Christian Drerup

Habitat Preference and Behavioural Ecology of Bobtail Squids (Sepiolidae)

UNIVERSIDADE DO ALGARVE
FACULDADE DE CIÊNCIAS E TECNOLOGIA
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Mestrado em Biologia Marinha

Trabalho efetuado sob a orientação de:

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Declaração de autoria de trabalho

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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ABSTRACT

Bobtail squids (Sepiolidae, Cephalopoda) have recently become popular in scientific studies as model organisms due to their symbiotic relationship with light producing bacteria. However, the overall knowledge on the behaviour of sepiolids is based on observations on just a few of the roughly 70 extant species and must still be considered as sparsely. As understanding their behavioural ecology is vital for establishing a good welfare when holding sepiolids in captivity, the present thesis aimed at improving the knowledge on the ecology of these cephalopods. In a first study, several behavioural aspects of the so far less-investigated bobtail squid species *Sepiola parva* were analysed, showing that the behavioural ecology of this sepiolid greatly resembled the observations reported for other bobtail squids. Furthermore, this study did not only present the first detailed information for sepiolids about activity and time budgets as well as the positioning towards the prey and the tentacle speed during hunting events; it also provided the first evidence for the ability to adhere a 'sand coat' in the genus *Sepiola* and the use of 'ink ropes' for sepiolids in general. In a subsequent study, the burying behaviour of *Sepiola* sp. was analysed with regards to the effect of different sediment types. It was shown that the mean grain size played a major role in both the duration and the number of body movements (funnel jets/ arm sweeps) in different burying characteristics. While the latency until the start of burying, the duration and number of funnel jets of phase 1 and the total burying duration was the shortest/lowest on medium grained sediment samples and correspondingly extended on finer and coarser sediment samples, the duration of phase 2 and the number of arm sweeps within phase 2 was the longest/highest on coarser sediment and decreased the finer the sediment was.

Keywords: Behavioural ecology, Bobtail squid, Burying behaviour, Cephalopod, *Sepiola*

RESUMO

Nas últimas décadas, algumas espécies de cefalópodes têm sido estudadas devido ao seu potencial enquanto novas espécies para aquacultura mas também devido aos seus repertórios comportamentais notáveis, pigmentação cutânea neuralmente controlada e habilidades cognitivas complexas. Entre estas encontram-se cefalópodes de maior porte e comercialmente valiosos, como polvos (Octopoda), lulas (Teuthida) e chocos (Sepiidae). No entanto, não existe muita informação acerca de outros géneros e da maioria das outras famílias de cefalópodes. Esta lacuna na literatura incide principalmente sobre a sua ecologia comportamental e deve-se essencialmente ao papel menor desempenhado enquanto espécies-modelo para investigação científica até ao presente. Neste grupo incluem-se as lulas bobtail (Sepiolidae, Cephalopoda), uma família de cefalópodes com distribuição global que inclui cerca de 70 espécies com valor comercial baixo ou nulo. Recentemente, o interesse em utilizar espécies desta família como organismos modelo tornou-se mais popular devido à relação simbiótica que possuem com bactérias que emitem luminescência e os órgãos emissores da mesma. No entanto, o conhecimento geral sobre o comportamento desta família é baseado em observações de apenas algumas espécies e deve ser considerado escasso. Devido à recente inclusão da classe Cephalopoda na Diretiva Europeia de bem-estar animal 2010/63/EU, sobre a proteção de animais utilizados para fins científicos, entender a sua ecologia comportamental não é apenas necessário para compreender os complexos padrões comportamentais dos cefalópodes, mas também é vital para assegurar um nível de bem-estar animal quando se mantêm estas lulas em cativeiro. Nesse sentido, a presente tese teve como objetivo ampliar o conhecimento sobre a ecologia comportamental desta família de cefalópodes.

Um primeiro estudo foi realizado no Instituto de Ciência e Tecnologia de Okinawa no Japão, no qual vários aspectos comportamentais de *Sepiola parva* foram estudados. Para tal foram capturados indivíduos selvagens e registrado o seu comportamento em cativeiro por um total de 41 dias e noites consecutivos. Adicionalmente, foram registados detalhes de seu comportamento de enterrar e caçar através de filmagens em tanques separados. Foi demonstrado que o comportamento de enterrar, caçar e acasalar, bem como as respostas de fuga desta espécie se assemelhavam muito a observações documentadas em outras lulas bobtail. No entanto, foram encontradas diferenças para outros géneros ou até mesmo para outras espécies do mesmo género principalmente no comportamento relacionado com o acasalamento. Ao contrário do que foi relatado para outras espécies, todo o ato de acasalamento de *S. parva* ocorre

na coluna de água e nem o macho nem a fêmea tocam o fundo em nenhum momento durante a cópula. Além do mais, este estudo não forneceu apenas as primeiras informações detalhadas para sepiólidos no que concerne orçamentos de atividades e de tempo, bem como de posicionamento em relação à presa e velocidade do tentáculo durante os eventos de caça; também contribuiu com novo conhecimento para o género *Sepiola* ou lulas bobtail de um modo geral. Demonstrou-se que *S. parva* é capaz de aderir uma camada de grãos de areia ao manto ('revestimento de areia'), característica comportamental até agora relatada apenas para sepiólidos do género *Euprymna*. Além disso, observou-se que *S. parva* ejeta uma 'corda de tinta', aproximadamente 4-5 vezes o comprimento do indivíduo, mantendo-o imóvel e uma coloração de corpo escura. Este tipo de comportamento foi apenas descrito até agora em lulas de profundidade, e pressupõe-se que o objetivo das 'cordas de tinta' de *S. parva* é o de mimetizar as folhas em forma de lâminas flutuantes de ervas marinhas, como parte de seu comportamento críptico.

Um estudo subsequente, realizado na estação de trabalho de campo da empresa HYDRA (Elba, Itália), foi analisado o efeito de sete amostras diferentes de sedimentos, com propriedades geológicas variadas, no comportamento de enterrar de lulas bobtail. Quatro dessas amostras de sedimentos foram recolhidas em baías onde existem lulas de cauda cortada com abundância, enquanto as três restantes foram preparadas artificialmente através da adição de partições de sedimentos do mesmo tamanho. Embora os indivíduos utilizados neste estudo possam ser identificados como *Sepiola* sp., devido à presença de órgãos de luz em forma de rim dentro de sua cavidade do manto; nem uma abordagem morfológica ou genética permitiram a identificação exata destas lulas, o que leva à assumpção que estas possam presumivelmente representar uma nova espécie, até agora não descrita. No sentido de se testar possíveis diferenças no procedimento de enterrar das lulas bobtail, relacionadas com as propriedades das amostras de sedimento utilizadas, o comportamento de vários indivíduos de *Sepiola* sp. foi filmado em laboratório e posteriormente analisado quanto à duração e execução de diferentes características de enterrar. Embora todos os indivíduos testados tenham realizado o mesmo comportamento de enterrar em duas fases em todas as amostras de sedimentos, não foi encontrada correlação entre o tamanho de *Sepiola* sp. e seu correspondente comportamento de enterrar. No entanto, os resultados obtidos demonstraram que o tamanho médio do grão afeta muito o comportamento de enterrar das lulas bobtail, pois a duração ou o número de movimentos do corpo (jatos de funil / varreduras de braço) por fase de enterrar foi significativamente alterado pelo tamanho médio do grão do sedimento usado. Foi demonstrado que a latência até o início do enterro, a duração da primeira fase, o número de jatos de funil

durante a primeira fase e a duração total do enterro foram os mais curtos / mais baixos nas amostras de sedimentos de grão médio e estendidos correspondentemente nos mais finos e amostras de sedimentos mais grossas. Por outro lado, a duração da segunda fase do comportamento de enterrar, bem como o número de varreduras de braço na segunda fase, foram os mais longos / mais altos nos sedimentos mais grossos e diminuíram quanto mais finos os sedimentos. Como todas as lulas bobtail observadas realizaram a mesma técnica de enterrar, independentemente do tamanho médio dos grãos da amostra de sedimentos e da duração ou número correspondente de movimentos corporais, o comportamento de enterrar de *Sepiola* sp. pode ser considerado como um padrão comportamental relativamente fixo, exibindo uma característica incomum para cefalópodes que são conhecidos por sua imensa flexibilidade comportamental.

Os resultados da presente tese indicam claramente que as lulas bobtail não devem ser investigadas apenas em termos de simbiose bacteriana e órgãos emissores de luz, mas também em termos da sua notável ecologia comportamental. Embora presumivelmente negligenciadas devido ao seu tamanho pequeno e estilo de vida noturno, o presente estudo demonstrou que as lulas bobtail também apresentam comportamentos complexos e que pode valer a pena incluí-las em estudos ecológicos adicionais para entender a notável biologia comportamental da classe Cephalopoda.

Palavras-Chave: Ecologia do comportamento, Lula bobtail, Comportamento de enterrar, Cefalópode, *Sepiola*

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ABREVIATIONS

AD	Attack distance
BORIS	Behavioural Observation Research Interactive Software
DML	Dorsal mantle length
FNE	Fetovaia North-East
FSW	Fetovaia South-West
MED	Medium sand
mgs	Mean grain size
OIST	Okinawa Institute of Science and Technology
SA4	Sant’Andrea 4 metre
SA8	Sant’Andrea 8 metre
SD	Standard deviation
TLA	Tentacle length at approach
TLS	Tentacle length at strike
TSS	Tentacle strike speed
VCO	Very coarse sand
VFI	Very fine sand

1 – Introduction

Habitat Preference and Behavioural Ecology of Bobtail Squids (Sepiolidae)

Master Thesis in Marine Biology

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1.1 – State of the Art

Many colleoid cephalopods have been intensively studied in the past as potential new species for aquaculture production (Sykes et al., 2014; Vidal et al., 2014), as well as in terms of their behaviour and nervous system due to their complex cognitive abilities, neurally controlled skin pigmentation and dynamic behavioural repertoires (Hanlon & Messenger, 2018). While this applies especially to groups of larger, commercially valuable and in general more 'popular' species such as octopuses (Octopoda), squids (Teuthida, thus Myopsida + Oegopsida) and cuttlefish (Sepiidae), there are families of rather small cephalopods, such as bobtail squids (Sepiolidae), bottletail squids (Sepiadaridae) and pygmy squids (Idiosepiidae), which have played a minor role in research up to now. Although those three families are similar in size and posture, they show different anatomical, morphological and behavioural attributes (Reid, 2005a, 2005b; Reid & Jereb, 2005).

Despite their names, bobtail squids and bottletail squids are closer related to cuttlefish than to squids, whereas the phylogenetic position of pygmy squids was for a long time unclear (Allcock et al., 2015) and some studies suggested indeed a closer relationship to squids (Bonnaud et al., 2005; Hylleberg & Nateewathana, 1991; Takumiya et al., 2005). However, recent studies have shown that pygmy squids are also closely related to cuttlefish (Yoshida et al., 2010) as well as to bobtail squids (Strugnell et al., 2017; Tanner et al., 2017).

While bottletail squids and pygmy squids can be found only in the western central Pacific and the Indo-Pacific, respectively, and each family consist of less than 10 species only (Reid, 2005a, 2005b), bobtail squids have a global distribution in tropical, temperate and polar waters where they inhabit the continental slope from intertidal zones to depths of more than 1600 m (Boyle & Rodhouse, 2008; Reid & Jereb, 2005). In total, the family of bobtail squids consist of three subfamilies (Sepiolinae, Rossiinae and Heteroteuthinae) with a total of more than 60 species. While the subfamily Sepiolinae with species of the genera *Euprymna*, *Inioteuthis*, *Rondeletiola*, *Sepietta* and *Sepiola* generally occurs in shallower areas, the subfamily Rossiinae consisting of species of the genera *Austrorossia*, *Neorossia*, *Semirossia* and *Rossia* is associated with colder waters and inhabits polar shelves and the deep sea from 200 up to 2000 m of depth. As contrary to the latter two subfamilies who generally exhibit a benthic or nektobenthic lifestyle (Reid & Jereb, 2005) with some species potentially undergoing vertical migrations of several tens of meters (Bello & Biagi, 1995), the third subfamily Heteroteuthinae including species of the genera *Heteroteuthis*, *Iridoteuthis*, *Nectoteuthis*, *Sepiolina* and *Stoloteuthis* can be found in oceanic and pelagic environments (Reid & Jereb, 2005) and is generally the less studied subfamily of the Sepiolidae. Although not commercially exploited, some bobtail squids represent an important bycatch of trawl fisheries, especially in the Mediterranean Sea where they are considered as a delicacy in some areas (Jereb et al., 1997).

Bobtail squids belong to the smallest cephalopods and commonly have oval-shaped mantles with lengths of less than 80 mm. Their fins are wide, semi-circular rounded or kidney-shaped, and attached to their body about midway along the mantle. Compared to their body size with respect to other cephalopod groups, their arms are rather short, whereas their eyes are large and covered by corneal membranes (Figure 1.1A-D). Internally, no cuttlebone is found, but a rudimentary, chitinous gladius may be present in some species (Reid & Jereb, 2005). Bobtail squids are commonly known for their light-emitting organs associated with their ink sac (Figure 1.1E) (Belcaid et al., 2019; Lee et al., 2009; McFall-Ngai, 1999; Nyholm & McFall-Ngai, 2004). The emitted, ventrally directed luminescence of these organs is used to obscure the silhouette of the sepiolid when located in the water column (counterillumination) as a mean of cryptic defence behaviour (Figure 1.1F) (Jones & Nishiguchi, 2004; Stabb, 2006). While in some of the genera within the subfamilies Sepiolinae and Rossiinae those light-emitting organs correspond to a mutualistic symbiosis with light-producing *Vibrio fischeri*-bacteria (Boletzky, 1995; Stabb, 2006), the light organs of all members of the subfamily Heteroteuthinae are rather autogenic than bacterial in origin (Dilly & Herring, 1978).

Considering potential habitat preferences and the behavioural ecology of different species within the bobtail squids, literature is scarce for the greater part. Whereas for several species the reproductive biology is moderately well-studied, most species lack detailed observations of different behaviours such as feeding, hunting, burying or escaping.

1.1.1 – Habitat preference

It is widely reported that bobtail squids of the two subfamilies Sepiolinae and Rossiinae occur on the continental slope from intertidal waters to a depth of more than 1600 m (Boyle & Rodhouse, 2008; Reid & Jereb, 2005). While in most studies sandy or muddy grounds are mentioned as the common habitat (e.g. Anderson & Mather, 1996; Arnold et al., 1972), little to no detailed information on substrate type, salinity, pH and other parameters is available (Appendix – Table 4.1). To the best of my knowledge, only Anderson (1987) conducted field surveys to establish habitat preferences for *Rossia pacifica*. The author reported that the aforementioned species was commonly found on moderately steep slopes (average 14°) in shallow waters (< 30 m) which continued to areas with depths of more than 300 m. Their sediment analysis showed that *R. pacifica* was always found on muddy sand containing both

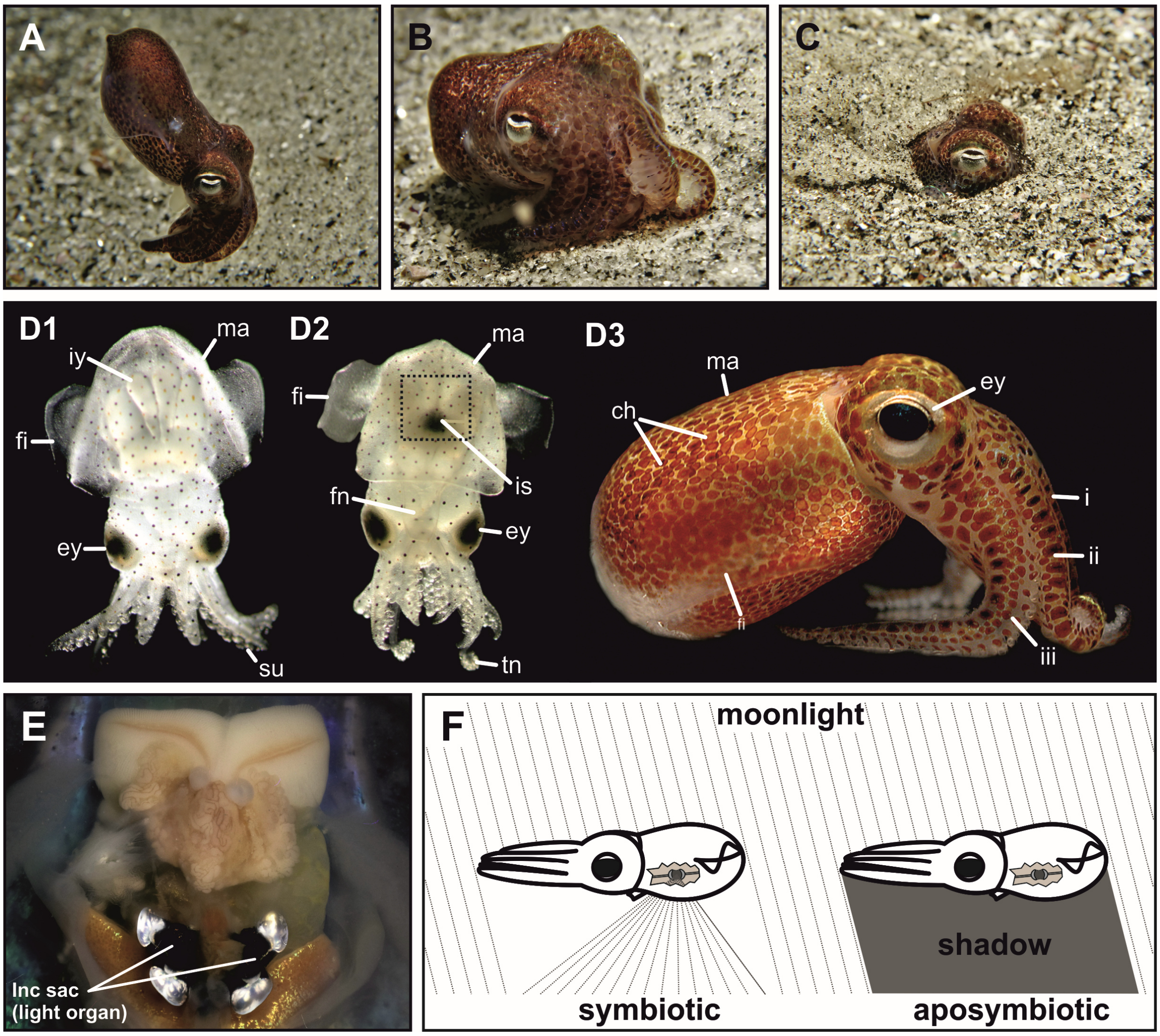

Figure 1.1 – Bobtail squid characteristics. **(A – C)** Photographs of a wild individual of *Sepiola* sp., **(A)** swimming in the water column, **(B)** resting on the sediment and **(C)** being partly buried in the sediment. All photographs taken by Christian Drerup. **(D)** Bobtail squid anatomy with **(D1)** dorsal and **(D2)** ventral view of a juvenile individual as well as **(D3)** lateral view of an adult individual of *Euprymna scolopes*. Anatomical features as follows: (i) arm I; (ii), arm II; (iii) arm III; (ch) chromatophore; (ey) eye; (fi) fin; (fn) funnel; (is) ink sac; (iy) internal yolk sac; (ma) mantle; (su) sucker; (tn) tentacle. Modified from Lee et al. (2009). The dashed box in **(D2)** indicates the location of **(E)**, which shows a ventrally dissected bobtail squid with its light organ associated with its inc sac. Modified from Suria (2019). **(F)** Counterillumination behaviour of bobtail squids. While an individual with bioluminescent symbiotic *Vibrio* bacteria in its light organ can obscure its silhouette with controllable, ventrally directed luminescence, an aposymbiotic (lacking symbionts) squid would cast a shadow. Redrawn from Stabb (2006).

fine sediments as well as organic material, whereas none of them occurred on pure mud or bare sand. The salinity and pH did not vary in between sites and averaged at 28.5 ppm and 7.8, respectively (Anderson, 1987). However, especially the low salinity measured in this study should not be considered as a general benchmark for bobtail squids, since the author conducted his field work in the Puget Sound (Washington, USA), a bay with strong freshwater inflows.

Likewise, barely any detailed information on habitat preference is available for bottletail squids and pygmy squids. While the former commonly occur on shallow soft sediment (Norman & Reid, 2000), pygmy squids are associated with seagrass meadows and mangrove roots to which they adhere using a glandular structure on the dorsal part of their mantle (Reid & Strugnell, 2018; von Byern et al., 2008), although observations on hard substrate such as rocks, coral and artificial objects (concrete and iron ramps) were reported by Moynihan (1983b).

1.1.2 – Feeding/hunting behaviour

Bobtail squids commonly spend the daytime buried in the sediment while emerging at night for hunting (Boletzky et al., 1971), although occasional feeding activity during the day was observed in captivity (Moynihan, 1983a; Shears, 1988). Their prey choice is not fully understood yet but consists mostly of crustaceans (e.g. mysids, euphausiids, decapods as well as shrimps and crabs), whereas fish or even other cephalopods may also be part of their diet (Boletzky & Hanlon, 1983; Orsi Relini & Massi, 1988).

For some bobtail squids, hunting and feeding patterns are described (Appendix – Table 4.1). One of the earliest yet detailed description for *Sepietta* spp. and *Sepiola* spp. was made by Boletzky et al. (1971). According to these authors, members of those species hunt with their arms forming an elongated cone (arms placed together and stretched out) pointing at the prey while circling around it. The prey is then caught by a swiftly approach going along with a simultaneous forward-shooting of their tentacles and pulled back to their body to be instantly grabbed by their arms (Figure 1.2). Additionally, the authors reported that very large prey is usually consumed while swimming, whereas prey of smaller size was mainly eaten while resting on the ground (Boletzky et al., 1971).

The same hunting behaviour was observed by Moynihan (1983a) for the Hawaiian bobtail squid *Euprymna scolopes*. However, an interesting feeding habit of the latter species was additionally mentioned by this author. To avoid the long, less nutritious appendages of a shrimp's head (e.g. the antennae), those were either broken off by the bobtail squid using its arms, or the shrimps were eaten 'tail-first', which represents an uncommon technique amongst carnivores. While smaller shrimps were usually fully consumed, the exoskeletons of larger individuals were not completely ingested and were commonly ejected after the internal content has been consumed. Additionally, Moynihan (1983a) reported that individuals of *E. scolopes* usually attacked from the water column, although a few strikes of resting or even partly buried animals were observed. The latter behaviour is contrasting to observations of Boletzky et al. (1971) who suggested that hiding in the sand is no mean of prey outwitting and solely purposes protection.

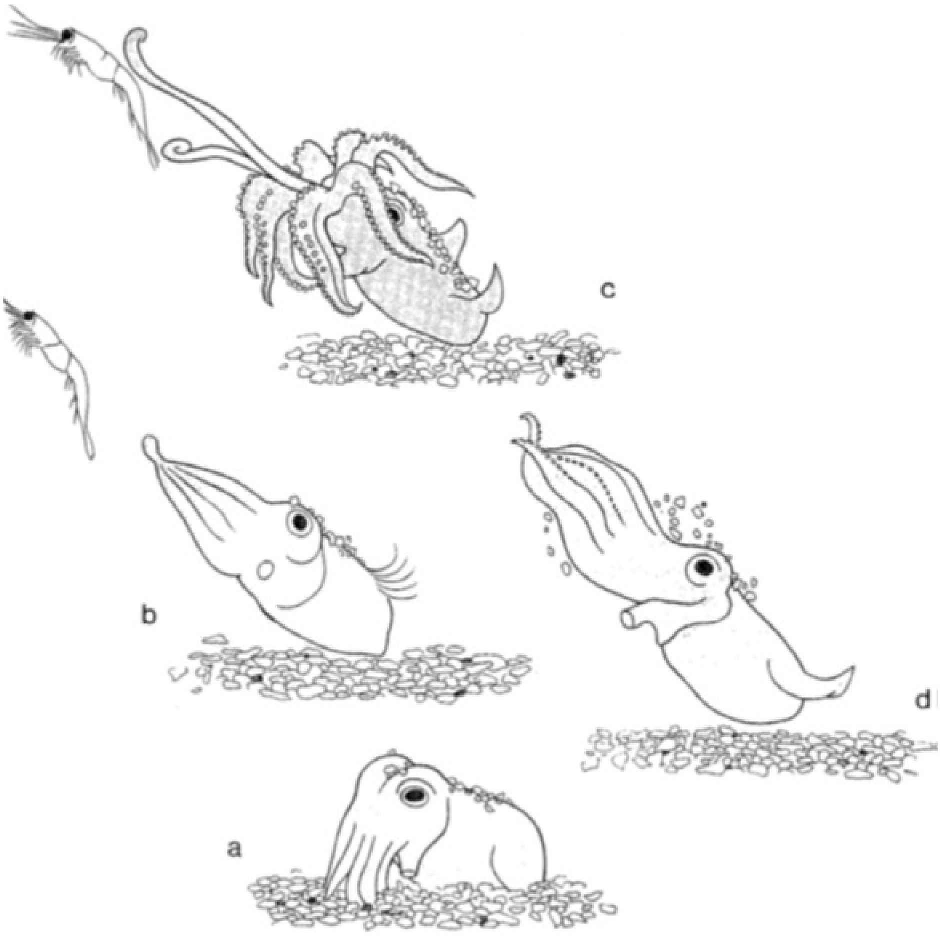

Figure 1.2 – Foraging behaviour of *Sepietta oweniana*. **(a)** Resting on the bottom; **(b)** positioning and circling around the prey; **(c)** attacking; **(d)** returning to bottom. Adapted from Bergström (1985)

The same hunting behaviour as described by Boletzky et al. (1971) and Moynihan (1983a) was also confirmed for *Sepietta oweniana* by Bergström (1985). Additionally, the latter author reported a change in the body colouration of *S. oweniana* when attacking prey. While this species usually has a dense reddish-brown pigmentation, it bleaches while circling around potential prey. Afterwards, when its tentacles eventually strike the prey, the reddish-brown pigmentation returns instantly.

While, to the best of my knowledge, no foraging behaviour is published yet for any bottletail squids, the hunting techniques of some pygmy squids such as *Idiosepius thailandicus* closely resemble those described for bobtail squids (Nabhitabhata, 1998), whereas Sato et al. (2016) further reported that *Idiosepius paradoxus* uses inking for prey distraction during foraging, which displays an uncommon use of ink in cephalopods (see 1.1.3). However, observations of some pygmy squids being attached to substrate while exhibiting a cryptic body colouration suggest an additional ambush strategy by catching passing prey (Reid & Strugnell, 2018).

Furthermore, an additional feeding behaviour was observed for *Idiosepius pygmaeus* by Moynihan (1983b) as this species also 'nibbles' on surfaces, potentially to graze microscopic settling invertebrates. As according to this author, juveniles of *I. pygmaeus* lack tentacles and therefore cannot catch moving prey, the grazing behaviour of adult individuals may be a carry-over from their juvenile feeding behaviour. Another remarkable observation regarding the feeding behaviour of pygmy squids was reported for *I. paradoxus*. This species appears to inject digestive enzymes, probably from a unique organ located in the outer lip, into the prey's flesh and eventually eats externally semi-digested pieces of flesh, while the exoskeleton of a shrimp or the bones of a fish, respectively, remain fully intact and untouched (Kasugai, 2001; Kasugai et al., 2004).

1.1.3 – Escape response

Considering the escape response of bobtail squids, most observations were made for the Hawaiian bobtail squid *E. scolopes* (Appendix – Table 4.1). In general, a discrepancy in the personality of this species between wild and captive individuals was found, with the former exemplars exhibiting an evasive escape response when approached by a diver (Anderson & Mather, 1996), whereas those kept in tanks showed a more passive and persistent behaviour and could be observed from close distance or even touched (Moynihan, 1983a). However, whether this discrepancy was caused by the use of diving lights in the wild (which cannot be considered as a natural condition for bobtail squids) or by a stress response of the captive individuals was not further discussed by those authors.

More detailed observations regarding the escape responses of *E. scolopes* were reported by Anderson & Mather (1996). According to these authors, individuals situated on sandy bottom would either remain stationery and shift to a deep red-brown colouration or bury themselves into the sand (see 1.1.4). When closer approached, they would either swim away for roughly 2 m and try burying themselves again or jet into the water column and hang there motionless while again exhibiting a red-brow colouration. The latter behaviour was also observed when individuals of *E. scolopes* were approached in mid-water, although some exemplars either jetted to the bottom or raised to the surface and stayed there motionless while potentially resembling floating bits of seaweed (Anderson & Mather, 1996).

