

First description of eggs, hatchlings and hatchling behaviour of *Enteroctopus megalocyathus* (Cephalopoda: Octopodidae)

NICOLÁS ORTIZ¹*, MARÍA EDITH RÉ¹ AND FEDERICO MÁRQUEZ²

¹CENTRO NACIONAL PATAGÓNICO (CONICET), BOULEVARD BROWN S/N, U9120ACV PUERTO MADRYN, ARGENTINA AND ²UNIVERSIDAD NACIONAL DE LA PATAGONIA SAN JUAN BOSCO, BOULEVARD BROWN 3700, 9120 PUERTO MADRYN, ARGENTINA

*CORRESPONDING AUTHOR: nicortiz@cenpat.edu.ar

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*As for many other commercially exploited octopodid species, there are no detailed descriptions of the eggs and hatchlings of *Enteroctopus megalocyathus* that can be used for the identification of individuals captured in plankton samples. Eggs, clutches and hatchlings are described here for the first time and compared with those of both other Patagonian octopodids and other *Enteroctopus* species. Relative to other *Enteroctopus*, hatchlings were large with total length (TL) 14.8–21.5 mm, mantle length (ML) 7–9.5 mm and arm length (AL) representing 90.5% of the mantle. Dorsal chromatophores were more abundant than ventral ones, and their shape and distribution created a very distinctive pattern. Eggs and hatchlings were found to have several characteristics that can be used for species identification. Although the mode of life of newly hatched cephalopods is often categorized as either planktonic or benthic, the hatchlings in aquaria showed no preference for swimming or settling. Additionally, the size of eggs and hatchlings which is correlated with the mode of life at hatching in other octopodid species, fitted both planktonic and benthic in *E. megalocyathus*. Furthermore, morphological and behavioural characteristics were similar to the pre-settlement stage of planktonic hatchlings of *Octopus vulgaris*. Therefore, we suggest that hatchlings of *E. megalocyathus* have an unusual, suprabenthic mode of life.*

INTRODUCTION

Difficulty in identifying the eggs and young of cephalopods is an impediment to progress in all fields of research on these molluscs, because the young forms of many species are still unknown. This is especially the case for many commercially valuable species upon which major fisheries are based and for which information on early life stages is required for the sustainable management of stocks (Rodhouse *et al.*, 1992; Sweeney *et al.*, 1992). Similarly, observation of egg masses may be the only tangible proof of spawning activity of a species in a given area over the year, and the description of its eggs provides important information about synecological relations among species (Boletzky, 1998; Barón, 2001).

In order to overcome these problems, hatchlings of several species of octopus have been described, and it has been shown that the number and distribution of

chromatophores on the skin over the arms, funnel, eyes, head, mantle and perivisceral epithelium (i.e. chromatophore fields) are species-specific. In addition, the number and relative size of suckers at hatching can be used to separate species (Young *et al.*, 1989; Hochberg *et al.*, 1992).

The Patagonian red octopus, *Enteroctopus megalocyathus* (Gould, 1852), is a large benthic species that is caught by artisanal fishermen at several localities along the Argentine Patagonian coast mainly between March and November. It is distributed in the Atlantic Ocean from the San Matías Gulf (41°30'S 64°40'W) to the Beagle Channel (54°53'S 67°50'W), Falkland (Malvinas) Islands (51°50'S 59°40'W) and Burdwood Bank (56°10'S 54°20'W) (Ré, 1984, 1998a, 1998b). In the Pacific Ocean, it constitutes the greater capture of octopuses to the south of region VI (34°20'S 72°00'W) of the Chilean

coast, reaching ~200 tonnes per year (Rocha and Vega, 2003). Although this species supports important local fisheries and, recently, culture projects have been initiated (Perez *et al.*, 2006), the general aspects of the life cycle are poorly known for both oceans, and the hatchlings have not been described.

The hatchlings of bottom-dwelling species of the family Octopodidae can develop an adult-like mode of life or, in a considerable number of species, change life style during the juvenile phase. The latter group exhibit first a planktonic post-hatching phase with short arms and continuous swimming; then they gradually switch to a benthic mode of life when the arms attain a length similar to the length of the mantle [i.e. relative arm length (AL)] (Itami *et al.*, 1963; Boletzky, 1987; Villanueva *et al.*, 1995).

In addition, in many species it has been shown that other characters related to the size of the eggs and hatchlings are correlated with the mode of life of newly hatched octopods or with the subsequent change in life style observed in small-scale aquarium cultures. These characters are the length of the eggs and the mantle length (ML) of the hatchlings, both relative to adult ML (termed egg index and relative size of the hatchling, respectively) (Boletzky, 1977, 1978–79, 2003). So, in general, egg lengths (ELs) >10 mm, egg indices of 10–12%, relative size of hatchlings of 6–20% and relative ALs >50% produce crawl-away (benthic) young. ELs of 1.5–5 mm, egg indices of 2–10%, relative size of hatchlings of 2–5% and relative ALs <50% give rise to planktonic hatchlings. Intermediate egg sizes, from 6 to 9 mm, may produce either benthic or planktonic young, depending on the specific egg index (Boletzky, 1977, 1978–79, 1987, 2003; Hochberg *et al.*, 1992; Boletzky *et al.*, 2002). This division may be very strict but it is useful for comparisons between young cephalopods (Boletzky, 1977).

There are only two reports on the eggs or hatchlings of *E. megalocyathus*. Chong *et al.* (Chong *et al.*, 2001), using an interviewing resource from artisanal fishermen in the Chilean regions X (41°30'S 72°50'W) and XI (44°60'S 73°30'W), obtained partial information about the eggs, although not enough for a correct identification. Vega *et al.* (Vega *et al.*, 2000) analysed young cephalopods captured by nets in Moraleda Channel (Southern Chile) and suggested that some specimens could be *E. megalocyathus*.

The aims of this study were (i) to describe qualitatively and quantitatively the eggs and egg strings of the Patagonian red octopus, *E. megalocyathus*, (ii) to describe the hatchlings, (iii) to compare the clutches of eggs and hatchlings with those of other Patagonian octopods and other species of the genus *Enteroctopus* and (iv) to

describe the mode of life of the young animals based on their morphological characteristics and observations in the aquarium.

