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Ethogram Analysis Reveals New Body Patterning Behavior of the Tropical Arrow Squid *Doryteuthis plei* off the São Paulo Coast

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Abstract. Squids can express several body patterns, aided by a variety of visual signals that are produced by chromatophore organs. However, for several squid species, body patterning behavior during reproductive activity is still not completely understood. For example, what are the specific patterning changes and other visual signals, how do they appear, and how long do they last? To test the hypothesis that distinct chromatic components appear at different durations on the skin of the tropical arrow squid *Doryteuthis plei* in the Southern Hemisphere, we identified and described its body patterning behavior. Specimen squids were obtained from off the South Brazil Bight, near the coast of the São Paulo shelf. Animals were maintained and monitored in circular tanks for 62 d over six observation periods, from 2011 through 2013. An ethogram was constructed showing 19 chromatic, 5 locomotor, and 12 postural components, or body patterns, associated with reproductive behavior. New chromatic components (*i.e.*, those not yet reported in the North Atlantic *D. plei* species), particularly those linked to female sexual maturity, were observed. A postural component, the "J-Posture," linked to defenses and alarm, also was noted. The average time spent for "light" components was 32 s. The corresponding "dark" components had an average duration of 28 s. Females displayed the chromatic components related to calm behavior longer than males. However, males appeared to be more dedicated to disputes over resources, and used rapid, miscellaneous visual signaling. In conclusion, new basic types of body patterns are described for *D. plei*. The repertoire of chromatic components reported in the ethogram is, to our knowledge, the first record for *D. plei* of the Southern Hemisphere.

Introduction

Squids can express multiple body patterns, each associated with specific behavioral responses. These visual signals are produced by chromatophore organs in the skin that are controlled by hormones and neurotransmitters through specific structures (Hanlon and Messenger, 1996; Messenger, 2001). In most cephalopods, body patterns are created by the simultaneous occurrence of chromatic, postural, and locomotor components. These affect the appearance of the animal and may be acute, with a duration lasting seconds, or chronic, extending for minutes. The composite is produced by groups of the chromatic units constructed from different elements (Hanlon, 1982; Hanlon *et al*., 1994, 1999; DiMarco and Hanlon, 1997). The variety of body patterns of each individual is used both for crypsis and communication.

The body pattern outline and the spread of chromatophores in the body are important tools for the study of ethology of cephalopods. These characteristics have been used to compare behavioral variation among loliginid squid species (Hanlon, 1982; Hanlon *et al*., 1994; Hanlon and Messenger, 1996). The pigmentation of cephalopod skin is contained within unique cellular chromatophore organs (Cloney and Florey, 1968). Chromatophores have a unique ability to rapidly change their shape through a specialized neuromuscular control system (Hanlon and Messenger, 1996).

The body pattern dynamics in loliginids have been investigated using captive and field studies in several parts of the world. The patterns are linked predominantly to courtship

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and mating during reproductive behavior, interspecific association, and competition for resources (Hanlon and Messenger, 1996). The following species have been investigated in previous behavioral studies: *Loligo vulgaris reynaudii* Orbigny, 1841 (Sauer and Smale, 1993; Sauer *et al*., 1997; Hanlon *et al*., 2002), *Doryteuthis pealeii* Lesueur, 1821 (Griswold and Prezioso, 1981; Hanlon *et al*., 1999; Sharsha and Hanlon, 2013), *Doryteuthis opalescens* (Berry, 1911) (Hurley, 1977; Hunt *et al*., 2000; Hanlon *et al*., 2004), *Loligo* spp. (Hanlon, 1998), *Sepioteuthis australis* Quoy & Gaimard, 1832 (Jantzen and Havenhand, 2003), and *Sepioteuthis sepioidea* (Blainville, 1823) (Arnold, 1965). There were also behavioral studies of *Doryteuthis plei* (Blainville, 1823) in captivity in the North Atlantic (Hanlon, 1982; Hanlon *et al*., 1983) and in the wild, *i.e.,* from a research submersible during night dives (Waller and Wicklund, 1968).

The number of chromatic components described for the genus *Loligo* is large and complex. *Loligo vulgaris reynaudii* demonstrates 23 chromatic signals (Hanlon *et al*., 1994), *Loligo forbesi* Steenstrup, 1856 uses 17 signals (Porteiro *et al*., 1990), and *D. pealeii* has 34 signals (Hanlon *et al*., 1999). Studies focused on *D. plei* have reported that the organization of chromatophores and iridophores is not constant, differing and specific in certain regions of the body. For example, larger brown chromatophores are located on the dorsal mantle and small yellow chromatophores appear on the arms or tentacles (Hanlon, 1982). Therefore, the final appearance of a certain chromatic component is not only the result of neural excitation of colored chromatophores, but is also due to the size and distribution (vertical and horizontal) of chromatophores in different parts of the body. According to Hanlon (1982), *D. plei* displayed 16 chromatic components that were produced through specific static, morphological, and chromatic units.

D. plei inhabits coastal and shelf waters in the Western Atlantic Ocean, from the coast of Florida in the United States (Hixon *et al*., 1980) to Rio Grande do Sul, Brazil (Perez *et al*., 2005). This species is an important fishing resource off the São Paulo coast and is mostly found at shallow depths (< 30 m) in coastal waters (Gasalla *et al.,* 2005). This squid spawns throughout the year, but its reproductive peaks occur during the summer months (Rodrigues and Gasalla, 2008; Postuma and Gasalla, 2010, 2014). In the South Brazil Bight, many studies have addressed the population biology of *D. plei*, including growth, reproduction, feeding, and fisheries oceanography (Martins *et al*., 2006; Martins and Perez, 2007; Gasalla *et al.,* 2010; Postuma and Gasalla, 2010, 2014). However, the behavioral and body patterns of this animal have rarely been described. In this study, we present illustrations and descriptions of behavioral and body patterning. We also provide details of the variety of patterns and duration of each chromatic component observed. It is noteworthy that recent phylogeographic studies suggest that the Brazilian population of *D. plei* is genetically distinct from *D. plei* in North America and Central America (Sales *et al*., 2013).

The aim of the study was to describe the components and body patterns of the squid *Doryteuthis plei*, which may aid in distinguishing species in the North Atlantic Ocean based on previous work by Hanlon (1982) and Hanlon *et al.* (1983). To this end, an ethogram of the signals of *D. plei,* especially those related to reproductive behavior, was constructed. The ethogram is based on quantification of the time and duration of each chromatic component, gender differences, and types of chromatic components (light or dark).

Materials and Methods

Animal capture

Seventy-eight specimens of the tropical arrow squid *Doryteuthis plei* were obtained using hand jigs and Japanese-style pound nets ("kaku-ami") off the Ubatuba coast (23° 51′ S; 45° 08′ W) in marine waters less than 10.9 m deep. Additional samples were collected in São Sebastião (23° 83′ S; 45° 44′ W) in 6-m depth. Animals were immediately transported to the laboratories of the research station at the northern coast of São Paulo, Brazil, using the research vessel, *Veliger II,* and the small boat, *Nautilus*. During transport, animals were held in a 300-l tank containing local seawater that was constantly aerated by a submersible pump capable of pumping 432 l h^{-1}. Transport to the laboratory required 5–40 min after each sampling, as the distance ranged from 1.3 to 3.5 nautical miles. All of these steps were taken to minimize the animals' stress and injury during collection and transport (Aguiar *et al*., 2012; Marian, 2012).

Experimental tank setup

In the laboratory, animals were held in two indoor circular tanks: a 2.3-m diameter, 3000-l tank with a closed seawater system, and a 1.8-m diameter, 1000-l tank with a flow-through system (Fig. 1). Both tanks contained gravel and sand substrates. The closed seawater system provided a continuous flow of seawater. A pump with a capacity of 10,000 l h^{-1} was used to circulate water through the sand filter and a UV sterilizing filter. Squids were exposed to ambient light during a 12:12 light:dark photoperiod. During the night periods, observations were aided by a low-intensity LED light (about 50 lumens/watt). Water quality monitoring included temperature, salinity (ppt), and dissolved oxygen, all of which were measured daily with a multiparameter probe. Temperatures ranged from 21.86–28.81 °C, salinity ranged from 34.71–35.83 ppt, and dissolved oxygen was never greater than 5.00 mg l^{-1}. The mean level of toxic ammonia was 0.020 ppm (range, 0.012–0.035, $n = 15$), and the mean level of total nitrite-nitrogen (NO_2) measured 0.5 mg l^{-1} (range 0.02–3.05, $n = 15$).

