日本近海における潜水艇及び無人探索機を用いる中層・近底層生物調査プログラムの開発，実施及び設立について

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ここでは潜水艇及び無人探索機技術を用いる日本近海の中層・近底層生を探索する生物調査プログラムの設立について報告する。過去にも海洋科学技術センターにおいて中層調査は行われたことはあったが，非常に断続的であった。このプログラムで新開発の採集器や採集した生物の長期生存を可能とする中層生物専用の水槽などを利用し，本格的な中層研究が可能となった。当論文では実験道具の開発や入手方法を説明し，中層・近底層生調査の目的やそれによって得られると期待される成果を述べ，そして中層における潜水艇及び無人探索機を用いた研究に関する文献をまとめる。当論文で述べる技術を利用することにより，実際に得られたデータや生物標本を紹介する。

キーワード：中層，近底層生，実験道具，技法

The development, implementation, and establishment of a Meso-pelagic and Bentho-pelagic biological survey program using submersibles in the seas around Japan

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This paper reviews the establishment of a program to explore the pelagic communities surrounding Japan using submersible technology. Although pelagic dives have been conducted at JAMSTEC intermittently in the past, this program will unify efforts through a more rigorous approach taking full advantage of new collecting equipment and aquaria for long term maintenance. The acquisition of this equipment, review of existing work, purpose and goals of establishing this program are reviewed here. Examples of data collected and specimens observed are also presented.

Key words : meso-pelagic, bentho-pelagic, equipment, methodology

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1. Introduction

The Japan Marine Science and Technology's Deep Sea Research Department has established a program to survey the meso-pelagic and bentho-pelagic communities around Japan. This program combines the unique technology of the submersibles at JAMSTEC with sampling gear used in similar meso-pelagic studies elsewhere, as well as newly developed collecting gear.

It is common knowledge that the earth is covered with far more water than "earth." It is less well known that the average depth of the world's oceans is over 4,000 meters. Therefore the single largest biome on our planet is the deep sea. Yet the vast majority of marine biological research has concentrated on the upper 200 meters – the epipelagic zone – particularly in areas over continental shelves and easily accessible by SCUBA divers.

Modern biological oceanography began systematically exploring the world's deep oceans using what are now traditional techniques, principally the towing of nets and of benthic sleds through deep water. The first net trawls damaged most animals and so were not very useful for behavioral ecology or life history studies, although they were useful for distributional work and systematic work. Recently, nets have become more sophisticated and proficient at collecting samples intact and will hopefully continue to improve.

However, the understanding of meso-pelagic biology and ecology has been greatly increased through live observation of specimens, particularly *in situ* observations using submersibles. Submersibles allow specific observations of animals in their environment. This provides an advantage over traditional survey techniques for two principle reasons : 1) the behavior of intact undamaged animals can be witnessed and 2) precise unambiguous data for the position (both vertical and horizontal distributions) and physico-chemical parameters can be collected. Furthermore, submersibles can collect specifically targeted specimens in pristine condition which allows critical shipboard and laboratory experimentation to be conducted. These experiments provide information on many aspects of the biology of deep water organisms as well as detailed information on their morphology.

The study of open ocean pelagic species, specifically meso-pelagic fauna, has been limited. Biological oceanographers of the past were instrumental in establishing the foundations of current understanding about midwater communities. However, key information was overlooked because the meso-pelagic community is dominated by fragile gelatinous forms including taxa such as siphonophores and appendicularians, which were difficult to examine using nets. This was first uncovered by the pioneering efforts of William Hamner who developed safe techniques for SCUBA diving in the open ocean, known as "blue-water" diving (Hamner, 1975). He and his associates realized the strength of studying animals live and in their natural environments, and of collecting specimens in good health, undamaged by nets. Using direct observations, new information was collected on animals which until then could not be observed live (Hamner *et al.*, 1975). This new information changed our understanding of the midwater community and has provided insight and inspiration for modern surveys of meso-pelagic species.

Such surveys, using submersible technology, continue to discover new species, behaviors, and ecological interactions of deep living animals. Using submersibles, biological oceanographers have discovered new species and other information about taxa including siphonophores (Pugh & Harbison, 1987 ; Pugh & Youngbluth, 1988a, 1988b), appendicularians (Barham, 1979 ; Hamner & Robison, 1992), ctenophores (Madin & Harbison, 1978 ; Matsumoto & Robison, 1992 ; Matsumoto & Harbison, 1993), jellyfishes (Mills *et al.*, 1987), salps (Wiebe *et al.*, 1979), holothurians (Robison, 1992), and fishes (Robison, 1995). Additionally, interactions between community members or with the environment peculiar to the deep sea have been investigated. Such areas of interest include marine snow (Silver & Alldredge, 1981) and bioluminescence (Widder *et al.*, 1989). This list is cursory and merely meant to give some examples of the kinds of research conducted across various taxa. A more specific review of each taxon could be given.

As an example, the use of submersibles to observe deep water animals has yielded various information about cephalopods (Clarke, 1990). The earliest studies had been limited to a discussion of locations and morphology collected in photographs (Church, 1971 ; Jahn, 1971 ; Pearcy and Beal, 1973) with some discussion of locomotion and postures (Clarke, 1970 ; Roper and Brundage, 1972). Submersible observations of the social behavior of loliginid squids (Milliman and Manheim, 1968 ; Waller and Wicklund, 1968 ; Vecchione and Gaston, 1985 ; Vecchione, 1988), the ecology of the paralarval form of *Loliguncula brevis* (Vecchione, 1991a), and the distribution of the cuttlefish *Sepia pharaonis* (Gutsall, 1989) have supplemented information on relatively well known cephalopods. Direct observation of cephalopods from submersibles has led to the description of the swimming behavior of cirrate octopuses (Aldred *et al.*, 1983), the differing tail morphology of the oegopsid squid *Chiroteuthis* (Vecchione *et al.*, 1992), and the taxonomic position of a cranchiid squid (Vecchione and Roper, 1992). Collection by submersibles of specimens in pristine condition has led to discoveries about cephalopod bioluminescence (Young and Roper, 1976 ; 1977 ; Young *et al.*, 1979a ; 1979b ; 1980 ; 1982 ; Young and Mencher, 1980), an improved method for gut-content analysis in paralarval squids (Vecchione, 1991b), the histology of the digestive gland in paralarval squids (Vecchione and Hand, 1989), and the life histories and behavioral ecology of midwater cephalopods (Hunt, 1996a). Further review of the use of submersibles to study cephalopods can be found in Vecchione and Roper (1991) and Hunt (1996b).

Submersibles are in high demand among biological oceanographers, and so studies for one particular area or research group are often intermittent and lack iterative rigor. One notable exception to this is the midwater survey program of the Monterey Bay Aquarium Research Institute (MBARI). This survey is lead by Dr. Bruce Robison who has been repeatedly surveying a single site in the Monterey Canyon for more than seven years. The MBARI program allows midwater surveys to be conducted two to four times per month, and the tools and methodology (see Robison, 1993) have yielded a variety of results (see Robison, 1995).

