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Spawning by jumbo squid *Dosidicus gigas* in San Pedro Mártir Basin, Gulf of California, Mexico

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ABSTRACT: Paralarvae of ommastrephid squid in the eastern Pacific Ocean are exceedingly difficult to identify at the species level due to extreme morphological similarities, fragmentary knowledge of early life history and ontogeny, and the co-occurrence of adults of 2 or more species in most areas. We employed molecular genetic methods to identify ommastrephid paralarvae and juveniles captured in the area of the San Pedro Mártir basin in the central Gulf of California. Sequence analysis of a mitochondrial gene, cytochrome *c* oxidase I, identified all specimens analyzed (3 paralarvae and 11 small juveniles) as *Dosidicus gigas* and definitively ruled out other candidate species, specifically *Sthenoteuthis oualaniensis*. Paralarvae of the complete developmental size range were taken (1 to 10 mm mantle length), the smallest being the size expected for *D. gigas* at hatching. In addition, pairs of coupled adult *D. gigas* engaged in putative mating behavior were observed. Taken together, these findings indicate that mating, spawning and early development of *D. gigas* occur in this area.

KEY WORDS: Jumbo squid · *Dosidicus gigas* · Paralarvae · Juveniles · Cytochrome *c* oxidase · Mating · Gulf of California

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INTRODUCTION

Squid of the family Ommastrephidae are pelagic predators that inhabit all the world's oceans, except the polar regions. Although there are only about 25 ommastrephid species, they constitute a biomass of about 50 million tons (Rodhouse & Nigmatullin 1996) and are extremely important ecologically as both predator and prey throughout their life cycles. Before the curtailment of commercial whaling, the biomass of just 1 ommastrephid species consumed by exploited sperm whales in the Eastern Pacific, *Dosidicus gigas* (jumbo squid), was estimated to be nearly 10 million tons (Clarke et al. 1988). Ommastrephids are also the primary targets of the world's squid fisheries, with landings approaching 1 million tons yr^{-1} (Boyle & Boletzky 1996).

Despite the great importance of these squid, relatively little is known of their behavior in the wild or of their life histories, especially concerning early life-stages. Details of spawning areas, mating behavior, egg masses and embryonic development in the wild are unknown for most ommastrephids, and morphological features for species-level identification of paralarvae and juveniles are ambiguous (Nesis 1979, Wormuth et al. 1992, Nigmatulin et al. 2001). This latter problem is particularly vexing, because paralarvae and juveniles of multiple ommastrephid species coexist in most areas.

Dosidicus gigas, the only species in the genus, is endemic to the eastern Pacific and inhabits waters associated with the California and Peru current systems, ranging from at least 45° N to 45° S along the continental slope and reaching 140° W in the equatorial region where these currents converge (Nigmatulin et al. 2001). This species supports major fisheries off Chile and Peru and in the Gulf of California, with combined landings approaching 400 000 tons in 2002

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and 2003 (ftp.fao.org/fi/stat/summary/a1e.pdf). A sizeable body of fisheries-related work has been carried out in the southern hemisphere over the last 25 yr and constitutes the basis for most of our knowledge of this species (Nesis 1983, Nigmatullin et al. 2001). Historically, work on *D. gigas* in the northern hemisphere has been much more limited (Klett 1982, Ehrhardt et al. 1983), but recent studies in the Gulf of California have helped clarify many questions concerning the following in that body of water: (1) feeding habits (Markaida & Sosa-Nishizaki 2003), (2) growth (Markaida et al. 2004), (3) seasonal migrations (Markaida et al. 2005) and (4) stock assessment (Morales-Bojórquez et al. 2001).

The questions posed above concerning spawning and early ontogeny of *Dosidicus gigas* remain open, largely because of the virtual impossibility of reliable identification of paralarvae and juveniles based on morphological criteria. Even though *Sthenoteuthis oualaniensis* is the only other adult ommastrephid species found in areas of the Eastern Pacific where *D. gigas* is abundant (Nigmatullin et al. 2001), difficulty in distinguishing paralarvae and juveniles of these 2 species has generally prevented definitive conclusions (Yamaguchi & Okutani 1990, Vecchione 1999, Yatsu 1999).

We report here on observations made in the central Gulf of California in May 2004. A series of plankton tows in an area of active upwelling resulted in the capture of ommastrephid paralarvae which, using molecular genetic methods, were subsequently positively identified as *Dosidicus gigas*. Juveniles were captured in the same region and were similarly identified. Small adults and putative mating pairs of mature adults were also observed. These findings indicate that spawning by *D. gigas* occurs within the Gulf of California, and represent the first species-level identification of ommastrephid paralarvae and juveniles using molecular methods.