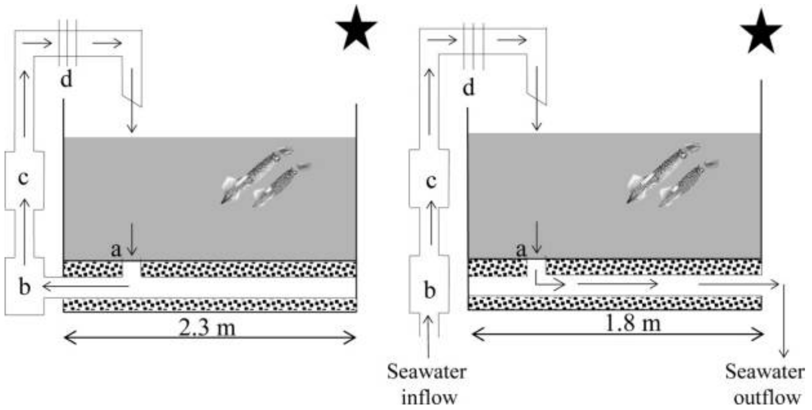

Figure 1. Two circular tank systems: left, a 2.3-m diameter, 2000-l tank with closed seawater system; right, a 1.8-m diameter, 1000-l tank with a flow-through system. Substrate bottom of the tank (a); pump (b); sand filter (c); UV sterilizing filter (d). Star shows observer site and camera location.

Animal care and husbandry

Specimens were monitored for 62 d over six observation periods conducted in November 2011 (15 d), February 2012 (7 d), and March 2012 (14 d) with the closed seawater system; and in November 2012 (13 d), February 2013 (19 d), and November 2013 (10 d) using the flow-through system tank.

The squids' survival period in both systems averaged 7 d; some animals survived up to 19 d in February 2013. Of the population of 78 *Doryteuthis plei* specimens maintained in captivity, there were 46 females and 32 males. Females were more frequent than males only during February 2012 (19 females, 0 males). Multiple combinations of male/female pairs were acclimated in the tanks (Table 1) for observation and identification of the components of the body patterns related to behaviors. Based on previous studies, we devised a components checklist, including calm and reproductive behaviors (*e.g.,* agonistic [males' dominance over females], courtship with displays of gonads, mating types, and egg-directed behaviors), which was used to categorize behavioral context (Roper, 1965; Hanlon *et al.,* 1983, 1994, 1999, 2002, 2004; Hanlon and Messenger, 1996; DiMarco and Hanlon, 1997; Shashar and Hanlon, 2013).

During the observation period, a male in the tank was considered "dominant" when, having been paired with a female for 10 min, won the disputes with other males considered "intruders." The definition of these terms and of the period were chosen based on a study of fighting tactics of *D. plei* in the Gulf of Mexico (DiMarco and Hanlon, 1997).

Mean mantle length (ML) for females was 144.23 mm (± 26.55 mm, SD; range 65–243 mm), and mean ML for males was 227.84 mm (± 45.73 mm, SD; range 99–299 mm). Food was offered *ad libitum* one to two times per day, and consisted of small fish, frozen or live, plus live crustaceans when available. The species offered during feeding were *Sardinella brasiliensis*, *Anchoa tricolor*, and *Anchoa* sp., measuring 45–89 mm. However, species not described as prey in the diet of *D. plei*, such as the crustacean *Callinectes danae* and fish (the barred grunt *Conodon nobilis*), also were offered. Observation periods at the 3000-l tank with the closed seawater system showed a mean daily mortality rate of 22.02% (range 0–33%); observation times at the tank with the 1000-l, flow-through seawater system had a mean daily mortality rate of 21.05% (range 0–47%). A number of factors, including water quality, space confinement, live feed, exposure to light, and low noise and

Table 1

Number of Doryteuthis plei *females and males with mean mantle length (mm) (in parentheses), sex ratio with Chi-square test: results during the six observation periods from 2011 through 2013*

Observation period	Females	Males	Sex ratio	Chi-square	*df*	*P*-value
November 2011	3 (144)	7 (209)	0.4286	1.600	1	0.21
February 2011	19 (140)	0	19.000	19.000	1	1.30E^{-05}
March 2011	6 (162)	5 (194)	1.200	0.090	1	0.76
November 2012	6 (143)	5 (210)	1.200	0.090	1	0.76
February 2013	8 (160)	7 (281)	1.142	0.060	1	0.79
November 2013	4 (145)	8 (210)	0.555	1.330	1	0.24
Total	46 (144)	32 (227)	1.516	2.510	1	0.11

Table 2

Video recordings (n) *during the six laboratory maintenance periods, with total video footage and mean, minimum, maximum, and standard deviation (h:min:s) of each video*

Observation period	*n*	Total video time	mean	min	max	SD
November 2011	462	13:27:20	0:55:55	0:01:26	0:28:33	0:35:21
February 2012	47	1:26:31	0:12:22	0:05:12	0:20:10	0:05:44
March 2012	197	8:53:14	0:38:05	0:05:42	0:38:49	0:56:07
November 2012	189	2:42:02	0:25:32	0:01:12	0:12:10	0:06:34
February 2013	159	1:12:06	0:29:51	0:03:45	0:45:22	0:21:54
November 2013	36	1:02:06	0:19:51	0:02:46	0:35:22	0:19:05
Total	1090	28:43:19	0:30:16	0:01:12	0:45:22	0:19:07

stress, were monitored to ensure the welfare of the study squids, as recommended for cephalopods by Moltschaniwskyj *et al*. (2007), and the ethical use of animals in applied ethology studies (Sherwin *et al*., 2003).

Experimental methods

To describe the organization of body patterns, we used the hierarchical classification developed for octopus by Packard and Sanders (1971) and Packard and Hochberg (1977), and reviewed by Hanlon and Messenger (1996). The classification hierarchy follows a top-down flow: (i) body patterns, (ii) components, (iii) units, and (iv) elements.

The chromatic, postural, and locomotor components were described and tabulated in a spreadsheet and compared for time, gender, and chromatic component (light and dark). Body patterns were classified into two categories: (1) chronic patterns and (2) acute patterns. The terminology used to name the components and body patterns was based on studies conducted on the behavior of other loliginids around the world, such as *Loligo vulgaris reynaudii* (Hanlon *et al*., 1994) and *Doryteuthis pealeii* (Hanlon *et al*., 1999), and on previous studies, particularly of *D. plei* in the North Atlantic (Hanlon, 1982; Hanlon *et al*., 1983).

Overall, 96 observations were made from the top of the tank (Fig. 1), averaging 3 h per observation, at a rate of approximately three observations per day, and totaling 530 h over the six observation periods. In addition, 1056 video frames were recorded at an average rate of 15 per day, totaling 28 h, 40 min of video recording time, with a mean filming time of 30 min each (range 1–45 min) (Table 2). The videos were focused on male and female pairs of *D. plei*, and especially on those that exhibited reproductive behavior. The videos were reviewed six times each, two times looking at one component category, following the methodology described by Hanlon *et al.* (1999). Observations started with the chromatic component (looking only for chromatic signals), followed by the postural components, and, finally, the locomotor components. Then each component of the body patterns was observed and noted for any squid at a given time during the observation periods. With respect to egg capsule deposition, the first hours of the day (00:00–06:00 am) were monitored, and observations of egg deposition were noted. Therefore, the observations were focused on a single female. We conducted "focal animal sampling" by following that female as long as possible, filming continuously to record the sequence of behaviors that preceded egg laying, after the methodology of Hanlon *et al.* (2004).

In analyzing the 1056 videos, we calculated in seconds the mean duration of each chromatic component. The duration of these components did not have a normal distribution (Shapiro-Wilk test); in fact, the duration in seconds was found to violate the criteria for normality. Therefore, the non-parametric Kruskal-Wallis test and post-hoc pairwise comparison tests (Siegel and Castellan, 1988) were applied to assess the influence of gender and type of chromatic component (light or dark) on the duration of each chromatic component. All statistical tests were considered significant when $P < 0.05$.

Results

Ethogram

An ethogram for *Doryteuthis plei*, based on our observations and the videos, is shown in Figures 2 and 3. The chromatic, locomotor, and postural components found in this study were used to build up body patterns and represent a segment of the behaviors for this species, especially those related to reproduction. The variation of each chromatic component is shown in Figure 2.

Locomotor components

The **hovering** position was caused by the interaction of fin movements and siphon fanning. This component was observed in solitary squids and in groups when the squids were calm. **Free swimming** occurred when the squid moved forward, with the head backward and raised slightly, and the arms somewhat depressed. During this component, the siphon and fins steered the animal during swimming. This posture was observed in calm squids preparing to mate.