Another escape response reported both for *E. scolopes* (Anderson & Mather, 1996; Moynihan, 1983a; Seehafer et al., 2018) as well as for *R. pacifica* (Shimek, 1983) was inking, which resembles a common defence behaviour in many cephalopods (Hanlon & Messenger, 2018). However, the distinctive use of ink while escaping varies between species. While other cephalopod families may produce large clouds ('smoke screens') behind which they can hide or escape (Hanlon & Messenger, 1988) or use the ink as a chemical defence (Derby, 2007, 2014) or as an intraspecific alarm cue (Wood et al., 2008), both aforementioned bobtail squid species turned dark and ejected a series of ink blobs, similar in size to themselves, which may have acted as a decoy to confuse potential predators (Anderson & Mather, 1996; Moynihan, 1983a; Shimek, 1983). Additionally, species of the genus *Euprymna* such as *E. scolopes* have a specialised epidermis to which sand adheres (von Byern & Klepal, 2006). This feature is used as crypsis during the day and can be dropped as a unit, potentially to confuse predators while escaping (Moynihan, 1983a; Shears, 1988; Singley, 1982).

Considering the escape response of bottletail squids and pygmy squids, hardly any information is available. However, observations on *I. pygmaeus* imply that at least pygmy squids do not show any burying behaviour to escape and rather attach themselves to objects while changing to a cryptic body colouration (Moynihan, 1983b).

1.1.4 – Burying behaviour

While many cephalopods hide by either camouflaging (Hanlon, 2007) or sheltering in crevices and holes (Katsanevakis & Verriopoulos, 2004), bobtail squids bury themselves in the sand to avoid predation. This can be considered as an less common escape response in cephalopods and has been observed in a similar fashion only in the cuttlefish *Sepia officinalis* (Hanlon & Messenger, 1988; Mather, 1986) and the octopus species *Amphioctopus burryi* (Hanlon & Hixon, 1980; Hanlon et al., 2010), *Eledone cirrhosa* (Guerra et al., 2006), *Macrotritopus defilippi* (Hanlon et al., 2010) and *Thaumoctopus mimicus* (Hanlon et al., 2008). Although the burying behaviour of sepiolids aroused interest since the closer beginning of cephalopod research (e.g. Racovitza, 1894), only incomplete observations were initially published (Jaeckel, 1958; Naef, 1923). The first detailed observations for a broad range of *Sepiola* spp. and *Sepietta* spp. were made by Boletzky & Boletzky (1970) who divided the burying of both genera into two phases: (1) blowing up sand with vigorous funnel jets to get almost completely covered by sand, followed by (2) dorsolateral arm movements to cover the remaining body parts with sand (Figure 1.3).

In (1), a depression in the sediment will be created by a gentle forward-directed funnel jet. This depression serves as a hold for a strong, backward-directed funnel jet to blow away the sand under the sepiolid's body. While the sand is dispersed in the water column, the body will be immersed in the sediment, and the descending sand will cover already most of it. This behaviour is followed by alternating forward- and backward-directed funnel jets, and some animals may be nearly completely covered by a total of four funnel jets, depending on their fitness and the given substrate (Boletzky & Boletzky, 1970).

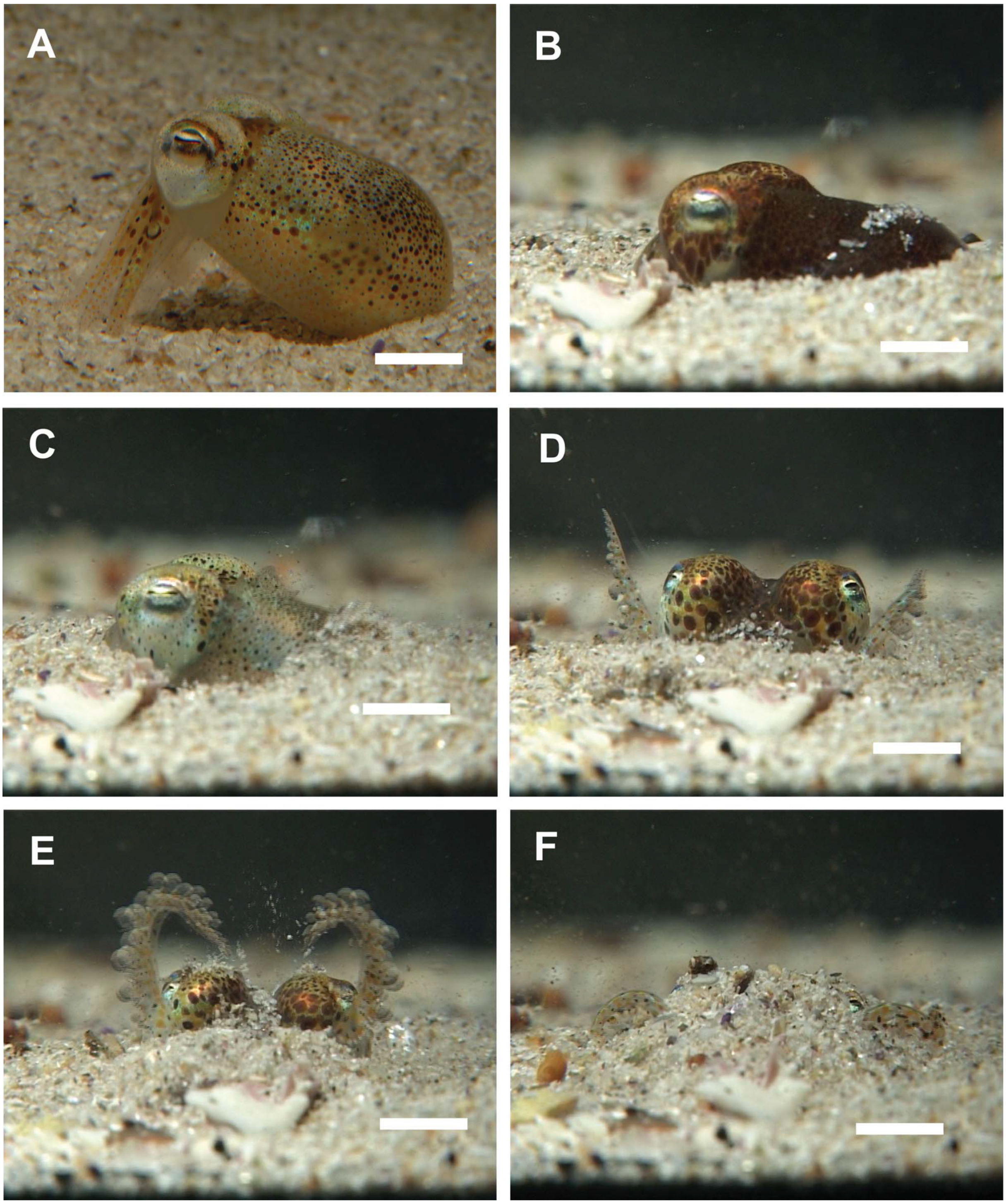

Figure 1.3 – Burying pattern in *Sepiola atlantica*. **(A)** Alert posture of *S. atlantica* settling on the substrate. **(B, C)** Continuous burying procedure by alternating forward- and backward-directed funnel jets. **(D, E)** Dorsolateral arm movements to cover the remaining body parts with sand. **(F)** The individual is almost completely buried in the sediment. Scale bars: (A) 10 mm; (B – F) 5 mm. Adapted from Rodrigues et al. (2010).

Either immediately after (1) or after a few seconds of rest, (2) starts by stretching out their dorsolateral arms above the sediment, followed by sweeping sand over their head and body with their arm tips pointing medially. This procedure may be repeated several times until the animal is fully covered by sand, whereby the arm movements are always closely synchronous. Fin movements may counteract the funnel jet thrust to maintain a steady position during (1) but do not contribute to the burying behaviour at any point (Boletzky & Boletzky, 1970; Boletzky, 1996) and are not used in a shovel-like fashion, as stated by Jaeckel (1958).

Those observations of a two-phase burying behaviour as observed in *Sepiola* spp. and *Sepietta* spp. (Boletzky & Boletzky, 1970) were later confirmed for *Sepiola atlantica* (Rodrigues et al., 2010) and identically observed for *R. pacifica* (Anderson et al., 2004). Although the burying behaviour for species of the genera *Euprymna* mostly resembles that of *Sepiola* spp., *Sepietta* spp. and *R. pacifica*, some details differ in *E. scolopes* (Anderson, 1997; Anderson et al., 2002; Moynihan, 1983a) and *Euprymna hyllebergi* (Nabhitabhata et al., 2005) (Appendix – Table 4.1). These species were observed to start their burying procedure with a backward-directed funnel jet instead of a forward-directed one (Anderson et al., 2002; Nabhitabhata et al., 2005). Additionally, *E. hyllebergi* performed the second phase of its burying behaviour with its ventrolateral arms rather than its dorsolateral arms (Nabhitabhata et al., 2005).

Two further peculiarities were reported by Boletzky & Boletzky (1970) regarding the burying of *Sepiola* spp. and *Sepietta* spp.. The authors mentioned that the respiration of buried individuals is not accomplished by mantle expansions and contractions, as it is common for unburied individuals and other cephalopods (Reid & Jereb, 2005), but rather by the funnel collar which acts as a diaphragm pushing water back and forth, in through the mantle slits and out through the funnel (Boletzky & Boletzky, 1970). The latter finding was further confirmed by Anderson et al. (2002, 2004) who observed small bursts of water coming from the sediment after individuals of *E. scolopes* and *R. pacifica*, respectively, became fully covered by sediment, resulting in fine, roughly 2 mm wide holes in the sediment.

Additionally, Boletzky & Boletzky (1970) documented that, as already observed but not further explained by Naef (1923), the eyeballs of *Sepiola* spp. and *Sepietta* spp. are rotated vertically when buried, resulting in the pupils facing upwards. Furthermore, Anderson et al. (2002, 2004) reported that completely buried individuals of both *E. scolopes* and *R. pacifica* were still able to see through the sediment. This was concluded from diffuse jets of ink ejected by buried individuals, evoked by sudden movements outside the tank.

Moreover, Anderson et al. (2004) and Rodrigues et al. (2010) documented an ‘alert posture’ (Figure 1.3A) in *R. pacifica* and *S. atlantica*, respectively, exhibited when settling on the ground before burying. Neither of these authors exactly explained the purpose of this posture, in which all arms are directed forward and raised off the sand, but according to Rodrigues et al. (2010), it may benefit individuals in the event of jetting away to escape.

For bottletail squids and pygmy squids, no information about any kind of burying behaviour is available. However, Moynihan (1983b) reported that captive *I. pygmaeus* generally avoided the bottom substrate, suggesting that this species does not exhibit a burying behaviour at all.

1.1.5 – Reproductive behaviour and egg characteristics

The reproductive biology of bobtail squids is the most elaborated feature of their ecology. While most publications cover aspects such as maturity or mating/spawning seasons of bobtail squids, only a small part focuses on their reproductive (courtship, mating and spawning) behaviour (Appendix – Table 4.2). Up to present, behavioural observations are available for the genera *Euprymna* (Hanlon et al., 1997; Moynihan, 1983a; Nabhitabhata et al., 2005; Singley, 1983; Squires et al., 2013), *Sepietta* (Bergstrom & Summers, 1983), *Sepiola* (Boletzky, 1983; Jones & Richardson, 2010; Racovitza, 1894; Rodrigues et al., 2009) and *Rossia* (Racovitza, 1894). While no prior pair formation or courtship behaviour was ever reported (Jones & Richardson, 2010; Nabhitabhata et al., 2005; Rodrigues et al., 2009; Squires et al., 2013), mating usually occurs as follows:

The male approaches a swimming female from below and initiates physical contact by grasping the female, usually at her mantle, followed by a shifted grasp to the female's neck. The female will then be pulled down to the substrate (Nabhitabhata et al., 2005; Rodrigues et al., 2009) where the copulation takes place by the male inserting his hectocotylus into the female's mantle cavity (Figure 1.4). Commonly, mating occurs in the 'male-to-female neck' position (Boletzky, 1983; Hanlon et al., 1997; Nabhitabhata et al., 2005; Rodrigues et al., 2009; Singley, 1983; Squires et al., 2013), although also mating positions such as 'male-parallel' (Brocco, 1971) or 'head-to-head' (Bergstrom & Summers, 1983) have been observed (for a review of mating positions in cephalopods, see Hanlon & Messenger (2018)).

Recently, Squires et al. (2013) reported for male *Euprymna tasmanica* a 'pumping behaviour' (increased mantle contractions) during the intercourse, which may be used to shoot jets of water into the female's mantle cavity to either dislodge spermatophores or flush out accessory seminal fluids from previous mates. Although this behaviour has also been documented for some cuttlefish species such as *Sepia apama* (Hall & Hanlon, 2002), *Sepia esculenta* (Wada et al., 2005) and *Sepia officinalis* (Hanlon et al., 1999), it has not been confirmed yet for other bobtail squid species.

Figure 1.4 – Mating in *Sepiola atlantica*. **(a)** The male (right) approaches the female and holds her with his arms by the ventral region of her mantle. **(b)** The male, located below the female, inserts his pair of dorsal arms in the mantle cavity of the female. **(c, d, e, f)** The male exhibits a generally darker colouration than the female during the mating process. **(g)** Before separation, the female displays a distinct white colouration. Scale bars: (a – d, f, g) 10 mm; (e) 5 mm. Adapted from Rodrigues et al. (2009).

During copulation, females show a pale or translucent (*S. atlantica*; Rodrigues et al., 2009), pale brown (*E. hyllebergi*; Nabhitabhata et al., 2005) or greyish colouration (*E. scolopes*; Moynihan, 1983a), whereas males exhibit a cream-yellowish background with a dark colouration (Moynihan, 1983a; Nabhitabhata et al., 2005; Rodrigues et al., 2009). Mating duration varies significantly in between species, with 7 – 10 min for *E. hyllebergi* (Nabhitabhata et al., 2005), 8 min for *Sepiola rondeletii* (Racovitza, 1894), 45 – 80 min and 30 – 50 min, respectively, for *E. scolopes* (Hanlon et al., 1997; Singley, 1983), 68 – 80 min and 49 – 77 min, respectively, for *S. atlantica* (Jones & Richardson, 2010; Rodrigues et al., 2009) and 45 – 184 min for *Euprymna tasmanica* (Squires et al., 2013), whereas pairs separate after the act (previous references).

Spawning generally occurs at night (Arnold et al., 1972; Boletzky et al., 1971; Jones & Richardson, 2010; Rodrigues et al., 2011a) or in the early morning (Boletzky et al., 1971; Hanlon et al., 1997; Nabhitabhata et al., 2005), although occasional spawning during daytime was also observed by Boletzky et al. (1971). The spawning period of an individual female ranges among species and may take from 1 – 20 days in *E. hyllebergi* (Nabhitabhata et al., 2005) over 3 – 30 days in *S. atlantica* (Rodrigues et al., 2011b), 1 – 61 days in *Sepiola affinis* (Gabel-Deickert, 1995), and up to over 4 months in *E. tasmanica* (Squires et al., 2013). Before laying their eggs, bobtail squids may investigate given substrata for attaching their eggs by touching it with their arms (Nabhitabhata et al., 2005). After finding a suitable substrate, several species were observed to adopt a vertical 'sitting' position while laying their eggs (Boletzky et al., 1971). Bobtail squids often attach their droplet-shaped eggs in several layers where the outer jelly capsule becomes leathery or turns opaque and rigid (Anderson & Shimek, 1994; Arnold et al., 1972; Boletzky & Boletzky, 1973; Choe, 1966; Nabhitabhata et al., 2005). No maternal care of the egg capsules was ever observed (Nabhitabhata et al., 2005) and females usually die within a few hours after their last spawning event (Boletzky et al., 1971; Nabhitabhata et al., 2005; Rodrigues et al., 2011b), whereas Jones & Richardson (2010) documented a survival for up to 8 days in *S. atlantica*.

While little to no information about the reproductive behaviour of bottletail squids is available, some publications cover this aspect for pygmy squids. No courtship behaviour was ever observed in species of the genus *Idiosepius* and mating can occur in 4 different patterns (Nabhitabhata, 1998; Nabhitabhata & Suwanamala, 2008; Sato et al., 2010) in the 'head-to-head' position (Kasugai, 2000; Nabhitabhata, 1998; Nabhitabhata & Suwanamala, 2008). Another difference to the reproductive behaviour of bobtail squids is the length of copulation in pygmy squids, which usually takes less than 10 seconds (Nabhitabhata, 1998; Nabhitabhata & Suwanamala, 2008; Sato et al., 2010). Spawning of *I. thailandicus* and *I. biserialis* occurs over a period of 4 – 21 days and females are reported to die within 6 hours to 2 days (Nabhitabhata & Suwanamala, 2008).

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2 – Manuscript

Behavioural aspects of the spotty bobtail squid *Sepiola parva* (Cephalopoda: Sepiolidae)

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Abstract

Bobtail squids (Sepiolidae, Cephalopoda) have recently become more popular in scientific studies due to their symbiotic relationship with light producing bacteria and their corresponding light emitting organs. However, the overall knowledge on the behaviour of sepiolids is based on observations on just a few of the roughly 70 extant species and must still be considered as sparsely. As understanding their behavioural ecology is not only beneficial to further grasp the complex behavioural patterns of cephalopods but also vital for establishing a good welfare when holding sepiolids in captivity, the present study dealt with several behavioural aspects of the spotty bobtail squid *Sepiola parva*. Although the burying, hunting and mating behaviour as well as the escape responses of this so far less investigated sepiolid species greatly resembled those of other observed bobtail squids, differences to sepiolids from other genera or even the same genus could be identified in the present study. Additionally, some findings on *S. parva* of the present study provide the first observations for the genus *Sepiola* or sepiolids in general. *S. parva* was able to adhere a sand grain layer to its mantle area ('sand coat'), which displays a behavioural feature up to now only reported for sepiolids of the genus *Euprymna*. Moreover, *S. parva* was observed to eject a stretch of ink ('ink rope'), potentially for masquerade, which has only been described for deep sea squids so far. Besides these novel observations, the present study also provides detailed information on daily time and activity budgets and the positioning towards the prey as well as tentacular strike speed during hunting, two up to now barely investigated behavioural aspects of the sepiolid ecology.

Keywords: Behavioural ecology, Bobtail squid, Cephalopod, *Sepiola parva*, Sepiolidae

2.1 – Introduction

In the past decades, colleoid cephalopods have been part of numerous studies as potential new sources in aquaculture (Sykes et al., 2014; Vidal et al., 2014) as well as in terms of their remarkable behavioural repertoires, their neurally controlled skin pigmentation and their complex cognitive abilities (Hanlon & Messenger, 2018). While this is especially true for larger and commercially valuable cephalopods such as octopuses (Octopoda), squids (Teuthida) and cuttlefish (Sepiidae), there are families of rather less investigated cephalopods such as bobtail squids (Sepiolidae) which have played a minor role in research until recently.

Sepiolids (= members of the family Sepiolidae) belong to the smallest known cephalopods with mantle lengths ranging between 1 and 8 cm and exhibit a global distribution in tropical, temperate and polar waters. Commonly reported to be found on sandy or muddy bottoms (e.g. Anderson & Mather, 1996; Rodrigues et al., 2010), they spend the daytime buried in the sediment while emerging at night for hunting (Boletzky et al., 1971) by forming their arms into an elongated cone while pointing at their prey. By forward-shooting their tentacles, the prey will then be caught and pulled back to their buccal area where it will be hold in position by their arms (Bergström, 1985; Boletzky et al., 1971). Their prey choices are not fully understood yet but might consist mostly of crustaceans, whereas fish or even other cephalopods may also be part of their diet (Boletzky & Hanlon, 1983; Orsi Relini & Massi, 1988).

When threatened, sepiolids show a variety of escape responses including jetting away in combination with ejecting a series of ink blobs potentially acting as a decoy to confuse predators (Anderson & Mather, 1996; Moynihan, 1983; Shimek, 1983). Furthermore, bobtail squids bury themselves in the sediment to avoid predation (Anderson & Mather, 1996). This can be considered as an less common defence behaviour in cephalopods and has been observed in a similar fashion only in the cuttlefish *Sepia officinalis* (Hanlon & Messenger, 1988; Mather, 1986) and the octopus species *Amphioctopus burryi* (Hanlon & Hixon, 1980; Hanlon et al., 2010), *Eledone cirrhosa* (Guerra et al., 2006), *Macrotritopus defilippi* (Hanlon et al., 2010) and *Thaumoctopus mimicus* (Hanlon et al., 2008). The burying procedure of sepiolids can be generally divided into two phases (Boletzky & Boletzky, 1970). While the first phase includes of a series of alternating forward- and backward-directed funnel jets to blow up sand into the water column to eventually cover the sepiolid's body almost completely, the second phase consists of sweeping arm movements to cover the still exposed body parts with sand (Anderson, 1997; Anderson et al., 2002, 2004; Boletzky & Boletzky, 1970; Rodrigues et al., 2010).

Contrarily to other cephalopod species, the reproductive behaviour of sepiolids appears to be less complex. Up to present, no prior pair formation or courtship behaviour was ever observed (Jones & Richardson, 2010; Nabhitabhata et al., 2005; Rodrigues et al., 2009; Squires et al., 2013). After approaching a swimming female from below, the male grabs the latter at her mantle and inserts his hectocotylus into the female's mantle cavity, followed by the male pulling down the female to the substrate where the copulation takes place (Nabhitabhata et al., 2005; Rodrigues et al., 2009). Commonly, mating occurs in the 'male-to-female neck' position (Boletzky, 1983; Hanlon et al., 1997; Nabhitabhata et al., 2005; Rodrigues et al., 2009; Singley, 1983; Squires et al., 2013), although the 'male-parallel' (Brocco, 1971) and 'head-to-head' (Bergstrom & Summers, 1983) positions have also been observed. The duration of the copulation varies significantly from 7 – 10 min in *Euprymna hyllebergi* (Nabhitabhata et al., 2005) up to more than 3 h in *Euprymna tasmanica* (Squires et al., 2013).

Spawning usually occurs at night (Arnold et al., 1972; Boletzky et al., 1971; Jones & Richardson, 2010; Rodrigues et al., 2011b) or in the early morning (Boletzky et al., 1971; Hanlon et al., 1997; Nabhitabhata et al., 2005). After investigating the substrate by touching it with their arms (Nabhitabhata et al., 2005), bobtail squids often attach their droplet-shaped eggs in several layers to the latter (Anderson & Shimek, 1994; Arnold et al., 1972; Boletzky & Boletzky, 1973; Choe, 1966; Nabhitabhata et al., 2005). No maternal care of the egg capsules was ever observed (Nabhitabhata et al., 2005) and females usually die within a few hours after their last spawning event (Boletzky et al., 1971; Nabhitabhata et al., 2005; Rodrigues et al., 2011a).

Over the last few years, some sepiolid species are beginning to become more popular in scientific studies as model organisms for host-microbe interactions (Mandel & Dunn, 2016; McAnulty & Nyholm, 2017) as well as for genetic studies (Belcaid et al., 2019; Bosch, 2019) due to their symbioses with light producing bacteria and their corresponding light emitting organs (McFall-Ngai, 1999; Nyholm & McFall-Ngai, 2004) used for counterillumination as a means of cryptic defence behaviour (Jones & Nishiguchi, 2004). Hence, understanding their behavioural ecology is not only interesting to further grasp the complex behavioural patterns of cephalopods, it is also vital for establishing a good welfare when holding these in captivity. However, literature about bobtail squids is scarce for the greater part and only a few species can be considered as well-elaborated in terms of their behavioural ecology. *Euprymna scolopes* holds the most extensive body of literature, including studies on its feeding and hunting behaviour (Hanlon et al., 1997; Moynihan, 1983; Shears, 1988), its escape response (Anderson & Mather, 1996; Moynihan, 1983; Seehafer et al., 2018), its burying behaviour (Anderson, 1997; Anderson et al., 2002; Moynihan, 1983) and its reproductive behaviour (Hanlon et al., 1997; Moynihan, 1983; Singley, 1983). Furthermore, different aspects of the behavioural ecology of *Euprymna hyllebergi* (Nabhitabhata et al., 2005), *Sepiola atlantica* (Jones & Richardson, 2010; Rodrigues et al., 2009, 2010; Yau & Boyle, 1996) and *Rossia pacifica* (Anderson, 1987; Anderson et al., 2004; Brocco, 1971; Shimek, 1983) have been addressed in detail in the given studies but their behavioural ecology must be still considered as inchoate. Besides these particular species, the behavioural ecology of other sepiolids has been partly well-investigated. For some members of the subfamily Sepiolinae, the burying and spawning behaviour was described in detail by Boletzky & Boletzky (1970) and Boletzky et al. (1971), respectively. Moreover, the most detailed studies on sepiolid reproductive behaviour were conducted for *E. tasmanica* (Franklin & Stuart-Fox, 2017; Franklin et al., 2012, 2014; Squires, 2013; Squires et al., 2012, 2013, 2014, 2015).

Despite these existing studies, the overall knowledge on the behavioural ecology of sepiolids is based on observations on a small number of the roughly 70 extant species only and must still be considered as incipient, which makes it difficult to generalize any findings to other less-studied sepiolids. One of the latter is the spotty bobtail squid *Sepiola parva*. After being described by Sasaki (1913), the only notable publication considering this sepiolid species is by Takayama & Okutani (1992), describing a method to identify it from the closely resembling sepiolid *Sepiola birostrata*, whereas, to the best of our knowledge, no behavioural observations have been published so far. By recording different behaviours of both wild and captive individuals of *S. parva* and compare those to existing observations on other sepiolids and cephalopods in general, this study aimed at improving the knowledge on the ecology of these cephalopods.

2.2 – Material & Methods

2.2.1 – Ethical statement

As cephalopods do not fall under the national "Act on Humane Treatment and Management of Animals" (Law No. 105, 1973) as national legislation in Japan (Ogden et al., 2017), this study followed the regulations of Directive 2010/63/EU (European Parliament & Council of the European Union, 2010) for cephalopods (Fiorito et al., 2015; Smith et al., 2013; Sykes et al., 2012). All animals in this study were kept according to established methods (Boletzky et al., 1971; Hanlon et al., 1997) and no specific procedures were applied to those animals. This manuscript was further prepared according to the ARRIVE guidelines (Kilkenny et al., 2010a, 2010b) for reporting animal research.

2.2.2 – Animal collection and husbandry

During February and March 2019, a total of 32 individuals of *S. parva* were collected from Seragaki Bay (Onna, Okinawa, Japan; 26°30'19.9"N 127°52'56.1"E) at night by SCUBA diving at depths between 0.5 and 8 m. Encountered individuals were caught using green, finely meshed hand nets and subsequently placed in a plastic container during the dive. Immediately after each survey, all caught individuals were transported to the Marine Science Station of the Okinawa Institute of Science and Technology (Onna, Okinawa, Japan; 26°30'35.6"N 127°52'12.7"E) and kept in rectangular 100 L aquaria (50 cm long x 50 cm wide x 40 cm deep) with a maximum stocking density of 40 animals/m^2, corresponding to a maximum of 10 sepiolids/aquaria. Each aquarium was connected to a flow-through system which was supplied by natural sea water, equipped with an additional air stone to provide a proper dissolved oxygen saturation, and with its bottom covered with a 3 cm layer of sediment taken from the collection spot. Water parameters were monitored using an aquarium controller (ProfiLux 3.1T eX, GHL Advanced Technology GmbH & Co. KG, Kaiserslautern, Germany) and are shown in Table 2.1. Rocks and clay-based flowerpots were added to the aquaria as shelter opportunities. Fluorescent tube lights (Colour temperature: 7800K, CRI: 80) were used and followed a 10:14 LD cycle with a light period from 8 am to 6 pm. However, due to its position inside the facility the aquaria were already mildly exposed to ambient sun light from sunrise on (approximately 6:30 am during the experimental period). Bobtail squids were fed daily before switching off the aquarium lighting *ad libitum* with the local mysid shrimp species *Neomysis japonica* and food remains (dead mysid shrimps and undigested appendages) were removed from the aquaria on the consecutive morning. Based on their morphological development and previously recorded size indication (Sasaki, 1913; Takayama & Okutani, 1992), individuals with a dorsal mantle length (DML) of less than 4 mm were considered as hatchlings, whereas bigger individuals were classified either as juveniles (DML between 4 to 9 mm) or adults (DML > 9 mm).

Table 2.1 – Water parameters during the experimental period

	Temperature	**pH**	**Conductivity**	**Redox**
Lowest value	20.1 °C	8.40	11.7 mS	290 mV
Highest value	22.7 °C	8.61	39.1 mS	474 mV
Average ± SD	21.5 ± 0.5 °C	8.47 ± 0.02	25.8 ± 6.0 mS	405.5 ± 13.9 mV

SD is an abbreviation for standard deviation.