MATERIALS AND METHODS

Sampling. Plankton tows for paralarvae were carried out in May 2004 near the San Pedro Mártir Basin in the central Gulf of California (Fig. 1) on board the FV 'Gus D' in conjunction with the 2004 Sea of Cortez Expedition and Education Project sponsored by Stanford University (described in more detail at www.seaofcortez.org). A dual bongo-type net (0.5 m diameter, 0.5 mm mesh) without bridles was towed from a steel cable (0.42 cm diameter) with a 23 kg lead counterweight in a manner similar to that described by Zeidberg & Hamner (2002). Temperature and depth profiles for most of the tows were obtained using: (1) a YSI 600XL Sonde for the first 50 m of the water column and (2) a Lotek 1100 archival electronic tag attached to the net frame. In some cases, tow depth was estimated based on previous calibrations of the winch using the archival tag. Bottom depth and position of each tow were determined using the vessel's echo sounder and GPS, respectively. Relevant details of all tows are given in Table 1.

For each tow, the entire sample from 1 of the 2 nets was visually sorted on board with the specific goal of identifying cephalopod paralarvae. Specimens thus identified were anesthetized in a 35 mm, plastic culture dish with seawater containing 1 to 2% ethanol,

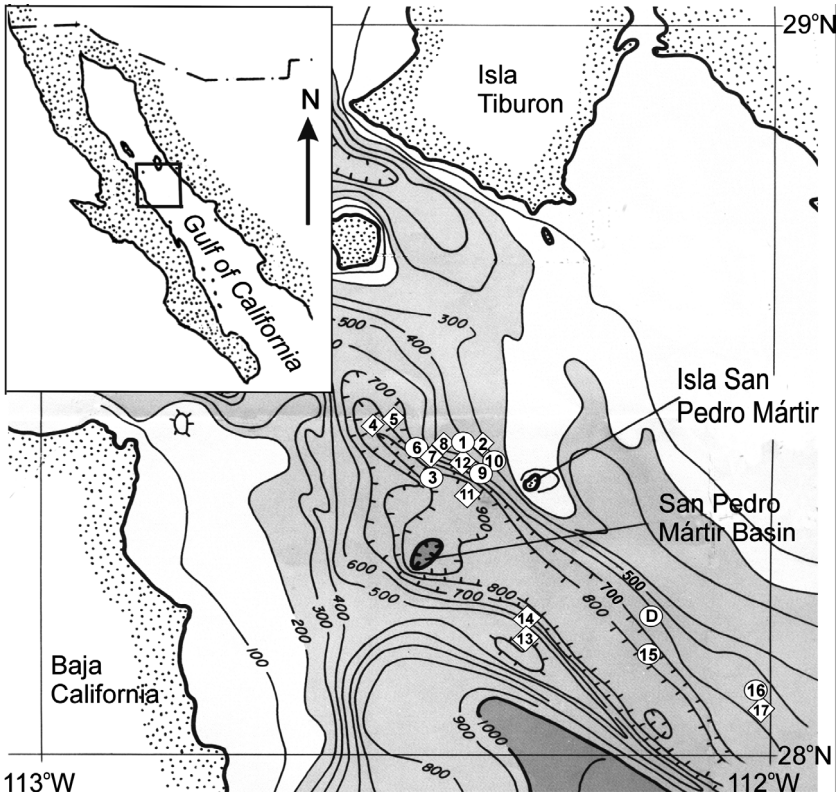

Fig. 1. Location of plankton tows and bathymetric features in San Pedro Mártir Basin, Gulf of California. 1 to 17: tows (coded as in Table 1) in NE edge of basin, west of Isla San Pedro Mártir. (○/◇) Tows in which ommastrephid paralarvae were collected/not collected, respectively. Some symbols displaced slightly for clarity (exact locations in Table 1). Ⓓ location where juvenile *Dosidicus gigas* were taken by dipnet. Map and bathymetry adapted from Bischoff & Niemitz (1980); depths in meters

Table 1. Summary data for ommastrephid paralarvae and other cephalopods captured in the series of plankton tows in 2004. Numbers (and sizes in mm) of individuals identified visually in live material and in formalin-fixed samples are tabulated separately. Formalin-fixed material was deemed not suitable for DNA extraction and was therefore not used for molecular investigation. Juvenile ommastrephids taken by dipnet on 6 May are also included; 11 of these were preserved in ethanol and 9 in formalin. Tow depth was estimated for those with no temperature data (nd)