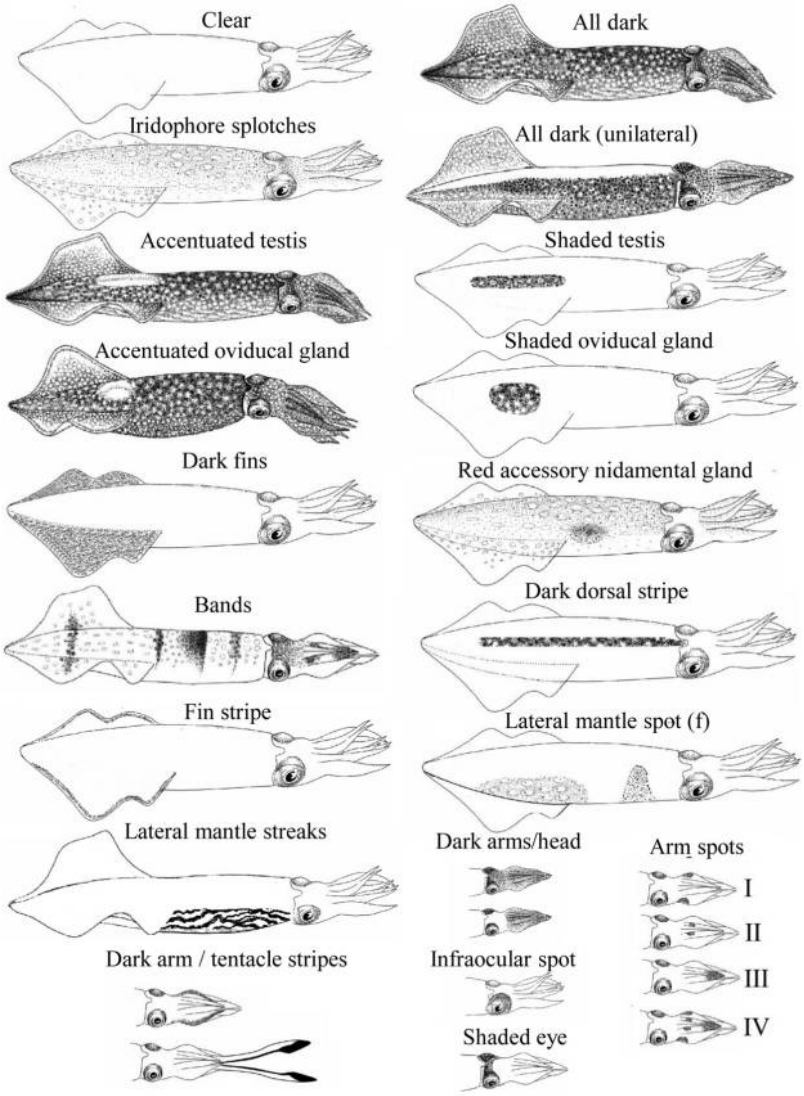

Figure 2. Illustrations of the chromatic components of body patterning found in the squid *Doryteuthis plei*.

Parallel positioning involved two animals hovering or swimming parallel to each another in the same direction, within one body length of the other. **Fin beating** a male-specific behavior, occurred during parallel positioning, when two males maneuvered into position to beat their fins against each other. This was a physical and escalating stage with an agonistic context, but it resulted in no obvious physical injury. These encounters lasted up to 10 s in disputes for females during mating.

The **chase** occurred when one squid (of either sex) vigorously pursued another squid by intraspecific competition or cannibalism in the tank. It occurred especially in moribund squid. This component was often observed during agonistic behavior in males. During the contests, a winner might chase the loser several times, with each chase lasting as long as 40 s. During feeding, squids also chased their prey; chases varied from a few seconds to minutes, when the squid's chase was failing. **Escaping** and **fleeing** occurred during intraspecific competition between winners and losers, especially among males during mating.

Jetting consisted of rapid body movements causing the expulsion of water from the siphon, producing rapid jet propulsion. Jetting usually stretched from 0.5 to 1 m in distance and was always performed backwards. Jetting was combined in escapes used to avoid both predators and conspecifics over spawning among females after mating and deposition of an egg capsule into the egg mop (assemblage of egg capsules on the substrate).

The **courting pairs** component occurred when a male initiated a parallel position to a mature female; agonistic encounters also began with this positioning. However, when squids were placed in the tank, there was no synchronic,

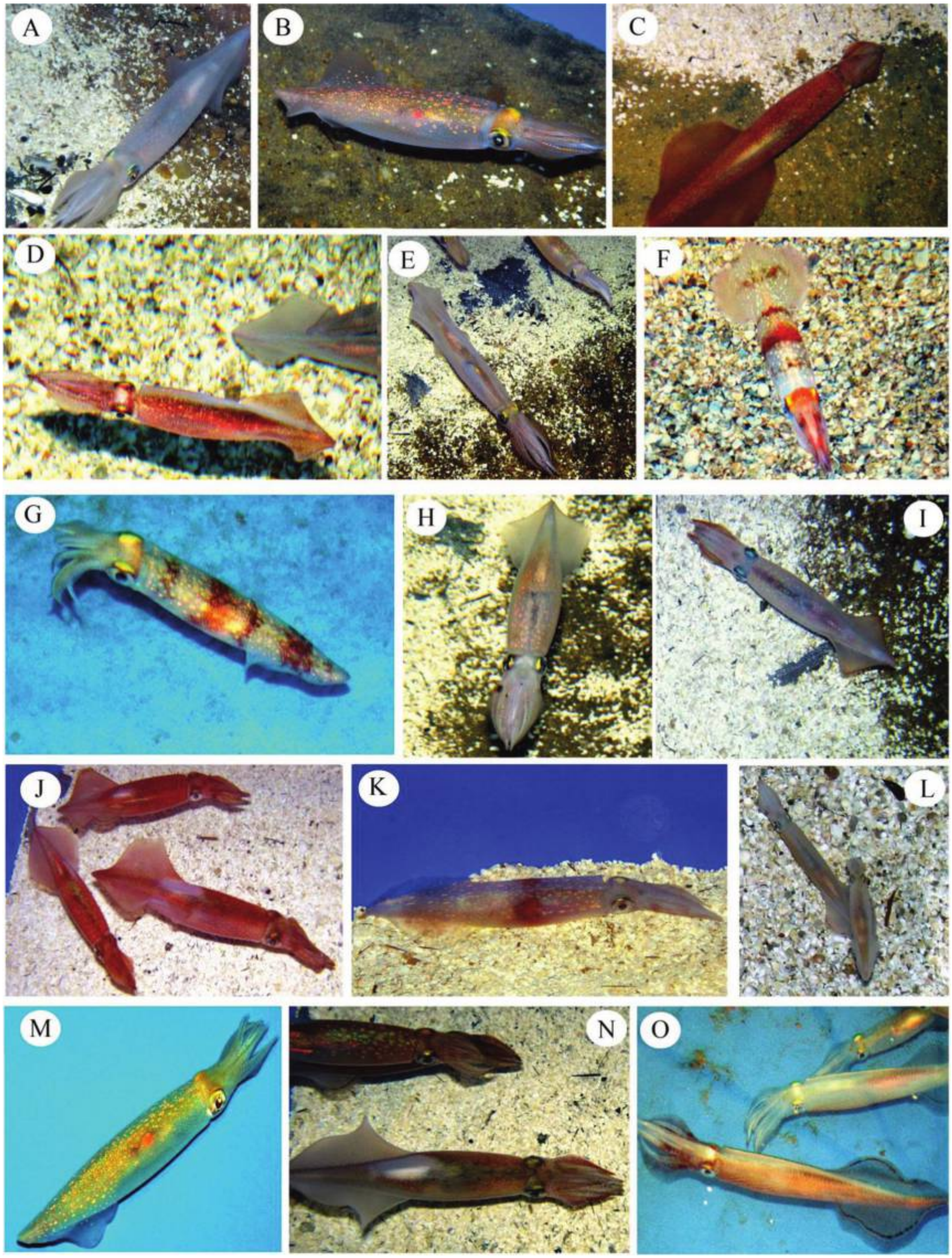

Figure 3. Underwater photographs of the components of *Doryteuthis plei* in captivity. (A) Chronic "clear" body pattern during the free swimming locomotor component. (B) Yellow iridophore splotches as seen on the dorsal mantle and head. (C) All dark coloration, and (D) all dark (unilateral). (E) Dark dorsal stripe with arm stripes. (F) Male with four bands, arm spots, and dark head; and (G) downward curl with three bands in females. (H) Arm spots with one side at the base of the third arms and a small spot on the second, and iridophore splotch sheen. (I) Arm stripes. (J) Arm stripes, noted only in females during courtship behavior, and accentuated oviducal glands with the chromatophores directly above the retracted glands. (K) Lateral mantle spot. (L) Shaded oviducal gland. (M) Red accessory nidamental gland. (N) Accentuated testis in males. (O) Fin stripe, lateral mantle streaks, and arm spots during agonistic behavior.

parallel swimming. At first, pairs did not swim in the same direction. However, within one day (24 h) of the experiment, they began to do so. **Swimming upwards** was observed in females when they swam up the tank. This action occurred before **male-parallel** mating. The female attracted the male to mating by **swimming upwards**. This movement was observed eight times in the total video footage, lasted an average of 15 s per episode.

