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**Feeding habits of juvenile slime flounder *Microstomus achne* in the coastal area of southern Hokkaido**

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Running title: Feeding habits and growth of juvenile slime flounder

Short report

10 text pages, 4 figures, and 1 table

**Abstract** A total of 45 juvenile [30.0–57.4 mm total Length (TL)] slime flounder *Microstomus achne* were collected in the coastal area of southern Hokkaido from April to July in 2001, and April to June in 2002. Their diets were analyzed. Slime flounder juveniles of 30.0–39.9 mm TL fed predominantly on small crustaceans (gammarid amphipods, harpacticoids and cumaceans), and 40.0–57.4 mm TL on gammarid amphipods, cumaceans and polychaetes. The major prey items changed with growth from small crustaceans (e.g., harpacticoids) to polychaetes, although gammarid amphipods were major prey items throughout the juvenile period (30.0–57.4 mm TL).

**Keywords** Diet shift · Feeding habits · Juvenile · Slime flounder

## **Introduction**

The slime flounder *Microstomus achne* is distributed from the northern part of the East China Sea, Yellow Sea, Bohai Sea, Japan Sea, southeastern Sakhalin, and the Pacific waters off northern Japan and along the Kuril Islands (Ishito 1972; Chen et al. 1992; Orlov and Tokranov 2007). It is commercially important in the Pacific waters off northern Japan in particular off the Tohoku area (Ishito 1977). The annual catch of this species by offshore bottom trawls was about 6,500 tons from the 1950s to 1970s in the Pacific waters off northern Japan, from the 1980s to early 1990s the catch amount was at a low level in the same area (about 65 tons) (Ishito and Hashimoto 1993), and in the late 1990s it drastically increased again although the reason for the recovery is not known (D. Kitagawa, unpublished data).

To reveal the cause of stock fluctuations, we first need to obtain information on its life history. For adult slime flounder, factors such as feeding habits (Fujita et al. 1995), growth (Chen et al. 1992), seasonal migration (Ishito 1962), and spawning (Chen et al. 1992) have been examined. However, there is only limited information about the early life stage in the field except for the egg and larval distribution (Ishito and Hashimoto 1993), and size at metamorphosis (19 mm standard length; Minami 2001). Ecological data during the benthic phase, i.e., on metamorphosed juveniles is limited due to the lack of samples in the field. In this study, a total of 45 juvenile slime flounder were collected. We have investigated the feeding habits during the juvenile period of slime flounder, particularly the growth related diet shift.

## **Materials and methods**

Juvenile slime flounders were collected with a sledge net (100 cm wide, 60 cm tall, and 5 mm mesh) or hand net using scuba offshore between 10–25 m depth in southern Hokkaido (area near to the Usujiri Fisheries Station Field Science Center for Northern Biosphere, Hokkaido University) from April to July 2001, and from April to June 2002 (Fig. 1). Water temperature depth at 10 and 20 m were measured approximately every 10 days as part of the regular sampling of Usujiri Fisheries Station Field Science Center for Northern Biosphere, Hokkaido University. Total length (TL) of slime flounder was measured to the nearest 0.1 mm scale with an electric slide caliper after being fixed in 90% ethanol solution (little shrinkage occurred in flatfish larvae >5 mm notochord length; Hjörleifsson and Klein-Macphee 1992; Joh et al. 2003). The stomachs were removed and then the total wet weight of the stomach contents was measured to the nearest 0.1 mg. The stomach contents were identified to the lowest possible taxa under a dissecting microscope, and the number and wet weight of each prey individual were measured. An index of the relative importance of prey taxa (*IRI*) was calculated for each taxon found in stomachs according to Pinkas et al. (1971). This index was calculated by the following equation:

$$IRI = (N\% + W\%) \times F\%$$

where *N%* is the percentage of the number of each food item to the total number of all food items identified; *W%* is the percentage of wet weight of each food item to the total wet weight of all food items identified; and *F%* is the frequency of occurrence for each food item in the total number of stomachs examined. The *IRI* of each food item was standardized to %*IRI* (Cortés 1997):

$$\%IRI = (IRI / \Sigma IRI) \times 100$$

Mann–Whitney *U* test and Steel–Dwass tests for non-parametric multiple comparisons were used for statistical analyses. Values were considered significant at the 5% level.

