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Effect of temperature and salinity on egg hatching and description of the life cycle of *Lernanthropus latis* (Copepoda: Lernanthropidae) infecting barramundi, *Lates calcarifer*

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ABSTRACT

The parasite *Lernanthropus latis* (Copepoda: Lernanthropidae) is a major threat to the sustained mariculture of barramundi, *Lates calcarifer* (Perciformes: Latidae). We investigated the effect of water temperature and salinity on egg hatching success of *L. latis* and describe the life cycle for the first time. Wild and sea-caged *L. calcarifer* examined in tropical north Australia exhibited similar parasite prevalence (range: 80–100%) and mean parasite intensity (range: 3–6), whereas land-based maricultured fish were not infected. Hatching success and time to first and last hatch was determined for a range of water temperature (22, 30, 32 and 34 °C) and salinity (0, 11, 22, 35 and 40‰) combinations representing current and predicted climate conditions. There was a significant interaction between water temperature and salinity on the hatching success of *L. latis* nauplii. Eggs hatched in all temperature treatments, with the greatest hatching success at 30 °C and 32 °C (98 and 92% success, respectively) in 35‰. Hatching did not occur at 0‰ and was severely reduced at 11‰ (1.6% success). Hatching began within 6 h at all water temperatures with >95% of eggs hatched within 30 h at 30, 32 and 34 °C and within 60 h at 22 °C. Adult parasites differed from the original description by the presence of the parabasal flagellum, small setae on the legs and caudal rami and minor incongruences regarding morphological measurements. The life cycle of *L. latis* includes three free living stages and five parasitic stages. Although *L. latis* exhibits broad environmental tolerance, freshwater can be used as an effective management strategy to break the life cycle in aquaculture.

1. Introduction

Barramundi (*Lates calcarifer* Bloch, 1790) is a catadromous teleost that inhabits freshwater rivers, lagoons, mangrove swamps and coastal bays throughout southern and southeast Asia, Papua New Guinea and northern Australia [1–3]. It is a species of considerable economic value for recreational and commercial fishers and aquaculture in Australasia [4–6]. Reliable hatchery production of *L. calcarifer* has enabled aquaculture expansion and global production is now ~26,000 tonnes per annum, with the majority cultured in the Indo-Pacific in brackish or marine systems [7].

The *L. calcarifer* aquaculture industry is facing severe challenges from parasitic copepod epizootics. Most parasitic copepods exhibit monoxenous (direct, single host) life cycles which facilitate the rapid completion of their life cycles and prolific reproduction in aquaculture environments [8,9]. Climate change is expected to exacerbate the frequency and intensity of disease epizootics by enabling some pathogens to complete their life cycles faster [10–12]. Several copepod species threaten *L. calcarifer* production, including, *Caligus chiastos* Lin & Ho, 2003, *Caligus epidemicus* Hewitt, 1971, *Caligus rotundigenitalis* Yü, 1933, *Caligus punctatus* Shiino, 1955 (see [13]) and *Lernanthropus latis* Yamaguti, 1954 (see [14,15]).

Species in *Lernanthropus* de Blainville, 1882 present considerable concern for sustained global production of finfish. Damage to fish occurs through the parasite's attachment appendages that constrict the flow of blood through the gills, and high burdens can cause anaemia and a loss in respiratory surface area [15–20]. Extensive research has been dedicated to *Lernanthropus kroyeri* (see [21–32]) which infects farmed fishes in the Mediterranean, but research on tropical *Lernanthropus* spp., such as *L. latis*, is comparatively sparse [14]. This study examined the hatching success of *L. latis* infecting *L. calcarifer* in current and predicted water temperature and salinity scenarios and provided the first description and illustrations of all life cycle stages.

2. Materials and methods

2.1. Fish and parasite collection

L. calcarifer examined for *L. latis* were collected from four localities in tropical north Australia between October 2010 and June 2011 (Table 1; Ethics approval number: A1579, James Cook University). Fish from the wild (Cleveland Bay, Townsville; 19.284331: 146.866419) were

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Table 1
Host localities indicating number of fish examined, parasite prevalence (P), mean intensity (I) and mean abundance (A) of adult *Lernanthropus latis*. WA = Western Australia. QLD = Queensland. Cone Bay, WA fish collected in March 2011, Cleveland Bay, QLD fish collected between March and June 2011, Hinchinbrook, QLD fish collected in October 2010 and Good Fortune Bay, QLD fish collected in May 2011. Measurements in millimetres (mm); mean followed by range in parentheses.

Location	Origin	# Fish	Fish size	Female			Male			Total		
				P	I	A	P	I	A	P	I	A
Cone Bay, WA	Sea-cage	10	438 (310–540)	100%	3	2.6	0%	0	0	100%	3	2.6
Cleveland Bay, QLD	Wild	20	515 (420–560)	80%	6	4.8	25%	1.8	0.7	80%	6	4.8
Hinchinbrook, QLD	Sea-cage	26	574 (515–650)	89%	6	5	8.6%	1.3	0.11	89%	6	5
Good Fortune Bay, QLD	Land based	10	491 (435–540)	0%	0	0	0%	0	0	0%	0	0

captured by a commercial fisherman using a gill net. Fish from two Queensland farms were captured from their sea-cage (Bluewater Barramundi, Hinchinbrook: 18.392292: 146.136547) or raceway (Good Fortune Bay, Bowen: 19.940108: 147.932147) using hand nets and cast nets, respectively. Freshly killed sea-caged fish from Western Australia (Marine Produce, Cone Bay: 16.472297: 123.543144) were refrigerated and freighted to the laboratory for examination. Gills were removed from fish and placed into individual petri dishes filled with filtered seawater. Gill arches were separated with dissection scissors and individual gill filaments were observed under a stereomicroscope (~20× magnification). Parasites were removed from filaments with fine forceps. Representative male and female specimens were fixed in 70% ethanol for morphology (see Section 2.4 below).