The establishment of a meso-pelagic and bentho-pelagic biological survey program at JAMSTEC will serve three purposes. First, JAMSTEC will compliment ongoing international research programs such as those by MBARI. This will provide data from Japanese waters to compare and contrast to other points around the world. Such broadly reaching studies are vital for a global understanding of meso-pelagic and bentho-pelagic community structure and ecology. Second, the JAMSTEC survey will complement work done by scientists throughout Japan also interested in midwater biology and oceanography. Submersible data differs from that which can be collected by traditional techniques. Such data lend themselves well to collaborative efforts as well as comprehensive reviews investigating similar areas of interest or specific fauna. For example, trawls and towed equipment can provide good information on overall abundance, vertical distributions, and taxonomy by collecting quantitative numbers of specimens from a given area. Submersibles can supplement this effort with good information about specific behaviors, feeding, reproduction, vertical migration, and precise morphology for taxonomic keys. These two types of surveys combine well, and will provide a stronger national program for open ocean biology here in Japan. Third, this program will provide new insights and revelations because it is being conducted in a different geographic area than other surveys, and more importantly its submersibles can dive deeper than any other institute in the world.

2. Materials

The most imporant tools are the vehicles themselves. JAMSTEC has a wide array of equipment, and there are four submersibles available for use. They include two remotely operated vehicles (ROVs) and two crewed submersibles. The ROVs are the "Dolphin 3 K" which is capable of diving to 3,300 m and the "Kaiko" which can dive to the deepest parts of the ocean and therefore has no depth "limit." The crewed submersibles are the "Skinkai 2000" and the "Shinkai 6500." These are capable of diving to 2,000 m and 6,500 m respectively (see Takagawa, 1995).

There are various samplers used in the collection of animals from submersibles. One sampler which has been developed for use on the Johnson-Sea-Link submersibles is called the detritus sampler or 'D'-sampler (Youngbluth, 1984). This sampler is a 7.5 liter tube with two hydraulically controlled hinged lids that move laterally in unison to open and close the tube. With the tube open, the submersible is maneuvered to pass the tube over the animal, whereupon the lids are closed and the animal is trapped inside.

Another sampler which was developed specifically for the "Shinkai 2000" uses a motor driven fan to slurp water through a tube. This sampler was developed by scientists at the Ocean Research Institute of the University of Tokyo. Six separate collection canisters allow collection of six specimens on a dive.

A third sampler which was developed at JAMSTEC for use by all submersibles is the gate valve sampler. The concept for the gate valve sampler was developed by Dr. William Hamner. This sampler uses gate valves which are sold commercially and designed to control irrigation for agriculture. The gate valve consists of a plastic tube which is sealed by a metal plate that blocks the tube transversely. This plate slides open and closed and acts as a "gate" to control the water flow. The plate slips between two rubber rings which act to seal the hole.

The gate valve sampler consists of a Plexiglas tube body with a single gate valve mounted on both ends (Fig. 1). One advantage of the gate valve sampler over other samplers is that the body can be made in any shape and size the scientist wishes, limited only by practicality of space and utility. Originally, the body was conceived of as a rectangular or box-shaped sampler which could be used directly as an aquarium thereby reducing stress to the captured animal. Gate valves could be removed from the sampler and theoretically attached to another sampler body for the next dive while the initial sampler body was transported to the laboratory for use as an aquarium for observation and experimentation. Our experience however suggests that the frontal wake created by the pressure of forward movement of the sampler hindered the collection of animals through the gate. This problem was originally minimized by using a funnel attached to the gate sampler. However, in successive generations of this sampler, we have opted to employ a cylindrical sampler body to reduce this effect.

On one recent dive ("Shinkai 2000" dive #945), the ORI suction sampler was combined with one gate valve sampler allowing gentle suction to draw a specimen into the gate sampler before the gates were closed. This system worked efficiently and effectively. It is our opinion

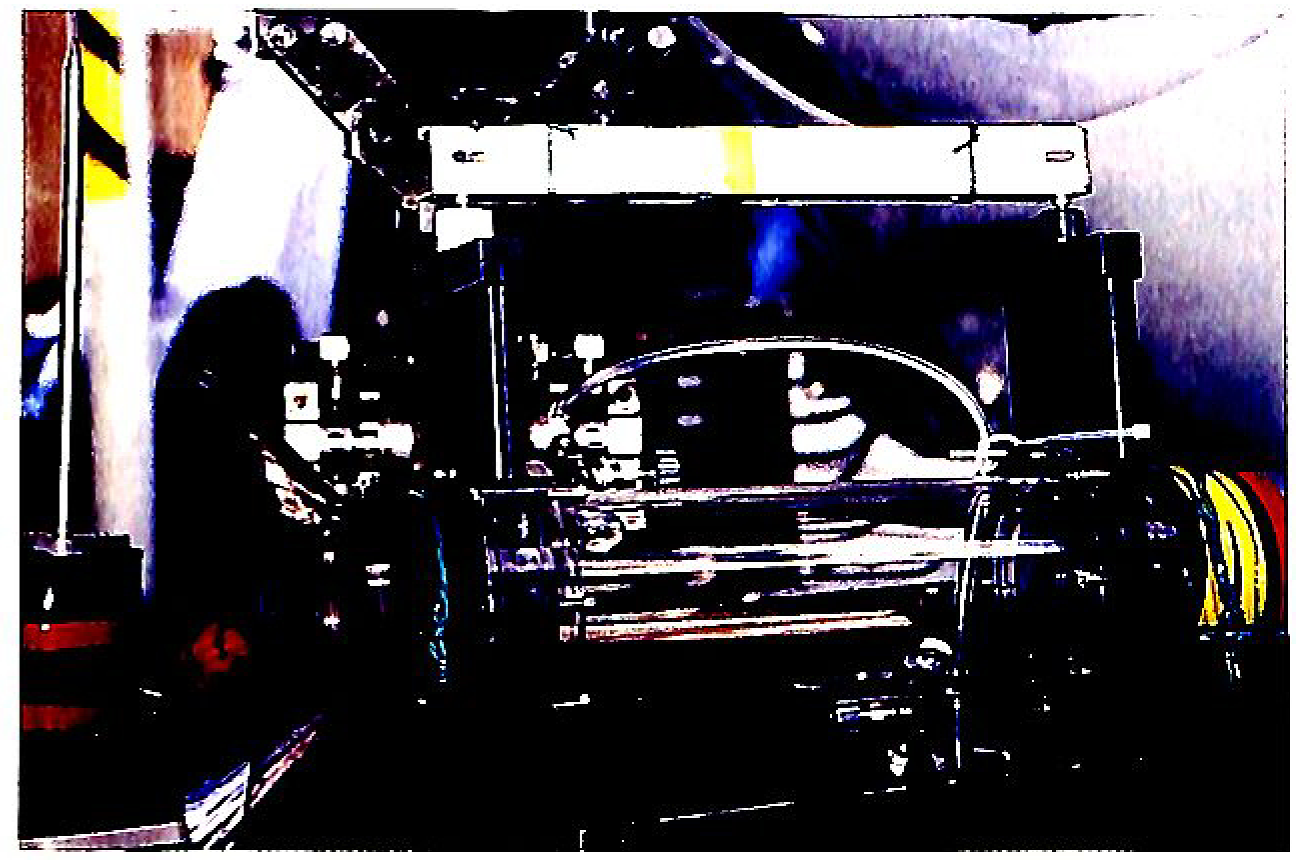

Fig. 1 Photograph of a gate valve sampler attached to the "Shinkai 2000." The sampler body (clear tube) is located between two gate valves (grey with metal bar connecting the top). The sampler is shown in the closed position.

that the use of gentle suction with gate valve samplers would adequately compensate for any frontal wake. Therefore, we wish to develop an array of gate valve samplers with box-shaped bodies, which could be used directly as aquaria, attached to a pump with variable speed suction capability. This system would allow for collection of specimens in pristine condition and would reduce the need for tampering with a specimen brought aboard the ship.