Head-to-head mating occurred when a male and female faced each other and the male grasped the female's arms. Spermatophores were placed in a seminal receptacle below the mouth. **Male-parallel** mating involved a male positioning himself under a female, then grasping her anterior mantle to pass spermatophores into the mantle cavity.

Oviposition consisted of a female taking a single, extruded egg capsule and affixing it to the substrate or to the existing communal egg mop. The female did not hold the egg mop for long periods. A female with a 180-mm mantle was observed sometimes depositing an egg capsule on the substrate. She then positioned herself vertically, that is, at 90 degrees to the substrate, and affixed the egg to it. Afterward, this squid performed a fast backwards movement similar to jetting, and showing an **all dark** chromatic component.

Bottom sitting occurred when a squid rested on the substrate. This position was observed in tired squids, and was accompanied by the chromatic component of arm spots and bands (see *Chromatic components* below). This component preceded the death of a squid and could last up to 1 min.

Egg touching consisted of contact with an egg mop by both males and females. Contact ranged from a brief exploratory touch to an embrace of the egg capsule with all of the squid's arms. Females usually laid eggs on the existing egg mop; touching may have been a way of assessing the egg-laying substrate. Males commonly touched eggs; however, touching was often followed by highly aggressive agonistic bouts, suggesting that the eggs provided a visual, tactile, or perhaps chemosensory stimulus.

Chromatic components

Light chromatic components. The **clear** chromatic component was the most common behavior, recorded 176 times during the entire video footage (Table 3). This component is caused by a retraction of all or a majority of the chromatophores. As a result, the squid's mantle appears almost transparent (Fig. 3A). In clear waters, when the squid was observed against a gravel background, translucency camouflaged the animal. Internal organs, such as the testis in males and the oviducal and nidamental glands in females, were readily visible in our specimens. During feeding, the stomach and the entire digestive system also were seen.

Iridophore splotches appeared on the dorsal mantle and the head. These splotches were a distinctive yellow or golden color, and they helped to produce general camouflage. Three iridescent components are thought to aid in crypsis: (i) **iridescent arm stripes**, (ii) **dorsal mantle splotches**, and (iii) **dorsal iridophore sheen**. This component was observed especially when the squid was calm and floating in the tank. This component occurred 61 times during the total video footage period and lasted from 2 s to 3 min, averaging 46 ± 36 s (SD) for the dorsal **iridophore splotches** (Fig. 3B).

Dark chromatic components. The **all dark** chromatic component usually occurred at night, together with agonistic behavior in males. This coloring is created by expansion of all of the chromatophore cells across the entire mantle, turning the squid entirely dark. Expansion of all of the chromatophore cells produced brown and red coloring (Fig. 3C). **All dark (unilateral)** coloring occurred six times, and was used during agonistic encounters between males and females (Fig. 3D). This coloring appeared on one side of the mantle, showing the chromatophores expanded in perfect lateral symmetry.

A **dark dorsal stripe** was observed in calm squids. This stripe is thought to aid in crypsis through counter-shading when viewed laterally, and through disruptive coloration when viewed from above, by covering some of the bright organs, such as the testis, oviducal glands, and ink sac (Fig. 3E). The transverse **bands** component was observed frequently in groups of large males (Fig. 3F), and appeared in four varieties. The component was found in crypsis behavior through disruptive coloring, as a warning sign when a squid moved close to a possible predator, or when the prey that were offered for feeding were equal to or greater than 50% of the mantle length of the squid. It also occurred in females at the bottom of the tank (Fig. 3G). The most commonly observed pattern was one band ($n = 37$), followed by a variation that included four **bands** ($n = 31$), during the total video footage (Table 3).

The **arm spots** component was observed at the base of the third arms, the second arms, or both sets of arms. This component was common and had several variations, but we were only able to highlight points on the arms on one side of the body (Fig. 3H). The **arm** and **tentacular stripes** components were most readily observed when the tentacles were extended. In most animals, the first or third pair of arms was darkened. The average duration of this component was 55 s (Fig. 3I).

The **infraocular spot** occurred in a circular shape near the eyes, roughly between the eye and the **arm spots** component. Both components were sometimes expressed simultaneously during alarm situations when coloring was rarely observed. The **shaded eye** component is a transverse head band of expanded chromatophores, and may aid crypsis as it covers the bright iridescent sclera of the eyes. The **fin stripe** component expressed during agonistic contests was also observed when the squid was transported to captivity. It was also noted in alarmed squids and especially in large males during fights. **Dark arm and head** coloring was

Table 3

Time (h:min:s) of expression and frequency of the chromatic components observed in the video footage of Doryteuthis plei *over the six observation periods from 2011 through 2013*

				Time			
Chromatic components	Observation frequency	Component variations		Mean	Min	Max	SD
Light chromatic components							
Clear	176		–	00:00:32	00:00:01	00:03:07	00:00:32
Iridophore splotches	61	*Dorsal iridophore sheen*	8	00:00:47	00:00:02	00:01:31	00:00:25
		Dorsal iridophore splotches	44	00:00:46	00:00:02	00:03:00	00:00:36
		Iridescent arm stripes	9	00:01:00	00:00:03	00:03:00	00:00:48
Accentuated oviducal gland	51		–	00:00:16	00:00:01	00:01:31	00:00:21
Accentuated testis	20		–	00:00:28	00:00:05	00:01:30	00:00:30
Dark chromatic components							
All dark	94	*All dark*	88	00:00:34	00:00:01	00:03:00	00:00:32
		All dark (unilateral)	6	00:00:46	00:00:05	00:02:25	00:00:53
Arm spots	155	*Arm spots I*	66	00:00:27	00:00:01	00:01:31	00:00:28
		Arm spots II	11	00:00:12	00:00:01	00:00:55	00:00:15
		Arm spots III	13	00:00:21	00:00:01	00:01:31	00:00:27
		Arm spots IV	65	00:00:27	00:00:01	00:02:25	00:00:29
Bands	75	*Bands I*	37	00:01:11	00:00:02	00:03:00	00:00:56
		Bands II	2	00:01:33	00:00:05	00:03:00	00:02:04
		Bands III	5	00:00:17	00:00:01	00:00:55	00:00:22
		Bands IV	31	00:00:39	00:00:01	00:02:11	00:00:34
Dark dorsal stripe	62	*Dark dorsal stripe*	42	00:00:26	00:00:01	00:02:10	00:00:30
		Dark dorsal stripe II	20	00:00:30	00:00:01	00:01:31	00:00:26
Lateral mantle streaks	54		–	00:00:31	00:00:01	00:02:41	00:00:38
Shaded oviducal gland	40		–	00:00:34	00:00:02	00:02:25	00:00:35
Shaded testis	32		–	00:00:35	00:00:01	00:02:25	00:00:39
Infraocular spot	21		–	00:00:41	00:00:03	00:02:05	00:00:28
Shaded eye	14		–	00:00:09	00:00:03	00:00:18	00:00:05
Fin strip	14		–	00:00:41	00:00:05	00:01:30	00:00:44
Dark arms/head	18		–	00:00:35	00:00:02	00:01:31	00:00:35
Dark arm stripes/tentacle stripes	10		–	00:00:39	00:00:01	00:02:55	00:00:52
Lateral mantle spot (f)	11		–	00:00:19	00:00:03	00:00:55	00:00:23
Dark fins	8		–	00:00:50	00:00:02	00:02:00	00:00:38
Red accessory nidamental gland	7		–	00:00:08	00:00:05	00:00:31	00:00:08

noted during intraspecific agonistic encounters, but was also seen during mating and care of the egg mop.

The **dark fins** component was caused by expanded chromatophores in the region above the fins, which darkened them. This component lasted 2 s on average and was rarely observed. The component was most commonly observed in females when displaying alarm behavior.

Females

Females exhibited the light chromatic components for longer periods than males, possibly as a result of their calm or courtship behaviors. The white **accentuated oviducal gland** of females was similar in appearance to the males' **accentuated testis** (see *Males* below), but it differed in shape, position, and frequency of expression (Fig. 3J, Table 3). The accentuated area was an ovate shape on the dorsal lateral region of the mantle. The oviducal glands were observed on the mantle in quick flashes for 2 s or, rarely, for a longer duration, *i.e.,* 1 min.

The **lateral mantle spot** was expressed as a bold side area of dark chromatophores near the head or in the middle of the mantle. The mantle side spot was observed only when the female was paired with the male during mating, and could indicate receptivity. This component occurred several times and passed relatively quickly after approximately 2 s. This pattern may also indicate the maturity of the animal (Fig. 3K). The **shaded oviducal gland** component preceded mating and occurred during parallel positioning. It was caused by the selective expansion of chromatophores over the oviducal gland (Fig. 3L).

