Mar Biol (2009) 156:753–761
DOI 10.1007/s00227-009-1126-0

ORIGINAL PAPER

Laboratory culture of the aeolid nudibranch *Spurilla neapolitana* (Mollusca, Opisthobranchia): life history aspects

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Received: 28 September 2008 / Accepted: 4 January 2009 / Published online: 28 January 2009

Abstract *Spurilla neapolitana* from the Eastern Mediterranean was cultured in a self-sustained, temperature-controlled laboratory culture system, and its life cycle is described. Adults were collected from three field sites situated 120 km apart, along the Israeli Mediterranean coastline, between March 2006 and August 2007. Cultures of the life-cycle stages were raised at 24°C. *S. neapolitana* deposited white, coiled, spiral egg masses containing zygotes. Veliger larvae hatched 3.0 ± 0.4 days post oviposition. The veliger larvae are obligatory planktotrophs, with a minimal larval phase of 22 days. In the lab, larvae settled and metamorphosed following exposure to metabolites derived from distinct prey sea anemone species. Reproductive maturity was reached 42 ± 5 days post metamorphosis, resulting in a laboratory generation time of 67 days (egg to egg). The average life span of reproductive specimens in this study was 157 ± 13 days post-oviposition and they reached a length of 7–10 cm. During this period, an average adult deposited ca. 40×10^6 zygotes. This species has several characteristics that suggest it will be a useful model for laboratory-oriented research.

Communicated by J.P. Grassle.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-009-1126-0) contains supplementary material, which is available to authorized users.

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Introduction

Various life-cycle stages of opisthobranch mollusks have served for research in such diverse areas as behavior, development, and ecology (Bonar and Hadfield 1974; Bonar 1976; Kandel 1979; Faucci et al. 2007). In particular, adult opisthobranchs have become premier models for neurobiological investigations, because neurons in their central nervous system are large and easily identifiable and manipulated (Kriegstein 1977a; Harrigan and Alkon 1978; Cohen et al. 2006).

Most nudibranch species produce planktonic larvae that remain in their larval phase for periods of minutes to months, sometimes dispersing over great distances before settling on suitable substrata (Hadfield and Miller 1987; Todd et al. 2001). Many marine invertebrate larvae are influenced to settle and metamorphose by specific chemical and physical cues, which ensure appropriate sites for juvenile growth and eventual reproduction (reviewed by Hadfield and Paul 2001).

Spurilla neapolitana is a cosmopolitan aeolid nudibranch species, occurring in the Mediterranean, West Atlantic (from Florida to Brazil), and East Pacific (Baja California) (Rudman 1999). According to McDonald and Nybakken (1996) and references therein, it has been reported to feed on 37 sea anemone (Anthozoa: Actinaria) species, yet not on cnidarians of other orders or on other epifaunal taxa. Virtually nothing is known of its life history.

Recently, we found *S. neapolitana* year-round at four sites along a 150-km strip of the Israeli Mediterranean coast. Three of the sites are characterized by a fouling community in which two sea anemone species, *Aiptasia diaphana* and *Aiptasiogeton hyalinus*, are common. The fourth site is characterized by an intertidal community in which two other sea anemone species, *Actinia equina* and *Anemonia viridis,* are common. Temperature data in the MEDATLAS/2002 database (MEDAR Group 2002) indicate an annual temperature range of 15–30°C at 10–m depth in our study area. Here we describe the life history of *S. neapolitana* from egg to egg at 24°C. In addition, we tested the effects of several diets on the veliger larvae, as well as the influence of settlement and metamorphic cues on the competent larvae.

Materials and methods

Collection of adult nudibranchs

Adult specimens of *S. neapolitana* (delle Chiaje 1823) (Fig. 1a) were collected from three field sites off the Israeli Eastern Mediterranean coastline, situated 120 km apart, between March 2006 and August 2007. Seawater temperatures at the sites ranged between 15 and 29°C. The northernmost site was situated near Haifa (32°42′25.55″N, 34°56′16.89″E), the central site near Michmoret (32°24′9.85″N, 34°50′49.92″E), and the southern site near Ashdod (31°50′14.35″N, 34°35′35.90″E). The sites were characterized by fouling communities on piers and pilings. Two sea anemone species were common at these sites: *Aiptasia diaphana* and *Aiptasiogeton hyalinus*. The specimens were collected by SCUBA diving at depths of 1–10 m.

Sea-anemone culture

A modular culture system was used to culture sea anemones (S.1), the nudibranchs' food source (Schlesinger et al. 2007). The sea anemones were collected from two sites and settled on portable mesh fabric (SEFAR NITEX, Switzerland) anemone substratum. The temperature-controlled culture system was located indoors, and connected to an open, flow-through, seawater system, which provided a constant supply of filtered (5 µm) seawater (SW). Sea anemones were cultured at the same temperatures as the nudibranch brood-stock, on a diet of *Artemia salina* nauplii and commercial fish food (NRD-INVE, Belgium).

