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SYMPOSIUM

Seasonal Polyphenism in Larval Type: Rearing Environment Influences the Development Mode Expressed by Adults in the Sea Slug *Alderia willowi*

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Synopsis Dimorphisms occur when alternative developmental pathways produce discrete phenotypes within a species, and may promote evolutionary novelty in morphology, life history, and behavior. Among marine invertebrates, intra-specific dimorphism in larval type (poecilogony) is notably rare, but should provide insight into the selective forces acting on larval strategies. Most established cases of poecilogony appear to be allelic polymorphisms, with local expression regulated by population-genetic processes. Here, we present evidence that dimorphic larval development in the sea slug *Alderia willowi* is a seasonal polyphenism; the type of larvae produced by an adult slug depends on the rearing environment in which that slug matured. In field surveys of 1996–1999 and 2007–2009, the population in Mission Bay, San Diego (California, USA) produced only short-lived lecithotrophic larvae in summer and early fall, but a varying proportion of slugs expressed planktotrophy in winter and spring. In laboratory experiments, slugs reared under summer conditions (high temperature, high salinity) produced the highest proportion of lecithotrophic offspring, whereas winter conditions (low temperature, low salinity) induced the lowest proportion of lecithotrophy. The shift to a nondispersive morph under summer conditions may be an adaptive response to historical closure of coastal wetlands during the dry season in southern California, which would inhibit dispersal by larvae of back-bay taxa. In most animal polyphenisms, a single larval type is produced and the rearing environment determines which adult phenotype develops. In contrast, alternative larval morphs are produced by *A. willowi* in response to seasonal cues experienced by the adult stage, varying the phenotype and dispersal potential of offspring. As the only known case of polyphenism in mode of larval development, *A. willowi* should become a model organism for mechanistic studies of dimorphism and the evolution of alternative life histories.

Introduction

Evolutionary processes can maintain alternative developmental pathways that produce morphologically distinctive and discrete adult forms within a species (Nijhout 1999, 2003; West-Eberhard 2003). Dimorphisms can be adaptive responses to disruptive selection, and may facilitate the exploration of morphospace, shifts in life cycles, and even speciation (West-Eberhard 2003; Fusco and Minelli 2010; Moczek 2010; Pfennig et al. 2010). Some dimorphisms are maintained by underlying allelic polymorphisms, and governed by population-genetic processes (Roff 1986; Joron et al. 2006). Alternatively, the trait state expressed by an individual may be a plastic response to its rearing conditions. A polyphenism occurs when the same genotype expresses different possible phenotypes depending on the environment experienced during development (Nijhout 2003; Leimar 2009). The switch between developmental pathways is often triggered by cues that presage impending bouts of selection, suggesting the adaptive value of polyphenism.

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Predictable fluctuations in the environment, such as annual hot–cold or wet–dry cycles, may favor the evolution of seasonal polyphenism in short-lived organisms (Shapiro 1976). For genetic polymorphisms, selection may favor alternative alleles in different seasons, but the population must bear a genetic load if transiently unfavorable alleles are to persist through their "bad" season. Polyphenisms escape such a load, and can increase the fitness of a genotype by producing a phenotypic match to either of two possible environments. However, there are costs and constraints on plasticity, and environmental factors that regulate phenotype may not always correspond to the agents of selection (DeWitt and Scheiner 2004). For an adaptive polyphenism to evolve, there must be a reliable environmental cue that (1) precedes change in the selective regime, (2) occurs during a developmental window in which the individual can respond, and (3) gives enough lead time for the organism to alter its phenotype before selection acts (Bradshaw 1973).

Harmless stimuli, such as gradual change in photoperiod or temperature, can reliably predict environmental stressors like the onset of harsh winter conditions, and allow anticipatory developmental responses. For example, temperature and/or photoperiod determine whether tropical butterflies develop their wet-season phenotype (colorful, and with eyespots that distract predators) or emerge as the dry-season morph (brown, and cryptic among leaf litter; Brakefield and Frienc h 1999; Simpson et al. 2011). The transition to shorter days can also induce development of a winged morph in some insects (Zera and Tiebel 1991; Zera and Denno 1997). Mechanistically, such environmental cues affect the (mis-)match between hormone titers and the timing of tissue-specific receptor-gene expression during larval development (Nijhout 2003).

In most polyphenisms, mothers produce one type of embryo and the interplay of development and environment determines the adult phenotype. In a few dimorphisms, the larval environment can be manipulated by the mother, adding a level of maternal control over offspring phenotype. In dung beetles, maternal provisioning of the dung ball determines larval growth potential; larger male offspring develop into horned fighters, whereas smaller males become noncombative "sneakers" (Moczek and Emlen 1999; Moczek 2010). The environment experienced by maternal plants, including seasonal cues (photoperiod, temperature) and herbivory, can alter the dormancy and germination requirements of their seeds (Donohue 2009). Plants can influence offspring phenotype by varying the mechanical properties of the seed coat, gene expression by endosperm, and maternal transcripts in the ovum. However, similar transgenerational cases of seasonal polyphenism (where maternal environment dictates offspring phenotype) are comparatively rare in animals.

Among marine invertebrates, higher clades have bimodal distributions of egg sizes that reflect the alternative life-history strategies of planktotrophy and lecithotrophy, which maximize either maternal fecundity or offspring survival, respectively (Strathmann 1985, 1990). Only a few species of annelid worms and gastropod molluscs express poecilogony, an intra-specific dimorphism in offspring type (Krug 2009, and other contributions from this symposium). In these taxa, planktotrophic larvae develop from small eggs and require an extended period of planktonic feeding during which extensive dispersal can occur. Lecithotrophic larvae either develop from larger eggs (gastropods, and the worm *Streblospio benedicti*) or consume nurse eggs before hatching (other annelids), and can settle immediately upon release from benthic egg masses or adult tubes (Levin 1984; Gibson 1997; Gibson et al. 2010; Vendetti et al. 2012). Thus, offspring type is a maternal effect controlled by investment in egg size or in nurse eggs, with cascading effects on the larval phenotype, lifespan, and dispersal ability.