Collecting animals in good condition is the first step to prolonged observation and analysis of deep water animals. Keeping animals healthy and in good condition is the second important step. To do this, we have built three plankton kreisels after the original design (Hamner, 1990). A plankton kreisel is a circular aquarium in which water current is directed around the inner periphery. The water traveling in the center of the tank spins more slowly than water near the edge of the tank, and so animals are shunted towards the center. This action tends to keep delicate specimens away from any hard surfaces of the aquarium and prevents them from being damaged. We have imported two large kreisels which are one meter in diameter, and 25 cm in depth. These kreisels are to be used for long term laboratory observations at JAMSTEC. Additionally, a smaller kreisel which is 80cm in diameter and 12cm in depth is carried aboard the ship for short term laboratory analyses and to transport specimens back to our research center in Yokosuka.

In order to collect physico-chemical data, we routinely employ a Seabird SBE16 CTD–dO meter. The CTD is attached directly to the submersibles. The data collected from the CTD and the dO meter are processed and analyzed. Video observations are correlated with physico-chemical data to provide information on the preferences and ranges of species over various parameters. For example, one species may live just below a pycnocline while another species lives above a thermocline. This kind of fine scale information is only possible using submersibles.

3. Methodology

In order to investigate the distributions and relative abundances of pelagic communities, profiles are conducted both vertically and horizontally. Vertical profiles are conducted simply by descending to the sea floor and recording the fauna observed. This also allows a full profile to be measured by the CTD–dO meter. Vertical distribution data is entered into a relational database and over time we hope to be able to discern trends and analyze patterns in water strata selection.

By traversing an area at a specific depth, information about the relative abundances of species is collected. Horizontal transects are usually run at specific depths, for example at 100m increments, or they are run at depths which correlate to some physico-chemical trend observed from CTD–dO data. For example, transects may be run above, within, and below a pycnocline or an oxygen minimum layer.

Baited traps have also been deployed on the submersibles. Such traps are simple constructions filled with fish or other bait. Baited traps will potentially show feeding behaviors and perhaps give some indication of food preferences for some pelagic fauna. Such information will aid in the care and maintenance of specimens caught by the samplers and transported to the surface.

4. Results

The purpose and scope of this paper is to discuss the establishment of our program and therefore specific quantitative results have been omitted. Such results are forthcoming, and data sets are still being processed and analyzed. It shall be instructive here to show the kinds of data we are collecting and the information and processes which have been implemented and form the core of our future research efforts.

The profiles shown for temperature and salinity (Fig. 2) and density and oxygen (Fig. 3) concentration are representative of the physico-chemical data collected by the SBE16 CTD–dO meter. These data were collected from the Suiyo Seamount in the Ogasawara Basin. These data show a thermal minimum at about 700m and the lack of a well defined oxygen minimum zone in this location. The oxygen continues to decline to the bottom, although it does approach a steady minimum beyond a depth of 1,000m. A noticeable reduction in the biomass of pelagic animals accompanied this area of low oxygen.

New information on behavior and morphology has

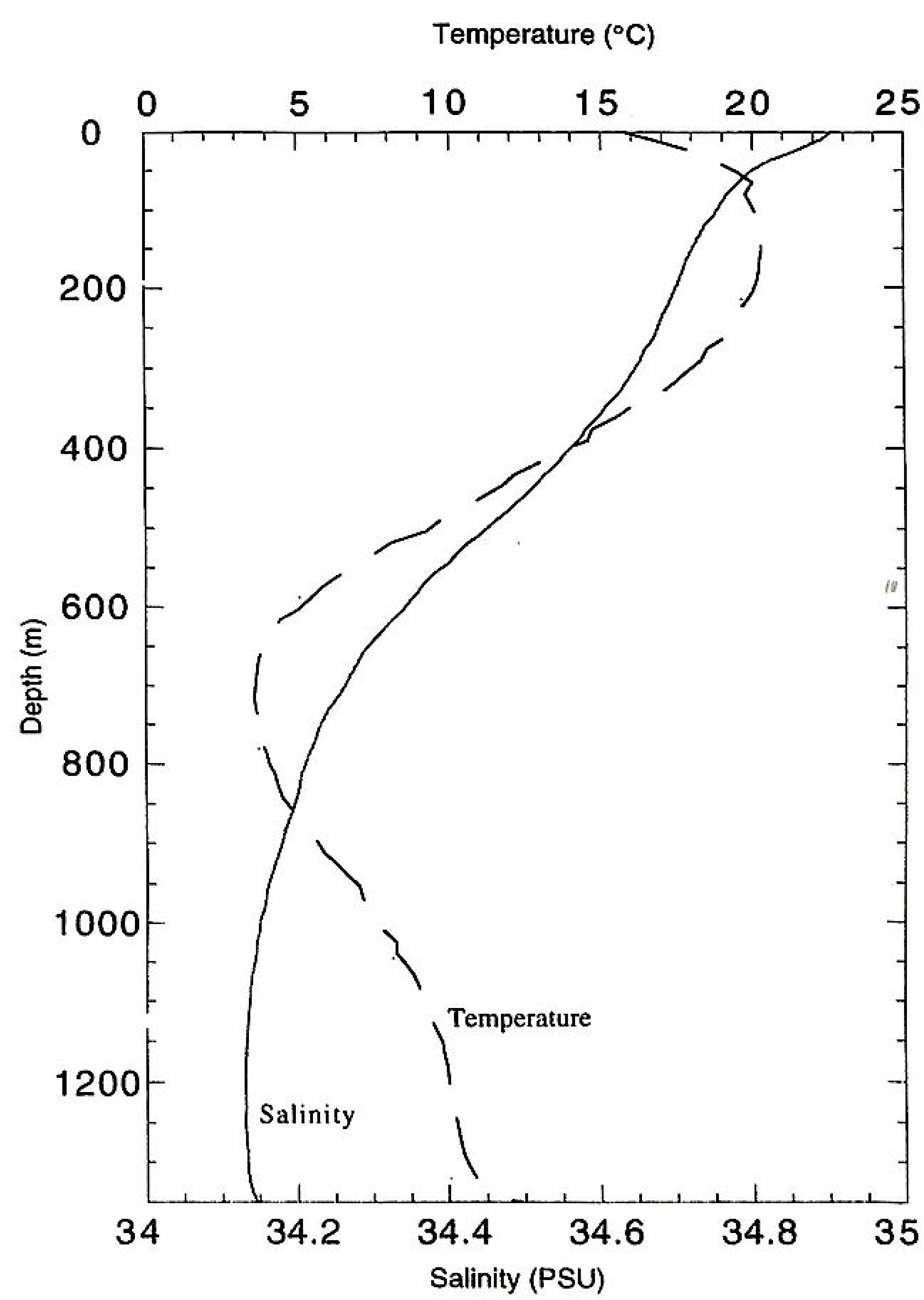

Fig. 2 CTD–dO profiles of the temperature and salinity of a typical submersible dive taken from the Suiyo Seamount of the Ogasawara Basin ("Shinkai 2000" dive #950).