2.2.3 – General behavioural sampling

To record different behavioural aspects as well as intraspecific interactions of *S. parva* on a long-term base, a 50 L aquarium (50 cm long x 25 cm wide x 40 cm deep) was set up identically as the housing aquaria mentioned in 2.2.2. Clay-based flowerpots were cut in half and added to the back of the aquarium as a shelter opportunity. By mounting an infrared-sensitive video camera (PXW-X70, Sony Corporation, Tokyo, Japan) in front of the aquarium, connected via HDMI to an external video recording device (Blackmagic Video Assist 4K, Blackmagic Design Pty. Ltd, Melbourne, Australia; Software: 2.3.1; recording quality: 720p/60fps), and placing an infrared light with a diffusor 60 cm above the water level, all bobtail squid activity inside the aquarium was recorded for a total of 41 days and nights and stored on external hard drives for later analysis. During this period, the aquarium was stocked with different compositions of juvenile and adult individuals of *S. parva*, based on availability. While most video material was filtered for specific, less frequently observed behaviours such as mating or spawning, a total of five 24h-periods of the recording time were used as follows:

Three activity patterns (consisting of the total time being partly buried, being located on the sediment and being located in the water column) as well as three swimming patterns (total time being located in the water column) within 24 h were determined for 11 adult individuals (6 females, 5 males) by scan-sampling (Altmann, 1974) at 5-min intervals for three consecutive periods from 12:00 noon to 12:00 noon on the next day (total recording time 72 h).

To further determine individual time budgets of different behavioural states of *S. parva* (being buried, being partly buried, being located on the sediment (= 'sitting') and being located in the water column (= 'swimming'); Table 2.2) as well as the occurrence of hunting events per individual within 24 h, the activities of two groups consisting two (1 female, 1 male) and six adult individuals (3 female, 3 male), respectively, were analysed by continuous focus-animal-sampling (Altmann, 1974) for two respective periods from 12:00 noon to 12:00 noon on the next day.

Table 2.2 – Ethogram of basic behaviour in sepiolids

Behaviour/State	**Description**
Buried	Fully buried in the sediment, no obvious activity can be observed
Partly buried	Partly buried in the sediment, the eyes or dorsal mantle are visible
Sitting	Located on the sediment, no body parts are immersed in the sediment
Swimming	Located in the water column, can be linked with other behaviours such as hunting or mating
Hunting	Orientation and approach to the prey followed by attempt to capture it
Feeding	Devouring of captured prey
Mating	Male approaches swimming female, grabs her and inserts his hectocotylus into the female's mantle cavity
Spawning	Female releases eggs, usually by attaching them to different kinds of substrate
Inking	Ejection of ink in a blob, rope or diffuse shape
Interaction	Interaction of two individuals, e.g. fighting or mating attempt

Only major behavioural states are listed. For an extensive list of detailed species-specific references of those behavioural aspects, see Appendix Table 4.1 + 4.2.

2.2.4 – Burying behaviour

A 0.8 L aquarium (10 cm long x 6.5 cm wide x 14.4 cm deep) with its bottom covered with a 3 cm layer of subtidal sediment was used to film the burying behaviour of *S. parva* in detail. A total of 21 individuals with DMLs between 2.9 mm and 12.2 mm (average DML = 8.1 mm) were individually recorded in the aquarium using a video camera (PXW-FS5 with SEL-35F14Z lens, Sony Corporation, Tokyo, Japan, recording quality: 1080p/60fps). Since burying behaviour during the day was commonly observed in sepiolids (Anderson et al., 2002, 2004; Rodrigues et al., 2010), all recordings were taken during daytime and under the stimulus of a light panel (RX-12 TD, FalconEyes Ltd., Hong Kong; white LEDs, Colour temperature: 3000K, CRI: 95) to increase illumination conditions. Each recording started shortly before introducing an individual into the aquarium and ended either 30 seconds after the last movement of a successful burying procedure, or 10 minutes after introducing the animal into the aquarium, which was considered as refusal to bury. After each recording, the aquarium water was exchanged for fresh sea water to ensure a pristine quality. Each observation was later examined for the burying characteristics displayed in Table 2.3.

Table 2.3 – Definition of burying characteristics

Burying sequence	Definition
Pre-Burying	
Water column (s)	Total time an individual spends in the water column before the start of burying.
Sediment (s)	Total time an individual spends resting on the sediment before the start of burying.
Latency (s)	Time between introducing an individual into the experimental aquarium and the start of burying (= total time spent in water column + total time spent on sediment).
Phase 1*	
Duration (s)	Time between the first and last recorded funnel jet, including potential resting periods.
Number of funnel jets	Total number of ejected funnel jets, including the initial funnel jet (= ‘depression’).
Resting period (s)	Resting period between the last observed funnel jet (end of phase 1) and the first observed arm sweep (beginning of phase 2).
Phase 2*	
Duration (s)	Time between the first and last recorded arm sweep, including potential resting periods.
Number of arm sweeps	Total number of observed arm sweeps.
Burying duration (s)	Time between the first recorded funnel jet (= ‘depression’) and the last observed arm sweep including all resting periods (= duration of phase 1 + time between phase 1 and phase 2 + duration of phase 2).

* *sensu* Boletzky & Boletzky (1970)

2.2.5 – Hunting Behaviour

The same experimental aquarium as in 2.2.4 was further used to record detailed observations of the hunting behaviour of *S. parva*, whereas for this experiment the sediment layer was removed. Additionally, a centimetre-grid was attached to the back of the aquarium as a size reference. Eleven adult *S. parva* which were not fed the night before and therefore fasted for approximately 43 to 48 h were individually placed in the aquarium. After 5 minutes of acclimatising, three to five mysid shrimps (*N. japonica*) were introduced into the aquarium and the hunting behaviour was filmed using a Sony PXW-FS5 with SEL-35F14Z lens (recording quality: 1080p/240fps). All recorded hunting events (n = 11; one per individual) were used to describe the general hunting behaviour in *S. parva*. Recordings of individuals hunting parallel to the camera along their dorsal plane (n = 3) were further analysed in terms of the positioning and distance of the sepiolid with respect to its prey.

By taking still images from the video material and inserting those into an image software (ImageJ 1.49v; https://imagej.nih.gov/ij/index.html; Schneider et al. (2012)), the DML of each sepiolid was measured. Subsequently, the hunting behaviour was analysed frame-by-frame using the latter image software, and the following characteristics were evaluated: (1) attack distance (AD; *sensu* Chen et al. (1996) and Sugimoto & Ikeda (2013)), defined as the distance between the distal tip of the tentacle and the targeted body part of the prey just before striking; (2) tentacle length during approach (TLA), defined as the length of the tentacles from their distal tips to their proximal end just before striking; (3) tentacle length during strike (TLS), defined as the length of the tentacles from their distal tips to their proximal end at the moment of prey capture, (4) tentacular strike speed (TSS; *sensu* Sugimoto & Ikeda (2013)), defined as the maximum elongation speed of the tentacles in the strike phase, calculated by the longest distance travelled by the tip of the tentacles within two consecutive frames (Figure 2.1). All these variables (1-4) were normalised to the DML of the corresponding sepiolid.

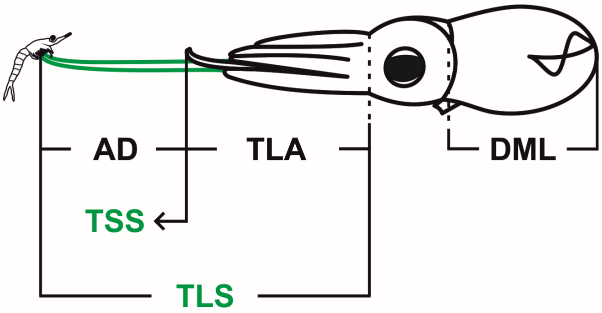

Figure 2.1 – Display of hunting behaviour variables (modified from Sugimoto & Ikeda, 2013). Measurements of attack distance (AD), tentacle length at approach (TLA), dorsal mantle length (DML), tentacular strike speed (TSS) and tentacle length at strike (TLS). See text for definitions.

2.2.6 – Video analysis

Video material gathered from all experiments (2.2.3 – 2.2.5) was analysed using the open-source software 'Behavioural Observation Interactive Research Software' (BORIS; http://www.boris.unito.it/; Friard & Gamba (2016)). In order to analyse the obtained video material of this study, an ethogram of potential behaviours of *S. parva* was established based on known behaviours and observed events from prior studies on other sepiolids as well as relatives such as cuttlefish (Sepiidae), bottletail squids (Sepiadaridae) and pygmy squids (Idiosepiidae) (simplified in Table 2.3, but see also Appendix Table 4.1 and 4.2). When new or altered behaviours were observed while analysing the video material, these were added to our ethogram for further consideration. All observations were collected and analysed by the same observer. The obtained BORIS-output from the time budget analysis as well as the burying and hunting observations was further imported into the open-source software 'Behatrix' (http://www.boris.unito.it/pages/behatrix) to analyse the corresponding behavioural sequences in terms of their transition probabilities and establish the latter in kinematic diagrams.

2.2.7 – Statistical analysis

All acquired data sets of this study were examined for potential outliers by using the 1.5 x IQR (interquartile range) rule (Tukey, 1977). If outliers were found, the mean ± standard deviation (SD) for both respective data sets (including and excluding outliers) were calculated.

The time budget data sets were tested for both normal distribution and homogeneity of variances using the Shapiro-Wilk and Levene's tests (Zar, 1999), respectively. As a normal distribution and a homogeneity of variances was achieved, an independent sample t-test (Zar, 1999) was used to establish potential differences among the mean time budgets of both tested groups (containing two and six individuals, respectively).

Potential body-size related relationships in the burying behaviour of *S. parva* were determined by testing each data set to meet the assumptions of a linear regression model (Quinn & Keough, 2002) and subsequently analysing the corresponding linear regressions of each data set against the DML. All above-mentioned statistical analyses were performed using SPSS® 21 (IBM, Armonk, New York, USA).

The significance of each transition probability in all presented kinematic diagrams was determined by running Behatrix' random permutation test with 100,000 permutations.

Statistical significance in all conducted test in this study was considered for $p < 0.05$.

2.3 – Results

2.3.1 – Habitat preference

All individuals of *S. parva* encountered in this study were found in shallow, coastal areas between 0.5 to 8 m depth, both on sandy bottoms and hard substrates. Sandy areas abundant in sepiolids were commonly not vegetated and had ripple marks, whereas areas of hard substrates mainly consisted of pebbles, shells and especially broken coral pieces. The water temperature during the survey period in February and March was on average 21.75 ± 0.53 °C, varying from 20 to 23 °C. While adult sepiolids were mainly found close to the bottom at night-time (with occasional findings in the water column and subsurface area), hatchlings and juveniles were usually encountered in the water column.

2.3.2 – Activity patterns and time budgets

The observed activity patterns and time budgets (Figure 2.2, 2.3 and 2.4, respectively) all indicate that *S. parva* spends daytime mostly buried in sediment and emerges shortly before sunset. However, brief observations of unburied animals during the day were made (Figure 2.2A-C; Figure 2.3B1). In general, 95 % of the observed animals tend to be active during the whole night (Figure 2.2; Figure 2.3), despite 29 % of them buried themselves during night-time for short periods of less than 1 h, followed again by an active phase (Figure 2.2B-C; Figure 2.3B5). While *S. parva* generally started to bury itself in the sediment from sunrise on (= end of active phase), 29 % of the observed individuals were still active in light conditions after switching on the aquarium lights (Figure 2.2A-C, Figure 2.3A-B), with one individual even unburying itself, swimming to another location within the aquarium and reburying itself immediately (Figure 2.3B3).

By analysing the individual time budgets of 8 sepiolids (Figure 2.3A-B), it was established that within 24 h *S. parva* spends on average 41.4 ± 1.2 % buried in the sediment (Table 2.4). While this value was moderately consistent in all 8 sepiolids, ranging from 40.0 to 43.5 % (Figure 2.3C), individual discrepancies were observed in the three remaining investigated state events. While *S. parva* was on average partly buried for 27.0 ± 15.9 % within 24h (Table 2.4), individual values reached from 7.3 up to 48.4 % (Figure 2.3C). Similar observations were taken for the average time of *S. parva* being located on the sediment, ranging from 6.0 to 38.7% within 24h (Figure 2.3C) with an average time of 22.6 ± 14.5 % (Table 2.4). While *S. parva* spend on average 9.0 ± 4.6 % in the water column (Table 2.4), individual values varied from 3.9 to 16.1 % (Figure 2.3C). No significant differences were observed in the average time budgets when keeping two (Figure 2.3A) or six individuals (Figure 2.3B) in the aquarium (Table 2.5).

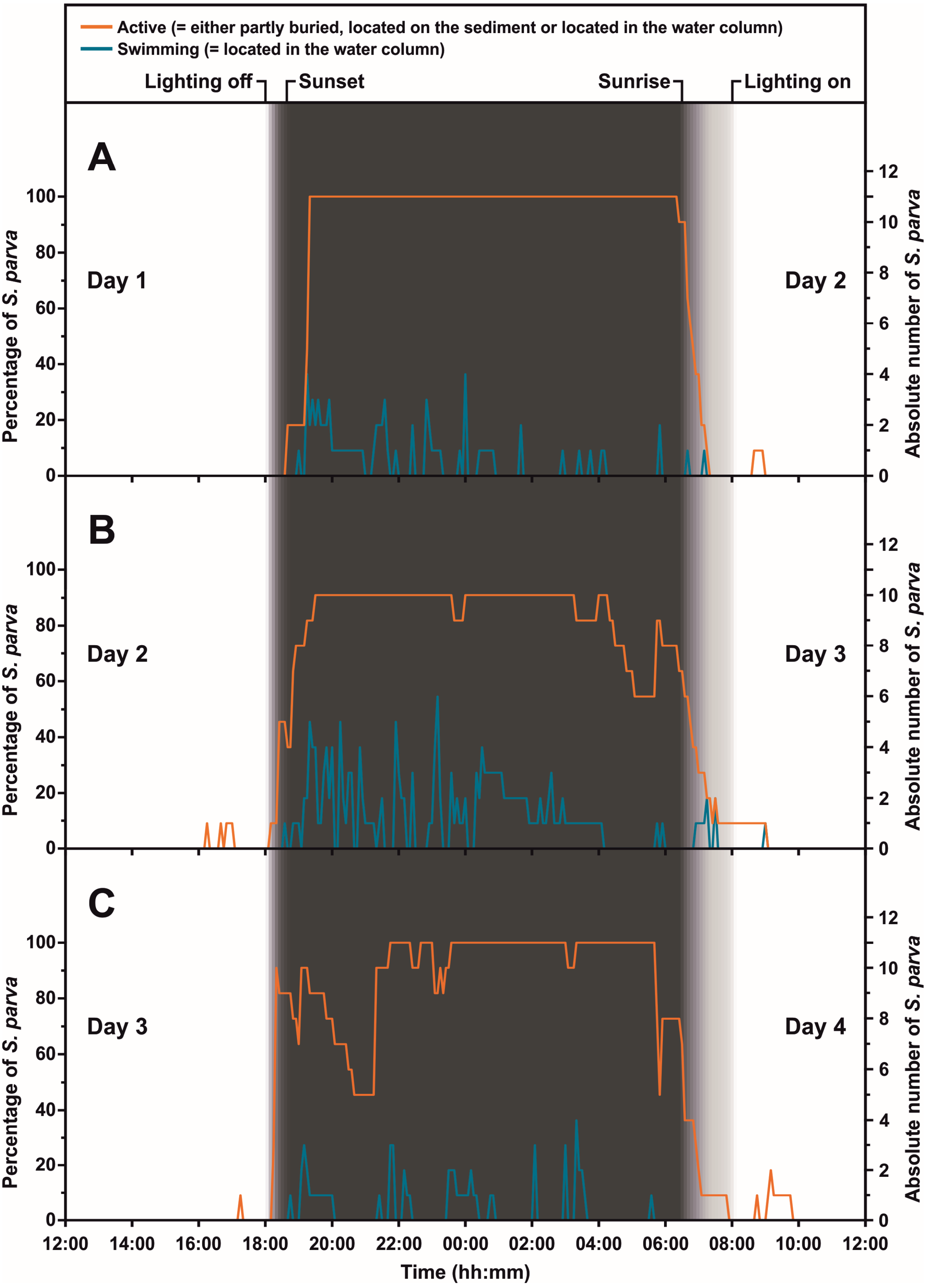

Figure 2.2 – Activity and swimming pattern of *Sepiola parva*. **(A-C)** Percentages and absolute numbers of active (= either party buried, located on the sediment or located in located in the water column) and swimming (= located in the water column) individuals of a group of eleven sepiolids (six females, five males) for three consecutive periods of 24 h (12:00 noon to 12:00 noon of the consecutive day). **(A)** Day 1 to Day 2, **(B)** Day 2 to Day 3 and **(C)** Day 3 to Day 4.

The time budgets results of the eight observed sepiolids were further analysed for their overall average percentage within 1 h increments, resulting in a prominent pattern (Figure 2.4). As already concluded from Figure 2.2 and 2.3, the observed bobtail squids started to become active between 18:00 and 19:00 h and spent a significant amount of time in the water column between 19:00 to 0:00 h, with some individuals swimming continuously up to 166.48 (Figure 2.3B2) or 170.95 min (Figure 2.3B3). During the same timeframe, resting animals also tended to spend more time on the sediment rather than being partly buried into the latter. From 0:00 h on, the time spent in the water column reduced for the greater part, and the observed sepiolids were mostly located on the sediment or partly buried into it. From 5:00 h on, more and more resting bobtail squids preferred to be partially buried and the average percentage of swimming individuals declined to values lower than 5 %. The average percentage of animals being buried increased in turn drastically from 8:00 h onwards with 33 % between 8:00 to 9:00 h, 98 % between 9:00 to 10:00 h and eventually 100 % from 10:00 h onwards (Figure 2.5).

Table 2.4 – Average values of individual time budgets

			Active		
Group	**n**	**Buried**	**Partly buried**	**Located on sediment**	**Located in water column**
A*	2	41.8 ± 1.1 %	28.6 ± 28.0 %	22.7 ± 22.6 %	6.9 ± 4.2 %
B$^+$	6	41.3 ± 1.3 %	26.5 ± 14.0 %	22.5 ± 13.9 %	9.7 ± 4.9 %
Total	8	41.4 ± 1.2 %	27.0 ± 15.9 %	22.6 ± 14.5 %	9.0 ± 4.6 %

*Group A consists of the two individuals depicted in Figure 2.3A. $^+$Group B consists of 6 the six individuals depicted in Figure 2.3B.

Table 2.5 – Statistical analysis of differences between the average time budgets of group A (consisting of 2 individuals) and group B (consisting of 6 individuals), as shown in Figure 2.3

Activity	**Test**	***t***	***df***	***p***
Buried	Independent sample t test	0.470	6	0.655
Partly buried		0.149	6	0.887
Located on sediment		0.014	6	0.989
Located in water column		- 0.714	6	0.502

df is an abbreviation for degrees of freedom.

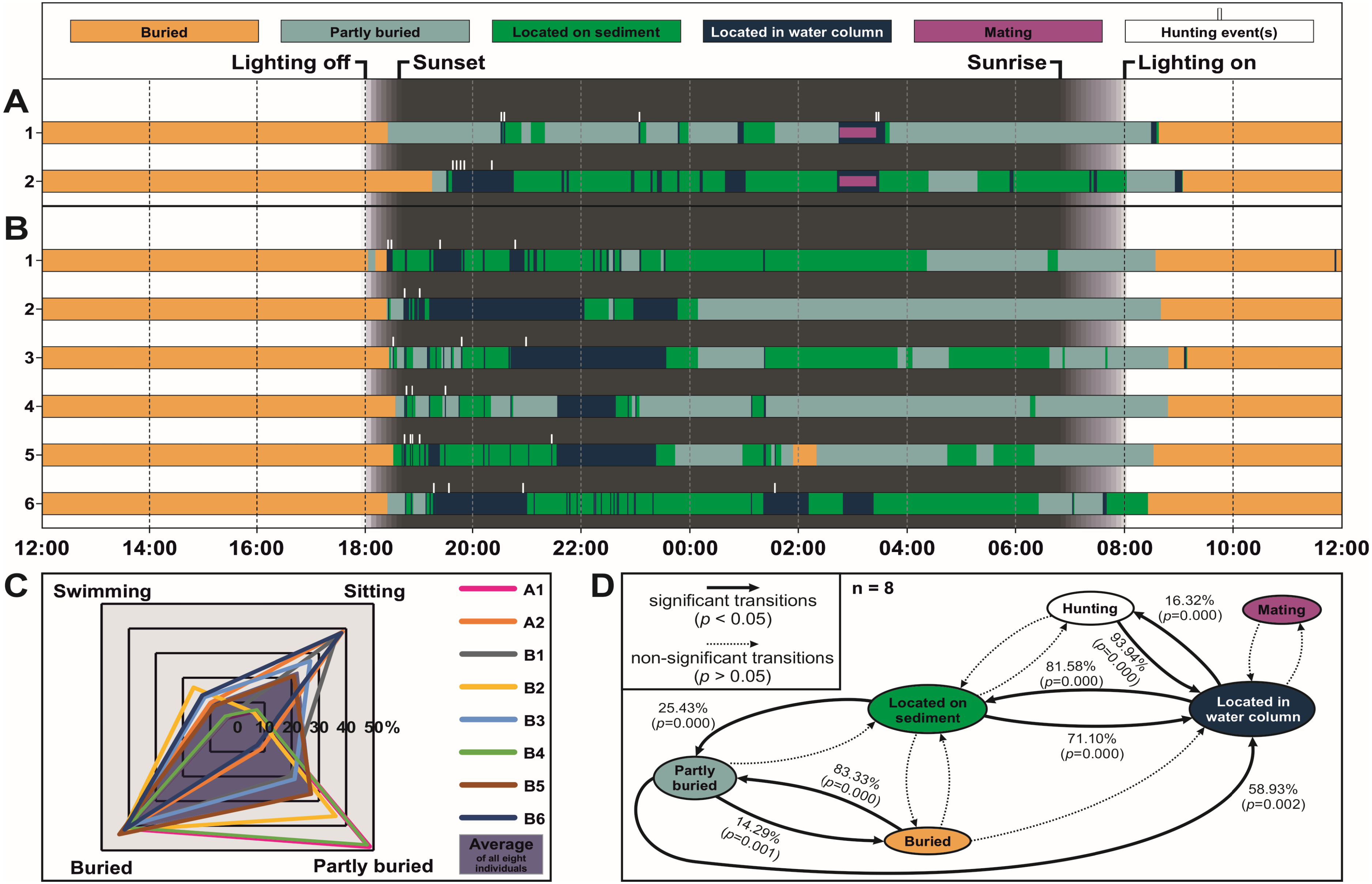
Buried
Partly buried
Located on sediment
Located in water column
Mating
Hunting event(s)
Lighting off
Sunset
Sunrise
Lighting on
A
1
2
B
1
2
3
4
5
6
12:00
14:00
16:00
18:00
20:00
22:00
00:00
02:00
04:00
06:00
08:00
10:00
12:00
C
Swimming
Sitting
Buried
Partly buried
0
10
20
30
40
50%
A1
A2
B1
B2
B3
B4
B5
B6
Average
of all eight individuals
D
significant transitions
(p < 0.05)
non-significant transitions
(p > 0.05)
n = 8
Hunting
Mating
Located on sediment
Located in water column
Partly buried
Buried
16.32%
(p=0.000)
93.94%
(p=0.000)
81.58%
(p=0.000)
71.10%
(p=0.000)
25.43%
(p=0.000)
83.33%
(p=0.000)
14.29%
(p=0.001)
58.93%
(p=0.002)

Figure 2.3 – Daily time budgets and hunting events of *Sepiola parva*. **(A-B)** Time budgets and hunting events of **(A)** two and **(B)** six individuals within a period of 24 h. **(C)** Percentual time budgets of the observed 24 h period for all individuals from **(A)** and **(B)** including the average value. **(D)** Kinematic diagram displaying significant and non-significant transitions of the daily activities in *S. parva*. For significant transitions, the corresponding transition probabilities are presented in %.

The kinematic diagram (Figure 2.3D) summarises the overall organisation of the above-mentioned behavioural observations. By focussing on the significant ($p < 0.5$) transitions only, a pattern could be observed. Buried sepiolids became partly buried (83.33 %) and entered the water column from the latter position (58.93 %). When located in the water column, the sepiolids either returned to the sediment (81.58 %) or hunting events took place (16.32 %). From the sediment, they either returned to the water column (71.10 %) or partly buried themselves into the sediment (25.43 %). From the latter position, they either reverted to the water column (again 58.93 %) or buried themselves completely (14.29%) (Figure 2.3D).

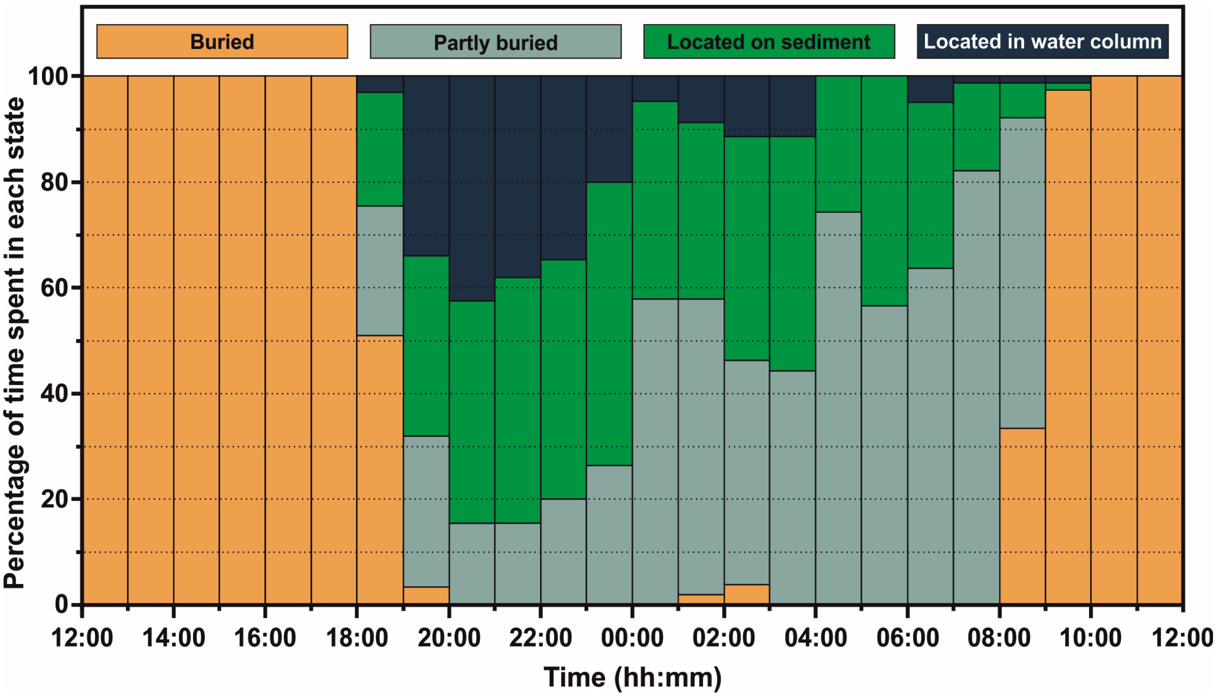

Figure 2.4 – Average behavioural activity per hour for 8 observed individuals of *Sepiola parva* within 24 h.

2.3.3 – Burying behaviour

Of the 20 tested sepiolids, 19 individuals (95.0 %) proceeded to bury themselves after being introduced into the aquarium within the observation limit of 10 min. For the subsequent analysis of their burying behaviour, only the observations on these 19 individuals will be considered. Due to the presence of outliers in all investigated burying characteristics except the total number of arm sweeps, results in this section will be presented excluding outliers, whereas in Table 2.6 both the results including and excluding outliers are provided.

S. parva spent in average 12.65 ± 7.87 s in the water column and 140.50 ± 129.38 s resting on the sediment before burying, resulting in a total average latency of 141.18 ± 115.65 s (Table 2.6). All tested *S. parva* exhibited their typical dark body colouration before burying (Figure 2.5A- B) which displayed a strong contrast to the whitish sediment.

The burying procedure was relatively consistent among all tested sepiolids. Phase 1 (*sensu* Boletzky & Boletzky, 1970) started with the creation of a depression in the sediment by tilting the body slightly forward and ejecting a gentle, forward-directed funnel jet (Figure 2.5C-D), whereas smaller individuals of *S. parva* were observed to remove coarser sediment with their arms beforehand. The depression then served as a hold for a strong, backward-directed funnel jet to swirl up sediment from the depression. While the sediment was dispersed in the water column, the sepiolid immersed its body in the depression, and the descending sediment particles covered the dorsal area of its body. This behaviour was followed by further alternating forward- and backward directed funnel jets to almost completely cover the animal's body with sediment (Figure 2.5E-F), whereas smaller individuals of *S. parva* were observed to occasionally remove coarser sediment simultaneously with a forward directed funnel jet. This behavioural sequence (creation of depression and subsequent funnel jets) took in average 9.19 ± 2.71 s (Table 2.6). Moreover, the alternating forward- and backward-directed funnel jets can be grouped in sets, as any burying behaviour in the first phase always started with a forward-directed funnel jet, directly followed by a backward-directed funnel jet. This behaviour was observed both at the beginning of phase 1 as well as after each resting period during this phase. Hence, the total number of funnel jets was even in all tested sepiolids (100 %). Additionally, one individual which was not considered for this burying analysis was observed to stop in between a set of funnel jets due to a disturbance (sudden movement in front of the experimental aquarium) and continued its burying behaviour with another forward-directed funnel jet, which supports the aforementioned observations. In total 17 out of the 19 tested sepiolids (89.5 %) conducted either 6 or 8 funnel jets, resulting in 7 ± 1.23 funnel jets on average (Table 2.6).

