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SURVIVAL AND DEVELOPMENT OF *CALIGUS EPIDEMICUS* HEWITT IN SEA WATER OF DIFFERENT SALINITY

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Abstract

Adult *Caligus epidemicus* can survive in 1.0 to 5 0 ppt. salinity for at least 40 h *in vitro*. Individuals can revive in normal salinity after short (15 min.) exposure to distilled water, but longer exposure is lethal. Eggs develop, hatch and moult successfully to nauplius and copepodid in salinity from 20 to 50 ppt. No embryos develop in 0 ppt. Eggs develop at 5 ppt but are not extruded; extruded eggs do not hatch at 10 ppt. Few eggs hatched and developed in 60 ppt salinity. These data are consistent with the euryhaline inshore and estuarime distribution of the copepod. Treatment of infection with fresh water would have to be done repeatedly and for extended periods.

Introduction

Caligus epidemicus is an important pathogen of captive (Roubal, 1995) and wild fish (Hewitt, 1971). The parasite occurs in inshore waters and estuaries of the western Pacific (Lin & Ho 1993). Studies have been done on some aspects of their developmental biology (Hewitt, 1971, Lin & Ho, 1993, Hallett & Roubal, 1995), but little is known about ecological factors that influence development and distribution. This study was done to investigate the salinity tolerance *in vitro* of adult parasites and their free-swimming larvae.

Materials and methods

Adults of *Caligus epidemicus* were collected from *Acanthopagrus australis* in the pond at Sea World, Gold Coast, Queensland and from fish caught previously in the wild and maintained in aquaria at 35 ppt. salinity. Fish were anaesthetised in 40 ppm benzocaine, adult parasites collected from the sediment and body surface, rimsed and maintained for up to four hours (h) in fresh sea water (30 ppt). Male and female copepods (n = 127) were placed individually in either 50 ml plastic jars or 4 cm (5ml) plastic Peri dishes filled with either diluted sea water (hyposaline: 5,10,15,20 ppt), distilled water (0 ppt) or 30 ppt sea water (control). Adult parasites (n = 16) were also exposed to hypersaline medium (40, 50, 60 ppt) made from artificial sea water. Salinity was measured with a hand-held refractometer. Penicillin (100 IU/ml), streptomycin (100µg/ml) and fungizone (0.25µg/ml)) were added to prevent bacterial growth. Preliminary observations found this medium not to be toxic. The activity and survival of adult parasites was monitored for up to 40 h at 24°C (±1°C). Activity was defined as the ability to swim or move limbs when disturbed by a gentle mechanical stimulus. Live, healthy adults are translucent whereas some inactive parasites became opaque and showed no limb movement and only occasional peristalsis of the gut and/or ducts in the genital segment. Dead parasites became opaque. Adult parasites (n = 20) were transferred from 30 ppt salinity to either 0 or 2 ppt for either 15-20 minutes or 1 h then returned to 30 ppt.; their ability to regain swimming activity was monitored.

Egg sacs (either attached to female or detached, n = 46) were placed in either hyposaline (0, 5, 10, 15, 20 ppt), control (30 ppt) or hypersaline (40, 50, 60 ppt) medium and their success to develop and moult was recorded for 40 h.

Results

The survival and activity of adult parasites in hyposalinity, control salinity (30 ppt) and hypersalinity are shown in Table 1. All adult *C. epidemicus* survived off the host in

Table 1. Salinity tolerance of adult *Caligus epidemicus*

		Hours			
Salinity (ppt.)	Sample size	0.25	1.0	16-20	40
0	10	0/10*	0/10	10X	10X
5	24	11/13	14/10	2/22	24X
10	30	24/6	24/6	23/7	17/13
15	27	27/0	27/0	25/2	24/3
20	21	21/0	21/0	21/0	18/3
30	15	15/0	15/0	15/0	15/0
40	6	6/0	6/0	6/0	4/2
50	5	5/0	4/1	4/1	3/2
60	5	0/5	0/5	0/3, 2X	5X

*, number active/number inactive; X, dead.

