

Effects of competition and egg predation on shelter use by *Octopus tehuvelchus* females

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ABSTRACT: Parental care in the ocean ranges from provision to eggs and embryos to protection from predators. In particular, nest attendance has several benefits associated with relatively higher survival rates, lower vulnerability to predation and parasitism, and faster development rates. The quality of shelters matters, and adults of many species have to compete for suitable shelters. Here, we studied whether shelter type for brooding and egg protection from predation could be a determinant of competition among adults in the cephalopod *Octopus tehuvelchus*. To this end, we (1) assessed the abundance of different types of shelters and preference under field and laboratory conditions, (2) tested the effect of intraspecific competition for shelters, (3) estimated density of potential sources of embryo mortality, and (4) determined the role of female protection in egg survival against predators. We found that in areas where shelters for development are scarce, females compete with males for the most suitable shelters. Fecundity depends on the female size, and shelters with higher volume are preferably selected by females. Excluding the brooding female from shelters resulted in increased egg mortality due to predation by octopus males and removal by chitons. We suggest that, in this species, shelter availability poses a constraint to brooding and affects total parental investment in reproduction and predation risk on eggs. This constraint may also take place in other marine invertebrates with similar life history traits, especially in areas where shelter is limited.

KEY WORDS: Shelter use · Competition · Predation on eggs · *Octopus tehuvelchus*

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INTRODUCTION

The widespread use of parental care clearly shows the benefits of protecting embryos and juveniles, despite the costs to parents (Clutton-Brock 1991, Alcock 1998, Tallamy & Brown 1999, Kokko & Jennions 2008). Although parental care in the ocean is less frequent (Trumbo 1996, Lee & Strathmann 1998, Fernández et al. 2002, Mank et al. 2005), it ranges from provision to the embryos, e.g. oxygen provision through either passive (Lee & Strathmann 1998) or

active behaviors (Blumer 1982, Diesel 1989, Crawford & Balon 1996, Baeza & Fernández 2002, Fernández & Brante 2003, Brante et al. 2008), to protection from predators (Balon 1990, Crawford & Balon 1996). Guarding parents in aquatic systems keep the embryos safe from predators, keep oxygen levels high by fanning water currents, and keep the area free from dead embryos and debris (Crawford & Balon 1996, Fernández & Brante 2003, Summers et al. 2007). Parents can protect the embryos until they hatch and often look after the larval stages as well;

therefore, the time spent guarding can range from a few days to several months (Crawford & Balon 1996).

Among the enormous diversity of parental behaviors to enhance offspring survival, nest attendance is exhibited among different taxa and in different environments. Nest attendance involves provision of heat, water, or oxygen (particularly in harsh environments) or protection from predators (Clutton-Brock 1991, Behringer & Butler 2010). The benefits from nest attendance include (1) that related species with different developmental modes (planktotrophic versus direct development) show dramatic differences in early mortality rates (Rivest 1983, Pechenik et al. 1984, Chaparro et al. 1999, Moran 1999, Moran & Emler 2001, Marshall & Keough 2007, Marshall et al. 2008), (2) that embryonic survival increases depending on the nest used to protect the offspring (Elwood & Kennedy 1988, Asakura 1995, Katsanevakis & Verriopoulos 2004, Boyle & Rodhouse 2005, Faraj & Bez 2007, Leporati et al. 2009), (3) that unguarded eggs of parental species tend to be more vulnerable to predation and parasitism than eggs of nonparental species (Anderson 1972, Blumer 1982, Tallamy & Brown 1999, Taborsky & Foerster 2004), (4) that oxygen provision lowers mortality and improves developmental rates (Tinbergen 1951, Fernández et al. 2006, 2007, Brante et al. 2008), and (5) that mouth-brooding cichlid fishes produce larger eggs than substrate-spawning or nest-tending cichlid fishes, which have lower yolk reserves and indirect development (Crawford & Balon 1996).

The behaviors associated with the protection of the brood in marine invertebrates are less known and understood. Studies on different marine invertebrates show the importance of the availability and quality of shelter to fecundity and survival (Iribarne 1990, Ramirez Llodra 2002, Fernández et al. 2006). Moreover, adults of many species have to compete for suitable shelters (Mather 1982a, Iribarne 1990, Debuse et al. 2003). The consequences of nest competition on the cost and benefits of parental care are particularly interesting in exploited marine species, given its effects on population dynamics and catches.