METHODS

Monthly scuba-diving surveys were conducted between November 2004 and November 2005 in San José Gulf, Argentina (42°15'S 64°14'W). Six clutches were encountered: three in March, one in April and two in August. Four of them were found with their brooding females. All the clutches and the females were collected with a hook used to capture Patagonian red octopus.

In the field, subsamples of clutches were preserved in 5% seawater formaldehyde solution for future identification of the embryonic stage of development. The remaining ones were kept indoors at the Centro Nacional Patagónico Laboratory, Puerto Madryn, Argentina in 5-L aerated glass aquaria with seawater previously filtered with 500- μ m filters. The strings were attached by thin threads hanging from the top of the container in order to keep the eggs away from the bottom and to maximize oxygen uptake. They were kept in darkness and cleaned with a fine brush twice a week. Incubation water temperatures ranged from 10 to 14°C (mean 11.7°C). To avoid thermal shock, we acclimated seawater to experimental conditions for a period of 12 h and changed it every 2 days.

Only one clutch of those taken in March was completely extracted, using the sampling method for removal of eggs. Because the brooding female had an empty ovary, we used this clutch to obtain the total number of eggs per clutch. In order to register the average length of strings and the average number of eggs per string, we used only complete strings. Eggs were measured with a Vernier caliper and characterized by their EL, egg width (EW) and chorion stalk length.

One of the clutches found in August began to hatch during collection, and the eggs kept hatching during the trip to the aquarium. Only 17 hatchlings arrived alive in the laboratory and were transferred to 80-L aerated seawater glass aquaria at 11.5 \pm 0.7°C. Our descriptions were based only on these hatchlings because laboratory-incubated embryos seemed to be premature as they were born with external yolk unlike the others.

As fixatives and preservatives might produce morphological modifications, measurements were taken in two different ways in order to make the results comparable with past and future studies. Freshly dead hatchlings were first measured with an ocular micrometer under a dissecting microscope and again 1 month later after fixation with 5% formalin in seawater.

For hatchling descriptions, the terminology and measurements recommended by Young *et al.* (Young *et al.*, 1989) and Hochberg *et al.* (Hochberg *et al.*, 1992) were used when possible. Hatchlings were weighed [total weight (TW)] to the nearest 0.01 g using a Mettler PC 440-Delta Range microbalance and characterized by their total length (TL), dorsal ML, mantle width (MW), AL, eye diameter (ED), funnel length, suckers number per arm and chromatophore fields.

Because some morphometric relations are associated with the mode of life of the young, the egg index and the relative size of hatchlings were calculated as a percentage of fully mature female size, using ML = 149 mm (Chong *et al.*, 2001).

Funnel length and AL were compared with ML of hatchlings. Wilcoxon paired-sample tests (Zar, 1996) were performed to compare the number of dorsal and ventral chromatophores and to compare AL with ML.

RESULTS

Eggs and egg strings

The large eggs had a pear-shaped chorion, devoid of other capsules, with a long chorion stalk that was enlarged at the free end (Fig. 1a). They were entwined with one another along the central axis, which was surrounded by secretions of the oviducal glands at the moment of laying, and forming strings of a variable number of eggs (Fig. 1b; Table I). In one complete clutch, 39 strings were counted, and only 15 of them were unbroken. The number of eggs in this clutch was 1469.

Clutches of eggs were found attached to the roof of holes that were located in low ledges (of up to 1.5–2 m

high) of submerged abrasion limestone platforms or in scattered holes of silty-clay sediments. They were found from 6 to 14 m depth and at surface water temperatures of 18°C in March, 15°C in April and 9.5°C in August. The mean TW, ML and TL of the four captured brooding females were 1240 g, 141.2 mm and 769 mm, respectively. The egg index is summarized in Table II.

External morphology and body proportions of hatchlings

The clutches from March and August did not hatch. The clutch from April did hatch, after being incubated for 88 days at a mean temperature of $11.7 \pm 0.8^\circ\text{C}$. However, as mentioned before, the following descriptions are based on hatchlings from August

A skin film densely surrounded by Kölliker's organs (seen as iridescent dots in alive animals and as white dots in dead ones) loosely covered the funnel, mantle, eyes and arms. This skin film was an unpigmented layer and was only interrupted by the funnel orifice, the mantle aperture and sucker openings. The internal yolk sac was not observed at the time of hatching. The mantle was broad, rounded and ventrally extended to the level of the lens. The funnel even extended to the base of the arms, and the free part of it represented 34.5% of the ML.

The arms were subequal. They were shorter than the mantle ($P < 0.01$) and represented 90.5% of the latter (Table II). A web was developed up to one-third of the length of the arms. The biserial suckers were arranged in a zigzag row and gently decreased in size from the mouth towards the tip. No development of sucker buds was found on the tip of the arms at the time of hatching. Morphological measures of *E. megalocyathus* hatchlings are summarized in Table III.

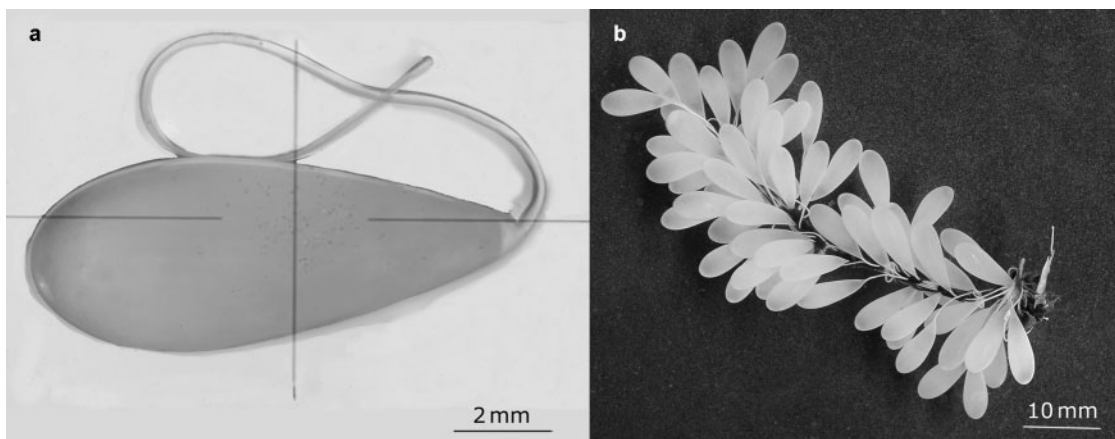


Fig. 1. (a) Egg and (b) egg strings of *Enteroctopus megalocyathus*.

