

***Elysia timida* (Risso, 1818) three decades of research**

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Giménez–Casalduero, F., Muniain, C., González–Wangüemert, M., Garrote–Moreno, A., 2011. *Elysia timida* (Risso, 1818) three decades of research. *Animal Biodiversity and Conservation*, 34.1: 217–227.

Abstract

Elysia timida (Risso, 1818) three decades of research.— During the last 30 years, studies on *Elysia timida* (Risso, 1818) have addressed various aspects related to food sources, photosynthetic efficiency of kleptoplasts, population genetics, chemical ecology and reproductive biology, both in the Mediterranean Sea and in the Mar Menor coastal lagoon. *E. timida* shows a strong specific interaction with *Acetabularia acetabulum*, retaining functional chloroplasts for at least 45 days and obtaining extra energy in periods when food resources are scarce. It shows control of parapodia, avoiding pigment photodestruction under oversaturated light conditions. The chemical ecological relationships established between *E. timida* and its potential predator fish, *Thalassoma pavo*, have also been evaluated, and it has been found that the extracts of the mollusc contain repellent and unpalatable polypropionate compounds. Population genetics has demonstrated the genetic divergence between populations showing high and significant values of FST and genetic distances, and at least six private alleles that are not shared with Mediterranean populations have been detected in lagoon populations. This sacoglossan is a poecilogonic species, and its lagoon populations show a greater reproductive output than Mediterranean populations; they produce a greater number of egg masses and embryos per individual, and the capsules have a wider diameter.

Key words: *Elysia timida*, Kleptoplasts, Environmental stress, Chemicals ecology, Genetic divergence, Poecilogonic specie.

Resumen

Elysia timida (Risso, 1818) tres décadas de investigación.— Durante los últimos 30 años los estudios sobre *Elysia timida* (Risso 1818) han abordado diversos aspectos relacionados con sus fuentes de alimentación, la eficacia fotosintética de los cleptoplastos, la genética de poblaciones, la ecología química y la biología reproductiva, tanto en Mar Mediterráneo como en la laguna costera del Mar Menor. *E. timida* presenta una fuerte interacción específica con *Acetabularia acetabulum*, reteniendo los cloroplastos funcionales durante al menos 45 días y obteniendo energía extra durante los periodos en que los recursos alimentarios escasean. Mediante el control de los parapodios evita la fotodestrucción de los pigmentos en condiciones de sobresaturación lumínica. También se han evaluado las relaciones ecológicas y químicas entre *E. timida* y su depredador potencial, el pez *Thalassoma pavo*, detectándose que los extractos del molusco contienen componentes polipropionados que son repelentes y de gusto desagradable. La genética de poblaciones ha demostrado la existencia de divergencia genética entre las poblaciones, presentando valores altos y significativos de FST y de distancias genéticas, detectándose al menos seis alelos privados en las poblaciones lagunares los cuales no son compartidos por las poblaciones del Mediterráneo. Este sacogloso es una especie poecilogónica, y sus poblaciones de la laguna muestran un mayor esfuerzo reproductivo que las poblaciones mediterráneas; producen un número mayor de masas de huevos y de embriones por individuo, y las cápsulas tienen un mayor diámetro.

Palabras clave: *Elysia timida*, Cleptoplastos, Estrés ambiental, Ecología química, Divergencia genética, Especie poecilogónica.

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Introduction

In recent decades, the sacoglossan *Elysia timida* (Risso, 1818) (Gastropoda, Opisthobranchia, Sacoglossa) has attracted the attention of numerous researchers (fig 1). The reasons for this growing interest are the easy accessibility of its habitat, the large number of individuals in a small areas (due to its gregarious behaviour associated with a low dispersal ability), the extraordinary physiological and ecological adaptations (great versatility and adaptability to stress conditions) and particular reproductive and survival strategies.

Results

The sacoglossan: habitat, interaction and geographical distribution

E. timida (Risso, 1818) is a gregarious opisthobranch mollusc that lives in shallow waters characterised by low-energy hydrodynamic conditions. These molluscs seek light in areas covered by stones or a thick layer of sand (Bouchet, 1984; Marín & Ros, 1988, 1992; Thompson & Jaklin, 1988; Giménez-Casalduero et al., 2002; Giménez-Casalduero & Muniain, 2006). They share these environments with the chlorophyceae algae, *Acetabularia acetabulum* (Linnaeus, 1758), along the Mediterranean coast, including coastal lagoons (Ortea et al., 1997).

E. timida occurs naturally in populations feeding on the fleshy, siphoned green alga, *A. acetabulum*, although it has been linked with other algae that are commonly found in photophilic environments such as *Padina pavonia* (Linnaeus) (Bouchet, 1984; Ballesteros, 1985), or *Ulva* sp., *Bryopsis* sp., *Enteromorpha* sp., and also phaeophyceae algae, such as *Halopectis filicina* (Gratoloup) and *Colpomenia sinuosa* (Mertens ex Roth) (Rahat, 1976). However, *A. acetabulum* is its optimal diet (Ros & Rodriguez, 1985; Marín, 1988; Marín & Ros, 1987, 1989, 1991, 1993). Some authors have suggested a co-evolution between the two species (Marín & Ros, 1992, 2004). *E. timida* populations associated with *A. acetabulum* meadows exceed densities of about 6 ind./m² (Marín & Ros, 1992). This gregarious nature is linked to a limited dispersion ability and a strong habitat preference. Lagoon environments are sometimes under conditions of extreme environmental stress due to significant changes in salinity and temperature, which may cause strong selective pressures on organisms (Gamito et al., 2005; González-Wanguemert et al., 2004, 2006, 2009). *E. timida* has been recorded from the Mediterranean Sea, Gibraltar Strait, Canary Islands, Cape Verde Island and Sao Tomé Island, including coastal lagoons (Bouchet, 1984; Ballesteros, 1979; García-Gómez, 2002; Giménez-Casalduero, 1997a, 1997b, 1999; Marín & Ros, 1987, 1988, 1991, 1992; Ortea et al., 1997; Rahat, 1976; Ros, 1976, 1977; Swennen, 1961; Templado, 1982; Thompson & Jaklin, 1988; Türkmen & Demirsoy, 2009; Wirtz & Anker, 2009). However, if we take into account the low dispersal

ability of this species and the genetic differentiation results found among populations at a small spatial scale (González-Wanguemert et al., 2006), the amphi-Atlantic status of *E. timida*, cited from the Western Atlantic coasts by Ortea et al. (1997) and Valdés et al. (2006), this does not seem consistent. It is more likely a new variety in the Western Atlantic, although this suggestion needs to be corroborated genetically.