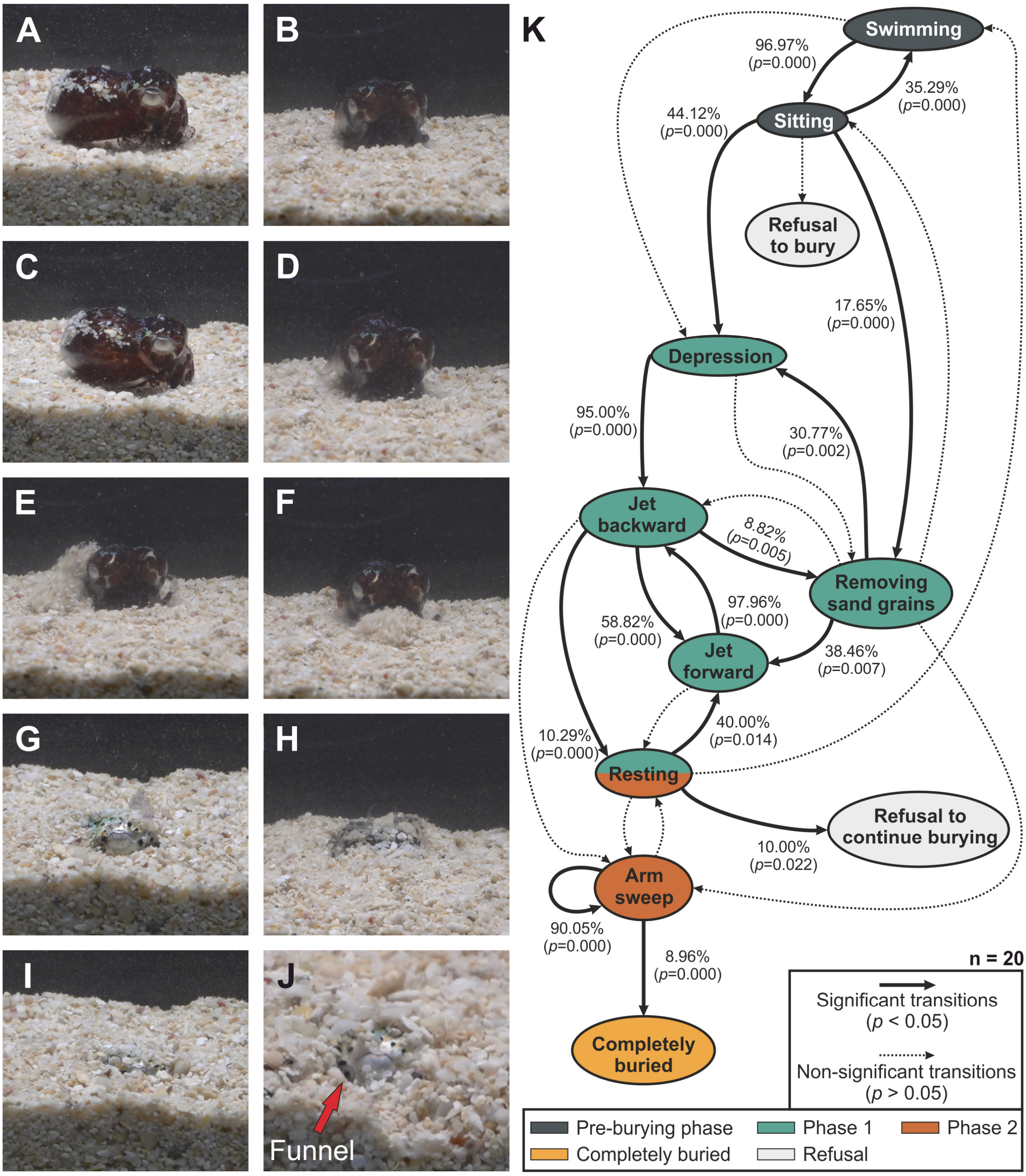

Figure 2.5 – Burying behaviour in *Sepiola parva.* After **(A, B)** settling on the sediment, the burying procedure starts with **(C, D)** the formation of a depression by tilting the body slightly forwards and ejecting a gentle forward-directed water jet. This depression serves as a hold for subsequent **(E, F)** alternating backward- and forward-directed water jets to cover nearly the whole body with sediment. Afterwards, the sepiolid starts **(G, H)** a series of arm sweeps to gather sediment from its circular vicinity to cover the remaining body parts. **(I, J)** Nearly fully buried animal with the positioning of the funnel for respiration shown in **(J)**. **(K)** Kinematic diagram displaying significant and non-significant transitions of the burying behaviour in *S. parva.* For significant transitions, the corresponding transition probabilities are presented in %.

Table 2.6 – Observations of the burying behaviour and the corresponding statistical analysis of correlation between the observations of each burying procedure and the DML of the tested sepiolids

Observation		**Observational data**		**Observational results**			**Linear regression**				
		Outliers	n	Ø ± SD	Min.	Max.	df_{reg}	df_{res}	F	p	r^2
Pre-Burying	Total time spent in water column (s)	yes	19	19.68 ± 25.44	2	116	1	17	0.325	0.576	0.019
		excluded	17	12.65 ± 7.87	2	25	1	15	2.312	0.149	0.134
	Total time spent on sediment (s)	yes	19	164.00 ± 162.18	5	587	1	17	1.276	0.274	0.070
		excluded	18	140.50 ± 129.38	5	460	1	16	1.205	0.289	0.070
	Latency until start of burying (s)	yes	19	183.68 ± 168.39	48	593	1	17	1.393	0.254	0.076
		excluded	17	141.18 ± 115.65	48	479	1	15	0.213	0.651	0.014
Phase 1	Duration (s)	yes	19	23.89 ± 40.89	4	140	1	17	5.838	**0.027**	0.256
		excluded	16	9.19 ± 2.71	4	13	1	14	0.336	0.572	0.023
	Total number of funnel jets	yes	19	7.16 ± 1.67	4	12	1	17	3.696	0.071	0.179
		excluded	18	6.89 ± 1.23	4	8	1	16	1.446	0.247	0.083
Time between Phase 1 and Phase 2 (s)		yes	19	11.21 ± 42.90	0	188	1	17	2.117	0.164	0.111
		excluded	15	0.27 ± 0.59	0	2	1	13	0.635	0.440	0.047
Phase 2	Duration (s)	yes	19	12.58 ± 3.24	8	20	1	17	0.838	0.373	0.047
		excluded	18	12.17 ± 2.77	8	17	1	16	3.003	0.102	0.158
	Total number of arm sweeps	no	19	11.32 ± 3.28	6	18	1	17	1.159	0.297	0.064
Total burying duration (s)		yes	19	47.68 ± 56.42	16	205	1	17	11.304	**0.004**	0.399
		excluded	16	24.38 ± 6.29	16	42	1	14	1.164	0.299	0.077

Ø + SD are an abbreviation for mean value and its corresponding standard deviation. Min and Max are abbreviations for minimal and maximal value, respectively. df_{reg} and df_{res} are abbreviations for degrees of freedom of the regression and residuals, respectively. Significant *p*-values are marked boldly.

The average resting time between phase 1 and phase 2 (*sensu* Boletzky & Boletzky, 1970) was 0.27 ± 0.59 s, and 12 of the tested sepiolids (63.2%) continued their burying procedure directly after their last funnel jet. After this potential resting time, phase 2 of the burying procedure (*sensu* Boletzky & Boletzky, 1970) started by stretching out a pair of dorsolateral arms above the sediment, followed by a series of sweeping arm movements to gather sediment from the circular vicinity in order to cover the remaining body parts (Figure 2.5G-H). If not altered by the presence of an obstacle (e.g. aquarium wall), these arm movements were always closely synchronous, whereby the arm tips were pointing medially towards the animal. All tested bobtail squids started their arm sweeps at the anterior end of their bodies and moved along both lateral axes until a posterolateral position. The average duration of the second phase was 12.17 ± 2.77 s and the number of arm sweeps ranged from 6 to 18 sweeps, with 11 ± 3.28 arm sweeps on average (Table 2.6).

Either by the end of the first phase or during the second phase, all observed sepiolids changed their body colouration to a pale background with dark chromatophores (Figure 2.5G-J). After being fully covered with sediment (Figure 2.5I), individuals of *S. parva* remained motionless except for positioning their funnel on either their left or right lateral side in order to maintain respiration (Figure 2.5J). The average duration for the whole burying procedure (phase 1 + phase 2 + the potential break in between) was 24.38 ± 6.29 s, ranging from 16 to 42 s.

No significant correlation with the DML was found for any obtained observations of the aforementioned burying characteristics (Table 2.6). Although admittedly for two of the burying characteristics a significant relationship between the corresponding data set and the DML was indicated (boldly marked in Table 2.6), the respective r^2 values displayed low correlation degrees only (Table 2.6).

The significant transitions ($p = 0.05$) of the kinematic diagram (Figure 2.5K) summarise the above-mentioned observations on the burying behaviour. After swimming, sepiolids settled on the sediment (96.97 %), followed by either returning to the water column (35.29 %) or starting the burying procedure by either creating a depression (44.12 %) or removing coarse sand grains (17.65 %), which in turn was then followed by the creation of a depression (30.77 %). The latter was followed by a backward-directed funnel jet (95.0 %). Subsequently, sets of forward and backward-directed funnel jets followed (58.82 and 97.96 %, respectively). In between these sets, sepiolids potentially rested after a backward-directed funnel jet (10.29 %) or removed more coarse sand grains (8.82 %), respectively followed by another forward-directed funnel jet (40.00 and 38.46 %, respectively) as part of set of forward-and backward-directed funnel jets. However, a resting period may have also led to a refusal to further continue burying (10.0 %).

No significant transition from phase 1 (depression + forward and backward-directed funnel jets) to phase 2 (arm sweeps) was established. Nevertheless, after the start of phase 2, an arm sweep was usually followed by another arm sweep (90.05 %) or the burying procedure ended as the animal was completely buried (8.96 %).

2.3.4 – Escape response

When encountered in the wild, *S. parva* displayed a variety of escape responses. The common means of escaping from the water column was ejecting a series of ink blobs, similar in size, shape and colouration to the corresponding individual, and subsequently jetting away in a straight or mild zig-zag line for about 2 m while maintaining their dark colouration. Alternatively, some individuals ejected an 'ink rope' (*sensu* Bush & Robison, 2007), approximately 4-5 times the length of the animal, and hold on to it motionless while maintaining a dark body colouration. However, the latter eventually let off the ink rope on closer approach and jetted away as well. Alternatively, they either moved to the sediment and started burying themselves or swam towards hard substrate such as rocks or corals and seek shelter under or in between those.

Bobtail squids sitting on the sediment or being partly buried generally showed two escape responses. Some individuals proceeded to further bury themselves but usually left their eyes uncovered while exhibiting a blue colouration between the latter (Figure 2.6A-B).

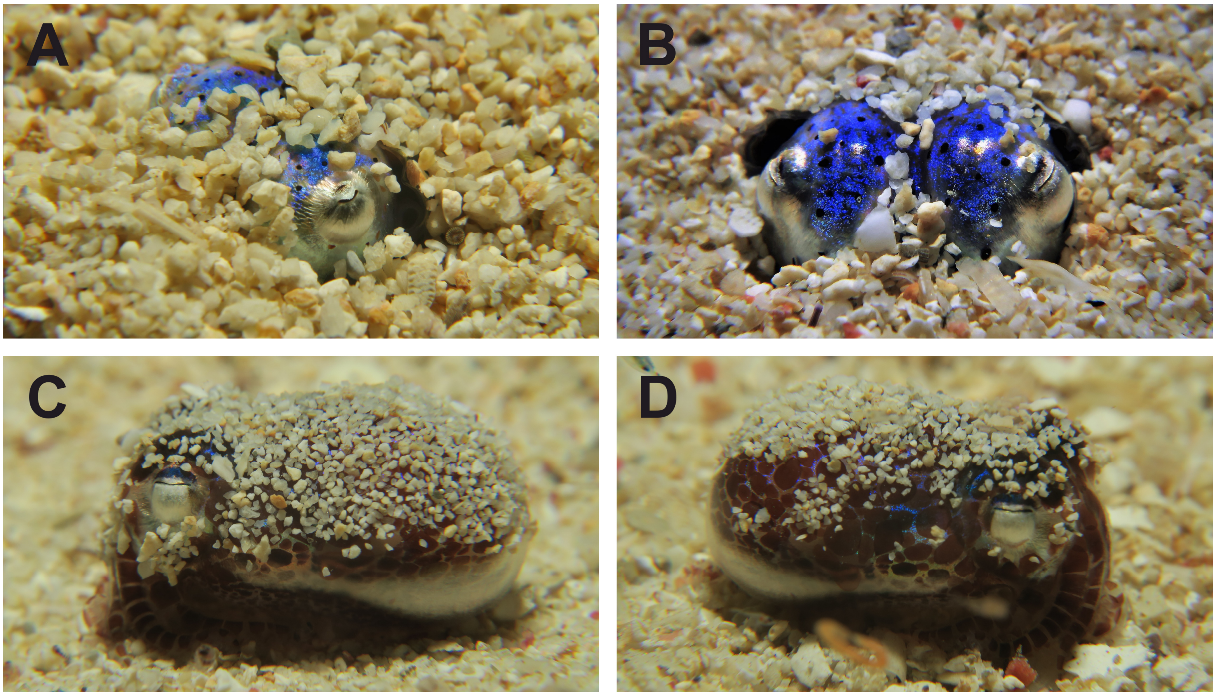

Figure 2.6 – Escape responses in *Sepiola parva* from the wild. **(A, B)** Blue colouration of buried individuals after escaping. **(C, D)** Sand grain layer (= sandcoat *sensu* Singley, 1982) after emerging from the sediment. **(A-D)** Photos taken by Christian Drerup.

Alternatively, some emerged roughly 10 cm from the sediment and swam away parallel to the bottom, either calmly or with abrupt water jets and potentially inking, and started to bury themselves again in the sediment, whereas some individuals also seek shelter under hard substrate, if available.

Individuals of *S. parva*, which have been (almost) completely buried, were frequently observed to keep the dorsal part of their bodies covered in a layer of sand grains when emerging from the sediment (Figure 2.6C-D). This layer could be maintained during calm body motions but often fell off when moving abruptly.

2.3.5 – Hunting & feeding behaviour

Hunting was observed both for sepiolids already situated in the water column for a longer period and sepiolids resting on the bottom of the aquarium, whereas the latter emerged to the water column shortly before hunting. Hunting behaviour was usually initiated by either active (swimming; 90.9 %) or passive (floating; 9.1 %) movements of the potential prey. The sepiolids then orientated themselves towards the prey so that the arms and tentacles were projected towards the latter, forming an elongated cone with the tips of the tentacles slightly protruding (Figure 2.7B-C). Afterwards, all tested *S. parva* swiftly approached the prey straight from their current location and positioned themselves for striking, whereas re-positioning was observed in one sepiolid (9.1 %) after it approached the prey too close. While in 90.9 % of the observations *S. parva* preferred to attack the prey upwards at about a 45 degree vertical angle, one individual (9.1 %) seized its prey close to the aquarium bottom using a horizontal approach. By characterising the attack distance (AD) and tentacle length at approach (TLA) for three individuals hunting parallel to the camera, a negative correlation regarding their DML was observed (Table 2.7).

After positioning, the sepiolids then seized the prey by striking their tentacles simultaneously towards the latter (Figure 2.7D-G). Either slightly before the tentacular strike or latest by the

Table 2.7 – Hunting characteristic analysis

	AD		TLA		TLS		TSS	
DML (mm)	**mm**	**Norm. to DML**	**mm**	**Norm. to DML**	**mm**	**Norm. to DML**	**mm/frame**	**m/s**
10.4	7.1	68 %	13.2	127 %	20.2	194 %	3.5	0.840
13.3	5.6	42 %	14.2	107 %	21.1	158 %	3.8	0.912
14.9	2.7	18 %	15.8	106 %	19.5	131 %	3.7	0.888

DML = dorsal mantle length. AD = attack distance. TLA = tentacle length at approach. TLS = tentacle length at strike. TSS = tentacular strike speed. Norm. to DML is an abbreviation for Normalised to dorsal mantle length. See text for definitions.

seizure of the prey, the still cone-formed arms were flung open (Figure 2.7H). The tentacle length at strike (TLS) for the three closer observed individuals again showed a negative correlation regarding their DML (Table 2.7). However, the tentacular strike speed of those three

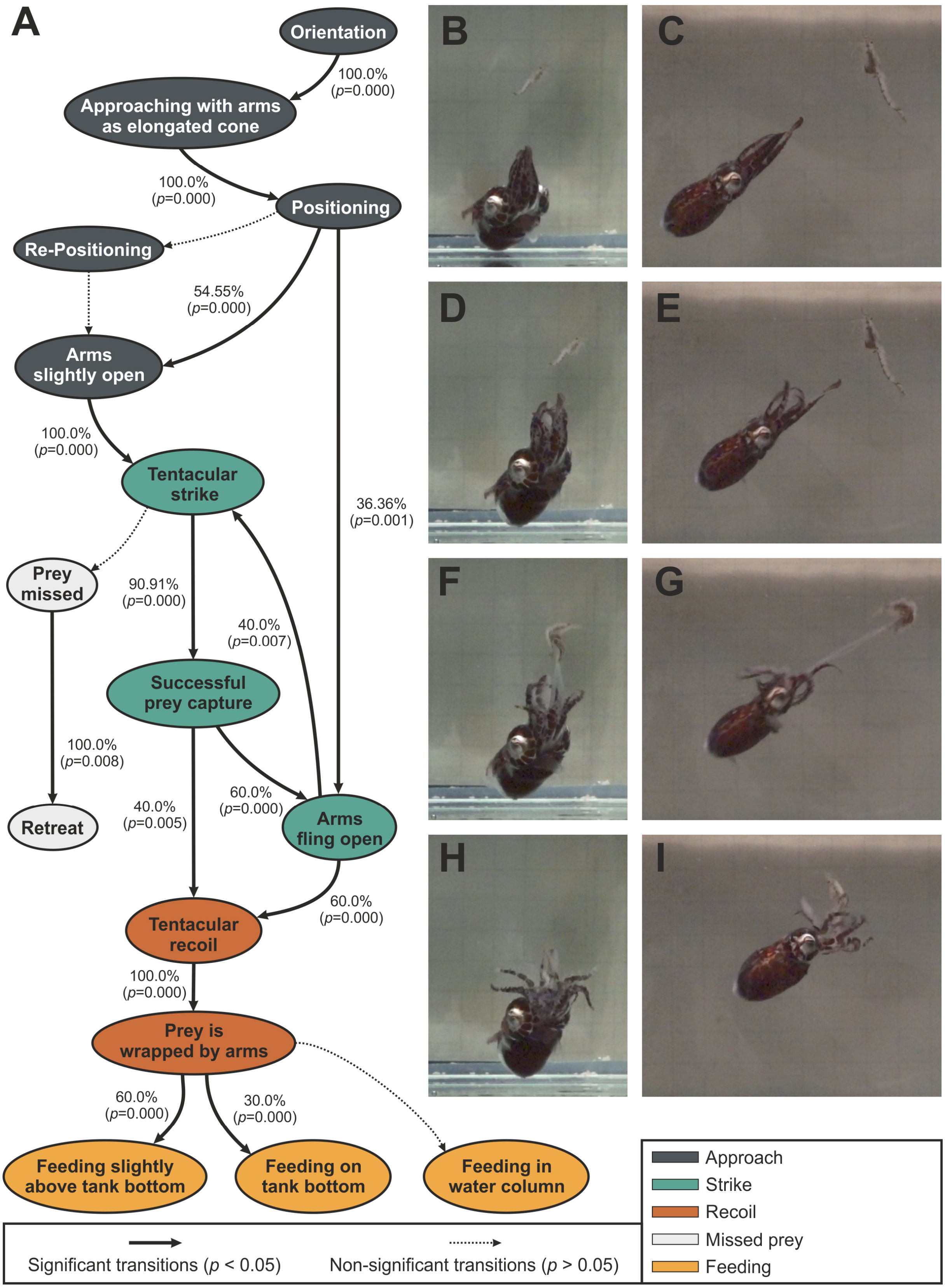

Figure 2.7 – Hunting behaviour in *Sepiola parva*. **(A)** Kinematic diagram displaying significant and non-significant transitions of the hunting behaviour in *S. parva.* For significant transitions, the corresponding transition probabilities are presented in %. **(B, C)** Approach: The sepiolid orientates itself with regards to its prey (in this case the mysid shrimp *Neomysis japonica*) and displays its hunting posture by pointing its arms formed as an elongated cone towards the prey. **(D, E, F, G)** Tentacular strike: The sepiolid **(D, E)** shoots its tentacle and **(F, G)** captures the prey successfully. **(H, I)** The sepiolid pulls the prey to its buccal cavity and wraps it with its arms.

sepiolids was remarkably close with 3.5, 3.7 and 3.8 mm/frame, resulting in a tentacular elongation of 0.840 to 0.912 m/s (Table 2.7). In the 11 observations, the prey (*N. japonica*) was caught 7 times by its abdomen (63.6 %) and 3 times by its carapax (27.3 %), with one sepiolid missing it with its tentacles (9.1 %), resulting in a capture success rate of 90.9 %. From the observations of 8 individuals of *S. parva* within 24h (Figure 2.3A-B), an average number of 3.9 ± 1.1 hunting events per night was established, with 90.3 % of them occurring before midnight. When the prey was successfully seized, the tentacles were retracted, pulling the prey into the buccal area where it was wrapped by the sepiolid arms and properly positioned for ingestion (Figure 2.7H-I). While the hectocotylus of male individuals was usually slightly splayed out, it could not be determined whether this was done intentionally or solely due to its bigger size compared to the remaining 7 arms. During the whole hunting procedure, the observed sepiolids maintained their dark body colourations. Individuals of *S. parva* were observed to ingest their prey either while hovering close to the aquarium bottom (60.0 %), resting on the latter (30.0 %) or swimming in the water column (10.0 %). While smaller mysid shrimps were usually fully consumed, the exoskeletons of larger individuals were commonly ejected, presumably after their contents had been devoured.

The significant transitions ($p < 0.05$) of the kinematic diagram (Figure 2.7A) summarise the above-mentioned observations on the hunting behaviour. After a sepiolid orientated itself towards the prey, it approached the latter with its arms formed as an elongated cone (100.0 %), followed by the positioning for the strike (100.0 %). Subsequently, the arms were either be slightly opened (54.55 %) or flung open (36.36 %), respectively, followed by the tentacular strike (100.0 and 40.0 %, respectively). After a successful prey capture (90.91 %), the tentacles were retracted (40.0 %) unless the arms were still only slightly opened. In this case, the arms were first flung open (60.0 %), which in turn was then followed by the tentacular recoil (60.0 %). After the retraction of the tentacles, the prey was wrapped by the arms (100.0 %) and the food was ingested either on the aquarium bottom (30.0 %) or slightly above the latter (60.0 %).

2.3.6 – Mating behaviour

A total of three full mating events were observed in the recorded aquarium within the 41 days of filming. Mating in this aquarium only occurred at the beginning of the recording period when stocking densities were low with a maximum of 3 sepiolids, corresponding to 24 animals/m². None of the observed mating events were accompanied by prior pair formation or any evident courtship behaviour. In all three events, the female sepiolid was swimming in the water column, whereas the male was either sitting on the sediment or partly buried into it. The male then approached the female by jetting towards her from below and initiated physical contact by grasping the female at her ventral mantle region. Subsequently, the male adjusted his position by shifting his grasp to the female's ventral neck area and copulation started (Figure 2.8). The three observed mating events lasted for 32, 35 and 40 min (average duration 35.66 ± 4.04 min), and each couple separated afterwards. In all three cases, the whole mating act took place in the water column, with neither the male nor the female touching the bottom at any point.

Furthermore, three additional initial mating events were recorded. However, shortly after the start of the copulation (4, 6 and 20 s), a second male interfered by jetting towards the pair and initiating physical contact. In all three cases, these disruptions took less than 5 s, resulting in the separation of all three sepiolids.

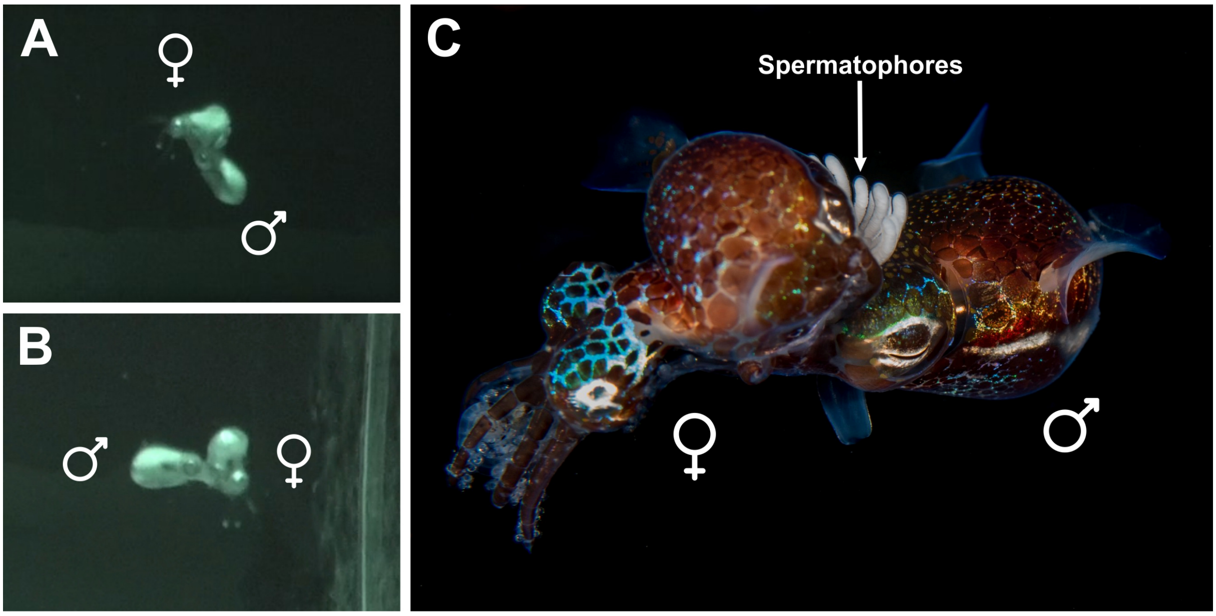

Figure 2.8 – Mating behaviour in *Sepiola parva*. **(A, B)** Still images from the copulation of two pairs from the recording aquarium. **(C)** Photograph of mating event from the field with the male transferring spermatophores (indicated) to the female (© Matt Rudoph).

2.3.7 – Spawning behaviour & egg characteristics

One spawning event was observed during the recording period. A total of 47 droplet-shaped eggs were laid at night-time by one female on the underside of a clay-based flowerpot. All eggs were individually attached to the flowerpot (Figure 2.9A) and covered in a sand grain layer (Figure 2.9A-B). However, the exact spawning duration and whether the sand grain layer was added intentionally could not be determined due to the video resolution. After this spawning event, no further maternal care was observed. All eggs remained in the aquarium for 22 days (water quality parameters in Table 2.1) before being moved to a 2 L acrylic tank (20 cm long x 10 cm wide x 10 cm deep; water temperature 20.0°C; pH of 8.3; salinity of 35 ppt; photoperiod 12:12 LD) on the main campus of the Okinawa Institute of Science and Technology which was supplied by a constant water flow from a 500 L water system with a filtration system containing a UV sterilizer, 2 protein skimmers, crushed corals and a 200 micron sock filter. After four days in the latter tank (26 days in total), the diameter of each egg capsule (excluding adhered sand grains; Figure 2.9C) reached ~ 2 mm and hatching started (Figure 2.9D).