In the few poecilogonous species studied to date, genetic polymorphisms appear to underlie dimorphisms in egg size. In *S. benedicti*, quantitative genetic studies indicate that poecilogony reflects an allelic polymorphism maintained by trade-offs between suites of correlated life-history traits (Levin and Creed 1986; Levin and Huggett 1990; Levin et al. 1991; Levin and Bridges 1994). In the sacoglossan gastropod *Elysia chlorotica*, breeding crosses between populations suggested a genetic basis for alternative development modes (West et al. 1984). In the sacoglossan *Costasiella ocellifera*, development varies at the population level and appears to be fixed over the lifetime of an individual (Ellingson 2006; P. Krug, unpublished data). These results suggest that in most cases of poecilogony, the maternal trait "offspring type" is controlled by a genetic polymorphism; disruptive selection acts on larval feeding or dispersal at a local scale, producing a geographic mosaic of development.

The sacoglossan sea slug *Alderia willowi* has an exceptionally flexible life history, even among poecilogonous species. Adults inhabit warm-temperate estuaries and reproduce year-round, laying benthic clutches of planktotrophic or lecithotrophic eggs (Ellingson and Krug 2006; Krug et al. 2007). Experiments with field-collected and laboratory-reared adults revealed that individuals can vary the larval type of their offspring, both within and among clutches; maternal condition also affects the propensity for lecithotrophic larvae to settle soon after hatching, in the absence of habitat cues (Krug 1998, 2001, 2007; Smolensky et al. 2009). Limited field surveys also suggested that the primary mode of development varies seasonally across the range of *A. willowi*, shifting from lecithotrophy in summer to a mixture of both types in winter, but no time-series data were reported previously (Ellingson and Krug 2006).

If its development mode were indeed environmentally cued, *A. willowi* would represent a novel model system for investigating environmental drivers of life-history transitions, and provide a valuable contrast with poecilogonous species in which larval type is genetically controlled. Here, we report monthly field surveys of larval type to test whether populations of *A. willowi* undergo a seasonal change in offspring phenotype. We then present results from laboratory rearing of slugs to test the hypothesis that temperature and/or salinity of the juvenile environment influences the type of larvae subsequently produced by an adult slug, to confirm that development mode is a transgenerational polyphenism.

Materials and methods

Organisms and field surveys of development mode

The sea slug *Alderia willowi* is restricted to the high intertidal zone of estuaries in southern and central California, USA, feeding exclusively on mats of the heterokont alga *Vaucheria longicaulis* (Krug 2007). Adults are simultaneous hermaphrodites that store sperm from prior matings, are reproductive year-round, and lay a clutch of eggs every 1–2 days under laboratory conditions (Smolensky et al. 2009). To determine the proportion of slugs producing offspring of each development mode in the field, surveys were conducted opportunistically in 1996–1999 and 2007–2009 in estuaries in southern California (Table 1). From February 1996 to July 1999, the Kendall-Frost marine reserve and Northern Wildlife refuge in Mission Bay, San Diego were sampled by P.J.K. 4–10 times per year, with a minimum of 1 month between sampling times. Mean number of slugs collected per survey was 113.3 ± 65.1 SD (range: 31–306). From 2007 to 2009, five sites in southern California were surveyed by M.R.R.; the Los Angeles and Long Beach sites were so geographically close that for graphical exploration of the data, samples from these two demes were pooled as one site. Mean sample size per collection was 117.4 ± 71.4 SD (range: 13–320).

All slugs found in a 30–60 min period were hand collected from the mudflat surface at low tide and brought back to the laboratory. Slugs were individually isolated in a Petri dish with 4 mL of 0.45 µm filtered seawater (FSW) for 2 days. The egg mass laid by each slug was typed for development mode by (1) egg size and (2) confirmation of metamorphic competence upon hatching for lecithotrophic clutches, according to Krug (1998, 2001). The proportion of mixed clutches containing intermediate-sized offspring was determined for samples from 1996 to 1999 only (Krug 1998); mixed clutches in 2007–2009 were treated as planktotrophic, the development mode of most intermediate-sized larvae.

Environmental induction of offspring type

Field surveys revealed a seasonal shift in the prevailing larval type produced by field populations in southern California (see "Results" section), suggesting poecilogony might be a seasonal polyphenism in *A. willowi.* We tested this hypothesis with laboratory-rearing experiments, manipulating the environment of maturing slugs to simulate seasonal trends in the field. Coastal wetlands in California experience predictable changes both in temperature and salinity over the year. Baywater salinity is normally slightly lower than seawater (~32‰), but can decrease dramatically during winter rainstorms from December to March, and to a lesser extent following spring snowmelt. To choose salinity levels that would represent wet- versus dry-season conditions, we examined historical trends for Mission Bay (Zedler 1982; Largier et al. 1997) and made *in situ* recordings with Star-Oddi salinity loggers (Star-Oddi; Reykjavik, Iceland) deployed over 5 years on mudflats in southern and central California (Garchow 2010; H. Koch and P. Krug, unpublished data). Winter rains episodically lower estuarine surface waters to <2‰ during storms, but mean daily salinity of baywater

Table 1 Sites and sampling periods for field surveys of developmental mode expressed by *Alderia willowi*, and collection of broodstock used to initiate rearing experiments in the laboratory (asterisks)

Location	Latitude, longitude	Sampling period
Cabrillo Marine Aquarium wetland, Los Angeles, CA, USA	33° 42′ 50″ N, 118° 17′ 5″ W	2007–2009
Golden Shore Marine Biological Reserve Park, Long Beach, CA, USA	33° 45′ 50″ N, 118° 12′ 12″ W	2009–2010*
Surfside, Seal Beach, CA, USA	33° 43′ 43″ N, 118° 4′ 58″ W	2008
Upper Newport Bay Ecological Reserve, Newport, CA, USA	33° 37′ 15″ N, 117° 53′ 35″ W	2007–2008
Kendall-Frost Mission Bay Marsh Reserve, San Diego, CA, USA	32° 47′ 36″ N, 117° 13′ 47″ W	1996–1999, 2008–2009

All sites are estuaries in southern California, USA.

during winter and spring was usually intermediate between fresh water and seawater. We thus chose 16‰ as a typical winter/spring salinity that could signal the onset of the rainy season, yet would not be stressful for *A. willowi*; in prior studies, field-collected slugs survived several days at salinities as low as 4‰, and suffered no significant mortality over a week at 16‰ (E. Shimer, H. Koch, and P. Krug, unpublished data). Based on 8 years of temperature recordings by Hobo data-loggers (Onset Corp.; Bourne, MA, USA), the annual temperature cycle is shifted relative to the wet–dry cycle on Californian mudflats. Highest temperatures were recorded in spring, when low tides exposed the mudflat throughout the day, and mean daily temperatures were consistently lower in fall than in spring (Willette 2006; Garchow 2010). Based on daily and monthly mean mud temperatures, we chose 16 and 24°C to represent fall/winter and spring/summer mud temperatures, respectively.