Tow code	Local time (h)	Lat. (°N)	Long. (°W)	Surface temp. (°C)	Tow temp. (°C)	Tow depth (m)	Ommastrephids (n) Live	Ommastrephids (n) Formalin	Other cephalopods (n) Live	Other cephalopods (n) Formalin
4 May										
SR1	18:49	28.433	112.418	20.4	18	40	0	2 (<1)	1	21
SR2	19:50	28.423	112.399	20.2	18.4	30	0	nd	4	nd
SR3	21:30	28.402	112.454	17.8	17.2	5	2 (4[a], 9[a])	1 (1)	0	7
5 May										
SR4	15:35	28.452	112.542	22.4	nd	30–40	0	0	0	0
SR5	16:32	28.452	112.537	21	nd	30	0	0	3	0
SR6	20:00	28.426	112.488	20.3	16	30–40	1 (8)	0	0	0
SR7	21:20	28.419	112.467	20.2	nd	30–40	0	0	0	2
6 May										
SR8	10:15	28.430	112.442	21.4	16	45	0	0	0	1
SR9	11:10	28.396	112.405	21.3	19.5	45	0	2 (<1, 1.5)	0	3
SR10	11:45	28.402	112.398	22.6	13.1	165	1 (2.5[a])	1(8)	0	1
SR11	14:00	28.402	112.413	21.8	13.1	175	0	0	0	0
SR12	14:35	28.402	112.421	22	17.4	35	0	0	0	24
SR13	16:40	28.184	112.339	22.7	14.4	70	0	0	3	6
SR14	17:40	28.174	112.342	22.8	17.5	35	0	0	1	9
SR15	19:20	28.136	112.176	23.2	18.4	25	0	1 (1)	6	12
Dipnet	22:00	28.192	112.170	21.9			11 (15–25)	9 (15–25)		
7 May										
SR16	6:51	28.169	112.027	22.7	nd	30	1 (7)	0	0	1
SR17	7:22	28.160	112.008	23.4	nd	100	0	0	0	0

[a]Ethanol-preserved paralarvae used for molecular analysis

and photographed alive. Half of these specimens were then preserved in 70% ethanol for molecular genetic identification, and half were fixed in seawater containing 4% formalin for morphological identification. The entire sample from the second net in each tow was also preserved in 4% formalin for morphological identification.

Juvenile squid were sampled from the surface at night by dipnet from a skiff. Adult squid were observed and photographed *in situ* from the deck of the Gus D. Adult specimens of *Dosidicus gigas* for molecular work were obtained on the commercial fishing grounds off Santa Rosalia (Baja California Sur [BCS], Mexico) in October 2004 using standard 30 cm jigs. Tissue samples were taken from an arm tip (1 to 2 cm segment) and immediately placed in 70% ethanol.

Molecular analysis. Molecular identification of all ethanol-preserved samples was carried out through analysis of the cytochrome *c* oxidase I gene (CO-I) following the procedures of Carlini & Graves (1999). Molecular analysis of formalin-preserved material was not attempted. DNA was extracted from tissue samples (~50 mg) from adult squid tissue following digestion with Proteinase K and treatment with CTAB (hexadecyl trimethylammonium bromide). Extractions were performed twice with equal volumes of phenol-chloroform and then with chloroform using the Phase Lock™ Gel system (Eppendorf) to facilitate layer separation. DNA was precipitated from the aqueous layer with 2 volumes of ethanol followed by (1) centrifugation at room temperature and (2) washing with 70% ethanol and (3) air-drying. The DNA was resuspended in TE (10 mM Tris, 1 mM EDTA, pH8), and the concentration was adjusted to yield an absorbance at 260 nm of 0.2 to 0.7 for subsequent PCR work.

Juvenile squid tissue was obtained from the posterior 3 to 4 mm from specimens of 15 to 25 mm in length. Larger paralarvae (SR3, Table 1) were treated in a similar manner, but the smallest paralarvae (SR10, Table 1) was digested *in toto*. These tissue samples were then processed as described above. Formalin-preserved paralarvae were not used for molecular analysis.

PCR amplifications were carried out using 2 to 4 U of *Taq* DNA polymerase and commercially supplied buffer (New England Biolabs) in 100 µl volumes with 200 µM dNTPs. Primer sequences were LCO 1490 (GGTCAACAAATCATAAAGATATTGG) and HCO 2198 (TAAACTTCAGGGTGACCAAAAAATCA), used at 250 nM (Folmer et al. 1994). After an initial denatu-

ration (7 min at 94°C), the reaction mixtures were subjected to 40 cycles of 94°C (10 s), 46°C (10 s) and 72°C (60 s) followed by a final extension at 72°C (7 min) using a themal cycler (MJ Research, PTC 2000). PCR products were purified by either by the Wizard™ Prep system (Promega) or by Qiaquick™ spin columns (Qiagen) following the manufacturer's protocols. Sequencing was accomplished with Big Dye™ v3.1 terminator chemistry (Applied Biosystems) using the primers indicated above and an ABI Model 3100 capillary electrophoresis sequencer. Sequences were aligned using Gene Works™ 2.0 software (Intelligenetics).

