FISHERIES SCIENCE 2006; **72**: 342–349

Molecular attempt to identify prey organisms of lobster phyllosoma larvae

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ABSTRACT: A molecular approach was adopted to investigate the natural diets of palinurid and scyllarid lobster phyllosoma larvae. The central domain of the 18S rDNA gene was amplified using nested polymerase chain reaction (PCR) and genomic DNA extracted from the larval hepatopancreas. The resulting 18S rDNA clones were screened using restriction fragment length polymorphism (RFLP) analysis, and then FASTA homology search and phylogenetic analysis were performed on the nucleotide sequences to identify the source of the eukaryotic organisms. The feasibility of this method was confirmed using the laboratory-reared phyllosoma larvae of the Japanese spiny lobster *Panulirus japonicus* that were fed either with common mussel *Mytilus edulis* gonads or with *Artemia* nauplii exclusively. Among the 804 clones isolated from five wild-caught mid- to late-stage phyllosoma larvae (three palinurids and two scyllarids), 0–132 clones per sample possessed restriction profiles distinct from those of the hosts. The Cnidaria and Urochordata DNA were identified from both the palinurid and the scyllarid larvae, which were thought to be prey animals for the mid- to late-stage phyllosoma larvae.

KEY WORDS: 18S rDNA, palinurids, phyllosoma larvae, prey organisms, scyllarids.

INTRODUCTION

Palinurid and scyllarid lobsters are important fishery resources. Their larvae, known as phyllosoma, are morphologically distinct from those of the other decapod crustaceans, possessing a flattened and highly transparent body (Fig. 1). Phyllosoma larvae also undergo a unique long planktonic period extending from several months to more than a year.[1–4] Although the predator-prey interaction may be vital to understanding larval ecology in terms of the dynamic food web, the prey organisms of the phyllosoma in the natural environment have been not determined. The scyllarid phyllosoma larvae exhibit the unique behavior of riding on jelly fish, known as 'piggyback riding'. This behavior may facilitate transportation and feeding simultaneously.[5,6] Sims and Brown[7] studied the fecal material of a scyllarid phyllosoma larva and found that it was comprised entirely of undigested nematocysts. Laboratory experiments offering a variety of plankton species as prey indicated that the *Panulirus interruptus* and *Jasus edwardsii* phyllosoma larvae preferred to feed upon medusae, ctenophores, chaetognaths and other soft-bodied animals.[8,9] However, phyllosoma larvae may change prey organisms as they grow and disperse over a wider range during the long larval period. Unlike the other decapod crustacean larvae, phyllosoma larvae may be a sucking predator (Murakami K., personal communication). Observations on mouthpart development suggested that the early stage *J. edwardsii* phyllosoma larvae would benefit from a diet comprised of soft gelatinous zooplankton.[10]

Although rearing phyllosoma larvae has been problematic, successful metamorphoses have been achieved in several palinurid species by using *Artemia* nauplii supplemented with common mussel gonads *Mytilus edulis* as prey.[9,11,12] However, their survival rate is still very low. Thus, information on natural diets for phyllosoma larvae may be crucial for dietary formulations in the aquaculture setting.

Recently, DNA-based approaches have been conducted to reveal prey organisms in the gut contents of several marine organisms.[13–17] These studies, however, dealt with adult predator and/or used polymerase chain reaction (PCR) primers designed

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Received 15 July 2005. Accepted 27 October 2005.

specifically for suspected targets or target prey organisms, thus restricting the number of taxa from which the sequence is amplified. In order to investigate and identify unknown prey organisms of lobster phyllosoma larvae, universal primers were designed for amplifying the relatively variable central domain of the 18S rDNA gene in this study. In this way, it is more likely to isolate a large number of prey taxa, thus giving a comprehensive view of the dietary preferences of wild-caught palinurid and scyllarid lobster larvae. In this study, the technique to detect foreign 18S rDNAs in the hepatopancreas is introduced, and results of our attempts using wild-caught phyllosoma larvae of palinurid and scyllarid lobsters are reported.

