## Note

## Efficient capture of deep-sea hyperbenthic calanoid copepods with baited traps

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The benthic boundary layers of the oceans harbor a unique biological assemblage with higher biomasses than in the overlying water column and a species composition different both from that in the upper water column and from the true benthic fauna (Grice & Hülsemann 1970; Wishner 1980a). The organisms in such layers, termed collectively the hyperbenthic or benthopelagic fauna, have been effectively collected from shallow habitats by SCUBA divers with various types of plankton nets and traps (see Mauchline, 1998, for review). For deep-sea habitats, plankton nets attached to a submersible (Grice & Hülsemann 1970; Childress et al. 1989; Toda et al. 1994) or to a deep-towed vehicle (Wishner 1980a, b; Toda et al. 1995) have been used to estimate benthopelagic faunal composition and biomass. However, ship time and equipment for this type of sampling is very expensive. Furthermore, the extended towing of nets often results in serious damage to specimens which are then unsuitable for experimental research. During submersible dives in Sagami Bay we deployed simple baited traps to collect deep-sea organisms and found that the method is effective in specifically collecting hyperbenthic copepods in good condition without expending much valuable survey time,

The traps were set and recovered from the sea bottom at a depth of 1445 m near the central part of Sagami Bay, central Japan ( $35^{\circ}00'$ N,  $139^{\circ}21.6'$ E) during Dives 723 and 724 (2 and 4 December 1993), respectively, of the crewed submersible *Shinkai 2000*. Twelve 1-liter cylindrical polyethylene bottles (diameter, 8 cm; height, 16 cm) were used as traps. The top of each bottle was sealed with a plastic screw cap through which either large (diameter, 10 mm), medium (5 mm) or small (2 mm) pores had been drilled in each of 4 bottles. The number of pores was 13 in the 10- and 5-mm pore traps and 20 in the 2-mm pore traps, resulting in total pore areas of 10.2, 2.6 and 0.6 cm<sup>2</sup>, respectively. A total of ca. 500 g of lead ballast was put in a plastic bag which was placed on the bottom of each bottle. Either Pacific saury (*Cololabis saira*) or Antarctic krill (*Euphausia superba*), both frozen and then thawed, were used

as bait. Whole Pacific saury were cut into round slices of ca. 1cm thickness; whole Antarctic krill were wrapped in a bag of nylon gauze (mesh size,  $95 \,\mu$ m). A bag containing ca.  $70 \,\text{g}$ (wet wt) of each bait was placed in 2 of the 4 traps with either large, medium or small pores, and the traps filled with filtered (Whatman GF/F) seawater. The 12 traps were placed in a PVC box (with 12 compartments, hinge covers on top, and a few pores on bottom for drainage; Kikuchi et al. 1990) which was then held in the payload-box of the Shinkai 2000. All traps were then sealed with a sponge-pad lining the inner surface of the lid of the box, thus protecting the traps from contamination during transportation through the water column. The traps were deployed during 1145-1215 h on 2 December and were retrieved during 1204-1235 h on 4 December 1993. Six traps (see Fig. 1 and Table 3 for the type of traps) were transferred with the manipulator arm from the compartment of the box to the seafloor, and placed at a distance of 20-30 cm from each other. The sediment consisted of pale, yellowish-gray mud with oxygenated surface layer of 2.0-3.0 cm (Ohga & Kitazato 1997). The traps sank into the surface flocculent layer to a greater of lesser degree, resulting the top of traps being 3-10 cm above the bottom. To see possible effect of the location of traps, the remaining 6 traps were left in the box which was placed on the seafloor ca. 6 m apart from the 6 traps outside the box. After 2 d the 6 traps outside the box were recovered by the manipulator and returned to the box. The cap of one trap (medium pore, Pacific saury) was broken accidentally on recovery, and the catch was therefore excluded from this analysis. Zooplankton was collected from 1227 to 1238 h on 2 December by towing an opening-closing net (mouth diameter, 20 cm; mesh size, 95  $\mu$ m; Kikuchi et al. 1990) attached to the lowermost side of the Shinkai 2000 at 1.5 kt in the layer ca. I m above the bottom (see Kikuchi et al., 1990, for performance of the net).

