Taxonomic study on crustacean parasites of the flathead grey mullet (*Mugil cephalus*) and red seabream (*Pagrus major*) in Hiroshima Bay, Japan

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**Abstract.** Crustacean fish parasites can be used as bio-indicators of pollution. This study examined the crustacean parasites of flathead grey mullet (*Mugil cephalus*) and red seabream (*Pagrus major*) landed at a fishing port in Hiroshima Bay. Specimens were collected from November 2015 to August 2016. Three species of parasitic crustaceans were identified. *Caligus* sp. (Copepoda: Siphonostomatoida: Caligidae) was collected from the gills of a male flathead grey mullet *Mugil cephalus*. The genus *Caligus* is the largest among the parasitic copepods, containing more than 250 species worldwide, and a total of 20 species have been found from marine fishes in Japan. *Lernanthropus atrox* Heller, 1865 (Copepoda: Siphonostomatoida: Lernaeopodidae) was collected from the gills of a female red seabream *Pagrus*. Copepods of the genus *Lernanthropus* are found attached to the gill filaments of marine fishes in the world. This species is known to occur in both Australian and Japanese waters; in Japan, it has been recorded from Tokyo Bay and the Seto Inland Sea. The cymothoid copepod *Ceratothoa verrucosa* (Schioedte and Meinert, 1883) (Isopoda: Cymothoidae) was found in the buccal cavity of a red seabream *Pagrus major*. Cymothoid isopods, including *Ceratothoa verrucosa*, are found in the buccal and gill cavities as well as on the body surface of fish hosts. This species is only known to occur in Japanese waters.

1. Introduction
The fishes of the family Mugilidae are economically important and are important food fishes. Their euryhalinity, eurythermality and simple diet as well as their rapid growth have made them the object of aquaculture in many parts of the world. They are of economic importance in various countries. Parasites have recently been highlighted as serious pathogenic problems in cultured mullets in marine and brackish waters. Among the parasites, copepods are commonly found on fishes cultured in brackish waters [1] and therefore a threat to the developing industry of finfish mariculture [2].

The flathead grey mullet *Mugil cephalus* Linnaeus, 1758 is the most widespread species among the family Mugilidae, which comprises 20 genera and 74 species, 11 of which belong to the genus *Mugil* [3]. One of the objectives of this study was to identify copepods found on *M. cephalus* in Hiroshima Bay, Japan. Copepods are aquatic crustaceans which are diverse and are the most numerous metazoans in the water community with habitats ranging from fresh waters to hypersaline conditions. Some copepod parasites may cause lesions that negatively affect the fisheries and aquaculture economies. Identification of copepod parasites on *M. cephalus* will provide better understanding of culture
condition for the species. This also will help to solve some of the problems of fish diseases that can negatively affect the culture of this fish.

The red seabream *Pagrus major* (Temminck and Schlegel, 1843) belongs to the family Sparidae and is a commercial important species found in the coastal waters of Japan. In this study, the lernaeopodid copepod *Lernanthropus atrox* (Heller, 1865) and the cymothoid isopod *Ceratothoa verrucosa* (Schioedte and Meinert, 1883) were found from the gills and mouth cavity and studied morphologically.

2. Materials and Methods

2.1. *Mugil cephalus* preparation

One individual of *Mugil cephalus* was collected by rod and line in a fishing port, Kure City, Hiroshima Prefecture, on 3 November 2015. The fish was transported in an ice box to the laboratory and examined for parasites. The body weight (BW) of the fish was taken using digital weighing balance and recorded. The standard length (SL) and total length (TL) were measured with the aid of meter rule. A stereo-microscopic observation was made for the presence of parasitic copepods on the body surface. Copepods were fixed and preserved in 70% ethanol. They were studied using both stereo and light microscopes. Selected specimens were examined with a series of magnification up to 40x/0.75. All drawings were made with the aid of a drawing to a compound microscope. In the description of the copepod found, the armature formula is shown using Roman and Arabic numerals.

2.2. Preparation of *Pagrus major*

One individual of *Pagrus major* was commercially caught in Hiroshima Bay on 2 August 2016. This fish was examined as described above, and isopods were found in the mouth cavity. They were fixed and preserved in 70% ethanol. Photographs were taken with a camera, and drawings were made using a microscope to identify them.