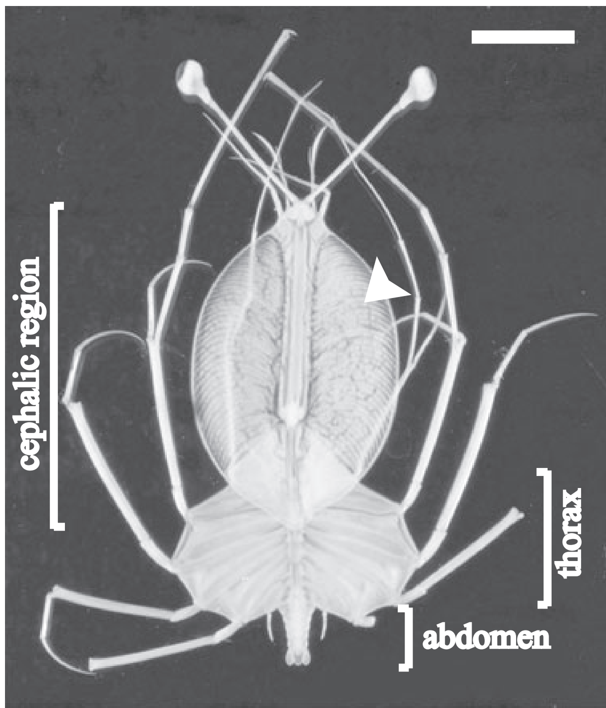

Fig. 1 Ventral view of stage VIII phyllosoma larva of *Panulirus longipes bispinosus* (ethanol preserved). Note the multiple radial grooves of the hepatopancreas in almost the entire cephalic region (arrowhead). Scale bar = 5 mm.

MATERIALS AND METHODS

Phyllosoma specimens and DNA extraction

Nine phyllosoma larvae of palinurid and scyllarid lobsters used in this study are shown in Table 1. The developmental stages of these phyllosoma larvae were determined according to previous descriptions.[3,18–20] Four were laboratory-reared phyllosoma larvae of the Japanese spiny lobster *Panulirus japonicus* hatched on July 2002 and kept at the Minami-izu Sea-Farming Center, Japan. The *P. japonicus* phyllosoma larvae were fed with gonad of common mussel *Mytilus edulis* and *Artemia* nauplii for 155 days after hatching, and were divided into two groups according to feeding conditions: (i) one was exclusively fed with common mussel gonads *M. edulis*; and (ii) the other with *Artemia* nauplii. Two individuals (Myt-1 and -2) from the mussel feeding condition and two (Art-2 and -3) from the *Artemia* feeding condition were sampled 160–175 days after hatching, frozen, transferred to the laboratory, and preserved in ethanol. Of the three wild-caught *Panulirus* phyllosoma larvae, two were from the Pacific Ocean (Pac-2 and -3) and the other one was from the Atlantic Ocean (Atl-2). Two scyllarid phyllosoma larvae were from the Atlantic (Atl-1) and the Pacific Oceans (Pac-1), which were found clinging on the longline gear and on the mid-water trawl net, respectively. Nucleotide sequence analysis on the mitochondrial cytochrome oxidase subunit I (COI) and/or 16S rDNA genes (data not shown), followed by GenBank homology search indicated that the three *Panulirus* species were *P. japonicus* (Pac-2), *P. longipes bispinosus* (Pac-3) and *P. echinatus* (Atl-2). One scyllarid larva (Atl-1) showing a high

Table 1 Palinurid and scyllarid lobster phyllosoma larvae used in this study

Source or locality	Family	Species	BL (mm)	stage	ID
Laboratory-reared					
Artemia feeding	Palinuridae	*Panulirus japonicus*	9.4, 10.6	VI	Art-2, -3
Mytilus feeding	Palinuridae	*Panulirus japonicus*	9.0, 9.4	VI	Myt-1, -2
Wild-caught					
Atlantic	Scyllaridae	*Parribacus* sp.	73.4	XI	Atl-1
	Palinuridae	*Panulirus echinatus*	20.3	VIII?	Atl-2
Pacific	Scyllaridae	*Scyllarus* sp.	19.3	VII?	Pac-1
	Palinuridae	*Panulirus japonicus*	11.5	VII	Pac-2
	Palinuridae	*Panulirus longipes bispinosus*	21.7	VIII	Pac-3

homology (98%) to *Parribacus antarcticus* in the COI sequence was denoted as *Parribacus* sp. in this study. However, the COI data poorly identified the other scyllarid larva (Pac-1) because of the small number of reference sequences in the data base. Thus, this larva was morphologically assigned to *Scyllarus* sp.[19,20]