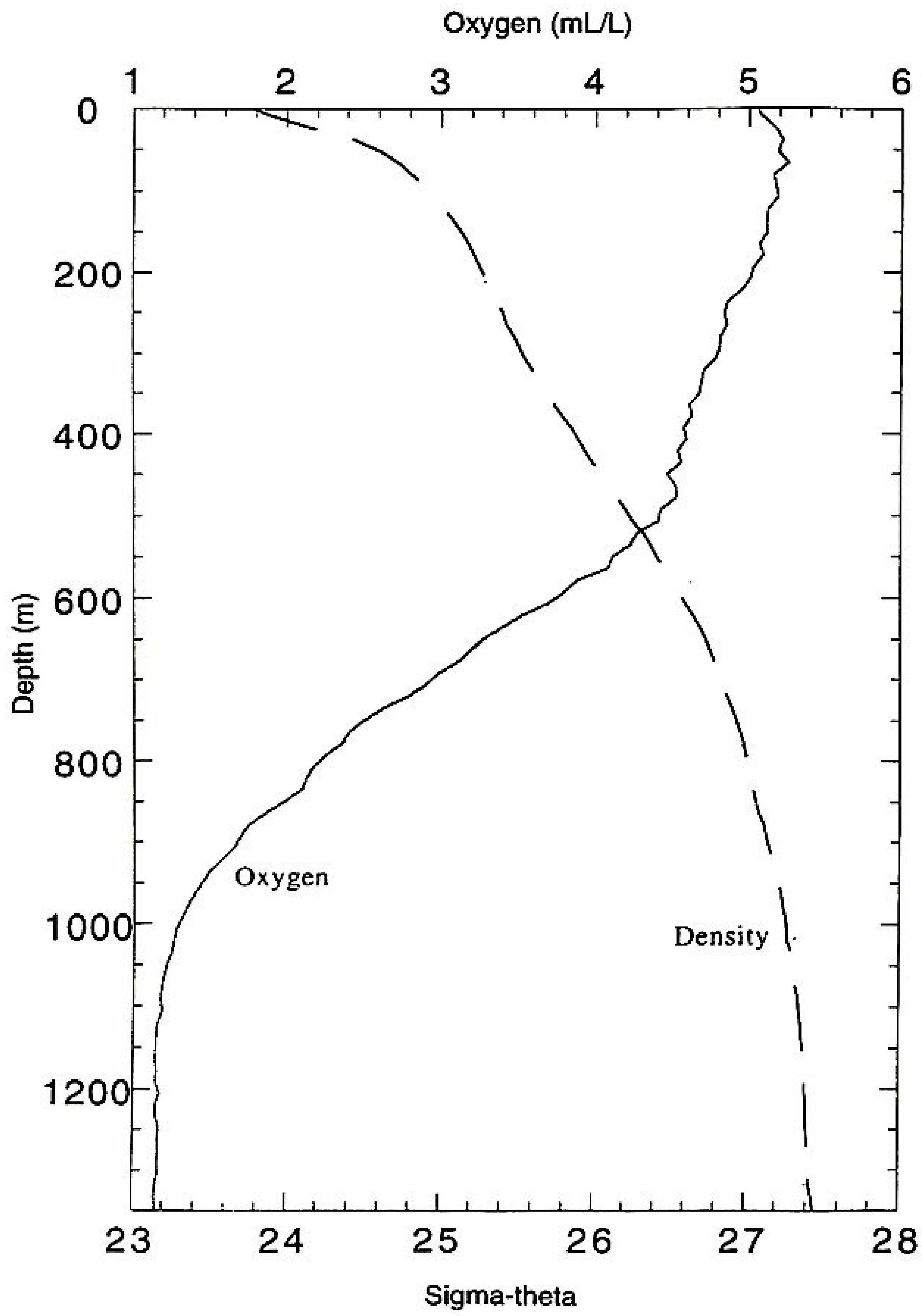

Fig. 3 CTD–dO profiles of the oxygen concentration and density of a typical submersible dive taken from the Suiyo Seamount of the Ogasawara Basin ("Shinkai 2000" dive #950).

been collected from a variety of taxa including : siphonophores, larvaceans, squids, fishes, polychaetes, pyrosomes, crustaceans and munnopsid isopods. Some of these animals are shown in video still figures accompanying this article. Fig. 4 shows a map of the three sites whence the specimens shown in the video stills came. The video stills (Fig. 5–12) present a cross section of the kinds of species we are able to observe and collect.