Another individual of *Pagrus major* was commercially caught in Hiroshima Bay on 21 December 2016. Copepods were found from the gills and fixed/preserved in 70% ethanol. They were identified by examining the isolated body parts and appendages under the compound microscope.

3. Results and Discussion

3.1. Description of male Caligus sp. (*Copepoda: Siphonostomatoida: Caligidae*)

Host and locality: *Mugil cephalus* from Hiroshima Bay (3 November 2015). Site on host: Gills.

Description: Proximal segment of antennule occupying length of antennule with 27 pinnate and 2 naked setae, distal segment with 12 naked setae and 2 aesthetases; proximal segment of antenna slender, middle segment largest; proximal segment of maxilliped large and unarmed, middle segment in distinct, basal segment bearing medial seta, claw with seta as base; proximal segment of maxilla with subdistal membrane of inner margin; coxa of leg 2 with large setae on inner posterior margin and setule on ventral surface, basis with small outer seta and one inner setule and membrane on inner part of posterior margin; protopod of leg 3 with small, plumose outer seta on posterior marginal membrane; protopod of leg 4 bearing outer distal corner, exopod 2-segmented, first segment with spine and 3 distal exopodal spines (Figures 1-2).

Armature on rami of legs 1-4 as follows:

- Leg 1: exopod 1-0; III, 1, 3; endopod (vestigial)
- Leg 2: exopod I-1; I-1; I, II, 5; endopod 0-1; 0-2; 6
- Leg 3: exopod I-0; I-1; III-4; endopod 0-1; 6
- Leg 4: exopod I-0; III; endopod (absent)

Remarks: Caligus is the largest genus in the family Caligidae and comprises more than 250 species [4]. The present material cannot be identified to the species level due to the limited observations.
Figure 1. *Caligus* sp., male. A, habitus, dorsal; B, maxillipeds; S, sternal furca; D, maxilla; E, leg 4; F, antenna; G, antennule; H, leg 1.
3.2. Description of female Lernanthropus atrox Heller, 1865 (Copepoda: Siphonostomatoida: Lernaeopodidae)

Host and locality: Pagrus major from Hiroshima Bay (21 December 2016). Site on host: Gills. Antennule with short setae; antenna small, pointed; maxilliped spinose at base; maxilla very elongated; leg 1 with hair on ventral side; leg 4 with 4 spines and short hairs close to inner margin. (Figures 3-5).
Figure 4. *Lernanthropus atrox* Heller, 1865, female. V, maxilliped (right), dorsal; W, maxilliped (left), dorsal; X, leg 1, dorsal; Y, maxillule, dorsal; Z, antennule (left), dorsal; 1, antennule (right), dorsal; 2, antenna, dorsal
Figure 5. *Lernanthropus atrox* Heller, 1865, female. 3, antennule, ventral; 4, maxillule, ventral 5, maxilliped, ventral; 6, maxilla, ventral; 7, leg 1, dorsal; 8, leg 2, ventral; 9, genital segment, abdomen, and caudal rami, ventral; 10, posterior extremely, ventral.

Remarks: Six species of *Lernanthropus* are known to occur in Japanese waters: *L. atrox*, *L. belones*, *L. chrysophyrs*, *L. cornutus*, *L. gisleri*, and *L. seriola* [5]. The present species has been described in detail by [6] from a specimen found in Japan.
3.3. *Ceratothoa verrucosa* (Schioedte and Meinert, 1883) (Isopoda: Cymothoidae) (Figures 6-7)

**Description of female:** Body oval, as long as wide, bilaterally symmetrical; cephalon visible from dorsal view; eyes small and hidden by antenna; antennula same length as antenna but comprised of 7 articles, extending to posterior margin of cephalon; antenna comprised of 9 articles; pereopods 1-7 of similar size, comprised of 6 articles; uropod lacks marginal setae.

**Host and locality:** *Pagrus major* from Hiroshima Bay (2 August 2016).

**Site on host:** Roof of the buccal cavity.

**Remarks:** Currently, 30 species of *Ceratothoa* are known worldwide [7]. The present species has been reported from Japan and surrounding islands [8].
Figure 7. *Ceratothoa verrucosa* (Schioedte and Meinert, 1883), female. H, pereopod 1, ventral; I, pereopod 2, ventral; J, pereopod 3, ventral; K, pereopod 4, ventral; L, pereopod 5, ventral; M, pereopod 6, ventral; N, pereopod 7, ventral.

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