Prior to DNA extraction, the surface of phyllosoma larvae was washed well with sterile distilled water to remove possible contaminants from the body surface. Since the hepatopancreas is a single chamber comprising numerous well-developed radial grooves (Fig. 1),[10] it was not possible to dissect out the contents. Therefore, hepatopancreas tissues including the contents were pipetted out from a small incision made in the cephalic region. This hepatopancreas content and a part of pereiopod were homogenized using a Teflon pestle followed by crude DNA extraction using a standard proteinase K digestion–phenol–chloroform extraction. The water used to wash all of the wild phyllosoma larvae was recovered, ethanol-precipitated and used as a negative control.

PCR primers to amplify 18S rDNA

Two sets of universal primers were designed to amplify the central domain of the 18S rDNA gene for nested PCR. The nucleotide sequences of the primers used for the first PCR were 5′-ATCCAAG GAAGGCAGCAGGC-3′ (18SU467F) and 5′-CTC CACCAACTAAGAACGGC-3′ (18SL1310R). Those used for the second PCR were 5′-CAAGTCTGGT GCCAGCAGCC-3′ (18SU615F) and 5′-GTGCCCT TCCGTCAATTCCT-3′ (18SL1170R). The labeled numbers of each primer correspond to the nucleotide positions of human 18S rDNA. The priming regions were highly conserved among eukaryotic organisms such that both primer combinations can amplify homologous regions of diatom *Chaetoceros* sp., lobster *Panulirus japonicus*, eels *Anguilla japonica* and *Muraenesox cinereus*, and human *Homo sapiens* (data not shown). The PCR mixture contained 0.2 U *Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA), 0.2 mM of each dNTP, 1.0 μL 10 × buffer with 15 mM $MgCl_2$, 10 pmol of each primer, and 10–50 ng of template DNA. The final volume of the reaction mixture was increased to 10 μL with sterile distilled water. The PCR included an initial denaturation at 95°C for 2 min, followed by 30 cycles of amplification (denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1.5 min, with a final extension at 72°C for 10 min). The first and second PCR products were electrophoresed through a 1.5–2.0% agarose gel in TBE buffer (50 mM Tris, 1 mM EDTA, and 48.5 mM boric acid) to confirm amplification.

Cloning, screening and sequencing of amplified fragments

Amplicons from the second PCR were directly subjected to cloning without further purification. Cloning was carried out using the pGEM-T Easy Vector System I (Promega, Madison, WI, USA). The transformed clones were first screened by colony-direct PCR with a primer set used for the second PCR. Since the preliminary restriction assays showed that three endonucleases (*Alu* I, *Hpy* 188I and *Mse* I) had multiple restriction sites in the phyllosoma larvae, the PCR products were digested with these enzymes. The digested samples were electrophoresed on an agarose gel to select clones possessing restriction profiles distinct from those of the hosts. One PCR product from each restriction type was selected arbitrarily and purified using ExoSAP-IT (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The sequencing reactions were conducted with the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems) with the 18SU615F primer, and nucleotide sequences were determined with an automated DNA sequencer (ABI 310, Applied Biosystems).

Homology search and sequence analysis

The obtained DNA sequences (~500 bp) were subjected to homology searches using the FASTA algorithm through the DNA Data Bank of Japan (DDBJ) to investigate sequence similarities and potential homologies among organisms. Among the FASTA-nominated sequences, the top five were selected and incorporated into a phylogenetic analysis. Alignments of the DNA sequences were performed with the ClustalW algorithm[21] followed by manual editing. The aligned sequences were imported into PAUP*[22] to infer the positions of the cloned DNA on a neighbor-joining (NJ) tree. The genetic distances among sequences were calculated under Kimura's two parameter (K2P) substitution model with the complete deletion option. Bootstrap probabilities with 1000 replications were calculated to assess reliability on each node of the NJ tree.

