

Note

Some diagnostic characters for the classification of two sympatric hyperiid amphipods, *Themisto pacifica* and *T. japonica*, in the western North Pacific

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Themisto spp. are the dominant amphipods in the pelagic realm and are widely distributed in high latitude seas (Vinoogradov et al. 1996). In the western subarctic Pacific and its marginal seas, *Themisto pacifica* and *T. japonica* are the most abundant species (Bowman 1960), and are an important prey component of salmon (Tadokoro et al. 1996), walleye pollock (Kooka et al. 1997; Yamamura et al. 2002), common squid (Okiyama 1965), and mesopelagic fishes (Moku et al. 2000; Uchikawa et al. 2001). The two species are not only similar morphologically but also co-occur widely in the western North Pacific (Bowman 1960; Semura et al. 1991). To distinguish them morphologically, Bowman (1960) described differences in the length of antennae 1 and 2 in adult females, but did not mention morphological differences in males. Semura et al. (1991) distinguished adult male species by the number of setae on the inner margin of the propod of the sixth pereopod and the number of segments of the exopod of the first pleopod. Unfortunately, they could not find appropriate morphological indices to separate the two species in specimens smaller than 5 mm. In this study, we document the morphological differences in juveniles, immature, and mature females and males of *T. pacifica* and *T. japonica*, and establish diagnostic features that allow these two species to be distinguished at all stages of maturity.

Specimens were collected using oblique tows of bongo nets (mouth opening 70 cm, mesh size 333 μ m) from about 500 m depth (range: 386 to 900 m) to the surface in the Oyashio region, within the rectangle defined by 41°30' to 42°30'N and 145°00' to 146°00'E (hereafter "Site H") between July 1996 and July 1998. Upon retrieval of the net, specimens were immediately preserved in 10% buffered formalin seawater for later observation. In the laboratory on land, *Themisto* specimens were sorted using the following morphological observations.

Themisto spp. have no larval stage, and hatchlings are mor-

phologically similar to adults. For hyperiid amphipods, a marked external (=secondary) sexual character of females is the development of oostegites, four pairs of which arise from the posterior inner margin of the coxa of pereopods 2 to 5 to form a chamber (=marsupium). The external sexual characters of males are an extended first or second antenna and an excavate organ on the first uropod rami (Kane 1963; Sheader 1981). In the present study, we classified the specimens into the following three maturity stages using Kane's (1963) criteria: "juveniles"—no sexual characters evident; "immature adults"—oostegites present, but not fully developed beyond the gill (females), or the first and second antennae are segmented but not fully extended (males); and "adults"—oostegites larger than gills (females), or fully extended first and second antennal flagellae (males). Body length (BL; mm) was measured as the maximal distance between the tip of the head and the distal end of uropod three of the straightened body under a dissecting microscope, to the nearest 0.05 mm. *Themisto* amphipods add one new pleopod-rami segment at each molting (Sheader 1981; Ikeda 1990); thus, the number of segments in the first pleopod rami can be used as an indicator of the instar number.

The size frequency distribution of mature and immature *Themisto* spp. collected at Site H between July 1996 and July 1998 is shown in Fig. 1. Mature females (Fig. 1A) were separated into groups, small (3.5 to 8.8 mm in BL) and large (9.6 to 16.2 mm); the former were numerous, but there were few of the latter. While antennae 1 and 2 were subequal in length in small females, antenna 2 was longer than antenna 1 in large females. Bowman (1960) noted that antennae 1 and 2 of female *T. pacifica* are subequal, and that the body length of adult females is 4.5 to 8.5 mm. On the other hand, in female *T. japonica*, antenna 2 is longer than antenna 1, and the body length of adult females is 9 to 17 mm. Considering Bowman's description, mature females less than 9 mm are *T. pacifica*, while those greater than 9 mm are *T. japonica*, at least in the western North Pacific.