Octopus tehuelchus (Octopodidae: Octopodinae) is a semelparous species that exhibits brood maternal care of eggs in shallow subtidal waters of northern Patagonia (Argentina) (Iribarne 1990). Females fix egg clutches to a hard substrate, usually a hole in the rock, empty shells, or artificial shelters, and protect the eggs for 3 or 4 mo, limiting other activities (Ré 1998). The shelters are hypothesized to be a limiting resource affecting fecundity and probably embryo survival (Iribarne 1990). This small octopus has been

targeted for >60 yr by an artisanal fishery that targets mostly females hidden in shelters in the rocky shore (Narvarte et al. 2006). The main goal of our study was to evaluate the role of shelters and female protection in embryo survival in this species and thus provide data for the management plans in marine protected areas as well as in the larger region where the fishery operates. To meet this goal, we (1) assessed the abundance of different types of shelters and preference under field and laboratory conditions, (2) tested the effect of intraspecific competition for shelters, (3) estimated the density of potential sources of embryo mortality in the field, and (4) evaluated the role of female protection in embryo survival through testing predation of unguarded eggs.

MATERIALS AND METHODS

Experimental species and study site

Octopus tehuelchus is a small-sized species (<150 g) with a short life span (<3 yr; Iribarne 1991, Storero et al. 2010). It inhabits rocky substrates from the low intertidal to the shallow subtidal zone between 20° and 40° S in the southwestern Atlantic Ocean. In its southern distribution limit, females of ~20 g are sexually mature and lay benthic eggs mainly during spring and summer (Ré 1998, Storero et al. 2010). The eggs are laid in clusters attached to rocky platforms and boulders in rocky areas and on hard substrates, such as shells, in sandy bottoms. The egg wall is fairly transparent, turning gray as the embryo develops (Pujals 1986). After ~3 or 4 mo of development, crawl-away juveniles (total length: 10 to 14 mm; total weight: 0.05 to 0.14 g) emerge and recruit to the population (Storero 2010). According to Iribarne (1990), (1) shelters on sandy bottoms consist mostly of shells of invertebrates; (2) small octopuses use shells of gastropod, barnacles, or clams, intermediate-size octopuses use mainly oyster shells, and large octopuses use a variety of shelters made up of parts of shells or other sources; (3) shells of the oyster *Ostrea puelchana* are the most important shelter for brooding females, with most eggs placed on the concave valve; and (4) the quality of the shelter affects the reproductive output, particularly that of larger females; in fact, the number of eggs per clutch is correlated with shelter volume and female size.

Samples and field experiments were conducted respectively in Banco Reparo and Banco Víbora, San Antonio Bay (40° 42' 30.74" S, 64° 58' 46.53" W; Fig. 1), a multi-use marine protected area (i.e. arti-

sanal fisheries, small industries, and tourism are permitted) since 1993 where sandy bottom dominates and natural shelters are scarce (Iribarne 1990). Nevertheless, octopus abundance is high throughout the year (Millán 2007). The mean annual seawater temperature ranges from 6 to 26°C (Bas et al. 2005).

Shelter availability and preference

To assess the availability of shelters in nature, 9 line transects (100 m long, 1 m wide, 20 m apart) were carried out in summer months (January and February) 1996. This sampling was performed 8 yr after that of Iribarne (1990), and it was important to verify whether changes in the availability or quality of shelters were significant. All potential shelters and sheltered octopuses were collected along transects. Potential shelters were defined as those of minimal size or volume used by any octopus in the field, i.e. shells of gastropods, bivalves, or barnacles. Previous observations indicate that minimal size is 54 mm of shell height for bivalves and that minimal volume is 13 ml for gastropods and barnacles. Octopuses with their respective shelters were individually placed in small bags. In the laboratory, the shelters were identified by origin (biological and others) and taxonomic group (e.g. gastropod, bivalve, or barnacles). Shelter volumes (ml) were calculated (measuring the volume of the water that each shelter could retain), and when occupied, the octopus, weight and sex were recorded. When females were brooding, the number of eggs and their developmental stage (only to verify if clutches were already completely deposited) were also recorded.

For each shelter type (clams, flat oysters, other mollusks, or others), the proportion of occupied and available shelters was estimated in each transect. Considering the results obtained by Iribarne (1990), who observed that the main shelter used was the flat oyster and the fecundity was highest in this shelter, we tested the response of the octopus when other shelters of 'high quality' (see Iribarne 1990) were available. Thus, we conducted field experiments to assess octopus preference for different types of shelter and to compare the octopus weights among shelter types. Shelters of biological origin were collected ~10 km from the study area. A total of 207 shell shelters (69 clams *Protothaca antiqua*, 69 oyster *Ostrea puelchana*, and 69 barnacles *Balanus*