RESULTS

Analysis of laboratory-reared phyllosoma larvae

Results of experiments using laboratory-reared larvae are summarized in Table 2. The transformation

Table 2 Results of food DNA detection using laboratory-reared phyllosoma larvae

Specimen ID	No. of amplification/PCR	RFLP profile		No. of types	Identified eukaryote (no. of clones)	
		host	non-host			
Art-2	58/62	54	4	3	*Artemia*	(1)
					unidentified	(3)
Art-3	58/63	57	1	1	*Panulirus*†	(1)
Myt-1	38/48	14	24	5	*Mytilus*	(6)
					Panulirus†	(1)
					Cryptomeria	(15)
					unidentified	(2)
Myt-2	53/60	41	12	5	*Mytilus*	(8)
					Panulirus†	(4)

†Supposed to be host variants.

efficiency was high for all samples (>80%). Of 48–63 white colonies per plate subjected to colony direct PCR using second PCR primers, amplicons were obtained in 38–58 colonies. The number of clones showing restriction profiles distinct from those of the hosts varied considerably, ranging from 1–24 (1.7–60.5%).

In the two phyllosoma larvae (Art-2 and -3) fed with *Artemia* nauplii, four and one clones with distinct restriction profiles, respectively, were detected. Three restriction profiles were observed in Art-2. The FASTA search indicated that the insert of one clone was *Artemia salina* (99% nucleotide identity). The other two clones (AB219772 and AB219773) were unidentified, because the sequence ranked first was uncultured ciliates (AF530529) with low nucleotide identity (<85%). A clone from Art-3 possessed a similar 18S rDNA sequence with *Panulirus* sp. with high identity (>95%), and was determined to be a host variant.

In the two larvae (Myt-1 and -2) fed with common mussel gonads, 24 and 12 clones, each with five restriction types were detected to have inconsistent restriction profiles from the host. Of the 24 clones we obtained from Myt-1, six were found to be *Mytilus* 18S rDNA, 15 were apparent terrestrial plant contaminants, Japanese cedar *Cryptomeria japonica*, and one was a host variant. Protista (AB022111) was nominated for the remaining two restriction types (AB219774 and AB219775), but the identity scores were very low (80%) and determined to be unidentifiable. Of the 12 clones from Myt-2, eight shared identical sequences with *Mytilus* 18S rDNA and the other four were host variants. Thus, the results obtained in the laboratory-reared larvae indicate that the experimental procedure presented in this study is powerful enough to detect gene sequences of fed organisms in the hepatopancreas of lobster phyllosoma larvae.

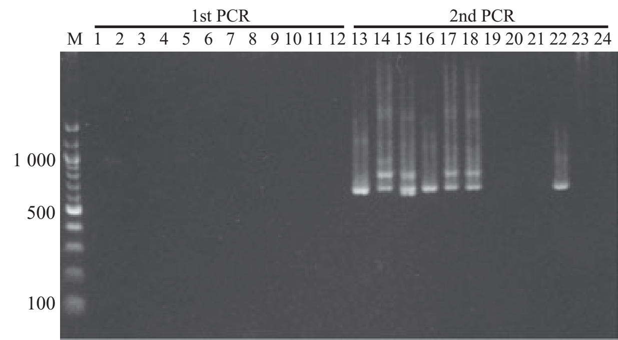

Fig. 2 Agarose gel electrophoresis showing results of nested PCR. Lanes 1–12: first PCR products, 13–24: second PCR products, 1–6 and 13–18: from foreguts, 7–12 and 19–24: from water washes (negative controls). The left end is a 100 bp DNA ladder (New England BioLabs, Ipswich, MA, USA), and molecular sizes are shown along the margin.

Analysis of wild-caught phyllosoma larvae

Strong amplification was obtained from the larval washing water in only one specimen of five phyllosoma larvae (Fig. 2), and all 81 clones analyzed from this individual shared a restriction profile that was identical to that of the host. These data indicated that cross-contamination from the body surface to the hepatopancreas of phyllosoma larvae was unlikely.