Aposematic versus cryptic coloration

The sacoglossan show a degree of feeding specialization that is similar to that of terrestrial insects. *E. timida* feeds with priority on the green alga, *A. acetabulum*. In the field, these animals are white and are conspicuous in winter or spring, but they are very cryptic when the algal food is calcified (Marín & Ros, 1992). The white colour of *E. timida* could serve two main purposes: chloroplasts exploitation and predation avoidance. The animal uses the white of the parapodia to control the amount of light used for the photosynthesis of kleptoplasts. The design of the mollusc coloration may appear relatively cryptic during periods of algal calcification (summer: white animal on white background) and relatively conspicuous during periods characterized by low algal calcification (autumn: white animal on green background).

Experiments conducted on feeding preference (Giménez-Casalduero, 1997b), have shown that the white models were more cryptic in the summer background than in the autumn background (fig. 2). This suggests that the optimal colour for a possible prey is white rather than green or red, in particular for *E. timida*, bearing in mind its defensive characteristics

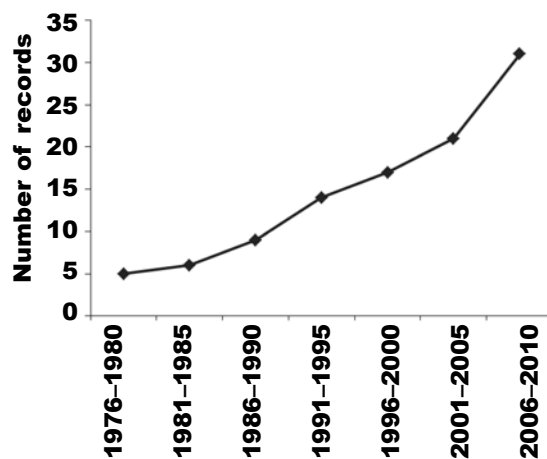


Fig. 1. Accumulative numbers of *Elysia timida* manuscripts pooled in five-year periods.

Fig. 1. Número acumulado de manuscritos de *Elysia timida*, agrupados en periodos de cinco años.

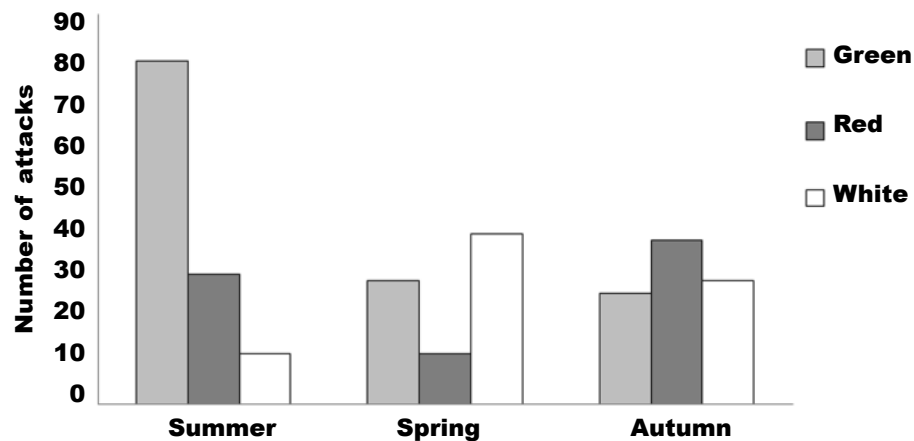


Fig. 2. Crypsis degree measured as number of attacks of the green, red and white artificial models over the three backgrounds (autumn, spring and summer), which represents the seasonal change of the algae *A. acetabulum* (modified from Giménez-Casalduero, 1997b).

Fig. 2. Grado de cripsis medido como el número de ataques a los modelos artificiales verdes, rojos y blancos, sobre los tres fondos (otoño, primavera y verano), los cuales representan el cambio estacional del alga *A. acetabulum* (modificado de Giménez-Casalduero, 1997b).

and population dynamics. The colour design of a prey is considered to be cryptic when the potential predator cannot distinguish it from the sea floor on which it lies.

This strategy is very common in many elysiidae, one example being *Bosellia mimetica* (Trinchese, 1891), which feeds on *Halimeda tuna* (Ellis et Solander) and

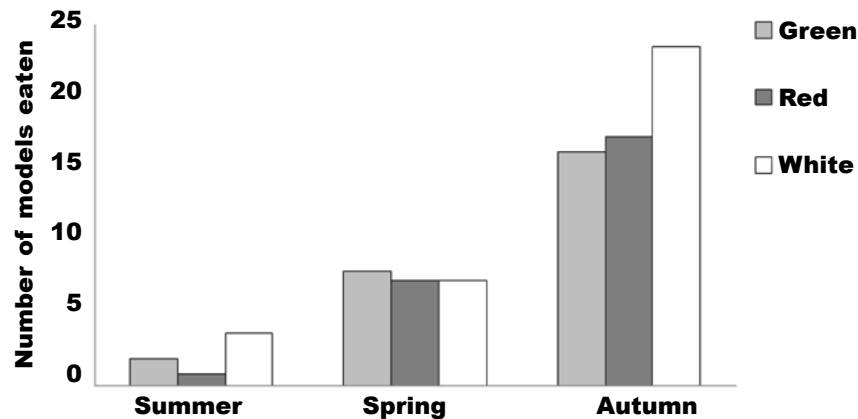


Fig. 3. The ability of algae to refuge measured as number of attacks of the green, red and white artificial models over the three backgrounds (autumn, spring and summer), which represent the seasonal change of the algae *A. acetabulum*. In this experiment the background was three-dimensional, simulating the algae structure with white plastic stick; the density of the stick was proportional to the alga density during the three seasons simulated (modified from Giménez-Casalduero, 1997b).

Fig. 3. Capacidad de refugio de las algas, medido como número de ataques a los modelos artificiales verdes, rojos y blancos, en las tres estaciones (otoño, primavera y verano), los cuales representan el cambio estacional del alga *A. acetabulum*. En este experimento el fondo fue tridimensional, simulando la estructura de las algas mediante palitos blancos de plástico; la densidad de los palitos fue proporcional a la densidad del alga durante las tres estaciones simuladas (modificado de Giménez-Casalduero, 1997b).

is virtually indistinguishable. Aposematic coloration is used as a warning to potential predators of their toxic or unpleasantness. The close relationship between *E. timida* and *A. acetabulum*, apparently favours a design that can be either cryptic or aposematic.

Gendron & Staddon (1983) showed that if the probability of detecting a prey is inversely related to both the search rate and the degree of crypsis, specialist herbivores could be subject to greater selective pressure by natural enemies than generalist animals. Laboratory experiments show that *E. timida* is less consumed when the calcified algal biomass increases (fig. 3) (Giménez-Casalduero, 1997b). The nutritional quality decreases, but it provides an effective refuge to avoid predation (Hacker & Steneck, 1990; Duffy & Hay, 1991).

Some sacoglossans however have adapted their diet to algae with chemical defence, because they subsequently avoid predation and accidental ingestion by macro-herbivores, or because the sequestered secondary metabolites from the algae protect them from their own predators (Hay, 1991; Hay et al., 1994). *A. acetabulum* contains no deterrent metabolites and *E. timida* must synthesize its own defence (Gavagnin et al., 1994).