Spurilla neapolitana culture and feeding

All cultures of *S. neapolitana* were raised in SW with a salinity of 39, at 24°C, under a 12-h light:12-h dark photoperiod, using commercially available T5 fluorescent tubes (Aqua Medic™ Planta illuminant, spectral peaks: 420 nm and 670 nm, and Aqua Medic™ Ocean White illuminant, spectral peak: 540 nm). During the study period our brood-stock was composed of different groups of 5–10 hermaphrodite adults, divided into sub-groups of 2–7 specimens. In order to test the preferred adult prey, *S. neapolitana* was introduced into cultures of four sea anemone species, *Aiptasia diaphana*, *Aiptasiogeton hyalinus*, *Anemonia viridis*, and *Actinia equina*, as well as to those of other coelenterates collected from the study sites, *Aglaophenia* sp. (Hydrozoa), *Pennaria* sp. (Hydrozoa), and *Oculina patagonica* (Anthozoa: Scleractinia).

Portable egg-laying substrates made of halved 40-mm diameter PVC tubes were positioned within the brood-stock culture tank and monitored daily for egg masses. After rinsing in SW, the egg masses were transferred to 1L sterile beakers containing gently aerated 0.22 µm-filtered seawater

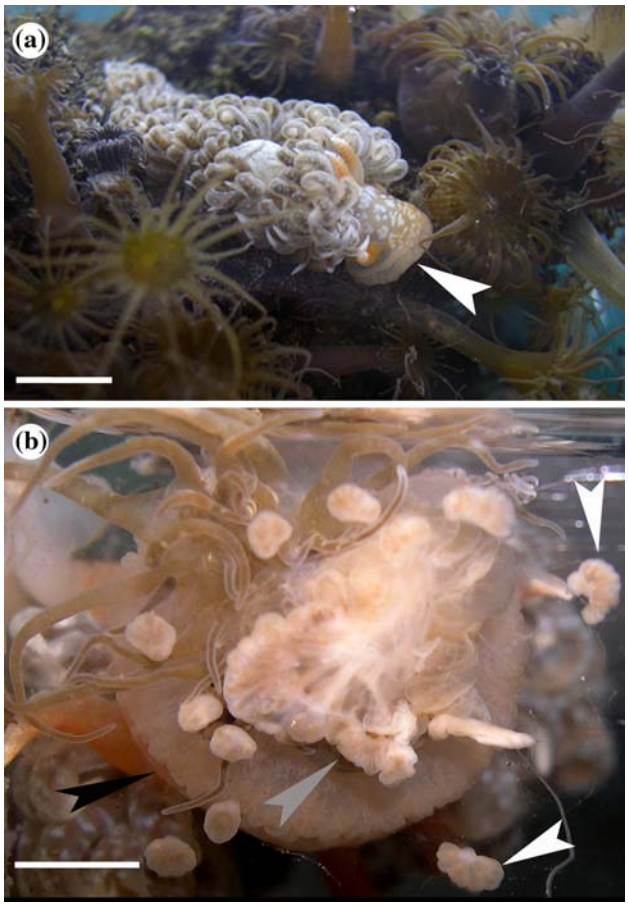

Fig. 1 *Spurilla neapolitana* predation on *Aiptasia diaphana*. **a** Grazing on sea anemones, note inflated pharynx (*arrow*)(scale bar = 2 cm). **b** Predation viewed at height of sea anemone pedal disc. Inflated pharynx (*black arrow*) engulfs anemone prey, exerting tearing forces on anemone tissues. During predation, pedal lacerates are generated by the anemone (*white arrows*) at pedal disc circumference (*gray arrow*) (scale bar = 5 mm)

(FSW) and maintained in an illuminated incubator (Binder, Germany). Each beaker was marked with the date of spawning and number of egg masses, and observed daily in order to monitor hatching.

Following release from the egg cases, the veligers were cultured in FSW containing antibiotics (streptomycin sulfate, 50 μg ml^{-1} and penicillin G, 60 μg ml^{-1}, Sigma) (see also Miller and Hadfield 1986; Miller 1993) in a double beaker culture chamber as described by Strathman (1987), at a density of 4 larvae ml^{-1}. The apparatus prevented larvae from adhering to the water surface tension by constantly pulling them down and away from the water surface. Water was changed every 3–4 days. Antibiotics and algae were added to the culture after each water change.