Results of the experiment using the hepatopancreas of wild-caught phyllosoma larvae are summarized in Table 3. The number of clones amplified ranged 151–178 per sample (84.8–99.3%), and all inserts had restriction site(s). The proportion of clones showing restriction profiles that were inconsistent from the hosts varied considerably among the samples ranging from 0 (Atl-1) to 74% (Pac-1). The number of restriction types

Table 3 Results of eukaryotes DNA detection using wild-caught phyllosoma larvae

Specimen ID	No. of amplification/PCR	RFLP profile		No. of types	Identified eukaryote (no. of clones)
		host	non-host		
Atl-1	151/152	151	0	0	all host
Atl-2	151/152	117	34	3	Urochordata[1†] (2)
					host variant[2] (32)
Pac-1	178/192	46	132	8	Urochordata[3] (55)
					Cnidaria[3] (59)
					chimera[1] (8)
					unidentified[1] (10)
Pac-2	168/184	164	4	2	pine tree[1] (1)
					chimera[1] (3)
Pac-3	156/184	150	6	2	Urochordata[1] (4)
					Cnidaria[1] (2)

†Number of restriction type.
‡Host type was not included.

observed in each sample was small, ranging 0–8. The nucleotide sequence alignments among the hepatopancreas-derived and FASTA-nominated sequences revealed eight Pac-1 and three Pac-2 clones to be chimeric artifacts (AB219788 and AB219790) between partial sequences of fungus and host 18S rDNA. These chimera sequences were not included in subsequent phylogenetic analysis. The remaining 15 sequences including host representatives (AB219776-AB219787, AB219789, AB219791 and AB219792) recovered from the phyllosoma hepatopancreas were assembled into four clades (pine tree, Cnidaria, Crustacea and Urochordata) supported by high bootstrap probabilities (>66%) within the phylogenetic tree (Fig. 3). Three sequences obtained from the three phyllosoma larvae (Atl-1, Atl-2 and Pac-1) grouped with palinurid and scyllarid lobster sequences, and were distinct from several other arbitrarily chosen Decapod crustacean species, *Nephrops norvegicus*, *Raninoides louisianensis* and *Helice tridens*. This distinction indicates that these host-like sequences may have originated from variant copies of the host 18S rDNA cluster. Pine tree DNA may be introduced into the laboratory environment from airborne pollen commonly found during certain seasons in Japan. Such is the case with the Japanese cedar observed in the laboratory-reared phyllosoma larvae. In the Cnidaria clade, one sequence (Pac-1 Cnidaria1) from Pac-1 was assigned to Scyphozoa and three (two from Pac-1 and one from Pac-3) were assigned to Hydrozoa. A sequence (Pac-1 Cnidaria3) (525 bp) was closely associated with Siphonophora *Nanomia bijuga* in the phylogenetic tree, with an identity score of 99.8%. In the Urochordata clade, one sequence (Pac-1 Urochordata1) from Pac-1 was assigned to Larvacea (appendicularia) and four (two from Pac-1 and one each from Atl-2 and Pac-3) to Thaliacea (salpa), respectively. Two sequences (Pac-1 Urochordata3 and Pac-3 Urochordata) from *Scyllarus* sp. and *P. longipes bispinosus* (538 and 556 bp, respectively) shared similar sequences (98.1% homology), although these samples were collected in different years.

DISCUSSION

The successful detection of food DNAs in the laboratory-reared lobster phyllosoma larvae indicates that the method presented in this study may be a promising tool to investigate natural diets of the larval form with totally unknown prey. Detection of prey DNA depends on the DNA degradation status and the amount of foreign tissue consumed and remaining in the gut.[23,24] Since laboratory-reared larvae were sampled during continuous feeding, it is likely that the amount of consumed food is different between the *Mytilus* gonad and *Artemia* nauplii feeding groups. The cephalic region of the phyllosoma fed with *Mytilus* gonad was yellowish, while that of *Artemia* was transparent. Detection of the host-variant DNA was not expected, because concerted evolution or molecular drive may have homogenized tandem copies of the ribosomal RNA gene cluster.[25] However, it is obvious that the 18S rDNA clusters of these lobsters are not homogenized. Since universal primers were used, it was expected that mostly host DNAs would be detected. However, in one case of wild-caught larva (Pac-1), the number of clones carrying host DNA fell far below those carrying non-host DNAs (Table 3). This finding indicates that efficient sam-