Photosynthetic efficiency of kleptoplasts

The sacoglossans are specialized herbivores that can retain chloroplasts intact within the cells of the gastrointestinal tract (Marín & Ros, 1988). The manipulation of unchanged foreign structures is a complex mechanism involving both cell recognition and acceptance, and physiological adaptation within the digestive glands. Also, its operation as a photosynthetic source is an extraordinary evolutionary feature (Marín & Ros, 1991; Rumpho et al., 2000, 2001).

From the point of view of chloroplasts' autonomy it is interesting to note the time period that chloroplasts retain their normal functions within their host cells. Several authors have demonstrated the photosynthetic capacity of *E. timida* that consists of their using symbiont chloroplasts (kleptoplasts) retained in their intestinal diverticula (Ros & Rodriguez, 1985; Marín & Ros, 1989; Wägele & Johnsen, 2001; Evertsen et al., 2007; Händerler et al., 2009, 2010). In addition, Greene (1970) and Greene & Muscatine (1972) not only described the release of organic compounds in chloroplast symbionts but noted that the metabolism of symbiotic chloroplasts can be modified by the association. They found abundant evidence showing the transfer of photosynthates from the chloroplast to the host tissue sacoglossans. In the case of isolated *Acetabularia*, chloroplasts have been reported that C^{14} labelled only chlorophyll joins the first 70', after which it is extracted from the algal tissue (Trench & Smith, 1970). The chloroplast-animal association is unable to synthesize chlorophyll, glycolipids, ribulose-bisphosphate carboxylase or membrane proteins. Therefore chloroplasts are unable to grow or have a real division when they are inside the host animal (Trench & Ohlhorst, 1976). This inability to synthesize DNA and RNA or regenerate plastidial proteins means that

elysoidea must obtain chloroplasts in each generation (Marín & Ros, 1993; Wägele et al., 2010).

Effectiveness in this symbiotic system depends on the ability of the host to incorporate active chloroplasts and retain them functionally active in its glands for as long as possible (a high turnover rate would not provide benefits). Holding capacity of active chloroplasts is revealed by analysing the changes in the proportion of *chlorophyll a* over time compared to other pigments within the body of sacoglossa. *Chlorophyll a* has been described as one of least stable pigments over time as compared to carotenes or degradation products from chlorophyll, which have an extraordinary resistance to destruction. The experiment carried out by Giménez-Casalduero (1997b) on *E. timida* showed a lower degradation rate for chlorophyll than for the remaining pigments during the experimental time (starved and dark conditions) (fig. 4). However, the absence of light seems to be a determining factor for the incorporation of new chloroplasts. This result was interpreted as the ability of sacoglossa to assimilate or eliminate the remaining pigments, because *E. timida* tends to retain and maintain high levels of *chlorophyll a*, declining its net concentration over time more slowly than the other pigments. In an effective system, the retained chlorophyll must endure being photosynthetically active for long periods of time. Gross oxygen production per milligram of chlorophyll reaches values between 2.910 and 3.397 mg O₂/mg of *chlorophyll a* in *E. timida* tissues and these levels are maintained over time, even in starvation periods (table 1) (Giménez-Casalduero, 1997b)

Metabolic benefits and consequences

As mentioned above, the chloroplasts retained in the intestinal diverticula of *E. timida* are able to perform photosynthesis (Ros & Rodriguez, 1985; Marín & Ros, 1989). The use of photosynthetic energy has also been demonstrated by Giménez-Casalduero & Muniain research (2006, 2008). These latter works studied the photosynthetic parameters of *E. timida* in the Mar Menor lagoon environment, and the researchers found that the results of P-I curves were explained by the model based on the kinetics of Michaelis-Menten (1913) and described by Pérez (1989). This model showed a rapid saturation of the photosynthetic apparatus at relatively low irradiance values, seeming to contradict the animal habitat (shallow and lit areas). However, this fast saturation might be influenced by a control of parapodia, which would avoid pigment photodestruction under oversaturated light conditions. During the experimental period (Giménez-Casalduero & Muniain, 2006), *E. timida* individuals were observed closing and opening their parapodia, illustrating the ability of sacoglossans to regulate the photosynthetic production described by Rahat & Monseline (1979), Monseline & Rahat (1980) and Jesus et al. (2010): parapodia were completely opened when individuals were exposed to low irradiance and gradually closed with increasing the irradiance values. Furthermore, in the field, the sacoglossan is commonly found

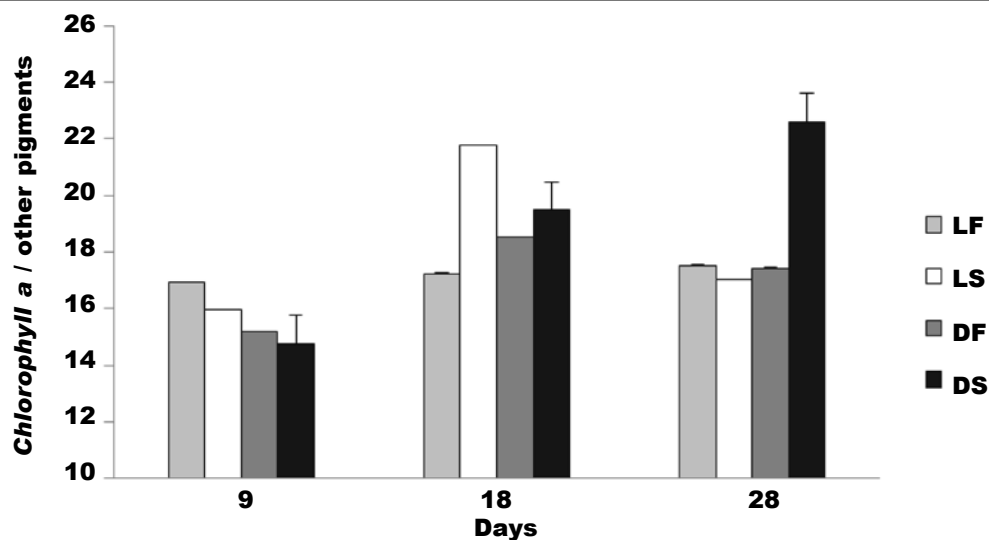


Fig. 4. Rate of *chlorophyll a* versus other photosynthetic pigments (\pm SE) in the tissue of *E. timida* specimens kept in a laboratory for 28 days at a constant temperature (25°C) with four treatments: LF. Light photoperiod 12:12 light and darkness feeding *A. acetabulum*; LS. Light photoperiod 12:12 light and darkness without food; DF. Continuous darkness feeding *A. acetabulum*; DS. Continuous darkness without food (modified from Giménez-Casalduero, 1997b).