The influence of distinct diets on veliger growth and development was tested. Sibling veligers (derived from one egg-mass culture) were cultured on different diets. The three algal diets tested were *Isochrysis galbana* 10^5 cells ml^{-1}(standard diet, SD) (see also Harrigan and Alkon 1978; Perron and Turner 1977), a high-biomass diet of *I. galbana* (HBD, 10^6 cells ml^{-1}), and SD fortified with an additive consisting of 1% (10^3 cells ml^{-1}) *Tetraselmis tetrathele* (SD+). The maximal shell diameter of 3–10 larvae from each culture was measured at release from the egg mass and every 3–7 days from eye-spot appearance until no live larvae were found in the culture. This experiment was repeated three times, using a total of eight egg masses.

Determination of metamorphic competence period

Metamorphic competence experiments were performed on sibling larvae, aged 18–48 days post-oviposition, which had not previously been exposed to the inducer. Eye spot appearance was confirmed for each culture tested. For each experiment, 15–20 larvae were removed from the culture, placed in 15-ml glass Petri dishes, and exposed to a settlement inducer. The settlement inducer was prepared by immersing 30 medium-sized (pedal disc diameter = 1 cm) individuals of *Aiptasia diaphana* in 500 ml of FSW for 24 h. The FSW was then sieved through a double layer of 30-μm SEFAR NITEX mesh in a 25-mm polypropylene Filter Holder (Andvantec MFS), and used directly (termed SAW). Sibling larvae were simultaneously exposed either to SAW, or to sieved FSW (control-SFSW). Metamorphosis was determined with the aid of a stereomicroscope.

Effect of potential prey metabolites on competent larvae

We exposed 30–40 day post-oviposition larvae to metabolites of additional sea anemone species. In order to determine whether potential adult prey metabolites affect larval metamorphosis, the effect of *Anemonia viridis* SAW was tested on larvae from eight cultures, derived from 19 egg masses, spawned by seven adults. In addition, the effect of *Actinia equina* SAW was tested on sibling larvae from three of the above cultures. All larvae in these experiments were derived from adult cultures that had not previously been exposed to *Anemonia viridis* or *Anemonia equina*. Veligers were all raised in *I. galbana*, 10^5 cells ml^{-1} + *T. tetrathele* 10^3 cells ml^{-1}. Conditioned anemone water was prepared as above, using *Anemonia viridis* (SAnW) and *Actinia equina* (SAcW). Each experiment included two control treatments designed to test the larvae for metamorphic competence: sieved FSW (the negative control), and sieved *Aiptasia diaphana* water (the positive control). The metamorphosis-induction experiments were conducted as described above.

Post-larva and juvenile cultures

Post-larvae and juveniles were cultured in crystallizing dishes, and their morphological development was monitored. The obligatory time period from metamorphosis to the onset of feeding was tested. Three post-larva cultures were maintained in SW. Their sibling cultures were maintained in SW and presented small (0.5-cm pedal disc diameter) polyps of *Aiptasia diaphana* 24 h post-metamorphosis; SW was changed every 3 days or sooner. The dates when the unfed nudibranchs died were recorded.

Juveniles were cultured in pairs in order to monitor the onset of spawning, i.e., the transition from juvenile to adult. The adults were then transferred to adult culture tanks, where egg masses were collected.

Results

Sea anemone culture

Predation on *Aiptasia diaphana* by *S. neapolitana* resulted in residual anemone tissues (pedal lacerates) (Fig. 1), enabling their continuous culture. In the modular culture apparatus (S1), the pedal lacerates fully regenerated within 40 days, i.e., reached sexual maturity (monitored on three anemone substrata, $n > 100$ anemones $substratum^{-1}$). The modular design of the culture system facilitated the transfer of anemones on NITEX mesh fabric from a sea anemone culture tank to an adult culture of *S. neapolitana*, and back. A reproducing pair of nudibranchs can consume up to 60 (0.5-cm pedal disc diameter) sea anemones d^{-1}.

Culture and feeding of *Spurilla neapolitana*

In our cultures *S. neapolitana* preyed on four anemone species, *Aiptasia diaphana*, *Aiptasiogeton hyalinus*, *Anemonia*

viridis, and *Actinia equina*, but not on the other coelenterates such as *Aglaophenia* sp. (Hydrozoa), *Pennaria* sp. (Hydrozoa), and *O. patagonica* (Anthozoa: Scleractinia) collected from the study sites.

Nudibranchs did not spawn on clean surfaces, but only on those surfaces that were submerged in SW for 2 days or more. The egg masses were strongly attached to the substratum at multiple points (Fig. 2b), and removing egg masses typically resulted in their damage. The egg-mass membranes are necessary to provide a physical barrier between the developing, susceptible embryos and the external environment. Halved 40 mm PVC pipes continuously submerged in SW attracted ovipositing adults, i.e., served as portable egg-mass substrates.