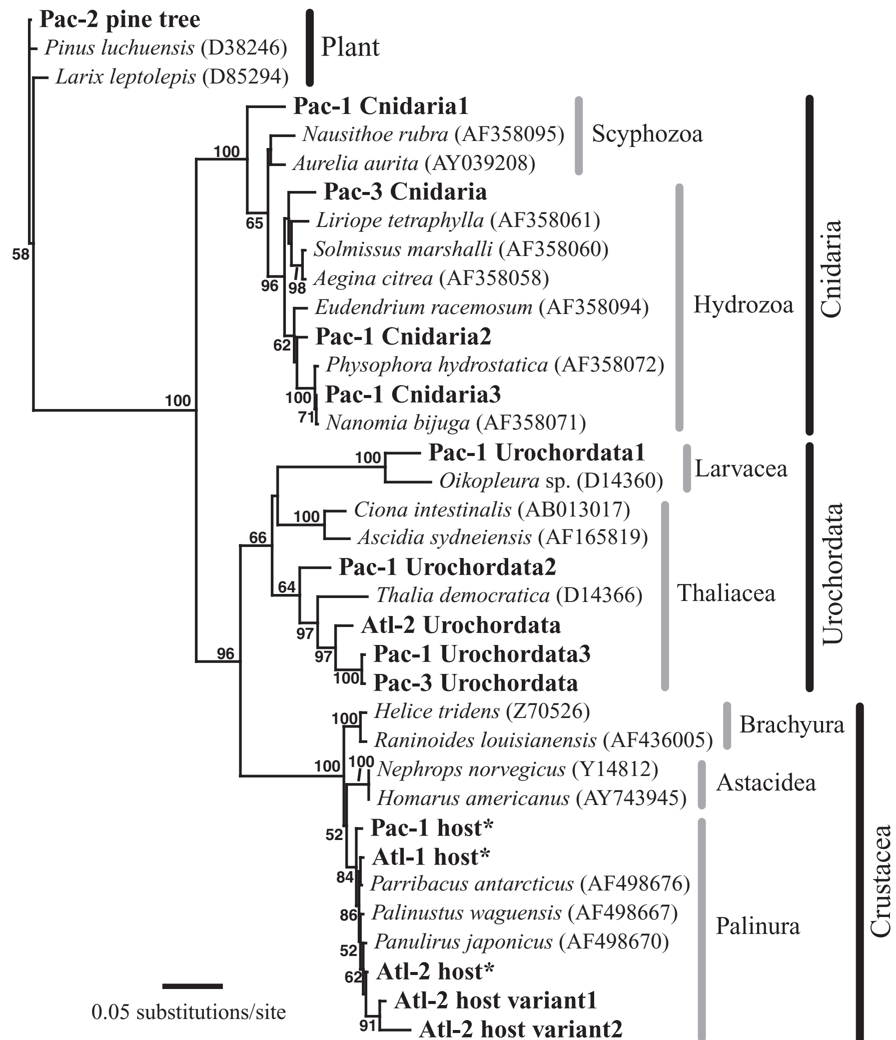

Fig. 3 Neighbor-joining tree for affiliating 18S rDNA sequences obtained from the phyllosoma hepatopancreas. Sequences obtained from the hepatopancreas are shown in bold with the host ID, and eukaryote 18S rDNAs nominated by the FASTA searches are in italic with the accession numbers in parenthesis. Sequences of several non-palinurid decapod crustacean species were incorporated with this phylogenetic tree to estimate the divergence of the host-like sequences. The numbers on the branches indicate bootstrap probabilities with 1000 replications.

pling of hepatopancreas contents with less of the contaminating host phyllosoma tissue may be possible.