## Results

**Environmental condition of the sampling site.** 30.0–57.4 mm TL slime flounder were collected from April to mid July in 2001 (collected with a sledge net, 8 samples; hand net using scuba, 22 samples), and from April to late June in 2002 (collected with a sledge net, 1 sample; hand net using scuba, 14 samples) (Fig. 2). Slime flounder juveniles were collected on sandy bottom areas where are several volcanic rocks that the size is like a fist or head of human. The mean bottom water temperature (measured at 10 and 20 m) gradually increased from early April to late June (main sampling period) 3.3–9.6°C in 2001, and 4.1–12.0°C in 2002 (Fig. 3). The mean bottom water temperature during early April to late June in 2001 and 2002 was  $6.0 \pm 2.59$  (mean  $\pm$  SD) and  $8.0 \pm 3.47^\circ\text{C}$ , respectively.

**Feeding habits.** Frequency of occurrence ( $F\%$ ) and  $N\%$  of juvenile slime flounder were calculated for 3 size classes (30.0–39.9, 40.0–49.9, and 50.0–57.4 mm TL) at each year. Two size classes (30.0–39.9, and 50.0–57.4 mm TL) did not have enough individuals to allow comparison of the difference between years, and 40.0–49.9 mm TL size class of their  $F\%$  and  $N\%$  had similar values among year. Therefore we showed the diet composition using the combined two years data (Table 1). The principal prey items of fish 30.0–39.9 mm TL were gammarids ( $\%IRI = 37.0$ , mainly *Pontogeneia rostrata*), harpacticoids (30.9, *Harpacticus nipponicus*), and cumaceans (17.6, *Hemilamprops californicus*), 40.0–49.9 mm TL were gammarids (54.2, mainly *P. rostrata*), cumaceans (28.9, *H. californicus*), and polychaetes (16.5, mainly *Prionospio membranacea*), 50.0–57.4 mm TL were gammarids (58.9, mainly *P. rostrata*), polychaetes (24.5, mainly *P. membranacea*), and cumaceans (15.7, *H. californicus*). Diet composition changed with growth from small crustaceans (such as harpacticoids) to polychaetes, although gammarids were a principal prey item throughout the juvenile period

(30.0–57.4 mm TL). Body weight of the three major prey items (harpacticoids, gammarids, and polychaetes) for juvenile slime flounder were not significantly different among the three size classes of juvenile slime flounder (Fig. 4). They did not increase with juvenile growth throughout the size range. Body weight of cumaceans (*H. californicus*) as the prey was significant difference among three size classes of juvenile slime flounder. The prey body weight of 30.0–39.9 mm TL size class for juvenile slime flounder was lower than that of 40.0–49.9, and 50.0–57.4 mm TL size classes. Median and ranges of body weight were 2.5 and 1.0–3.0 mg, and 1.0 and 0.2–3.0 mg for harpacticoids (principal prey for 30.0–39.9 mm TL), and polychaetes (principal prey for 40.0–57.4 mm TL), respectively. Body weight for harpacticoids was significantly higher than that of polychaetes (Mann–Whitney *U* test,  $P < 0.05$ ).

