Caligus longipedis Infection of Cultured Striped Jack, Pseudocaranx dentex (Teleostei: Carangidae) in Japan

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A caligid copepod was collected from the body surface of striped jack, Pseudocaranx dentex cultured in net cages in western Japan. It is identified as Caligus longipedis Bassett-Smith, 1898. Adults as well as all the developmental stages are described; morphologically they are divided into 2 nauplius, 1 copepodid, 4 chalimus, 1 preadult and 1 adult stages. Newly hatched nauplii moulted two times into copepodids in about 19 h at 22.5 ± 2.0°C. Copepodids survived in sea water 7 days at most at 20°C. Chalimus larvae were found on the fins and body surface of fish. When free-swimming larvae were exposed to uninfected fish, they were transformed into adults on Day 10 (10 days after exposure to uninfected fish), and possessed egg sacs on Day 12 at 21.2 ± 0.6°C. Nauplii were recovered from the rearing water on Day 13. It is thus estimated that the life cycle was completed in about 2 weeks.

The striped jack, Pseudocaranx dentex, is extensively cultured in net cages in western Japan, with the annual production of 959 metric tons according to the latest governmental statistics in 1990. With increasing production, caligid copepod infections have been encountered in many culture sites. Fish were sometimes heavily infected with several hundreds of copepods, often unmarketed because of bruises on the body surface caused by the caligids.

In this study, the parasite has been identified, adults as well as all the developmental stages have been described, and some data on the development have been obtained by incubating free swimming larvae and exposing them to uninfected fish.

Materials and Methods

Identification of the Caligid and Description of Developmental Stages on the Fish

Fresh adults and chalimus larvae were collected on 19 August and 4 November from the striped jack cultured in Shizuoka Prefecture, 1988 and fixed in 70% alcohol. Some additional adult specimens were obtained from the same species of host from Kochi Pref. fixed in January and March, 1989 and from Oita Pref. in October 1989. They were cleared in lactic acid and observed microscopically. Drawings were made with the aid of a camera lucida. Terminology on the copepod structure was based on Kabata (1979).

Developmental Stages from Nauplius to Copepodid

Egg sacs were removed from female adults and kept in a small Petri dish filled with filtered sea water. Newly hatched nauplii were immediately transferred individually into a cell-well (24-well plate; Corning Glass Works, U. S. A.), each containing about 2 ml sterilized sea water, which was not changed during the observation. The time of moults was recorded by confirming the presence of sloughs in the water by a stereomicroscope. Water temperature was 22.5 ± 2.0°C. Newly moulted copepodids consisting of three groups (19, 22 and 26 individuals each) were individually kept in a cell-well as above. They were observed daily under a stereo-microscope to record the survival time. Sea water was changed daily at the time of observation. Copepodods which did not react to gentle water currents generated by a Pasteur pipet or did not make any move for more than 30 sec were con-
sidered to be dead. Water temperature was 20.0 ± 0.5°C.

Attachment Sites of Chalimus Larvae on the Host
Chalimus-stage larvae were detected by the naked eye from 15 striped jack of the year (standard length 15.8–21.0 cm), and sites of attachment were recorded.

Infection Experiments
In the first trial, about 170 copepodids obtained from the rearing experiments of newly hatched nauplii were used. In the next trial, nauplius and copepodid stage larvae were collected from filtrates of effluent for 24 h from a 1.5 ton running-water tank containing ten heavily infected fish. Experimental fish had been slightly infected with the caligid; after eradication of the parasites by dipping them in fresh water for about 10 min, they were transferred into a 1.5 ton tank. Subsequently, the experimental fish (25 fish in the first and 18 in the second experiment) were exposed to the parasite larvae for 24 h. The water was aerated but no fresh sea water was added into the tank during the time of exposure. The fish were sampled six times in the two-week experiment time. Parasites detected on the experimental fish were fixed in 70% alcohol, and observed as described above. Water temperature during the experiments was 21.2 ± 0.6°C.