A fully factorial design was then implemented to test the hypothesis that temperature and/or salinity of the environment in which a slug matured would affect the type of offspring it subsequently produced. A series of three experimental runs were performed from 2009 to 2010, each with the same four temperature × salinity combinations ($n = 3$–6 replicate dishes per run), loosely corresponding to the four seasons. Dishes of juveniles were reared in incubators at a constant temperature of either 16°C or 24°C, and in a constant salinity of either 16‰ or 32‰. Each run used the offspring from a separate collection of adults sampled in late winter or spring (run 1: February 2009; run 2: April 2009; run 3: March 2010); the same experimental procedure was then followed. To begin a run, adults collected from Los Angeles area mudflats were pooled overnight to obtain 50–100 lecithotrophic clutches, representing the diverse genotypes present in the population at that time. All clutches were held together in FSW at 16°C until hatching, when filaments of the adult host alga *Vaucheria longicaulis* were added to induce metamorphosis (Krug 2001). After 2 days, juveniles were subsampled and haphazardly assigned to a replicate dish ($n = 7$–10 slugs per dish; all replicates in a given experimental run held the same number of juvenile slugs).

Natural seawater was obtained from the Cabrillo Marine Aquarium (San Pedro, CA, USA) and filtered to 0.45 µm prior to use. To adjust salinity, a solution of FSW was warmed to 25°C and ultrapure water was gradually added to lower the salinity to 32‰ or 16% (±0.2‰), based on the readings from a calibrated conductivity probe (Mettler-Toledo; Columbus, OH, USA). Juvenile slugs were reared on an *ad libitum* supply of laboratory-cultured *Vaucheria litorea* obtained from S.K. Pierce, grown at 16°C on a modified low-salinity f/2 medium (260 µl nutrient solution, 0.21 g MOPS buffer, 10 g sea salt per liter of water). Food and water were changed in replicate dishes every 1–2 days.

Approximately 2 weeks after metamorphosis, laboratory-grown slugs reached reproductive maturity and began depositing egg masses. Starting from the time of first reproduction, dishes were scored daily for deposition of egg masses. Each clutch was isolated and typed as lecithotrophic if the majority of hatching larvae metamorphosed in the presence of *Vaucheria*. One week after first reproduction, body length was measured for 3–7 specimens per replicate dish using an ocular micrometer on a Zeiss Stemi 2000C stereomicroscope (Carl Zeiss Microscopy LLC, Thornwood, NY, USA) calibrated with a hemocytometer grid. Mean length of the slugs in each dish was included as a covariate in analyses (as all individuals are egg-producing). Length was included to control for potential differences in growth rate across runs, as maternal size at the point of first reproduction could influence what type of offspring a slug produced (in addition to environmental cues). The proportion of lecithotrophic clutches deposited over the next 2 weeks was calculated for each dish, as an estimate of the proportion of adults laying lecithotrophic eggs.

Statistical analyses

Data were analyzed in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) using a general linear model including main effects of temperature and salinity as fixed factors, experimental run as a random factor to represent different starting collections of adults, and mean body length per dish as a covariate. The unit of replication was the dish, and the response variable was percentage of lecithotrophic clutches laid. Percentages were arcsine (square-root)-transformed to increase normality prior to analysis. All possible two-way and higher-order interaction terms were included in the initial model. During sequential rounds of model simplification, nonsignificant terms were removed one at a time starting with the largest *P*-values, with a cut-off of $P < 0.1$ for retaining terms in the final model (Quinn and Keough 2002). Based on field surveys, our *a priori* expectation was that the proportion of lecithotrophic clutches would be highest for slugs maturing under summer conditions, lowest for winter, and intermediate for spring and fall. Interaction plots were used to explore the basis for significant higher-order interactions.

Results

Seasonal shifts in mode of larval development

From 1996 to 1999, specimens of *A. willowi* from Mission Bay, San Diego produced almost exclusively lecithotrophic clutches in summer months (Fig. 1). Slugs began laying planktotrophic clutches in October–November, and up to 67% of the population produced planktotrophic larvae in winter and spring. The maximum proportion of planktotrophy for a year was typically observed in March–April. Mixed clutches of intermediate-sized offspring were produced by <5% of slugs and only in months when at least some slugs were planktotrophic (Fig. 1). Limited sampling one decade later also detected <20% planktotrophy in Mission Bay at any time except March 2008 (Fig. 2). Over 3 years, only 2 out of 1533 slugs sampled in Mission Bay from June to September were planktotrophic.

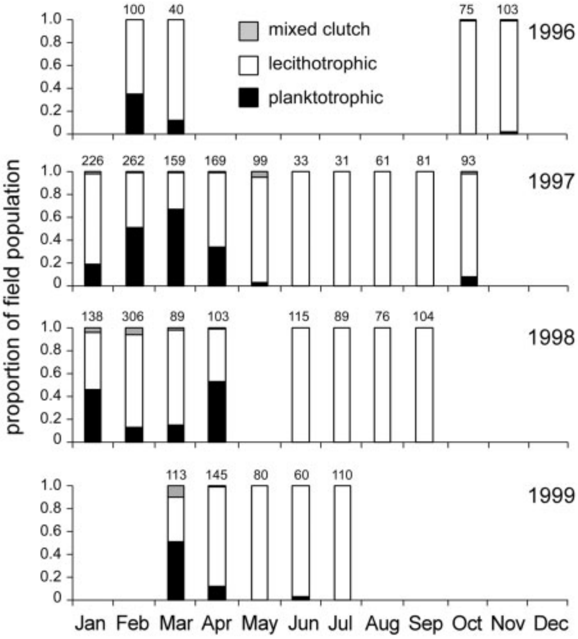

Fig. 1 Proportion of field-collected specimens of *Alderia willowi* that produced egg masses of three developmental types in Mission Bay (San Diego, CA, USA). Mixed clutches contain eggs that are intermediate in size between planktotrophic and lecithotrophic eggs. Sample size is given above the bar for a given month.