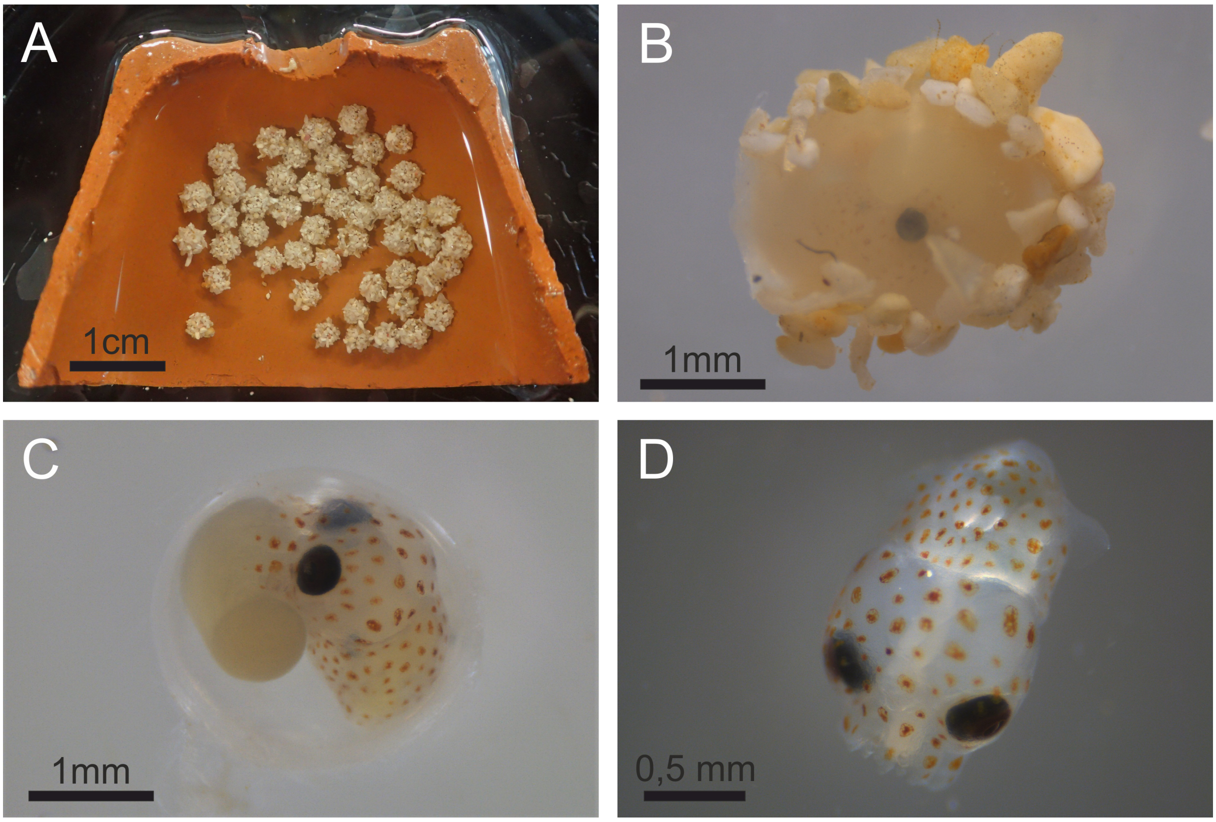

Figure 2.9 – Eggs and hatchlings of *Sepiola parva*. **(A)** A spawning batch of a single individual, containing 47 droplet-shaped eggs which were laid on the underside of its artificial shelter, a clay-based flowerpot. **(B)** A single egg with its outermost layer covered in sand grains. The part without any sand grains corresponds to the attachment point on the flowerpot **(C)** The same egg with the sand grains removed. **(D)** A newly hatched juvenile of *S. parva*.

2.4 – Discussion

The behavioural ecology of *S. parva* mostly resembled those of previously investigated bobtail squids. However, a few variances in its behaviour or even undescribed behavioural aspects compared to other sepiolid species were observed in this study. As to the best of our knowledge no peer-reviewed literature about the ecology of *S. parva* exists, the only available information about this species' habitat preference can be found in the FAO guides (Reid & Norman, 1998; Reid & Jereb, 2005) and in Okutani (2015). According to these authors, *S. parva* prefers a hard substrate, unlike the closely resembling species *S. birostrata* which usually occurs on sandy bottoms. Generally, most observations on other sepiolid species also reported their occurrence on sandy (e.g. Anderson, 1997; Anderson & Mather, 1996; Anderson et al., 2002, 2004; Arnold et al., 1972; Jones & Richardson, 2012; Kimbell et al., 2002; Mauris, 1989; Rodrigues et al., 2009, 2010, 2011a, 2011b, 2012) or muddy substrates (Anderson, 1987, 1997; Arnold et al., 1972; Jones & Richardson, 2012), whereas a habitat with at least occasional hard substrates was only mentioned by Moynihan (1983) and Anderson & Mather (1996) for *E. scolopes*. However, during our field surveys, individuals of *S. parva* were found both on unvegetated sand patches as well as in areas with hard substrates, demonstrating that this species can be found in both types of habitat.

The observed discrepancy between hatchlings and juveniles of *S. parva* being largely found in the water column during SCUBA surveys, contrarily to adult individuals being mainly found closer to the bottom, resembles the field observations of Anderson & Mather (1996) for *E. scolopes*. As shown in laboratory rearing conditions for *E. scolopes* (Hanlon et al., 1997) and *E. hyllebergi* (Nabhitabhata et al., 2005), hatchlings of the latter two species were actively planktonic during the first 30 days of their life which might explain the aforementioned discrepancy in *S. parva*.

From the established activity patterns and individual time budgets, *S. parva* can be considered nocturnal, with occasional activity in light conditions. Similar observations were made for *E. scolopes* (Hanlon et al., 1997; Moynihan, 1983; Shears, 1988) and *E. hyllebergi* (Nabhitabhata et al., 2005). Shears (1988) further reported that approximately 96 % of *E. scolopes'* activity was nocturnal, with activity peaks just after sunset and just before sunrise. Although the author's data cannot be directly compared to our results due to another definition of an 'activity event', it still underlines the nocturnal lifestyle of sepiolids in general. However, 9% of the individuals of *S. parva* in the present study showed behavioural activities shortly before switching off the aquarium lighting and 29 % of them after switching on the aquarium lighting.

While activity shortly before switching off the aquarium lighting can potentially be explained by the corresponding feeding time in this study (as suddenly available food potentially triggered hungry individuals to leave the sediment and start foraging) (Hanlon et al., 1997), no clear evidence was found explaining any general activity after sunrise (approximately 6:30 am) and especially after switching on the aquarium lighting (8:00 am). Bobtail squids of the genus *Sepiola* are known to possess light organs (Fidopiastis et al., 1998; Herring et al., 1981; Kishitani, 1928) like the well-studied sepiolid *Euprymna scolopes* (McFall-Ngai & Montgomery, 1990; Nyholm & McFall-Ngai, 2004; Ruby, 1996). These light organs are used at night to emit ventrally directed luminescence matching the downwelling moonlight or starlight and thereby obscuring their shadow to reduce the risk of predation, a behaviour commonly known as counterillumination (Jones & Nishiguchi, 2004; Stabb, 2006). As this behaviour will be less effective during daytime as light levels will potentially be too bright to efficiently obscure their shadow (Jones & Nishiguchi, 2004), *S. parva* might become more prone to predation in light conditions. Although the aquarium was already mildly exposed to ambient sunlight before switching on the lighting in the morning, these light levels seem to be too low to trigger the burying behaviour of some of the recorded sepiolids. However, as all individuals of *S. parva* were already hold in captivity for at least 9 days before the activity and time budget recordings, some individuals might have habituated to their new environment going along with a different photoperiod and the absence of predators in general (Fiorito et al. 2015). For other nocturnal cephalopods, there are to the best of our knowledge barely any observations of activity in light conditions. In the few studies on activity patterns of the latter, individuals of the octopus species *Eledone cirrhosa* (Cobb et al., 1995a, 1995b), *Octopus macropus* (Meisel et al., 2006) and *Octopus vulgaris* (Brown et al., 2006; Kayes, 1973; Wells et al., 1983; but see also Meisel et al., 2006) were observed to return to their dens shortly after sunrise while showing little to no activity during daylight, potentially using these resting period for sleeping (Brown et al., 2006; Meisel et al., 2011). However, activity patterns of cephalopods might change throughout the year based on environmental synchronizers, as shown for the cuttlefish *S. officinalis* by Oliveira et al. (2017). Hence, further research is needed to understand any bobtail squid activity in light conditions, especially those which is not caused by hunting behaviour.

The burying performance of *S. parva* resembled the observations reported for other sepiolids of the genera *Rossia*, *Sepietta* and *Sepiola* (Anderson et al., 2004; Boletzky & Boletzky, 1970; Rodrigues et al., 2010), whereas minor differences were found compared to species of the genus *Euprymna* (Anderson et al., 2002; Moynihan, 1983; Nabhitabhata et al., 2005). While *S. parva* (present study) as well as *R. pacifica* (Anderson et al., 2004), *S. atlantica* (Rodrigues et al., 2010) and a variety of other tested *Sepietta* spp. and *Sepiola* spp. (Boletzky & Boletzky, 1970) started their burying behaviour with a forward-directed funnel jet, an initial backward-directed funnel jet was reported in *E. hyllebergi* (Nabhitabhata et al., 2005) and *E. scolopes* (Anderson et al., 2002). Additionally, *S. parva* was observed to use its dorsolateral arms during phase 2 of its burying behaviour, as identically reported for the above mentioned species of the genera *Rossia*, *Sepietta* and *Sepiola* (Anderson et al., 2004; Boletzky & Boletzky, 1970; Rodrigues et al., 2010). Contrarily to these observations, Nabhitabhata et al. (2005) stated that *E. hyllebergi* rather used its ventrolateral arms during phase 2 of its burying. While Anderson et al. (2002) did not mentioned the arm use preference of *E. scolopes* in their study, Moynihan (1983) reported for this species even the use of its long tentacles for sweeping sand during phase 2 of its burying behaviour. However, it might be possible that the latter author mistook the tentacles for the actual arms of *E. scolopes* due to their thin appearance while being stretched out, as generally postulated before by Boletzky & Boletzky (1970).

The recorded average times of the tested individuals of *S. parva* spent in the water column and on the sediment before the start of burying in the present study (12.65 ± 7.87 s and 140.50 ± 129.38 s, respectively) were comparable to those of *R. pacifica* (17 s and 222 s, respectively; Anderson et al., 2004), whereas *E. scolopes* (13 and 13 s, respectively; Anderson et al., 2002) spent on average significantly less time on the sediment. However, the latency until the start of burying must be interpreted with due caution since a single animal might show different reactions when exposed to the same sediment for several times (Boletzky & Boletzky, 1970). Additionally, the initially shown behaviour of sepiolids introduced to an experimental tank might be heavily altered by their previous level of handling, their corresponding stress level or simply by the experimental setup itself, e.g. the size of the used tank which varied between 1.3 L in the present study and 40 L in both studies of Anderson et al. (2002, 2004).

The first phase of the burying behaviour of *S. parva* in the present study took on average 9.19 ± 2.71 s, and the observed sepiolids used 7 ± 1.23 funnel jets. This resembles similar values as recorded by Rodrigues et al. (2010) for *S. atlantica* (12.2 ± 4.37 s with 6.3 jets on average), whereas the slightly longer duration could potentially be explained by a different sediment type or grain size distribution. The latter authors also observed no pause between the first and second phase of *S. atlantica*'s burying behaviour, which is similar to the observation for *S. parva* in the present study with an average resting time of 0.27 ± 0.59 s between both phases. However, these two observations differ from the on average 83 s long pause observed in *Rossia pacifica* (Anderson et al., 2004). Although the duration of phase 2 as well as the total duration of *S.*

parva's burying behaviour in this study (12.17 ± 2.77 s and 24.38 ± 6.29 s, respectively) was similar to those recorded for *S. atlantica* (10.2 ± 2.95 s and 21.9 ± 4.93 s, respectively; Rodrigues et al., 2010), the average number of arm sweeps in the present was nearly twice as high (11.32 ± 3.28) as reported for *S. atlantica* (6.00 ± 0.79) by Rodrigues et al. (2010). This indicates a higher arm sweep frequency in *S. parva* compared to *S. atlantica*. However, no hard evidence was found to explain this observation.

Boletzky & Boletzky (1970) further reported that in species of the genus *Sepiola* the first phase of the burying behaviour is longer than the second one. While this was later confirmed for *S. atlantica* by Rodrigues et al. (2010), the present results cannot confirm these observations (phase 1: 9.19 ± 2.71 s; phase 2: 12.17 ± 2.77 s). Furthermore, Boletzky & Boletzky (1970) stated that with an increasing DML the duration of the first phase declines whereas the second phase extends in *Sepiola robusta*. While Rodrigues et al. (2010) reported no significant relationship between the burying behaviour of *S. atlantica* and its DML, the results of the present study also cannot confirm those observations by Boletzky & Boletzky (1970) as no correlation between the DML and any of the observed burying characteristics was found. Moreover, previous studies document a variety of pre- or post-burial behaviours among sepiolids. Anderson et al. (2004) and Rodrigues et al. (2010) documented an 'alert posture' in *R. pacifica* and *S. atlantica*, respectively, exhibited when settling on the ground before burying. However, *S. parva* did not show this or any other different body postures compared to its regular resting posture. Furthermore, an angling behaviour (= protruding of one arm vertically out of the sediment) was reported for buried or partly buried individuals of *R. pacifica* (Anderson et al., 2004) and *E. hyllebergi* (Nabhitabhata et al., 2005) but has not been observed in the present study for *S. parva*.

Besides the burying behaviour, other escape responses and defence behaviours commonly seen in sepiolids were observed for *S. parva* in the present study. The most frequently observed escape response in the wild was ejecting ink, usually as a series of blobs similarly sized, shaped and coloured as the sepiolid. This behaviour was also reported for the sepiolids *E. scolopes* (Anderson & Mather, 1996; Moynihan, 1983) and *R. pacifica* (Shimek, 1983) and is further documented in other cephalopods such as octopuses (e.g. Caldwell, 2005) and squids (Bush & Robison, 2007; Bush et al., 2009; Hall, 1956). The corresponding authors all stated that these ink blobs may serve as a decoy ('pseudomorph') for potential predators. An observation from the lab in which an individual of *S. parva* either attacked or attempted to mate with one of these blobs which has previously been ejected by another individual supports this theory (as the attacking sepiolid may have mistaken the ink blob for one of its conspecifics) and further arouses the question if these ink blobs feature not only visually but also olfactory characteristics of the ejected sepiolids. Hence, further research needs to be addressed to the exact characteristics and purpose of these ink blobs. Additionally, individuals of *S. parva* were observed to eject 'ink ropes' (*sensu* Bush & Robison, 2007), approximately 4 – 5 times the length of the animal, and hold on to them motionless. To the best of our knowledge, this inking behaviour has not been documented for any other sepiolid species and was only reported for deep sea squids which remained in most cases close to their ejected ink ropes (Bush & Robison, 2007; Bush et al., 2009). While Bush & Robison (2007) suggested that these long ink ropes may be used by deep sea squids as mimicry and resembled the form of stinging siphonophores, the use of ink ropes by *S. parva* might rather be a masquerade by resembling floating seagrass leaves.

Another escape response of *S. parva* observed in the wild was burying. However, most observed individuals left their eyes uncovered and exhibited a blue colouration in the dorsal head area. While sepiolids are able to rotate their eyeballs vertically, resulting in upwards facing pupils (Boletzky & Boletzky, 1970; Naef, 1923), they are able to see to a certain degree when buried. This was concluded from diffuse jets of ink ejected by completely buried individuals in captivity, evoked by sudden movements outside the tank (Anderson et al., 2002, 2004). As it was never determined to what extent buried sepiolids are able to perceive visual stimuli, it can by hypothesised that they might be able to perceive sudden differences in brightness only. This might explain that escaping individuals of *S. parva* leave their eyes uncovered when buried to keep their predators in fully sight.

Furthermore, animals both in the wild and in captivity were frequently observed to wear a sand grain layer when emerging from the sediment. While this so-called 'sandcoat' (*sensu* Singley, 1982) was frequently observed in *E. hyllebergi* (Nabhitabhata et al., 2005) and *E. scolopes* (Moynihan, 1983; Shears, 1988; Singley, 1982, 1983), it has to the best of our knowledge never been reported for members of the genus *Sepiola.* According to Singley (1982) and von Byern & Klepal (2006), individuals of *E. scolopes* possess multiple adhesive glands in the dorsal epidermis by which they are able to adhere sand grains to their mantle. However, identical or similar adhesive mechanisms among cephalopods have been found in only four cephalopod genera so far, namely *Nautilus*, *Euprymna*, *Idiosepius* and *Sepia* (Cyran et al., 2010; von Byern & Klepal, 2006), whereas there are again to the best of our knowledge no records of adhesive structures reported for *Sepiola*. Hence, further research is needed to determine how exactly *S. parva* achieves to adhere sand grains to its dorsal mantle.

The observed hunting behaviour of *S. parva* partly matches observations for other sepiolids.

While the hunting posture and actual capture of the prey, followed by the retraction of the tentacles resembled observations for species of the genera *Sepietta* and *Sepiola* (Bergström, 1985; Boletzky et al., 1971), those authors further reported that the observed sepiolids were searching for an ideal attacking position (Bergström, 1985) or even circled around the prey to attack it from a different position (Boletzky et al., 1971). This differed to *S. parva* which in the present study generally attacked the prey directly with a straight approach despite re-positioning was observed occasionally. While Bergström (1985) further reported a bleaching in the body colouration of *Sepietta oweniana*, no such change was seen for *S. parva* as all observed individuals maintained their dark body colouration throughout the whole hunting event.

By analysing the AD, TLA and TLS, a negative correlation with increasing DML was found. To the best of our knowledge, comparable studies were conducted for the cuttlefish *Sepia pharaonis* (Sugimoto & Ikeda, 2013) and the squids *Loligo opalescens* (Chen et al., 1996) and *Sepioteuthis lessoniana* (Sugimoto & Ikeda, 2013) only, whereas no other sepiolids were tested upon this up to present. Nevertheless, the results of the present study cannot be compared directly with those above-mentioned as the authors evaluated the hunting behaviour of these species with regards to their age rather than their DMLs. However, by assuming that older individuals of the before-mentioned species exhibit larger DMLs, a similar correlation was found for the AD in all three species, whereas TLA and TLS were either not measured or differently defined in those studies and therefore not comparable (Chen et al., 1996; Sugimoto & Ikeda, 2013). Regarding the TSS, again to the best of our knowledge, no data is available for other sepiolids. However, the calculated TSS for *S. parva*, ranging from 0.840 to 0.912 m/s, corresponds to roughly 72 % of the TSS measured in the squid *Lolliguncula brevis* (1.23 m/s; Jastrebsky et al. (2017) and to 38 % of the TSS recorded in the squid *Loligo pealei* (2.3 m/s; Kier & van Leeuwen (1997)). These differences correspond positively to the size of the three species and are probably caused by varying morphological and physiological properties of the tentacle tissue between these species. Although the tentacular strikes of *S. parva* were not in detail perceptible for the human eye and high-speed cameras were needed to record the latter, the above presented TSSs of cephalopods can easily be surpassed by other movements with higher speeds in the marine environment (Sakes et al., 2016), such as the discharge of nematocytes in cnidarians with up to 18.6 m/s (Nüchter et al., 2006) or the strike of the feeding appendages in stomatopods with up to 23 m/s (Patek et al., 2004).

The mating behaviour of *S. parva* resembled the observations of other sepiolids for the greater part. However, all mating events in this study occurred solely in the water column, which seems to be uncommon amongst sepiolids as *E. hyllebergi* (Nabhitabhata et al., 2005), *E. scolopes*

(Moynihan, 1983) and *S. atlantica* (Rodrigues et al., 2009) were observed to mate on the sediment. No courtship behaviour or prior pair formation was seen in this study which matches with the observations for *E. hyllebergi* (Nabhitabhata et al., 2005), *E. scolopes* (Moynihan, 1983), *E. tasmanica* (Squires et al., 2013) and *S. atlantica* (Jones & Richardson, 2010; Rodrigues et al., 2009) in which no such behaviour was seen either. However, Rodrigues et al. (2009) suggested that courtship behaviour might still occur in the wild and that the captive environment might alter the reproductive behaviour of sepiolids in general. In all three observed mating events in this study, the copulation occurred in the 'male-to-female neck' position, which appears to be the common mating positions in sepiolids (Boletzky, 1983; Hanlon et al., 1997; Nabhitabhata et al., 2005; Rodrigues et al., 2009; Singley, 1983; Squires, 2013) despite occasional reports of other mating positions such as 'male-parallel' (Brocco, 1971) or 'head-to-head' (Bergstrom & Summers, 1983). The mating events of the present study lasted for 32 – 40 min which represent similar durations as recorded for *E. scolopes* (30 – 50 min; Hanlon et al. (1997). However, mating durations in sepiolids may depend on several not yet entirely investigated factors such as sperm competition (Squires et al., 2013; Squires et al., 2014, 2015), as the duration of mating varies significantly amongst sepiolids from 7 – 10 min in *E. hyllebergi* (Nabhitabhata et al., 2005) and 8 min in *Sepiola rondeletii* (Racovitza, 1894) up to more than 3 h in *E. tasmanica* (Squires et al., 2013). In three additional mating events in the present study, male-male competition was observed due to a second male interfering in an ongoing copulation 4 – 20 s after the latter started. While agonistic behaviour is frequently observed in other cephalopods (Hanlon & Messenger, 2018), to the best of our knowledge only one observation of two males briefly fighting after simultaneously grabbing a female is documented for sepiolids (Hanlon et al., 1997). However, it could not be determined whether the second male attacked the copulating male or also tried to mate with the female. Hence, further detailed observations need to be taken to draw a conclusion on the mating behaviour in *Sepiola parva* and other sepiolids in general.

In one occasion, a total of 47 eggs were attached by one female to the underside of her shelter (clay-based flowerpot), which closely resembles the average number of spawned eggs per batch (48.54) in *S. oweniana* (Bello & Deickert, 2003) and lies within the range of 1 – 89 eggs per batch observed in *S. atlantica* (Rodrigues et al., 2011a). All spawned eggs in this study were covered with a layer of sand grains, probably to assume a cryptic colouration. While this characteristic was also reported for the eggs of *E. scolopes* (Hanlon et al., 1997; Singley, 1983) and the cuttlefish *Sepia esculenta* (Natsukari & Tashiro, 1991), neither in the present nor in the latter two studies it could be determined how exactly the eggs got covered with sand grains.

In summary, the behavioural ecology of the spotty bobtail squid *S. parva* greatly resembles those of other observed sepiolids, although minor differences to sepiolids from other genera or even the same genus have been identified in the present study. Additionally, new behavioural aspects for the genus *Sepiola* ('sand coat') or sepiolids in general ('ink rope') were reported in the present study, emphasising that further research on both well-investigated as well as so far less-noticed sepiolids is needed to improve the knowledge about these cephalopods.

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3 – Manuscript

The burying behaviour of *Sepiola* sp. (Sepiolidae: Cephalopoda) on different sediment types

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Abstract

Burying in soft sediment is a widespread behaviour of animals inhabiting the marine environment. In cephalopods, it can be seen in octopus, cuttlefish and especially bobtail squids. While the latter usually spend the daytime buried in the sediment, they emerge at night for hunting and mating. Although they have become popular organisms for scientific studies in recent years, little is known about the ecology of bobtail squids. Hence, understanding their needs and requirements when holding them in captivity is vital for a proper welfare, especially since the inclusion of cephalopods in the EU welfare directive 2010/63/EU on the protection of animals used for scientific purposes. Bearing this in mind, this study tested the effect of seven sediment samples, differing in their mean grain sizes, grain size distribution and origin (natural/artificially made), on the burying behaviour of *Sepiola* sp. (Sepiolidae, Cephalopoda). All tested individuals performed the same two-phased burying behaviour on all sediment samples. While no correlation was found among the size of *Sepiola* sp. and its burying behaviour, the respective duration and number of body movements (funnel jets/ arm sweeps) per burying phase was significantly altered by the mean grain size of the used sediment samples. The latency until the start of burying, the duration of phase 1, the number of funnel jets during phase 1 and the total burying duration was the shortest/lowest on medium grained sediment samples and correspondingly extended on finer and coarser sediment samples. Contrarily, the duration of phase 2 and the number of arm sweeps within phase 2 was the longest/highest on coarser sediment and decreased the finer the sediment was. These results imply that *Sepiola* sp. should be kept on sediment types with a mean grain size between 125 to 250 µm when hold in a captive environment in order to minimise the burying duration and energetic effort.

Keywords: Bobtail squid, Burying behaviour, Mean grain size, Sediment, *Sepiola*

3.1 – Introduction

Burying and burrowing to minimize the risk of detection and predation is a widespread behaviour among marine organisms such as fish (e.g. Gibson & Robb, 1992; Lü et al., 2018), crustaceans (e.g. Bellwood, 2002; McGaw, 2005), holothurians (e.g. Purcell, 2010) or molluscs (e.g. Taylor, 1976). Also seen in cephalopods (Boletzky, 1996), burrowing is here defined as ‘the construction of a semi-permanent den or refuge where none existed previously’, whereas burying can be considered as ‘the act of covering oneself with the substrate (or diving into it), resulting in temporary concealment’ (Hanlon & Messenger, 2018). While burrowing in cephalopods can be found in octopus only (Ambrose, 1982; Montana et al., 2015; Yarnall, 1969), burying behaviour has been observed in some octopus species, such as *Amphioctopus burryi* (Hanlon & Hixon, 1980; Hanlon et al., 2010), *Eledone cirrhosa* (Guerra et al., 2006), *Macrotritopus defilippi* (Hanlon et al., 2010) and *Thaumoctopus mimicus* (Hanlon et al., 2008); in the cuttlefish *Sepia officinalis* (Hanlon & Messenger, 1988; Mather, 1986) and especially in several bobtail squid species, such as *Euprymna scolopes* (Anderson, 1997; Anderson et al., 2002; Moynihan, 1983), *Euprymna hyllebergi* (Nabhitabhata et al., 2005), *Rossia macrosoma* (Boletzky & Boletzky, 1973), *Rossia pacifica* (Anderson et al., 2004), *Sepiola atlantica* (Rodrigues et al., 2010), *Sepiola parva* (Drerup et al, Chapter 2) and a variety of other species of the genera *Sepiola* and *Sepietta* (Boletzky & Boletzky, 1970). However, the used burying technique varies among those orders (Boletzky, 1996).

In bobtail squids, the burying procedure can generally be divided into two phases (*sensu* Boletzky & Boletzky, 1970). In phase 1, an initial depression will be created in the sediment by a gentle funnel jet, followed by a strong, oppositely-directed funnel jet to blow up sediment from underneath the sepiolid's bodies. While the sediment is dispersed in the water column, the animal immerses its body in the sediment which will subsequently get covered by descending sediment particles. This behaviour is followed by further alternating forward- and backward-directed funnel jets until the bobtail squid is almost completely covered with sediment (Boletzky & Boletzky, 1970).

Either immediately or shortly after the last funnel jet, phase 2 (*sensu* Boletzky & Boletzky, 1970) starts by stretching out a pair of arms above the sediment, followed by several sweeps of the latter to cover the remaining body parts of the animal. If not altered by the presence of an obstacle, these arm movements are always closely synchronous, whereby the arm tips are pointing medially towards the body (Boletzky & Boletzky, 1970).

Whether the burying behaviour, technique or duration is affected either by the size of a sepiolid or by the characteristics of the sediment was barely investigated up to now. Boletzky & Boletzky (1970) stated for *Sepiola robusta* and *Sepietta obscura* that the duration of the first phase decreases with animal size (dorsal mantle length = DML), whereas the duration of the second phase increases. Those observations were not confirmed for *Sepiola atlantica* (Rodrigues et al., 2010) and *Sepiola parva* (Drerup et al., Chapter 2), as in both studies no significant relationship between DML and burying behaviour was found.

Considering the sediment characteristics, Boletzky & Boletzky (1970) reported for different *Sepietta* and *Sepiola* species that the duration of the first phase is the shortest on medium grained sand and correspondingly longer on both finer and coarser grained sediment types (see

Blott & Pye (2001) for definitions of descriptive terminologies of sand types). Additionally, Anderson et al. (2002) found for *Euprymna scolopes* that not only the latency until burying, the burying duration and the refusal to bury was significantly higher on coarser sediment samples, but also that the latency until the start of burying and the burying duration significantly varied between subtidal and intertidal sand, although both sediment types had a similar mean grain size. In a subsequent study, the authors confirmed for *Rossia pacifica* that the latency until burying correlates with the mean grain size of the tested sediment types (Anderson et al., 2004). While these findings indicate that the mean grain size might affect the burying behaviour of sepiolids, the studies of Anderson et al. (2002, 2004) tested sediment types taken from the natural environment of sepiolids against sediments which do not normally occur in the habitat of those animals (e.g. construction and cryolite sand), with the latter potentially featuring unnatural mean grain sizes or grain size distributions. Up to now, it has not been reported that sepiolids analyse the sediment before burying. However, the cuttlefish *S. officinalis* which burying pattern closely resembles phase 1 of the burying behaviour of sepiolids (Mather, 1986) was observed to hover above the substrate while touching it with its arms, potentially to evaluate the sediment in terms of its characteristics and feasibility for burying (Hanlon & Messenger, 1988; Mather, 1986).