Immediately upon retrieval to the ship's deck all samples were fixed in 2% formaldehyde/seawater solution buffered with sodium tetraborate. Specimens from all traps were sorted, enumerated and identified to major taxonomic groups. The

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body height of the tharybid copepods and amphipods were measured under a compound- and a stereo microscope, respectively; for the amphipods randomly sampled specimens from each trap were measured. Some amphipods from the traps were dissected and their gut contents examined under a compound microscope.

The catch of the traps was dominated by amphipods  $(30-1.6 \times 10^3 \text{ indiv. trap}^{-1})$  followed by copepods which were much less abundant  $(0-63 \text{ indiv. trap}^{-1})$  (Table 1). Other minor constituents were, in order of abundance, gastropods, isopods, bivalves and a chaetognath. The amphipods consisted of single species (species not determined) belonging to the genus *Euonyx* (Family Lysianassidae; Vallet, personal communication). Most copepods from the traps were calanoids of the family Tharybidae (genera *Tharybis* and *Undinella*) belonging to hitherto unknown species, taxonomic description of which is now in progress (Toda & Ohtsuka, unpublished data). Although fewer in number, harpacticoids (genera *Para*-

 Table 1. Total number of organisms collected with baited traps and plankton net.

Taxon	Net	Trap
Amphipoda	0	6639
Copepoda		
Calanoida	22	129
Poecilostomatoida	3	0
Harpacticoida	4	33
Isopoda	0	16
Nauplius larva	l	0
Unidentified Crustacea	0	4
Cnidaria	1	0
Chaetognatha	4	1
Polychaeta	7	0
Gastropoda	0	22
Bivalvia	0	4
Eggs	1	0
Unidentified organisms	0	1
Total	43	6849

calanus and Clausocalanus were also collected (Table 2).

There were considerable differences in the total number of amphipods and tharybid copepods collected with traps of different pore sizes or with different baits (Table 3, Fig. 1). The catches of amphipods by traps with pore sizes of 5 mm and 10 mm were significantly larger than those from the 2-mm pore traps (Mann–Whitney U-test, p < 0.01), regardless of the type of bait (saury or krill), while the opposite was true for the catch of tharybids (p < 0.01). Additionally, amphipods were collected in significantly larger numbers (p < 0.01) by the traps containing saury as a bait than by those containing krill, regardless of the pore size and the location of the trap (directly on the bottom or in the box for the 10-mm and 2-mm pore traps; not examined for the 5-mm pore traps). The tharybids were collected in large numbers only in the 2-mm traps and the catches in the krill-traps were 7-15 times larger than in the saury-traps (Fig. 1). When plotted for all traps, the numbers of amphipods and tharybids co-occurring in the same traps showed a significant negative correlation (Kendall's rank correlation coefficient,  $\tau = -0.58$ , p < 0.01).

The body height of the tharybids ranged from 0.3 to 0. 6 mm for all traps. The body height of amphipods in the 2-mm pore traps were less than 2.5 mm, while those in the 10- and 5- mm pore traps reached 5 to 6 mm (Fig. 2). The modal abundance maxima for the amphipods were in the 0.5-2.0 mm

Table 2. Taxonomic composition of calanoid copepods collected with plankton net and traps (total number; carcasses excluded).

Genus	Net	Trap
Paracalanus	10	2
Clausocalanus	1	1
Eucalanus	1	0
Ryocalanus	2	0
Aetideopsis	l	0
Chiridius	6	0
Tharybis/Undinella	1	126
Total	22	129

Table 3. Number of amphipods and copepods collected with baited traps. Bait type: K, Antarctic krill; S, Pacific saury.

– Taxon –	Pore size of trap										
	Large (10 mm)			Medium (5 mm)		Small (2 mm)					
	K-1*	К-2	S-1*	S-2	K-3	K-4	S-3*	K-5*	K-6	S-4*	S-5
Amphipoda Copepoda	144	431	1139	1127	602	317	1612	40	30	636	561
Calanoida	2	1	0	1	2	2	0	63	46	3	9
Poecilostomatoida	0	0	0	0	0	0	0	0	0	0	0
Harpacticoida	0	1	0	0	1	0	0	16	13	2	0

\* traps set outside the box; others left in the box.