The **red accessory nidamental gland** occurred more than 10 times during the entire video footage (Table 3), usually during the daytime, when the females positioned themselves parallel to the males. In *Doryteuthis plei,* this gland is large and bright, and it can be observed through the mantle either laterally or from below (Fig. 3M). It may also signal female sexual maturity, because it turns red only upon attainment of full maturation.

Males

The **accentuated testis** component occurred 20 times during the total video footage (Table 3) in mature, mating males with mantle lengths of 200–299 mm (Fig. 3N). It was seen during courtship and parallel swimming immediately prior to or during mating. The **accentuated testis** appeared when the chromatophores directly above the testis were retracted and the mantle darkened completely. When the mantle is entirely dark, the sexual organ whitens laterally to the mantle and assumes an elongated shape.

The **lateral mantle streaks** component is produced by longitudinally oriented rows of partly expanded chromatophores (Fig. 3O). This phenomenon was observed during agonistic behavior. **Shaded testis,** caused by the selective expansion of chromatophores over the testis, preceded mating and occurred during parallel positioning.

Frequency and duration of chromatic component expression

Nineteen chromatic (4 light and 15 dark), 5 postural, and 12 locomotor components were identified in our compiled videos and correlated with different body patterns. We observed 923 displays of chromatic components in the skin of squids during the total video footage (Table 3). Most chromatic signals were significantly more frequent during the day than at night (73% of observations; Chi-square test, $\chi^2 = 3.38$, $P = 0.05$, df $= 1$). At night, the **all dark** chromatic components were observed more frequently (Table 3). Data in Figure 4 show the frequency of the chromatic components identified. The **clear** chromatic component was observed most often ($> 13\%$), and it occurred together with the **arm spots** (11%) and **dark dorsal stripe** (7%) components. **All dark** occurred in 9% of the observations during the total video footage, followed by **bands** (8.5%). The chromatic components were more evident in the body patterns; however, some components were rarely observed ($< 2\%$). The **red accessory nidamental gland, lateral mantle spots, infraocular spots, dark fins,** and **shaded eye** were rare in females.

The average durations of the chromatic components differed significantly (Kruskal-Wallis test, $P < 0.05$). A pairwise comparison test showed that mean durations were significantly higher in the most enduring components, such as **clear, iridophore splotches,** and **bands** ($P < 0.05$), than in the most short-lived components: **arm spots, lateral mantle spot** in females, **fin stripe, accentuated oviducal gland, lateral mantle streaks,** and **dark dorsal stripe** (Fig. 5, Table 4). The **infraocular spot** component was seldom displayed; however, it showed a significant difference in time from the **accentuated oviducal gland,** a fast component. The average time of the chromatic components of light (*e.g.,* **clear** and **iridophore splotches**) and dark (*e.g.,* **arm spots, lateral mantle spot**; see Table 4) also showed a significant difference ($\chi^2 = 13.55$, df $= 1$, $P < 0.05$). The light components had a longer duration (mean $= 32 \pm 25.2$ s, SD) than the dark components (mean $= 28 \pm 32$ s, SD). Significant differences based on gender also were noted ($P < 0.05$, Table 4, Fig. 6).

Postural component

Drooping arms in a swimming squid is a posture in which all of the arms appear relaxed and suspend downwards. This component preceded catching prey and occurred soon after relaxation. The squid maintains rigid arms, pointing at prey before capturing it. A **splayed arm** is a posture in which all eight arms are spread and flattened on the horizontal plane. This posture was expressed by both sexes when the chromatophores on the sides of the arms could also be observed. It occurred when a squid was defending a resource such as an egg mop, or when in the presence of another male during courtship or mating. The **raised arms** posture was a strong signal of alarm, and was used when a rival male was near. It also occurred when the animal was detected by a predator; it assumed a threatening posture by raising its arms to another animal. It was rarely observed in females.

The **downward curling** position consists of all of the arms and tentacles curled downward at 90 degrees. It is accompanied by four transverse **bands** on the mantle (Fig. 3G). This position was observed in aggressive encounters and in courtship; it was usually displayed at the bottom of the tank, next to the substrate. Females were more likely to use this position. The **J-posture** is characterized by **raised arms** at an angle of about 45 degrees, resembling the letter "J", such that the tips were close to the anterior dorsal margin of the mantle. This position, lasting about 5–7 s, relates to defense and alarm, and was observed in both sexes.

Body patterning

Chronic patterns. Patterns in this category can extend for seconds or minutes. For example, when squids were calm, they had a **clear body pattern** and the chromatophores were retracted over the mantle. This patterning leads to chromatic components such as the **dorsal stripe, arm spots,** and the **iridophore splotches** located on the mantle, fin, and head. These patterns were observed frequently in normal laboratory conditions. Calm animals were usually swimming forwards and backwards, or swimming in place (*i.e.,* the **free swimming** and **hovering** locomotor components), at which time the postural component of **drooping arms** was observed.

The **bands** body pattern was associated with alert or alarmed behavior, and can be considered a chronic pattern

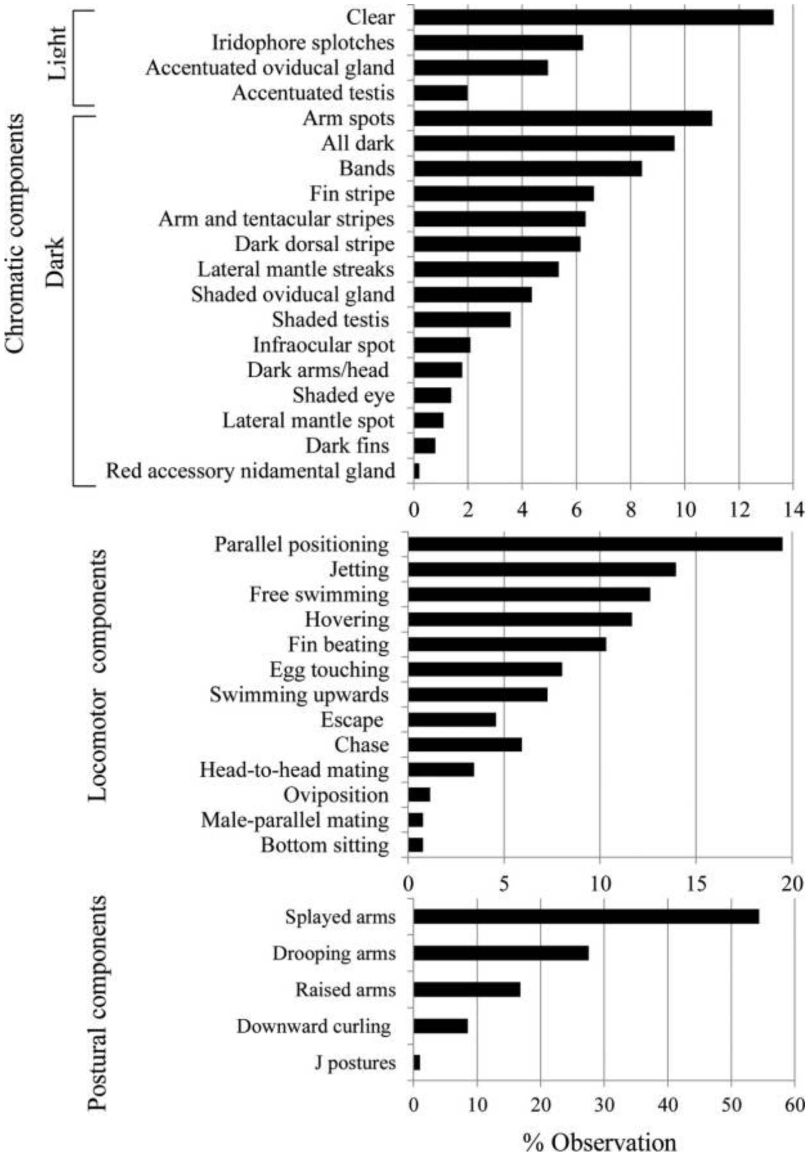

Figure 4. Percentage of observations of each chromatic (light and dark), postural, and locomotor component and body pattern observed in *Doryteuthis plei*.

because it occurred frequently for periods ranging from 20 s to 1 min. This pattern can take place when prey or predators are near or when the squid is alone. This body pattern occurred together with other chromatic components (see the male showing a **band** with **arm spots** in Fig. 3F; and females with **bands, dorsal iridophore sheen,** and **downward curling** in Fig. 3G).

The **all dark** body pattern was considered chronic because, during the nighttime observation periods, the squids were mostly totally dark or brown (90% of the night observations). In two situations during the daytime observation period, an entire school of squids appeared **all dark.** Video analysis showed that one squid darkened when it detected a predator, prey, or observer above the tank. This event caused all of the squids in the school to become **all dark** for 20 s to 2 min. At night, the squids were **all dark** and moved fast together, using the **jetting** locomotor component. **Jetting** occurred when the squid was alone or when prey in a tank was larger than 50% the size of the mantle; at these times, the squid likely believed the prey to be a potential predator. The **all dark** component was also used for hunting live prey (fish) or as nighttime camouflage.