The seasonal trend observed in San Diego was not as pronounced in estuaries sampled >100 km farther north (Fig. 2). About 25% of the slugs in Newport Bay were planktotrophic in summer 2007, far fewer than the 73% expressing planktotrophy in January 2008. No seasonal trend was apparent in the Los Angeles population, with some slugs expressing planktotrophy in all months sampled, including summer 2007 and 2008 (Fig. 2).

Effects of the rearing environment on production of lecithotrophic larvae

When slugs were reared in the laboratory under different temperature and salinity regimes, there was significant variation among treatments in the proportion of sexually mature slugs that produced lecithotrophic offspring (Fig. 3 and Table 2). Results of a general linear model revealed a significant four-way interaction among temperature, salinity, adult size, and experimental run ($P < 0.01$). As expected, slugs reared under summer conditions (high temperature,

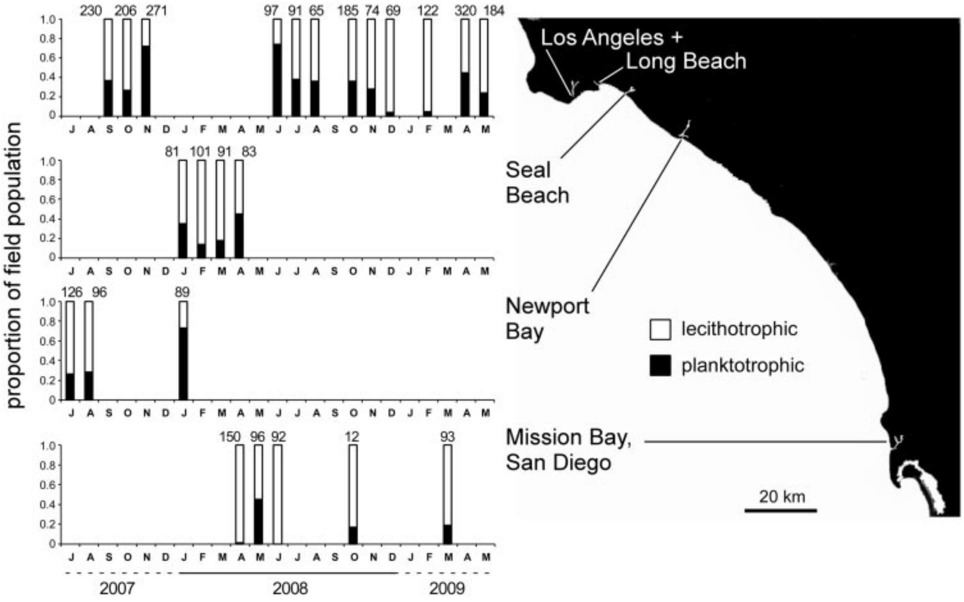

Fig. 2 Proportion of field-collected specimens of *Alderia willowi* that produced planktotrophic versus lecithotrophic egg masses in four field sites in southern California. Sample size is given above the bar for a given month.

high salinity) laid the highest proportion of lecithotrophic clutches in all three runs of the experiment (Fig. 3). However, the proportion of lecithotrophy under summer conditions varied considerably among runs, ranging from 38.1% (±2.2% SE) in run 1 to 95.9% (±2.7% SE) in run 3, and contributing to the three- and four-way interactions involving temperature, salinity, and run. Slugs reared under winter conditions consistently laid fewer lecithotrophic clutches, and the mean percentage of lecithotrophy differed only by ~10% among runs (26.4 ± 2.4% to 35.1 ± 8.7%).

Complex higher-order interactions were partly driven by inconsistent effects of intermediate treatments (high temperature and low salinity, or low temperature and high salinity) among runs. In run 1, both spring and fall conditions produced low levels of lecithotrophy, comparable to winter conditions (Fig. 3). In contrast, a relatively high proportion of lecithotrophy (compared to summer conditions) was induced by fall conditions in run 2, and by spring conditions in run 3. Effects of size also varied across runs and treatments, resulting in three- and four-way interactions involving size. Size had no clear effect on the proportion of lecithotrophy in run 1. Large size and high salinity were positively associated with lecithotrophy in run 2, whereas large size and high temperature were associated with lecithotrophy in run 3.

Discussion

Seasonal polyphenism in larval development mode

In contrast to all other marine animals, *Alderia willowi* toggles seasonally between alternative larval types, expressed as a maternal effect through a shift between two discrete size-classes of eggs. Prior studies indicated that the proportion of slugs laying lecithotrophic clutches was greater (often 100%) in summer, whereas both larval types were produced in all populations surveyed in winter months (Ellingson and Krug 2006). The present study confirms that over 3 years, slugs in San Diego exclusively produced lecithotrophic larvae from May to September, whereas a variable proportion of slugs laid planktotrophic eggs in winter and spring. Rearing experiments confirmed that temperature and salinity typical of summer conditions induced proportionally more adults to produce lecithotrophic clutches, including nearly all slugs in summer treatments from one experimental run. In treatments with lower temperature and/or low salinity, more slugs matured to produce planktotrophic offspring, consistent with the seasonal trend detected in field surveys.