**Fig. 1.** Numbers of amphipods and tharybid copepods taken in traps of different pore sizes and baits. Asterisks indicate those traps set outside the box; others left in the box.

body-height range for all traps (Fig. 2). Of the 14 amphipods examined, 4 specimens contained fragments of tharybid copepods in their guts. The sample from the plankton net contained 22 calanoid copepods, this being the dominant group collected (Table 1), including one tharybid and some specimens of the deep-sea genera *Ryocalanus*, *Aetideopsis* and *Chiridius*, but no amphipods (Table 2).

Baited traps have been used to collect hyperbenthic organisms, including copepods, under conditions not conducive to conventional net sampling, such as under sea ice (Bradford and Wells 1983; Ohtsuka et al. 1998) and in the deep sea (Schultz 1990; Kikuchi et al. 1990). The present results demonstrate that baited traps are effective in collecting copepods of particular groups, as well as in the capture of large numbers of amphipods in bottom- or near bottom habitats in the deep sea, through the application of selected pore sizes and bait types.

It is evident that the amphipods and the tharybid copepods were attracted to the bait, considering their abundance in the traps, their negligible occurrence in the plankton net towed just above the seafloor during the interval of trapping, and their total absence from the core samples of bottom sediments taken from the present collection site during a monthly sampling program over a 2-year period (Shimanaga, personal communication). The amphipods of the genus *Euonyx* and other lysianassids have been collected with baited traps on deep-sea bottom of 300–1015 m off the Pacific coast of Japan, as well as



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**Fig. 2.** Distribution of body heights of amhipods in traps with different pore sizes (10 mm: A, B, G, H; 5 mm: C, D, I; 2 mm: E, F, J, K) and baits (krill: open; saury: shaded).

from similar depths of the world oceans (Sekiguchi & Yamaguchi 1983, and literature therein). In addition, attarction of large numbers of abyssal amphipods, because of their sensitive chemoreceptive adaptaion, to baited traps has been reported from the central part of the Arctic Ocean and the Philippine Trench (George 1981). The specialized mouthpart setae, presumed to be chemosensory, in the tharybids as well as in related detritivorous genera (Nishida and Ohtsuka 1997) are assumed to function in detecting chemicals from the baits (see Ohtsuka et al. 1998). The occurrence of tharybids and related families, Phaennidae, Diaixidae, Scolecitrichidae and Parkiidae, from deep-sea near-bottom habitats (<ca. 10 m above bottom) has been reported (see Table 77 in Mauchline 1998). The absence from the traps of other calanoid copepods that were collected in the near-bottom net and those known from benthopelagic layers (see Table 77 in Mauchline 1998) suggests that the present bait was less attractive to them than to the tharybids, that missing calanoids were less closely associated to the bottom than the tharybids, or that they were absent from the collection site.

Pacific saury appears to be much more attractive to the amphipods than Antarctic krill, as indicated by the marked difference in catches between the traps with the different baits. The higher abundances of amphipods in the 10- and 5-mm pore traps than in the 2-mm pore traps is probably due to the combined effect of larger total aperture area and greater pore sizes relative to the size of the amphipods in the former 2 traps, as indicated by their body-height distributions in each trap (Fig. 2). The apparent negative correlation between the catches of amphipods and tharybids is presumably due to predation within the traps of the tharybids by the amphipods, as suggested by the occurrence of the tharybids in the amphipod guts. Hence, the higher abundances of tharybids in the 2-mm traps with krill bait than in those with saury bait appear to be the result of lower predation pressure in the former, rather than a case of higher attractivity to krill than saury for the tharybids, which is still an open question. In this respect, the use of a nylon-gauze wrapping for the bait had a dual effect: (1) the bait, after recovery, did not show any apparent indication of being eaten by the trapped animals, and thus they would have been decomposed primarily through microbial activity, which should be low at the ambient temperature (2-3°C), making them advantageous as a source of chemical stimuli over a particularly long (days to weeks) deployment; (2) the chemicallyattracted animals, after being trapped, had no direct access to the bait and therefore became starved as time passed, as a result, eating the other animals within the trap. While the traps of 2-mm pore diameter were effective in collecting tharybid copepods, pore sizes of less than 1 mm are recommended for more effective collection, considering the body height of the tharybids ( $\leq 0.6$  mm) and the need for effective elimination of amphipods (modal body height: 0.5-2 mm) from the traps.