Reproductive behavior

Acute patterns. These patterns occurred quickly and were linked to intra- and interspecific interactions during reproductive behavior, such as agonistic behavior during fights

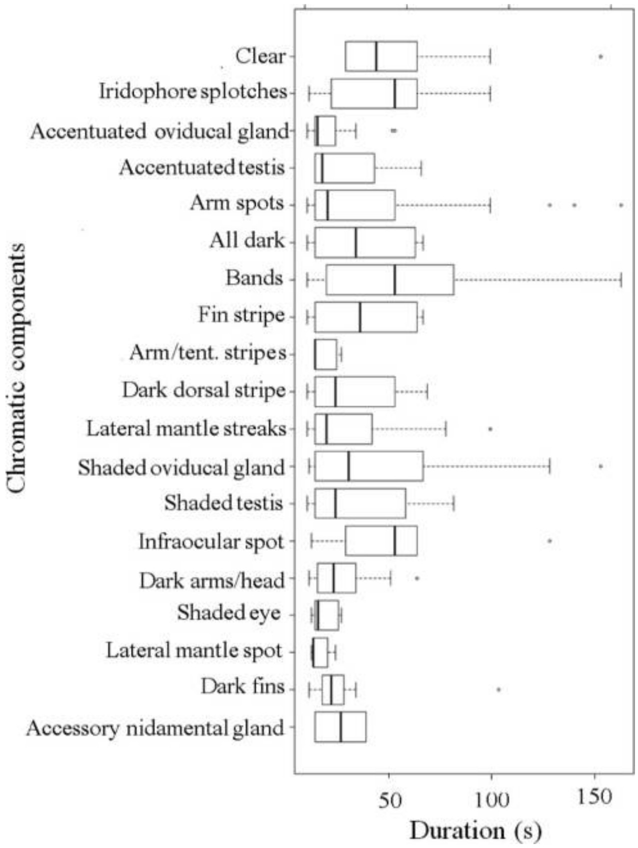

Figure 5. Duration of chromatic components (s) identified for males and females of *Doryteuthis plei*. The solid black line represents medians; open boxes are 25% and 75% of the observations, and bars indicate the range of durations. Dots show moderate outliers.

for a mate or during courtship, mating, spawning, and egg touching. These body patterns occurred for seconds only.

Figure 7 summarizes the behavioral sequence observed during the study. The dashed arrows represent the flow of behaviors over time. After the animals' acclimation period (1 or 2 d), observed behaviors became more complex. During reproductive behaviors, a combination of chromatic components was noted in agonistic activity, and included **arm spots** and **lateral mantle streaks** (only males). Mating activity included **accentuated testis** and **oviducal gland, red accessory nidamental gland, mantle spot** in females, and **shaded testis,** observed together with the locomotor components of **parallel positioning, fin beating, courting pairs,** and **oviposition** (during the spawning process).

Agonistic behavior

In the first days of the observations, a male established dominance and then protected the females from other males in disputes. During agonistic behavior displays, multiple chromatic components were displayed, such as **arm spots, lateral mantle streaks** (only males), **infraocular spot, dark fins,** and **arms/tentacular stripes** together with **fin beating, chase** (winner), and **flee** (loser) accompanied by **raised arms** or **splayed arms** (Fig. 3O). The **lateral display** patterns were easily observed immediately after the **clear** body patterning. The displays occurred for a fraction of a second and were repeated two or three times.

The **dark flashing** pattern occurred in a situation of high stress or an alarm signal during spawning and egg touching, and in response to non-specific threats such as the presence of people around the tank or noises made nearby. A strong alarm signals the squids to darken completely and to assume the **J-posture** component in both sexes. After mating, the females swim using jet propulsion; their mantle color is completely dark. The jetting is of short duration.

Courtship behavior

The **gonads display** body pattern was the most frequently shown pattern for reproduction during courtship behavior. Males and females formed pairs, during which the male pursued the female and protected her from other males. Video analysis showed that this behavior occurred 159 times, on an average of two events per day. In most cases (90%), the male began courtship by pairing with a female and moving forwards and backwards in parallel positioning for a lengthy time (> 3 min); the gonads are highlighted between the animals, accompanied by slight touching. However, less frequently, females initiated courting by swimming upwards.

Mating behavior

Two types of mating were observed in our study, and each differed in positioning, duration, and frequency (Table 5). **Head-to-head** mating was most commonly seen ($n = 18$), lasting 5–41 s (mean = 17 ± 10.24 s, SD). In this position, the body pattern was dark, the mate category was "sneakers", and females showed faster displays. Male-parallel mating occurred less often ($n = 4$), and lasted 10–15 s (mean = 12.2 ± 2.06 s, SD). During this type of mating, the fourth pair of male arms was totally dark. The mating category was "consorts."

Discussion

Reduction and internalization of the shell is a key trait in the evolution of cephalopods. It has allowed for an active life in the water column and an ability to compete with vertebrates (Packard, 1972). But this lifestyle has also made these animals more exposed and vulnerable to predatory attacks. In response, they have developed sophisticated mechanisms for camouflage that include the use of chromatophores (Messenger, 2001). A system of neurally controlled chromatophores is supremely well adapted for

Table 4

Pairwise comparison test for the average time (s) of the expression of chromatic components between females and males, light and dark, and among the 19 components observed from 2011 through 2013 for Doryteuthis plei *in the South Brazil Bight*

Factors		Observed difference	Critical difference	*P*-value
Females	Males	59.830	29.110	<0.05
Light component	Dark component	69.7656	31.272	<0.05
Clear	Accentuated oviducal gland	311.958	163.567	<0.05
Clear	Arm spots	183.532	114.425	<0.05
Clear	Fin stripe	337.264	292.921	<0.05
Clear	Dark dorsal stripe	183.417	155.506	<0.05
Clear	Lateral mantle streaks	211.561	159.289	<0.05
Clear	Lateral mantle spot	384.121	304.739	<0.05
Iridophore splotches	Accentuated oviducal gland	276.297	167.525	<0.05
Iridophore splotches	Arm spots	147.871	120.014	<0.05
Iridophore splotches	Fin stripe	301.604	295.149	<0.05
Iridophore splotches	Lateral mantle streaks	175.900	163.350	<0.05
Iridophore splotches	Lateral mantle spot	348.460	306.881	<0.05
Bands	Accentuated oviducal gland	320.248	174.072	<0.05
Bands	Arm spots	191.822	128.996	<0.05
Bands	Fin stripe	345.554	298.914	<0.05
Bands	Dark dorsal stripe	191.706	166.521	<0.05
Bands	Lateral mantle streaks	219.850	170.059	<0.05
Bands	Lateral mantle spot	392.410	310.504	<0.05
Infraocular spot	Accentuated oviducal gland	284.110	251.086	<0.05

The observed differences that were higher than a critical difference are considered statistically significant at ($P < 0.05$).

signaling. Many shallow-water cephalopods also use chromatophores to form both inter- and intraspecific visual signals (Hanlon and Messenger, 1996; Messenger, 2001).

This study is the first concerted effort to analyze the behavioral components of *Doryteuthis plei* in the South Brazil Bight and in the Southern Hemisphere. The behavior of *D. plei* is complex and presents a variety of chromatic, postural, and locomotor components. Chromatophores can alter visual appearance in response to stimuli (Hanlon and Messenger, 1996). Color changes occurred when a male intruder approached a dominant male to fight for a mating female. During the study, we observed that the light chromatic components (**clear** and **iridophore splotches**) had a longer duration than the dark chromatic components, especially those associated with calm behavior. Squid chromatophores are neurally controlled, allowing the animal quickly to select and demonstrate various body patterns. With this quick polymorphism, squids can rapidly hide from predators.

The locomotor components include a variety of movements using the siphon, arms, and fins. However, more attention is required to observe the postural components because they involve the body's position and arms. The locomotor and postural components observed in this study were earlier described for other squids (Hanlon, 1978; Hanlon *et al*., 1983, 1994, 1999, 2000, 2002; Hanlon and Messenger, 1996; Jantzen and Havenhand, 2003; Buresch *et al*., 2004; Pham *et al*., 2009; Shashar and Hanlon, 2013).