Nowadays, bobtail squids are more and more kept in captivity, either in public aquariums or in research facilities as model organisms for host-microbe interactions (Mandel & Dunn, 2016; McAnulty & Nyholm, 2017) as well as for genetic studies (Belcaid et al., 2019; Bosch, 2019) due to their symbioses with light producing bacteria associated with their light emitting organs (McFall-Ngai, 1999; Nyholm & McFall-Ngai, 2004). Due to the inclusion of cephalopods in the EU welfare directive 2010/63/EU on the protection of animals used for scientific purposes (European Parliament & Council of the European Union, 2010), understanding the needs and requirements of particular species when hold in captivity is vital for a good welfare, as reviewed in the first guidelines for welfare of cephalopods (Fiorito et al., 2015; Smith et al., 2013; Sykes et al., 2012). To extend the knowledge about the ecology of bobtail squids and understand their needs when kept in a captive environment in relation to their natural habitat preferences, this study aimed at testing the effect of different sediment samples (both taken from locations known to be abundant in sepiolids as well as artificially made) on the burying behaviour of *Sepiola* sp..

3.2 – Material & Methods

3.2.1 – Ethical statement

The research in the present study was performed in accordance with Directive 2010/63/EU (European Parliament & Council of the European Union, 2010) for cephalopods (Fiorito et al., 2015; Smith et al., 2013; Sykes et al., 2012). No specific procedures were applied to bobtail squid individuals. This manuscript was further prepared according to the ARRIVE guidelines (Kilkenny et al., 2010a, 2010b) for reporting animal research.

3.2.2 – Animal collection & husbandry

In May 2019, a total of 33 *Sepiola* sp. were collected from the Bay of Fetovaia (Elba, Italy; 42°43'54.6"N 10°09'18.5"E) at night by SCUBA diving at depths between 2 and 7 m. When an individual was encountered, it was caught using a green, finely meshed hand net and subsequently placed in a plastic container during the survey. Immediately after each survey, all caught sepiolids were transported individually in 1 L plastic containers to the field station of the HYDRA Institute (Fetovaia, Elba, Italy; 42°44'02.4"N 10°09'21.9"E) and kept individually in round HD-PE containers. Each container had a volume of 10.4 L and was filled with fresh sea water, equipped with an air stone to provide a proper dissolved oxygen saturation, and with its bottom covered with a 3 cm layer of local subtidal sediment. Bobtail squids were fed *ad libitum* after placing them in their HD-PE container with local mysid shrimps. All tested bobtail squids were kept to a maximum of 20 h and burying experiments for each individual took place on the consecutive day after collecting it from the ocean. To maintain a proper water quality in each container, approximately 80 % of the total volume was exchanged with fresh seawater 7 times per day between 9 am and 6 pm.

3.2.3 – Sediment collection and analysis

Two coastal areas, the Bay of Fetovaia and the Bay of Sant'Andrea (42°48'28.7"N 10°08'37.4"E), both known to be abundant in sepiolids, were chosen for sediment sampling. The Bay of Fetovaia is exposed in a South-Eastern direction to the Mediterranean Sea, with a prominent peninsula shielding its western and south-western part from strong, seasonal, northward-directed winds, leading to different sediment compositions on a shore-parallel axis. Therefore, sediment samples were taken from the North-Eastern part (42°43'56.2"N 10°09'22.0"E; hereinafter referred to as FNE (= Fetovaia North-East)) and the South-Western part (42°43'49.8"N 10°09'19.6"E, hereinafter referred to as FSW (= Fetovaia South-West)) of the bay, both at a depth of 4 m (Figure 3.1). In contrast, the widely-shaped Bay of Sant'Andrea, pointing in a northern direction to the Mediterranean Sea, is equally exposed to strong, seasonal, southward-directed winds, leading to different sediment compositions along a shore-perpendicular axis (Wright, 1995). Hence, sediment samples were taken from two locations in the western part of this bay, at 4 m (42°48'28.9"N 10°08'30.1"E; hereinafter referred to as SA4 (= Sant'Andrea 4 m)) and at 8 m (42°48'31.4"N 10°08'31.0"E; hereinafter referred to as SA8 (= Sant'Andrea 8 m)) (Figure 3.1).

At all four locations (FNE, FSW, SA4, SA8), 3 samples (V = 150 cm³, equalling 225 – 250 g dry sediment per replicate) of the upper 5 cm sediment layer were taken at the corners of a 3 m wide equilateral triangle as replicates for a corresponding grain size distribution analysis by using a sediment corer and subsequently stored in containers. Additionally, 6 containers (V = 250 cm³) were filled with sediment from the upper 5 cm layer from the centre of the sampling triangle for the burying experiments, again by using a sediment corer.

While the sediment samples collected for the burying experiments were not altered in any way, the 3 sediment replicates per location sampled for analysing their corresponding grain size

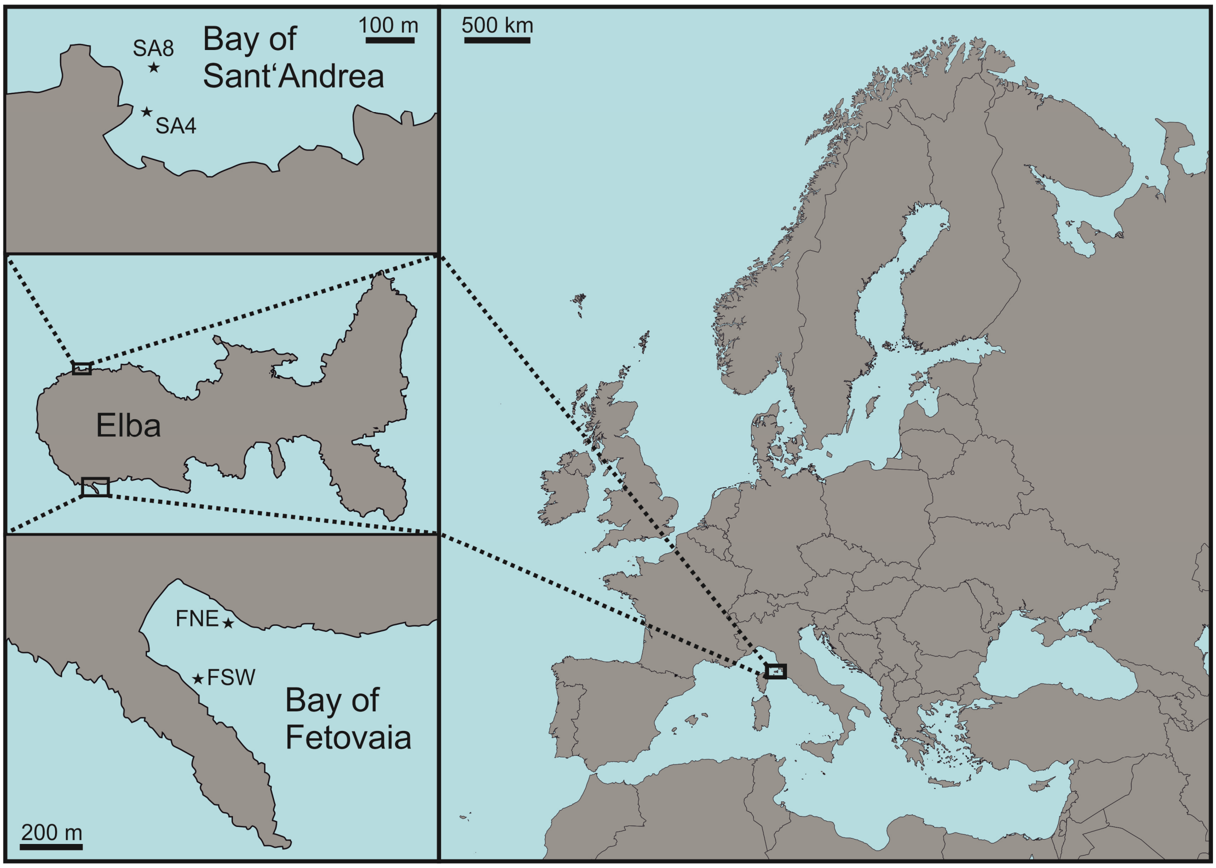


Figure 3.1 – Sediment sampling sites of the present study. The stars display the sampling locations of the four natural sediment samples SA8 (Sant'Andrea 8 m) and SA4 (Sant'Andrea 4 m) as well as FNE (Fetovaia North-East) and FSW (Fetovaia South-West).

distributions were subsequently washed with Milli-Q water until reaching a salinity of 0 PSU. Afterwards, they were transferred to individual aluminium trays, weighed and subsequently dried in a sediment drying oven (U10, Memmert GmbH + Co. KG, Schwabach, Germany) at a constant temperature of 80 °C. After every 60 minutes, the loss of water was determined by weighing each replicate. A sediment sample was considered fully dried when no weight loss was observed in two consecutive weightings. Each replicate was then put into a sieving machine (S-S, Retsch GmbH, Haan, Germany), containing sieves of different consecutive sizes (2000, 1000, 500, 250, 125, and 63 µm, respectively) as well as a pan (collecting sediment particles < 63 µm), and sieved at a constant speed (internal setting 1) for 10 min. Afterwards, the weight of the sediment partition collected in each sieve was measured using a micro scale and imported into GRADISTATv8 (Blott & Pye, 2001) to calculate the grain size distribution per sediment replicate, resulting in average values per sampling location.

Besides the four natural sediment samples directly taken from the Bay of Fetovaia as well as Bay of Sant'Andrea, three additional sediment samples were made artificially by adding up sediment partitions collected in the sieves of the sizes 63 µm, 250 µm and 1000 µm, respectively, corresponding to homogeneous sediment samples of very fine, medium and very coarse sand, according to Wentworth (1922) and Blott & Pye (2001). These samples are hereinafter referred to as VFI (= very fine sand), MED (= medium sand) and VCO (= very coarse sand).

3.2.4 – Burying experiments

To test the burying behaviour of *Sepiola* sp. on the seven sediment samples, the bottom of a 1.3 L glass aquarium (13 cm long x 8 cm wide x 15 cm deep) was covered with 3 cm of a corresponding sediment sample, and the tank was afterwards filled with fresh sea water. To minimise both visual and tactile disturbances (Boletzky & Boletzky, 1970), a visual barrier and a rubber mat were placed around and below the tank, respectively. On each consecutive day after collecting individuals of *Sepiola* sp., the latter were individually introduced into the experimental tank and their burying process was filmed in ambient sun light between 10:00 and 17:00 h using a digital camera (G7Xi, Canon Inc., Tokyo, Japan). Each recording was stopped 30 seconds after the last movements of a successful burying procedure, whereas a refusal to bury was set at a maximum of 10 minutes, similar as in Anderson et al. (2004). The tank water was exchanged at least every 25 min during the experimental period to maintain pristine seawater quality.

A total of 33 sepiolids were tested in this study, with at least 11 individuals on each sediment type. After the burying observations, the dorsal mantle length (= DML) of each individual was determined by placing the sepiolid in a tank with a centimetre grid on its bottom and photographing it with a digital camera (TG5, Olympus K.K., Tokyo, Japan). Video material gathered from the burying experiments was analysed using the open-source software 'Behavioural Observation Interactive Research Software' (BORIS; http://www.boris.unito.it/; Friard & Gamba (2016)) and subsequently examined for the burying characteristics presented in Table 3.1. All observations were collected and analysed by the same observer.

The obtained BORIS-output was further imported into the open-source software 'Behatrix' (http://www.boris.unito.it/pages/behatrix) to analyse the corresponding behavioural sequences in terms of their transition probabilities and subsequently establish the latter in kinematic diagrams.

Table 3.1 – Definition of burying characteristics

Burying sequence	Definition
Pre-Burying	
Water column (s)	Total time an individual spends in the water column before the start of burying.
Sediment (s)	Total time an individual spends resting on the sediment before the start of burying.
Latency (s)	Time between introducing an individual into the experimental tank and the start of burying (= total time spent in water column + total time spend on sediment).
Phase 1*	
Duration (s)	Time between the first and last recorded funnel jet, including potential resting periods.
Number of funnel jets	Total number of ejected funnel jets, including the initial funnel jet (= 'depression').
Resting period (s)	Time between the last observed funnel jet (end of phase 1) and the first observed arm sweep (beginning of phase 2).
Phase 2*	
Duration (s)	Time between the first and last recorded arm sweep, including potential resting periods.
Number of arm sweeps	Total number of observed arm sweeps.
Burying duration (s)	Time between the first recorded funnel jet (= 'depression') and the last observed arm sweep (= duration of phase 1 + time between phase 1 and phase 2 + duration of phase 2).

**sensu* Boletzky & Boletzky (1970)

3.2.5 – Statistical analysis

All acquired data sets of this study were examined for potential outliers by using the 1.5 x IQR (interquartile range) rule (Tukey, 1977). If outliers were found, the mean ± standard deviation (SD) for both respective data sets (including and excluding outliers) were calculated.

Potential body-size related relationships in the burying behaviour of *Sepiola* sp. were determined by testing each data set to meet the assumptions of a linear regression model (Quinn & Keough, 2002) and subsequently analysing the corresponding linear regressions of each data set against the DML.

The data sets of the burying experiments (excluding outliers) were further tested for both normal distribution and homogeneity of variances using the Shapiro-Wilk and Levene's tests (Zar, 1999), respectively. When a normal distribution and/or homogeneity of variances failed, non-parametric or robust tests were used, depending on the variance similarity criteria (Quinn & Keough, 2002). When differences were found in any statistical tests, Tukey HSD and Dunn-Bonferroni *post hoc* tests were used to establish homogenous groups, also considering the homogeneity criteria.

Furthermore, potential relationships between the mean grain size of each sediment sample and the burying behaviour of *Sepiola* sp. were determined by analysing each data set (excluded outliers) for linear or quadratic curve estimation regression models. All above-mentioned statistical analyses were performed using SPSS® 21 (IBM, Armonk, New York, USA).

The significance of each transition probability in all presented kinematic diagrams was determined by running Behatrix' random permutation test with 100,000 permutations.

Statistical significance in all conducted test in this study was considered for $p < 0.05$.

3.3 – Results

3.3.1 – Sediment analysis

The four natural sediment samples differed both in their geometric mean grain size (following Folk & Ward, 1957) and their grain size distribution (following Blott & Pye, 2001). All samples exhibited a bell-shaped grain size distribution, each with one large prominent partition enclosed by the corresponding second and third largest partitions (Figure 3.2; Table 3.2). In all four natural sediment samples, these three adjacent partitions corresponded to at least 95.93 % of the total weight of the respective sediment sample (FNE: 95.93 %; FSW: 99.28 %; SA4: 98.29 %; SA8: 96.69 %).

The sediment sample FSW exhibited the lowest mean grain size of all samples (131.2 µm; 2.930 Φ) with the largest partition within its grain size distribution being > 125 µm (67.53 %), followed by > 63 µm (18.43 %) and > 250 µm (13.32 %) (Figure 3.2; Table 3.2). Therefore, FSW was considered as fine sand (following Folk & Ward, 1957).

The sediment samples FNE as well as SA4 were analysed to have a mean grain size of 360.8 µm (1.471 Φ) and 299.1 µm (1.742 Φ), respectively, with both exhibiting the largest partition of the grain size distribution at > 250 µm (FNE: 57.80 %; SA4: 76.15%). While the second (> 500 µm) and third (> 125 µm) largest partition of SA4 were almost equally large with 11.10 % and 11.04 %, respectively, the corresponding partitions of FNE differed more strongly (> 500 µm: 24.88 %; > 125 µm: 13.25 %) (Figure 3.2; Table 3.2). Nevertheless, both sediment samples were classified as medium sand (following Folk & Ward, 1957).

The sediment sample with the largest mean grain size was SA8 with 567.7 µm (0.817 Φ), whereby 50.80 % of its grains were retrained in the partition > 500 µm, followed by > 250 µm with 29.74 % and > 1000 µm with 16.07 % (Figure 3.2; Table 3.2). Hence, the sediment sample SA8 was considered as coarse sand (following Folk & Ward, 1957).

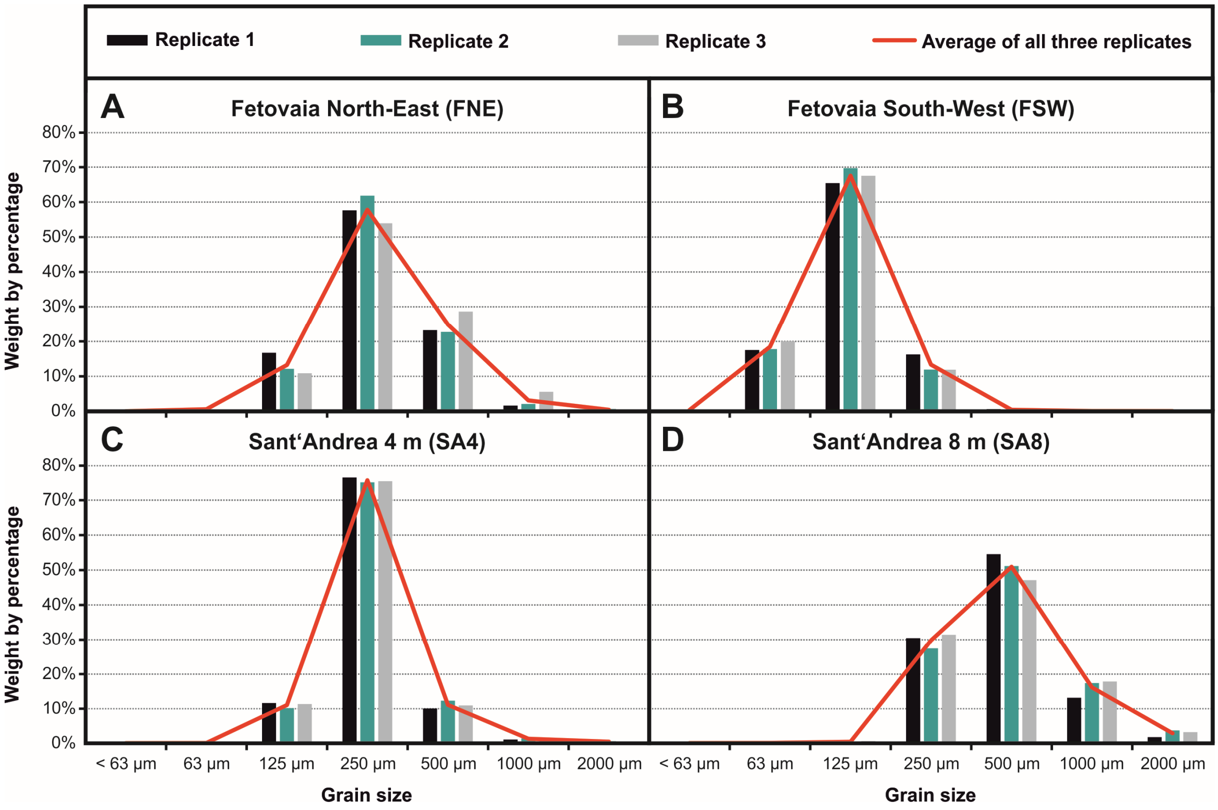


Figure 3.2 – Grain size distribution of the natural sediment samples. Depicted are the percentages by weight of each replicate (n = 3 per sediment sample) and the average grain size distribution of the sediment samples **(A)** Fetovaia North-East (FNE), **(B)** Fetovaia South-West (FSW), **(C)** Sant’Andrea 4 metre (SA4) and **(D)** Sant’Andrea 8 metre.

Table 3.2 – Characterisation of the sediment samples used in the present study

Sediment sample	Grain size distribution (in %)[a]							Mean grain size[b]		Type of sand[b]
	>2000 µm	>1000 µm	>500 µm	>250 µm	>125 µm	>63 µm	<63 µm	Geom. (µm)	Log. (Φ)	
FNE	0.41	3.08	24.88	**57.80**	13.25	0.57	0.01	360.8	1.471	Medium
FSW	0.02	0.02	0.36	13.32	**67.53**	18.43	0.32	131.2	2.930	Fine
SA4	0.40	1.26	11.10	**76.15**	11.04	0.05	0.00	299.1	1.742	Medium
SA8	2.84	16.07	**50.88**	29.74	0.38	0.08	0.01	567.7	0.817	Coarse
VFI	0.00	0.00	0.00	0.00	0.00	**100.00**	0.00	75.3	3.731	Very coarse
MED	0.00	0.00	0.00	**100.00**	0.00	0.00	0.00	297.9	1.747	Medium
VCO	0.00	**100.00**	0.00	0.00	0.00	0.00	0.00	1183.2	-0.243	Very fine

Bold values indicate the largest partition of the corresponding sediment sample. [a] Following GRADISTATv8 (Blott & Pye, 2001) [b] Following GRADISTATv8 (Blott & Pye, 2001) but based on the Folk & Ward Method (Folk & Ward, 1957). Geom. and Log. are abbreviations for geometric and logarithmic, respectively. The logarithmic mean grain size (Φ) is calculated using the expression $\Phi = -\log_2(d)$, where d is the geometric mean grain size in mm (Krumbein, 1934).

3.3.2 – General burying behaviour

Burying behaviour of *Sepiola* sp. started after an individual settled onto the sediment, whereas some swimming individuals were observed to actively touch the sediment with the tips of their arms beforehand (Figure 3.3A). After settling down (Figure 3.3B), *Sepiola* sp. tilted its body slightly forward and ejected a gentle forward-directed funnel jet to create a depression in the sediment. This depression subsequently served as a hold for a strong, backward-directed funnel jet to blow up sediment particles. While these particles were dispersed in the water column, the sepiolid immersed its body in the depression and let its dorsal body area get covered by the descending sediment particles. This behaviour was followed by further alternating forward- and backward directed funnel jets to almost completely cover the sepiolid's body with sediment (Figure 3.3C-D), whereas especially smaller individuals of *Sepiola* sp. were occasionally observed to further remove coarser sediment grains with their arms simultaneously with a forward directed funnel jet. Afterwards, *Sepiola* sp. stretched out its dorsolateral arms above the sediment and gathered sediment particles from the circular vicinity around its body with sweeping arm movements to cover the remaining body parts with sediment (Figure 3.3E) until being fully buried (Figure 3.3F). During the burying procedure, the body colouration of *Sepiola* sp. shifted from its typical brown pattern (Figure 3.3B) to a pale pigmentation (Figure 3.3D).

3.3.3 – Experimental outline

From a total of 123 burying observations on the seven sediment samples, 115 resulted in a successful burial (93.5 %) whereas on eight occasions (6.5 %) the tested sepiolid did not show any burying behaviour (Table 3.3). No significant difference was found among the different

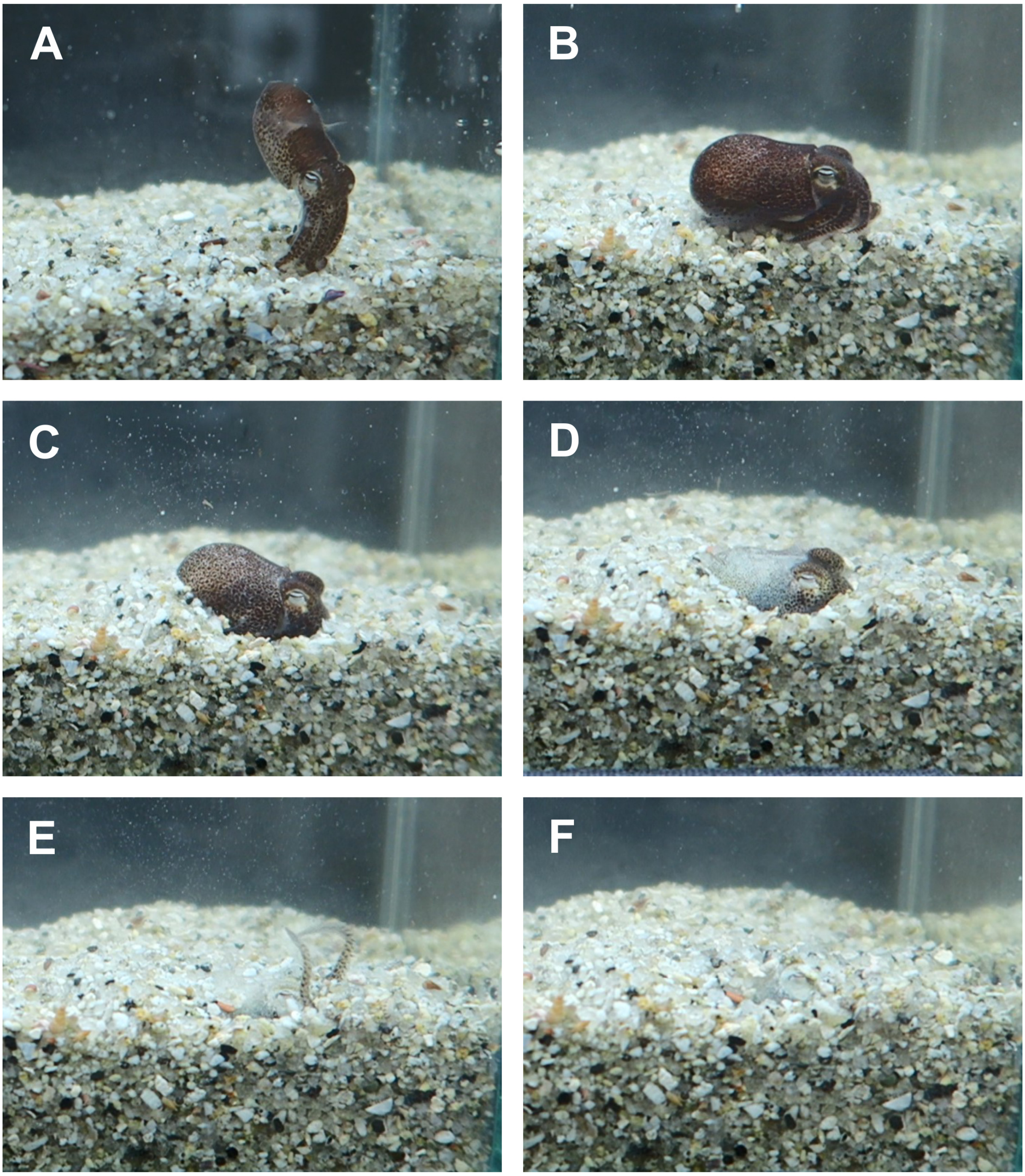


Figure 3.3 – Burying behaviour in *Sepiola* sp. **(A)** Some individuals touch the sediment actively with the tips of their arms before settling onto the it. **(B)** Settling/resting posture before the start of burying. **(C, D)** Phase 1 of the burying behaviour (*sensu* Boletzky & Boletzky, 1970). After the formation of a depression in the sediment by tilting the body slightly forwards and ejecting a gentle forward-directed water jet, alternating backward- and forward-directed water jets are ejected to cover nearly the whole body with sediment. **(E)** Phase 2 of the burying behaviour (*sensu* Boletzky & Boletzky, 1970). A series of arm sweeps is conducted to gather sediment from its circular vicinity to cover the remaining body parts. **(F)** Fully buried individual. During the burying procedure, **(D)** the animal shifts from a dark to a pale body colouration.

Table 3.3 – Number of burying experiments and dorsal mantle length of the used sepiolids

Sediment type	Number of burying experiments				DML of the used sepiolids (in mm)		
	Total	Successful burials	Refusal to bury	Refusal rate	Ø ± SD	Smallest individual	Largest individual
FNE	19	17	2	10.53 %	15.8 ± 4.8	5.7	20.7
FSW	17	17	0	0.00 %	16.1 ± 4.3	8.0	20.3
SA4	25	22	3	12.00 %	14.8 ± 5.0	5.7	20.7
SA8	25	23	2	8.00 %	14.8 ± 5.0	5.7	20.7
VFI	11	10	1	9.09 %	16.4 ± 2.7	12.8	20.7
MED	13	13	0	0.00 %	16.3 ± 3.1	10.8	20.7
VCO	13	13	0	0.00 %	16.3 ± 3.1	10.8	20.7
Overall	123	115	8	6.50 %	15.6 ± 4.3	5.7	20.7

DML = Dorsal mantle length. Ø = mean value. SD = standard deviation.

sediment samples in terms of their refusal rate ($H(6) = 4.911$, $p = 0.555$). Only the observations of successfully buried animals were considered for the subsequent analysis of the burying behaviour on different sediment samples, unless otherwise noted. Due to the presence of outliers in almost all investigated burying characteristics, results in this section are presented excluding outliers, whereas all results (including and excluding outliers, respectively) are listed in Table 3.4).

The tested sepiolids spent on average 13.60 ± 14.06 s in the water column and 62.93 ± 69.28 s on the sediment before the start of their burying procedure, resulting in a total average latency until the start of burying of 88.25 ± 84.78 s (Table 3.4). While no statistical differences were found among the seven sediment types for the average time spent on the sediment and the total latency, the average time spend in the water column did not differ significantly among SA4, FNE, FSW, SA8, VCO and MED as well as among FSW, SA8, VCO, MED and VFI (Table 3.5). Furthermore, both for the time spend in the water column and the total latency until the start of burying, a quadratic regression model could be applied (Table 3.6). These models showed that the tested sepiolids spent less time in the water column and in the tank in general before burying on the sediment samples considered as medium sand (following Folk & Ward, 1957), while correspondingly spending more time on finer or coarser sediment samples (Figure 3.4A+C). Contrarily, no regression model could be applied to the time spend on the sediment before burying (Table 3.6, Figure 3.4B).