We hypothesize that expression of lecithotrophy in summer months is an adaptive response to the seasonal cycle of estuarine opening and closing that characterized coastal wetlands in southern California. Low input of freshwater during the dry season, and

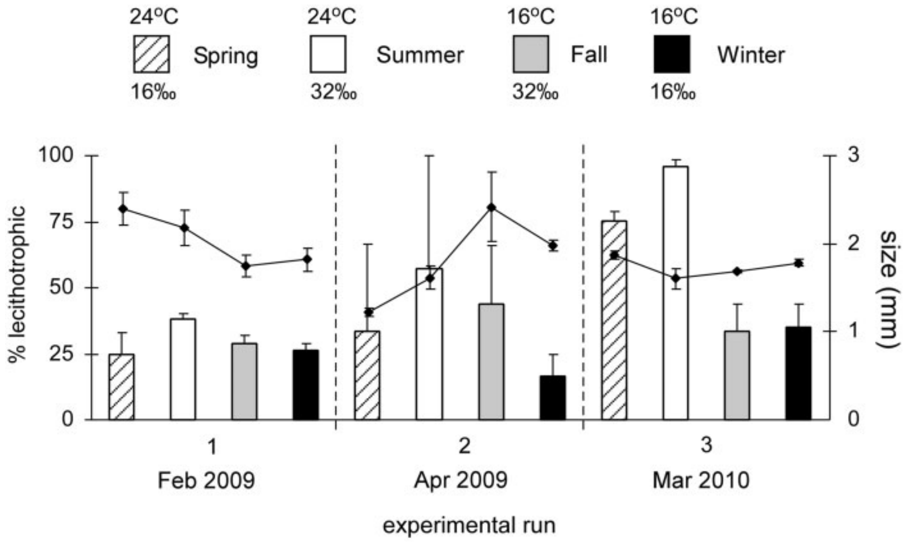

Fig. 3 Effects of the rearing environment on the type of offspring subsequently produced by adult specimens of *Alderia willowi*. Newly metamorphosed juveniles were raised in the laboratory under four conditions in a 2 × 2 design, testing the effects of high versus low temperature (given above boxes in the legend) and salinity (below boxes in the legend). The four treatment combinations approximate field conditions in the indicated season. Bars are the mean percentage (+1 SE) of lecithotrophic clutches produced over a 2-week period by slugs raised under the indicated conditions. The corresponding line graph gives mean size of adults (±SE) measured at the start of the egg-laying period. Results are shown for three independent runs of the experiment, each using eggs from a different starting batch of parents as a source of juveniles.

Table 2 General linear model testing the effects of rearing environment on the type of offspring produced by adult *A. willowi*

Source of variance	*df*	MS	*F*	*P*
Salinity	1	0.545	9.851	0.004
Error	27.958	0.055		
Salinity × size	2	0.338	7.625	0.004
Error	18	0.044		
Salinity × run × size	4	0.279	6.303	0.002
Error	18	0.044		
Salinity × temperature × run	10	0.109	2.451	0.047
Error	18	0.044		
Salinity × temperature × size	2	0.159	3.583	0.049
Error	18	0.044		
Salinity × temperature × run × size	4	0.203	4.586	0.010
Error	18	0.044		

Predictors were retained after sequentially removing nonsignificant terms by model simplification.

along-shore transport of sediment, frequently led to the formation of sand berms that closed the mouths of coastal embayments in summer and fall (Zedler 1982; Swift et al. 1989; Zedler 1996; Lafferty et al. 1999). During the winter rainy season, freshwater discharge into wetlands pushed open bay mouths and restored connectivity with the coastal ocean. Obligate mudflat fauna like *A. willowi* thus historically inhabited a variable environment that seasonally but predictably cycled between closed-lagoon and open-bay phases, although many estuaries are now permanently dredged open. This cycle likely affects the colonization dynamics and phylogeography of estuarine organisms in southern California (Dawson et al. 2002; Jacobs et al. 2004; Earl et al. 2010). Seasonal polyphenism in larval type expressed by *A. willowi* may be the first life-history adaptation to seasonal closure yet documented for the fauna of California's coastal wetlands.

Increasing back-bay temperatures and/or salinities in early summer could reliably indicate impending estuarine closure, and provide an environmental cue to mudflat animals. There would be little chance for larvae to escape from enclosed lagoons, negating the dispersal advantage of planktotrophy; lecithotrophy may then confer a selective advantage by increasing offspring survival in such closed systems. A shift to planktotrophy during the rainy season would subsequently allow long-lived larvae to exit an embayment and disperse via along-shore currents to other estuaries, facilitating colonization of ephemeral habitat patches, and maintaining gene flow between demes separated by uninhabitable stretches of open coast. Analyses of mitochondrial DNA spanning the known range of *A. willowi* revealed high genetic connectivity among populations (Ellingson and Krug 2006). Annual re-colonization events at the northern range edge also demonstrate demographic connectivity among estuaries (Garchow 2010). Thus, the production of planktotrophic larvae in winter and spring may capitalize on the seasonal opening and flushing of estuaries to carry larvae out of bays and facilitate along-shore transport.

An alternative hypothesis is that poecilogony is a trophic dimorphism, with planktotrophy allowing slugs to take advantage of phytoplankton blooms during periods of high productivity in coastal waters. The San Diego population expressed almost 100% lecithotrophy by May of 1997 and 1999, suggesting that in March–April, rising temperatures and salinities-induced slugs to produce larvae with no feeding requirement. Time-series data for chlorophyll levels in near-shore waters of San Diego show that the spring phytoplankton bloom occurred each May during the 1980s (Kim et al. 2009). Thus, in many years, the San Diego population of *A. willowi* appears to express lecithotrophy prematurely, shifting away from its feeding larval morph at the very time when larval food levels are maximal. A negative correlation between the frequency of planktotrophy and abundance of phytoplankton argues against poecilogony as an adaptation for larvae to exploit food resources. However, phytoplankton levels were highest from February to March in the late 1990s when the San Diego population of *A. willowi* was in fact producing a high proportion of feeding larvae, and lecithotrophy was expressed after phytoplankton levels declined. Recent trends could therefore allow feeding larvae to capitalize on spring phytoplankton blooms. However, earlier spring blooms have not been accompanied by associated shifts in water temperature that could serve as environmental cues of phytoplankton abundance (Kim et al. 2009). Seasonal polyphenism requires a reliable cue for a developing organism to anticipate the environment into which it will emerge (or reproduce, in the case of *A. willowi*). There is no clear mechanism by which benthic adults could anticipate seasonal trends in phytoplankton abundance, given the lack of correlated change in climatology or oceanography (Kim et al. 2009). Since the historical pattern of late-spring phytoplankton blooms in San Diego is not well correlated with the seasonal expression of planktotrophy in *A. willowi*, we hypothesize that selection against dispersal favored the evolution of a dry-season shift to lecithotrophy.