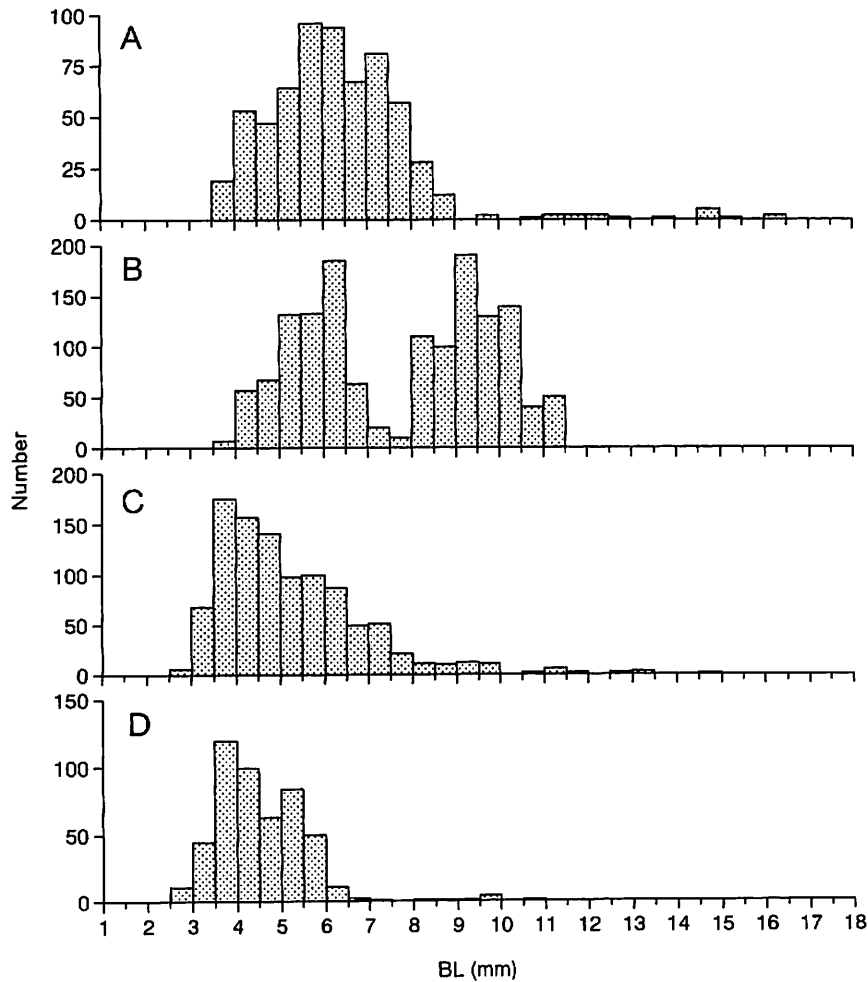


Fig. 1. Size (=BL) frequency distribution of specimens in which *Themisto pacifica* and *T. japonica* are mixed: (A) mature females, (B) mature males, (C) immature females and (D) immature males. All data collected during the period from July 1996 and July 1998 at Site H, western North Pacific, are pooled.

The size frequency distribution of mature males (Fig. 1B) was also bimodal, with a boundary at around 7 mm; small specimens were 3.8 to 6.9 mm long, and large specimens were between 7.1 and 11.4 mm long. According to Semura et al. (1991), *Themisto japonica* has one or two more setae on the inner margin of the propod of pereopod 6 than *T. pacifica* (Fig. 2B, 2D). Small mature males collected at Site H had five or fewer setae, while large males had six or more, suggesting that the number of setae on the inner margin of the propod of pereopod 6 is a useful character to distinguish mature males of *T. pacifica* from mature males of *T. japonica*.

The body sizes of immature *Themisto japonica* and *T. pacifica* specimens overlapped (Fig. 1C, D). Semura et al. (1991) noted that *T. japonica* had one or two more long setae on the inner margin of the carpus of pereopod 4 than *T. pacifica*. In our sample of immature females, those that had the same characters as *T. japonica* (antenna 1 shorter than antenna 2, see above), had six or more setae on the inner margin of the carpus of pereopod 4, whereas those with the characters of *T. pacifica* (antenna 1 = antenna 2, see above), had five or fewer setae (Fig. 2A, C). Immature male specimens with the characters of *T.*

japonica (six or more setae on the inner margin of the propod of pereopod 6, see above) had six or more setae on pereopod 4, while those with the characters of *T. pacifica* (less than five setae on pereopod 6, see above), had five or fewer setae on pereopod 4 (Fig. 2A, C). Consequently, we conclude that specimens with six or more setae on pereopod 4 are *T. japonica*, and those with five or fewer setae on pereopod 4 are *T. pacifica*.