Table I: Numerical and morphometric characteristics of strings (A) and eggs (B) of *Enteroctopus megalocyathus* from a female with a mantle length of 135 mm and a total weight of 1075 g

		Mean ± SD	Range	n
A	Number of eggs per string	48 ± 13.49	32-86	15
	Length of strings (mm)	58.02 ± 9.73	45-74	15
B	Eggs width (mm)	3.52 ± 0.33	3-4.7	166
	Eggs length (mm)	10.71 ± 0.46	9.5-12	165
	Length of chorion stalks (mm)	20.85 ± 1.34	17-24	153

Table II: Comparison of characteristics of planktonic or benthic hatchlings with the characteristics of hatchlings of *Enteroctopus megalocyathus* and the classification expected for *E. megalocyathus* using mean measurements

	Planktonic species	Benthic species	<i>E. megalocyathus</i>	Classification for <i>E. megalocyathus</i>
Egg length (mm)	1.5-5	>10	10.71	B
Egg index (%)	2-10	10-12	7.18	P
Relative arm length (%)	<50 or AL < ML	>50 or AL ≥ ML	90.5 or AL < ML	B or P
Relative size of hatchling (%)	2-5	6-20	5.62	B or P

Categorization of planktonic versus benthic species based on Boletzky (Boletzky 1977,1978-79,1987,2003, Boletzky *et al.* (Boletzky *et al.*, 2002) and Hochberg *et al.* (Hochberg *et al.*, 1992).

AL, arm length; B, benthic mode of life; egg index, egg length × 100/adult mantle length; ML, mantle length; P, planktonic mode of life; relative arm length, AL of hatchlings × 100/mantle length of hatchlings; relative size of hatchlings, mantle of hatchlings × 100/mantle length of adult.

Table III: Morphological measurements of freshly dead *Enteroctopus megalocyathus* at hatching (present work) and of anaesthetized *Octopus vulgaris* at 50 and 60 days old (Villanueva, 1995)

	<i>Enteroctopus megalocyathus</i>			<i>Octopus vulgaris</i>	
	At hatching			50 days (planktonic)	60 days (benthic)
	Mean ± SD	Range	n	Range	
Total length (mm)	18.32 ± 1.74	14.8-21.5	26	10.1-15.2	15.2-22.2
Mantle length (mm)	8.38 ± 0.69	7-9.5	27	4.9-7.5	7.8-9.3
Mantle width (mm)	7.38 ± 0.74	5.3-8.8	27		
Arms length (mm)	7.59 ± 0.99	5.8-9.2	26	3.55-5.8	6.6-8.4
Funnel length (mm)	3.11 ± 0.58	2.5-4.5	11		
Eye diameter (mm)	2.33 ± 0.26	1.7-2.8	24	1.30-1.80	1.80-2.00
Total weight (mg)	111.1 ± 17.1	90-150	18	43.5-78.67	160.2-182.1
Suckers per arm	21.19 ± 2.66	16-27	26	18.2-26.0	27.0-34.3

The measurements of freshly dead and fixed individuals were different. The mantle (ML and MW) was 12-22% shorter in fixed specimens than in freshly dead ones; the arms and funnel were reduced by 12 and 8%, respectively, and TL reduction was about 17%.

Chromatophore shape and arrangement

The shape of a given chromatophore changed according to the degree of its expansion. Despite the variability of this feature among individuals, when they were expanded, patterns in discrete regions of the body (mantle, head, arms and funnel) could be distinguished (Fig. 2).

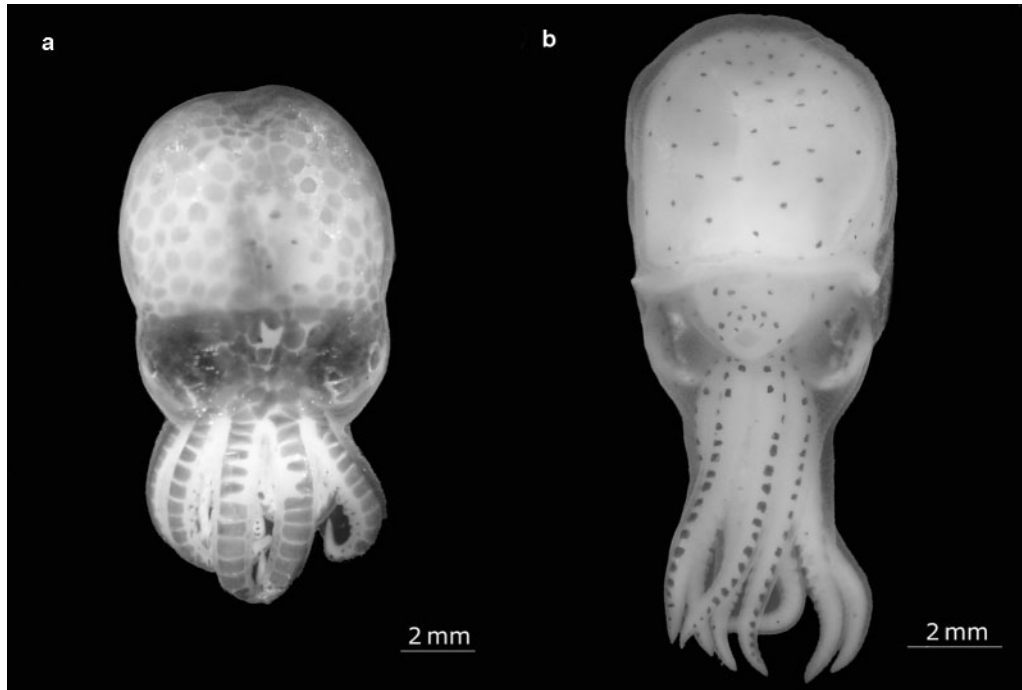


Fig. 2. Newly hatched *Enteroctopus megalocyathus*. Both specimens are fixed. **(a)** Dorsal side with chromatophores expanded and unnaturally contracted because of formaldehyde fixation. Note that the chromatophore fields are very distinctive in mantle, head and arms. **(b)** Ventral side showing the skin covering the whole animal and chromatophore fields of mantle, funnel and arms. This animal kept the original proportions.