Results
Description of Adult Stage
Caligus longipedis Bassett-Smith, 1898

Synonyms: Caligus longipes Basset-Smith, 1899
Caligus scabiei Gnanamuthu, 1950
Caligus amplifurcus Pearse, 1953
Caligus rugosus Shiino, 1959
Caligus lucidus Heegaard, 1962

1) Female (based on 10 specimens; Figs. 1a, 2–12):
Body 4.10–4.61 mm long. Cephalothorax: orbicular, almost equally long and wide (2.58–2.94 × 2.45–2.92 mm); frontal plates indented centrally; lunules well developed, 0.29–0.36 mm in diameter. Fourth leg-bearing segment short, 0.24–0.33 mm long by 0.50–0.76 mm wide. Gen-

Fig. 1. Caligus longipedis. a, adult female; b, adult male. Scale: 1 mm.
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Caligus longipedis female. 2, first antenna; 3, second antenna (a), postantennary process (b) and first maxilla (c); 4, sternal furca; 5, mandible; 6, second maxilla (a, whole; b, tip with a higher magnification); 7, maxilliped; 8, first leg; 9, second leg, ventral; 10, third leg, ventral; 11, fourth leg; 12, genital segment (posterior end), abdomen and caudal ramus. Scale I for 3, 4, 6a and 8-12: 0.4 mm; scale II for 2, 6b and 7: 0.2 mm; scale III for 5: 0.05 mm.

First antenna (Fig. 2): two-segmented; basal segment with 27 setae on anterior margin; terminal segment with 12 setae. Second antenna (Fig. 3a) with prominent process of second segment. Postantennary process (Fig. 3b) slender, mildly curved. First maxilla (Fig. 3c) subtriangular, with blunt tip. Sternal furca (Fig. 4) with almost parallel tines, 0.25-0.33 ×
Table 1. Armature of thoracic leg I-IV of Caligus longipes

<table>
<thead>
<tr>
<th>Leg</th>
<th>Surface</th>
<th>Interpodal plate</th>
<th>Sympod</th>
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<td>1rh</td>
<td>4H</td>
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<td>c</td>
<td>3P</td>
<td>c, c</td>
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<tr>
<td>I</td>
<td>Outer</td>
<td>1s, f, 1s</td>
<td>f, 1H'</td>
<td>1H', 1Q, 1Q'</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>Inner</td>
<td>1P, f, 1s</td>
<td>c, 1P</td>
<td>c, 1P</td>
<td>c, 2P, 4P</td>
</tr>
<tr>
<td>III</td>
<td>Outer</td>
<td>f, 1p</td>
<td>1H', 1s</td>
<td>c, 1H</td>
<td>c</td>
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<tr>
<td></td>
<td>Inner</td>
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<td>1P</td>
<td>1p, 3P</td>
<td>1P, 6P</td>
</tr>
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Abbreviations: c, hairy margin; cc, hairy area; f, membraneous flange; H, larger simple spine; h, smaller simple spine; H', hook-like spine; p, longer plumose seta; P, shorter plumose seta; Q, spine hairy on one side, rimmed with membrane on the other; Q', spine rimmed with membrane on one side; rh, tiny simple spinele; s, hair-like seta.

0.14–0.16 mm. Mandible (Fig. 5) with 12 teeth. Second maxilla (Fig. 6): calamus about 3 times as long as canna; canna doubly edged with serrated rim and calamus armed with 3 rows of more finely serrated filaments, which coiled several times toward tip; outer border of anterior half of brachium serrated, with small rounded protrusion at anterior end; flabellum in distal half of brachium on inner margin, large with tip almost reaching anterior end of brachium. Maxilliped (Fig. 7) with subchela about half length of corpus.