2.2. Incubation and hatching of L. latis eggs

Live ovigerous females from wild hosts collected at Cleveland Bay, Townsville between March and June 2011, were used for hatching and infection experiments (see Section 2.3 below). Over the sampling period, sea surface temperature ranged between 22 and 27 °C. *L. latis* bear uniserate egg sacs in which disc-shaped eggs are closely packed. Paired egg sacs were gently detached from the genital complex using fine-tipped forceps and placed in filtered sea water (35‰). Eggs within the sac were counted under a stereomicroscope using a hand-held counter. Egg sacs were randomized and placed in 100 mL plastic containers with 80 mL of 0, 11, 22, 35 or 40‰ saline solution and were incubated at 22, 30, 32 or 34 °C (Sanyo: ML-351 Versatile Environmental Incubation Chamber). Each treatment had four replicates.

The water temperature treatments represented the average winter ocean temperature (22 °C) and average summer ocean temperature (30 °C) and the maximum summer ocean temperature ever observed (32 °C) at Lucinda, Queensland (approximately 100 km north of Cleveland Bay and the location of the temperature measuring station closest to Cleveland Bay, Townsville) [33]. The maximum water temperature (34 °C) represented the average summer ocean temperature predicted by 2050 at Lucinda, Queensland [34]. A two degree water temperature increase is predicted under the IPCC (Intergovernmental Panel on Climate Change) scenario of 'business as usual' emission levels by 2050 [34,35]. The salinity treatments represented normal seawater environments (35‰), extreme hyposaline and hypersaline environments (0‰ and 40‰) and pulse rainfall events (11‰, 22‰). Hypersaline solution was prepared through the addition of Mermaid Marine salt to 35‰ filtered seawater. Hyposaline solutions (11‰, 22‰) were prepared by mixing distilled water and filtered seawater to the desired concentration in a sterile container. Salinities were determined using a refractometer.

Incubators were lit with fluorescent lighting for a 12:12 day:night cycle. The water was aerated throughout the incubation process using a battery powered aerator. Eggs were monitored every 6 h. Monitoring ceased following a 24 h period without hatching in any treatment. Following hatching, a representative sample of five individual nauplii was aspirated with a pipette every 6 h and fixed in 70% ethanol to document larval development. Hatching success was defined as the number of eggs that hatched into swimming nauplii.

Proportional hatching data were arcsine transformed and analysed by PERMANOVA in PRIMER 6.0. A PERMANOVA with 9999 permutations based on Euclidean distance, followed by pairwise comparisons was performed. The advantage of using a permutation approach to data analysis is that the resulting test is 'distribution free' and does not assume normality or homogeneity of variance. Significance was accepted at $p < 0.05$.

2.3. Infection of L. calcarifer

Four *L. calcarifer* mean 180 mm L_T (100–220 mm), purchased from Good Fortune Bay barramundi farm, Townsville, Queensland, were acclimated to seawater for 1 h. To establish an infection, the four fish were placed in two 20 L containers (two fish per container) containing 5 L of 35‰ filtered seawater held at ambient temperature with constant aeration. Approximately 400 stage I copepodids (incubated at 30 °C, 35‰) were introduced using a glass pipette. Stage I copepodids were obtained by hatching eggs from eggs sacs removed from live ovigerous females, detached from wild hosts collected at Cleveland Bay, Townsville on the 16th of September, 2011. Approximately one third of the seawater was changed each day after an initial 48 h period to allow the copepodids time to attach to the gill filaments of the fish. One fish was removed three days following first exposure and euthanized with Aqui-S (1:5000). One fish was euthanized every two days thereafter. The gills were removed from the fish and each arch was separated with dissection scissors. Gill filaments were observed for parasites using a stereomicroscope. When found, the filament bearing a parasite was detached from the arch and placed in a petri dish of fresh, filtered seawater. The parasite was removed from the filament using fine-tipped forceps and placed onto a microscope slide for morphological examination.

2.4. Morphology

Parasite specimens were cleaned from debris using a fine-bristle paintbrush and cleared in lactophenol. The antennules, parabasal flagella, antennae and legs were dissected from adult specimens using entomological dissection pins and fine-tipped forceps. Measurements were made from photographs taken of morphology with the use of a micrometre and ImageJ 1.44p (Java 1.6.0_20). Measurements indicate the mean in μm, followed by the range in parentheses. Representative adults, nauplii and copepodites were accessioned in 70% ethanol to the crustacean collection in the South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia. The type series for *L. latis* was requested from the Meguro Parasitological Museum, Japan, for comparative purposes (accession number 20784) and Director Kazuo Ogawa provided digital photographs for review.

3. Results

3.1. Prevalence, intensity and abundance

No *L. latis* infected fish from the land-based marine raceway in Bowen, Queensland (Table 1). Prevalence of infection by female parasites