The mantle was spotted with oval to round tegumental chromatophores. The dorsal side was densely covered (11 across) except for the mid-region, which was more sparsely covered (more evident in fixed specimens than in fresh ones) (Fig. 2a). On the ventral surface, chromatophores were uniformly but less densely distributed (nine across) (Fig. 2b). Chromatophore numbers are summarized in Table IV. Dorsal chromatophores were significantly more abundant than ventral ones ($P < 0.01$).

There was a denser arrangement of extrategumental chromatophores on the dorsal head than on the mantle, where each chromatophore (eight across) did not have a defined shape, although with straight or round edges. In almost all cases, a single row of dorsal chromatophores (range 5–10) on the sides of one or both eyes stood out, whereas over them they were always present in two or three rows (Fig. 3a).

The aboral side of the arms had rectangular tegumental chromatophores in a single line decreasing in size towards the tip. On the tip, an additional line of two or three smaller chromatophores could be found. On each side of the arms, or sometimes distributed on the oral side of the middle portion of the arms, a variable number of chromatophores (one to seven) could be seen. Tegumental chromatophores of the funnel were

arranged on the ventral side, near the lip of the funnel orifice and in a complex stripe (one or two rows plus a few dispersed ones) (Figs 2b and 3b).

On all parts of the body, the chromatophores were brown although when they were slightly expanded they could appear somewhat orange. Expanded chromatophores were lighter in colour and less discrete than retracted ones, which also appeared as dots. When retracted and in freshly dead animals, dorsal visceral extrategumental chromatophores could be seen like a suite of disordered dots over the visceral mass. This structure is the darkest, and the shape of its chromatophores is like those of the head when the animals are alive and either swimming or resting. When fixed, the transparent mantle of fresh animals became white, hiding the chromatophores over the visceral mass.

Behaviour

Newly hatched animals would swim in the water column or remained at the bottom of the aquaria. Strong negative-buoyancy allowed the animal to swim slowly, backwards, with loose arms, in oblique body position and with the posterior end pointing diagonally towards the surface or straight to it. When jet propulsion stopped, the animal drifted along the water column, with its mantle

Table IV: Hatchling chromatophore numbers of *Enteroctopus megalocyathus* (n = 27) in different body areas

	Dorsal		Ventral		Aboral	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Mantle	105.77 ± 18.08	85-161	67.70 ± 8.9	50-80		
Visceral	143.88 ± 28.44	100-210	47.66 ± 17.94	15-72		
Head	59.66 ± 8.87	35-74	13.59 ± 3.33	7-21		
Eyes	13.98 ± 1.64	9-18				
Funnel			15.60 ± 1.52	11-18		
Arms					19.59 ± 1.58	14-23

pointing towards or parallel to the bottom and its arms loose. It could start swimming again both in the middle of the water column or when reaching the bottom. This behaviour was very frequently observed, and it lasted for long periods, up to several hours.

With similar regularity, adhesion to the glass surface of the aquarium was observed. On the bottom, animals usually sat with the head uppermost and the arms spread out, attaching themselves to the glass with their suckers. They could either remain inactive in that position or begin to crawl along the sidewall with a coordinated action of the arms for short distances. When disturbed, animals responded in two ways, swimming (and sometimes ejecting ink) or crawling with their chromatophores

expanded. These two behavioural patterns were seen in the hatchlings of April and August

Under starvation conditions, hatchlings of August survived between 5 and 15 days (mean = 9.5, SD = 3.5, n = 17) at 11.5 ± 0.7°C, whereas premature hatchlings of April lived for 1-5 days (mean = 2.4, SD = 0.8, n = 293) at the same temperature. Cannibalism was not observed in either group.

DISCUSSION

The octopodid species recorded for the north Patagonian Atlantic coast are *Octopus tehuelchus*,

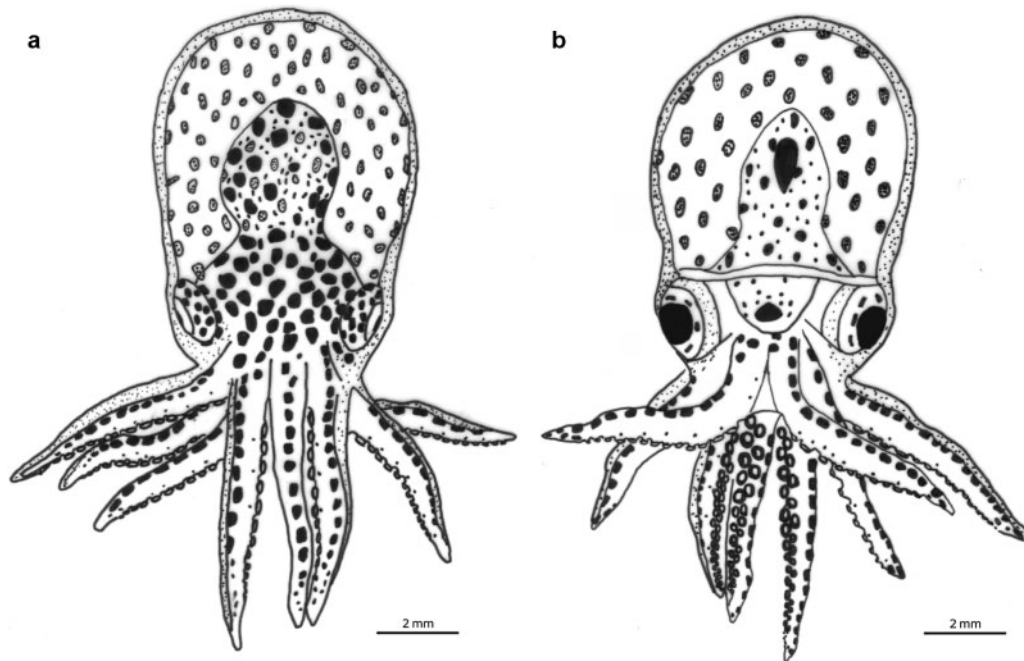


Fig. 3. Schematic drawing of *Enteroctopus megalocyathus* at hatching. (a) Dorsal side and (b) ventral side.