The occurrence of intact specimens belonging to the genera *Paracalanus* and *Clausocalanus* in both the traps and the net is notable, since these genera are known to be primarily epipelagic. While both the samplers were equipped to avoid contamination from the water column before and after deployment, neither possibility, contamination through leakage into the mouth of the net or under the lid of the trap-box, nor that of true existence of these copepods in the near bottom habitat, can be excluded at present.

Most of the amphipods and the tharybids were actively moving/swimming in the traps at the time of recovery onto the ship's deck, and could have provided a set of live animals for experimental studies, although they were fixed immediately in the present study. Live tharybids collected during recent dives using the same traps have been reared in the laboratory for more than six months.

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## **Literature Cited**

- Bradford, J. M. & J. B. J. Wells 1983. New calanoid and harpacticoid copepods from beneath the Ross lee Shelf, Antarctica. *Polar Biol.* 2: 1–15.
- Childress, J. J., D. L. Gluck, R. S. Carney & M. M. Gowing 1989. Benthopelagic biomass distribution and oxygen consumption in a deep-sea benthic boundary layer dominated by gelatinous organisms. *Linnol. Oceanogr.* 34: 913–930.
- George, R. Y. 1981. Functional adaptations of deep-sea organisms, p. 280-332. In *Functional Adaptations of Marine Organisms* (eds. Vernberg, F. J. & W. B. Vernberg). Academic Press, New York.
- Grice, G. D. & K. Hülsemann 1970. New species of bottom-living calanoid copepods collected in deepwater by the DSRV Alvin. *Bull. Mus. Comp. Zool. Harvard* 139: 185–230.
- Kikuchi, T., T. Toda, T. Nemoto & S. Ohta 1990. Preliminary survey on the deep-sea near bottom zooplankton by means of deep-sea submersible "SHINKAI 2000". *JAMSTEC Deepsea Res.* (1990): 115–122. (In Japanese with English abstract.)
- Mauchline, J. 1998. The biology of calanoid copepods. *Adv. Mar. Biol.* 33: 1–710.
- Nishida, S. & S. Ohtsuka 1997. Ultrastructure of the mouthpart sensory setae in mesopelagic copepods of the family Scolecitrichidae. *Plankton Biol. Ecol.* 44: 81–90.
- Ohga, T. & H. Kitazato 1997. Seasonal changes in bathyal foraminiferal populations in response to the flux of organic matter (Sagami Bay, Japan). *Terra Nova* 9: 33–37.
- Ohtsuka, S., I. Takeuchi & A. Tanimura 1998. Xanthocalanus gracilis and Tharybis magna (Copepoda: Calanoida) rediscovered from the Antarctic Ocean with baited traps. J. Nat. Hist. 32: 785–804.
- Schulz, K. 1990. Pterochirella tuerkavi, new genus, new species, an unusual calanoid copepod from the deep Gulf of Aden (Indian Ocean). *Mitt. Hamb. Zool. Mus. Inst.* 87: 181–189.
- Sekiguchi, H. & Y. Yamaguchi 1983. Scavenging gammaridean amphipods from the deep-sea floor. Bull. Fac. Fish., Mie Univ. No. 10: 1–14.
- Toda, T., T. Kikuchi, J. Hashimoto & M. Terazaki 1995. A survey of deepsea benthopelagic organisms by a multiple plankton sampler attached to the deep tow system, p. 171–178. In Proceedings of the NRCT-JSPS Joint Seminar on Marine Science, December 2–3, 1993 (eds. Snidvongs, A., W. Utoomprukporn & M. Hungspreugs). Chulalongkorn Univ., Bangkok.
- Toda, T., T. Kikuchi, S. Ohta & S. Gamo 1994. Benthopelagic zooplankton from a deep-sea cold-seep site in Sagami Bay. *Bull. Plankton Soc. Jpm* 41: 173–176.
- Wishner, K. F. 1980a. The biomass of the deep-sea benthopelagic plankton. *Deep-Sea Res.* 27A: 203–216.
- Wishner, K. F. 1980b. Aspects of the community ecology of deep-sea, benthopelagic plankton, with special attention to gymnopleid copepods. *Mar. Biol.* 60: 179–187.