Fig. 4. Proporción de clorofila a frente a otros pigmentos fotosintéticos (\pm ES) en el tejido de especímenes de *E. timida* mantenidos en el laboratorio durante 28 días a una temperatura constante (25°C) con cuatro tratamientos distintos: LF. Fotoperiodo lumínico 12:12 de luz y oscuridad alimentándose de *A. acetabulum*; LS. Fotoperiodo lumínico 12:12 de luz y oscuridad sin alimento; DF. Oscuridad continuada alimentándose a base de *A. acetabulum*; DS. Oscuridad continuada sin alimento (modificado de Giménez-Casalduero, 1997b).

with completely closed parapodia on a well-lit day (Giménez-Casalduero & Muniain, 2006).

The production versus respiration relationship (P/R) was initially used to estimate compensation irradiance for symbiotic associations between corals and zooxanthellae: (i) P/R = 1 indicates that most of the fixed carbon during photosynthesis is consumed on respiration by the host and the 'guest'; (ii) P/R > 1 means that the amount of fixed carbon by photosynthesis exceeds the basal metabolic requirements of the host; (iii) P/R < 1 indicates that the host has to feed on other sources to fulfil the amount of carbon required for its basal metabolic needs (McCloskey et al., 1978). Theoretically, the estimated mean P/R values of *E. timida* populations from the Mar Menor coastal lagoon are above 0.91, these values are considered high in the coral-zooxanthellae symbioses. If we consider that sacoglossans are more active than corals and possess higher respiration values, then production values of chloroplasts should also be higher to obtain similar indices. Therefore, photosynthetic efficiency of symbiont chloroplasts on *E. timida* is fairly high. The parameters of the *P-I* curve obtained by Giménez-Casalduero & Muniain (2006) provide a useful baseline information to perform further studies on photosynthetic energy generated from the relationship between the mollusc and the

acquired chloroplasts, as well as on the importance of such energy for primary metabolism of lagoon sacoglossans (Giménez-Casalduero & Muniain, 2006; Jesus et al., 2010).

Poecilogony is an intraspecific variation in the mode of larval development and can be found in single or different populations (Bouchet, 1989; Krug, 1998; Krug et al., 2007). *E. timida* is considered a poecilogonic species showing direct and lecithotrophic development in the Mediterranean area (Rahat, 1976; Marín & Ros, 1993; Garrote-Moreno, 2007). Direct development increases the likelihood of offspring survival in the absence of food limitation. However when the food is scarce, lecithotrophic development allows exploration of new areas with *A. acetabulum*. So, is there coevolution between *E. timida* and *A. acetabulum*? Or, conversely, is it an adaptive response of *E. timida* to environmental factors, such as the greater or lesser availability of food?

Energy invested in reproduction is also affected by the presence of 'extra energy' from symbiotic chloroplasts. In general, the number of spawn is greater if animals have extra energy input; a disturbance of normal conditions initiates a massive reproductive activity and the number of eggs per spawn is greater in starved conditions (Giménez-Casalduero & Muniain, 2008).

Table 1. Gross oxygen production (GOP, mg O₂/mg *chlorophyll a*) in *Elysia timida* for specimens kept in a laboratory under starved conditions for 28 days at a constant temperature (25°C) with two treatments: GOP–L. With light photoperiod (12:12 light and darkness); GOP–D. Continuous darkness (modified from Giménez-Casalduero, 1997b).

Tabla 1. Producción total de oxígeno (GOP, mg O₂/mg de clorofila a) en *Elysia timida*, para especímenes mantenidos en el laboratorio sin recibir alimento durante 28 días a una temperatura constante (25°C) bajo dos tratamientos: GOP–L. Con fotoperiodo lumínico (12:12 luz y oscuridad); GOP–D. Oscuridad continuada (modificado de Giménez-Casalduero, 1997b).

Days	GOP–L	GOP–D
0	2.91	2.91
9	2.25	1.31
18	2.47	0.68
28	3.397	1.58

The sacoglossan reaction in a starved situation is to invest energy in reproduction, using a mating behaviour and a particularly highly efficient co-occurrent sperm transfer (Schmitt et al., 2007). Reproductive effort is very high the first few days, using both photosynthetic and metabolic energy (which explains a decrease of up to 20% in size the first 18 days) (Giménez-Casalduero, 2008).

Adaptations to environmental stress conditions

Estuaries and coastal lagoons constitute transitional environments whose main characteristic is the instability of their physical-chemical parameters, mainly the concentration of salt (Cognetti & Maltagliati, 2000; Gamito et al., 2005). Physiological adaptations are very important for survival in these extreme environments. These adaptations imply a high energetic cost that is reflected in growth and reproduction rates; thus genetic selection aimed at minimizing such costs can be assumed (Wright, 1977; Remmert, 1988). When environmental conditions change unpredictably in space and time, variation in life-history traits can be an adaptive response to selection (Meyers & Bull, 2002). For instance, stable dispersal dimorphisms can evolve when the quality of habitat patches varies over time and there is spatial heterogeneity in environmental fluctuations (Mathias et al., 2001). Two morphs of *E. timida* have been described in the Mar Menor coastal lagoon and shallow environments of the Southwest Mediterranean coast, showing differences in body

size, colour and chlorophyll concentration (Giménez-Casalduero, 1997a). It is important to stress, however, that differences in these parameters have also been found between lagoon (regardless of morphotype) and Mediterranean populations (Giménez-Casalduero, 1999). For example, simultaneously comparing the mean dry weight and mean *chlorophyll a* concentration from two lagoon morphotypes and the Mediterranean population, we recognized differences between all of them (fig. 5). These differences have recently been confirmed by Jesus et al. (2010).

Moreover, changes have been observed in the spawn of populations from Mar Menor lagoon and the Mediterranean Sea, with the reproductive effort in lagoon sacoglossa being higher than in Mediterranean populations (Giménez-Casalduero, 1997b, 1999). The variability in the number of eggs per spawn is smaller in the Mediterranean population than the lagoon one which varies from 88 to 725 eggs per spawn (fig. 6). In some species, such as *Elysia viridis* (Montagu, 1804) the increase in reproductive success may result from a change in salinity of environment (Hagerman, 1970). High salinity of the Mar Menor lagoon could influence the increase in *E. timida* reproductive output, but it does not explain the differences among environments or among morphs.

According to Giménez-Casalduero (1997a, 1997b), the differences between brown and green lagoon animals, could have diverse explanations: i) We could be facing a new variety of *E. timida*, considering the Mar Menor lagoon as a shelter for this species (high salinity, extreme temperature changes, isolation from the Mediterranean Sea, few predators) and the capacity of this elysoidea to have a direct larval development and being a strong candidate to develop genetic adaptations in lagoon conditions; ii) Another possible scenario would be the higher versatility concerning food in this species, with a great capacity to adapt to new food resources.