For juveniles smaller than 5 mm, Semura et al. (1991) found no reliable characters to separate the two species. They also mentioned that although the number of setae on the inner margin of the carpus of pereopod 4 increased with increasing body length, the total number of setae was one or two greater in *Themisto japonica* than in *T. pacifica* (Fig. 2A, C). In the present study, instar 3 specimens had two or three setae on pereopod 4, instar 4 specimens had four or five, and instar 5 specimens had five or six. These results show that juvenile *Themisto* amphipods can be distinguished as *T. pacifica* or *T. japonica* by using the number of long setae on the inner margin of the carpus of pereopod 4.

Thus, *Themisto* amphipods in the western North Pacific can

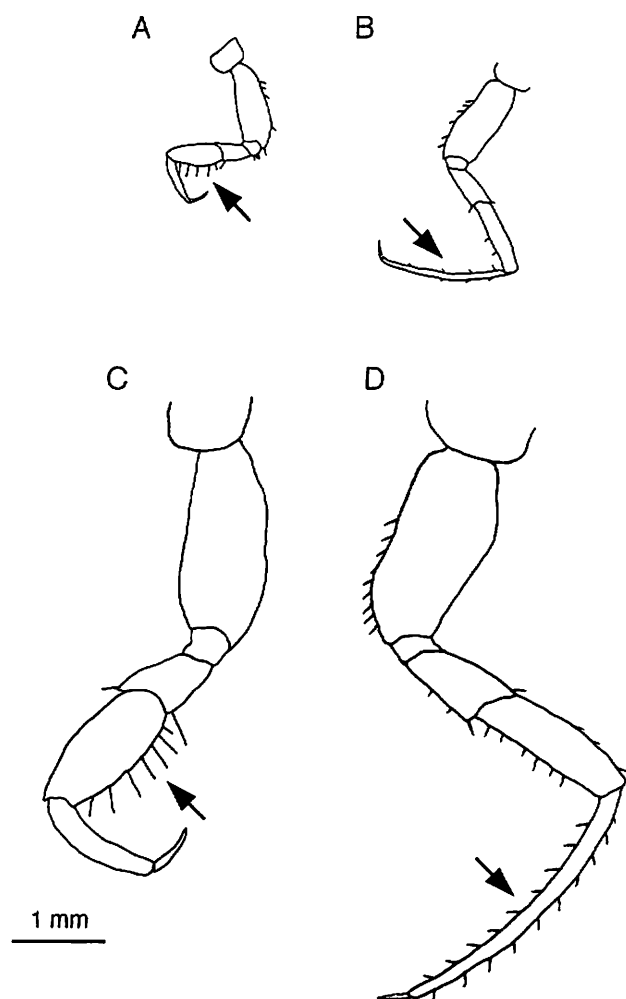


Fig. 2. Pereopod 4 (A) and 6 (B) of *Themisto pacifica*, as compared with Pereopod 4 (C) and 6 (D) of *T. japonica*. Note the differences in the number of setae (arrowed).

be classified as *Themisto pacifica* or *T. japonica* using the following criteria. First, *Themisto* amphipods need to be divided into three maturity stages: adult, immature, and juvenile. Adult female specimens less than 9 mm in BL are *T. pacifica*, and those longer than 9 mm are *T. japonica*. Adult male specimens less than 7 mm in BL are *T. pacifica*, and those longer than 7 mm are *T. japonica*. Immature female and male specimens with five or fewer setae on the inner margin of the carpus of pereopod 4 are *T. pacifica*, and those with six or more setae are *T. japonica*. Juvenile specimens at the same instar stage that have lesser setae on the inner margin of the carpus of pereopod 4 are *T. pacifica*, and those with greater setae are *T. japonica*.