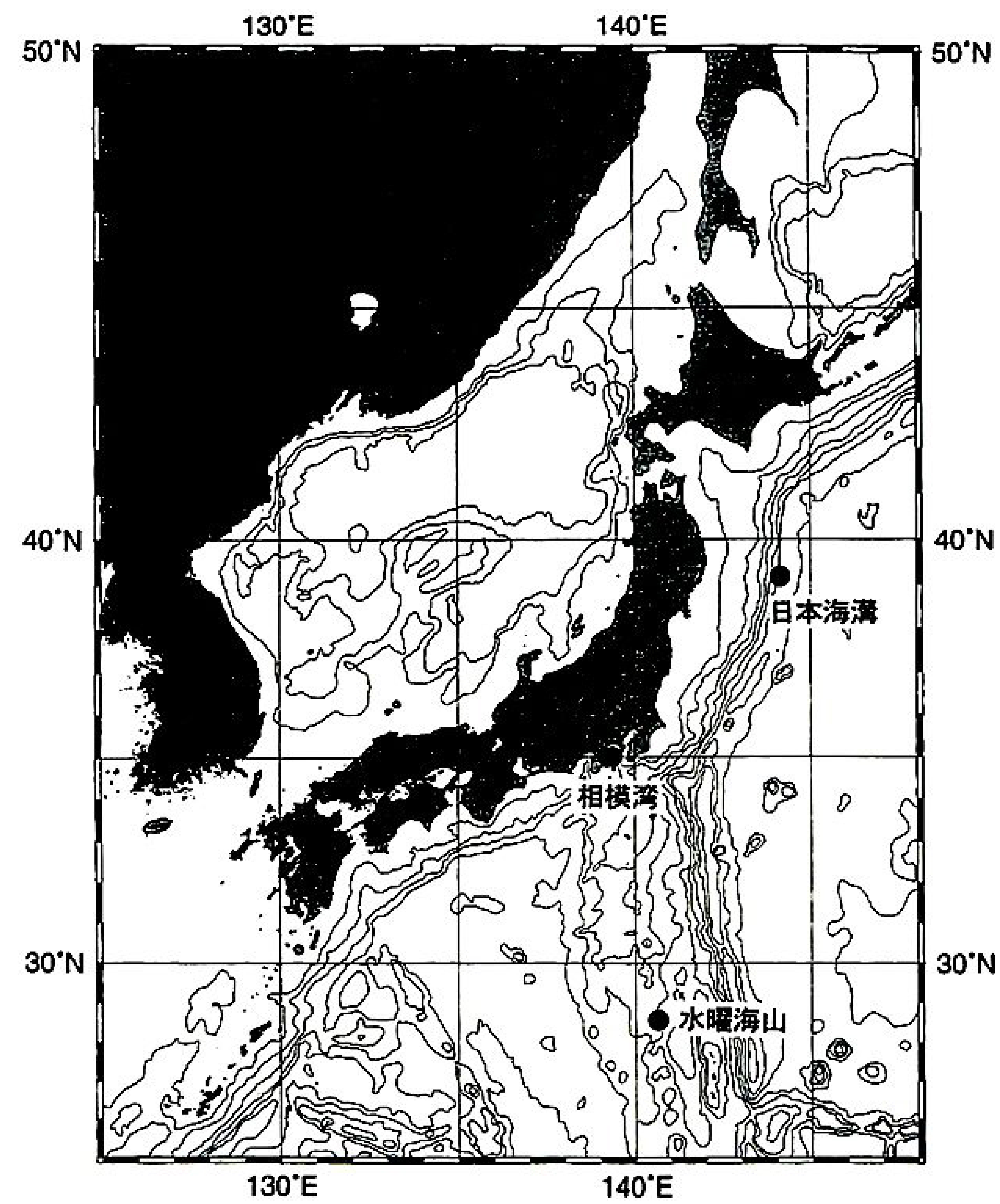

Fig. 4 Map showing the dive locations of the accompanying video still pictures.

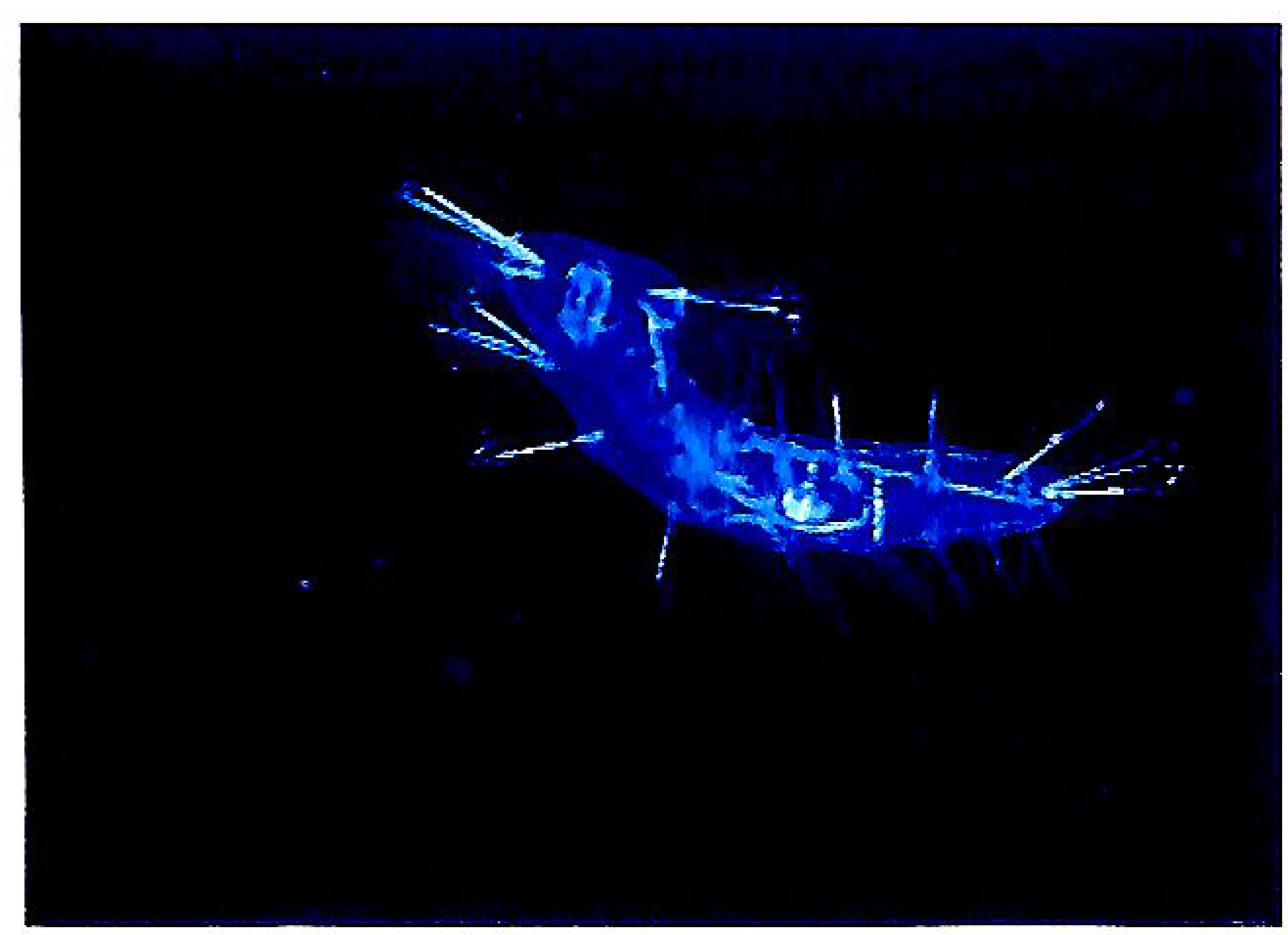

Fig. 5 New species of polychaete worm found at 6,495 m in the Japan Trench ("Shinkai 6500" dive #372).

5. Discussion and future studies

The use of submersibles to study the meso-pelagic and bentho-pelagic community around Japan has begun to yield some interesting information. The water column of Sagami Bay, for example, does not seem to contain as many oegopsid cephalopods as in other similar bays such as the Monterey Bay off the western coast of the United States (Hunt, pers. obs.). Observations on Japanese species of jellyfishes in the same genera as those studied elsewhere have revealed similar behavioral traits. *Atolla* from Japanese waters trail one tentacle in a way similar to those seen elsewhere in the world (Hunt, pers. obs.). *Colobonema* from Sagami Bay also autotomize their tentacles when threatened as do those