Phase 1 of the burying behaviour (*sensu* Boletzky & Boletzky, 1970) of *Sepiola* sp. took on average 10.04 ± 5.37 s, ranging from 5.27 ± 1.27 s on the sediment sample MED to 42.67 ± 36.84 s on the sample VCO (Table 3.4) with statistically similar average values among MED, SA4, SA8 and FNE, among SA4, SA8, FNE and FSW, among SA8, FNE, FSW and VFI as

Table 3.4 – Observational results of the burying experiments and the statistical analysis of the relationship between the dorsal mantle lengths and the corresponding observations (durations and number of funnel jets/arm sweeps) per burying sequence

Burying sequence		**Sediment type**	**Observational data**		**Observational results**			**Linear regression**				
			Outliers	**n**	**Ø ± SD**	**Min.**	**Max.**	df_{reg}	df_{res}	F	p	r^2
Pre-Burying	Water column (s)	FNE	yes	17	11.18 ± 12.04	2	38	1	15	0.994	0.335	0.062
			excluded	14	6.36 ± 5.80	2	24	1	12	1.049	0.326	0.080
		FSW	yes	17	19.06 ± 27.41	1	114	1	15	0.127	0.726	0.008
			excluded	16	13.13 ± 12.77	1	36	1	14	0.659	0.430	0.045
		SA4	yes	22	9.27 ± 12.77	1	57	1	20	1.121	0.302	0.053
			excluded	19	5.05 ± 3.08	1	13	1	17	1.076	0.314	0.060
		SA8	yes	23	15.43 ± 14.81	3	63	1	21	0.057	0.814	0.003
			excluded	21	11.86 ± 8.77	3	36	1	19	2.139	0.160	0.101
		VFI	no	10	59.00 ± 44.23	2	130	1	8	2.370	0.162	0.229
		MED	yes	13	27.15 ± 38.01	3	144	1	11	0.774	0.398	0.066
			no	12	17.42 ± 15.21	3	52	1	10	0.004	0.950	0.000
		VCO	no	13	17.77 ± 17.96	2	53	1	11	0.003	0.957	0,000
		Overall	yes	115	19.54 ± 26.77	1	144	1	113	0.214	0.644	0.002
			excluded	107	13.60 ± 14.06	1	57	1	105	0.947	0.333	0.009
	Sediment (s)	FNE	no	17	89.59 ± 104.51	1	290	1	15	1.326	0.267	0.081
		FSW	yes	17	79.35 ± 118.17	1	439	1	15	0.835	0.375	0.053
			excluded	15	42.80 ± 52.42	1	156	1	13	0.394	0.541	0.029
		SA4	yes	22	71.14 ± 99.5	0	410	1	20	2.119	0.161	0.096
			excluded	21	55.50 ± 66.90	0	235	1	19	0.967	0.338	0.048
		SA8	no	23	86.61 ± 86.71	1	350	1	21	4.048	0.057	0.162
		VFI	no	10	50.20 ± 46.01	1	127	1	8	0.002	0.964	0.000
		MED	yes	13	108.08 ± 167.61	1	576	1	11	0.222	0.646	0.020
			excluded	11	43.45 ± 36.34	1	113	1	9	5.669	0.041	0.386
		VCO	no	13	110.38 ± 113.96	1	317	1	11	13.631	**0.004**	0.553
		Overall	yes	115	84.97 ± 107.53	0	576	1	113	9.894	**0.002**	0.081
			excluded	107	62.93 ± 69.28	0	278	1	105	6.706	**0.011**	0.060

Table 3.4 – Continued

Burying sequence		Sediment type	Observational data		Observational results			Linear regression				
			Outliers	**n**	**Ø ± SD**	**Min.**	**Max.**	df_{reg}	df_{res}	F	p	r^2
Pre-Burying	Latency (s)	FNE	no	17	100.76 ± 103.22	3	302	1	15	1.684	0.214	0.101
		FSW	yes	17	79.35 ± 118.17	2	464	1	15	0.586	0.456	0.038
			excluded	16	75.56 ± 87.91	2	274	1	14	0.579	0.459	0.040
		SA4	yes	22	82.23 ± 101.75	2	416	1	20	2.255	0.149	0.101
			excluded	21	66.33 ± 70.96	2	239	1	19	1.089	0.310	0.054
		SA8	yes	23	102.04 ± 90.70	5	377	1	21	3.835	0.064	0.154
			excluded	22	89.55 ± 69.67	5	207	1	20	1.963	0.177	0.089
		VFI	no	10	109.20 ± 80.26	3	244	1	8	0.644	0.446	0.074
		MED	yes	13	135.23 ± 186.93	20	599	1	11	0.361	0.560	0.032
			excluded	11	60.36 ± 36.26	20	140	1	9	6.165	**0.035**	0.407
		VCO	no	13	128.15 ± 120.52	3	357	1	11	10.923	**0.007**	0.498
		Overall	yes	115	104.85 ± 115.09	2	599	1	113	9.119	**0.003**	0.075
			excluded	110	88.25 ± 84.78	2	357	1	108	9.642	**0.002**	0.082
Phase 1	Duration (s)	FNE	yes	17	35.59 ± 56.73	3	190	1	15	0.736	0.404	0.047
			excluded	14	11.79 ± 8.75	3	37	1	12	0.004	0.952	0.000
		FSW	yes	17	15.71 ± 14.13	4	65	1	15	0.318	0.581	0.021
			excluded	15	11.40 ± 4.26	4	18	1	13	2.850	0.115	0.180
		SA4	yes	22	16.86 ± 24.00	4	88	1	20	0.022	0.884	0.001
			excluded	19	8.16 ± 4.40	4	20	1	17	1.011	0.329	0.056
		SA8	yes	23	14.87 ± 16.22	6	71	1	21	0.191	0.666	0.009
			excluded	19	8.68 ± 2.00	6	13	1	17	3.056	0.098	0.152
		VFI	no	10	23.60 ± 17.21	9	59	1	8	0.358	0.566	0.043
		MED	yes	13	11.54 ± 15.68	3	54	1	11	1.180	0.301	0.097
			excluded	11	5.27 ± 1.27	3	8	1	9	0.163	0.696	0.018
		VCO	yes	13	53.00 ± 51.31	8	177	1	11	0.090	0.770	0.008
			excluded	12	42.67 ± 36.84	8	108	1	10	0.788	0.396	0.073
		Overall	yes	115	23.13 ± 33.66	3	190	1	113	0.211	0.647	0.002
			excluded	93	10.04 ± 5.37	3	31	1	91	0.604	0.439	0.007

Table 3.4 – Continued

Burying sequence		Sediment type	Observational data		Observational results			Linear regression				
			Outliers	**n**	**Ø ± SD**	**Min.**	**Max.**	df_{reg}	df_{res}	F	p	r^2
Phase 1	Number of funnel jets	FNE	no	17	8.71 ± 2.11	4	12	1	15	0.621	0.443	0.040
		FSW	yes	17	10.24 ± 2.63	6	16	1	15	2.059	0.172	0.121
			excluded	16	9.88 ± 2.25	6	12	1	14	1.239	0.284	0.081
		SA4	no	22	8.00 ± 1.51	6	12	1	20	7.606	**0.012**	0.276
		SA8	yes	23	11.48 ± 3.68	8	26	1	21	1.936	0.179	0.084
			excluded	22	10.82 ± 1.92	8	14	1	20	0.228	0.638	0.011
		VFI	no	10	13.80 ± 4.37	10	22	1	8	0.018	0.896	0.002
		MED	no	13	8.00 ± 1.41	6	10	1	11	0.001	0.970	0.000
		VCO	yes	13	26.31 ± 12.22	14	52	1	11	2.000	0.185	0.154
			excluded	11	22.00 ± 6.75	14	38	1	9	0.384	0.554	0.041
		Overall	yes	115	11.70 ± 7.26	4	52	1	113	0.000	0.993	0.000
			excluded	100	9.50 ± 2.42	4	16	1	98	3.037	**0.085**	0.030
Resting period (s)		FNE	yes	17	20.18 ± 62.17	0	256	1	15	0.762	0.397	0.048
			excluded	14	1.50 ± 1.83	0	6	1	12	4.805	**0.049**	0.286
		FSW	yes	17	4.41 ± 3.48	0	15	1	15	0.409	0.532	0.027
			excluded	16	3.75 ± 2.24	0	7	1	14	0.303	0.591	0.021
		SA4	yes	22	6.00 ± 13.08	0	61	1	20	1.403	0.250	0.066
			excluded	20	2.70 ± 3.47	0	13	1	18	4.228	**0.055**	0.190
		SA8	yes	23	24.35 ± 68.35	0	196	1	21	0.077	0.784	0.004
			excluded	19	1.32 ± 1.49	0	5	1	17	6.435	**0.021**	0.275
		VFI	yes	10	1.10 ± 2.08	0	5	1	8	0.439	0.526	0.052
			excluded	8	0.13 ± 0.35	0	1	1	6	1.367	0.287	0.186
		MED	yes	13	7.92 ± 12.47	1	48	1	11	0.916	0.359	0.077
			excluded	12	4.58 ± 3.40	1	11	1	10	0.115	0.741	0.011
		VCO	no	13	0.46 ± 0.66	0	2	1	11	0.100	0.758	0.009
		Overall	yes	115	10.70 ± 39.70	0	275	1	113	0.002	0.960	0.000
			excluded	104	2.21 ± 2.61	0	11	1	102	6.286	**0.014**	0.058

Table 3.4 – Continued

Burying sequence		Sediment type	Observational data		Observational results			Linear regression				
			Outliers	**n**	**Ø ± SD**	**Min.**	**Max.**	df_{reg}	df_{res}	F	p	r^2
Phase 2	Duration (s)	FNE	no	17	13.18 ± 3.26	8	18	1	15	4.939	**0.042**	0.248
		FSW	yes	17	14.59 ± 19.37	6	89	1	15	2.122	0.166	0.124
			excluded	16	9.94 ± 2.86	6	14	1	14	0.639	0.437	0.044
		SA4	no	22	13.77 ± 3.38	7	20	1	20	1.184	0.290	0.056
		SA8	no	23	17.65 ± 3.79	10	25	1	21	3.387	**0.080**	0.139
		VFI	no	10	9.30 ± 1.95	7	12	1	8	0.012	0.914	0.002
		MED	no	13	10.08 ± 1.98	6	13	1	11	0.601	0.455	0.052
		VCO	no	13	16.31 ± 3.09	13	21	1	11	0.143	0.713	0.013
		Overall	yes	115	14.06 ± 8.24	6	89	1	113	7.352	**0.008**	0.061
			excluded	113	13.30 ± 4.16	6	24	1	111	6.712	**0.011**	0.057
	Number of arm sweeps	FNE	no	17	5.76 ± 2.22	2	9	1	15	17.217	**0.001**	0.534
		FSW	no	17	4.94 ± 1.60	2	9	1	15	4.504	0.051	0.231
		SA4	no	22	6.41 ± 1.94	3	10	1	20	12.896	**0.002**	0.392
		SA8	no	23	8.43 ± 1.83	4	12	1	21	7.660	**0.012**	0.267
		VFI	yes	10	4.50 ± 1.43	2	7	1	8	1.366	0.276	0.146
			excluded	9	4.22 ± 1.20	2	6	1	7	0.058	0.816	0.008
		MED	no	13	5.92 ± 0.95	4	7	1	11	5.688	**0.036**	0.341
		VCO	no	13	9.15 ± 1.28	7	11	1	11	0.069	0.798	0.006
		Overall	no	115	6.59 ± 2.28	2	12	1	113	25.580	**0.000**	0.185
Burying duration (s)		FNE	yes	17	68.94 ± 82.55	14	305	1	15	0.000	0.992	0.000
			excluded	14	35.07 ± 25.49	14	107	1	12	3.064	0.106	0.203
		FSW	yes	17	34.71 ± 22.01	12	101	1	15	0.571	0.461	0.037
			excluded	15	27.67 ± 8.75	12	44	11	13	0.018	0.896	0.001
		SA4	yes	22	36.64 ± 26.81	14	99	1	20	0.703	0.412	0.034
			excluded	18	24.83 ± 7.33	14	40	1	16	0.925	0.350	0.055
		SA8	yes	23	56.87 ± 67.85	17	299	1	21	0.232	0.635	0.011
			excluded	21	37.33 ± 17.86	17	83	1	19	1.053	0.318	0.053
		VFI	no	10	34.00 ± 18.38	16	70	1	8	0.220	0.651	0.027

Table 3.4 – Continued

Burying sequence	**Sediment type**	**Observational data**		**Observational results**			**Linear regression**				
		Outliers	**n**	**Ø ± SD**	**Min.**	**Max.**	df_{reg}	df_{res}	F	p	r^2
	MED	yes	13	29.54 ± 23.94	13	96	1	11	1.340	0.272	0.109
		excluded	11	20.18 ± 4.03	13	26	1	9	0.184	0.678	0.020
	VCO	yes	13	69.77 ± 53.01	19	198	1	11	0.100	0.757	0.009
		excluded	12	59.08 ± 38.03	19	126	1	10	0.837	0.382	0.077
	Overall	yes	115	47.89 ± 51.68	12	305	1	113	0.463	0.497	0.004
		excluded	101	32.30 ± 17.23	12	85	1	99	0.758	0.386	0.008

Ø = mean value. SD = standard deviation. Min. and Max. are abbreviations for the minimal and maximal value of the corresponding data set. df_{reg} and df_{res} are abbreviations for the regression degrees of freedom and the residual degrees of freedom, respectively. Significant *p*-values are marked boldly.

Table 3.5 – Statistical analysis of the differences between the obtained observations (excluding outliers) of each burying sequence and each sediment sample

Burying sequence	Test	SS	*d.f.1*	*d.f.2*	Statistic	*p*	Homogeneous groups
Pre-Burying							
Water column (s)	Kruskal-Wallis	n.s.	6	-	$H = 22.482$	0.001	SA4, FNE, FSW, SA8, VCO, MED < FSW, SA8, VCO, MED, VFI
Sediment (s)	Kruskal-Wallis	n.s.	6	-	$H = 5.713$	0.456	
Latency (s)	Kruskal-Wallis	n.s.	6	-	$H = 5.284$	0.508	
Phase 1							
Duration (s)	Kruskal-Wallis	n.s.	6	-	$H = 47.537$	0.000	MED, SA4, SA8, FNE <SA4, SA8, FNE, FSW < SA8, FNE, FSW, VFI < FNE, FSW, VFI, VCO
Number of funnel jets	Kruskal-Wallis	n.s.	6	-	$H = 60.183$	0.000	SA4, MED, FNE, FSW < FNE, FSW, SA8 < FSW, SA8, VFI < VFI, VCO
Resting period (s)	Kruskal-Wallis	n.s.	6	-	$H = 33.774$	0.000	VFI, VCO, SA8, FNE, SA4 < SA8, FNE, SA4, FSW, MED
Phase 2							
Duration (s)	Kruskal-Wallis	n.s.	6	-	$H = 57.268$	0.000	VFI, MED, FSW, FNE < MED, FSW, FNE, SA4 < FNE, SA4, VCO, SA8
Number of arm sweeps	Welch	n.s.	6	42.951	$F = 21.127$	0.000	VFI, FSW, FNE, MED < FSW, FNE, MED, SA4 < SA8, VCO
Burying duration (s)	Kruskal-Wallis	n.s.	6	-	$H = 24.868$	0.000	MED, SA4, FSW, VFI, FNE < SA4, FSW, VFI, FNE, SA8 < FSW, VFI, FNE, SA8, VCO

SS = Sum of the Squares. *d.f.1* = degrees of freedom (n-1). *d.f.2* = degrees of freedom (n-k). n.s. = not supplied by the SPSS programme. Kruskal Wallis is an abbreviation for Kruskal Wallis H test. Welch is an abbreviation for Welch's Robust ANOVA. Groups underlined with the same line do not exhibit a significant difference ($p < 0.05$).

Table 3.6 – Statistical relationship between the mean grain size of the tested sediment samples and the obtained results (excluding outliers) of the burying characteristics

Burying characteristic		**Model**	df_{reg}	df_{res}	F	p	r^2	**Regression equation**
Pre-Burying	Water column	Linear	1	5	2.644	0.165	0.346	$y = 8.308x + 4.181$
		Quadratic	2	4	8.652	**0.035**	**0.812**	**$y = 7.109x^2 – 17.09x + 16.46$**
	Sediment	Linear	1	5	10.352	**0.024**	**0.674**	**$y = -16.87x + 97.74$**
		Quadratic	2	4	6.857	0.051	0.774	$y = 4.783x^2 – 33.96x + 106$
	Latency	Linear	1	5	0.492	0.514	0.090	$y = -5.602x + 99.75$
		Quadratic	2	4	6.036	**0.062**	**0.751**	**$y = 11.22x^2 – 45.69x + 119.1$**
Phase 1	Duration	Linear	1	5	0.670	0.450	0.118	$y = -3.461x +21.97$
		Quadratic	2	4	25.623	**0.005**	**0.928**	**$y = 6.676x^2 – 27.31x + 33.50$**
	Number of funnel Jets	Linear	1	5	1.122	0.338	0.183	$y = -1.644x + 14.46$
		Quadratic	2	4	174.467	**0.000**	**0.989**	**$y = 2.538x^2 – 10.71x + 18.85$**
Resting time		Linear	1	5	0.144	0.720	0.028	$y = 0.2143x + 1.690$
		Quadratic	2	4	2.156	0.232	0.519	$y = -0.6605x^2 + 2.574x + 0.5487$
Phase 2	Duration	Linear	1	5	13.254	**0.015**	**0.726**	**$y = -2.142x + 16.62$**
		Quadratic	2	4	5.331	0.074	0.727	$y = 0.0613x^2 – 2.361x + 16.73$
	Number of arm sweeps	Linear	1	5	40.873	**0.001**	**0.891**	**$y = -1.293x + 8.657$**
		Quadratic	2	4	21.653	**0.007**	**0.915**	**$y = 0.1578x^2 – 1.857x + 8.929$**
Burying duration		Linear	1	5	3.024	0.143	0.377	$y = -5.934x + 44.36$
		Quadratic	2	4	20.063	**0.008**	**0.909**	**$y = 5.197x^2 – 24.50x + 53.34$**

df_{reg} and df_{res} are abbreviations for the regression degrees of freedom and the residual degrees of freedom, respectively. Bold values indicate significant relationships ($p < 0.05$).

well as among FNE, FSW, VFI, VCO (Table 3.5). The tested sepiolids ejected an average of 10 ± 2.42 funnel jets during phase 1, whereas these observations ranged from 8 ± 1.41 funnel jets on the sediment sample MED up to 22 ± 6.75 funnel jets on the sediment sample VCO (Table 3.4), resulting in statistically similar numbers of funnel jets found between SA4, MED, FNE and FSW, between FNE, FSW and SA8, between FSW, SA8 and VFI as well as between VFI, VCO (Table 3.5). For both burying characteristics, a quadratic regression model could be applied (Table 3.6), displaying that *Sepiola* sp. needed less time and less funnel jets on medium sediment samples to accomplish phase 1 of its burying behaviour, whereas both the duration and number of funnel jets increased on finer and coarser sediment samples (Figure 3.4D+E). In between phase 1 and phase 2 of their burying procedure, the tested sepiolids rested on average 2.21 ± 2.61 s, ranging from 0.13 ± 0.35 s (VFI) to 4.58 ± 3.40 s (MED) (Table 3.4). While statistically similar values were found among VFI, VCO, SA8, FNE and SA4 as well as among SA8, FNE, SA4, FSW and MED (Table 3.5), no regression model could be applied to this data set (Table 3.6; Figure 3.4F).

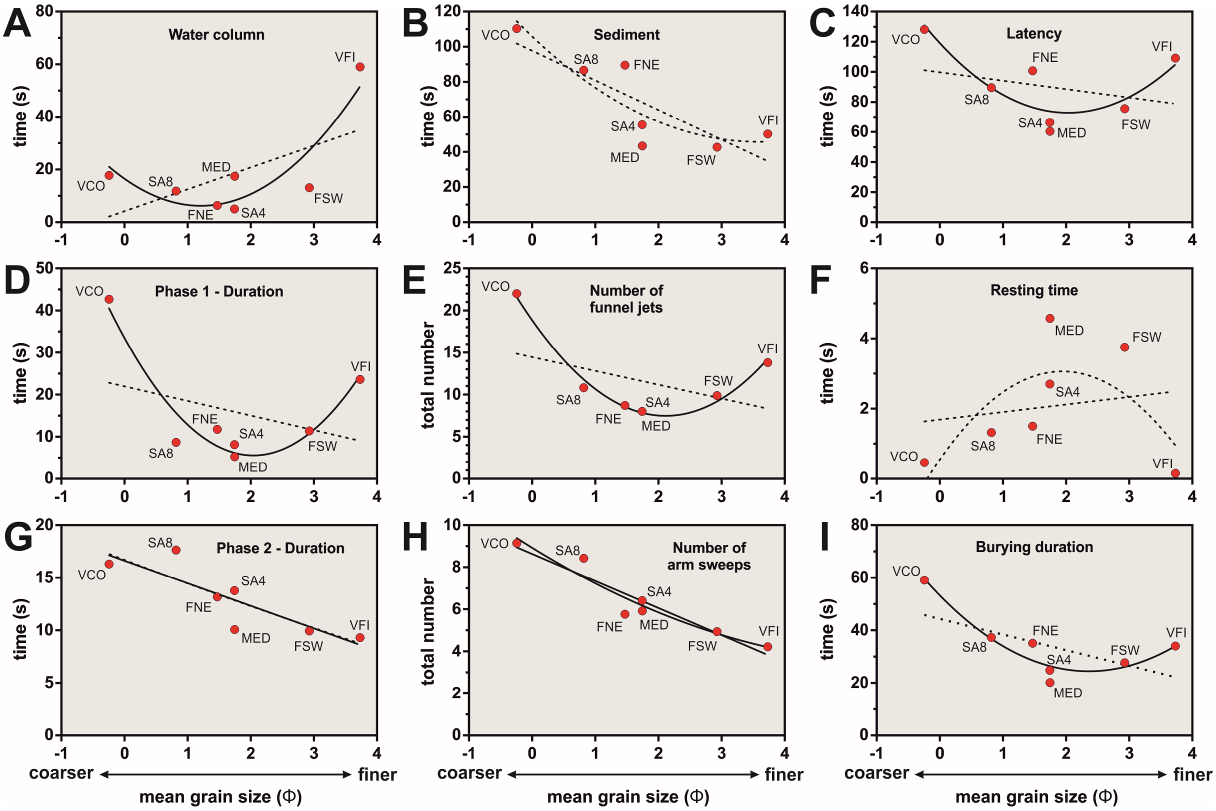


Figure 3.4 – Average burying characteristics per sediment sample. (**A** – **I**) see Table 1 for definitions. Depicted are also significant (solid lines) and non-significant (dashed lines) linear and quadratic regression models for the relationship between the mean grain size (Φ) of each sediment sample and the correspondingly obtained observations per burying characteristics (Table 3.6).

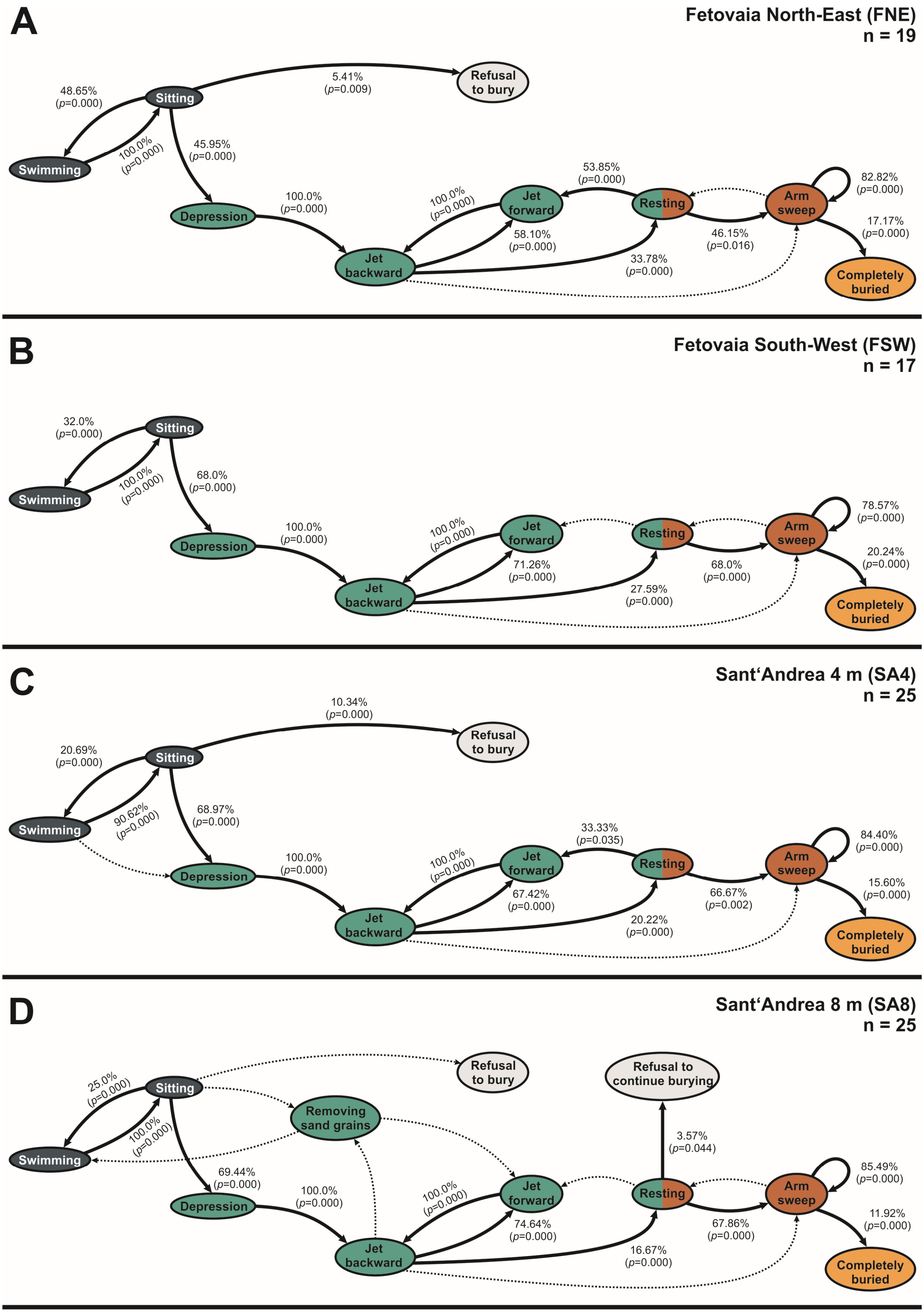
The duration of phase 2 of the burying behaviour (*sensu* Boletzky & Boletzky, 1970) lasted on average 13.30 ± 4.16 s while ranging from 9.30 ± 1.95 s (VFI) to 17.65 ± 3.79 s (SA8). During this phase, the tested sepiolids needed on average 7 ± 2.28 arm sweeps to cover their bodies with sediment, although this value different from 4 ± 1.20 arm sweeps on VFI up to 9 ± 1.28 arm sweeps on VCO (Table 3.4). Consequently, statistically similar durations of phase 2 were established among VFI, MED, FSW and FNE, among MED, FSW, FNE and SA4 as well as among FNE, SA4, VCO and SA8, whereas the number of arm sweeps did not differ significantly among the sediment samples VFI, FSW, FNE, MED, among FSW, FNE, MED and SA4 as well as among SA8 and VCO (Table 3.5). Additionally, linear regression models could be applied to both characteristics (Table 3.6), showing that both the duration of phase 2 as well as the number of arm sweeps decreased the finer the tested sediment samples were (Figure 3.4G+H). For the number of arm sweeps, the same correlation could also be shown with a quadratic regression model (Table 3.6; Figure 3.4H).