First leg (Fig. 8) with 2 of 4 apical spines forked. Second leg (Fig. 9): distal segment of endpod with two small flap-like projections on lateral surface. Third leg (Fig. 10) with expanded sympod, distinctly three-segmented exopod and two-segmented endpod; basal segment of exopod armed with stout, blade-shaped hook, 0.065–0.079 mm long, with membrane on lateral surface and blunt tip. Fourth leg (Fig. 11) with sympod and two-segmented exopod; exopod with 4 slender spines: one at laterodistal end of basal segment, 0.16–0.21 mm long and 3 at tip of distal segment, 0.14–0.17 mm, 0.19–0.25 mm and 0.23–0.30 mm long (measured from inner to outer order); outermost spine being longest and curved inward at tip, all provided with large pecten basally. Fifth leg (Fig. 12) vestigial.

Armature of four pairs of legs is listed in Table 1.

2) Male (based on 10 specimens; Figs. 1b, 13–16):

Body 3.35–6.60 mm long. Cephalothorax: widened posteriorly, 2.05–4.58 × 2.03–5.45 mm, almost equally long and wide in small males but wider than long in large males. Lunules 0.25–0.50 mm in diameter. Fourth leg-bearing segment short, 0.19–0.42 × 0.54–1.06 mm. Genital complex (Fig. 16) barrel-shaped, 0.50–0.99 × 0.50–1.00 mm, with very thick cuticle (9–17 μm in thickness). Division between genital complex and abdomen distinct. Abdomen narrow and indistinctly two-segmented, 0.33–0.80 × 0.30–0.64 mm. Caudal ramus 0.23–0.55 × 0.16–0.35 mm.

Second antenna (Fig. 13a): second segment inflated with two adhesion pads. Sternal furca (Fig. 14) 0.26–0.48 mm × 0.13–0.25 mm. Maxilliped (Fig. 15): much more robust than in female; corpus equipped on inner border with two conical processes and a bulge with flattened top between them.

Developmental Stages from Nauplius to Copepodid

First nauplius (Fig. 17), oval in shape, 0.33–0.45 mm long (N: 87). Second nauplius (Fig. 18), slightly more slender, 0.44–0.51 mm long (N: 31). Copepodid (Fig. 19) 0.45–0.63 mm long (N: 20), consisting of cephalothorax and four posterior segments.

First nauplii moulsted 2.1–2.5 h after hatch to second nauplii, which were then transformed into
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copepodids after 16.5–17.2 h from the first moult. These were based on 17 individual observations.

Copepodids survived in sea water 7 days at most after the moult. Fifty percent survived approximately 4 days (Fig. 28; based on observations of 67 individuals).

Description of Developmental Stages on the Fish

Developmental stages of the copepod collected from the fish were divided into 6: 4 chalimus, 1 preadult and 1 adult stages, according to their morphology and body sizes.

First chalimus (Fig. 20): considerably larger than copepodid, 0.75–0.89 mm long (N: 14); segmentation less distinct. Second chalimus (Fig. 21): 1.05–1.56 mm long (N: 21); cephalothorax incorporating third thoracic segment. Sexes differentiated from third chalimus.

Caligus longipedis male. 13, second antenna (a), postantennary process (b) and first maxilla (c); 14, sternal furca; 15, maxilliped; 16, genital segment (posterior end), abdomen and caudal ramus. Scale I for 13–15 : 0.4 mm; scale II for 16 : 0.4 mm.

Developmental stages of Caligus longipedis-I. 17, first nauplius; 18, second nauplius; 19, copepodid; 20, first chalimus; 21, second chalimus. Scale: 1 mm.

Developmental stages of Caligus longipedis-II. 22, third chalimus (a, female; b, male); 23, fourth chalimus (a, female; b, male); 24, preadult (a, female; b, male). Scale: 1 mm.

Figs. 17–21.

Figs. 22–24.
Fourth thoracic segment incorporated into cephalothorax at this stage. Females (Fig. 22a): 1.87–2.07 mm long (N: 14); second antenna still unsegmented; genital complex with small posterior indentations on both sides of genital complex. Males (Fig. 22b): 1.83–2.04 mm long (N: 9); second antenna two-segmented, slightly larger than that of female; genital complex without posterior outgrowths.