Although lecithotrophy is expressed earlier in the year than would be expected from seasonal trends in productivity, low coastal productivity in summer and fall could still select against the expression of planktotrophy. However, several lines of evidence argue against a general-sense selective advantage of lecithotrophy during Californian summers. First, summer phytoplankton blooms are common, if episodic, in southern California, suggesting selection against planktotrophy would be weak and inconsistent (Kim et al. 2009). Second, the vast majority of marine heterobranchs (sea slugs) are planktotrophic in Californian waters, but inhabits the open coast (Goddard 2004). Both factors argue that lecithotrophy is an adaptation to the estuarine environment of southern California. Finally, the lecithotrophic morph of *A. willowi* is capable of facultative feeding, but does not need to feed to complete development (Botello and Krug 2006). Thus, selection has greatly curtailed the dispersal potential but not the feeding ability of the larger larval morph in *A. willowi*, consistent with the hypothesis that lecithotrophy is a response to selection against a prolonged larval stage during the dry season.

"Mixed" clutches were produced in Mission Bay only during spring and fall, when some proportion of the population was transitioning between development modes. Mixed clutches contain a normal distribution of offspring sizes centered in between the extremes of planktotrophy and lecithotrophy, which occupy the tails of the distribution; most larvae are thus substantially larger than normal planktotrophs, but still must feed to complete development (Krug 1998). In long-term rearing studies, a few mixed clutches were transiently produced by individuals switching between larval types (Smolensky et al. 2009). Field data are consistent with the hypothesis that mixed clutches are produced by the ovotestes of slugs that are transitioning from one egg size to the other, and do not reflect a stable, intermediate life-history strategy. The fact that *A. willowi* can produce intermediate egg sizes, yet normally does not, is consistent with life-history theory predicting strong selection against in-between strategies that maximize neither fecundity nor larval survival (Vance 1973; Smith and Fretwell 1974).

The Mission Bay population consistently shifted to lecithotrophy each summer from 1996 to 1999. A decade later, the Mission Bay population similarly switched from 45% planktotrophic in May 2008 to 100% lecithotrophic in June. Seasonal trends were less pronounced in the Los Angeles area, with a variable proportion of slugs expressing planktotrophy in summer. One possible explanation is that slugs experience more extreme summer conditions in Mission Bay than in Los Angeles, because latitudinal gradients in temperature and rainfall intensify the expression of seasonal polyphenism (Zedler 1982; http://www.nodc.noaa.gov). However, the wet/dry seasonal shift in development was noted in populations north of Los Angeles (Ellingson and Krug), indicating the polyphenism is not unique to the Mission Bay population. Slugs from Los Angeles and Newport also live closer to the mouth of the bay, whereas Mission Bay slugs inhabit the back bay, which should intensify differences between summer and winter conditions; re-circulating back-bay waters are warmer than the coastal ocean and less saline after rainfall (e.g., Levin 1983; Largier et al. 1996, 1997). Monthly sampling of northern sites and additional populations in San Diego County should confirm whether the latitude or the geography of Mission Bay drive the pronounced seasonal shift in larval type in the resident population of *A. willowi*. However, we hypothesize that under equivalent conditions, slugs from any population will exhibit polyphenism in development mode.

There was considerable variation in the response to rearing conditions by offspring from different batches of adults collected from the Los Angeles area. Using laboratory-cultured algae, we attempted to keep conditions constant across replicate runs. However, the starting collection of egg masses was a random factor that sampled unknown genotypes from the standing diversity of the meta-population in southern California. Variance among runs may reflect genotype × environment interactions, with some genotypes having a higher temperature–salinity threshold for production of lecithotrophic eggs. Analogous results have been observed for seasonal maternal effects in plants: Flowering time (under maternal control) determines the temperature at which seeds mature, but temperature regime and genotype interactively determine germination success (Donohue et al. 2008). In *A. willowi*, accumulation of genotypes with a lower threshold in Mission Bay may contribute to the marked seasonal shift in larval type in this population. This possibility could be tested by concurrently rearing offspring from Mission Bay and Los Angeles to determine whether more offspring from San Diego grow up to express lecithotrophy under summer conditions.

Alternatively, variable expression of lecithotrophy among rearing trials could result from differences in the environment experienced by the parents of laboratory-reared slugs. In diverse systems, transgenerational effects of maternal or even grand-maternal condition can fine-tune the match between offspring phenotype and the environment (Badyaev and Uller 2009). Field-collected slugs of unknown age and condition were the source of the offspring reared in laboratory experiments, and may have epigenetically modified the threshold for expression of lecithotrophy in their laboratory-reared descendants. "Larval type" could thus be a maternal effect that integrates over many aspects of a slug's experience (including seasonal cues, food levels, age, and population density), and that of its parents as well. Further rearing studies that manipulate environmental factors across two or more generations are needed to ascertain whether the environment or genotype of the mother plays a greater role in mediating polyphenism in larval type.

Trans-generational plasticity in larval type may also alter life-history trajectories and population dynamics through its influence on local recruitment and hence density-dependent processes. Maternal effects on egg size in mites (Plaistow et al. 2004, 2006; Benton et al. 2005) and seed germination in plants (Donohue 2009) can affect phenology or population processes for two or more generations. In *A. willowi*, lecithotrophic offspring mature ~27 days faster than planktotrophic larvae, conferring the potential for rapid self-seeding and population growth (Krug 2007). Factors influencing the expression of lecithotrophy should thus influence local population density; if crowding influences the type of offspring a slug produces, density-dependence could initiate feedback cycles lasting several generations (e.g., Benton et al. 2005). Even the type of larva that an individual itself was may influence the type of offspring it will later produce as an adult. In aphids, wings can be induced by temperature, density, or host cues, but wing induction is more likely if the mother or grandmother lacked wings (reviewed in Braendle et al. 2006). Further research is needed to determine if lecithotrophy is more (or less) likely to be expressed by an individual that was itself a lecithotrophic larva, or whose parents experienced a particular environment. Such complex maternal effects have the potential to influence population dynamics in *A. willowi*, and should offer insight into how developmental processes evolve to respond adaptively to fluctuating environments.