psittacus) were arranged along 3 experimental long-lines. As we ignored the home range of the octopus, we distributed these long-lines in habitats with known high octopus density (Iribarne 1990) and that had low availability of natural shelters. To match the density of available shelters to those in natural conditions, existing shelters next to the long-lines were completely removed. In the case of bivalves, 'cluckers' (shells still joined by their ligament) or both valves arranged as cluckers were used. The 3 types of shelters were systematically deployed along each long-line at the study site, assigning 69 shelters (23 of each type) per transect. The volumes (means \pm SD) of these shelters were not significantly different among types (barnacle: 45.7 ± 11.7 ml; clam: 47.6 ± 3.3 ml; oyster: 42 ± 2.3 ml; ANOVA, $F_{2,206} = 0.83$, $p > 0.05$). After 1 wk, the number of occupied shelters and the octopus weight, sex, and female condition (brooding and non-brooding) were recorded. Preferences for the 3 types of shelter, by male, female, and brooding female, were evaluated with chi-squared tests. In addition, mean octopus weight was compared between shelter types and sexes using a 2-way ANOVA.

Shelter competition between sexes

To test the outcome of competition between sexes in captivity, we used males and females of similar weight (differences in weight < 10 g) collected in nature. Octopuses were kept in captivity individually for 24 h before the test and for 24 h together during the experimental trials. After the trials, octopuses were sacrificed. All lab experiments were conducted in transparent aquaria (50 \times 50 \times 30 cm high), aerated

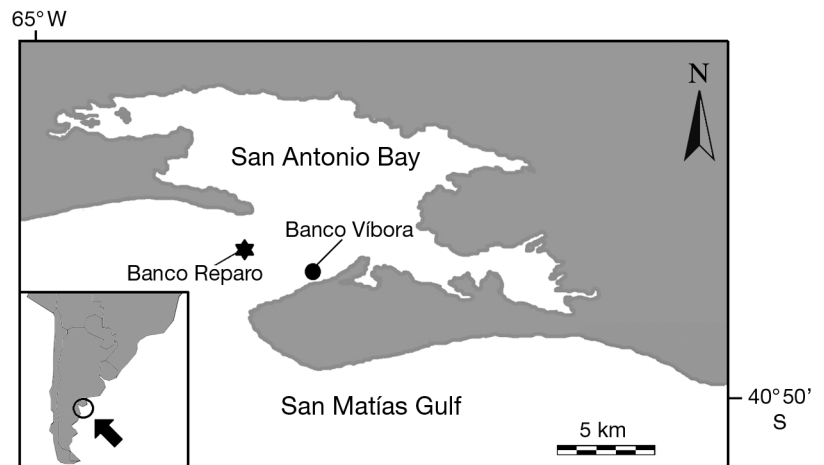


Fig. 1. San Antonio Bay (Patagonia, Argentina) showing the experimental sites Banco Reparó and Banco Víbora

and illuminated (photoperiod 14 h light: 10 h dark; 36 W fluorescent tube). Temperature and salinity were similar to those in the natural habitat (22°C and 36 to 37), and water was changed daily. Each aquarium was covered with uniform sand level (5 cm) on the bottom and filled with clean sea water. In each case, the shelter used was that preferred by octopuses as determined in the previous lab experiment. Twelve replicates were used. The lack of difference in the number of winning events per sex was assessed using a chi-squared test.

Sources of egg removal in the field

Preliminary results suggested that chitons *Chae-topleura isabellei* (Placophora) could remove eggs from the shelters when feeding on the microalgae attached to hard substrates. Additionally, cannibalism has also been reported for *Octopus tehuetchus* (Ibáñez & Keyl 2010). Therefore, we recorded the densities of octopus, chitons, and other macrofauna potentially harmful for egg clutches (considering those that could prey upon or remove eggs; see Appendix 1). A total of 8 of the 9 transects were surveyed to estimate the mean density of species that could either prey upon (including cannibalism) or remove eggs from the shelter. For these estimates, only the organisms attached to valves or natural shelters were considered. These data were used to perform laboratory experiments on egg removal.

Assessment of potential egg removal in the laboratory and female protection

Preliminary observations of shelters with egg clutches in aquaria allowed us to determine that after 4 d, egg-loss by natural detachment of stalks was negligible (mean percentage \pm SD = 0.51 ± 0.13 , $n = 8$). Thus, we evaluated detachment of eggs due to cannibalism or removal by chitons (as no other predators or egg removers were noted). To evaluate whether the presence of the brooding female diminished the rate of egg removal by 'defending' the shelter, we evaluated 2 potential sources of removal: (1) egg removal by adult conspecifics and (2) egg removal by chitons. Shelters (mainly bivalve cluckers) containing eggs were collected in the field. In each assay, 1 shelter with eggs (48.1 ± 19.5 ml, $n = 35$) was placed in the aquaria. The number of eggs in the shelter was counted at the beginning (Egg_{beg}) and at the end of the experiment (Egg_{end} , 72 h after), and the

rate of egg removal was calculated as $(Egg_{\text{beg}} - Egg_{\text{end}}) \cdot Egg_{\text{beg}}^{-1}$.