González-Wangüemert et al. (2006) estimated the degree of genetic divergence between populations living inside and outside the Mar Menor coastal lagoon. They showed genetic identity values ranging from 0.9 to 0.87 in the Mediterranean and coastal lagoon populations. These values indicated a subspecies-rank separation according to several authors (Avise, 1974; Thorpe, 1983; Mariani et al., 2002). However, it is important to stress that there are different methods of delimiting species and subspecies (Sites & Marshal, 2003). However, significant genetic differences (F_{ST} values and Nei's genetic distances) were found between lagoon and marine populations, confirmed by the principal component analysis. This genetic differentiation (Mar Menor/Mediterranean Sea populations) has been confirmed using other molecular markers and species (González-Wangüemert & Pérez-Ruzafa, in review; Vergara-Chen et al., 2010a, 2010b). Nevertheless, no genetic differences were found between the two *E. timida* morphs previously described (fig. 7). In fact, according to data from Giménez-Casalduero (1999), both morphs can occur either in the Mediterranean or Mar Menor populations, which implies

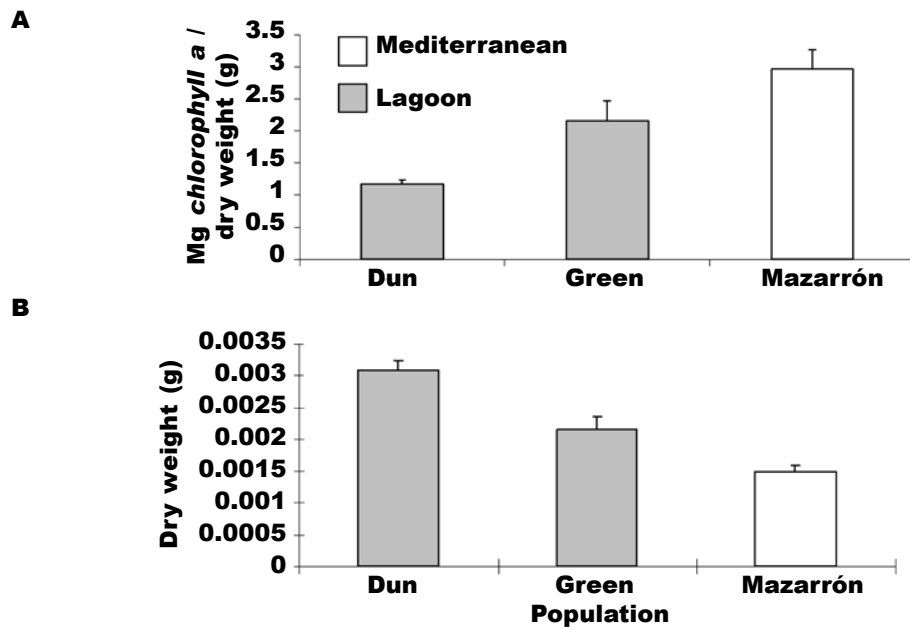


Fig. 5. Mean chlorophyll a (A) and mean dry weight (B) concentration values in the tissues of Mediterranean and lagoon populations of *Elysia timida* (modified from Giménez-Casalduero, 1997b, 1999).

Fig. 5. Concentración media de clorofila a (A) y peso seco medio en los tejidos de las poblaciones mediterránea y de laguna de *Elysia timida* (modificado de Giménez-Casalduero, 1997b, 1999).

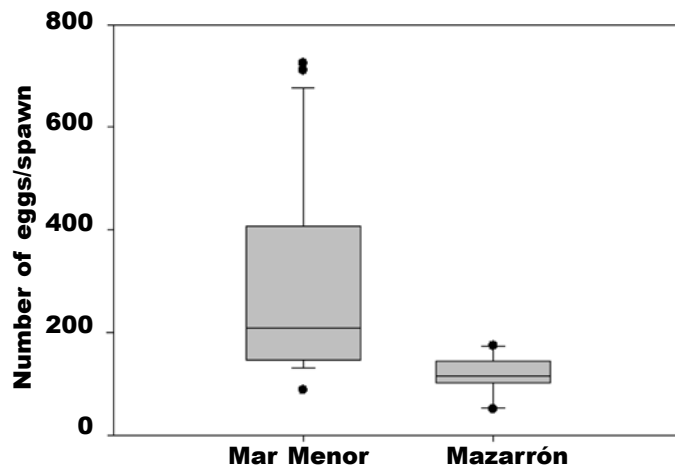


Fig. 6. Box plot of number of eggs per spawn in the Mediterranean and lagoon (Mar Menor) *E. timida*. The box itself represents 50% of all cases, and extends from the 25th to the 75th quartiles. The line inside the box shows the media. Points beyond the whiskers (outliers) are drawn individually. (Source of data from: Marín & Ros, 1993; Giménez-Casalduero, 1997a, 1997b, 1999; unpublished data, 2007).

Fig. 6. Diagrama de cajas del número de huevos por puesta de *E. timida* en el Mediterráneo y en la laguna (Mar Menor). La caja por sí misma representa el 50% de todos los casos, y se extiende desde el cuartil 25 al 75. La línea del interior de las cajas representa la media. Los puntos situados más allá de las líneas (datos atípicos o outliers) se han dibujado individualmente. (Procedencia de los datos: Marín & Ros, 1993; Giménez-Casalduero, 1997a, 1997b, 1999; datos no publicados, 2007).

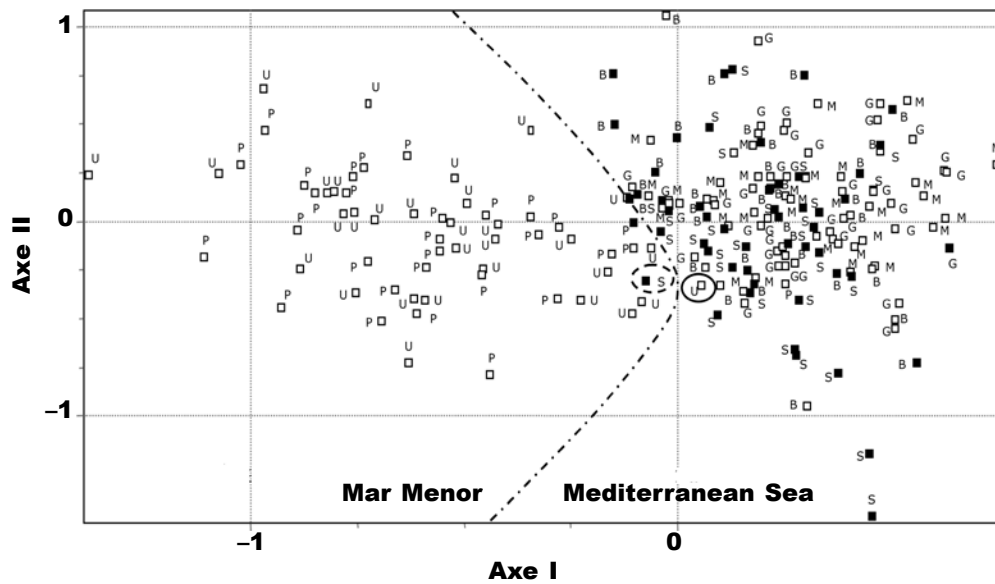


Fig. 7. Factorial correspondence analysis on *E. timida* genotypes: ■ Dun individuals; □ Green individuals. Mediterranean Sea: B. Tabarca Beach; S. South Tabarca; M. Mazarrón Harbour; G. Gachero. Mar Menor coastal lagoon: P. Perdiguera Island; U. Urrutias. The dashed line separates Mar Menor from Mediterranean samples (modified from Gonzalez-Wangüemert et al., 2004).