Eledone massyae, *Robsonella fontaniana* and *E. megalocyathus* (Ré, 1998a). The eggs of *O. tehuelchus* (9–12 × 3–4 mm) are different from those of *E. megalocyathus* because their chorion stalks are shorter (3–5 mm) and cemented individually or in small clusters of two to three eggs, with 230 eggs as a maximum per clutch (Iribarne, 1991; Ré, 1998a). They produce benthic hatchlings with a ML of 6.64 mm and TL of 14.23 mm (Ré, 1998a). The eggs and hatchlings of *E. massyae* are still unknown. The oocyte length (10–11.9 mm) is similar to that of *E. megalocyathus*, but its estimated potential fecundity is only 27–126 eggs (Perez and Haimovici, 1991), whereas fecundity for *E. megalocyathus* is 2500–2900 oocytes, depending on the weight of females at an advanced maturity stage (815–1400 g TW) (Ré, 1998a). The eggs of *R. fontaniana* are 4.3 × 2.0 mm; hatchlings are planktonic with a ML of 3.31 mm and TL of 5.22 mm (Ré and Ortiz, unpublished data). Hence, clutches and eggs of *E. megalocyathus* differ from all other co-occurring species. In our study, four clutches were found together with a brooding female, but these differences allow for the assignment of clutches to *E. megalocyathus* even in the absence of a brooding female.

Ré (Ré, 1984) suggested two separate breeding seasons for the north Patagonian Atlantic coast, one in winter and one in summer, whereas on the Pacific coast Chong *et al.* (Chong *et al.*, 2001) found a prolonged spawning season extending from late winter through spring to the end of summer. Because we observed clutches from late summer to late winter, we suggest that (at least in the Northern Atlantic Patagonian part of its distribution and in shallow waters), *E. megalocyathus* has an extended breeding season that also includes autumn. However, the low number of clutches found during the sampling period suggests that the regular spawning grounds could be deeper than the fishing grounds (more than 20–25 m depth), in accordance with a decline in abundance in the shallow waters during summer (Ré, 1984).

The genus *Enteroctopus* includes *Enteroctopus dofleini*, *Enteroctopus magnificus*, *Enteroctopus zealandicus* and *E. megalocyathus* (Hochberg, 1998; Sweeney, 2001). Only for *E. dofleini* are the spawned eggs known, measuring 6–8 × 3 mm, with a chorion stalk of 11 mm and a clutch size ranging from 30,000 to 180,000 eggs (Hochberg *et al.*, 1992). For *E. zealandicus*, the sizes of mature oocytes are 12.5 × 2.3 mm (O'Shea, 1999), whereas for *E. magnificus* the length of immature oocytes is 6–8 mm, with an ovary containing about 20,000 of them (Villanueva *et al.*, 1991). All of them share long chorion stalks. Thus, characteristics of *E. megalocyathus* eggs, including mature oocytes, are consistent with those found for the genus.

Both *E. dofleini* and *E. zealandicus* have planktonic hatchlings (Gabe, 1975; O'Shea, 1999), whereas those of *E. magnificus* are unknown. In morphometric terms, hatchlings of *E. megalocyathus* are not comparable with those of *E. dofleini* (the only species of the genus with fully described hatchlings), whose hatchlings have a ML = 3.51 mm and an AL = 1.77 mm (Gabe, 1975). Comparing their chromatophore fields, we found that they share only a few similarities: the single line on the aboral side of arms, the funnel pattern complex and the two rows on the dorsal surface of the eyes (Kubodera, 1991; Hochberg *et al.*, 1992).

In practical terms, these features, in addition to a very high number of chromatophores on the whole animal (with ventral chromatophores 45% less numerous than dorsal ones) (Fig. 3; Table IV), could be a useful preliminary tool to distinguish the hatchlings of *E. megalocyathus* from those of other species within its Atlantic distribution range. In this sense, the *Octopus* sp., described by Rodhouse *et al.* (Rodhouse *et al.*, 1992), from the shelf around the Falkland (Malvinas) Islands, caught close to the seabed with nets would belong to *E. megalocyathus*. They shared two rows of suckers on the arms, an ED of ~30% ML, chromatophores more densely concentrated over the head than over the mantle and denser on the dorsal surface than on the ventral mantle surface and a single row of chromatophores along both sides of the arms and along the aboral side of each arm. However, the lower limit of the ML range (3–13 mm) does not correspond to our measurements of *E. megalocyathus*, so perhaps another species is involved. The same could be true for the young cephalopods found by Vega *et al.* (Vega *et al.*, 2000) that appear to belong to *E. megalocyathus* but with a ML range of 2.1–8.3 mm.

The presence of a skin film covering the body in *E. megalocyathus* was also observed by Kubodera (Kubodera, 1991) in *E. dofleini* as a 'thin gelatinous tissue'. However, at hatching, this structure may not be unique for the genus *Enteroctopus*, as it also seems to be present in some species of the suborder Incirrina. From the drawings published in Hochberg *et al.* (Hochberg *et al.*, 1992), a similar skin could be observed at least in *Octopus bimaculatus* from the eastern North Pacific and in the '*Octopus defilippi* complex' from Hawaiian waters. Additionally, Diekmann *et al.* (Diekmann *et al.*, 2002) also drew this structure for *Argonauta argo* and an undetermined species of *Octopus* collected in the subtropical eastern North Atlantic. The transient Kölliker's organs (Brocco *et al.*, 1974) spread over this structure in *E. megalocyathus* have been found in hatchlings of all other species studied until now, with the exception of the benthic young of *Octopus briareus* and *Octopus maya* (Boletzky, 1977). The function at hatching for a skin film (if any) is unknown but, at least in

E. megalocyathus, it may be an early skin that later will be pigmented in juvenile and adult animals.

Three phases of development have been recognized for several octopodid species that have a planktonic or micro-nektonic post-hatching phase: fully planktonic, planktonic/benthic (pre-settlement stage) and truly benthic (Hanlon *et al.*, 1985; Villanueva, 1995; Villanueva *et al.*, 1995). In other species, the benthic behaviour of adults is evident at hatching. Planktonic young cephalopods are continually in motion (having an exclusive swimming existence), intermittently sinking to the bottom for a short time or attaching to the substrate under laboratory conditions (Boletzky, 1974, 1977; Overath and Boletzky, 1974; Gabe, 1975; Forsythe and Hanlon, 1985). Benthic hatchlings rarely swim, although they can do so if needed, and they are able to crawl or rest with their arms outstretched on the ground. Hence, benthic hatchlings display an adult-like mode of life from the outset of post-embryonic development (Messenger, 1963; Tranter and Augustine, 1973; Boletzky, 1977). Summarized, in newly hatched planktonic octopodids the tendency to swim clearly dominates over the tendency to settle (Boletzky, 1977), whereas for benthic young the opposite may be expected.