Differences in the egg-removal rate by octopuses depending on the presence of the brooding female and the sex of the cannibal octopus were evaluated with a 2-way ANOVA. Also, differences in the egg-removal rates were compared between sexes and sizes of the cannibal octopus (similar to, smaller, or larger than the brooding female) using a 2-way ANOVA. In this last case, we considered a difference of 25 ± 3 g (mean \pm SD) for the 'smaller or larger' trait and of 4 ± 1 g for the 'similar' trait.

Removal of eggs by chitons was also evaluated under laboratory conditions. For experiments, chitons of uniform lengths (mean \pm SD = 3.68 ± 1.06 cm, $n = 30$), and similar to the sizes observed in the natural environment, were used. Differences in the egg-removal rate depending on the presence of the brooding female and the number of chitons (1 or 2) were evaluated using ANOVA. Eight replicates per treatment were considered.

All removal rates were arcsine-transformed to meet ANOVA assumptions.

RESULTS

Shelter availability and preferential use

A total of 33 shelters along transects were occupied by octopuses. Mollusk shells were the most common shelters selected by octopus, with oyster cluckers being the most important ones (mean \pm SD = $51.5 \pm 12.2\%$), followed by clam cluckers ($24.2 \pm 7.3\%$) and other bivalve shells (*Atrina seminuda* or *Aequipecten tehuetchus*, $12.1 \pm 2.5\%$). Gastropod and barnacle shells and crevices in rocks completely covered by *Corallina* sp. and Bryozoa constituted the remaining $15.2 \pm 4.4\%$ of the occupied shelters. In total, 50% of the mollusk shells available were empty. Males were 2-fold more abundant than females in the occupied shelters, and 76.7% of the bivalve shelters were occupied by brooding females and males (both immature and mature) (Fig. 2). Brooding females occupied exclusively oyster cluckers.

The random sample of occupied shelters yielded similar results: 66% of the shelters with brooding females were oyster cluckers, and 33% were broken penshell (*Atrina seminuda*) cluckers. Eggs were evenly distributed between the 2 valves in penshells ($\chi^2_{(4)} = 1.38$; $p > 0.05$), but a clear preference for concave valves was observed in octopuses occupying the

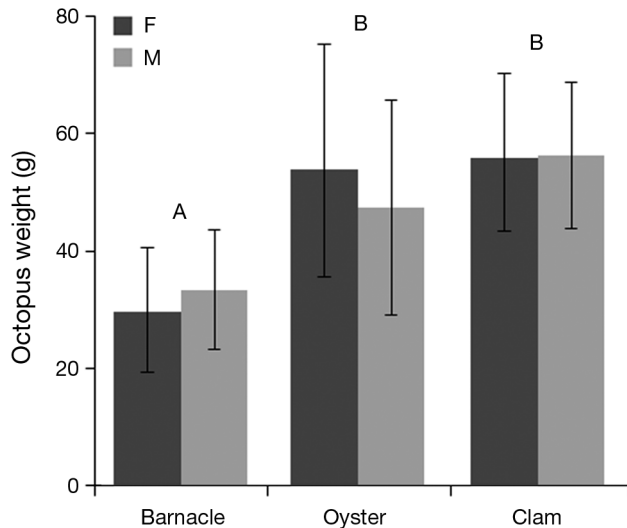


Fig. 2. Weight (mean \pm 1 SD) of octopus females (F) and males (M) in different types of shelter (barnacle, $n = 32$; oyster, $n = 18$; clam, $n = 62$) in the shelter selectivity experiment performed in the field. Different letters indicate significant differences ($p < 0.05$)

oyster shells ($\chi^2_{(19)} = 253.22$; $p < 0.05$). In the latter, $84.42 \pm 7.12\%$ ($n = 19$) of eggs were laid on the concave valve.

The volume of the occupied shelters in the field (i.e. naturally occurring shells) varied significantly between sexes (ANOVA, $F_{2,46} = 9.49$; $p < 0.001$). Males occupied the smallest shelters (20.05 ± 3.86 ml), while adult and brooding females occupied the largest ones (26.22 ± 4.06 ml and 29.42 ± 4.54 ml, respectively). The smallest shelter containing eggs was an oyster clucker of 22 ml.