## **Discussion**

The major prey item changed with growth from small crustaceans (e.g., harpacticoids) to polychaetes, although gammarid amphipods were major prey items throughout the juvenile period (30.0–57.4 mm TL). Several authors have suggested a diet shift during juvenile development, and diets of flatfishes have been closely correlated with growth (e.g., Aarnio et al. 1996; Takatsu 2003). Lockwood (1984) found that plaice *Pleuronectes platessa* of 20 to 40 mm TL basically had a diet such as harpacticoids, whereas one year old plaice (c.a. 80 mm TL) fed mainly on prey such as gammarid amphipods, polychaetes and cumaceans. The main food organisms of marbled sole *Pleuronectes yokohamae* juveniles (>30 mm SL) changed from harpacticoids to small benthic crustaceans and polychaetes (Nakagami et al. 2000). Marbled sole juveniles feed on harpacticoids which live in the surface or sub-surface layer of

sandy bottom sediment, and sometimes in the near bottom water, the main food organisms may change smoothly from planktonic to bottom prey items (Nakagami et al. 2000). Thus flatfish tend to feed on harpacticoids frequently in the transition period from pelagic to benthic feeding.

Harpacticoids were important dietary items for <40 mm TL of juvenile slime flounder; however, their importance decreased as body size of juvenile slime flounder increased. This result was similar to other flatfishes (e.g., plaice, marbled sole) that have a shorter period until metamorphosed and a smaller body size at settlement. Prey selection of animals is determined by many factors, including prey perceptibility, prey escape ability, handling time, prey volume and caloric value, nutrition, defense, probability of successful capture, preference, past memories of prey, and the predator's physical condition (Stephens and Krebs 1986). In any case the diet may shift from small crustaceans (harpacticoids) to polychaetes in order to optimize their energy gain. We observed the relationship between size of juvenile slime flounder and the prey size of four principal prey items. The body weight of prey did not increase with the juvenile growth throughout the size range (30.0–57.4 mm TL), except for cumaceans. Moreover, body weight for harpacticoids (principal prey for 30.0–39.9 mm TL) was significantly higher than for polychaetes (principal prey for 40.0–57.4 mm TL). Thus, the diet of juvenile slime flounder may not shift in relation to body weight of prey, but to benthic life adaptation. Density of gammarids in sandy areas of southern Hokkaido is usually high (Nakaya et al. 2004). Juvenile slime flounders consumed gammarid amphipods over an extended period, possibly due to their availability of gammarid amphipods. In this study, we reveal the feeding habits of juvenile slime flounder, however we have no data on the relative prey abundance in the field. We need further investigations about the relationship between the frequency of prey items in stomachs and their abundance in the field to reveal the food preference of juvenile slime flounder.



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**Fig. 1** Location of the sampling area of juvenile slime flounder *Microstomus achne* in the coastal area of southern Hokkaido. Inside of *dotted line* is the sampling area

**Fig. 2** Total length of juvenile slime flounder *Microstomus achne* from April to mid-July in 2001, and April to late June in 2002 on each sampling day

**Fig. 3** Variation of mean water temperature (°C) that was measured at 10–20 m depths near by the sampling site of juvenile slime flounder *Microstomus achne* from 2001 to 2002

**Fig. 4** Prey size (mg) in juvenile slime flounder *Microstomus achne* for each total length (TL) size class. *Different letters* indicate significant differences at  $P < 0.05$  in the same prey item by the Mann–Whitney  $U$  test between two TL size classes and by the Steel–Dwass test between three TL size classes. *Superscripts* indicate sample sizes

**Table 1** Percent frequency of occurrence (*F%*), percent by number (*N%*), percent by weight (*W%*) and index of relative importance (*%IRI*) of food items in stomachs of slime flounder juveniles collected in the coastal areas of southern part of Hokkaido from April to July in 2001, and from April to June in 2002