Because no tendency was observed in *E. megalocyathus* hatchlings, we could not assign the behaviour to either a truly planktonic or a benthic mode of life. Moreover, our observations on both premature and non-premature hatchlings approximate to the pre-settlement stage defined by Villanueva (Villanueva, 1995) for *Octopus vulgaris*. These individuals were predominantly planktonic and could swim but intermittently could adhere or crawl along the wall or bottom of the rearing tanks. The inverse transition from benthic hatchlings to an adult pelagic mode of life has never been reported (Boletzky, 1987, 2003).

There have been a few studies on the process of the settlement of octopods. Itami *et al.* (Itami *et al.*, 1963) and Villanueva (Villanueva, 1995) have shown under experimental conditions how the body structures of *O. vulgaris* change during this period. Despite the interspecific differences with *E. megalocyathus*, behaviour and measurements were very similar between the pre-settlement stage of *O. vulgaris* and the hatchlings of *E. megalocyathus*. Specifically, hatchlings of *E. megalocyathus* have comparable ranges for TL, ML, AL, TW, ED and number of suckers per arm as *O. vulgaris* at 50–60 days old in the transition between the planktonic and benthic mode of life of this species (Villanueva, 1995) (Table III). Additionally, similarities exist in the number of suckers with the benthic young of *Octopus joubini* (25–26 suckers) within the first month after hatching, which was suggested to have a ‘necto-benthic’

way of life during the first 2 weeks (Boletzky and Boletzky, 1969).

Marliave (Marliave, 1981) described a neustonic feeding behaviour for planktonic hatchlings of *E. dofleini* born in aquaria. These hatchlings showed arm extension and adherence to the water surface (using the strength of the surface tension) when looking for food. This behaviour was observed both in the presence and absence of food, until the animal grew from 3 to 4 mm (measured as head width), when the tendency to show this behaviour was reduced. We did not provide our hatchlings with floating food to test whether they could adhere to the water surface and feed, but we never observed the same behaviour as in *E. dofleini*. *E. megalocyathus* seems unable to display this behaviour due its large size, heavy weight and its strongly negative-buoyancy observed in aquaria. Also, the sampling area is characterized by prevailing westerly winds during the entire year (Prohaska, 1976) that produces much wave action and a mixed layer of tens of metres thick (Knauss, 1978). As a consequence, it may prevent *E. megalocyathus* hatchlings from making use of the surface tension.

The planktonic–benthic mode of life observed in *E. megalocyathus* could correspond to a mode of life often defined as suprabenthic (also called hyperbenthic). The suprabenthic includes all bottom-dependent animals that perform, with varying regularity, daily or seasonal vertical migrations above the bottom, such as mysids, cumaceans, isopods and amphipods (Brunel *et al.*, 1978). As far as we know, this term has not been applied to cephalopod hatchlings before, but *Opisthoteuthis calypso*, *Opisthoteuthis masyae* and *Sepietta oweniana* all seem to feed in the suprabenthic habitat (Bergstroem, 1985; Villanueva and Guerra, 1991; Villanueva *et al.*, 2002).

The arms seemed to play an important role in the planktonic–benthic coupling observed in *E. megalocyathus*. They limited the individuals’ mobility, pulling them down, influencing their swimming capacity and probably their time in the water column. Although significantly shorter than the mantle, the arms of *E. megalocyathus* were only 9.5% shorter than a length that would indicate a benthic life style. Similarly, the number of suckers of ≥ 20 per arm is characteristic of benthic young (Boletzky, 1977). When we consider the other features of eggs and hatchlings of other octopodid species in relation to their mode of life, it seems that at the outset of post-embryonic development *E. megalocyathus* fits both the planktonic and benthic life style. Moreover, when the range is taken into account instead of only the means, measurements overlap with both expected modes of life (Tables II and III).

On the basis of all these findings, we suggest that *E. megalocyathus* hatchlings may be hyperbenthic for a

short period until they reach a benthic mode of life. Consequently, they may have a short time of dispersal because an earlier settlement process is related to a restricted potential for dispersal (Hanlon *et al.*, 1985; Villanueva *et al.*, 1995; Villanueva, 1995). They may resemble more closely to *O. joubini*, which has an initial period of swimming just after hatching, than many planktonic young cephalopods whose dispersal is strongly influenced by different oceanographic features (Boletzky and Boletzky, 1969; Kubodera, 1991; Rodhouse *et al.*, 1992; Boyle and Boletzky, 1996; Rocha *et al.*, 1999).

Generally, hatchling cephalopods are obtained in two ways: from net samples or from eggs hatched in aquaria. Because we obtained specimens at sea at the moment they were hatching, we assume that the morphological descriptions made here resemble more closely what happens in the sampling area. Despite this, the body size at hatching may vary within species depending on the female body weight, the actual egg size, the individual hatching conditions and temperature during embryonic development (Bouchaud and Daguzan, 1990; Gowland *et al.*, 2002; Sakaguchi *et al.*, 2002; Vidal *et al.*, 2002; Barón, 2003; Boletzky, 2003). In this context, an increase in temperature may result in hatchlings emerging at smaller sizes and a decrease in temperature in bigger ones (Gowland *et al.*, 2002; Vidal *et al.*, 2002; Barón, 2003). Such a temperature effect could exist in the sampling area where the temperature at the end of summer and the end of winter differs by $\geq 8.5^{\circ}\text{C}$ or between the Atlantic and Pacific coasts. Even more, siblings reared under identical conditions still display a wide size range (Pecl *et al.*, 2004) which is also reflected in the range of *E. megalocyathus* hatchling measurements. Therefore, owing to these morphological variations at hatching, the length of the 'suprabenthic period' may be individually adjusted until the body proportions are suitable for a benthic mode of life. In this context, a larger number of animals, from hatching to stages of advanced development, and their extent of dependence on features of the bottom (e.g. prey, refuges and bottom-related boundary layer) should be examined in the field and aquarium to confirm this potential suprabenthic condition.

It has been suggested that the planktonic mode of life at the juvenile phase is a common ancestral feature that has evolved into a holobenthic mode of life in Octopodidae (Boletzky, 1987, 2003). If the suggested suprabenthic mode of life observed in *E. megalocyathus* were confirmed, then it might constitute a useful species to study the evolutionary origin of the benthic life in this family.