Shelter selectivity

A total of 112 of the 207 shelters offered were found occupied by octopuses after 1 wk (59 by males and 53 by females). Because differences in the proportion of shelters occupied were low between long-lines, we could pool all of the data. Shelter selection was independent of the sex ($\chi^2_{(2)} = 1.87$; $p > 0.05$), and differences in the type of shelter selected were significant ($\chi^2_{(2)} = 27.07$; $p < 0.05$). The most preferred shelters were clam shells (55.4%), followed by barnacle (28.9%) and oyster (16.1%) shells. Brooding females were observed only in barnacle and clam shells, and the latter were differentially selected ($\chi^2_{(2)} = 12.82$, $p < 0.05$). The weight of octopuses sheltered in barnacle shells was significantly lower than that of octopuses sheltered in oysters and clams (ANOVA, $F_{2,111}$,

$p < 0.0001$, Fig. 2) regardless of the sex (ANOVA, $F_{1,111} = 0.06$, $p = 0.801$). The interaction was not significant ($F_{2,111} = 0.79$, $p = 0.4575$).

Shelter competition between sexes

The experiment of competition on shelters between males and females (of similar weights) showed that females won the shelter in most of the cases ($\chi^2_{(1,11)} = 5.34$; $p < 0.05$; Fig. 3) regardless of the shelter type. When the shelter was an oyster clucker, females won in 100% of the events.

Sources of egg removal and the role of female protection

Among the species that may remove eggs, *Tegula patagonica* showed the highest density (19.2 ± 8.1 ind. 100 m^{-2}) followed by *Chaetopleura isabellei* (10.7 ± 3.9 ind. 100 m^{-2}), *Octopus tehueltchus* (4 ± 1.1 ind. 100 m^{-2}), *Fissurellidea hiantula* (0.3 ± 0.5 ind. 100 m^{-2}), and *Crepidula* sp. (0.8 ± 1.3 ind. 100 m^{-2}), all means \pm SD. From the 2 most abundant chitons, only *C. isabellei* were observed inside egg-containing shelters at densities ranging from 1 to 2 ind. (3 chitons were observed only once).

Removal by conspecifics

The egg-removal rate showed significant differences depending on the presence of the brooding female (ANOVA, $F_{1,65} = 8.98$, $p = 0.004$) and the sex

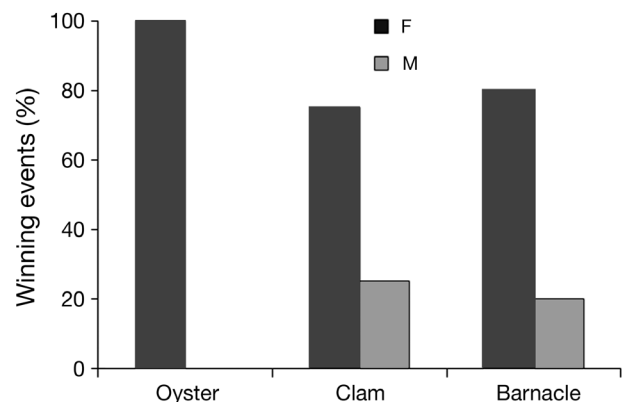


Fig. 3. Shelter competition between sexes for 3 kinds of shell. Individual weights of males (M) and females (F) for each shelter type were similar (oyster: F 62.4 ± 6.0 g, M 56.6 ± 1.4 g; clam: F 60.4 ± 9.2 g, M 59.0 ± 9.9 g; barnacle: F 18.8 ± 4.5 g, M 20.8 ± 6.7 g)

of the cannibal octopus (ANOVA, $F_{1,65} = 16.89$, $p < 0.001$). In this case, the interaction was significant ($F_{1,65} = 8.03$, $p = 0.006$); only the egg-removal rate by males when the brooding female was absent was relevant (Fig. 4). The egg-removal rates were near zero when the brooding female was present, both in the comparison between sexes and between females of different size. These differences were not significant (ANOVA, $F_{1,60} = 1.90$, $p = 0.170$).

Egg removal by chitons

The egg-removal rate by chitons showed significant differences depending on the presence of the brooding female (ANOVA, $F_{1,14} = 9.58$, $p = 0.010$, Fig. 5). However, this result should be interpreted cautiously, given that the power of the performed test

