose Green were most heavily infested with *C. parasitica*, which tended to occur in large individuals particularly those with damaged or eroded shells. Naidu (1971) similarly found that larger, older *Placopecten magellanicus,* especially those with damaged or deformed shells, were more susceptible to algal infestation probably by *C. parasitica*, whilst in Norway the cyanobacterium *Microcystis* was most prevalent in damaged *Mytilus edulis* (Meixner, 1984).

Within *M. e. chilensis, C. parasitica* is most abundant within those tissues which are most exposed to light, either directly at the posterior mantle edge when the shell valves gape, or indirectly in areas where the shell is particularly thin or badly eroded and, consequently, partly translucent. In the anterior regions of the mussel shell where the shell is thicker and/or more protected within the sediment and mussel bed matrix, light penetration to the tissues is minimal and levels of infestation are reduced. Light has also been considered to be an important factor controlling the distribution and abundance of *C. parasitica* within the tissues of *P. magallenicus* (Naidu, 1971).

Coccomyxa parasitica cells are probably ingested as part of the normal diet of the bivalve host after which they are phagocytosed by leukocytes and distributed throughout the host tissues via the circulatory system (Stevenson & South, 1975). The subsequent establishment and reproduction of *C. parasitica* occurs within those host tissues where there is adequate penetration of light usually as a consequence of shell thinning by erosion (Stevenson & South, 1975). The significantly reduced flesh weight in infested *M. e. chilensis* during the summer (November), when mussels in this region are normally in peak reproductive condition (Gray *et al*., 1997a), suggests that *C. parasitica* might have an adverse effect on fecundity. It is uncertain whether poor condition of the host is due to the presence of the parasitic alga or whether *C. parasitica* infests only those mussels that are already in poor condition.

Larger, older scallops, especially those with damaged or deformed shells are particularly

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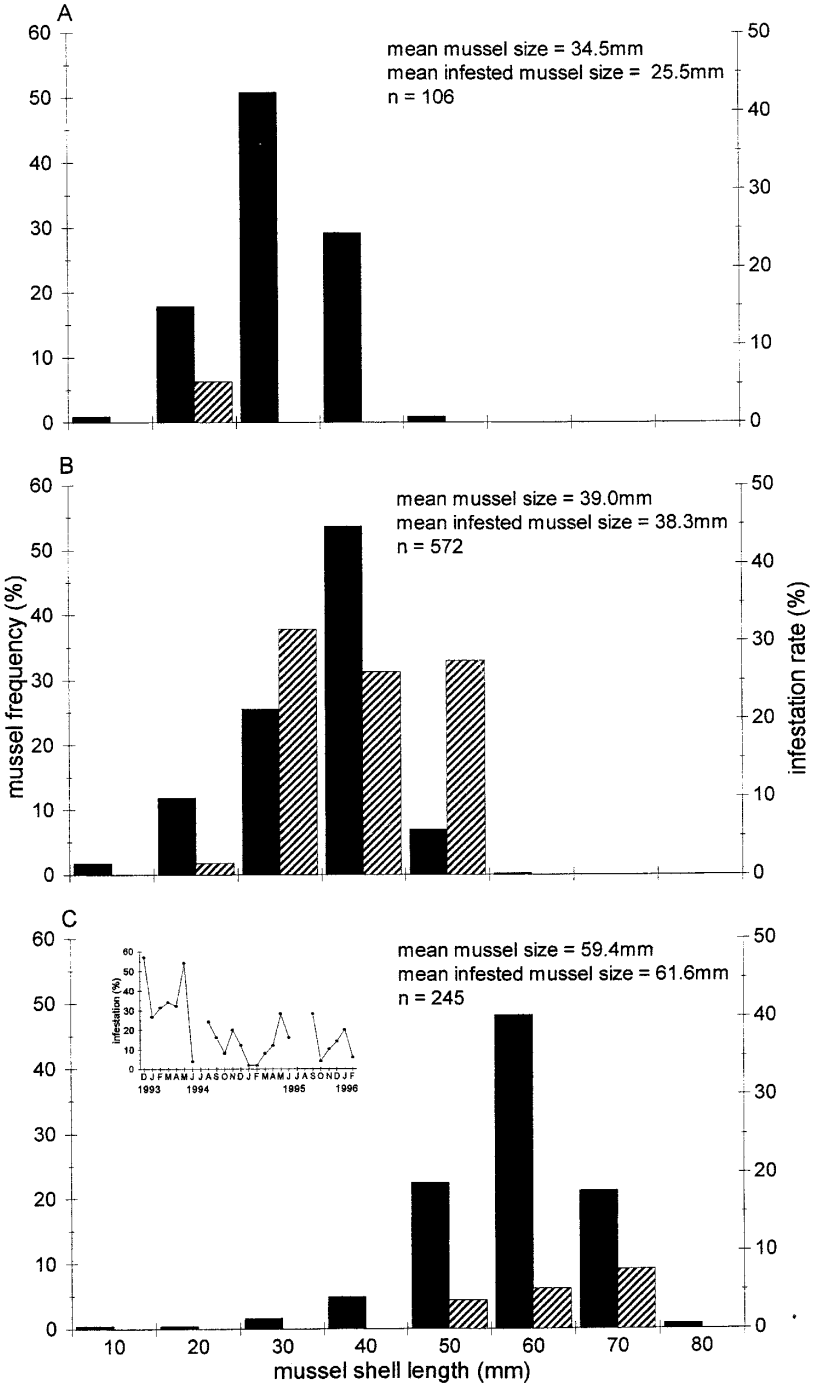

Figure 2. Length frequency distributions (filled bars) of *Mytilus edulis chilensis* together with infection rates (hatched bars) by *Coccomyxa parasitica* at Goose Green in November 1995. **A.** High mussel zone, **B**. mid zone and **C**. low zone. **C.** Inset. Seasonal variation in the infection rate of *Coccomyxa parasitica* within the host mussel *Mytilus edulis chilensis*, from the mid zone of the mussel population.

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susceptible to the alga and this together with the reduced body condition of infected *P. magellanicus* led Naidu (1971) to conclude that the endozoic alga (now believed to be *C. parasitica*) is a parasite. Stevenson and South (1974) further suggested that this relationship was probably facultative since the isolated alga could be grown successfully on an inorganic medium. Clearly further information on this interesting and unusual relationship between *M. e. chilensis* and *C. parasitica* is required.

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