Egg mass and veliger culture

Embryos were deposited in white, coiled, spiral ribbons of ca. 3–5 cm diameter (Fig. 2b, c), with the diameter of the ribbon being correlated with adult size. An average 5-cm egg mass released ca. 10^6 larvae. Each capsule in an egg mass contained 1–5, 50-µm eggs, and development was not always synchronous. Veliger larvae hatched 3.0 ± 0.4 days post-oviposition ($n = 83$ egg masses), with an average shell diameter of 117.9 ± 8.3 µm ($n = 198$ larvae from 83 egg masses), a pair of statocysts, a spiral, Type One veliger shell (as defined by Thompson 1961), and no propodium (Fig. 2e), as is typical of planktotrophic veliger larvae (Hadfield and Switzer-Dunlap 1984).

We tested the effect of three algal diets on veliger growth and development, and recorded day of eye appearance, size at eye appearance, maximum size, and duration of larval survival (Table 1). Veligers fed a high-biomass diet (HBD, *I. galbana*, 10^6 cells ml^{-1}) survived for only 17.0 ± 4.4 days (average $\pm$ SD, $n = 3$ veliger cultures), after which the larvae died within a few days. They did not develop eyes although the survivorship lay within the time period for eye development in their siblings cultured in the other two food treatments.

The maximum shell diameter of the veligers fed *I. galbana*, 10^5 cells ml^{-1} (standard diet, SD) was significantly smaller than siblings fed with *I. galbana*, 10^5 cells ml^{-1} + *T. tetrathele* 10^3 cells ml^{-1}(standard diet+, SD+) (Student's *t* test, $T_{(33)} = 2.1$, $P < 0.05$). However, the addition of 1% *T. tetrathele* to the standard diet had no significant effect on the day of eye appearance or on the duration of larval survival in the cultures (Student's *t* test, $P > 0.05$, $n = 6$ cultures, Fig. 2d, Table 1). Moreover, the sibling veligers' size at eye appearance was not diet dependent ($n = 48$ veligers; Student's *t* test, $P > 0.05$). We observed an increase in the average shell diameter with time in both SD and SD+ treatments, with no significant effect of diet on growth rate (S.3). However, the addition of 1% *T. tetrathele* to the standard diet resulted in a more homogenous growth curve (S.3).