Most of the chromatic components observed in this study occurred during diurnal periods, which is easily explained by the difficulty of nighttime observations due to the lack of light in the tank. When the LED light was turned on in the tank, squids kept to the periphery of the light. Several of the 19 chromatic components identified in our study of *D. plei* in the Southern Hemisphere were identical to those previously described for other loliginids, including *Loligo vulgaris* (Hanlon *et al*., 1994), *Doryteuthis pealeii* (Hanlon *et al*., 1999), and *Doryteuthis opalescens* (Hunt *et al*., 2000). However, in comparison to *D. plei* in the North Atlantic (see Hanlon, 1982, 1988; Hanlon *et al*., 1983), a greater variety of chromatic components was observed during our study. Hanlon (1982) describes only 16 chromatic components in *D. plei* of the North Atlantic (USA). However, the chromatic components that were seen in our study in relation to the sexual maturity of females were not reported by the author studying *D. plei* of the North Atlantic (*e.g.*, **accentuated oviducal gland;** (Fig. 3J)); **dark arms/head, dark fins,** and **infraocular spot** components also were not mentioned. Possibly, maintenance conditions, such as color, depth, and bottom type of the tank used in this study, influenced the observed patterns of skin coloration of the squids.

Body patterns and behavior

Calm behavior. Calm behavior in this species was scored when squids did not haphazardly strike the tank walls, avoiding significant injury to skin and fin (Hanlon *et al*., 1983). Through video analysis, we observed that at the beginning of each maintenance period, squids adapted to the tank conditions and showed calm behavior. This state was also easily identified through the clear chromatic component and free swimming. During parallel positioning, a calm state was noted when the postural component was relaxed, with drooping arms; squids appeared to be at ease, and there was no threat in the tank. The **clear** chromatic component was the most frequently observed display in this study (see Table 3). Our observations support the notion that the *D. plei* squid mantle is transparent or has pale coloration. Unlike the **all dark** pattern, this pattern (*i.e.*, **clear** pattern = calm and **all dark** pattern = alarmed) has been found for this species (Boycott, 1965; Hanlon *et al.*, 1983) and other loliginids around the world (*e.g.*, *Loligo forbesi* in Europe, Porteiro *et al.*, 1990; *Loligo vulgaris reynaudii* in South Africa, Hanlon *et al.*, 1994; *Doryteuthis pealeii* in Massachusetts, Hanlon *et al.*, 1999; *Doryteuthis opalescens* on the California coast (USA), Hunt *et al.*, 2000; and *Sepioteuthis australis* in Australia, Jantzen and Havenhand, 2003).

Alarm behaviors. During the "dark" (*vs.* light) observation period, alarm and jetting behaviors were observed through display of all chromatophores on the mantle. This component may also be used as camouflage to catch prey at night. When large or uncommon prey was placed in the tank, it caused a rapid expansion of chromatophores, turning the squid **all dark** during the day period. However, this pattern is also used intraspecifically during agonistic encounters

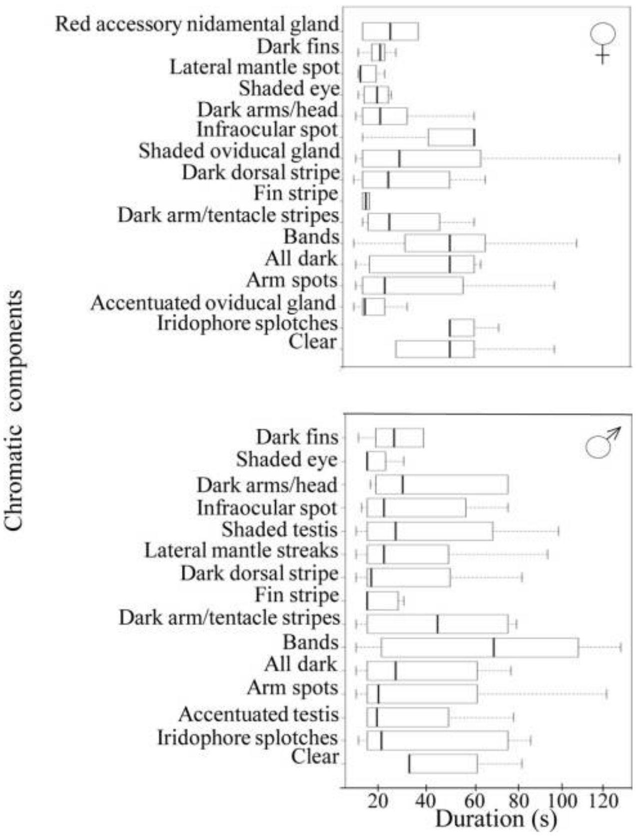

Figure 6. Duration of chromatic components (s) identified for males and females of *Doryteuthis plei*. The solid black line represents medians; open boxes are 25% and 75% of the observations, and bars indicate the intervals of durations range.

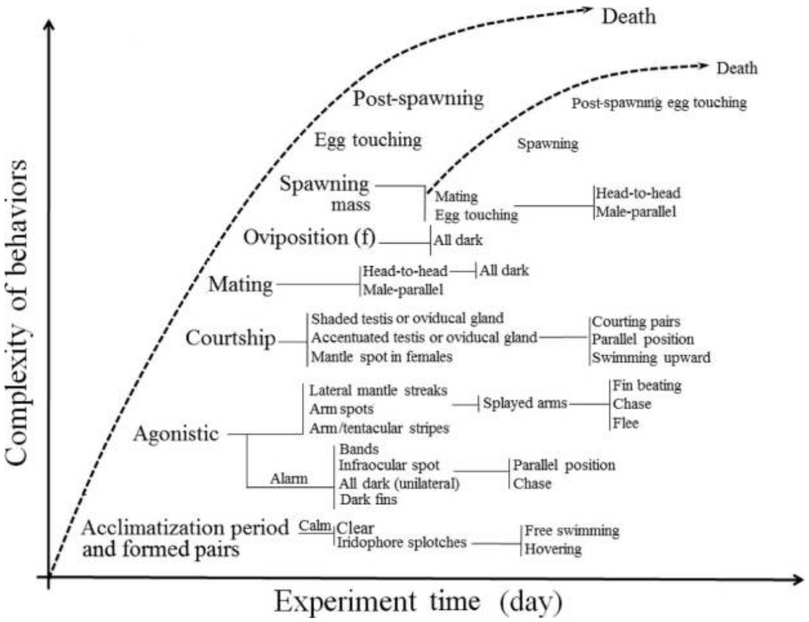

Figure 7. Schematic representation of the observed behaviors of *Doryteuthis plei*. Dashed arrows represent the flow of behaviors over time.

Table 5

Types of mating and their characteristics observed for Doryteuthis plei.

Date	Period	Light	T °C	Sal ppt	% DO	n (f - m)	Mating type	Mating duration (s)	Category of mate
13/11/2011	Daytime	N	27.2	35.2	81.9	8 (4 - 4)	Head-to-head	15	Sneakers
13/11/2011	Daytime	N	27.4	35.2	77.3	8 (4 - 4)	Head-to-head	25	Sneakers
13/11/2011	Daytime	N	27.4	35.2	77.3	8 (4 - 4)	Head-to-head	06	Sneakers
13/11/2011	Nightly	Y	27.1	35.1	77.4	8 (4 - 4)	Male-parallel	15	Consort
13/11/2011	Nightly	Y	27.1	35.1	77.4	8 (4 - 4)	Male-parallel	12	Consort
13/11/2011	Nightly	Y	27.1	35.1	77.4	8 (4 - 4)	Head-to-head	13	Sneakers
13/11/2011	Nightly	Y	27.1	35.1	77.4	8 (4 - 4)	Head-to-head	14	Sneakers
13/11/2011	Nightly	Y	27.1	35.1	77.4	8 (4 - 4)	Head-to-head	08	Sneakers
13/11/2011	Nightly	Y	27.1	35.1	77.4	8 (4 - 4)	Head-to-head	09	Sneakers
14/11/2011	Daytime	Y	26.0	35.4	83.2	7 (3 - 4)	Head-to-head	17	Sneakers
17/11/2011	Daytime	Y	24.4	35.5	86.1	4 (2 - 2)	Head-to-head	20	Sneakers
17/11/2011	Nightly	N	24.4	35.5	86.1	4 (2 - 2)	Male-parallel	10	Consort
17/11/2011	Nightly	N	24.4	35.5	86.1	4 (2 - 2)	Male-parallel	12	Consort
16/03/2012	Nightly	N	27.50	35.00	87.6	10 (5 - 5)	Head-to-head	05	Sneakers
17/03/2012	Daytime	N	26.70	34.80	93.3	10 (5 - 5)	Head-to-head	10	Sneakers
18/03/2012	Nightly	Y	24.90	34.90	95.1	7 (5 - 2)	Head-to-head	11	Sneakers
19/03/2012	Daytime	N	24.90	34.70	97.4	6 (4 - 2)	Head-to-head	25	Sneakers
19/03/2012	Nightly	Y	25.90	34.70	90.5	6 (4 - 2)	Head-to-head	41	Sneakers
19/03/2012	Nightly	N	25.90	34.80	90.1	6 (4 - 2)	Head-to-head	37	Sneakers
20/03/2012	Daytime	N	26.30	34.90	87.3	5 (3 - 2)	Head-to-head	12	Sneakers
20/03/2012	Nightly	N	27.00	34.80	90.1	5 (3 - 2)	Head-to-head	21	Sneakers