Conclusions

Advances in understanding dimorphic development at the molecular level have come chiefly from arthropods (Brisson 2010; Simpson et al. 2011; Snell-Rood et al. 2011). Understanding the genetic changes and developmental mechanisms producing dimorphisms in non-ecdysozoan animals will require novel model systems, such as sacoglossan gastropods or spionid polychaetes that express alternative larval types. Poecilogony represents an opportunity to examine the genetic architecture underlying divergent developmental pathways, both in maternal ovarian tissue controlling egg size, and in embryos developing into distinct larval morphs. Since larval type is canalized for the overwhelming majority of marine species, only in poecilogonous taxa can different developmental morphs be contrasted with no confounding effects of inter-specific comparisons (Marsh and Fielman 2005; McCain 2008; Gibson et al. 2010; Pernet and McHugh 2010). Larval development mode is remarkably plastic in *A. willowi*, changing seasonally in response to environmental cues and with sweeping implications for offspring dispersal. No other marine animal is known to toggle between larval morphs, and no other case of poecilogony reflects a polyphenism determined by the environment in which the mother matures. We therefore expect *A. willowi* to become a valuable model system for exploring the mechanisms that underlie seasonal expression of alternative developmental pathways, with a view toward better understanding evolutionary transitions in marine life histories.

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References

Badyaev AV, Uller T. 2009. Parental effects in ecology and evolution: mechanisms, processes and implications. Phil Trans R Soc B 364:1169–77.

Benton TG, Plaistow SJ, Beckerman AP, Lapsley CT, Littlejohns S. 2005. Changes in maternal investment in eggs can affect population dynamics. Proc R Soc B 272:1351–6.

Botello G, Krug PJ. 2006. Desperate larvae revisited: age, energy and experience affect sensitivity to settlement cues in larvae of the gastropod *Alderia* sp. Mar Ecol Prog Ser 312:149–59.

Bradshaw WE. 1973. Homeostasis and polymorphism in vernal development of *Chaoborus americanus*. Ecology 54:1247–59.

Braendle C, Davis GK, Brisson JA, Stern DL. 2006. Wing dimorphism in aphids. Heredity 97:192–9.

Brakefield PM, French V. 1999. Butterfly wings: the evolution of development of color patterns. BioEssays 21:391–401.

Brisson JA. 2010. Aphid wing dimorphisms: linking environmental and genetic control of trait variation. Phil Trans Roy Soc B 365:605–16.

Dawson MN, Barlow M, Louie KD, Jacobs DK, Swift CC. 2002. Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. Mol Ecol 11:1065–75.

DeWitt TJ, Scheiner SM. 2004. Phenotypic plasticity: functional and conceptual approaches. Oxford: Oxford University Press. p. 1–9.

Donohue K. 2009. Completing the cycle: maternal effects as the missing link in plant life histories. Phil Trans Roy Soc B 364:1059–74.

Donohue K, Heschel MS, Butler CM, Barau D, Sharrock RA, Whitelam GC, Chiang GC. 2008. Diversification of phytochrome contributions to germination as a function of maternal environment. New Phytol 177:367–79.

Earl DA, Louie KD, Bardeleben C, Swift CC, Jacobs DK. 2010. Rangewide microsatellite phylogeography of the endangered tidewater goby, *Eucyclogobius newberryi* (Teleostei: Gobiidae), a genetically subdivided coastal fish with limited marine dispersal. Conserv Gen 11:103–14.

Ellingson RA. 2006. Variable development versus cryptic speciation: phylogeography and evolutionary history of the sea slugs Alderia and Costasiella (Opisthobranchia: Sacoglossa) [M.S. Thesis]. [Los Angeles (CA)]: California State University.

Ellingson RA, Krug PJ. 2006. Evolution of poecilogony from planktotrophy: cryptic speciation, phylogeography, and larval development in the gastropod genus *Alderia*. Evolution 60:2293–310.

Fusco G, Minelli A. 2010. Phenotypic plasticity in development and evolution: facts and concepts. Phil Trans Roy Soc B 365:547–56.

Garchow MN. 2010. Population dynamics at the range boundary between sister species of the estuarine sea slug genus *Alderia*: role of the physical environment versus larval supply [M.S. Thesis]. [Los Angeles (CA)]: California State University. 57 pp.

Gibson GD. 1997. Variable development in the spionid *Boccardia proboscidea* (Polychaeta) is linked to nurse egg production and larval trophic mode. Invert Biol 116:213–26.

Gibson GD, MacDonald K, Dufton M. 2010. Morphogenesis and phenotypic divergence in two developmental morphs of *Streblospio benedicti* (Annelid, Spionidae). Invert Biol 129:328–43.

Goddard JH. 2004. Developmental mode in benthic opisthobranch molluscs from the northeast Pacific Ocean: feeding in a sea of plenty. Can J Zool 82:1954–68.

Jacobs DK, Haney TA, Louie KD. 2004. Genes, diversity and geologic process on the Pacific coast. Ann Rev Earth Planet Sci 32:601–52.

Joron M, Papa R, Beltrán M, Chamberlain N, Mavárez J, Baxter S, Abanto M, Bermingham E, Humphray SJ, Rogers J, et al. 2006. A conserved supergene locus controls colour pattern diversity in *Heliconius* butterflies. PLoS Biol 4:e303.

Kim H-J, Miller AJ, McGowan J, Carter ML. 2009. Coastal phytoplankton blooms in the Southern California Bight. Prog Oceanogr 82:137–47.

Krug PJ. 1998. Poecilogony in an estuarine opisthobranch: planktotrophy, lecithotrophy, and mixed clutches in a population of the ascoglossan *Alderia modesta*. Mar Biol 132:483–94.

Krug PJ. 2001. Bet-hedging dispersal strategy of a specialist marine herbivore: a settlement dimorphism among sibling larvae of *Alderia modesta*. Mar Ecol Prog Ser 213:177–92.

Krug PJ. 2007. Poecilogony and larval ecology in the gastropod genus *Alderia*. Am Malacol Bull 23:99–111.

Krug PJ. 2009. Not my "type": larval dispersal dimorphisms and bet-hedging in opisthobranch life histories. Biol Bull 216:355–72.