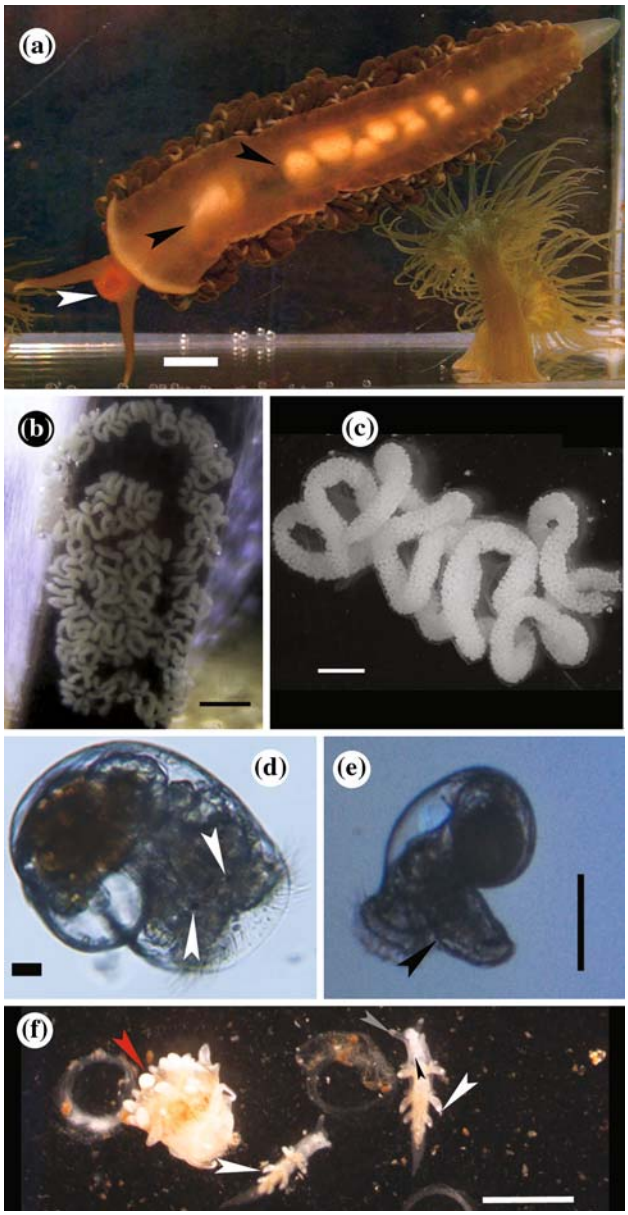

Fig. 2 *Spurilla neapolitana* life history. **a** Ventral view of reproductive adult, pharynx (*white arrow*), egg masses (*black arrows*), (scale bar = 1 cm). **b** Right-spiral egg mass attached at multiple points to operating aeration device (scale bar = 1 cm). **c** Supercoiled egg cord, capsules containing 1–5 eggs encased within layer of protective tubing, (scale bar = 200 µm). **d** Veliger eye-spots, at 17 days post-oviposition (notated by *white arrows*, scale bar = 10 µm). **e** Well-developed veliger propodium (*black arrow*, scale bar = 100 µm) 41 days post-oviposition. **f** Juvenile morphology 17 days post-metamorphic induction. Cnidosac tipped cerrata notated by white arrows, oral tentacle by gray arrow, and eye spot by *black arrow*, partially consumed sea anemone marked by red arrow (scale bar = 5 mm)

Survival of *S. neapolitana* through metamorphosis was 8% when cultured on the mixed diet, slightly higher

Table 1 Effect of diet composition on growth and development of sibling veliger larvae

	Standard diet	High-biomass diet	Standard diet (+)
Age at eye appearance (dPO)	20 ± 4.0	NA	15.7 ± 1.1
Length at eye appearance (μm)	159.7 ± 9.0 (n = 28)	NA	161.2 ± 6.3 (n = 20)
Survival duration (dPO)	34.8 ± 3.0	17 ± 4.4	37.7 ± 7.6
Max. shell length (μm)	*175 ± 26.7 (n = 16)	138.5 ± 14 (n = 8)	*189 ± 9.5 (n = 19)

The experiment was conducted in triplicate (n = 3 larvae cultures) for each diet. Sibling larvae were fed one of the following diets: standard diet: *I. galbana* 10^5 cells ml^{-1}, High biomass diet: *I. galbana* 10^6 cells ml^{-1}, fortified standard diet (+): *I. galbana* 10^5 cells ml^{-1} + *T. tetrathele* 10^3 cells ml^{-1}. Survival duration was defined as period from ovipostion until <5% of the larvae culture was viable (swimming). Age was measured in days post-oviposition (dPO). Max. shell length was average of max. shell diameters measured at end of culture period as defined above. Significant differences between standard and standard+ diets are marked*

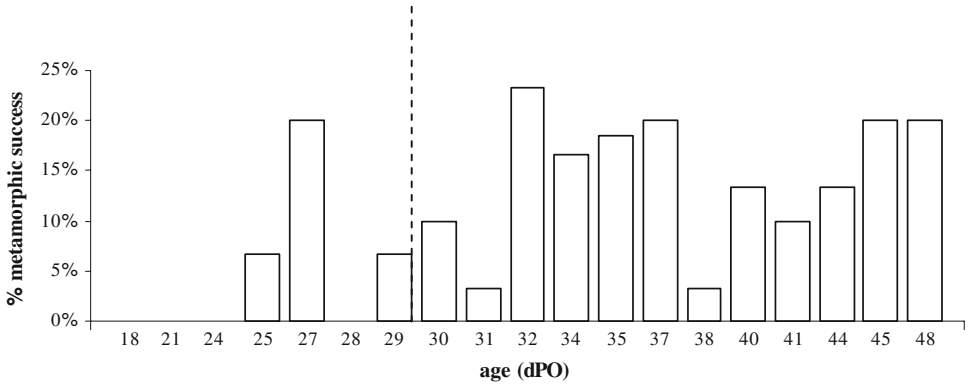

Fig. 3 Age range of metamorphic competence. Percentage metamorphic success for larvae aged 18–48 days post-oviposition (dPO). Total of 49 larvae metamorphosed, aged 25–48 dPO. *Dashed line* end of larval growth period

than the larvae of the aeolid nudibranch *Hermissenda crassicornis* (5%) (Harrigan and Alkon 1978). Larvae were obligatory planktotrophs, i.e., veligers did not develop or survive without an algal food source.

Metamorphic competence

During the growth period, larvae were evenly dispersed within the culture container. As they approached metamorphic competence, they tended to concentrate at the bottom, slowly swimming or contracted into their shells. A total of 46 settlement-induction experiments were performed on larvae aged 18–48 days post-oviposition, from a total of 69 egg masses, spawned by 15 adults. Forty-nine larvae metamorphosed, five in the negative control, sieved FSW, and 44 in sieved FSW treated with *Aiptasia diaphana*. Larvae metamorphosed from 25 days post-oviposition until 48 days post-oviposition, which was the maximum veliger age in this study. In the described experimental setup metamorphic competence developed in *S. neapolitana* ca. 7 days after eye-spot development, at 25 days post-oviposition (Fig. 3). Metamorphic success was 10%; much lower than the 85% reported for the aplysiid larvae of *Aplysia californica* (Kriegstein et al. 1974) and the 90–100% in the nudibranch *Phestilla sibogae* (Hadfield 1984).