Fourth chalimus (Fig. 23) 2.57–3.17 mm long in females (N: 13) and 2.88–3.27 mm in males (N: 10). First leg: sympod with two small plumose setae; exopod unsegmented with 3 unarm ed setae on posterior margin and 4 spines on distal margin; only innermost spine armed with minute prickles; endopod vestigial. Second leg: sympod unsegmented without membrane; exopod unsegmented without membrane, armed with 4 spines on lateral margin and 7 unarmed setae on posterior margin; endopod two-segmented; distal segment with 8 unarmed setae and basal segment with an unarmed seta. Third leg: exopod two-segmented with a hook-like spine at basal segment and 9 unarmed setae at distal segment; endopod unsegmented with 7 unarmed setae. Fourth leg unsegmented with 3 setae at distal end and a seta on posterior margin, all unarmed and unaccompanied by pectens.

Female preadult (Fig. 24a) (3.51–3.98 mm long; N: 38) was distinguished from the adult female by having relatively thin cuticle and poorly developed genital complex. Body length of male preadult (Fig. 24b) varied widely from 3.19 to 5.38 mm (N: 37). This stage was distinguished in general appearance from the adult male by having relatively thin cuticle. This was most prominent in the genital complex; 9–17 μm in adults vs 2–3 μm in preadults. Three out of 37 specimens observed still carried the frontal filament, their body length ranging from 4.32 to 4.50 mm (Figs. 24b & 27). No difference was found in the segmentation and armature of the appendages between the preadult and adult.

Frequency distribution of body length of chalimus stage larvae (Fig. 25) showed four clear peaks according to the number of chalimus stages. No sexual dimorphism was observed in these stages. In the preadult and adult, body length in males was much more widely distributed than in female, and males finally grew bigger than females (Figs. 26 & 27). Body length of adult females and males ranged 3.53–4.77 mm (N: 202).
340) and 3.16-6.58 mm (N: 62), respectively.

Attachment Sites of Chalimus Larvae on the Host
A total of 70 chalimus larvae were recorded for their attachment sites on the external surface of the host; 42 (60%) were attached on the body surface and 28 (40%) were on the fins (pectoral, dorsal, anal and caudal fins). Fig. 29 illustrates the attachment sites of individual larvae collected from the left side of the body (N: 40). No larvae were found on the gills, inner surface of opercula or buccal cavity wall.

Infection Experiments
In the first trial, only 4 chalimus larvae were obtained 6 days after the start of exposure (Day 6). In the second trial, most of the fish were infected and a total of 91 specimens of different developmental stages were collected from Day 3 to Day 14. Chalimus larvae were collected from the fins and body surface and preadults and adults were from the body surface. Since no difference was found in the development between the two trials, the results were put together (Fig. 30).

On Day 3 only first chalimus larvae were collected (N: 8; 0.70-0.84 mm in body length). On Day 5, both second chalimus (N: 4; 1.15-1.37 mm long) and third chalimus (12 females and 14 males; 1.79-2.07 mm long), and on Day 6, both third chalimus (2 females and 1 male; 2.01-2.06 mm long) and fourth chalimus (2 females and 2 males; 2.81-3.28 mm long) were collected.

On Day 10, all specimens were young adults. All females had already been impregnated, but possessed no egg sacs yet. Females (N: 14) were 3.75-4.09 mm long, and males were 3.44-3.71 mm long. On Day 12, females (N: 6) were 4.06-4.42 mm long, and males (N: 6) were 3.39-4.20 mm long. Four out of 6 females had a pair of egg sacs containing 22-24 eggs each. On Day 14, females (N: 12) were 4.07-4.43 mm long and males (N: 7) were 3.50-3.90 mm long. Number of eggs in each egg sac was 32-41.