During the course of our analysis, we discovered that the CO-I gene is duplicated in the mitochondrial genome of *Dosidicus gigas*, as it is in another ommastrephid squid, *Todarodes pacificus* (Yokobori et al. 2004). Duplication in *D. gigas* was initially suspected because of an occasional doublet in the sequencing electropherograms for several of the PCR products, and was subsequently confirmed (data not illustrated) by independent amplification and sequencing of the 2 copies using primers to the predicted flanking sequences that code for highly conserved tRNAs in *T. pacificus*. Both CO-I copies were found to be identical in 28 of the 34 adult *D. gigas* sequences included in this study. In the other 6 cases, only 1 base (0.016% of 630 total) differed between the 2 copies. Sequences from these 6 individuals were excluded from the analysis in 'Results'. Of 11 juvenile specimens, 1 was also found to have a single difference between the 2 CO-I copies. Both CO-I copies were identical in each of the 3 paralarvae analyzed.

RESULTS

Although Isla San Pedro Mártir lies more than 100 km north of the major commercial fishing zones for *Dosidicus gigas* (Santa Rosalia, BCS, Guaymas, Sonora), where large populations are well known, we hypothesized that adult *D. gigas* would be abundant near the island, because this area supports a high density of sperm whales (Jaquet & Gendron 2002). These whales are thought to preferentially feed on adult *D. gigas* where this squid is abundant (Clarke et al. 1988, Ruiz-Cooley et al. 2004). Indeed, we observed numerous sperm whales west of the island during the day, and *D. gigas* was regularly seen near the surface at night. Furthermore, the San Pedro Mártir area is a highly productive upwelling zone, and remote-sensing data received on board the vessel by satellite phone revealed an intense upwelling event to the west of the island. We therefore chose this site to search for *D. gigas* paralarvae, because local convergences and eddies concentrate zooplankton, including squid paralarvae (Zeidberg & Hamner 2002). The site of the upwelling event corresponded to the NE edge of the San Pedro Mártir basin on bathymetric charts (Fig. 1) and was locally recognizable by dense fog banks and by steep thermal gradients of up to 5°C in surface temperature over a horizontal distances of hundreds of meters.

Ommastrephid paralarvae captured in plankton tows

Conventional plankton tows were carried out primarily at depths of < 50 m in the area described above, and the location and general outcome of all tows are indicated in Fig. 1 (see also Table 1). Of 17 tows conducted over a period of 4 d, 7 yielded a total of 12 ommastrephid paralarvae, with approximately equal numbers occurring in the 2 nets.

Bottom depth at the tow sites varied from 250 to 1000 m, but depth was not correlated in any obvious way with the presence or absence of cephalopods taken in the tows.

Photographs of the live ommastrephid paralarvae identified on board ship are provided in Fig. 2, and the characteristic fused tentacles are readily apparent in each case. The size range encountered in our samples essentially spans the complete time course expected for the development of *Dosidicus gigas* paralarvae development (~1 mm to 1 cm dorsal mantle length, ML) given by Wormuth et al. (1992) and Yatsu et al. (1999). Of the 5 paralarvae illustrated in Figs. 2 & 3 (i.e. all those preserved in ethanol) were subsequently identified as *D. gigas* (see later subsection) by molecular genetic methods. A total of 14 tows yielded 104 other cephalopod specimens (see Table 1), most commonly *Pterygioteuthis giardi* (not illustrated). Only 3 tows yielded no cephalopods.

Ommastrephids were generally captured in the same tows and sometimes in comparable numbers as other cephalopod species, but most often in nondaylight hours. Other cephalopods, especially *Pterygioteuthis giardi*, were regularly taken in shallow tows during the day. Results from all tows are summarized in Fig. 3. Examination of temperature profiles in the upper 50 m of the water column did not reveal any strong correlation between cold, upwelled water at the surface and the presence of ommastrephid paralarvae captured in the tows (data not illustrated).

Juvenile squid

Numerous juvenile squid (1.5 to 2.5 cm ML) were observed at the surface near the vessel on the night of May 6 and were collected by dipnet from a skiff. They