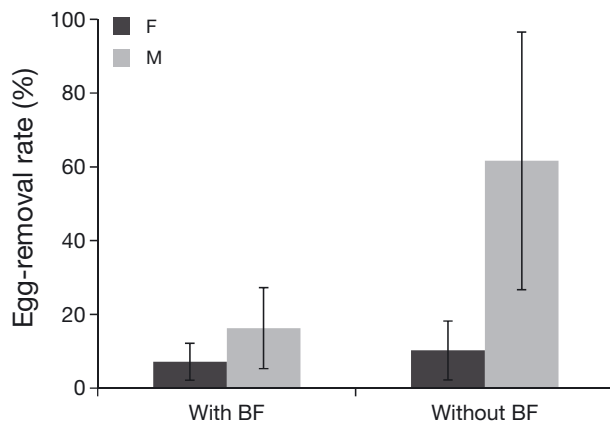


Fig. 4. Egg-removal rates (mean percent ± 1 SD) observed in laboratory experiments by sexes (M: males, F: females), in the presence (with) or absence (without) of the brooding female (BF)

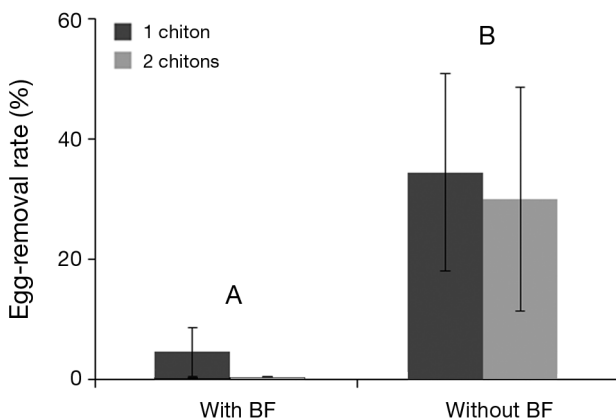


Fig. 5. Egg-removal rate (percent ± 1 SD) by 1 or 2 ind. chitons of *Chaetopleura isabellei* in the presence (with) or absence (without) of the brooding female (BF) within the shelter. Different letters indicate significant differences ($p < 0.05$)

(0.52) is below the desired minimum power of 0.80 (e.g. Steidl et al. 1997). Indeed, the egg-removal rate by chitons shows a trend (Fig. 5) with lower values in presence of the brooding female. The removal rate (%) was low ($8.0 \pm 4.0\%$, $n = 8$) when the brooding female was present and increased to $58.2 \pm 10.1\%$ ($n = 8$) when the brooding female was absent. There was no difference in the egg-removal rate between 1 or 2 chitons (ANOVA $F_{1,14} = 0.39$, $p = 0.547$; Fig. 5). The interaction was not significant ($F_{1,14} = 0.06$, $p = 0.808$).

DISCUSSION

Our results can be divided into 3 categories: (1) evidence of shelter limitations (quality and volume) in sandy bottoms, (2) evidence that shelter competition exists and that the most suitable shelters are predominantly occupied by females, and (3) the importance of the brooding female in defending the shelter (and consequently eggs) against predators and enemies.

Shelter limitations in sandy bottoms

The use of a shelter for the attachment of eggs is key to the ecology of several coastal cephalopods, which seek secluded locations for spawning (Boyle & Rodhouse 2005). For some species, the scarcity of suitable shelters for egg deposition may be an important limitation for the breeding female population (Boyle 1980, 1990, Iribarne 1990, Anderson 1997). In our experiments of shelter selectivity, large octopuses (of both sexes) selected clam and oyster shells, while small octopuses selected mainly barnacle shells. As we discard the hypothesis that there may be more small barnacles (the barnacle density in the field census was quite low), there are 2 possible explanations: (1) barnacle shells may offer a better protective function for juveniles, or (2) bivalves are occupied first by large octopuses, and thus, juveniles can only select the small, less adequate, remaining shelters. It seems that competition may be relevant at adult stages, which may have less protection in lower-quality shelters for brooding their eggs. Previous research with octopuses has shown a clear positive relationship between shelter volume and octopus size (Hartwick et al. 1984, Iribarne 1990, Oosthuizen & Smale 2003). In our study, when shelters of higher volume (as empty clam shells taken from neighboring areas) were offered, the larger females preferred them. Shelter availability has been

mentioned to be a limiting factor for octopus distribution, and several studies in different octopus species have described shelter choice and suggested some characteristics used in the selection of hiding places (Altman 1967, Hartwick & Thorarinsson 1978, Mather 1982b, Forsythe & Hanlon 1997, Katsanevakis & Verriopoulos 2004).