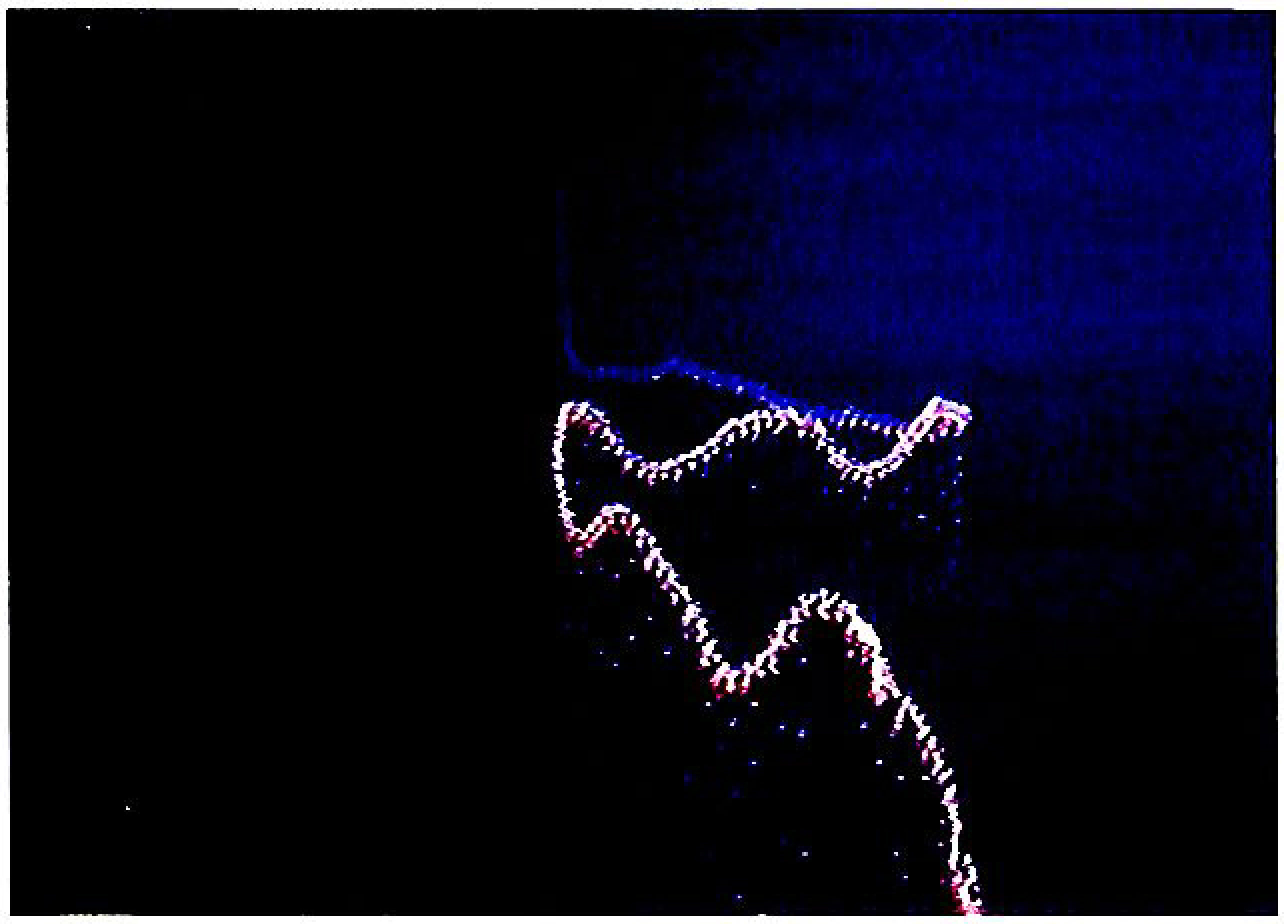

Fig. 6 Physonect siphonophore from Sagami Bay with tentacles deployed ("Dolphin 3 K" dive #327).

Fig. 7 Physonect siphonophore from Sagami Bay with tentacles retracted ("Dolphin 3 K" dive #323).

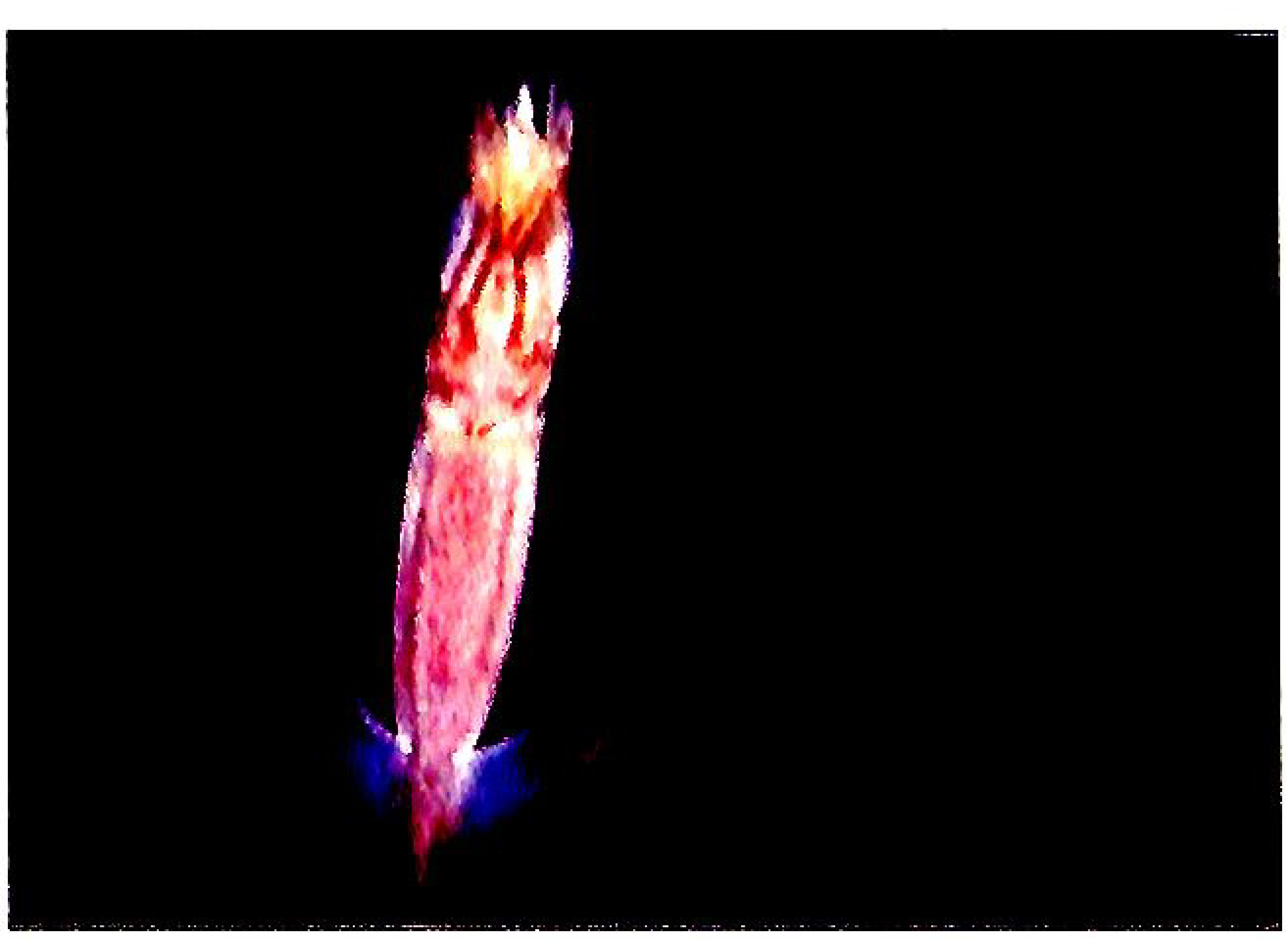

Fig. 8 Ommastrephid squid swimming down in the Sagami Bay ("Shinkai 2000" dive #945).