Prey items	Size class (mm in total length)												
	30.0–39.9				40.0–49.9				50.0–57.4				
	<i>F%</i>	<i>N%</i>	<i>W%</i>	<i>%IRI</i>	<i>F%</i>	<i>N%</i>	<i>W%</i>	<i>%IRI</i>	<i>F%</i>	<i>N%</i>	<i>W%</i>	<i>%IRI</i>	
Harpacticoida <i>Harpacticus nipponicus</i>	50.0	27.6	30.7	30.9	10.5	1.8	2.5	0.4	12.5	0.7	0.7	0.3	
Cumacea <i>Hemilamprops californicus</i>	44.4	23.2	14.3	17.6	68.4	24.1	28.7	28.9	37.5	14.1	15.0	15.7	
Ostracoda <i>Euphiomedes sordida</i>	0	0	0	0	10.5	0.6	0.1	0.1	0	0	0	0	
Calanoida	5.6	1.0	0.2	0.1	5.3	0.3	1.8	<0.1	0	0	0	0	
Gammaridea <i>Pontogenesia rostrata</i>	61.1	27.1	28.4	35.9	78.9	55.0	29.2	53.2	50.0	58.5	20.5	56.8	
	<i>Photis reinhardi</i>	16.7	3.0	3.4	1.1	21.1	1.2	4.7	1.0	12.5	0.7	11.1	2.1
Mysidacea <i>Neomysis czerniawskii</i>	0	0	0	0	0	0	0	0	12.5	1.4	2.1	0.6	
Polychaeta <i>Priomospio membranacea</i>	33.3	17.7	22.9	14.3	52.6	16.2	22.3	16.2	37.5	21.1	20.4	22.4	
	<i>Pseudopolydora</i> sp. 1	0	0	0	0	5.3	0.3	5.3	0.2	12.5	3.5	7.9	2.1
	<i>Pseudopolydora</i> sp. 2	0	0	0	0	5.3	0.3	0.2	<0.1	0	0	0	0
Bivalvia		5.6	0.5	0.1	<0.1	0	0	0	0	0	0	0	0
Other organisms		0	0	0	0	5.3	0.3	0.1	0	0	0	0	0
Number of samples	18				19				8				