Table 2 Effect of sea anemone metabolites on competent larvae

Treatment	Culture no. 2 (%)	Culture no. 3 (%)	Culture no. 5 (%)
SAcW	0	20	0
SAnW	20	80	10
SAW	0	80	10
SFSW	0	10	0

Percentage metamorphic success resulting from exposure of ten larvae to sea anemone solutes and seawater, from the three metamorphically competent larval cultures. *SAcW* sieved *Actinia equina* water, *SAnW* sieved *Anemonia viridis* water, *SAW* sieved *Aiptasia diaphana* water (positive control), *SFSW* sieved filtered seawater (negative control)

Effect of potential prey metabolites on larval metamorphosis

A total of 23 veligers from three (representing nine egg masses) of the eight larval cultures metamorphosed in the sea anemone metabolites, and one veliger metamorphosed in sieved filtered SW (Table 2). No significant difference was found between the treatments and the controls (Fig. 4).

Juvenile to adult culture

Newly metamorphosed juveniles were pale orange in color with visible eyespots. A tail-like posterior extension of the

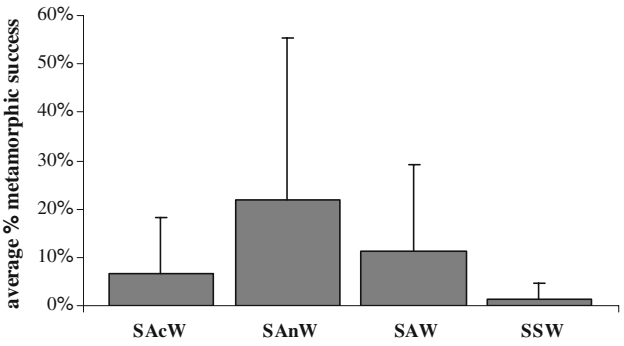

Fig. 4 Induction of metamorphosis. Average percentage of metamorphosis +SD resulting from exposure of competent larvae to sea anemone metabolites. *SAcW* sieved *Actinia equina* water, 2 larvae metamorphosed, 30 larvae tested. *SAnW* sieved *Anemonia viridis* water, 11 larvae metamorphosed, 50 larvae tested. *SAW* sieved *Aiptasia diaphana* water (positive control), 9 larvae metamorphosed, 80 larvae tested. *SFSW* sieved filtered sea water (negative control), 1 larva metamorphosed, 80 larvae tested. No significant differences were found between the treatments and the controls

foot was visible within 24 h after metamorphosis; and rhinophore rudiments were visible within 4 days, anterior to the eye spots. Cerrata and cnidosacs developed 15 days post-metamorphosis (Fig. 2f).

Post-larvae in unfed cultures survived for a maximum of 4 days, with mortality occurring simultaneously on day 4. Sibling cultures supplied with sea anemones 24 h after metamorphosis survived and grew. During the first 2 weeks, juveniles were often observed undulating in a viscous substance at the base of the anemone column. Sea anemones appeared to be consumed by 17 days post-metamorphic juveniles, whose feeding spares the pedal lacerates. Consumption of sea anemones increased as juveniles grew and matured, and peaked at oviposition. Copulation was first observed 37 days post-metamorphosis, and oviposition occurred 2 days later. Reproductive maturity in laboratory-reared *S. neapolitana* was reached 42 ± 5 days post-metamorphosis ($n = 4$), at a body length of 3 cm. Generation time (egg to egg) was ca. 67 days.

Adults reached a length of 7–10 cm (Fig. 2a). They laid 2–3 egg masses week^{-1} containing ca. 10^6 larvae each. An ovipositing pair consumed up to 60 *Aiptasia diaphana* daily, depending on the prey size. Senescence in the nudibranch was evident with the cessation of oviposition, which was followed by the nudibranch's death. The average ($n = 4$) longevity of laboratory-cultured *S. neapolitana* in this study was 157 ± 13 days post-oviposition. During this period, an average specimen spawned ca. 40×10^6 zygotes. Growth and development parameters (i.e., shell growth, eye appearance) in F_1 larvae did not differ from the wild-type larvae raised under the same conditions (data not shown).