Fig. 7. Análisis factorial de correspondencia de los genotipos de *E. timida*: ■ Individuos pardos; □ Individuos verdes. Mar Mediterráneo: B. Playa de Tabarca; S. Sur de Tabarca; M. Puerto de Mazarrón; G. Gachero. Laguna costera del Mar Menor: P. Isla Perdiguera; U. Los Urrutias. La línea discontinua separa las muestras del Mar Menor de las muestras mediterráneas (modificado de González-Wangüemert et al., 2004).

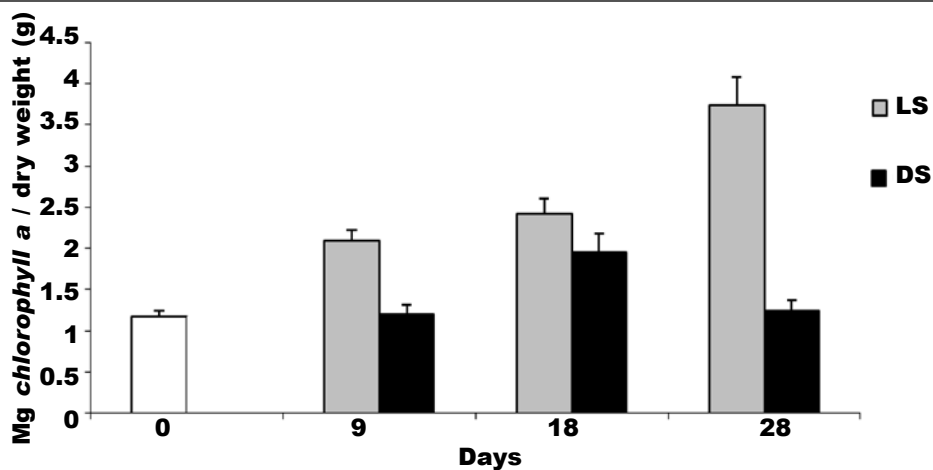


Fig. 8. Mean *chlorophyll a* concentration values (\pm SE) in the tissue of *E. timida* specimens kept in a laboratory for 28 days at a constant temperature (25°) and fed with *A. acetabulum*, for treatment light photoperiod 12:12 light and darkness (LS) and continuous darkness (DS) (modified from Giménez-Casalduero, 1997b).

Fig. 8. Concentración media de clorofila a (\pm EE) en los tejidos de especímenes de *E. timida* mantenidos en el laboratorio durante 28 días a una temperatura constante (25°C) y alimentados con *A. acetabulum* con los tratamientos fotoperiodo lumínico 12:12 de luz y oscuridad (LS) y oscuridad continua (DS) (modificado de Giménez-Casalduero, 1997b).

that the existence of morphotypes is not related to the lagoon environment.

A sign of the versatility of the animal food was observed in the course of an experiment (Giménez-Casaldueiro, 1997b) in which animals kept in laboratory conditions for 28 days and feeding on *A. acetabulum* showed an increased concentration of chlorophyll from the start day supply of algae, while in dark conditions the values remained more or less constant over time (fig. 8). The interpretation of these data could be the possible ability of this sacoglossa to feed on different algal species at least during stress situations (Ros & Rodríguez, 1985; Marín & Ros, 1989, 1993), and this would explain the different proportion of pigment found in dun animals as compared to the remaining populations analysed.

The hypothesis of a certain food versatility is somewhat striking, given the close relationship between *E. timida* and *A. acetabulum*. It is likely that the presence of the dun morph was due to degradation of pigments in aging chloroplasts. However, the animal can feed on other algae during periods of food shortages, according to some observations in the laboratory (unpublished data).