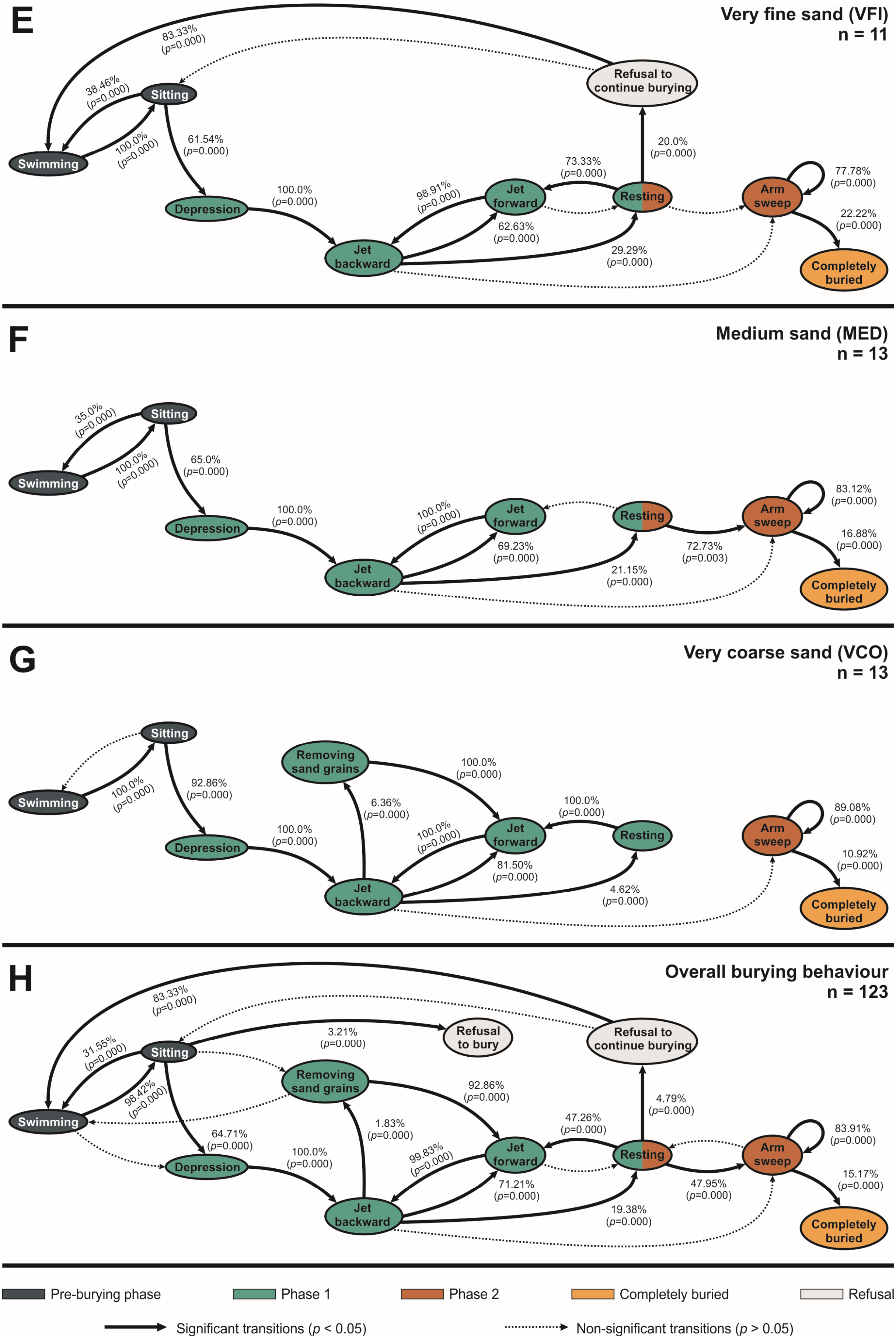
The total duration of the burying behaviour of *Sepiola* sp. lasted on average 32.30 ± 17.23 s, ranging from 20.18 ± 4.03 s on the sediment sample MED to 59.08 ± 38.03 s on the sediment sample VCO (Table 3.4). No statistical differences were found among MED, SA4, FSW, VFI and FNE, among SA4, FSW, VFI, FNE and SA8 as well as among FSW, VFI, FNE, SA8 and VCO (Table 3.5). Subsequently, a quadratic regression model could be applied to this data set (Table 3.6), demonstrating that the duration of the whole burying procedure of *Sepiola* sp. was the shortest for medium to fine sediment samples while it extended for very fine and coarse sediment samples, respectively (Figure 3.4I).

No significant correlation with the DML was found for any obtained observations of the burying characteristics in general or on a specific sediment sample (Table 3.4). Although admittedly for some of the burying characteristics a significant relationship between the corresponding data set and the DML was indicated (boldly marked in Table 3.4), the respective r^2 values displayed low correlation degrees only (Table 3.4).

When analysing the kinematic diagrams of the burying behaviour of *Sepiola* sp. (Figure 3.5), a moderately consistent pathway of significant ($p < 0.05$) transitions was found on all sediment types, whereas especially non-significant observations varied between the sediment samples. By focussing on significant transitions only, it could be seen for each sediment sample that settled individuals of *Sepiola* sp. might either return to the water column or start phase 1 of the burying procedure by creating a depression in the sediment. Interestingly, the significant transitions during phase 1 of the burying behaviour differed among the sediment samples. While the tested sepiolids on the sediment samples FSW (Figure 3.5B), SA8 (Figure 3.5D) and MED

(Figure 3.5F) continued their burying behaviour with alternating forward- and backward-directed funnel jets, followed by a resting period before starting phase 2 of their burying





E
Very fine sand (VFI)
n = 11
Refusal to continue burying
Sitting
Swimming
Depression
Jet backward
Jet forward
Resting
Arm sweep
Completely buried
83.33% (p=0.000)
38.46% (p=0.000)
100.0% (p=0.000)
61.54% (p=0.000)
100.0% (p=0.000)
98.91% (p=0.000)
62.63% (p=0.000)
73.33% (p=0.000)
20.0% (p=0.000)
29.29% (p=0.000)
77.78% (p=0.000)
22.22% (p=0.000)
F
Medium sand (MED)
n = 13
Sitting
Swimming
Depression
Jet backward
Jet forward
Resting
Arm sweep
Completely buried
35.0% (p=0.000)
100.0% (p=0.000)
65.0% (p=0.000)
100.0% (p=0.000)
100.0% (p=0.000)
69.23% (p=0.000)
21.15% (p=0.000)
72.73% (p=0.003)
83.12% (p=0.000)
16.88% (p=0.000)
G
Very coarse sand (VCO)
n = 13
Sitting
Swimming
Depression
Removing sand grains
Jet backward
Jet forward
Resting
Arm sweep
Completely buried
100.0% (p=0.000)
92.86% (p=0.000)
100.0% (p=0.000)
100.0% (p=0.000)
6.36% (p=0.000)
100.0% (p=0.000)
100.0% (p=0.000)
81.50% (p=0.000)
4.62% (p=0.000)
89.08% (p=0.000)
10.92% (p=0.000)
H
Overall burying behaviour
n = 123
Refusal to bury
Refusal to continue burying
Sitting
Swimming
Depression
Removing sand grains
Jet backward
Jet forward
Resting
Arm sweep
Completely buried
83.33% (p=0.000)
31.55% (p=0.000)
98.42% (p=0.000)
3.21% (p=0.000)
64.71% (p=0.000)
100.0% (p=0.000)
1.83% (p=0.000)
92.86% (p=0.000)
99.83% (p=0.000)
71.21% (p=0.000)
47.26% (p=0.000)
4.79% (p=0.000)
19.38% (p=0.000)
47.95% (p=0.000)
83.91% (p=0.000)
15.17% (p=0.000)
Pre-burying phase
Phase 1
Phase 2
Completely buried
Refusal
Significant transitions (p < 0.05)
Non-significant transitions (p > 0.05)

Figure 3.5 – Kinematic diagrams of the burying behaviour in *Sepiola* sp.. Depicted are significant (solid lines) and non-significant (dashed lines) transitions for the four natural sediment samples **(A)** Fetovaia North-East (FNE), **(B)** Fetovaia South-West (FSW), **(C)** Sant'Andrea 4 metre (SA4) and **(D)** Sant'Andrea 8 metre (SA8) as well as the three artificially made sediment samples **(E)** very fine sand (VFI), **(F)** medium sand (MED) and **(G)** very coarse sand (VCO). In **(H)**, the overall burying behaviour, consisting of all recorded burying observations is presented. For significant transitions, the corresponding transition probabilities are presented in %.

procedure, significant resting periods during phase 1 were observed in the sepiolids of the remaining sediment samples FNE (Figure 3.5A), SA4 (Figure 3.5C), VFI (Figure 3.5E) and VCO (Figure 3.5G). However, once phase 2 of the burying procedure was started, no significant resting periods were observed on any of the tested sediment samples (Figure 3.5A-G).

The removal of coarser sediment particles was only observed for sepiolids burying on the two sediment samples with the largest mean grain size, SA8 and VCO. On SA8, the removal of sediment particles could be seen both between settling on the sediment and the start of the burying procedure as well as during phase 1, whereas no significant transitions were established (Figure 3.5D). Contrarily, this behaviour was found be to significant during phase 1 of the burying behaviour of the tested sepiolids on the sediment sample VCO but could not be observed at all before the start of the burying procedure on the same sediment sample (Figure 3.5G).

Based on the significant transitions in Figure 3.5H, the overall burying procedure of *Sepiola* sp. can be summarised as follows: After individuals of this species settled on the sediment (98.42 %), they either returned to the water column (31.55 %), did not bury at all (3.21 %) or started the burying procedure by creating a depression (64.71 %), followed by a backward-directed funnel jet (100.0 %). Subsequently, alternating forward and backward-directed funnel jets were conducted (71.21 % and 99.83 %, respectively), potentially intermitted by the removal of sand grains (1.83 %) or a resting period (19.38 %). However, a resting period may have also led to a refusal to continue burying (4.79 %), followed by the sepiolid re-entering the water column (83.33 %), or to the beginning of the second phase, hence an arm sweep (47.95 %). Arm sweeps were then repeated (83.91 %) until the animal was completely buried.

3.3 – Discussion

The sepiolids used in the present study were identified as *Sepiola* sp. by the presence of kidney-shaped light organs inside their mantle cavities (Bello, 1995; Reid & Jereb, 2005). However, an exact identification of the species could not be achieved. Although the collected individuals shared close anatomical and morphological features with two species from the *Sepiola atlantica*-group (*sensu* Naef, 1923), namely *Sepiola intermedia* as well as *Sepiola bursadhaesa*, they differed in at least one significant identification characteristic with each of the two species (Bello, 1995, 2013). In *S. intermedia*, two enlarged proximal suckers can be found on the inner row of the distal part of the hectocotylus (Bello, 2013), whereas all individuals of the species of the present study possessed three of those enlarged suckers. While the same number of enlarged suckers can also be found in *Sepiola bursadhaesa*, this species additionally exhibits a prominent semi-circular groove perpendicular to the arm axis (Bello, 2013) which could not be found in the sepiolids used in the present study.

Furthermore, tissue samples of the used individuals were sent to the Institute of Environmental Science of the University of Koblenz-Landau (Germany). After extracting DNA from these samples, a 595 bp fragment of the cytochrome oxidase subunit I (COI) was amplified using standard 'Folmer primers' (Folmer et al., 1994) and subsequently sequenced. The obtained sequence was compared to the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). The best match with 100 % query coverage and 99.66 % similarity was identified as *Sepiola affinis* (Accession number AY557523.1; Lindgren et al. (2004)). However, the latter species could be morphologically excluded due to differently pronounced enlarged suckers on the inner row of the distal part of the hectocotylus (Bello, 1995). Nevertheless, the high similarity with *S. affinis* as well as *Sepiola tridens* (100% query coverage; 91.11% similarity) and *Sepiola rondeletii* (100% query coverage; 90.44 % similarity) further supports the assumption that the species used in the present study belongs to the *Sepiola atlantica*-group to which the genetically similar species *S. affinis*, *S. tridens* and *S. rondeletii* as well as the morphologically similar species *S. intermedia* and *S. bursadhaesa* belong (Bello, 2013; Naef, 1923). Bello (2013) stated that 'because of the […] combination of factors that facilitates speciation [in *Sepiola*], it is likely that other, still undescribed species of this genus await discovery', which is further emphasised by recent findings of previously undescribed sepiolids in European waters (Bello, 2013; Bello & Salman, 2015; de Heij & Goud, 2010; Groenenberg et al., 2009). While the species used in the present study also potentially represents an up to now undescribed *Sepiola* species, it could still be clearly distinguished from another not closer identified sepiolid species caught twice during the conducted SCUBA surveys based on the above-mentioned anatomical features.

The burying behaviour of *Sepiola* sp. greatly resembled the observations reported for other members of the genus *Sepiola* (Boletzky & Boletzky, 1970; Drerup et al., Chapter 2; Rodrigues et al., 2010), *Sepietta* (Boletzky & Boletzky, 1970) and *Rossia* (Anderson et al., 2004) while it slightly differed to members of the genus *Euprymna* (Anderson et al., 2002; Moynihan, 1983; Nabhitabhata et al., 2005). While *Sepiola*, *Sepietta* and *Rossia* begin their burying procedure with a forward directed funnel jet (Anderson et al., 2004; Drerup et al., Chapter 2; Boletzky & Boletzky, 1970; Rodrigues et al., 2010), both for *Euprymna hyllebergi* and *E. scolopes* an initial backward-directed funnel jet was reported (Anderson et al., 2002; Nabhitabhata et al., 2005). Similar to the first phase, a disparity among the genera can be also found in phase 2. Identically to the tested species of the genera *Sepiola*, *Sepietta* and *Rossia* (Anderson et al., 2004; Drerup et al., Chapter 2; Boletzky & Boletzky, 1970; Rodrigues et al., 2010), *Sepiola* sp. used its dorsolateral arms during phase 2 of their burying pattern in the present study. Contrarily to these observations, Nabhitabhata et al. (2005) stated that *E. hyllebergi* performed phase 2 with its ventrolateral arms, while Moynihan (1983) reported for *E. scolopes* even the use of its long tentacles for sweeping sand during phase 2 of its burying behaviour. However, it might be possible that the latter author mistook the tentacles for the actual arms of *E. scolopes* due to their thin appearance while being stretched out, as generally postulated before by Boletzky & Boletzky (1970).

In the present study, individuals of *Sepiola* sp. exhibited a dark colouration when settling on the sediment as well as at the beginning of their burying procedure, which displayed a strong contrast to the rather white sediment samples. As already suggested for *E. scolopes* by Anderson et al. (2002), a dark colouration might be beneficial in terms of a sudden escape response, as sepiolids are known to eject a series of dark ink blobs when jetting away as a decoy for predators (Anderson & Mather, 1996; Drerup et al., Chapter 2; Moynihan, 1983; Shimek, 1983). However, as the escape responses of *Sepiola* sp. have not been investigated in this study, further research is needed to confirm this hypothesis.

Although in this study the latency until the start of burying was recorded and even a correlation between the mean grain size and the latency as well as the mean grain size and the total time spend in the water column was found, this data must be interpreted with due caution. As already mentioned by Boletzky & Boletzky (1970), the behaviour and reaction of a bobtail squid might vary when exposed to the same kind of sediment for several times. Furthermore, the latency shown by an individual might also be heavily affected by its stress level, e.g. altered by the previous level of handling before introducing it into the experimental tank or simply the experimental setup itself. Nevertheless, in the present study *Sepiola* sp. spent on average 13.60 ± 14.06 s in the water column, which is similar to *E. scolopes* (13 s; Anderson et al., 2002), *Rossia pacifica* (17 s; Anderson et al., 2004) and *Sepiola parva* (12.65 ± 7.87 s; Drerup et al., Chapter 2). Contrarily, the total average time spent on the sediment (62.93 ± 69.28 s) was longer than observed for *E. scolopes* (13 s; Anderson et al., 2002) but significantly shorter than for *S. parva* (140.50 ± 129.38 s; Drerup et al., Chapter 2) and *R. pacifica* (222 s; Anderson et al., 2004).

In the present study, phase 1 of the burying behaviour of *Sepiola* sp. took on average 10.04 ± 5.37 s during which the observed sepiolids conducted 10 ± 2.42 funnel jets. While for *S. atlantica* (Rodrigues et al., 2010) and *S. parva* (Drerup et al., Chapter 2) similar durations were reported with 12.2 ± 4.37 s and 9.19 ± 2.71 s, respectively, the average number of funnel jets was evidently lower in this species (*S. atlantica* 6.3 funnel jets, *S. parva* 7 ± 1.23 funnel jets). Phase 2 of *Sepiola* sp. in the present study lasted with on average 13.30 ± 4.16 s slightly longer compared to *S. atlantica* (10.2 ± 2.95 s; Rodrigues et al., 2010) and *S. parva* (12.17 ± 2.77 s; Drerup et al., Chapter 2). While the average number of arm sweeps (6.59 ± 2.28) was alike to those reported for *S. atlantica* (6.00 ± 0.79; Rodrigues et al., 2010), it only corresponded to roughly 60 % of the observed 11.32 ± 3.28 arm sweeps in *S. parva* (Drerup et al., Chapter 2). In sum, the total duration of the burying behaviour in *Sepiola* sp. in the present study (32.30 ± 17.23 s) was shorter than observed for *E. scolopes* (48 and 44 s, respectively; Anderson, 1997; Anderson et al., 2002) and *R. pacifica* (213 s; Anderson et al., 2004) but lasted longer than reported for *S. atlantica* (21.9 ± 4.93 s; Rodrigues et al., 2010) and *S. parva* (24.38 ± 6.29 s; Drerup et al., Chapter 2).

Boletzky & Boletzky (1970) reported that the duration of phase 1 is always longer than phase 2 of the burying behaviour in species of the genus *Sepiola*. This was later confirmed for *S. atlantica* by Rodrigues et al. (2010) but refuted for *S. parva* by Drerup et al. (Chapter 2). The present study also generally refutes these observations as phase 1 was on average shorter than phase 2 (phase 1: 10.04 ± 5.37 s; phase 2: 13.30 ± 4.16 s). Contrariwise, for the two finest sediment samples FSW and VFI as well as the coarsest sediment sample VCO the opposite was observed. Boletzky & Boletzky (1970) further stated for *S. robusta* and *S. obscura* that with an increasing DML the duration of the first phase declines whereas the second phase extends. However, these observations could neither be confirmed in previous studies with *S. atlantica* (Rodrigues et al., 2010) and *S. parva* (Drerup et al., Chapter 2) nor in the present study. Additionally, neither a pre-burial alert posture as reported for *R. pacifica* (Anderson et al., 2004) and *S. atlantica* (Rodrigues et al., 2010) nor any angling behaviour (= protruding of one arm vertically out of the sediment) as documented for buried or partly buried individuals of and *E. hyllebergi* (Nabhitabhata et al., 2005) and *R. pacifica* (Anderson et al., 2004) was observed for *Sepiola sp.* in this study. However, several individuals of *Sepiola* sp. were observed to actively touch the sediment with the tips of their arms before settling, potentially to analyse the sediment in terms of its characteristics and composition. To the best of our knowledge, this behaviour has never been reported in sepiolids before and is otherwise only known in a similar fashion in the cuttlefish *S. officinalis* (Mather, 1986).

Up to present, only a few studies dealt with the burying behaviour of sepiolids on different sediment types. Anderson et al. (2002) analysed the burying behaviour of *E. scolopes* on subtidal (mean grain size (mgs) = 0.47 mm), intertidal (mgs = 0.48 mm) and magmatic sand (mgs = 0.85 mm) as well as aquarium gravel (mgs = 4.9 mm). The authors found not only that the latency until burying, the burying duration and the refusal to bury was significantly higher in the two sediment types with the highest mgs (magmatic sand and gravel), but also that the latency until start of burying and the burying duration significantly varied between subtidal sand (mean latency 13 s; mean burying duration 48 s) and intertidal sand (31 s; 80 s), although both sediment types had a similar mean grain size. A direct comparison with the observations from the present study is not feasible as the tested sediment types of Anderson et al. (2002) exhibited different mean grain sizes and no information about the corresponding grain size distributions are available. However, the results of both studies still follow for the greater part a similar trend to the present results, as both the latency until the start of burying and the burying duration was generally longer on coarser sediment samples than on samples considered as medium grained sand. Additionally, sediment samples with nearly identical mean grain sizes (SA4 and MED in the present study, subtidal and intertidal sand in Anderson et al. (2002)) caused different average latencies as well as burying durations. While Anderson et al. (2002) considered the different silt proportions of the subtidal and intertidal sediment samples to be the reason for these observations, the differences between SA4 and MED in the present study are potentially even caused by several factors. While SA4 is a natural sediment sample with a naturally occurring grain size distribution and microbial community, MED was made up artificially of other washed, dried and subsequently sieved sediment samples and therefore does neither contain a grain size distribution nor a microbiome which can be accounted as natural. In a subsequent study, Anderson et al. (2004) tested the burying activity of *Rossia pacifica* on subtidal sand (mgs = 0.468 mm), construction sand (mgs = 0.56 mm), cryolite sand (mgs = 2.2 mm) and quartz gravel (mgs = 5.2 mm). Again, the authors found that the latency until burying correlated with the mean grain size, whereas on both cryolite sand and quartz gravel no burying activity was observed at all. Furthermore, Boletzky & Boletzky (1970) reported for *S. robusta*, *S. affinis* and *S. obscura* that the duration of the first phase is shorter on medium sand with a mean grain size of 0.6 mm than on fine (mgs = 0.15 mm) or coarse (mgs = 1.6 mm) sand, respectively. These observations were generally confirmed for *Sepiola* sp. in the present study although the investigated sediment samples slightly differed in their mean grain sizes.

While prior studies on other marine organisms with a prominent burying behaviour such as flatfish (Gibson & Robb, 1992; Lü et al., 2018), crustaceans (Nel et al., 1999; Pinn & Ansell, 1993) or bivalves (Alexander et al., 1993; de la Huz et al., 2002; Nel et al., 2001) have shown that the sediment grain size plays an important role in the burying procedure of these animals, the data of the present study confirm these observations for the sepiolid species *Sepiola* sp. by revealing for almost all of the previously defined burying characteristics either a linear or quadratic relationship concerning the mean grain sizes of the tested sediment samples. Although *Sepiola* sp. conducted on the coarsest sediment sample VCO more than three times as many funnel jets as on the medium sediment samples (FNE, SA4, MED) and more as twice as much arms sweeps as on the finest sediment sample (VFI), all observed individuals still performed a moderately fixed burying procedure on all sediment samples, consisting of phase 1 and phase 2 (*sensu* Boletzky & Boletzky, 1970), and displayed hardly any variations besides the removal of sand grains with their arms on coarser sediment. Despite the high behavioural flexibility found among cephalopods (Hanlon & Messenger, 2018), the moderately fixed burying behaviour in *Sepiola* sp. can be considered as a modal action pattern (*sensu* Barlow, 1977), as already suggested in previous studies on the burying behaviour of sepiolids (Anderson et al., 2002, 2004) and cuttlefish (Mather, 1986).

As the burying behaviour of sepiolids is evidently a means of protection (Boletzky et al., 1971), a proper, species-specific sediment assessments may increase the welfare of bobtail squids in captivity (Moltschaniwskyj et al., 2007). The results of the presents study imply that *Sepiola* sp. should be kept on fine to medium grained sediment types with a mean grain size between 125 to 250 µm (2 – 3 Φ) when hold in a captive environment in order to minimise the burying duration and energetic effort.

In conclusion, the burying behaviour of *Sepiola* sp. greatly resembles those of other sepiolids, but the duration and execution of different burying characteristics can be significantly prolonged by certain sediment characteristics. While this study showed that the mean grain size might be the major aspect evoking these differences in the burying procedure, further research needs to be addressed to test the effect of other sediment characteristics such as the microbial community or the organic content.

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APPENDIX

Appendix to the thesis:

Habitat Preference and Behavioural Ecology of Bobtail Squids (Sepiolidae)

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Table 4.1 – Literature dedicated to different ecological aspects (habitat preference, feeding/ hunting behaviour, escape response and burying behaviour) of bobtail squids (Sepiolidae) and pygmy squids (Idiosepiidae).

Ecological aspect	**Family**	**Subfamily**	***Species***	**References**
Habitat preference	Sepiolidae	Rossiinae	*Rossia pacifica*	Anderson (1987)
	Idiosepiidae	-	*Idiosepius pygmaeus*	Moynihan (1983a)
Feeding/ hunting behaviour	Sepiolidae	Sepiolinae	*Euprymna scolopes*	Hanlon et al. (1997), Moynihan (1983a), Shears (1988)
			Sepietta/Sepiola spp.	Boletzky et al. (1971)
			Sepietta oweniana	Bergström (1985), Orsi Relini & Massi (1988)
			Sepiola affinis	Mauris (1989)
		Rossiinae	*Rossia pacifica*	Brocco (1971)
	Idiosepiidae	-	*Idiosepius paradoxus*	Kasugai (2001), Kasugai et al. (2004), Sato et al. (2016)
			Idiosepius pygmaeus	Moynihan (1983b)
			Idiosepius thailandicus	Nabhitabhata (1998)
Escape response	Sepiolidae	Sepiolinae	*Euprymna scolopes*	Anderson & Mather (1996), Moynihan (1983a), Seehafer et al. (2018)
		Rossiinae	*Rossia pacifica*	Shimek (1983)
	Idiosepiidae	-	*Idiosepius paradoxus*	Sato et al. (2016)
			Idiosepius pygmaeus	Moynihan (1983b)
Burying behaviour	Sepiolidae	Sepiolinae	*Euprymna scolopes*	Anderson (1997), Anderson et al. (2002), Moynihan (1983a)
			Euprymna hyllebergi	Nabhitabhata et al. (2005)
			Sepiola atlantica	Rodrigues et al. (2010)
			Sepiola/Sepietta spp.	Boletzky & Boletzky (1970)
		Rossiinae	*Rossia macrosoma*	Boletzky & Boletzky (1973)
			Rossia pacifica	Anderson et al. (2004)

Table 4.2 – Literature dedicated to reproductive behaviour and egg characteristics of bobtail squids (Sepiolidae), bottletail squids (Sepiadaridae) and pygmy squids (Idiosepiidae).

Family	**Subfamily**	***Species***	**References**
Sepiolidae	Sepiolinae	*Euprymna berry*	Choe (1966[d])
		Euprymna hyllebergi	Nabhitabhata et al. (2005[a, b, c, d])
		Euprymna scolopes	Arnold et al. (1972[d]), Hanlon et al. (1997[b, c]), Moynihan (1983a[b]), Singley (1983[b])
		Euprymna tasmanica	Franklin & Stuart-Fox (2017)[e], Franklin et al. (2012[e], 2014[e]), Squires (2013[e]), Squires et al. (2012[e], 2013[a, b, c, d], 2014[e], 2015[e])
		Rondeletiola minor	Czudaj et al. (2013*), Olmos-Pérez et al. (2018*), Önsoy et al. (2013*), Salman & Katagan (1996*)
		Sepietta/ Sepiola	Olmos-Pérez et al. (2018*), Boletzky et al. (1971[c])
		Sepietta spp.	Deickert (2009*)
		Sepietta neglecta	Lefkaditou & Kaspiris (1998*)
		Sepietta oweniana	Bello & Deickert (2003[c, d]), Bergstrom & Summers (1983[b]), Czudaj et al. (2012*), Deickert & Bello (2005[d]), Salman (1998*)
		Sepiola spp.	Salman & Önsoy (2004*)
		Sepiola affinis	Gabel-Deickert (1995[c, d])
		Sepiola atlantica	Jones & Richardson (2010[b, c, d], 2012*), Rodrigues et al. (2009[a, b], 2011[c, d], 2012*), Yau & Boyle (1996*)
		Sepiola intermedia	Salman (2014*)
		Sepiola robusta	Boletzky (1983[b])
		Sepiola rondeletii	Racovitza (1894[b])
	Rossiinae	*Neorossia caroli*	Cuccu et al. (2007[d]), Salman & Önsoy (2010*)
		Rossia sp.	Aldrich & Lu (1968[d])
		Rossia/Neorossia spp.	Laptikhovsky et al. (2008*)
		Rossia macrosoma	Mangold-Wirz (1963[b]), Racovitza (1894[b]), Salman & Önsoy (2010*), Boletzky & Boletzky (1973[d])
		Rossia mollicella	Okutani & Sasaki (2007[d])
		Rossia pacifica	Anderson & Shimek (1994[d]), Brocco (1971[b]), Mangold et al. (1998[d])
		Semirossia patagonia	Önsoy et al. (2008*)
	Heteroteuthinae	*Heteroteuthis dispar*	Hoving et al. (2008*)
Sepiadariidae	-	*Sepiadarium austrinum*	Hooper et al. (2016[e]), Wegener et al. (2013a[e], 2013b[e])
Idiosepiidae	-	*Idiosepius biserialis*	Nabhitabhata & Suwanamala (2008[b, c, d])
		Idiosepius paradoxus	Kasugai (2000[a, b, c]), Kasugai & Ikeda (2003[d]), Natsukari (1970[c, d]), Sato (2017)[e], Sato et al. (2010[e], 2013a[e], 2013b[e], 2014a[e], 2014b[e], 2017[e], 2018[e])
		Idiosepius pygmaeus	Lewis & Choat (1993[c, s])
		Idiosepius thailandicus	Nabhitabhata (1998[b, c, d]), Nabhitabhata & Suwanamala (2008[b, c, d])

[a, b, c, d, e] Information on [a] courtship behaviour, [b] mating behaviour, [c] spawning behaviour, [d] eggs and [e] other reproductive behaviour. References marked with an asterisk (*) are not dedicated to a particular reproductive behaviour but provide useful information on the corresponding species' reproductive biology.

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