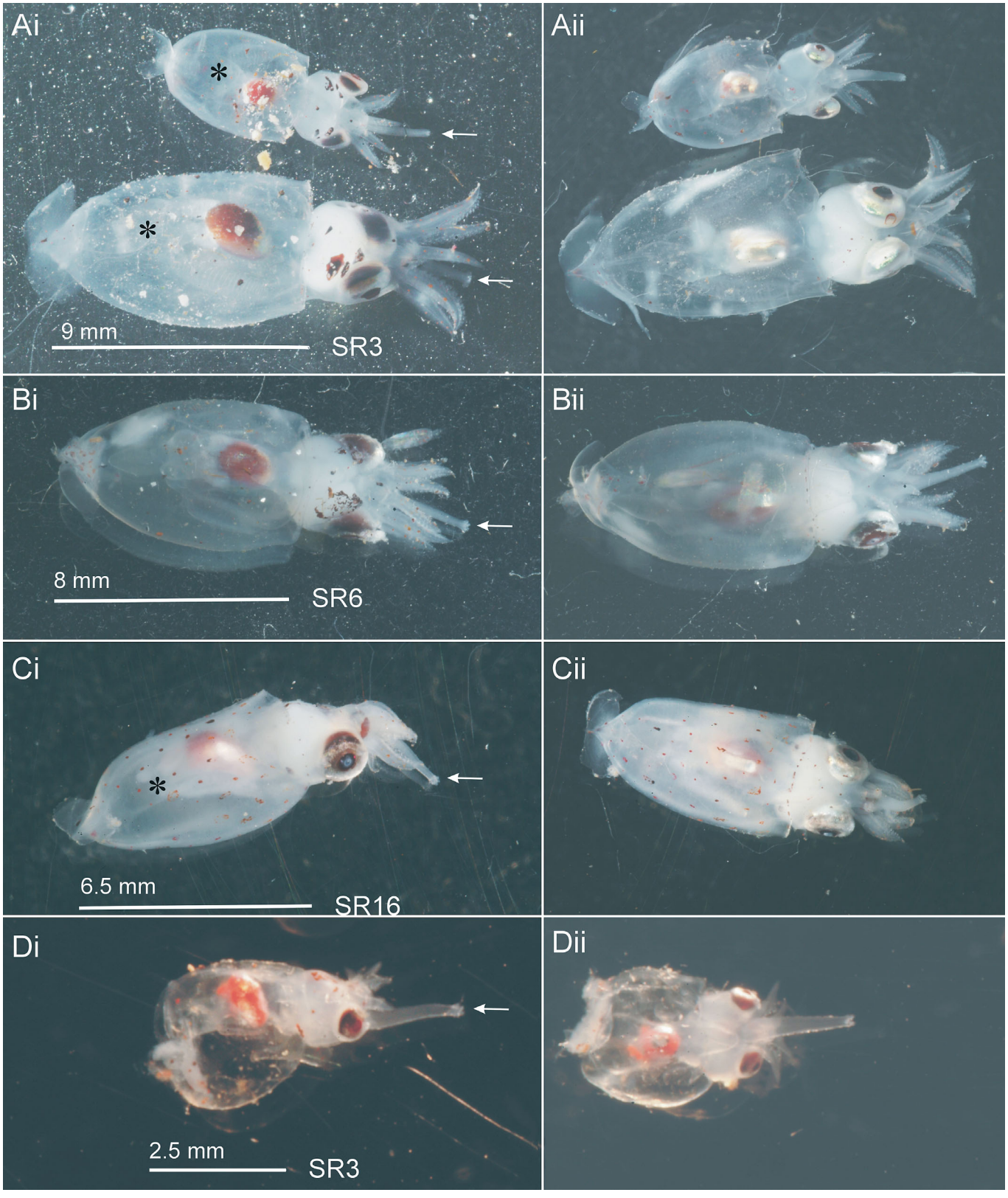

Fig. 2. Live ommastrephid paralarvae photographed on board the research vessel. Dorsal (Ai, Bi, Ci) lateral (Di) and ventral (Aii–Dii) views are shown. Arrows indicate fused tentacles characteristic of ommastrephid paralarvae. Tow codes as in Table 1. Asterisks in Ai and Ci: 3 specimens subsequently identified as *Dosidicus gigas* by molecular genetic methods

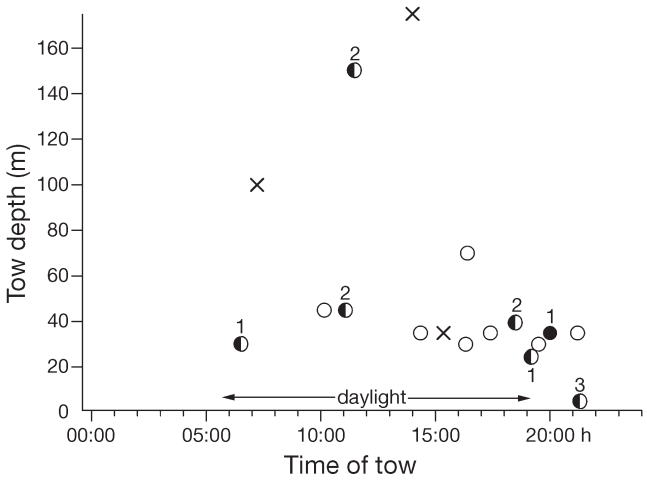

Fig. 3. Overall summary of collection data for all plankton tows (samples sorted on board research vessel plus formalin-preserved material examined later). Each symbol represents tow in Series SR1 to SR17 (Table 1). Depth at which each tow was carried out is plotted vs. time of day. (×) Tow in which no cephalopods were identified; (○) tows which captured cephalopods but no ommastrephid paralarvae; (●) Tow SR6, the only tow in which an ommastrephid paralarvae was the only cephalopod captured; (◐) tows in which both ommastrephid paralarvae and other cephalopods were taken. Number next to a symbol indicates number of ommastrephid paralarvae captured in that tow