References

- Avise, J. C., 1974. Systematic value of electrophoretic data. *Systematic Zoology*, 23: 465–481.
- Ballesteros, M., 1979. *Bosellia mimetica* Trinchese, 1891 y *Elysia timida* Risso, 1818, dos ascoglossos nuevos para la fauna ibérica. *Publicaciones Dpto. Zoología Barcelona*, 4: 13–17.
- 1985. Contribución al conocimiento de los Sacoglossos y Nudibranchios (Mollusca: Opisthobranchia). Estudio anatómico, sistemático y faunístico de las especies del mediterráneo español. Ph. D. Thesis, Univ. de Barcelona.
- Bouchet, P., 1984. Les Elysiidae de Méditerranée (Gastropoda, Opisthobranchiata). *Annales de l'Institut Oceanographique (Paris)*, 60: 19–28.
- 1989. A review of poecilogony in gastropods. *Journal of Molluscan Studies*, 55: 67–78.
- Cognetti, G. & Maltagliati, F., 2000. Biodiversity and adaptative mechanisms in brackish water fauna. *Marine Pollution Bulletin*, 40: 7–14.
- Duffy, J. E. & Hay, M. E., 1991. Food and shelter as determinants of food choice by an herbivorous marine amphipod. *Ecology*, 72: 1286–1298.
- Evertsen, J., Burghardt, I., Johnsen, G. & Wägele, H., 2007. Retention of functional chloroplasts in some sacoglossans from the Indo-Pacific and Mediterranean. *Marine Biology*, 151: 2159–2166.
- Gamito, S., Gilabert, J., Marcos, C. & Pérez-Ruzafa, A., 2005. Effects of changing environmental conditions on lagoon ecology. In: *Coastal Lagoons: Ecosystem Processes and Modelling for Sustainable Use and Development*: 193–229 (I. E. Gonenç & J. Wolfiin, Eds.). CRC Press Boca Raton, Florida.
- García Gómez, J. C., 2002. Paradigmas de una fauna insólita. Los moluscos opisthobranchios del estrecho de Gibraltar. *Instituto de Estudios Gibraltareños Algeciras (Cádiz) (Serie Ciencias)*, 20: 397.
- Garrote-Moreno, A., 2007. Estudio de la ovipostura del sacogloso *Elysia timida* (Risso, 1818) en la laguna del Mar Menor. Memoria de DEA, Univ. de Alicante.
- Gavagnin, M., Spinella, A., Castelluccio, F., Cimino, G. & Marin, A., 1994. Polypropionates from the Mediterranean mollusk *Elysia timida*. *Journal of Natural Products*, 57: 298–304.
- Gendron, R. P. & Staddon, J. E. R., 1983. Searching for cryptic prey: the effect of search rate. *American Naturalist*, 121: 172–186.
- Giménez-Casaldueiro, F., 1997a. Diferencias entre dos poblaciones de *Elysia timida* (Risso, 1818) de Mar Menor (Murcia, SE de España). *Actas del Segundo Congreso de la Naturaleza de la Región de Murcia*: 79–86.
- 1997b. Relaciones y estrategias tróficas de los opisthobranchios: aposematismo, defensa química y retención de cloroplastos. Ph. D. Thesis, Univ. de Murcia.
- 1999. Estudio comparativo en diferentes poblaciones de *Elysia timida* (Risso, 1818) (Gastropoda, Opisthobranchia, Sacoglossa) en mar abierto y ambiente lagunar. *Iberus*, 17: 137–146.
- Giménez-Casaldueiro, F. & Muniain, C., 2006. Photosynthetic activity of the solar-powered lagoon mollusc *Elysia timida* (Risso, 1818) (Opisthobranchia: Sacoglossa). *Symbiosis*, 41: 151–158.
- 2008. The role of kleptoplasts in the survival rates of *Elysia timida* (Risso, 1818) (Sacoglossa: Opisthobranchia) during periods of food shortage. *Journal of Experimental Marine Biology and Ecology*, 357: 181–187.
- Giménez-Casaldueiro, A., Muniain, C. & García-Charton, J. A., 2002. *Elysia timida* (Risso, 1818) (Gastropoda, Opisthobranchia): relationship and feeding deterrence to a potential predator on the south-western Mediterranean coast. *Marine Biology*, 141: 1051–1057.
- González-Wangüemert, M., Cánovas, F., Marcos, C. & Pérez-Ruzafa, Á., 2009. Phospoglucose isomerase variability of *Cerastoderma glaucum* as a model for testing the influence of environmental conditions and dispersal patterns through quantitative ecology approaches. *Biochemical Systematics and Ecology*, 37: 325–333.
- González-Wangüemert, M., Giménez-Casaldueiro, F. & Pérez Ruzafa, A., 2006. Genetic differentiation of *Elysia timida* (Risso, 1818) populations in the Southwest Mediterranean and Mar Menor coastal lagoon. *Biochemical Systematics and Ecology*, 34: 514–527.
- González-Wangüemert, M., Pérez-Ruzafa, A., Rosique, M. J. & Ortiz, A., 2004. Genetic differentiation in two cryptic species of Ostreidae, *Ostrea edulis* (Linné, 1758) and *Ostreola stentina* (Payraudeau, 1826), in the Mar Menor lagoon (SW Mediterranean). *The Nautilus*, 118:103–111.
- Greene, R. W., 1970. Symbiosis in sacoglossan opisthobranchs: Symbiosis with algal chloroplasts. *Malacologia*, 10: 357–368.
- Greene, R. W. & Muscatine, L., 1972. Symbiosis in

- sacoglossan opisthobranchs: photosynthetic products of animal–chloroplast associations. *Marine Biology*, 14: 253–259.
- Hacker, S. D. & Steneck, R. S., 1990. Habitat architecture and the abundance and body–size–dependent habitat selection of a phytal amphipod. *Ecology*, 71: 2269–2285.
- Hagerman, L., 1970. The influence of low salinity on survival and spawning of *Elysia timida* (Montagu) (Opisthobranchia, Sacoglossa). *Sarsia*, 42: 1–6.
- Händeler, K., Grzybowski, Y., Krug, P. J. & Wägele H., 2009. Functional chloroplasts in metazoan cells—a unique evolutionary strategy in animal life. *Frontiers in Zoology*, 6: 28.
- Händeler, K., Wägele, H., Wahrmund, U., Rüdinger, M. & Knoop, V., 2010. Slugs' last meals: Molecular identification of sequestered chloroplasts from different algal origins in Sacoglossa (Opisthobranchia, Gastropoda). *Molecular Ecology Research*, 10: 968–978.
- Hay, M. E., 1991. Marine terrestrial contrasts in the ecology of plant chemical defenses against herbivores. *TREE*, 6: 362–365.
- Hay, M. E., Kappel, Q. E. & Fenical, W., 1994. Chemical defenses against different marine herbivores: interaction of chemistry, calcification and plant quality. *Ecology*, 75: 1714–1726.
- Jesus, B., Ventura, P. & Calado, G., 2010. Behaviour and functional xanthophyll cycle enhance photo-regulation mechanisms in the solar–powered sea slug *Elysia timida* (Risso, 1818). *Journal of Experimental Marine Biology and Ecology*, 395: 98–105.
- Krug, P. J., 1998. Poecilogony in an estuarine opisthobranch: Planktotrophy, lecithotrophy, and mixed clutches in a population of the estuarine opisthobranch *Alderia modesta*. *Mar Biol* 132: 483–494.
- Krug, P. J., Ellingson, R. A., Burton, R. A. & Valdés, Á., 2007. A new poecilogonous species of sea slug (Opisthobranchia: Sacoglossa) from California: Comparison with the planktotrophic congener *Alderia modesta* (Lovén, 1844). *Journal of Molluscan Studies*, 73: 29–38.
- McCloskey, L. R., Wetthey, D. S. & Porter, J. W., 1978. *Coral Reefs: Research Methods*. D. R. Stoddart & R. E. Johannes, Eds.). UNESCO, Paris.
- Mariani, S., Ketmaier, V. & de Matthaëis, E., 2002. Genetic structuring and gene flow in *Cerastoderma glaucum* (Bivalvia: Cardiidae): evidence from allozyme variation at different geographic scales. *Marine Biology*, 140: 687–697.
- Marín, A., 1988. Moluscos gasterópodos del sureste español. Faunística, ecología y estudio de la simbiosis con algas. Ph. D. Thesis, Univ. de Murcia.
- Marín, A. & Ros, J. D., 1987. Catálogo preliminar de los gasterópodos marinos del sudeste español. *Iberus*, 7(1): 137–145.
- 1988. Los sacoglossos (Mollusca, Opisthobranchia) del sudeste ibérico. Catálogo de las especies y presencia de cloroplastos algales en las mismas. *Iberus*, 8(1): 25–49.
- 1989. The chloroplast–animal association in four Iberian sacoglossan opisthobranchs: *Elysia timida*, *Elysia translucens*, *Thuridilla hopei* and *Bosellia mimetica*. *Scientia Marina*, 53: 429–440.
- 1991. Presence of intracellular zooxanthellae in Mediterranean nudibranchs. *Journal of Molluscan Studies*, 57(4 suppl.): 87–110.
- 1992. Dynamics of a peculiar plant–herbivore relationship: The photosynthetic ascoglossan *Elysia timida* and the chlorophycean *Acetabularia acetabulum*. *Marine Biology*, 112: 677–682.
- 1993. Ultrastructural and ecological aspects of the development of chloroplast retention in the sacoglossan gastropod *Elysia timida*. *Journal of Molluscan Studies*, 59: 95–104.
- 2004. Chemical defenses in sacoglossan opisthobranchs: Taxonomic trends and evolutive implications. *Scientia Marina*, 68: 227–241.
- Mathias, A., Kisdí, E. & Olivieri, I., 2001. Divergent evolution of dispersal in a heterogeneous landscape. *Evolution*, 55: 246–259.
- Meyers, L. A. & Bull, J. J., 2002. Fighting change with change: adaptive variation in an uncertain world. *Trends in Ecology & Evolution*, 17: 551–557.
- Michaelis, L. & Menten, M. L., 1913. Der chinetik der invert wirkung. *Biochemestry Z*, 49: 333–369.
- Monseline, E. B. & Rahat, M., 1980. Photobiology of *Elysia timida* (Mollusca: Opisthobranchia): Observations in the sea. *Israel Journal of Zoology* 29: 125–128.
- Ortea, J., Moro, L. & Espinosa, J., 1997. Nuevos datos sobre el género *Elysia*, 1818 (Opisthobranchia: Sacoglossa) en el Atlántico. *Revista de la Academia Canaria de Ciencias*, 9: 141–155.
- Pérez, M., 1989. *Fanerógamas marinas en sistemas estuárico: producción, factores limitantes y algunos aspectos del ciclo de nutrientes*. Ph. D. Thesis, Univ. de Barcelona.
- Rahat, M., 1976. Direct development and symbiotic chloroplasts in *Elysia timida* (Mollusca: Opisthobranchia). *Israel Journal of Zoology*, 53: 186–193
- Rahat, M. & Monseline, E. B., 1979. Photobiology of the chloroplast hosting mollusc *Elysia timida* (Opisthobranchia). *Journal of Experimental Biology*, 79: 125–128.
- Remmert, H., 1988. *Ecología, autoecología, ecología de poblaciones y estudio de ecosistemas*. Blume, Barcelona.
- Risso, A., 1818. Memoire sur quelques Gasteropodes nouveaux, Nudibranches et Tectibranches observes dans la mer de Nice (1). *Journal de Physique, de Chimie, d'Histoire naturelle et des Arts (Paris)*, 87: 368–377.
- Ros, J. D., 1976. Catálogo provisional de los opisthobranchios (Gastropoda: Euthyneura) de las costas ibéricas. *Miscel-lània Zoològica*, 3: 21–51.
- 1977. La defensa en los opisthobranchios. *Investigación y Ciencia*, 12: 48–60.
- Ros, J. D. & Rodríguez, J., 1985. La simbiosis algal en *Elysia timida* Risso, 1818. Primeros resultados. *Anales de Biología*, 4: 37–47.
- Rumpho, M. E., Summer, E. J., Green, B. J., Fox, T. C. & Manhart, J. R., 2001. Mollusc–algal chloroplast symbiosis: how can isolated chloroplasts continue to function for months in the cytosol of a sea slug in the absence of the algal nucleus?