Day period (daytime, nightly), presence of light (yes, Y; no, N), temperature (T, °C), salinity (sal, ppt), percentage of dissolved oxygen (% DO), duration of mating (s), squid in the tank (n), number of females (f), and number of males (m).

and between males and females when one squid is alarmed (Boycott, 1965; Hanlon, 1978, 1982; Hanlon *et al.*, 1983, 1994, 1999). **Downward curling** and **J-posture** were less frequently observed postures, but **downward curling** (Fig. 3H) was noted more often than the **J-posture.** Both postures were observed in males and females, and were related to aggressive behavior or alarm. These two postural components are commonly observed among other squids. The **J-posture** was reported in *D. opalescens* (Hunt *et al.*, 2000) and *Lolliguncula brevis* (Blainville, 1823) (Martins and Perez, 2006). Other equivalent components are found in the following squids: *Ornithoteuthis antillarum* Adam, 1957 (Vecchione and Roper, 1991); the J-curl in *Gonatus onyx* Young, 1972 (Hunt and Seibel, 2000); arms flexed dorsally in *Octopoteuthis megaptera* (Verrill, 1885) (Vecchione *et al.*, 2002); upward curl in *Sepioteuthis australis* (Quoy & Gaimard, 1832) (Jantzen and Havenhand, 2003); and dorsal arm curl in the deep-sea squid *Octopoteuthis deletron* Young, 1972 (Bush *et al.*, 2009). This posture was related to deimatic behavior in cephalopods (Hanlon and Messenger, 1996). **Downward curling** has been reported for *D. plei* (Hanlon, 1978) and other loliginids (Hanlon *et al.*, 1994, 1999; Hunt *et al.*, 2000; Jantzen and Havenhand, 2003).

Reproductive behavior in Doryteuthis plei

The reproductive behavior in *D. plei* includes a variety of skin colorations, movements, and postures (Hanlon *et al.*, 1983; DiMarco and Hanlon, 1997). Shoaling squids have ample opportunity for social communication with conspecifics throughout most of their lives, and some species have established elaborate behavioral sequences, including agonistic, courtship, and mating behaviors (Hanlon and Messenger, 1996).

Agonistic behavior. In our study, the male initiated courtship and immediately established and maintained a dominant relationship over females. Fighting between large males was a conspicuous event during their reproductive behavior. The behaviors also included threats, **chases,** and **fleeing** during **fin beating;** together with the presence of **lateral mantle streaks** and **arm splotch,** these behaviors are easily identified. The most noted posture that we observed is **splayed arms** during agonistic behavior. The locomotor component of **fin beating** is also easily recognized and represents the escalation of an agonistic encounter by involving physical contact (Porteiro *et al.*, 1990; Hanlon and Messenger, 1996; DiMarco and Hanlon, 1997; Hanlon *et al.*, 2002; Jantzen and Havenhand, 2003; Pham *et al.*, 2009; Shashar and Hanlon, 2013). The courtships are interrupted by large, lone males or intruders, as previously reported (DiMarco and Hanlon, 1997; Hanlon *et al.*, 2002), that engage the paired consorts in agonistic contests, often resulting in successful takeovers. The agonistic behavior of this species was described in detail by DiMarco and Hanlon

(1997), who observed various aspects of the behavior mainly in laboratory studies. This behavior occurred similarly in other loliginids (Hanlon *et al.*, 1994, 1999; Hunt *et al.*, 2000).

Courtship behavior. In this study, most squids formed mate pairs (females and males), and the duration of mate pairing lasted for a long period. In the experiment of November 2011, we observed that in the first 2 d of maintenance, squids performed **free swimming** inside the tank. However, by the third day they had formed pairs. The pairs were generally formed after the agonistic contests. In females, the **red accessory nidamental gland** and **oviducal gland** were often visible, as in some species of *Loligo* (Hanlon *et al.*, 1994, 1999, 2002; Hunt *et al.*, 2000).

Mating behavior. Mating of *Doryteuthis plei* in this study occurred in two positions and was similar in duration and positioning to what was reported for other loliginids (Hanlon *et al.*, 1994, 1999; Sauer *et al.*, 1997; Jantzen and Havenhand, 2003; Zeidberg, 2009; Sharsha and Hanlon, 2013). The first position was **head-to-head mating** with a sneaker male, which was observed more often than the **male-parallel** position with a large consort male. Hanlon (1998) and Hanlon *et al.* (2002), in studying the behavior of *Loligo* sp. and *Loligo vulgaris reynaudii* in South Africa, used the term "sneakers" for the smaller males and "consorts" for the larger males that formed different reproductive disputes during spawning on the seafloor. The **male-parallel** mating always occurred soon after eggs were deposited on sand at the bottom of the tank. Waller and Wicklund (1968) observed a larger natural spawning shoal in the sea, noting that nearly all squids were paired, and mating in the **male-parallel** position was followed almost immediately by egg laying (**oviposition**). Shashar and Hanlon (2013) detailed the multiple mating tactics during copulation in *Doryteuthis pealeii.*

Egg-directed behavior. The **oviposition** component was rarely observed in the filming (*i.e.,* only twice). Egg deposition of females occurred in a completely darkened lab or in the early hours of the day. Egg depositions that occurred during the night were filmed with the aid of an LED light. The egg mop developed rapidly, and the first paralarvae appeared 10 d after eggs were deposited at the bottom of the tank. This result is similar to findings reported by Roper (1965) for this species. **Oviposition** was also observed in shallow waters during field studies of this species (Waller and Wicklund, 1968), for *D. opalescens* in Monterey Bay California USA (Hanlon *et al.*, 2004), and for *Loligo vulgaris reynaudii* in South Africa (Sauer and Smale, 1993; Hanlon *et al.*, 1994). However, **egg touching** was observed for long periods among *D. plei* males and females. **Egg touching** is common in captivity and can be artificially stimulated by inserting an egg capsule, as described for *D. pealeii* by Arnold (1962). This action was most common in males guarding an egg mop.

Conclusion

This study reports the first findings of body patterning behavior of *Doryteuthis plei* in the Southern Hemisphere. Currently, it represents the only ethogram with quantitative analysis of a myopsid cephalopod in South America. Our results showed that most behaviors observed for *D. plei* are similar to those of other squids around the world, both in captivity and in the field. However, some differences were found between *D. plei* investigated here and previous studies in the North Atlantic. For example, the chromatic component of the female **accentuated oviducal glands** was readily observed during pairing or courtship in our study (Fig. 3J). However, the glands were not observed by Boycott (1965) in Bermuda, in natural habitats in the Bahamas (Waller and Wicklund, 1968), or in captivity in Massachusetts (Hanlon, 1982, 1988; Hanlon *et al.*, 1983). We did not observe chromatic components such as **infraocular spots** or **dark arms/head** for long periods, as was reported only for *Loligo vulgaris* (Hanlon *et al.*, 1994) and *Doryteuthis pealeii* (Hanlon *et al.*, 1999).

Recent phylogeographical studies of *D. plei* have suggested that the Brazilian population is genetically distinct from *D. plei* in North America and Central America (Sales *et al.*, 2013). For this reason, behavior is another attribute that can assist in taxonomic identification and phylogenetic analyses (Hanlon, 1988; Hanlon *et al.*, 1999). The genetic description, coupled with detailed behavioral aspects such as those reported in this study, should provide insights into the variability of reproductive behaviors and the potential for differences between the various populations of *D. plei* in the Atlantic Ocean.

In summary, the squid *D. plei* has a large repertoire of body patterns, including many combinations of skin coloration, body postures, and swimming movements, that are used specifically for communication during reproductive behavior. Our results showed that the duration of each chromatic component differed significantly, suggesting that these components are connected to behaviors performed during the short life cycle, for example, agonistic behavior or mating. Females displayed the chromatic components for longer durations than the males, which may have been the result of their calm behavior or display of their gonads. The males were dedicated to winning the females in the first days of the observation periods. **Head-to-head** mating was more frequent and longer lasting than **male-parallel** mating. Egg-directed behaviors occurred during nighttime periods only.

Our evidence supports the theory that the elaborate sensorial system in cephalopods, allowing for rapid chromatophore activity and skin-based communication skills for intra- and interspecific relationships, is complex and highly evolved, even in small-size nektonic species. The particularly ritualized reproductive behavior found in *D. plei,* with the gonadal displays during courtship and the immediate expansion of some groups of chromatophores and retraction of others, seems to be one of the most complex and interesting body patterning behaviors noted in the marine realm.

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