A vast amount of 18S rDNA data over a wide range of eukaryote taxa has been accumulated in the database, and is available for investigating the taxonomic affinity of unknown sequences. However, the sequence variation of the 18S rDNA gene within a species is not typically investigated, and sequence information among closely related species may not be abundant, making it difficult to find exact matches. Further, in the 18S rDNA region that was selected, sequence divergence among closely related species may be miniscule in certain taxa. Nevertheless, using the nuclear 18S rDNA detection approach may be advantageous to investigate gut contents, especially when the prey organisms are unknown, because the universal primers used may amplify homologous regions of almost all eukaryotes. Further, multiple copy sequences of prey DNA may survive digestion for a considerable period in the gut of the predator.[26]

The present study may shed light on what these lobster phyllosoma larvae consume during their development and migration. It is proposed that Cnidaria and Urochordata are major prey organisms for the late-stage phyllosoma larvae of the species studied here. This finding corresponds to that by Sims and Brown[7] who observed nematocysts in the fecal mass of a scyllarid phyllosoma. It is possible that the tissue and/or body fluid of these gelatinous animals were forced into the phyllosoma hepatopancreas in the cod-end of plankton nets. This situation may have occurred in our three *Panulirus* phyllosoma larvae and with Sims and Brown's[7] scyllarid larva caught by the plankton net. However, the two scyllarid lobster larvae used in the present study were found clinging on the longline and trawl net, and did not experience the 'plankton jumble' in the net. Further, if the *Panu-*

lirus phyllosoma larvae were distended with other organisms in the net, the universal primers would be able to detect them. A wide variety of eukaryotes (algae, Protista, Protozoa and Stramenopila) have been detected in the gut contents of the deep sea bivalve *Lucinoma aequizonata*[27] using 18S rDNA gene analysis similar to that in the present study. Supposing that phyllosoma larvae may be a sucking predator as observed in the laboratory, their secondary predation and/or scavenging intake may be inconsequential, corresponding to the reduced variety of organisms observed in the present study. The present technique should be applied to earlier stages of phyllosoma larvae and to larvae collected in different seasons and locations to investigate the dynamics of prey organisms.

ACKNOWLEDGMENTS

Thanks to K. Konishi, National Research Institute of Aquaculture, for valuable information on taxonomy and ecology of lobster larvae. Thanks to H. Yamada and K. Satoh, National Research Institute of Far Seas Fisheries, to the members of RV Shunyo-Maru and RV Shoyo-Maru for assistance in sample collection, and to H. Hasegawa and M. Michibayashi for superb technical assistance in DNA analyses. This work was supported in part by grants from the Japanese Society for the Promotion of Science, the Ministry of Agriculture, Forestry, and Fisheries of Japan, and a Grant-in-Aid for Scientific Research on Priority Areas (B) (No. 15380137) from the Ministry of Education, Science, Sports and Culture.