probably were attracted by zooplankton that had been drawn to the surface by the deck lights from the vessel, and they appeared to be organized in small schools and feeding on unidentified euphausids. Fig. 4 illustrates 2 of these live squids. All 11 juvenile specimens preserved in ethanol were subsequently identified as *Dosidicus gigas* (see later subsection) using molecular methods.

Putative mating behavior of adult squid

On 2 occasions (nights of 5 and 6 May), pairs of adult squid were observed engaged in a behavior that appeared to be related to head-to-head mating. In each case, a large squid, presumably female, held a much smaller (presumptive male) squid inside the circle of arms, as shown in Fig. 5A. This engagement took place at the surface, and half of the larger squid's fins in Fig. 5A were actually protruding above the surface. During this coupling, an arm-tip of the presumptive male periodically emerged from between the female's left arms II and III and stretched over and touched the flanged region of left arm III (arrow in Fig. 5B). This behavior lasted for up to 15 min before the still-engaged pair sank slowly beneath the surface. At no time was there any visible sign of rapid motion, strong jetting, struggling or aggression.

Molecular identification of *Dosidicus gigas* adults, juveniles and paralarvae

DNA of adult *Dosidicus gigas* was extracted from ethanol-preserved arm tips, and 630 bp of the sequence (within a 709 bp PCR-generated fragment) of the mitochondrial gene encoding cytochrome *c* oxidase I (CO-I) was analyzed from 28 individuals captured on the commercial fishing grounds off Santa Rosalia, BCS. Identical CO-I sequences were found in 10 individuals, and this sequence was deemed canonical for the purposes of this study (GenBank Accession No. DQ191367); 2 additional identical sets also occurred, each comprising 2 individuals, that differed from the canonical sequence at 1 or 2 bases. The remaining 14 individual sequences were unique, differing at 1 to 5 bases. Maximum genetic variability in the CO-I sequence observed for adult squid (0.79%) is thus consistent with all samples coming from a single, well-defined species (Herke & Foltz 2002).

The CO-I sequences from juvenile squid (11 individuals captured by dipnet) and the 3 ommastrephid paralarvae preserved in ethanol were analyzed in the same manner: 4 of the juveniles and 1 of the paralarvae yielded the canonical adult sequence. The remainder of the juvenile and paralarvae samples differed by only 1 or 2 bases. In comparison, the published sequences for other ommastrephid species (*Sthenoteuthis oualanienesis* and *Ommastrephes bartrami*: Carlini & Graves 1999), differed from our canonical sequence for *Dosidicus gigas* at 80 bp (13% overall) and 92 bp (15% overall), respectively.

DISCUSSION

This study makes 2 major points. First, it clearly demonstrates the power and utility of molecular genetics for species-level identification of squid paralarvae. Second, it shows that spawning of the jumbo squid *Dosidicus gigas* takes place in the central Gulf of California.

Molecular identification of squid paralarvae

Extreme difficulty with species-level identification of ommastrephid paralarvae has been a major problem in previous studies of the early life-history of these ecologically and economically important pelagic squids (Yamaguchi & Okutani 1990, Vecchione 1999, Yatsu 1999). This study is the first to employ molecular genetic methods to positively identify paralarvae and juveniles of any ommastrephid squid species.