Krug PJ, Ellingson RA, Burton RA, Valdés A. 2007. A new poecilogonous species of sea slug (Opisthobranchia: Sacoglossa) from California: Comparison with the planktotrophic congener *Alderia modesta* (Lovén, 1844). J Moll Stud 73:29–38.

Lafferty KD, Swift CC, Ambrose RF. 1999. Extirpation and recolonization in a metapopulation of an endangered fish, the tidewater goby. Conserv Biol 13:1447–53.

Largier JL, Hearn CJ, Chadwick DB. 1996. Density structures in low inflow estuaries. In: Aubrey DG, Friedrichs CT, editors. Buoyancy effects on coastal dynamics, Vol. 53. Washington, DC: American Geophysical Union. p. 227–42.

Largier JL, Hollibaugh JT, Smith SV. 1997. Seasonally hypersaline estuaries in mediterranean-climate estuaries. Estuar Coast Shelf Sci 45:789–97.

Leimar O. 2009. Environmental and genetic cues in the evolution of phenotypic polymorphism. Evol Ecol 23: 125–35.

Levin LA. 1983. Drift tube studies of bay-ocean water exchange and implications for larval dispersal. Estuaries 6:364–71.

Levin LA. 1984. Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three coasts of North America. Biol Bull 166:494–508.

Levin LA, Bridges TS. 1994. Control and consequences of alternative developmental modes in a poecilogonous polychaete. Am Zool 34:323–32.

Levin LA, Creed EL. 1986. Effect of temperature and food availability on reproductive responses of *Streblospio benedicti* (Polychaeta: Spionidae) with planktotrophic or lecithotrophic development. Mar Biol 92:103–113.

Levin LA, Huggett DV. 1990. Implications of alternative developmental reproductive modes for seasonality and demography in an estuarine polychaete. Ecology 71: 2191–208.

Levin LA, Zhu J, Creed E. 1991. The genetic basis of life-history characters in a polychaete exhibiting planktotrophy and lecithotrophy. Evolution 45:380–97.

McCain ER. 2008. Poecilogony as a tool for understanding speciation: early development of *Streblospio benedicti* and *Streblospio gynobranchiata* (Polychaeta: Spionidae). Invert Reprod Dev 51:91–101.

Marsh AG, Fielman KT. 2005. Transcriptome profiling of individual larvae of two different developmental modes in the poecilogonous polychaete *Streblospio benedicti* (Spionidae). J Exp Zool B 304B:238–49.

Moczek AP. 2010. Phenotypic plasticity and diversity in insects. Phil Trans Roy Soc B 365:593–603.

Moczek AP, Emlen DJ. 1999. Proximate determination of male horn dimorphism in the beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). J Evol Biol 12:27–37.

Nijhout HF. 1999. Control mechanisms of polyphenic development in insects. Bioessays 49:181–192.

Nijhout HF. 2003. Development and evolution of adaptive polyphenisms. Evol Dev 5:9–18.

Pernet B, McHugh D. 2010. Evolutionary changes in the timing of gut morphogenesis in larvae of the marine annelid *Streblospio benedicti*. Evol Dev 12:618–27.

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol Evol 25:459–67.

Plaistow SJ, Lapsley CT, Beckerman AP, Benton TG. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. Proc R Soc B 271:919–24.

Plaistow SJ, Lapsley CT, Benton TG. 2006. Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. Am Nat 167:206–15.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. Cambridge: Cambridge University Press.

Roff DA. 1986. The evolution of wing dimorphism in insects. Evolution 40:1009–20.

Shapiro AM. 1976. Seasonal polyphenism. Evol Biol 9: 259–333.

Simpson SJ, Sword GA, Lo N. 2011. Polyphenism in insects. Curr Biol 21:R738–49.

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. Am Nat 108:499–506.

Smolensky N, Romero MR, Krug PJ. 2009. Evidence for costs of mating and self-fertilization in a simultaneous hermaphrodite with hypodermic insemination, the opisthobranch *Alderia willowi*. Biol Bull 216:188–99.

Snell-Rood EC, Cash A, Han MV, Kijimoto T, Andrews J, Moczek AP. 2011. Developmental decoupling of alternative phenotypes: insights from the transcriptomes of horn-polyphenic beetles. Evolution 65:231–45.

Strathmann RR. 1985. Feeding and nonfeeding larval development and life history evolution in marine invertebrates. Ann Rev Ecol Syst 16:339–61.

Strathmann RR. 1990. Why life histories evolve differently in the sea. Am Zool 30:197–207.

Swift CC, Nelson JL, Maslow C, Stein T. 1989. Biology and distribution of the tidewater goby, *Eucyclogobius newberryi* (Pisces: Gobiidae) of California. Contributions in science 404. Los Angeles: Natural History Museum of Los Angeles County.

Vance RR. 1973. More on reproductive strategies in marine invertebrates. Am Nat 107:353–61.

Vendetti JE, Trowbridge CD, Krug PJ. 2012. Poecilogony and population genetic structure in *Elysia pusilla* (Heterobranchia: Sacoglossa), and reproductive data for five sacoglossans that express dimorphisms in larval development. Int Comp Biol 52:138–50.

West HH, Harrigan JF, Pierce SK. 1984. Hybridization of two populations of a marineopisthobranch with different developmental patterns. Veliger 26:199–206.

West-Eberhard MJ. 2003. Developmental plasticity and evolution. New York: Oxford University Press.

Willette DA. 2006. Larval supply to the "muddy intertidal": Variation at small spatial scales and molecular methods for species identification. [M.S. Thesis]. [Los Angeles (CA)]: California State University.

Zedler J. 1982. The ecology of southern California coastal salt marshes: a community profile. FWS/OBS-31/54. U.S. Fish and Wildlife Service, Biological Services Program, Washington, DC, USA.

Zedler J. 1996. Coastal mitigation in southern California: the need for a regional restoration strategy. Ecol App 6:84–93.

Zera AJ, Denno RF. 1997. Physiology and ecology of dispersal polymorphism in insects. Ann Rev Entomol 42:207–30.

Zera AJ, Tiebel KC. 1991. Photoperiodic induction of wing morphs in the waterstrider *Limnoporus canaliculatus* (Gerridae: Hemiptera). Ann Entomol Soc Am 84:508–516.