Based on these criteria, we classified all maturity stages and corresponding instar numbers of *Themisto pacifica* and *T. japonica* collected at Site H (Table 1). Newly hatched juveniles of both species (instar 1) develop until instar 2 in the female's marsupium, and are released into the surrounding water at instar 3. The external sexual characters of females and males, respectively, are evident from instars 6 and 5 in *T. pacifica*, and from instars 7 and 7 in *T. japonica*, respectively. Ma-

Table 1. Maturity stages, instar number and body length (BL: mm, means \pm SD) of *Themisto pacifica* and *T. japonica* at Site H, western North Pacific.

Stage	<i>T. pacifica</i>		<i>T. japonica</i>	
	Instar (n)	BL	Instar (n)	BL
In marsupium				
Egg	(5)	0.35 \pm 0.002	(5)	0.35 \pm 0.004
Juveniles	1 (5)	0.83 \pm 0.006	1 (5)	0.83 \pm 0.009
	2 (5)	0.96 \pm 0.004	2 (5)	0.95 \pm 0.027
Free living				
Juveniles	3 (27)	1.62 \pm 0.14	3 (5)	1.59 \pm 0.12
	4 (104)	1.90 \pm 0.16	4 (4)	2.40 \pm 0.17
	5 (243)	2.39 \pm 0.20	5 (4)	2.68 \pm 0.16
			6 (10)	2.86 \pm 0.17
Females	6 (13)	2.94 \pm 0.16	7 (15)	3.56 \pm 0.12
Immature	7 (37)	3.24 \pm 0.15	8 (70)	4.07 \pm 0.14
	8 (190)	3.87 \pm 0.27	8 (73)	4.79 \pm 0.19
	9 (122)	4.67 \pm 0.35	10 (112)	5.59 \pm 0.23
			11 (92)	6.40 \pm 0.27
			12 (50)	7.19 \pm 0.18
			13 (33)	8.12 \pm 0.36
			14 (13)	9.17 \pm 0.22
Mature	8 (47)	4.06 \pm 0.37	13 (3)	10.07 \pm 0.57
	9 (86)	4.68 \pm 0.39	14 (3)	11.37 \pm 0.25
	10 (152)	5.55 \pm 0.38	15 (5)	12.50 \pm 0.62
	11 (125)	6.47 \pm 0.51	16 (2)	14.70 \pm 0.28
	12 (118)	7.34 \pm 0.53	17 (6)	15.22 \pm 0.71
	13 (27)	7.92 \pm 0.73		
	14 (2)	9.03 \pm 0.18		
Males	5 (2)	2.65 \pm 0.01	7 (9)	3.55 \pm 0.23
Immature	6 (4)	2.98 \pm 0.10	8 (22)	3.86 \pm 0.28
	7 (42)	3.33 \pm 0.29	9 (47)	4.68 \pm 0.39
	8 (109)	3.88 \pm 0.26	10 (46)	5.50 \pm 0.31
	9 (73)	4.72 \pm 0.38	11 (5)	6.19 \pm 0.55
			12 (2)	7.60 \pm 0.85
Mature	8 (21)	4.10 \pm 0.22	12 (10)	7.87 \pm 0.40
	9 (99)	4.57 \pm 0.36	13 (36)	8.98 \pm 0.36
	10 (259)	5.57 \pm 0.44	14 (29)	10.13 \pm 0.36
	11 (162)	6.21 \pm 0.39	15 (4)	11.25 \pm 0.13

ture females and males were seen from instars 8 to 14 (females) and 8 to 11 (males) in *T. pacifica*, and instars 13 to 17 (females) and 12 to 15 (males) in *T. japonica*, respectively. Assuming that hyperiid amphipods have sequential broodings separated by moltings (Kane 1963; Sheader 1981), *T. pacifica* and *T. japonica* females have seven and five successive broods in their lifetime, respectively. Considering that *T. pacifica* matures to a smaller body size than *T. japonica*, *T. pacifica* may breed several times within their shorter life span, whereas *T. japonica* take a long time to begin reproduction. These differences in maturation and reproduction might be important factors in separating their life history traits.

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