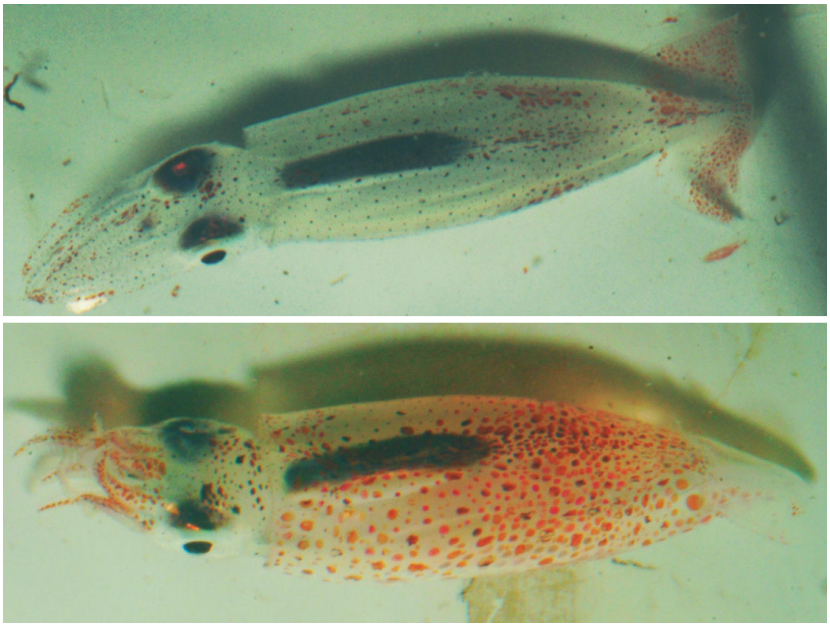

Fig. 4. *Dosidicus gigas*. Live juveniles captured by dipnet at the surface; dorsal mantle length is about 2 cm. All juveniles captured that were analyzed by molecular methods (11 of 20) were positively identified as *D. gigas*. Captured at Ⓓ in Fig. 1

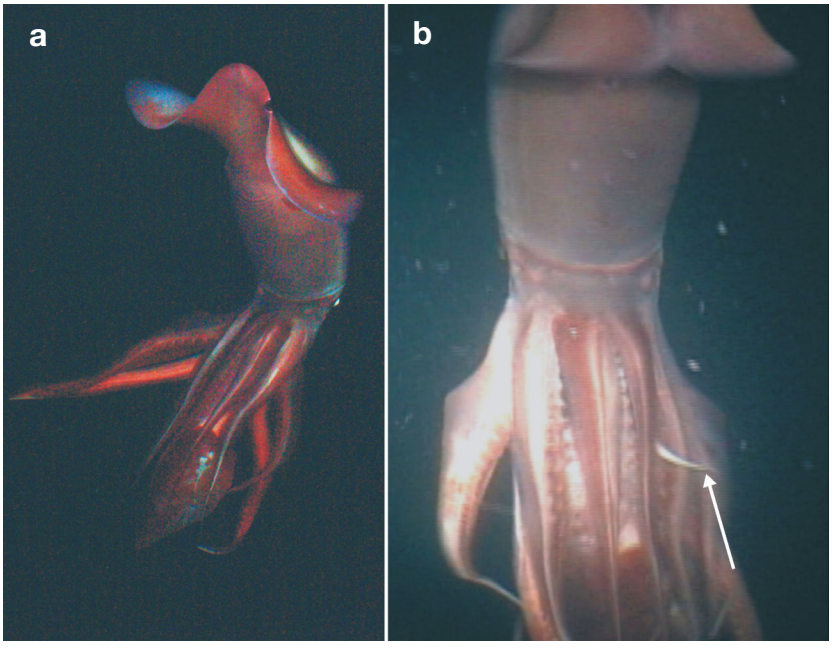

Fig. 5. *Dosidicus gigas*. Presumptive mating behavior of adults observed at night near location of Tow SR6 (Fig. 1, Table 1). (A) Pair of squid coupled in head-to-head fashion; larger squid (presumably female) is oriented vertically with posterior tip of mantle and about half the fins emerging from the water. (B) Detail of video frame showing arm tip of the presumed male squid (arrow) emerging from within female's circle of arms; this arm tip periodically stroked flange on female's leftmost arm (extreme right of photograph). Mantle length of larger squid is about 70 cm

All specimens of adult (n = 34), juvenile (n = 11) and larval (n = 3) *Dosidicus gigas* analyzed in the present study differed by less than 1% across 630 bp of the mitochondrial CO-I gene, consistent with all being of the same species. The only other ommastrephid species (*Sthenoteuthis oualanienesis*) that may occur in the southern Gulf of California (Roper et al. 1995, R. Brusca pers. comm.) differs over this same region by almost 15% (Carlini & Graves 1999).

We have subsequently sequenced many samples of adult *Dosidicus gigas* (n = 315 total) from a variety of locations along the north Pacific coast (Magdalena Bay, Baja California Sur, Mexico to Sitka, Alaska). All sequence data thus far are also consistent with a single species (i.e. variation is always <1%; W. F. Gilly & C. A. Elliger unpubl. data). The canonical sequence submitted to GenBank occurred in about 43% of all sequences analyzed and at all geographical locations.

Although molecular analysis can easily differentiate paralarvae of *Dosidicus gigas* from those of *Sthenoteuthis oualaniensis*, published morphological criteria seem problematic. In particular, the presence of ocular and visceral photophores in our material appeared to be a variable feature. *D. gigas* is supposed to have photophores only as small juveniles (Nigmatullin et al. 2001), and the absence of these organs in paralarvae differentiates this species from *S. oualaniensis*. As expected, our identified juvenile *D. gigas* had photophores, and paralarvae did not, including the largest individual (SR3, 9 mm ML; Table 1). However, 2 large, formalin-preserved paralarvae (8 mm ML) had ocular photophores, but visceral photophores were somewhat vague, i.e. SR6 (Fig. 2B) and SR10 (not illustrated). This type of ambiguity raises the possibility of variability in the ontogenetic timing of photophore development in *D. gigas* and points directly to the need for combined molecular and morphological analyses on individual specimens.