Understanding shelter selection by different life-history stages of marine invertebrates could contribute to our understanding of the ecological costs and benefits involved in this particular strategy. Iribarne (1990) showed that the mean number of eggs per shelter was related to the size of the female, with the females in turn selecting shelters according to their volume, and the fecundity of *Octopus tehuelchus* was higher in high-quality (large-volume) shelters. Then, the close relationship between shelter type and octopus size could be explained in terms of shelter size and volume preference. Our results confirm the importance of large and secure shelters mainly for females. Oosthuizen & Smale (2003) found that small and large *Octopus vulgaris* show different behaviors: while small ones generally occupy holes sunk perpendicular into the substrate, larger ones excavate shelters beneath rocks and ledge. Alterations in shelter, such as removing rock and sand and bringing items to block the aperture, seem to be important for octopus females (Mather 1994, Anderson 1997). The fact that adult octopuses, both males and brooding females, selected all the available good shelters (bivalve) and occupied a small fraction of other available non-bivalve shelters (Iribarne 1990, present study) corroborates the idea that good shelters are limiting for *O. tehuelchus*. Many authors have suggested that shells are limiting resources, which restricts the occupancy of other invertebrate populations besides cephalopods (Vance 1972, Fotheringham 1976, Kellogg 1976). The evidence available indicates that (1) empty suitable shelters are rarely found in locations inhabited by those species (Provenzano 1960), (2) empty shelters added to sites are readily inhabited by those species, increasing the population density (Vance 1972, Wicksten 1977, Blackstone & Joslyn 1984), and (3) those species have evolved specialized behaviors that enable them to choose and compete for shelters (Hazlett 1966). In agreement with the results of Beck (1998), our results indicate that large individuals are the most likely size class to be shelter-limited. Potential behavioral artifacts that could confound interpretation of the results from this experimental design include ontogenetic changes in preferred shelter types, and some shelters considered available (ana-

lyzed by us in the laboratory) may have not been so in the field for octopus use, in which case occupancy rates may be underestimated.

Shelter competition and occupation of most suitable shelters by females

If octopuses of both sexes and similar sizes compete for the same shelter, females win the shelter. This may be related to the reproductive stage of each particular female, as observed in other species for which clumps of 1 female and >3 males were described (Hanlon and Messenger 1996). Contest over the occupancy of a shelter has also been observed in other octopus species (*Octopus dofleini*, Hartwick et al. 1984; *O. briareus*, Aronson 1986), and it is possible that these species exhibit a degree of individual preference in shelter selection (Cigliano 1993, Mather 1994). In the case of *O. tehuelchus*, considering that females use the shelter to brood and care for the offspring, the use of the same shelter may last >2 mo. Thus, it is expected that octopuses in San Antonio Bay face shelter limitations and compete for the same shelter in a more important way during the reproductive period. During the brooding period, females do not feed, but when they are not brooding eggs, they may be more active searching for food and should change shelter more often. Experiments on pairs of male *O. vulgaris* in which 2 shelters were provided have shown that usually one was preferred and was most likely occupied by the larger animal (Boyle 1980). A size-biased dominance hierarchy in the use of space has been described in other octopus (Mather 1980) and *Sepia* species (Adamo et al. 2006), with similar results.

Importance of the brooding female in defending the shelter (and consequently eggs)

Parental care in marine invertebrates includes a behavior that enables a parent (the male or the female) to promote offspring survival by ameliorating conditions that are harmful to offspring. Our results suggest not only that the ventilation and aeration activity of the female on the eggs is essential for their survival (Hanlon & Messenger 1996) but also that the presence of the female 'defending' the eggs against predators or removal is highly important. In the laboratory, when the brooding female was absent, egg-removal rates by males were ~60% and those by chitons were ~40%. In contrast, removal rates by any

octopus sex were negligible when the brooding female was in the shelter. Moreover, removal by chitons was practically null when the female was present and increased significantly in the absence of the brooding female (however, in this last case, the power analysis was below the minimum desirable power of 0.80; see Fig. 5). Chitons remove eggs while feeding on the microalgae within the shelter, and do not predate on eggs (the eggs were found free on the bottom of aquaria). Thus, 1 or 2 chitons caused similar effects. In nature, chitons may change more often from one shelter to another and to natural pebbles, and consequently, the effect may be less important; however, the removal of eggs may have important consequences on embryo survival if females are absent. Also, if chitons spatially concentrate at high densities in a suitable habitat in nature, their removal effect should be considerable. Indeed, other factors, which were not the objective of the present study, such as oxygen deprivation, siltation, or predation by other organisms not counted in the transects (e.g. carnivorous crabs, fishes, or gastropods) would also have an effect on egg survival rates.