REFERENCES

1. Chittleborough RG, Thomas LR. Larval ecology of the Western Australian marine crayfish, with notes upon other palinurid larvae from the Eastern Indian Ocean. *Aust. J. Mar. Freshwat. Res.* 1969; **26**: 177–196.
2. Lesser JHR. Phyllosoma larvae of *Jasus edwardsii* (Hutton) (Crustacea: Decapoda: Palinuridae) and their distribution off the east coast of the North Island, New Zealand. *NZ J. Mar. Freshwat. Res.* 1978; **12**: 357–370.
3. Inoue M. Studies on the cultured phyllosoma larvae of the Japanese spiny lobster, *Panulirus japonicus* (V. Siebold). *Special Rep. Kanagawa Pref. Fish. Exp.* 1981; **1**: 1–91.
4. Kittaka J, Kimura K. Culture of the Japanese spiny lobster *Panulirus japonicus* from egg to juvenile stage. *Nippon Suisan Gakkaishi* 1989; **55**: 963–970.
5. Shojima Y. Scyllarid phyllosomas habit of accompanying the jelly-fish (preliminary report). *Nippon Suisan Gakkaishi* 1963; **29**: 349–353.
6. Thomas LR. Phyllosoma larvae associated with medusae. *Nature* 1963; **198**: 208.
7. Sims HW, Brown CL. A giant Scyllarid phyllosoma larva taken north of Bermuda (Palinuridea). *Crustaceana Suppl.* 1968; **2**: 80–82.
8. Mitchell JR. Food preferences, feeding mechanisms and related behavior in phyllosoma larvae of the California spiny lobster, *Panulirus interruptus* (Randall). Master's Thesis, San Diego State College, San Diego, 1971.
9. Kittaka J. Culture of phyllosomas of spiny lobster and its application to studies of larval recruitment and aquaculture. *Crustaceana* 1994; **66**: 258–270.
10. Johnston DJ, Ritar A. Mouthpart and hepatopancreas ontogeny in phyllosoma larvae of the spiny lobster *Jasus edwardsii* (Decapoda: Palinuridae). *Mar. Freshwat. Res.* 2001; **52**: 1375–1386.
11. Kittaka J. Culture of larval spiny lobsters: a review of work done in northern Japan. *Mar. Freshwat. Res.* 1997; **48**: 923–930.
12. Kittaka J, Ono K, Booth JD. Complete development of the green rock lobster, *Jasus verreauxi* from egg to juvenile. *Bull. Mar. Sci.* 1997; **61**: 57–71.
13. Asahida T, Yamashita Y, Kobayashi T. Identification of consumed stone flounder, *Kareius bicolonatus* (Basilewsky), from the stomach contents of sand shrimp, *Crangon affinis* (De Haan) using mitochondrial DNA analysis. *J. Exp. Mar. Biol. Ecol.* 1997; **217**: 153–163.
14. Jarman SN, Gales NJ, Tierney M, Gill PC, Elliott NG. A DNA-based method for identification of krill species and its application to analysing the diet of marine vertebrate predators. *Mol. Ecol.* 2002; **11**: 2679–2690.
15. Rosel PE, Kocher TD. DNA-based identification of larval cod in stomach contents of predatory fishes. *J. Exp. Mar. Biol. Ecol.* 2002; **267**: 75–88.
16. Saitoh K, Takagaki M, Yamashita Y. Detection of Japanese flounder-specific DNA from gut contents of potential predators in the field. *Fish. Sci.* 2003; **69**: 473–477.
17. Jarman SN, Deagle BE, Gales NJ. Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. *Mol. Ecol.* 2004; **13**: 1313–1322.
18. Matsuda H, Yamakawa T. The complete development and morphological changes of larval *Panulirus longipes* (Decapoda, Palinuridae) under laboratory conditions. *Fish. Sci.* 2000; **66**: 278–293.
19. Johnson MW. The phyllosoma larvae of slipper lobsters from the Hawaiian Islands and adjacent areas (Decapoda, Scyllaridae). *Crustaceana* 1971a; **20**: 77–103.
20. Johnson MW. On Palinurid and Scyllarid lobster larvae and their distribution in the South China Sea (Decapoda, Palinuridea). *Crustaceana* 1971b; **21**: 247–282.
21. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 1994; **22**: 4673–4680.
22. Swofford DL. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, 2000.
23. Wallace SK. Molecular gut analysis of carabids (Coleoptera: Carabidae) using aphid primers. MSc Thesis, Montana State University, Bozeman, 2004.
24. Deagle BE, Tollit DJ, Jarman SN, Hindell MA, Trites AW, Gales NJ. Molecular scatology as a tool to study diet: analy-

sis of prey DNA in scats from captive Steller sea lions. *Mol. Ecol.* 2005; **14**: 1831–1842.
25. Arnheim N. Concerted evolution of multigene families. In: Nei M, Koehn RK (eds). *Evolution of Genes and Proteins*. Sinauer, Boston. 1983; 38–61.
26. Zaidi RH, Jaal Z, Hawkes NJ, Hemingway J, Symondson WOC. Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? *Mol. Ecol.* 1999; **8**: 2081–2087.
27. Blankenship LE, Yayanos AA. Universal primers and PCR of gut contents to study marine invertebrate diets. *Mol. Ecol.* 2005; **14**: 891–899.