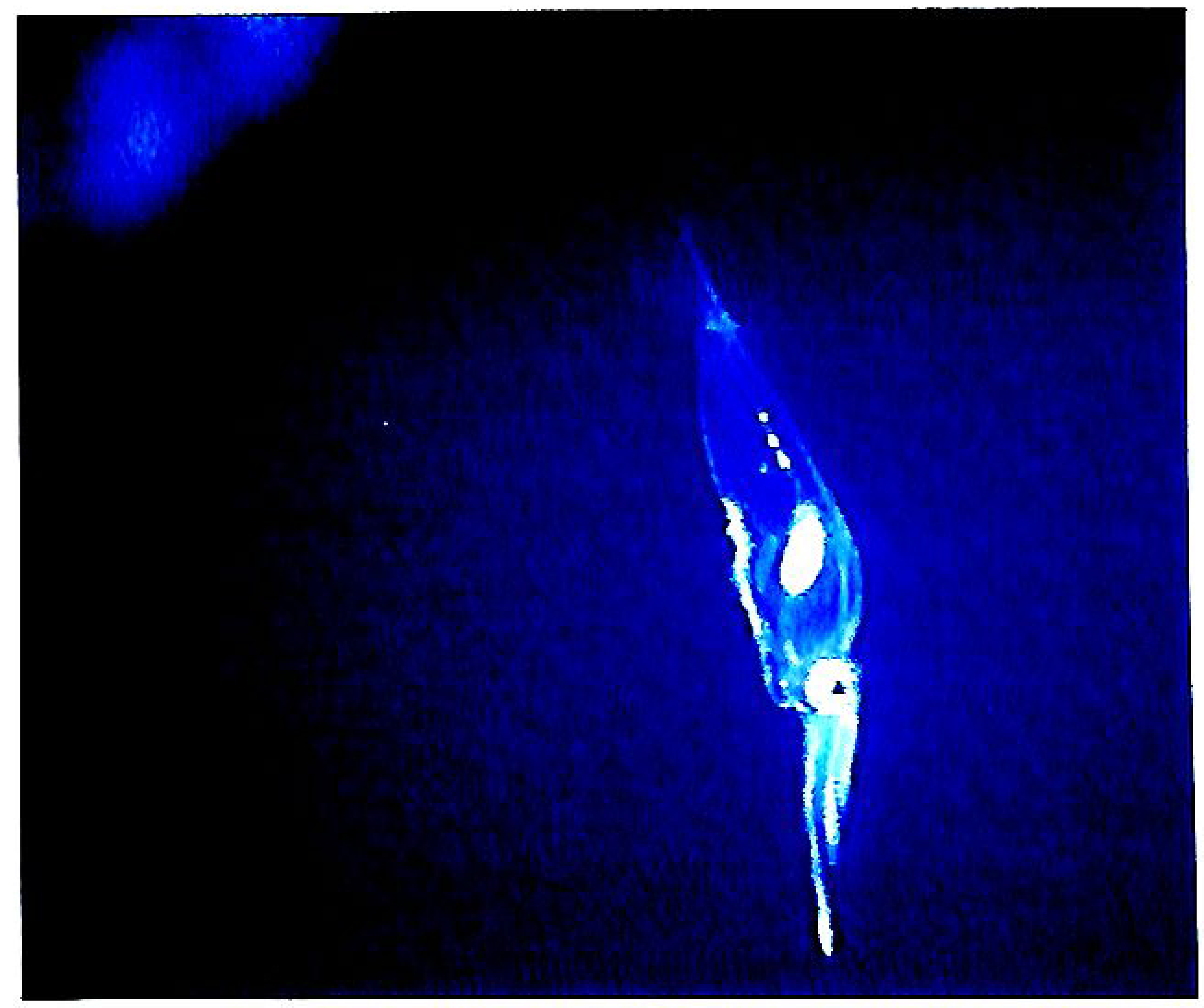

Fig. 9 Cranchiid squid from Suiyo Seamount ("Shinkai 2000" dive #950) with a healing wound on its ventral side (whitish scar on the left).

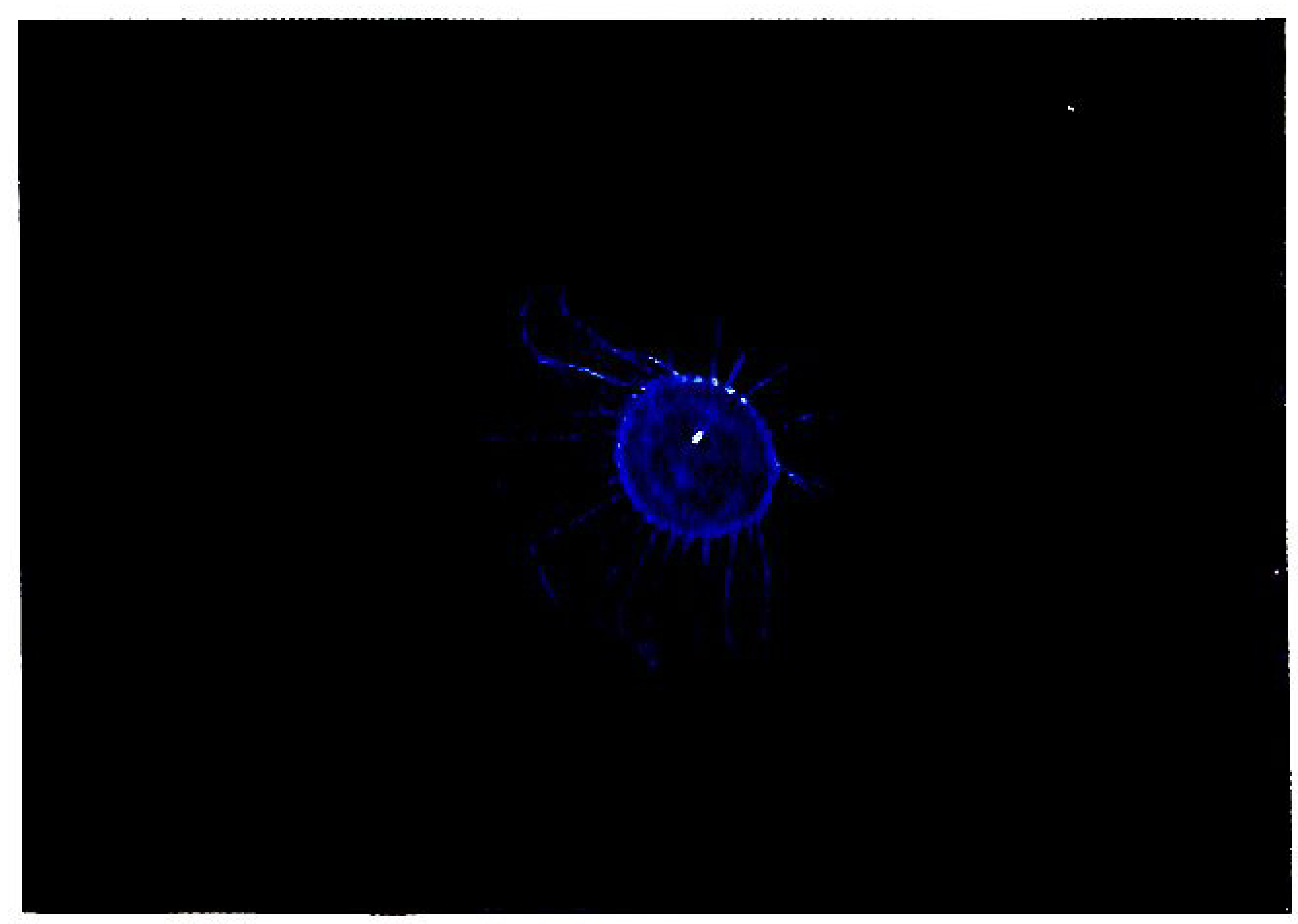

Fig. 10 Purple-ringed Solmissus sp. taken from Sagami Bay ("Shinkai 2000 dive" #945).