Discussion

In general, *S. neapolitana* is characterized by the major life-cycle stages that Kriegstein (1977b) noted for *Aplysia californica*, i.e., they develop via (1) embryonic, (2) planktonic, (3) metamorphic, (4) juvenile, and (5) adult stages. Adult *S. neapolitana* fed on several anemone species but not on other cnidarian groups. This trophic restriction is in agreement with the conjecture that nudibranchs as a group are food specialists (Todd et al. 2001). While there are exceptions, such as *H. crassicornis* (Harrigan and Alkon 1978), many species are monophagous, e.g., *P. sibogae* on species of the coral genus *Porites* (Faucci et al. 2007); or stenophagous, e.g., *Aeolidia papillosa*, which feeds on a number of sea anemone species (Todd et al. 2001). *S. neapolitana* therefore falls somewhere within the stenophagous group of predators, feeding only on sea anemones (Anthozoa: Actinaria).

Like many other opisthobranchs and especially aeolid nudibranchs, *S. neapolitana* spawns spiral, cylindrical, coiled cords filled with egg capsules (Type 2, see Hurst 1967). The egg mass bears a strong resemblance to that of the aeolid nudibranch *H. crassicornis* (Kuzirian et al. 1999) except in color, which in *S. neapolitana* varies with the color of the anemone prey.

The number of embryos produced per spawning in opisthobranchs is largely dependent on size, ranging from 3 to 140×10^6 (Hadfield and Switzer-Dunlap 1984). Many opisthobranchs deposit egg masses containing hundreds to tens of thousands of eggs. *S. neapolitana* is among the truly prodigious species, spawning throughout the entire year (Yavetz 2007), millions of embryos at a time. The total number of eggs laid by an individual is also a function of its lifespan. Only a few species have been followed throughout an entire lifetime. Switzer-Dunlap and Hadfield (1979) found lifetime egg production of the anaspidean *Aplysia juliana* to be 272×10^6, while Chia (1971) reported 1000–2250 eggs per individual lifetime for very small sacoglossans. In laboratory culture *S. neapolitana* produced 40×10^6 eggs in a lifetime, very similar to the egg production of *H. crassicornis* (Kuzirian et al. 1999). This high reproductive effort may be especially advantageous for a species that colonizes ephemeral fouling communities, taking advantage of a short time period in order to produce a vast number of offspring.

The developmental mode of *S. neapolitana* is indirect, i.e., it includes free-living larval development of feeding (planktotrophic) veliger larvae (with shell and velum). The embryonic period of 3.0 ± 0.4 days is short but within the range for species with planktotrophic larvae. The larval growth factor (final diameter/initial diameter) of 1.49 is characteristic of species with similar larval periods; yet shell diameters at hatching (114 μm) and at settling (170 μm) are smaller than most other opisthobranch larvae

that have been reared in the laboratory (Hadfield and Switzer-Dunlap 1984).

The larval diet significantly affected both the final diameter of the larval shell and larval growth rate (S.3). *I. galbana* (cell diameter = 5 μm), commonly and successfully used as a food source in many veliger cultures, was found to be adequate for larval rearing. The addition of *T. tetrathele*, a flagellated alga with a diameter of 15 μm, which is larger than the typical unicellular strains used to culture veligers, enhanced larval growth (S.3). If we assume that the algae are spherical, and have similar densities, the addition of 1% *T. tetrathele* cells actually raised the available diet biomass by 27%. Additionally, *T. tetrathele* complements *I. galbana* in EPA (eicosapentaenoic acid) fatty acid content (M. Ucko, personal communication). Larvae of many obligatory planktotroph opisthobranch species have been successfully cultured at lower larval and alga densities than those used in this study (Kriegstein 1977a; Switzer-Dunlap and Hadfield 1977). We tested high algal densities because field populations of *S. neapolitana* were often found associated with aquaculture facilities, in nutrient-enriched environments. Testing lower concentrations of algal cells or, alternatively, culturing larvae in a flow-through system, i.e., in a constant state of dilution, may be expedient in improving larval survival and metamorphic success rates. The true relationship between larval growth and development rates in the field cannot be predicted from our laboratory cultures, since we supplied the larvae with algal cell densities two to four orders of magnitude greater than those they might encounter in natural, near shore Eastern Mediterranean waters unaffected by nutrient enrichment (Kress et al. 2005; Zohary et al. 2005).

The larval period in *S. neapolitana* consists of two phases, a 30-day growth phase, followed by an 18-day non-growth phase (S.3). Shell growth is accompanied by other morphological changes, including the appearance of eyes (Fig. 2d, S.2). In many other species the growth phase is followed by an interval during which larvae are not yet competent to metamorphose (Kriegstein 1977b; Switzer-Dunlap and Hadfield 1977; Harrigan and Alkon 1978). In larvae of *S. neapolitana* metamorphic competence appears to be reached directly at the end of the growth phase or slightly before (Fig. 3). In addition to morphological development, there are behavioral changes that occur with larval age. As they approach metamorphic competence, the dispersal pattern of the larvae changes, and they tend to concentrate at the bottom, slowly swimming or contracted into their shells. This behavior has also been recorded in aplysiid larvae (Kriegstein 1977b; Switzer-Dunlap and Hadfield 1977).