Spawning of *Dosidicus gigas* in the Gulf of California

Paralarvae of the size expected for *Dosidicus gigas* at hatching (1.1 mm ML; Yatsu et al. 1999) constitute strong evidence for spawning of adults in the same general area. Although 5 of the 12 ommastrephid paralarvae sampled were of this size (Table 1), the smallest molecularly identified specimen was 2.5 mm ML (SR10, Table 1). Even though the time course of neither embryonic development nor post-hatching growth in nature is known, a rough estimate of the age of our smallest identified specimen is possible.

Artificially fertilized eggs of *Dosidicus gigas* hatch under laboratory conditions in 7 d at 18°C, and post-hatching growth probably proceeds at a rate between 4% ML d^{-1} (no feeding, 18°C; Yatsu et al. 1999) and 8% ML d^{-1} (conditions not specified; Nigmatullin et al. 2001). The smallest ommastrephid paralarvae collected in this study were about 1 mm ML. Thus egg deposition leading to the smallest specimens probably occurred about 7 d before they were captured. Assuming a daily growth rate of 6% ML (midpoint of values cited above), a 2.5 mm paralarvae would be about 14 d old. Thus, egg deposition leading to the smallest molecularly identified specimen may have occurred 2 to 3 wk before capture.

Although the complex circulation currents of the Gulf are incompletely understood (Álvarez-Borrego 2002, Makarov & Jiménez-Illescas 2003), a northward-flowing circulation current of up to 20 cm s^{-1} (8.6 km d^{-1}) might be expected along the Baja coast of the Guaymas basin during early May. With an estimated 2 to 3 wk of total development time, the 2.5 mm paralarva sampled could have conceivably drifted as far as 100 to 200 km from the site of egg deposition. Even so, the site of egg deposition would probably lie within the Guaymas basin (where a large *Dosidicus* population exists year-round: Markaida et al. 2005) and well within the Gulf of California.

Local mating and spawning of *Dosidicus gigas* in San Pedro Mártir basin

A suite of circumstantial facts indicates that mating and spawning by *Dosidicus gigas* probably takes place in the general area of our plankton tows. First, the full size range of *D. gigas* paralarvae was present, and paralarvae were not uncommon in relation to other cephalopod species in many tows (Table 1). Second, small juvenile *D. gigas* were also present. Third, adult *D. gigas* were directly observed while engaged in presumptive mating behavior. Taken together, these observations strongly suggest that this species mates and deposits eggs in this area and that paralarvae hatch and develop into juveniles here as well.

Mating in *Dosidicus gigas* has never been definitively documented, but our observations are in fair agreement with the only published description (Nigmatullin et al. 2001): 'Mating was only observed once at a night light station while the boat was drifting. A male … swam slowly near a female … for around 20 s. The male then approached the female and both animals interweaved arms in the head-to-head position, gently moving their fins. This lasted for approximately 50 s and then the squid separated.'

In our case, we came upon the squid after they had already coupled, and the pair remained embraced in the head-to-head position for much longer at the surface (up to 15 min). Although mating behavior is further suggested by periodic emergence of the male's arm tip from the lateral surface of the female's surrounding arms, the functional importance of this behavior is unclear. The hectocotylized male appendage used for spermatophore insertion is ventralmost in *Dosidicus gigas*, and spermatopohores are implanted on the buccal membrane of females (Nigmatullin et al. 2001, W. F. Gilly unpubl. obs.), so this activity would not be expected to be visible in the dorsal view of the squid in Fig. 4. We can only speculate that the periodic stroking of the female's lateral arm by the male's snaking arm tip is part of mating behavior.

Rapid responses to remotely sensed oceanographic events

Identification of an active upwelling event by remote sensing in the present study led directly to the exploration of a relatively remote site by a vessel already at sea in the general area. Although we discovered *Dosidicus gigas* paralarvae at this site and elsewhere in the area within a time period of several days, a rigorous causal connection cannot be made between this success and the guidance provided by remote-sensing. Specifically, we did not exhaustively explore other regions, and it is conceivable that paralarvae occur throughout much of the Guaymas basin, where commercial fishing is concentrated. Indeed, most large mature females sampled in the Guaymas basin show evidence of mating (Markaida & Sosa-Nishizaki 2001). Nonetheless, the use of a small research vessel in conjunction with remote sensing to rapidly target oceanographic investigations to discrete localized events, such as upwelling or phytoplankton blooms (Beman et al. 2005), promises to open up new possibilities for real-time analysis of acute events.

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