from the same genus in Monterey Bay, California, although they seem to do it less readily (Hunt, Pers. obs.). A new bentho-pelagic species of polychaete worm was found in the Japan Trench at depth range extending to 6,495 m. Although the head of this polychaete looked like that of a tomopterid, it possessed rigid glass-like parapodia unlike the flaccid parapodia observed in tomopterids commonly found on shallower living species (Hunt, Pers. obs.). There appear to be more siphonophores and pyrosomes in Sagami Bay than net trawling would indicate (Lindsay, pers. obs.). Tuna

Fig. 11 Cydippid ctenophore from Sagami Bay shown with tentacles deployed widely ("Shinkai 2000" dive # 945).

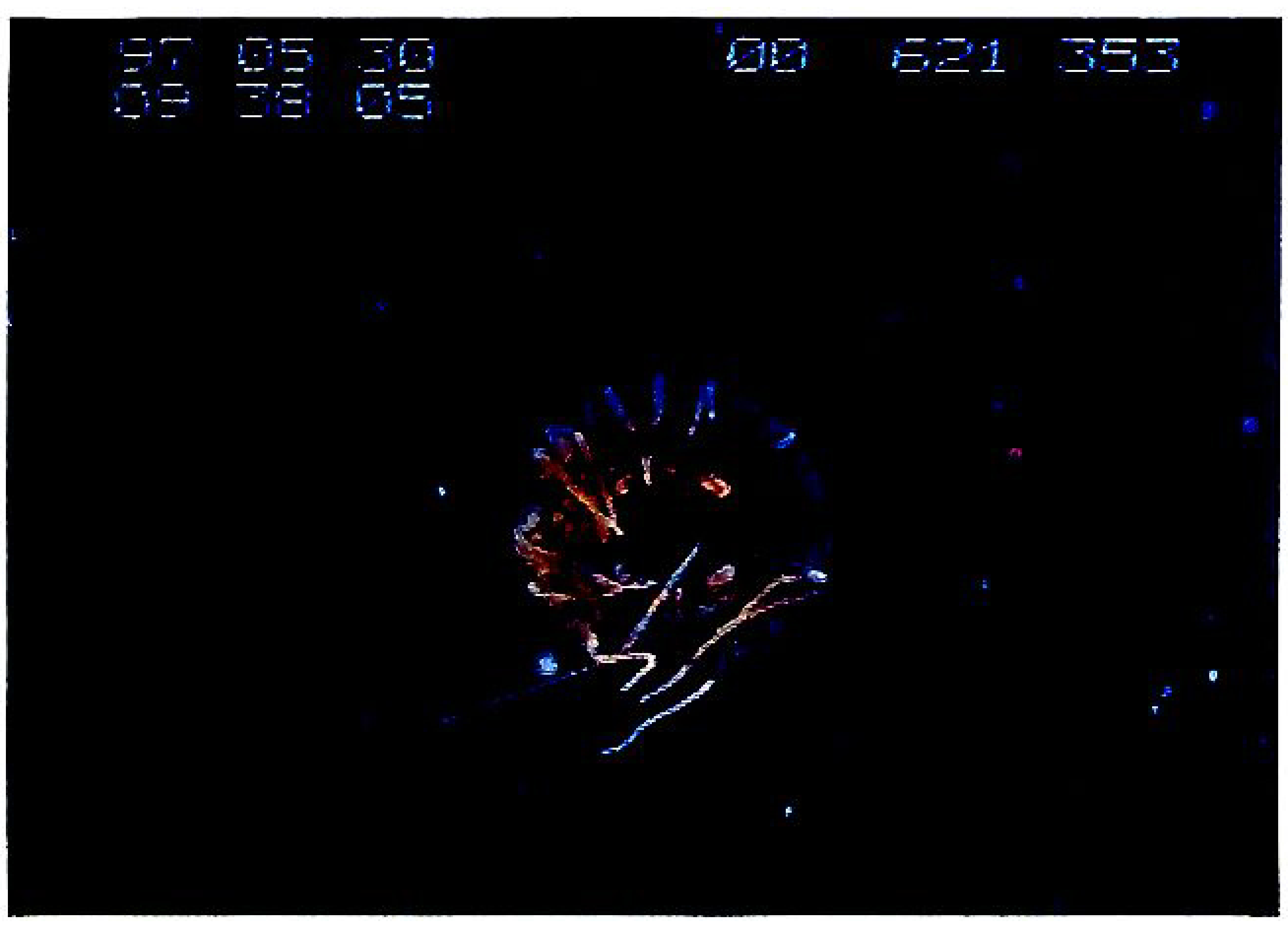

Fig. 12 Atolla sp. from Sagami Bay shown swimming with one tentacle deployed ("Dolphin 3 K" dive #326).

have been confirmed feeding on squid at great depth (560 m ; Lindsay, pers. obs.). Midwater shrimps will sometimes alight on pyrosomes, perhaps as a place to rest or perhaps to feed on organisms trapped on the surface of the pyrosome (Lindsay, pers. obs.).

These kinds of observations are anecdotal at this time due to the relatively few meso-pelagic dives made. As time passes, specific questions can be targeted and repeated observations (or lack thereof) should support (or refute) anecdotal information. But such observations serve to highlight the utility of submersible research. Information on the behavior of deep-sea animals can best be acquired using submersibles or animals collected with submersibles. Human or video observation of animals *in situ* provide the most detailed morphological data including the color and markings of species. Finally, observations from submersibles can validate speculative information about behavior and provide precise information about location and activities.

Having established a survey program and acquired the basic equipment, we hope to continue our studies of the pelagic fauna of Japan and to expand our capabilities. Live observation and collection of healthy specimens is crucial to this task. In order to further develop this program, we wish to acquire more samplers, and to attach them to all the available submersibles. We also hope to collect CTD-dO data and possibly video data from the dives of other researchers using the "Shinkais" and the "Dolphin 3 K". This could be accomplished by videotaping the water column on ascent, or descent, or both depending on the discretion of the chief scientist of each dive. Finally, we wish to begin a study of deep scattering layers in the waters around Japan. Therefore, we shall attempt to acquire appropriate sonar which could be used in concert with the submersibles to establish clearly for the first time which animals are contributing most to scattering data and what effects the submersibles themselves are having on estimates of the abundances of pelagic species.

The use of submersibles has provided biological oceanographers with a new means of collecting data that supplements information collected by traditional means as well as providing new information which could not be collected by any other method. Because submersible research is expensive and time consuming, and because dive time is limited worldwide, there is excellent opportunity for nationwide and international collaboration. We hope this program will provide a catalyst to bring together scientists from around Japan and strengthen ties internationally. In doing so, we have a good chance to assemble a functional and comprehensive knowledge of one of the least understood animal communities in the world.

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