Octopus tehuetchus cannibalism is important during the summer and autumn, when adults prey on eggs and juveniles (Ré & Gómez-Simes 1992). An extraordinary case of cannibalism is that of *O. maorum* in Tasmania, in which cannibalized small conspecifics and egg clutches (Anderson 1997) are one of the most important components of the diet and have the highest weight percentage of all of the prey items (Ibáñez & Chong 2008, Ibáñez & Keyl 2010). Similarly, *O. mimus* (Cortez et al. 1995, Grubert & Wadley 1999) feeds on juveniles, and females cannibalize their eggs (i.e. filial cannibalism) during brood care. It has been assumed that the reason for this behavior is that they eat nonviable or sick eggs to prevent further infections, as observed in *Bathypolypus arcticus* (Wood et al. 1998). Due to the monocylic life history of most cephalopods, this type of cannibalism seems to occur only in the case of brood-caring species of octopuses and polycyclic nautilus (Arnold & Carlson 1986, Ré & Gómez-Simes 1992, Cortez et al. 1995, Wood et al. 1998, Grubert & Wadley 1999, Ibáñez & Chong 2008).

Cannibalism on eggs by females was null while that by males was important (sometimes up to 90%), suggesting that competition over one particular good-quality shelter may exist between sexes more intensely than among individuals of the same sex. In fact, our experiments of shelter competition showed that females always won the shelter. Probably, the presence of eggs within the shelter represents a risk

to a male occupying it (i.e. that the female is returning soon), and the only alternative for males is to predate on the eggs to diminish this risk, similarly to the process described for guarding fishes (see the review by Magnhagen 1992) or birds (Arnold et al. 2012). An alternative explanation is that eggs are simply available food, and males take advantage of it. Since the female never leaves the shelter after having deposited eggs (Hanlon & Messenger 1996), chances to find good-quality empty shelters during the reproductive season are low. However, fishing, if performed using lines of shelter traps, could act as an additional source of offspring mortality by removing brooding females and triggering cannibalism. Finally, because better-quality shelters are supplied through natural mortality of source species (dead bivalves), an additional effect of fisheries on octopus abundance is the bivalve exploitation in sandy habitats, which may contribute to the decrease in shelter availability. In addition, habitat deterioration by the growing human population could contribute to the shelter depletion in coastal areas.

The contribution of parental care to the viability of offspring has been studied in a wide range of taxonomic groups (Charrassin et al. 1998, Eggert et al. 1998, Aubret et al. 2005, Cockburn 2006, Kokko & Jennions 2008). Brooding behavior has been widely observed among marine invertebrates, such as amphipods (Dick et al. 2002, Thiel 2003), crabs (Fernández et al. 2000), oysters (Ó Foighil & Taylor 2000) sea stars (Strathmann et al. 1984, Bosch & Slattery 1999, Byrne 2005), and polychaetes (Sella 1991, Sella & Lorenzi 2000). In general, brooding behavior in marine invertebrates has been associated with adverse environmental conditions, such as low oxygen availability within the embryo mass (Baeza & Fernández 2002, Lardies & Fernández 2002, Brante et al. 2008), although protection of offspring from predation, such as observed in *O. tehuetchus*, is arguably one of the strongest drivers for the evolution of parental care (Aoki & Kikuchi 1991, Kutschera & Wirtz 2001, Thiel 2003, Taborsky & Foerster 2004).

Our results confirm the importance of incorporating shelter selectivity for development as a key factor to identify trade-offs in the life histories of marine invertebrates. Shelter availability poses a constraint to brooding among different taxa of marine invertebrates and has an effect on total parental investment in reproduction (Fernández et al. 2000), on parental investment per offspring (Lee & Strathmann 1998), and on egg predation risk (our study). The effect on egg predation risk may be related to a compromise between the capacity of adults to invest in searching

for appropriate shelters and providing oxygen to the brood (Lardies & Fernández 2002), which may reduce the egg predation risk. Thus, the availability of adequate shelters for brooding and further development could be one of the critical factors that cause variations in the fitness of brooding species. In this sense, habitat deterioration is one of the threats against shelter availability for brooding. Bivalve fisheries, which is usually removing shells as biogenic shelters, would also have an effect on discounting good-quality shelters.

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Appendix 1. Possible egg predators or removers found along transects in Banco Reparó (San Antonio Bay, Patagonia, Argentina)

Table A1. Density (means \pm SD) of potential egg predators or removers observed along the studied transects

Group/species	Density \pm SD (ind. 100 m ⁻²)
Mollusca, Gastropoda	
<i>Buccinanops globulosum</i>	1.5 \pm 0.7
<i>Buccinanops cochlidium</i>	7.5 \pm 3.5
<i>Tegula patagonica</i>	19.2 \pm 8.0
<i>Fissurellidea hiantula</i>	0.3 \pm 0.6
<i>Crepidula</i> spp.	0.8 \pm 1.3
Crustacea, Brachyura	
<i>Rochinia gracilipes</i>	3.5 \pm 0.7
<i>Coristoides chilensis</i>	1.3 \pm 0.5
<i>Peltarion spinosulum</i>	1.2 \pm 0.5