20 - 40 ppt. for up to 20 h and all controls for 40 h. Most copepods survived for 40 h in the 10 to 50 ppt salinity range. In 5 ppt salinity, about half were active for up to 1 h although 2 were active after 20 h; all were dead by 40 h. Copepods rapidly became inactive at 0 ppt but had internal organ movement after 1 h; all were dead by 20 h. A similar pattern was found in 60 ppt although 3 copepods had internal activity at 20 h; all were dead by 40 h.

Eight of 12 adults exposed to 0 ppt salinity for less than 15 minutes eventually regained mobility on return to 30 ppt sea water. Seven of eleven copepods exposed to 0 ppt for 20-25 minutes also regained mobility. Exposure to 0 ppt salinity for more than 1 hour was lethal. During exposure to low salinity, the copepod body became opaque, but returned to its normal translucent state in those copepods that revived in 30 ppt sea water.

The success of the embryo to develop, hatch and moult is shown in Table 2. Development was normal in 20 and 30 ppt, but hatching and moulting success were reduced at 15 ppt. More than half of the eggs were extruded at 10 ppt but none hatched, and no eggs were extruded at 5 ppt. Developing embryos in distilled water degenerated in the egg sac and nauplii exposed to distilled water died in 1 - 2 minutes with a ruptured cuticle. Eggs were extruded and hatched at 40 and 50 ppt and a few moulted to the copepodid stage in 50 ppt salinity. Few eggs developed or hatched in 60 ppt salinity although a few larvae developed to the second nauplius stage.

Table 2. Success of development by eggs and moulting in different salinity.

Salinity (ppt)	Embryos develop	Eggs extruded	Nauplius I hatches	Nauplii 1, 11 moult
0	-	-	-	-
5	++	-	-	-
10	++++	+++	-	-
15	++++	++++	++	+
20	++++	++++	++++	++++
30	++++	++++	++++	++++
40	++++	++++	++++	++++
50	+++	+++	++	+
60	+++	+	+	-

success rate: - 0%, + < 10%, ++ < 30%, +++ < 60%; ++++ > 60%

Discussion

Caligid copepods in warm water can withstand a wide range of salinities. Thus, *Caligus minimus* from two species of *Dicentrarchus* tolerated a salinity of 20 - 55 ppt but 20 ppt and 55 - 60 ppt were detrimental to the copepodids. *Caligus pageti* was found on grey mullets in salinities from 40 to 80 ppt whereas *Pseudocaligus apodus* was found on grey mullet in 20 to 70 ppt salinity (Paperna, 1977). *Caligus epidemicus* lives on a wide range of hosts in inshore and estuarine environments (Hewitt, 1971). These are subjected to hyposalinity associated with the tidal cycle and seasonal rain periods, but the fish may also enter inshore lagoons and other embayments that are subjected to evaporative loss and mcreased salinity. There will be selection for a euryhaline capability in the parasite.

Gradual acclimatisation of the host and parasite to either high and low salinity may extend the tolerance of the parasite to extreme salinities. Finstad *et al.* (1995) found that *Lepeophtheirus salmonis* attached to Arctic charr could survive for up to three weeks in fresh water. Attached L. *salmonis* were able to survive longer in fresh water than were detached parasites, presumably the result of salt replenishment from the mucus and host tissue (Hahnenkemp & Fyhn, 1985).

The fully developed embryo within the egg sac of *C. epidemicus* is enclosed in several membranes (cuticle, egg membranes, egg sac). Once extruded from the sac, the embryo is enclosed in egg membranes as well as the cuticle (Lin & Ho, 1993) whereas the hatched nauplius is covered in cuticle only. This may explain how embryos can survive and are extruded from the egg sac at a lower salinity than that tolerated by the nauplius.

Landsberg *et al.* (1991) found a dip in freshwater for 20 minutes killed all copepods of *Caligus elongatus* on red drum, *Sciaenops ocellatus.* Some *Caligus epidemicus* would survive such a treatment, although most would be likely to detach from the host. A longer bath in freshwater would be needed to kill attached and detached adult copepods. Future work needs to examine the survival in different salinities of chahmus, pre-adult and adult stages of *C. epidemicus* attached to different host species.

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