- Zoology*, 104: 303–312.
- Rumpho, M. E., Summer, E. J. & Manhart, J. R., 2000. Solar-powered sea slugs. Mollusc/algal chloroplast symbiosis. *Plant Physiology*, 123: 29–38.
- Sites, J. W. & Marshal, J. C., 2003. Delimiting species: a renaissance issue in systemic biology. *Trends in Ecology and Evolution*, 18: 462–470.
- Schmitt, V., Anthes N. & Michiels N. K., 2007. Mating behaviour in the sea slug *Elysia timida* (Opisthobranchia, Sacoglossa): hypodermic injection, sperm transfer and balanced reciprocity. *Frontiers in Zoology*, 4: 17.
- Swennen, C., 1961. On a collection of Opisthobranchia from Turkey. *Zoologische Medelingen, Leiden*, 38: 41–75.
- Templado, J., 1982. Datos sobre los opisthobranchios del Cabo de Palos (Murcia). *Bollettino Malacologico*, 18(9–12): 247–254.
- Thompson, T. E. & Jaklin, A., 1988. Eastern Mediterranean Opisthobranchia: Elysiidae (Sacoglossa, Ascoglossa). *Journal of Molluscan Studies*, 54: 59–69.
- Thorpe, J. P., 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In: *Protein Polymorphism: Adaptive and Taxonomic Significance*: 131–152 (G. S. Oxford & D. Rollison, Eds.). Academic Press, London.
- Trench, R. K. & Ohlhorst, S., 1976. The stability of chloroplasts from siphonaceous algae in symbiosis with sacoglossan molluscs. *New Phytologist*, 76: 99–109.
- Trench, R. K. & Smith, D. C., 1970. Synthesis of pigment in symbiotic chloroplasts. *Nature*, 227: 196–197.
- Türkmen, A. & Demirsoy, A., 2009. Contributions to the Eastern Mediterranean Opisthobranchia (Mollusca: Gastropoda) Fauna of Turkey. *Turkey Journal of Zoology*, 33: 57–68.
- Valdés A., Hamann J., Behrens D. W. & DuPont A., 2006. *Caribbean sea slugs. A field guide to the opisthobranchs mollusks from the tropical north-western Atlantic*. Sea Challengers Natural History Museum Books, Gig Harbor, Washington.
- Vergara-Chen, C., González-Wangüemert, M., Marcos, C. & Pérez-Ruzafa, A., 2010a. Genetic diversity and connectivity remain high in *Holothuria polii* (Delle Chiaje 1823) across a coastal lagoon–open sea environmental gradient. *Genetica*, 138: 895–906.
- 2010b. High gene flow promotes the genetic homogeneity of *Pomatoschistus marmoratus* (Risso, 1810) from Mar Menor coastal lagoon and adjacent marine waters. *Marine ecology: an evolutionary perspective*, 31: 270–275.
- Wägele, H., Deusch, O., Händeler, K., Martin, R., Schmitt, V., Christa, G., Pinzger, B., Gould, S.B., Dagan, T., Klusmann-Kolb, A. & Martin, W., 2010. Transcriptomic evidence that longevity of acquired plastids in the photosynthetic slugs *Elysia timida* and *Plakobrachus ocellatus* does not entail lateral transfer of algal nuclear genes. *Molecular Biology and Evolution*, 28: 699–706.
- Wägele, H. & Johnsen, G., 2001. Observations on the histology and photosynthetic performance of 'solar-powered' opisthobranchs (Mollusca, Gastropoda, Opisthobranchia) containing symbiotic chloroplasts or zooxanthellae. *Organism Diversity and Evolution*, 1: 193–210.
- Wirtz, P. & Anker, A., 2009. Range extension for *Elysia timida* (Opisthobranchia: Sacoglossa) to São Tomé Island (Eastern Central Atlantic), with a film showing the curious locomotion of the species. *Marine Biodiversity Records*, 2: e144.
- Wright, S., 1977. Evolution and genetics of populations. Volume 3. *Experimental results and evolutionary deductions*. Univ. of Chicago Press, Chicago.
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