On Day 13, nauplii were collected from filtrates of the effluent from the experimental tank.
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Fig. 30. Growth of *Caligus longipedis* on experimentally infected fish. Vertical bars represent ranges of body length, with a mean shown by a transverse slit in the bar.

Discussion

Kubota and Takakuwa (1963) reported *Caligus amplifurcus* from cultured *Caranx delicatissimus* (=*Pseudocaranx dentex*) in Mie Prefecture, Japan; this species was synonymized with *C. longipedis* by Lewis (1967). Outside Japan, *C. longipedis* has so far been known to be parasitic on fishes of the genera *Caranx, Cantherhines, Trichiurus* and *Seriola* (?) from different localities like Gulf of Aden, Gulf of Mexico, the Indian and Pacific Ocean and the Tasmanian Sea (Basset-Smith, 1898; Gnanamuthu, 1950; Pearse, 1953; Shiino, 1959; Heegaard, 1962; Lewis, 1967), while in Japan, it has been found only on striped jack.

The development of this species has been divided into 2 nauplius, 1 copepodid, 4 chalimus, 1 preadult and 1 adult stages. A substantial difference between this result and the general account on the stages by Kabata (1981) lies in that in the latter, there is another preadult stage in all the members of the family Caligidae. Developmental stages are finally determined by confirming moults, but, for practical reasons, moults were observed only in the naupliar stages in this study. All the descriptions on the stages of *Caligus* species have so far been based mainly on the morphological characteristics. Among them, exactly the same stages were described in *C. clemensi* on pink salmon, *Oncorhynchus gorbuscha* by Kabata (1972) as in *C. longipedis* in this study, and 9 stages comprising 2 nauplius, 1 copepodid, 3 chalimus, 2 preadult and 1 adult stages were described in *C. spinosus* from the yellowtail, *Seriola quinqueradiata* by Izawa (1969), and 9 stages (2 nauplius, 1 copepodid, 5 chalimus and 1 adult) were described in *C. orientalis* on the Mozambique tilapia, *Tilapia mossambica* (=*Oreochromis mossambicus*) by Hwa (1965). It is interesting to note that they all have 9 stages. When stages are compared between this species and a closely related caligid *Lepeophtheirus salmonis* which has 10 stages as defined by Kabata (Johnson and Albright, 1991), fourth chalimus was much the same in morphology, suggesting that, in *C. longipedis*, no stage was missed up to fourth chalimus. A clear difference, however, lies in the morphology of preadult; even a very young preadult of this species corresponded to the second preadult of *L. salmonis*. A total of 75 preadults (38 females and 37 males) were used in this study, and not a single specimen corresponding to the first preadult stage of *L. salmonis* was found. All these make it clear that only one stage of preadult was included in the present material. It seems likely that one developmental stage (=first preadult in *L. salmonis*) is suppressed in *C.
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longipedis.

There are several reports on the duration of moults in the free-swimming stages. In Caligus curtus, it took 24 h in the moult from first nauplius to second, and another 24 h from second nauplius to copepodid at 6–8°C (Heegaard, 1947), much longer time than in C. longipedis in this study, apparently because of much lower temperature in the former species. In C. spinosus, it took 4–6 h and 12–18 h at 20°C, respectively (Izawa, 1969); it was about twice as long as the time in C. spinosus from hatch to the first moult, implying that time required for each developmental stage may be different among species.

In the infection experiments, specimens collected on Day 10 were all in the adult stage suggesting that they underwent at least 6 moults in 10 days. Observations on the fecundity on Day 10, 12 and 14 indicate that females had egg sacs for the first time on Day 12 and some of those for the second time on Day 14. This means that eggs hatch in about two-day interval. The average number of eggs in a pair of egg sacs was about 70; about 35 nauplii hatched from one female every day. Nauplii appeared in the rearing water on Day 13. It is thus estimated that the life cycle was completed in about 2 weeks at 20°C.

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References