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REFERENCES

- Barón, P. J. (2001) First description and survey of the egg masses of *Loligo gahi* (d'Orbigny, 1835) and *Loligo sanpaulensis* (Brackoniecki, 1984) from coastal waters of Patagonia. *J. Shellfish Res.*, **20**, 289–295.
- Barón, P. J. (2003) The paralarvae of two South American sympatric squid *Loligo gahi* and *Loligo sanpaulensis*. *J. Plankton Res.*, **20**, 289–295.
- Bergstroem, B. I. (1985) Aspects of natural foraging by *Sepietta oweniana* (Mollusca, Cephalopoda). *Ophelia*, **24**, 65–74.
- Boletzky, S. V. (1974) The "larvae" of Cephalopoda: a review. *Thalassia Jugosl.*, **10**, 45–76.
- Boletzky, S. V. (1977) Post-hatching behaviour and mode of life in cephalopods. *Symp. Zool. Soc., Lond.*, **38**, 557–567.
- Boletzky, S. V. (1978–79) Nos connaissances actuelles sur le développement des Octopodes. *Vie Milieu*, **28–29**, 85–120.
- Boletzky, S. V. (1987) Reproduction. In Boyle, P. R. (ed.), *Cephalopod Life Cycles*. Vol. II. Academic Press, London, pp. 157–200.
- Boletzky, S. V. (1998) Cephalopod eggs and egg masses. *Oceanogr. Mar. Biol., Annu. Rev.*, **36**, 341–371.
- Boletzky, S. V. (2003) Biology of early life stages in Cephalopod molluscs. *Adv. Mar. Biol.*, **44**, 143–203.
- Boletzky, S. V. and Boletzky, M. V. V. (1969) First results in rearing *Octopus joubini* Robson, 1929. Institute of Marine Sciences, University of Miami, (Contribution No. 1056), pp. 56–61.
- Boletzky, S. V., Fuertes, M. and Offner, N. (2002) Developmental features of *Octopus macroopus* Risso, 1826 (Mollusca, Cephalopoda). *Vie Milieu*, **52**(4), 209–216.
- Bouchaud, O. and Daguzan, J. (1990) Étude expérimentale de l'influence de la température sur le déroulement embryonnaire de la seiche *Sepia officinalis* L. (Céphalopode, *Sepioidae*). *Cah. Biol. Mar.*, **31**, 131–145.
- Boyle, P. R. and Boletzky, S. V. (1996) Cephalopod population: definition and dynamics. *Philos. Trans. R. Soc. Lond., B*, **351**, 985–1002.
- Brocco, S. T., O'Clair, R. M. and Cloney, R. A. (1974) Cephalopod integument: the ultrastructure of Kölliker's organs and their relationship to setae. *Cell Tissue Res.*, **151**, 293–308.
- Brunel, P., Besner, M., Messier, D. *et al.* (1978) Le traîneau supra-benthique Macer-GIROQ: appareil amélioré pour l'échantillonnage quantitatif étagé de la petite faune nageuse au voisinage du fond. *Int. Rev. Gesamten Hydrobiol.*, **63**, 815–829.
- Chong Lay-Son, J., Cortés, N., Galleguillos, R. *et al.* (2001) Estudio biológico pesquero del recurso pulpo en la X y XI regiones. *Informes Técnicos Del Fondo de Investigación Pesquera (FIP-IT No. 99–20)*, Universidad Católica de la Santísima Concepción, Chile, p. 211.
- Diekmann, R., Piatkowski, U. and Schneider, M. (2002) Early life and juvenile cephalopods around seamounts of the subtropical eastern