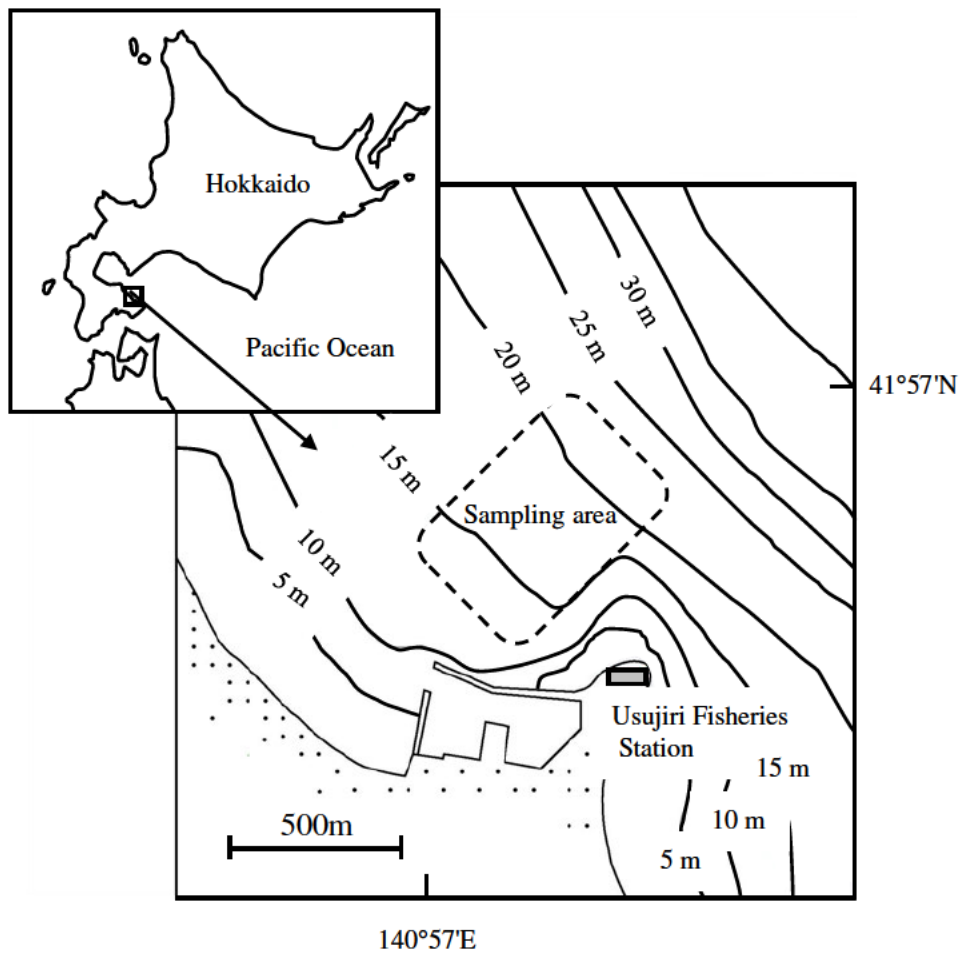


Fig. 1

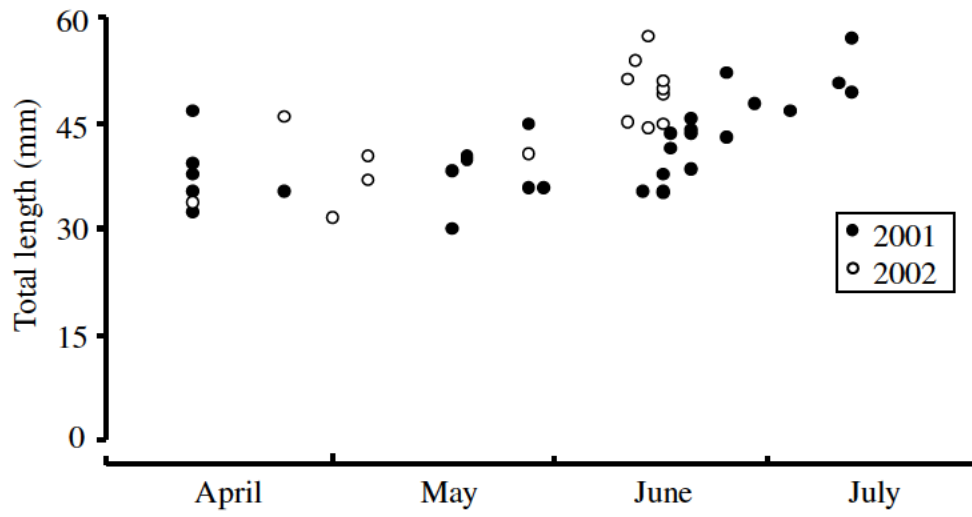


Fig. 2