Species of the tropical aeolid nudibranch genus *Phestilla* (Gastropoda, Opisthobranchia) feed and reproduce on specific scleractinian corals, and their planktonic larvae require coral-specific chemical cues in order to settle onto their host coral, metamorphose, and complete their life cycle almost entirely on their specific coral host (Hadfield and Pennington 1990). Host specificity has recently been shown to play an important role in the mechanism underlying speciation in this genus (Faucci et al. 2007).

For the most part, larvae of *S. neapolitana* settled and metamorphosed in seawater containing metabolites from distinct sea anemone prey species (Fig. 4). Less than 10% ($n = 6$) of the larvae that settled did so in untreated seawater, indicating that the presence of the prey species provides a signal for settlement and metamorphosis. Since a priori the veliger cultures varied in the percentage of metamorphically competent larvae (Table 2), it was difficult to obtain significant data on the effect of distinct prey species on larval metamorphosis. For most nudibranchs whose complete development has been observed in the laboratory, the specific prey organism of the adult nudibranch is necessary in order to elicit metamorphosis (Hadfield and Switzer-Dunlap 1984). While generalists will settle in response to different prey species (Harrigan and Alkon 1978), monophagous species only settle upon their specific host. Despite this, Todd (1991) showed that newly-settled juveniles of a barnacle-eating nudibranch, *Onchidoris bilamelata*, live on detritus before growing large enough to prey on barnacles. *Berghia verrucicornis*, an anemone predator, has been reported to settle in the absence of a habitat-specific inducer, yet this species may also undergo direct development (Carroll and Kempf 1990). Although our results do not show a significant difference between the effect of metabolites from different anemone species on veliger settlement, >90% ($n = 66$) of the metamorphic events that we documented in our experimental system occurred in seawater containing sea anemone metabolites. Moreover, two of the anemone species tested were ones to which the parents had not been previously exposed. Therefore it appears that larvae of *S. neopolitana* respond to cues that induce settlement and ultimately recruit to several sea anemone species, of both fouling and inter-tidal rocky shore communities.

Our results imply that, once metamorphosed, a crawling post-larva (250 μm long) must find a food source within 4 days. Thus, it must settle and metamorphose a very short distance from its settlement cue. During the transition from phytoplanktivore to predator, the juveniles (until 15-day post- metamorphic induction) appeared to feed upon and coat themselves with the mucous secretion of their sea anemone prey. This behavior may provide a means of defense from the anemone nematocysts, by acting as a "chemical camouflage," i.e., "blinding" the nematocyte chemoreceptors and disabling their discharge. This differs from the resistance to sea anemone toxins that has evolved in some anemone fishes (*Amphiprion* spp.) and from the protection provided by their skin's mucus layer (Mebs 1994).

Nudibranch molluscs, lacking a protective shell, may sequester and concentrate prey-derived secondary metabolites to serve in their own defense (e.g., latrunculine, an anticancer agent in sponges, Ilan 1995), making the nudibranchs a potentially useful supply of pharmaceutically valuable compounds. Like other aeolid nudibranchs, *S. neapolitana* ingests and retains undischarged nematocysts (venom-containing stinging organelles). Some are incorporated and stored in the tips of the nudibranch cerata in organs termed cnidosacs, and are thought to serve as a defense mechanism for these organisms (Greenwood and Mariscal 1984; Frick 2003). Recently, we have shown that *S. neapolitana* can be used to isolate active nematocysts from four sea anemone species upon which it preys (Schlesinger et al. 2009). Thus, the laboratory co-culture of this nudibranch species, and possibly others, with distinct cnidarian prey may facilitate and advance the investigation of cnidarian venoms.

Acknowledgments The authors would like to thank H. Ohana from the Ruppin Academic Center, School of Marine Sciences, Michmoret for assistance with the sea water systems, S. Kelly and C. Tran from the Kewalo Marine Laboratory, Hawaii for their helpful tutorials on nudibranch culture, R. Holtzman and S. Einbinder for advice on the statistical analysis and R. Yavetz for assistance with the field work. We would also like to thank M. Ucko at the National Centre for Mariculture, Eilat for the alga inoculates and invaluable advice. We would like to thank N. Paz for helpful commenting on this manuscript. The critical reading and constructive suggestions of three anonymous reviewers are gratefully acknowledged. This work was supported in part by the Tel-Aviv University Fund for Applied Research and in part by the Raynor Chair for Environmental Conservation Research (to YL). All experiments undertaken within this study comply with the current laws of Israel, the country in which they were performed.

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