- North Atlantic: illustrations and a key for their identification. *Berichte aus dem Institut für Meereskunde an der Christian-Albrechts-Universität*, Kiel. No. 326, p. 42.
- Forsythe, J. W. and Hanlon, R. T. (1985) Aspects of egg development, post-hatching behavior growth and reproductive biology of *Octopus burryi* Voss, 1950 (Mollusca: Cephalopoda). *Vie Milieu*, **35**, 273–282.
- Gabe, S. H. (1975) Reproduction in the giant octopus of the North Pacific, *Octopus dofleini martini*. *Veliger*, **18**, 146–150.
- Gowland, F. C., Boyle, P. R. and Noble, L. R. (2002) Morphological variation provides a method of estimating thermal niche in hatchlings of the squid *Loligo forbesi* (Mollusca: Cephalopoda). *J. Zool.*, **258**, 505–513.
- Hanlon, R. T., Forsythe, J. W. and Boletzky, S. V. (1985) Field and laboratory behavior of 'macrotritopus larvae' reared to *Octopus dofleini* Verany, 1851 (Mollusca: Cephalopoda). *Vie Milieu*, **35**, 237–242.
- Hochberg, F. G. (1998) Class Cephalopoda. In Scott, P. V. and Blake, J. A. (eds), *Taxonomic Atlas of the Benthic Fauna of Santa Barbara Basin and the Western Santa Barbara Channel*. The Mollusca Part I: The Aplousobranchia, Polyplacophora, Scaphopoda, Bivalvia, and Cephalopoda. Santa Barbara Museum of Natural History, Santa Barbara, California, 8, pp. 175–236.
- Hochberg, F. G., Nixon, M. and Toll, R. B. (1992) Octopoda. In: Sweeney, M. J., Roper, C. F. E., Mangold, K. M., Clarke, M. R. and Boletzky, S. V. (eds), *"Larval" and Juvenile Cephalopods: A Manual for Their Identification*. *Smithson. Contrib. Zool.*, **513**, 213–280.
- Iribarne, O. (1991) Life history and distribution of the small south-western Atlantic octopus, *Octopus tewelchus*. *J. Zool., Lond.*, **223**, 549–565.
- Itami, K., Izawa, Y., Maeda, S. *et al.* (1963) Notes on the laboratory culture of the octopus larvae. *Bull. Jpn. Soc. Sci. Fish.*, **29**, 514–520.
- Knauss, J. A. (1978) *Introduction to Physical Oceanography*. Prentice Hall, Englewood Cliffs, New Jersey.
- Kubodera, T. (1991) Distribution and abundance of the early life stages of octopus, *Octopus dofleini* Wülker, 1910 in the North Pacific. *Bull. Mar. Sci.*, **49**, 235–243.
- Marliave, J. B. (1981) Neustonic feeding in early larvae of *Octopus dofleini* (Wülker). *Veliger*, **23**, 350–351.
- Messenger, J. B. (1963) Behaviour of young *Octopus briareus* Robson. *Nature*, **197**, 1186–1187.
- O'Shea, S. (1999) Subfamily Octopodinae. In Gordon, D. P. (ed.), *The Marine Fauna of New Zealand: Octopoda (Mollusca: Cephalopoda)*. NIWA Biodiversity Memoir 112. National Institute of Water and Atmospheric Research, Wellington, pp. 114–191.
- Overath, H. and Boletzky, S. V. (1974) Laboratory observations on spawning and embryonic development of a blue-ringed octopus. *Mar. Biol.*, **27**, 333–337.
- Pecl, G. T., Steer, M. A. and Hodgson, K. E. (2004) The role of hatchling size in generating the intrinsic size-at-age variability of cephalopods: extending the Forsythe Hypothesis. *Mar. Freshw. Res.*, **55**, 387–394.
- Perez, J. A. A. and Haimovici, M. (1991) Sexual maturation and reproductive cycle of *Eledone massyae*, Voss 1964 (Cephalopoda: Octopodidae) in Southern Brazil. *Bull. Mar. Sci.*, **49**, 270–279.
- Perez, M. C., Lopez, D. A., Aguila, K. *et al.* (2006) Feeding and growth in captivity of the octopus *Enteroctopus megalocyathus* Gould, 1852. *Aquac. Res.*, **37**, 550–555.
- Prohaska, F. (1976) The climate of Argentina, Paraguay and Uruguay. In Schwerdtfeger, W. (ed.), *Climates of Central and South America*. World Survey of Climatology. Elsevier, Amsterdam, pp. 13–72.
- Ré, M. E. (1984) Maduración sexual en *Enteroctopus megalocyathus* (Cephalopoda, Octópoda). *Centro Nacional Patagónico, Consejo Nacional de Investigaciones Científicas Y Técnicas*, Contribución No. 93. Puerto Madryn, Argentina, p. 28.
- Ré, M. E. (1998a) Pulpos Octopódidos (Cephalopoda: Octopodidae). In Boschi, E. E. (ed.), *El Mar Argentino y sus Recursos Pesqueros. Tomo 2: Los moluscos de interés pesquero. Cultivos y estrategias reproductivas de bivalvos y equinoideos*. Publicaciones especiales INIDEP, Mar del Plata, Argentina, pp. 69–98.
- Ré, M. E. (1998b) Pesquerías de Pulpos. In Boschi, E. E. (ed.), *El Mar Argentino y sus Recursos Pesqueros. Tomo 2: Los moluscos de interés pesquero. Cultivos y estrategias reproductivas de bivalvos y equinoideos*. Publicaciones especiales INIDEP, Mar del Plata, Argentina, pp. 99–114.
- Rocha, F., Guerra, A., Prego, R. *et al.* (1999) Cephalopods paralarvae and upwelling conditions off Galician waters (NW Spain). *J. Plankton Res.*, **21**, 21–33.
- Rocha, F. and Vega, M. A. (2003) Overview of cephalopod fisheries in Chilean waters. *Fish. Res.*, **60**, 151–159.
- Rodhouse, P. G., Symon, C. and Hatfield, M. C. (1992) Early life cycle of cephalopods in relation to the major oceanographic features of the southwest Atlantic Ocean. *Mar. Ecol. Prog. Ser.*, **89**, 183–195.
- Sakaguchi, H., Araki, A. and Nakazono, A. (2002) Factors influencing body size of hatchlings, and relationships between fecundity and body weight of female, *Octopus vulgaris*. *Bull. Jpn. Soc. Fish. Oceanogr.*, **66**, 79–83.
- Sweeney, M. (2001) Current classification of recent Cephalopoda. <http://www.mnh.si.edu/cephs/newclass.pdf>.
- Sweeney, M. J., Roper, C. F. E., Mangold, K. M. *et al.* (eds) (1992) *"Larval" and Juvenile Cephalopods: A Manual for Their Identification*. *Smithson. Contrib. Zool.*, **513**, viii + 282 pp.
- Tranter, D. J. and Augustine, O. (1973) Observations on the life history of the blue-ringed octopus *Hapalochlaena maculosa*. *Mar. Biol.*, **18**, 115–128.
- Vega, M. A., Rocha, F. J. and Osorio, C. (2000) Variaciones espaciales y temporales de las paralarvas de cefalópodos en el Canal Moraleda (43° S), sur de Chile. *Cienc. Tecnol. Mar.*, **23**, 69–82.
- Vidal, E. A. G., DiMarco, P. F., Wormuth, J. H. *et al.* (2002) Influence of temperature and food availability on survival, growth and yolk utilization in hatchlings squid. *Bull. Mar. Sci.*, **71**, 915–931.
- Villanueva, R. (1995) Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to settlement. *Can. J. Fish. Aquat. Sci.*, **52**, 2639–2650.
- Villanueva, R., Collins, M., Sanchez, P. *et al.* (2002) Systematics, distribution and biology of the cirrate octopods of the genus *Opisthoteuthis* (Mollusca, Cephalopoda) in the Atlantic Ocean, with description of two new species. *Bull. Mar. Sci.*, **71**, 933–985.
- Villanueva, R. and Guerra, A. (1991) Food and prey detection in two deep-sea cephalopods: *Opisthoteuthis agassizi* and *O. vossi* (Octopoda: Cirrata). *Bull. Mar. Sci.*, **49**, 288–299.
- Villanueva, R., Nozais, C. and Boletzky, S. V. (1995) The planktonic life of octopuses. *Nature*, **377**, 107.
- Villanueva, R., Sánchez, P. and Compagno Roeleveld, M. A. (1991) *Octopus magnificus* (Cephalopoda: Octopodidae), a new species of large octopod from the southeastern Atlantic. *Bull. Mar. Sci.*, **49**, 39–56.
- Young, R. E., Hartman, R. F. and Hochberg, F. G. (1989) Octopodid paralarvae from Hawaiian waters. *Veliger*, **32**, 152–165.
- Zar, J. H. (1996) *Biostatistical Analysis*, 3rd edn. Prentice Hall, Upper Saddle River, New Jersey.