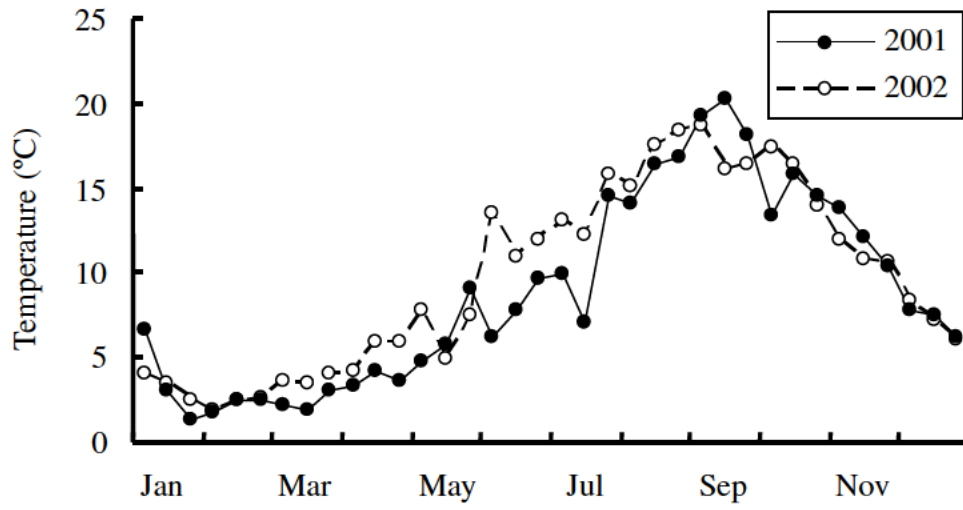


Fig. 3



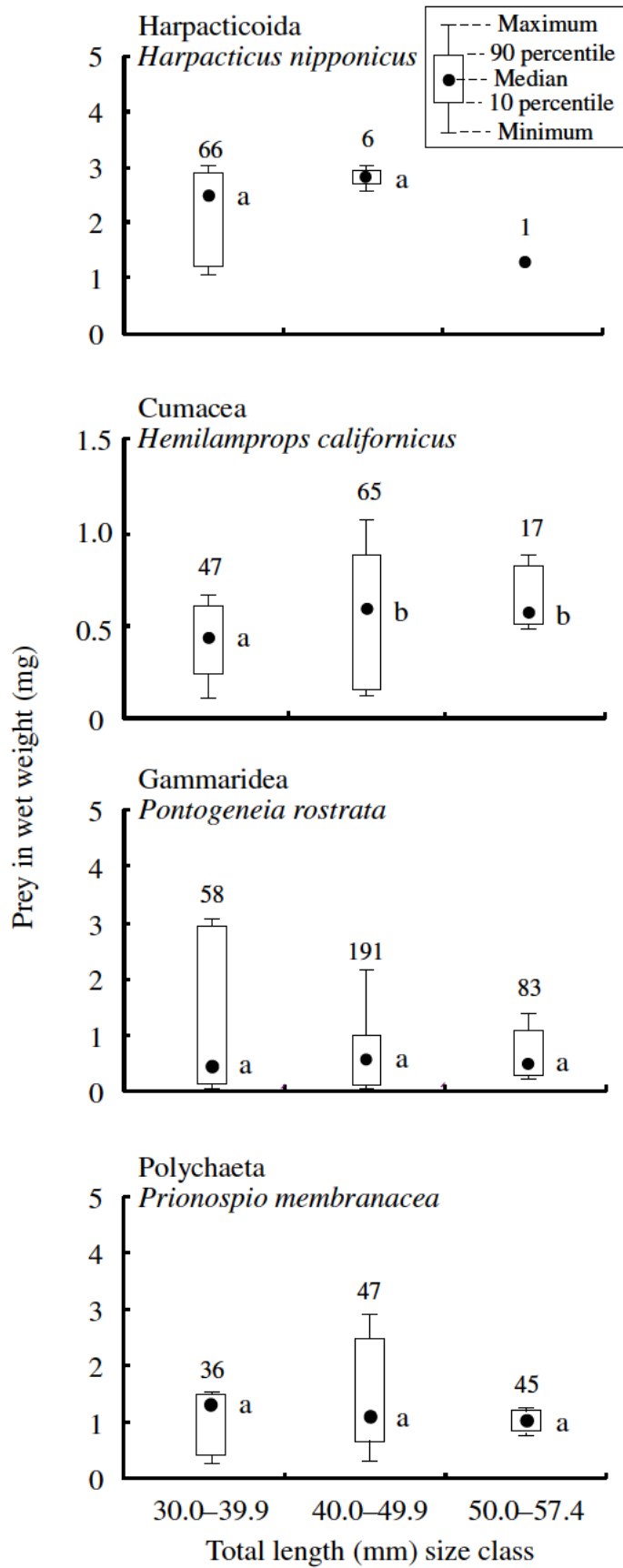


Fig. 4